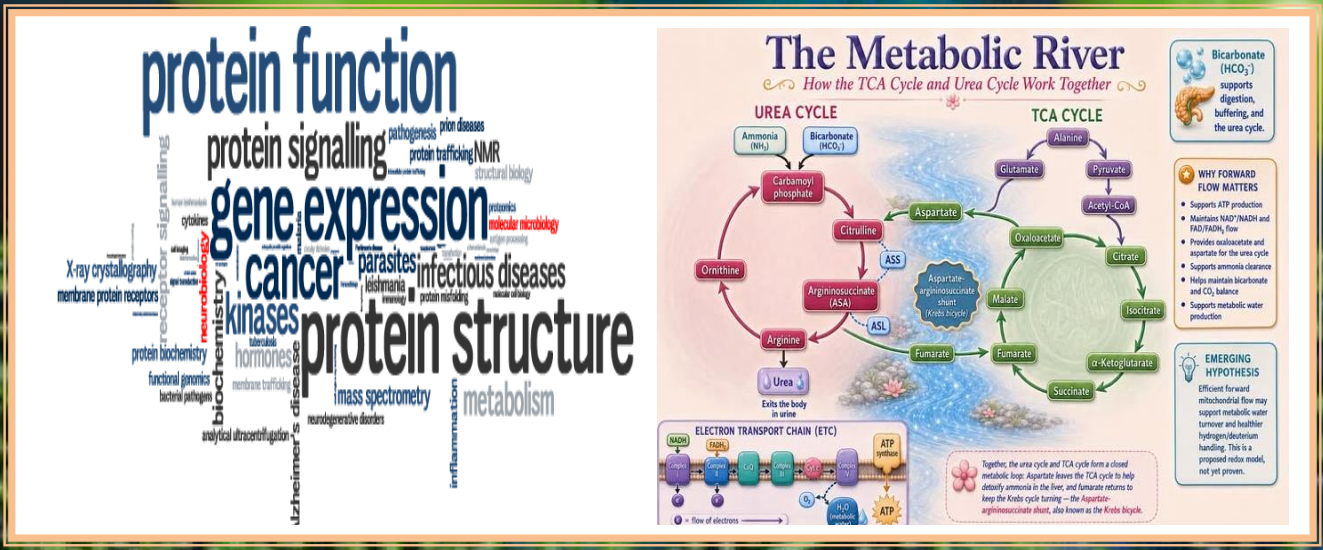


Université Ferhat Abbas –Sétif- 1
Faculté des Sciences de la
Nature et de la Vie
Département des études de base

DEPARTMENT OF COMMON CORE

BIOCHEMISTRY



Prepared by:

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***Level*: 2nd Year (LMD)**

Academic year :

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Semester: 3rd Semester
UE: Fundamental Teaching Unit 2
Subject 1: Biochemistry
Objectives of the teaching
This course aims to provide instruction on the fundamental principles of biochemistry and the concepts of enzymology, and to familiarize students with biochemical techniques.
Recommended prior knowledge (brief description of the knowledge required to follow this course – Maximum 2 lines).
The student must have some knowledge of chemical bonds (weak and strong) and the physicochemical properties of organic molecules.
Content
1. Chemical bonds
1.1. Strong bonds
1.2. Weak bonds
2. Structure and physicochemical properties of carbohydrates
2.1. Simple sugars
2.2. Oligosides
2.3. Polyholosides, heterosides.
3. Structure and physicochemical properties of lipids
3.1. Simple lipids
3.2. Complex lipids
4. Structure and physicochemical properties of amino acids, peptides, and proteins
4.1. Amino acids, peptides, proteins
4.2. Structure (primary and secondary, tertiary and quaternary)
4.3. Properties and effects of treatments (solubility, electrophoretic behaviour, denaturation.)
4.4. Separation of proteins
5. Concepts of Enzymology
5.1. Definition, classification
5.2. Mechanisms of action 5.3. Active site 5.4. Enzyme kinetics and types of representation
5.5. Enzymatic inhibition
5.6. Allosteric phenomenon
6. Concepts of bioenergetics
6.1. Types of chemical reaction
6.2. The respiratory chain and energy production 6.3. Phosphorylation and redox reaction
7. Carbohydrate metabolism
7.1. Catabolism (glycolysis, glycogenolysis, pentose phosphate pathway, Krebs cycle, energy balance)
7.2. Anabolism (gluconeogenesis and glycogenesis)
7.3. Regulation
8. Lipid metabolism
8.1. Catabolism of fatty acids (Beta-oxidation)
8.2. Catabolism of sterols
8.3. Biosynthesis of fatty acids and triglycerides
8.4. Biosynthesis of sterols
8.5. Regulation
9. Metabolism of peptides and proteins
9.1. Catabolism of amino groups
9.2. Catabolism of carboxylic groups
9.3. Catabolism of the side chain

9.4. Glucogenic and ketogenic amino acids
9.5. Biosynthesis of essential amino acids
9.6. Nitrogen elimination, urea cycle 9.7. Example of peptide biosynthesis (case of biologically active peptides) 9.8. Example of protein biosynthesis
9.9. Regulation

10. Structure and metabolism of other biologically relevant compounds

10.1. Vitamins
10.2. Hormones

Foreword

This modest handout, designed as a collection of several references, aims to present the basics of structural and metabolic biochemistry to support second-year LMD common core students in natural and life sciences within the framework of the biochemistry module.

Throughout its preparation, I have tried to present the information in the simplest and most didactic way possible.

In the first part dedicated to structural biochemistry, five fundamental chapters have been covered, namely: chemical bonds, carbohydrates, lipids, amino acids, peptides and proteins, enzymes as well as vitamins and coenzymes. In the physicochemical properties of these different constituents of living matter, I have tried to provide and emphasize the purpose of the existence of such reactions in the organism and their uses for analytical purposes.

After understanding the structure and properties of biological macromolecules, the student is led, through the second part devoted to metabolic biochemistry, to explore all the chemical and energetic transformations that take place within the cell.

The main objective of this second part is to make complex metabolic pathways accessible, often perceived as abstract, by highlighting the molecular and bioenergetic logic of living organisms.

Its content is structured around several major axes:

Bioenergetics: Introduction to energy transfers and the central role of molecules like ATP.

Carbohydrate Metabolism: Study of catabolic pathways (glycolysis) and anabolic pathways (gluconeogenesis), as well as regulatory cycles.

Lipid and Protein Metabolism: Analysis of fatty acid degradation (beta-oxidation) and the fate of amino acids.

Each chapter has been written with clarity in mind, enriched with reaction diagrams and illustrations to facilitate memorization and understanding of mechanisms. Links are also established between different pathways, illustrating the constant interconnection of intermediate metabolism.

Hopefully, this educational tool will serve as an effective work support for your success.

Dr. Sihem SOUFANE

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INTRODUCTION TO THE STUDY OF BIOCHEMISTRY

Biochemistry is the scientific discipline that studies the chemical reactions occurring within cells. It is divided into two groups: static biochemistry (studies composition and physicochemical properties) and dynamic biochemistry (studies transformations and chemical reactions).

The term was created in 1903 by Carl Neuberg based on the Greek root Βιοχημεία (biochēmeia). The main categories of molecules studied in biochemistry are carbohydrates, lipids, proteins, and nucleic acids. These molecules are primarily composed of carbon, oxygen, and nitrogen. These classes of molecules represent the fundamental elements of the construction and functioning of the cell.

The study of the structure of these molecules, their properties, the mode of linkage, and the properties of the constituent molecules is the domain of ***structural biochemistry***. ***Metabolic biochemistry*** focuses on the study of all chemical reactions and energy transfers that occur in living cells. It analyses how organisms transform matter to produce, maintain, and use the energy essential for their survival. This field of study is based on two fundamental and complementary mechanisms:

Catabolism: It involves the breakdown of large complex molecules (carbohydrates, lipids, proteins) into simpler molecules. This process releases energy (often stored in the form of ATP).

Anabolism: Also called biosynthesis, it uses the energy produced by catabolism to assemble simple molecules and create the components necessary for growth and cellular function. These reactions are not isolated but organised into metabolic pathways, a succession of steps where the product of one reaction becomes the substrate of the next. Each step is controlled by a specific enzyme.

CHAPTER I :

CHEMICAL BONDS



CHAPTER I Chemical bonds

Atoms tend to acquire the most stable electronic structure, which is that of the nearest noble gas in the periodic table. To acquire this electronic structure, atoms can either gain or lose electrons to form ions or form chemical bonds by sharing one or more pairs of electrons, which are called bonding pairs; they are represented by a dash between the two atoms.

I. 1. Chemical Bonds

A chemical bond is an attractive interaction between atoms, at a distance that allows the system to stabilize and a new chemical entity to form. This bond is very strong. Breaking strong chemical bonds requires the use of energies of several hundred kilojoules per mole (through a thermal or chemical treatment, using enzymes, for example).

Bonds are classified into two categories: high-energy bonds and low-energy bonds. Covalent, coordination, metallic, and ionic bonds are high-energy bonds. Van der Waals, hydrogen, hydrophobic, etc., bonds are low-energy bonds.

I. 1. 1. Covalent bond

The electrons in the outermost shell of an atom are called valence electrons. A covalent bond between two neighboring non-metallic atoms A and B results from the sharing of these electrons. This bond can be non-polar, if the atoms have practically the same electronegativity, or polar if the difference between their electronegativities remains less than 1.7 (the conventional value). Above this value, the interaction is said to be ionic. A covalent bond (polarized or non-polar) can be single (Fig. 1), double, or triple. To simplify the representation of molecules, a covalent bond is represented by a single line (which is doubled for a double bond).

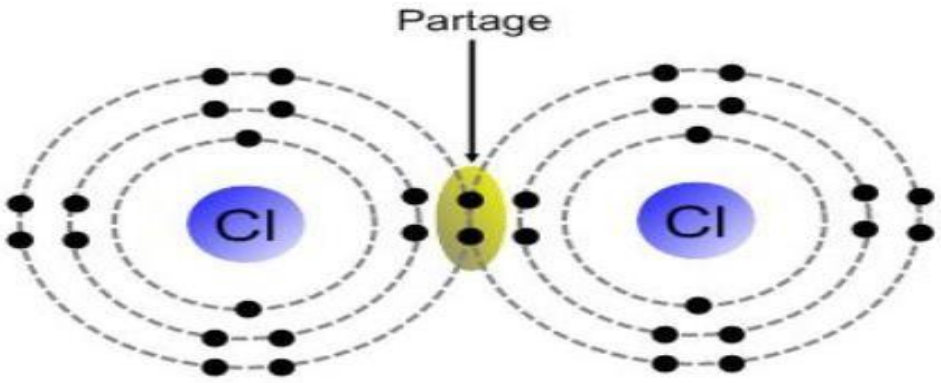


Fig. 1. Example of Cl_2 covalent bond.

The formation of covalent bonds results in an outer shell occupancy comparable to that of the nearest noble gas in the same period. The corresponding number of electrons is 2 for hydrogen, which therefore adopts the electron configuration of helium. All other noble gases have 8 electrons in their outer shell; elements tend to fill their outer shell with 8 electrons (octet rule), as is the case with carbon, nitrogen, and oxygen. The constituents of common biological molecules can form from 1 to 6 covalent bonds.

I. *1. 2. Coordinate Bond, Dative Bond, or Donor-Acceptor Bond*

This is the sharing of two electrons between two atoms A and B. One of the atoms provides the two electrons.

I. 1.3. Metallic Bond

This is the sharing of all valence electrons within the metal. This results in a conduction band. This is why a metal is capable of conducting electrical energy.

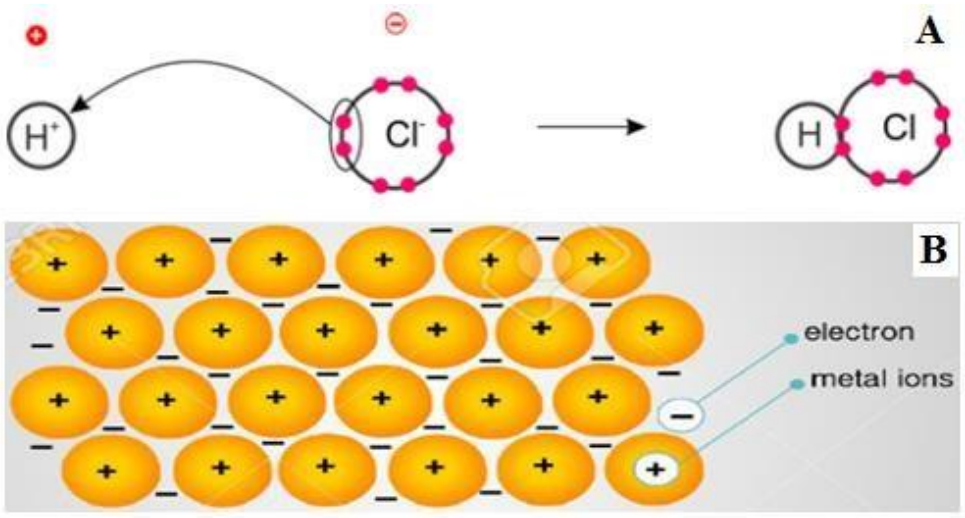


Fig. 2. Example of HCl coordination bond (A), metallic bond (B).

I. 1.4. Ionic Bonding

Some atoms do not share a pair of electrons, as in a covalent bond, but instead lose or gain one or more electrons to complete their valence electron shell. Consequently, the sum of the protons is no longer equal to the sum of the electrons. The atom then exhibits a net electric charge. It is an ion.

By losing one or more electrons, it becomes a cation (a positively charged atom or group of atoms). By gaining one or more electrons, it becomes an anion (a negatively charged atom or group of atoms). According to the basic principle of electricity, opposite charges attract while like charges repel. Thus, a cation can associate with an anion through a bond called an ionic bond. This is the case with sodium chloride (NaCl), i.e., table salt (Fig. 2).

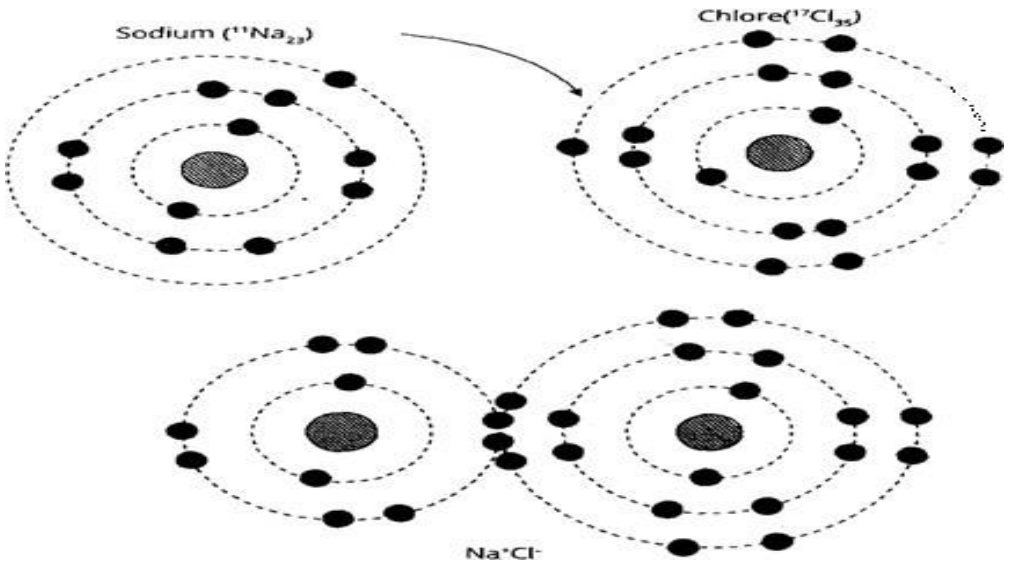


Fig. 3. Formation of an ionic bond between sodium and chlorine.

When an ion carries a single charge (Na^+, Cl^- it is said to be monovalent). If it carries two charges (Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}), it is said to be divalent.

I. 1.5. Hydrogen bonding

This interaction occurs when a hydrogen atom, covalently bonded to an electronegative atom (D for "donor"), is located near a second electronegative atom (A for "acceptor"). Due to its electronegativity, the donor atom (most often O or N) attracts the electrons of the covalent bond and carries a partial negative charge (d–), compensated by a partial positive charge at the hydrogen (d+): this bond is said to be polarized.

I.1. 6. Van der Waals' Forces

Van der Waals bonds are named after the Dutch theoretical physicist Johannes Diderik Van der Waals (1837-1923). They are an intermolecular bond formed between molecules of a substance (unlike covalent bonds, which are intramolecular because they are formed between atoms of the same molecule). More precisely, this bond is a weak electrical interaction between molecules with a dipole moment. By definition, these molecules are overall neutral but have a positive pole (center of partial positive charges located on the least electronegative atoms) and a negative pole (center of partial negative charges). An overall attractive electrical force is exerted between the

The poles of opposite poles of the different molecules. Van der Waals forces are of three types:

• **Permanent dipole-permanent dipole interactions:** Electrostatic forces exist between two polar molecules. The positive pole of one molecule attracts the negative pole of a neighboring molecule. These interactions contribute to the cohesion of the systems (Fig. 4A).

• **Permanent dipole – induced dipole interactions:** This type of interaction can also exist between a polar molecule and a non-polar molecule. The polar molecule creates an electromagnetic field around itself. The positive pole of the polar molecule attracts the negative charges of the non-polar (neighboring) molecule towards its side (Fig. 4B).

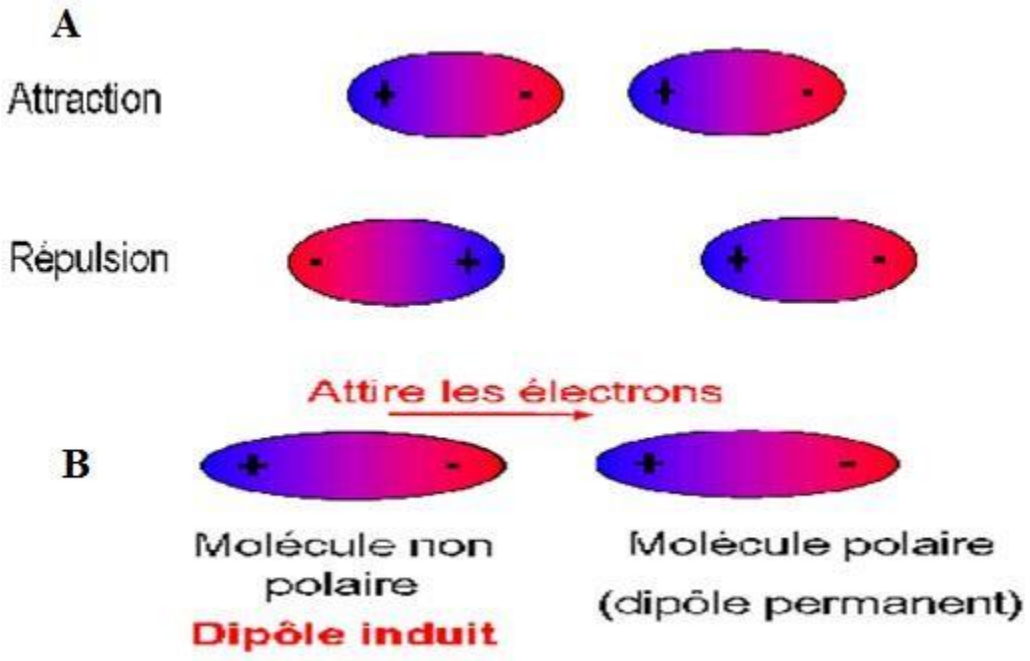


Fig. 4. Interactions of 2 permanent dipoles (A) and induced dipole – permanent dipole (B).

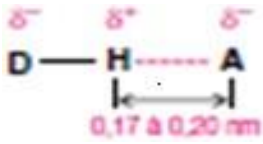
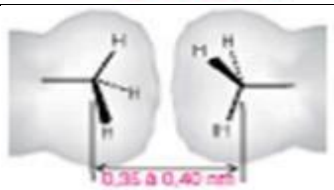
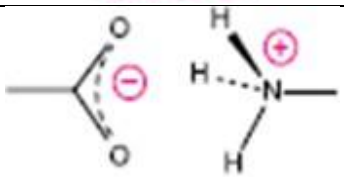
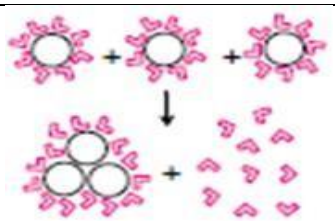
Instantaneous dipole-instantaneous dipole interactions: In neutral and nonpolar atoms and molecules, electrons "move," creating instantaneous dipoles. Interactions can exist between these dipoles and contribute to the cohesion of the molecule.

I. 1.7. Hydrophobic Interaction

Nonpolar and weakly polarizable molecules tend to cluster together, creating a hydrophobic bond. These are interactions between molecules or groups that have very little affinity for the solvent in which they are dissolved (water). The groups will position themselves to present the smallest possible surface area in contact with the water.

Indeed, when salad dressing is shaken vigorously, the oil gradually separates from the vinegar: this phase separation phenomenon is a consequence of the hydrophobic interaction that occurs at the molecular level. Shaking disperses the nonpolar groups (Table 1) in a polar medium in which they are insoluble (for example, water): the water molecules organize themselves (hydrogen bonds) around the nonpolar groups. This situation spontaneously evolves towards a more stable state, where the nonpolar molecules come together (van der Waals forces) and, little by little, form a distinct phase. At the same time, water molecules initially bound around the nonpolar groups are released into the surrounding medium.

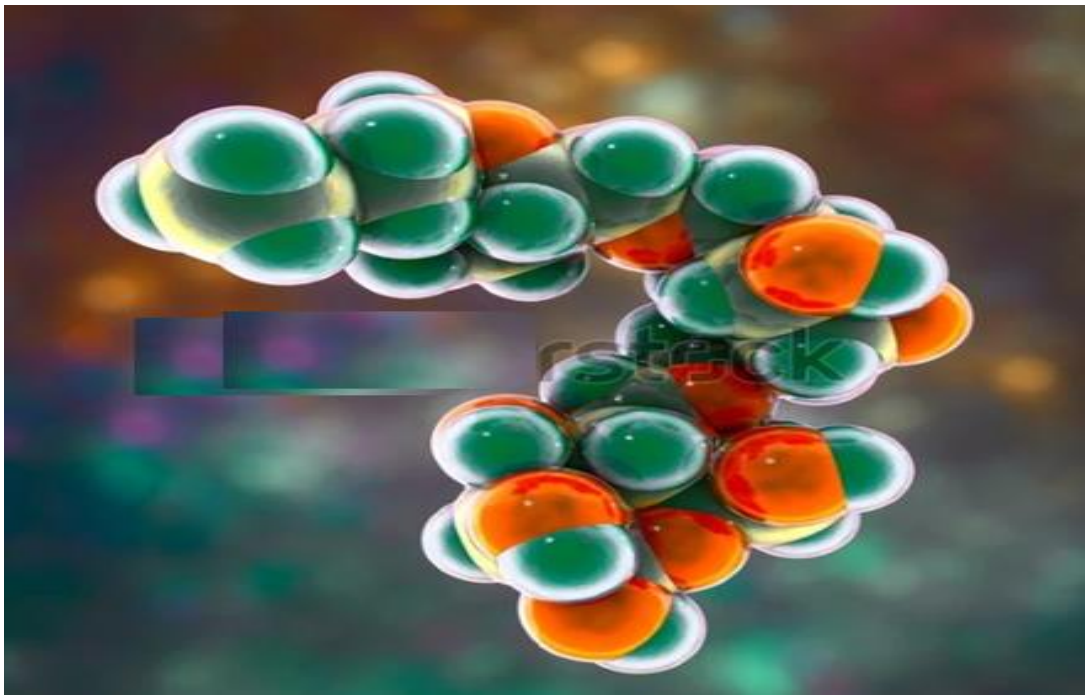
Table 1: The different types of connections.

Interaction	Examples	Δ, G° (kj.mol^{-1})	Intervention
H Bond		-10 to -40	Nucleic acids (base pairing) Proteins (secondary structures)
Van der Waals force		-4	Biological membranes Protein folding DNA (stacking of base pairs)
Ionic bond		-40	Proteins (salt bridges)
Hydrophobic interaction		-4 to -13	Proteins (folding stabilization) Biological membranes

CHAPTER II :

CARBOHYDRATES:

Structure and physicochemical properties



CHAPTER II Carbohydrates : Structure and physicochemical properties

II. 1. Definition

Carbohydrates (from the Latin glucis: "sweet") formerly called hydrates of carbon because of their basic generic formula $C_n(H_2O)_n$, are organic molecules characterized by the presence of carbon chains bearing hydroxyl groups, aldehyde or ketone functions, and possibly carboxyl or amine functions.

These molecules play a wide variety of functions within living organisms. In animals and plants, carbohydrate polymers (glycogen, starch) serve as energy reserves. Other polymers (cellulose, chitin, etc.) are also found in cell walls (protective role). Carbohydrate derivatives are found in a large number of biological molecules such as nucleic acids, DNA and RNA.

II. 2. Classification:

Structurally, carbohydrates can be divided into monosaccharides and polysaccharides (Fig. 5).

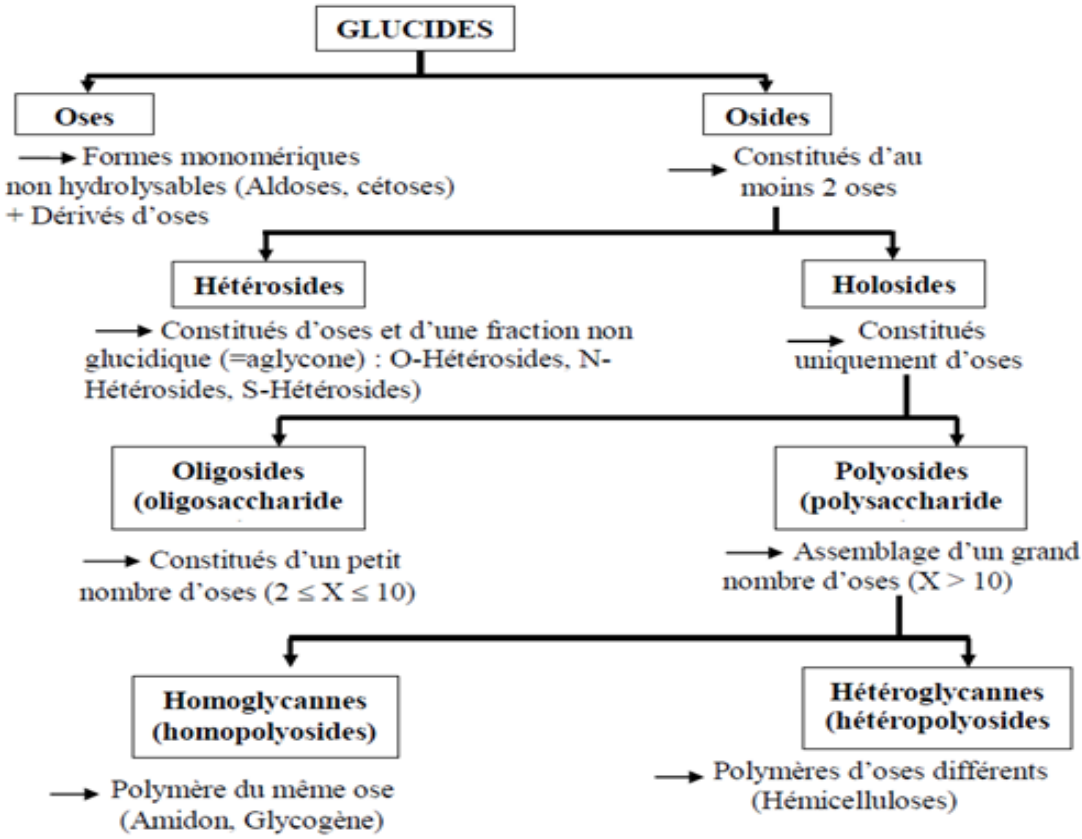


Fig. 5. Carbohydrates classification.

From a physiological perspective, carbohydrates can also be classified according to their digestibility (whether they are assimilated or not) into two categories:

- **Digestible carbohydrates** (digestible by our own enzymes): these are disaccharides (maltose, isomaltose, sucrose, lactose) and certain polysaccharides (starch, glycogen).
- **Non-digestible carbohydrates** (not digestible by our own enzymes): often grouped under the term dietary fiber. These include cellulose, hemicellulose, pectin…

II. 3. Sugars (simple sugars or monosaccharides)

Sugars can be divided into two categories: simple sugars and sugar derivatives (osamines, uronic acids, sialic acid).

II. 3. 1. Linear Structure

Simple sugars (monosaccharides) constitute the structural unit of carbohydrates. They have an unbranched carbon chain with single bonds between carbon atoms, an aldehyde group at C1 (aldoses) or a ketone group at C2 (ketoses), and an alcohol group on each of the other carbons. They are also called trioses, tetroses, pentoses, hexoses, etc., depending on the number of carbons (3, 4, 5, and 6, respectively). The designation of a sugar combines the two prefixes: aldopentose, ketohexose, etc.

II. 3. 2. Fisher projection representation

The simplest monosaccharides consist of three carbon atoms (trioses). Two of these are functional isomers: glyceraldehyde and dihydroxyacetone (Fig. 6). The carbon atoms of a monosaccharide are numbered starting from the most oxidized carbon.

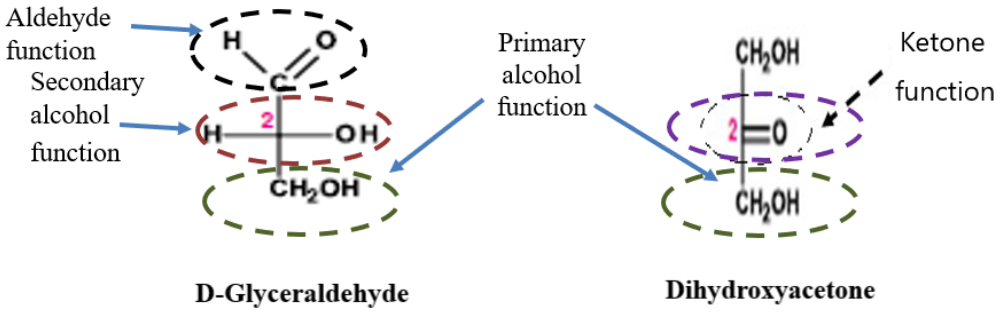


Fig. 6. Structure of glyceraldehyde and dihydroxyacetone.

For sugars with a longer carbon chain and therefore a greater number of asymmetric carbons, the Fisher representation has become the standard. According to this representation, the longest carbon chain is aligned vertically, with the most oxidized terminal carbon (bearing the carbonyl group) at the top and the non-carbon substituents horizontally. The asymmetric carbon is placed in the plane of projection (Fig. 7).

The configuration of the penultimate carbon (C_{n-1}) defines the membership of the sugar in the D series or L. A sugar belongs to the Fischer series D if the OH group is on the right at the C* (n-1) and to the L series if the OH group is on the left at the C* (n-1). Almost all natural sugars belong to the D series (the L series is rarely found, in certain plants and microorganisms).

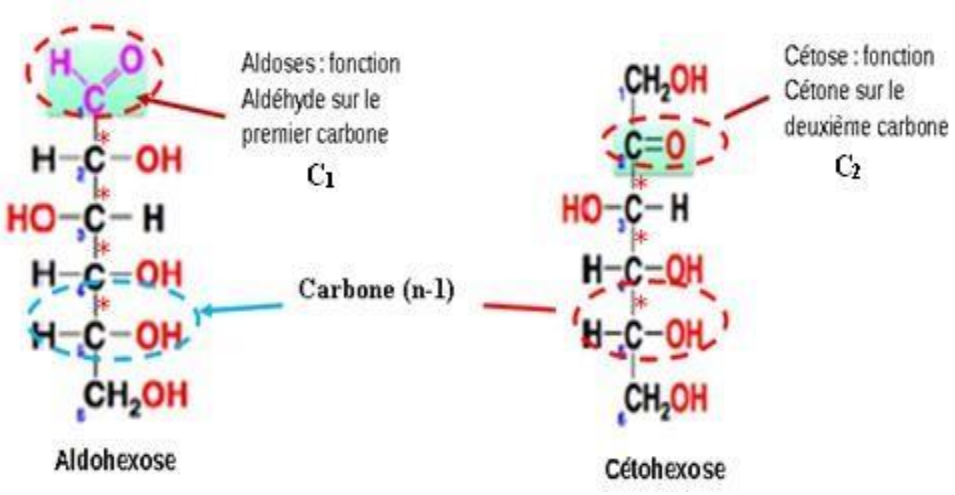


Fig. 7. Representation of sugars in Fisher projection.

II. 3. 3. Stereoisomerism and chirality

II. 3. 3. 1. Stereoisomerism (configurational isomerism)

This term refers to spatial arrangement isomers, that is, molecules of identical constitution (they have the same semi-developed formula) but whose spatial organization of atoms is different.

A molecule is said to be ***chiral*** if and only if it is not superimposable on its mirror image. It is a molecule possessing a single asymmetric center (an atom "A" bonded to 4 different substituents), denoted **A***.

In the glyceraldehyde molecule (Fig. 8), the carbon atom 2 bearing four different substituents is said to be ***asymmetric*** (often denoted C*). Only dihydroxyacetone does not possess an asymmetric carbon.

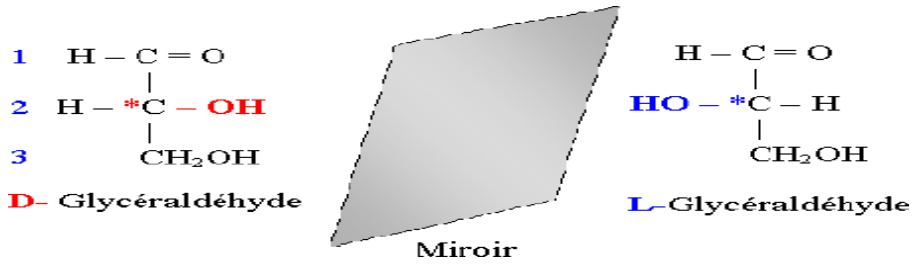


Fig. 8. The glyceraldehyde enantiomers.

II. 3. 3. 2. Forms of Isomerism

a. Enantiomerism: Enantiomers are non-superimposable isomers that differ in the absolute configuration of all their asymmetric carbons and are mirror images of each other. This is the case, for example, of D- and L-glucose (Table 2). The chemical and physical properties of enantiomers are generally identical, with the exception of optical rotation.

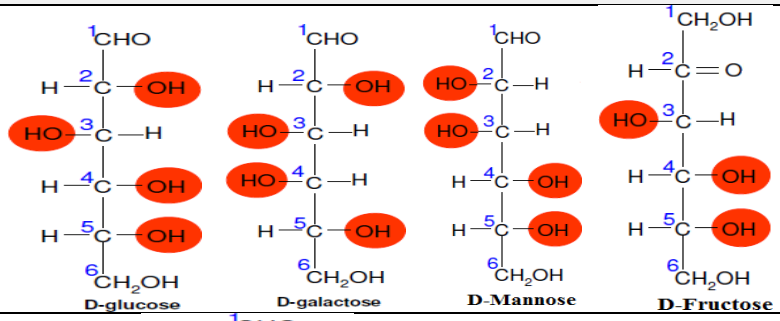
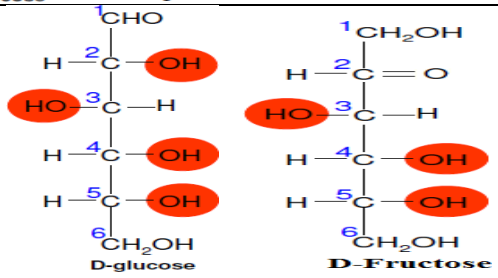
b. Epimeria: Two sugars that differ by the configuration of a single asymmetric carbon atom are epimers. Indeed, D-galactose and D-glucose are epimers at position 4 (Table 2).

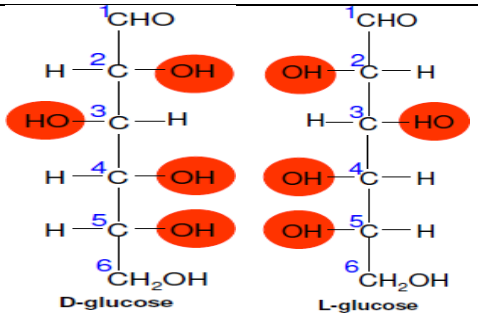
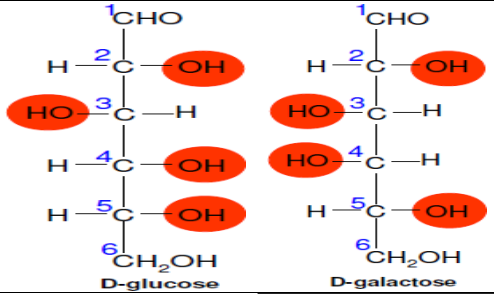
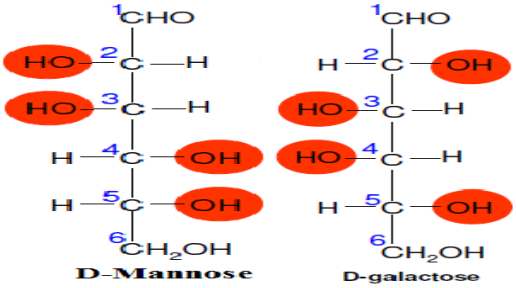
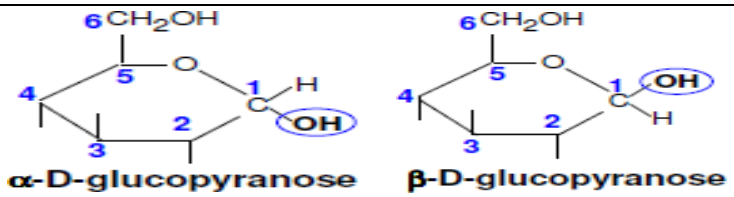
c. Diastereoisomerism: Two isomers are said to be diastoisomers if the difference lies in the number of carbon atoms (C*) between 1 and their total number n of C* atoms.

Example: mannose and galactose (Table 2) are diastereomers because they differ in the configurations of 2 from 4 of their C* atoms.

In general, for **n** asymmetric carbons, we will have $\mathbf{2^n}$ stereoisomers. For aldoses with n carbon atoms, we have **(n-2)** C* and therefore **2(n-2)** stereoisomers. For ketoses with n carbon atoms, we have **(n-3) C*** and therefore **2(n-3)** stereoisomers.

Table 2. The different types of sugar isomerism.

Isomerism	Example
Stereoisomers: Compounds having identical chemical formulas, but different spatial structures.	
Functional isomers: Even chemical formula with functions different (aldehyde/ketone)	

Enantiomers: 2 stereoisomers are mirror images of each other and are not superimposable.	
Epimers: The same chemical formula but differ only in the configuration of one asymmetric C.	
Diastereomers: The same chemical formula but the difference relates to a number of C* (**lower** to the total number of C* and greater than one C*).	
Anomers: The same chemical formula but differ in the spatial position of the OH of the anomeric C.	

II. 3. 4. Lineage of the oses

The lineage of sugars is established by adding carbon atoms to the carbon chain of the two precursor trioses of sugars (glyceraldehyde and dihydroxyacetone). This yields different sugars with secondary alcohol groups in different positions.

II. 3. 4. 1. Kiliani-Fischer Hydrocyanic Synthesis

It is a succession of several chemical addition reactions. It consists of adding to the carbon chain of an aldose (n carbons) an asymmetric carbon atom bearing an alcohol function allowing it to pass to its higher homologue (n+1) (Fig. 9).

This synthesis proceeds through the following steps (Fig. 9): The (n-carbon) aldose reacts with hydrogen cyanide (HCN) via its aldehyde group, leading to the formation of two epimeric cyanohydrin molecules. In the presence of water, the cyanohydrin molecule adopts a carboxyl function and it is transformed into an aldonic acid. The aldonic acid undergoes reduction by lithium borohydride ($LiBH_4$) or Na amalgam, which transforms the carboxyl function into an aldehyde function and thus, an aldose with (n+1) carbon is obtained.

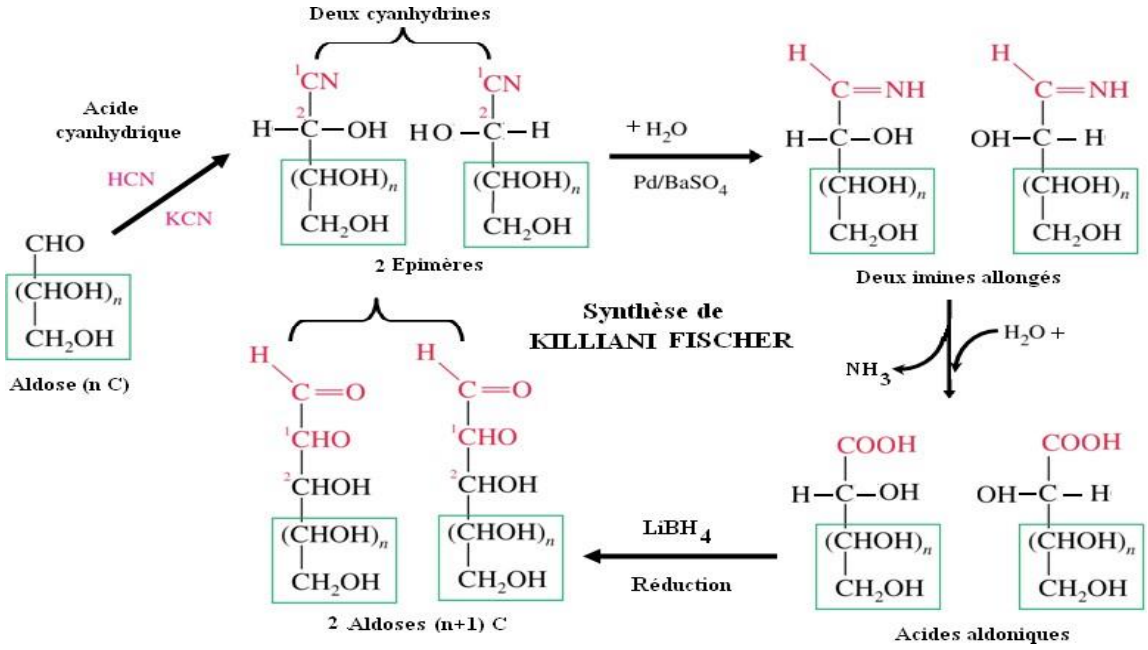


Fig. 9. Kiliani-Fischer synthesis pathway.

II. 3. 4. 2. Degradation of WOHL-ZEMPLEN

In contrast to the cyanide synthesis method, there is a degradation method called the Wohl degradation, which allows the conversion of a sugar into its lower-order counterpart (with one less carbon atom). This transforms a sugar with n carbon atoms into a sugar with (n-1) carbon atoms. The used reagent is hydroxylamine (NH_2OH) (Fig. 10).

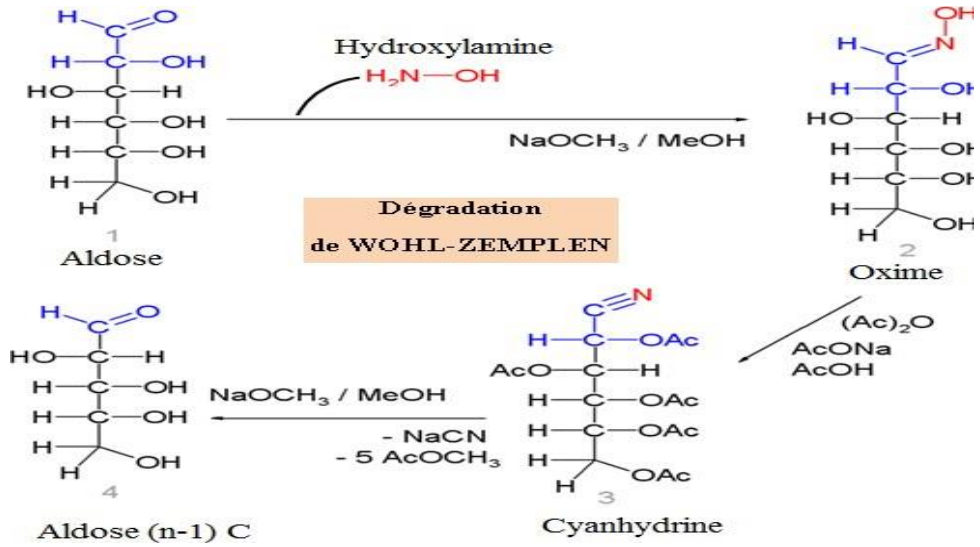


Fig. 10. WOHL-ZEMPLEN degradation pathway.

The principle is as follows (Fig. 10): In the first step, D-glucose (1) is converted to glucose oxime (2) by reaction with hydroxylamine and sodium methoxide. In a second step, the oxime is reacted with acetic anhydride in sodium acetate under heating. This produces acetylation of all alcohol groups (esterification) as well as the oxime group (3), yielding an unstable compound. Finally, the oxime acetate is converted to a nitrile, pentaacetyl glycononitrile (4).

II. 3. 4. 3. Relationship between aldoses and ketoses

Figures 11 and 12 respectively show D-aldoses and D-ketoses and indicate their stereochemical relationships. The process from D-glyceraldehyde or dihydroxyacetone to tetroses, then to pentoses, and finally to hexoses involves adding, at each step, a tetrahedral carbon atom bearing a hydroxyl group and a hydrogen atom, directly below the carbon atom of the carbonyl group. This carbon atom thus becomes a new chiral center, with two possible relative orientations of the substituents, creating a new pair of stereoisomers.

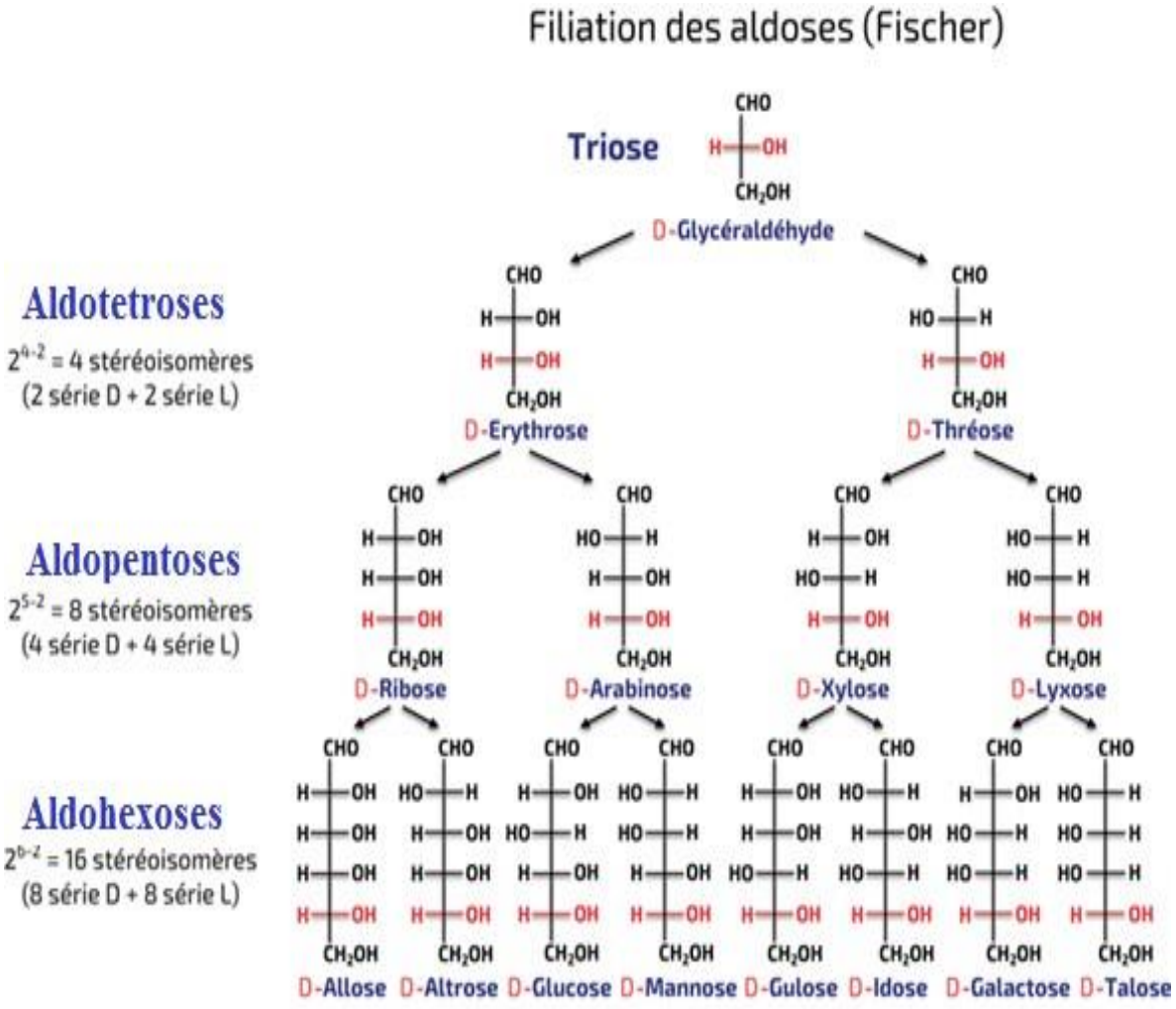


Fig. 11. Structure and stereochemistry of D-aldoses.

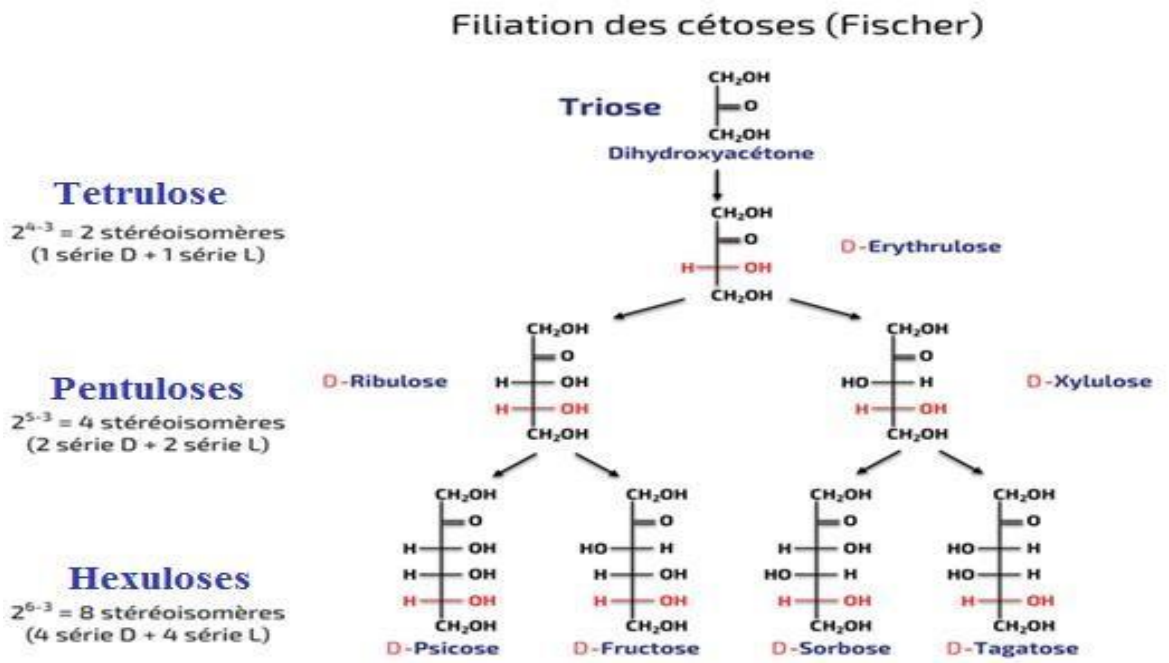


Fig. 12. Structure and stereochemistry of D-ketoses.

II. 3. 5. Objections to the linear form

The structure of sugars has been described as a linear carbon chain, but this representation is not satisfactory because it does not explain a number of observations (Table 3).

II. 3. 5. 1. Cyclic structures, anomericity and conformations

II. 3. 5. 1. 1. Cyclic structure of sugars

To interpret the anomalies described in the previous paragraph, Tollens put forward in 1883 a hypothesis to explain them and arrive at a cyclic representation of sugars: a glycosidic bridge is established by the formation of an internal hemiacetal bond between C1 (aldehyde function) and one of the alcohol functions of the same sugar, thus forming a ring and creating a new C* (Fig. 13).

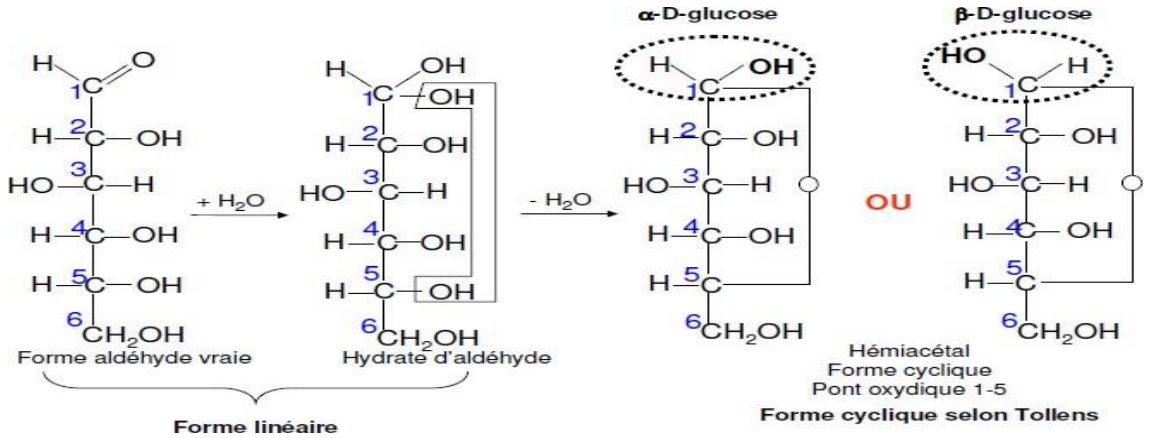
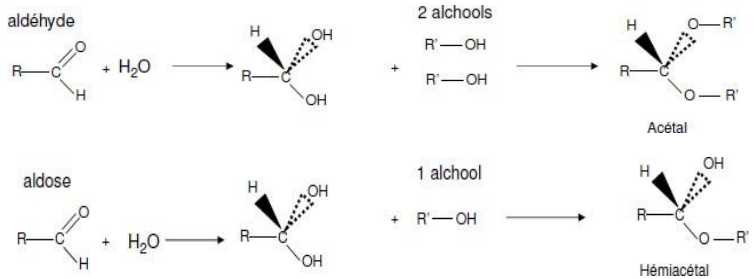
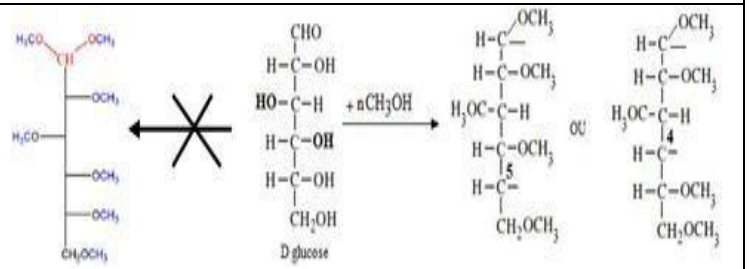
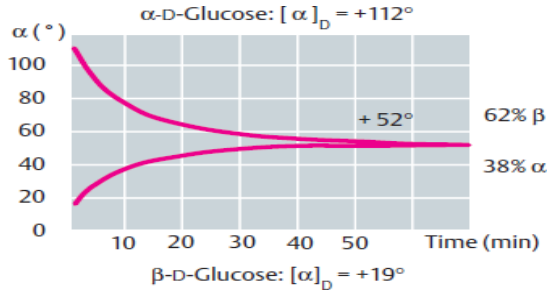


Fig. 13. Conversion of D-glucose according to TOLLENS from the linear form to the glucopyranose form (α and β isomers).

Table 3: The various objections to the linear sugars structure.

Objections to linear form	Experimental observations and explanations
1-Aldehydes and ketones stain with fuchsin decolorized by Schiff's reagent (Fuchsin decolorized by SO_2).2) ingiving a red colored product.	 + Fuchsine décolorée par le SO_2 ⟶ Produit de couleur rouge Ose (N≥4) + Fuchsin decolorized by SO_2 ⟶ Sugars do not change color.
2-Aldehydes and ketones in hydrated form react with two alcohol molecules to give acetals, while sugars combine with only one alcohol molecule to give a hemiacetal.	The objection to obtaining an acetal indicates that the aldehyde function would therefore be unavailable for these reactions and thus the existence of a new C* at the carbonyl level. 
3-Taking into account the linear structure of oses, a hydrated aldohexose (e.g., Glucose n=6), can fix theoretically 7 methyl -CH_3 groups (n+1 methyls) and form a heptamethylated molecule.	Experimentally, the hydrated aldohexoses molecules can only bind with 5 methyl groups (n-1 methyls) and form a pentamethylated molecule. 
4- Optical rotation is a constant characteristic of a substance.	The rotatory power of glucose solution freshly prepared change when observed with a polarimeter (mutarotation phenomena). This change reflects a modification of structure. 

II. 3. 5. 1. 2. HAWORTH projection

Haworth's perspective representation facilitates the depiction of various cyclic forms. The cycle is perpendicular to the plane of the sheet, its forward links are thickened. The most oxidized carbon is positioned at the right end. The position of the hydroxyl groups depends on their position in the Fisher projection. H and OH groups located on the right in the Fisher projection will be below the plane of the ring, and OH groups located on the left will be above the plane of the ring in the Haworth projection.

This cyclization makes the carbon **C1** ***asymmetric***. The relative spatial positions of the four substituents define two stereoisomeric configurations, the **α and β anomers**. Carbon C1 is designated as the anomeric carbon. For the ***α anomer***: the new (–OH) group on the anomeric carbon and carbon 6 are in a ***trans configuration*** (projecting to opposite sides of the ring plane in the Haworth projection). In the case of the ***β anomer***: the new (–OH) group on the anomeric carbon and carbon 6 are in a ***cis configuration*** (projecting to the same side of the plane).

a. Cyclization mechanism

The carbonyl group is sufficiently reactive to allow the aldehyde/alcohol reaction to occur when placed near a hydroxyl group. The cyclization begins with a 90° rotation of the hydroxyl group on carbon 5 (below the ring) around the bond between carbons 4 and 5. Consequently, carbon 6 undergoes a similar rotation and ends up above the ring. This rotation brings the hydroxyl group on carbon 5 closer to the aldehyde group on carbon 1, leading to the formation of an oxide bridge (Figs. 14, 15).

For an aldose, this intramolecular hemiacetalization can occur with the **C5-C1** or **C4-C1** carbon pairs to form a monooxygenated heterocycle known as pyran with 6 atoms (5 carbons and 1 oxygen) or furan with 5 atoms (4 carbons and 1 oxygen) (Fig. 14). Only sugars with 5 or 6 carbons give stable cyclic forms. Tetroses exist in solution in their open form.

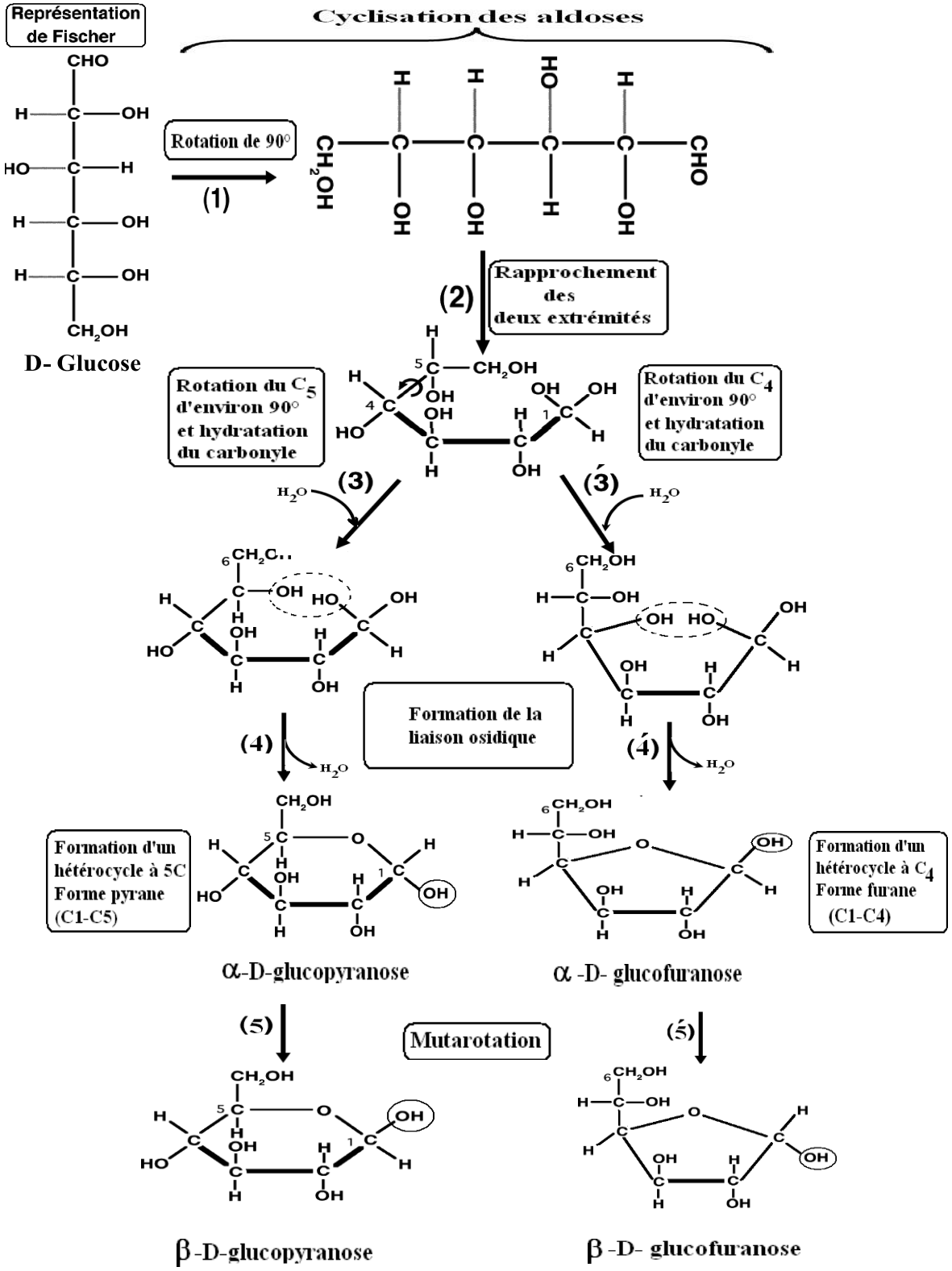


Fig. 14. Cyclization of aldoses.

In the case of ketoses, carbon **C2** is the anomeric carbon. Intramolecular hemi-acetalization can take place with the **C2-C6** carbon pair to form a 6-point oxygen heterocycle or with the **C2-C5** carbon pair to form a 5-point oxygen heterocycle (Fig. 15).

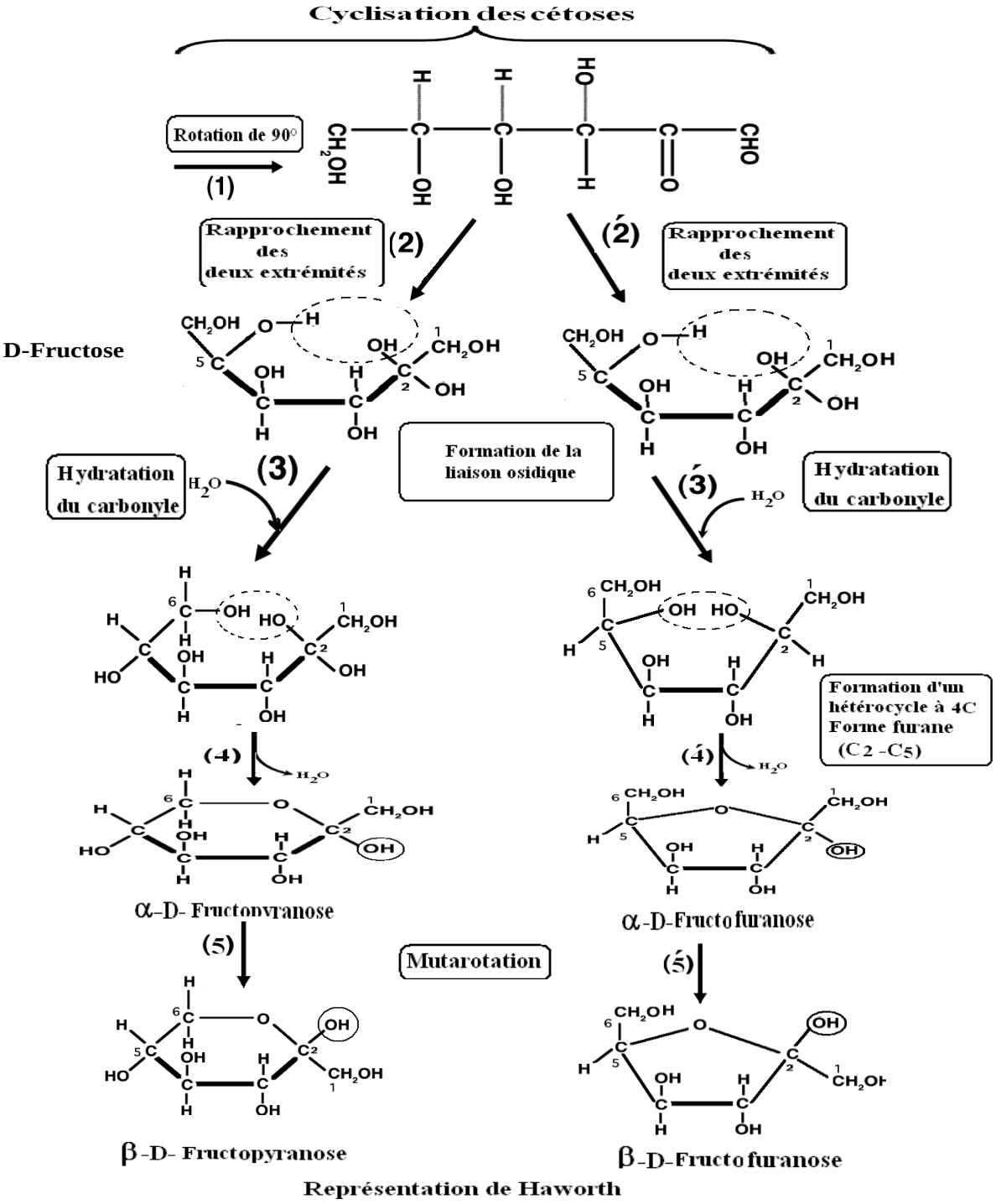


Fig. 15. Ketosis cyclization.

For an aldohexose, it is the configuration of the C_5 which determines the D or L series in the Fisher representation. Therefore, in the Haworth projection, the enantiomer of a cyclic sugar (Fig. 16) can be obtained using either a horizontal or vertical mirror. The resulting enantiomer differs in the series (D or L) but not in the anomeric orientation (α or β). The L form obtained after using a vertical mirror is characterized by a 180° vertical rotation of the chemical bonds while maintaining the positions of the hydroxyl groups (OH) and (CH_2OH). The L form obtained with a horizontal mirror reverses the positions of the hydroxyl groups (OH) and (CH_2OH) and maintains the same positions of the atoms forming the ring in the D form.

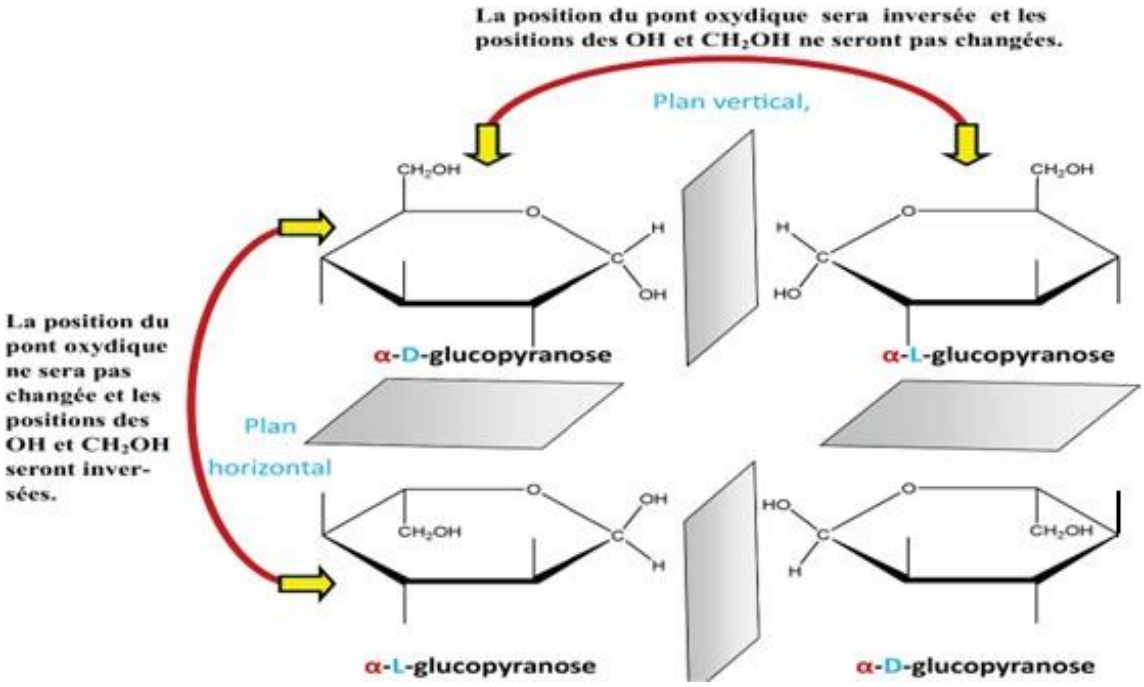


Fig. 16. The enantiomer of a cyclic sugar.

II. 3. 5. 1. 3. Conformation of cyclic structures

The conformation of 6- or 5-atom heterocycles is not planar. The atoms of pyranic and furanic rings adopt various conformations due to C–C or C–O bond rotations. Pyranoses can adopt "chair" or "boat" conformations (Fig. 17), the first ones being the most stable; example: β-D-glucopyranose chair (A) and boat (B).

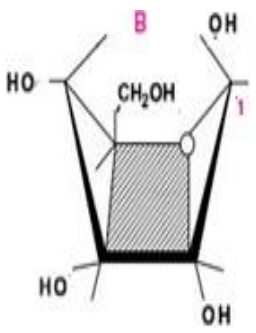
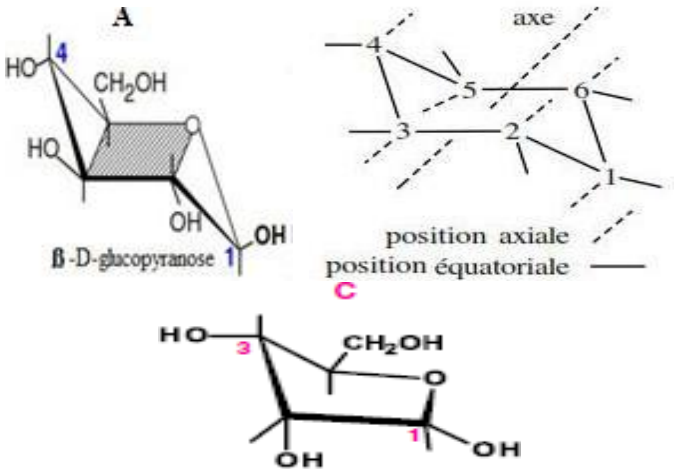
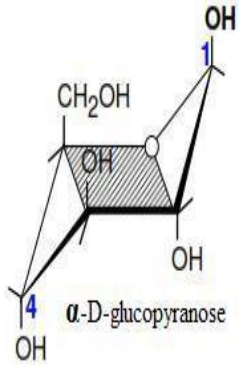
Boat shape	Chair shapes	
		
Unstable	**Stable**	**Bulky**

Fig. 17. Spatial conformation of cyclic structures.

They will be more stable the more the bulky substituents on the asymmetric carbons are in equatorial positions. In β-glucopyranose, the OH of the anomeric carbon (C_1) is in an equatorial position, while in α-glucopyranose it is in an axial position: β-glucopyranose will be a more stable molecule than α-glucopyranose. In the most stable conformations of furanoses, four atoms, including the oxygen, are found in the same plane; for example: β-D-ribofuranose (C).

II. 3. 6. Physical properties of sugars

II. 3. 6. 1. Optical rotation

In solution, the enantiomeric forms of a molecule bearing an asymmetric carbon atom possess optical activity. Each enantiomer specifically rotates the plane of polarization of a monochromatic polarized wave. The plane of polarization is rotated by an angle α equal in absolute value but opposite in direction. If the rotation is clockwise (right, $\alpha > 0$): the molecule is **dextrorotatory** (from the Latin *dexter*: right), denoted (+). If the rotation is counterclockwise (left, $\alpha < 0$): it is **levorotatory** (from the Latin *laevus*: left), denoted (-). These two enantiomers are also called **optical isomers**. An equimolar mixture of two enantiomers is optically inactive: it is called a **racemic mixture**.

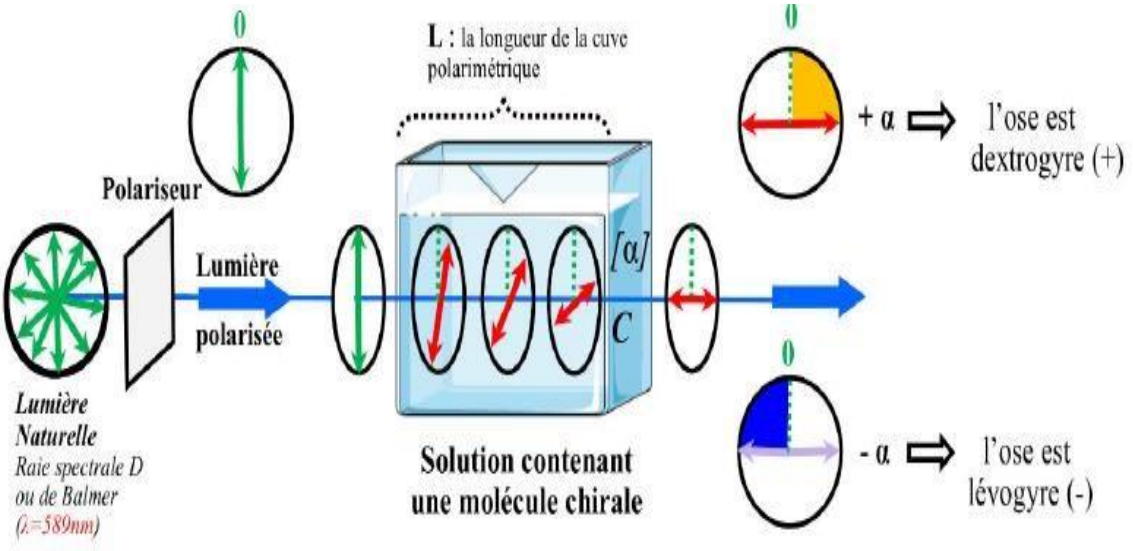


Fig. 18. Diagram of a polarimeter.

The angle α can be measured using a device called a polarimeter (Fig. 18). It depends on the nature of the substance, its concentration, and the length of the optical path (polarimetric cuvette). It follows a linear law known as **Biot's law**.

$$\alpha_{\text{of a solution}} = [\alpha]^{D}_{20°C\text{solut}} \times l \times C$$

α: is the angle of deviation of polarized light (in°).

$[\alpha]^{D}_{20°C\text{soluté}}$**:** is the **optically specific rotation** of the active solute (in °. Kg^{-1}. L. dm^{-1}). It is a characteristic constant of the solute measured at a temperature of 20°C using a wavelength of polarized light belonging to the D or Balmer spectral line (λ = 589nm).

L : is the length of the polarimetric cuvette or optical path length (in dm).

C: the concentration of the optically active solution (in Kg. L^{-1}).

In the presence of a mixture of different sugars, the optical rotation is the sum of the optical rotations of each sugar:

$$\alpha\text{mixture} = \alpha 1 + \alpha 2 + \alpha 3 + \cdots \alpha n \text{ ou } \alpha = \sum(\alpha i \times l \times Ci)$$

II. 3. 6. 2. Mutrotation phenomenon

The crystallization of D-glucose in different solvents (ethanol, pyrimidine) doesn't lead to a single product but to two products with different optical rotations. These two so-called **anomeric forms** have been designated the **α form** (+112°), crystallization in ethanol, and the **β form** (+19°), crystallization in pyrimidine.

For each of the forms placed in aqueous solution, a change in optical rotation is observed over time (Fig. 19), reaching the same value for each form.

At +52.5°C, 64% of the glucose molecule is in the β-D-glucopyranose form (the most stable) and 36% in the α-D-glucopyranose form, with a very small amount in the open form (0.01%). The change in optical rotation that accompanies the transformations between pyran and furan rings and between the α and β anomers is called **mutarotation**, a phenomenon described by the chemist Augustin-Pierre Dubrunfaut in 1846.

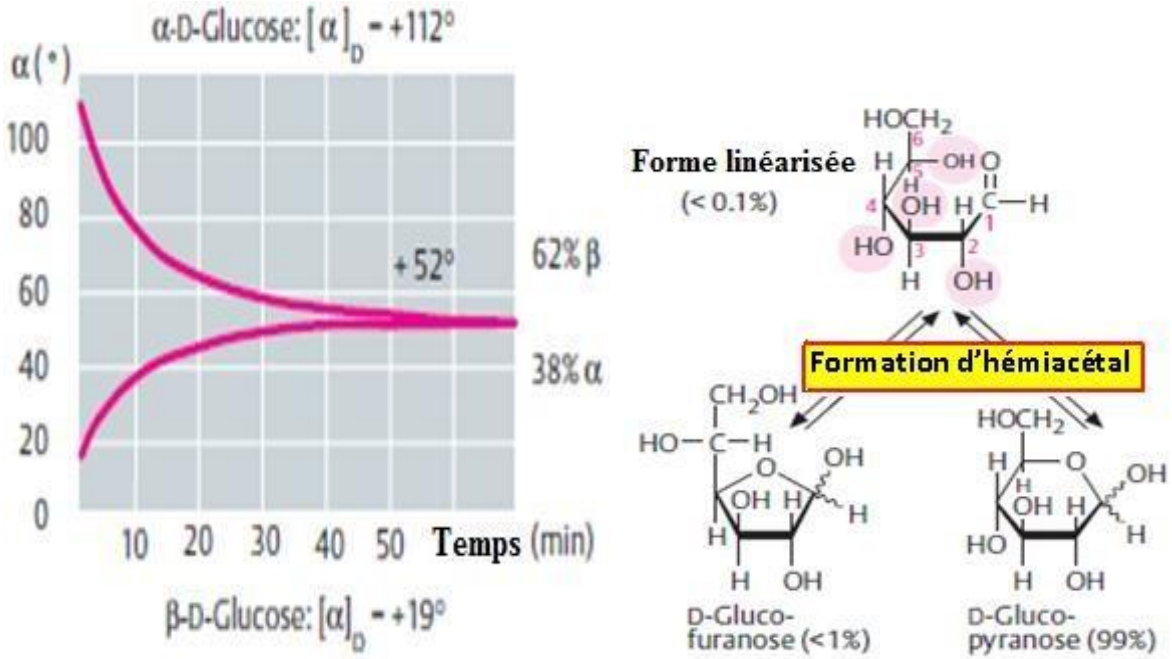


Fig. 19. The phenomenon of mutarotation.

II. 3. 6. 3. Optical properties

They are limited to optical rotation and refractive index. Thus, each sugar has a specific optical rotation that allows for its identification and quantification. For glucose, the optical rotation is a positive value of +52.5° (dextrorotatory molecule); for fructose, the optical rotation is -92° (dextrorotatory molecule).

The refractive index of water relative to air is 1.333 at 20°C. In the presence of a dissolved sugar or glycoside, this index increases with increasing concentration of the carbohydrate solution.

II. 3. 6. 4. Spectral properties

Sugars absorb radiation from the infrared spectrum, but not from the UV or visible spectrum. This is why they generally occur in the form of white crystals.

II. 3. 6. 5. Solubility

Sugars are molecules that possess multiple hydroxyl (OH) groups, giving them polar properties and the ability to form multiple hydrogen bonds with water and other biomolecules (proteins). They are highly water-soluble molecules, up to a concentration of 3M (glucose molar mass = 180 g/L, or 540 g/L), resulting in highly viscous aqueous solutions called syrups.

The solubility of carbohydrates in organic solvents varies. However, they are soluble in pyridine, slightly soluble in ethanol, and insoluble in ether. Therefore, they can be separated by thin-layer partition chromatography.

II. 3. 7. Chemical properties of sugars

Their chemical properties are characteristic of alcoholic hydroxyl groups and carbonyl groups.

II. 3. 7. 1. Properties due to the carbonyl function

II. 3. 7. 1. 1. Reduction of sugars: obtaining alditols (ositols)

Aldoses and ketoses are irreversibly reduced by alkali borohydrides ($NaBH_4$, $LiBH_4$) either chemically or enzymatically, yielding polyalcohols called glycitols or alditols (from 4C). The names of alditols are obtained by replacing the suffix **-ose** with **-itol**. For example, D-glucose yields D-glucitol (D-sorbitol), and D-mannose yields D-mannitol, and so on.

The formation of alditol from an aldose involves exclusively the open-chain form of the aldose. The reduction of D-fructose (a ketose) by $NaBH_4$ gives an equimolar mixture of D-glucitol and D-mannitol, C_2 epimeric alditols (Fig. 20).

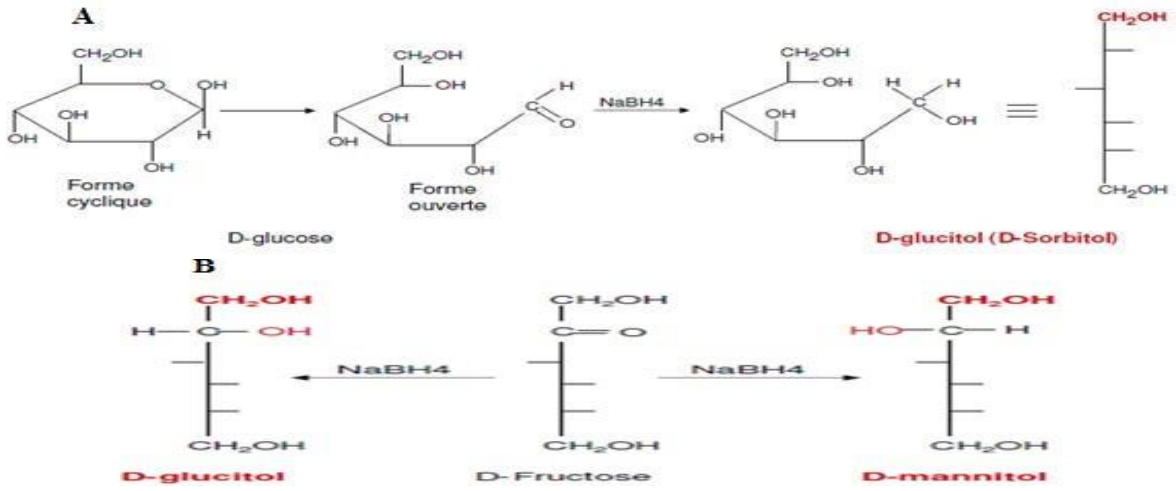


Fig. 20. Formation of alditol from an aldose (A) and epimeric alditols from a ketose (B) during reduction reactions.

II. 3. 7. 1. 2. Oxidation of sugars

II. 3. 7. 1. 2. 1. Oxidation in an alkaline medium and at low temperatures (Gentle or controlled oxidation)

The aldehyde group R-CHO of aldoses is oxidized to carboxylic groups R-COOH in the presence of mild oxidants such as bromine (Br_2), iodine (I_2), and dilute nitric acid (NHO_3) in an alkaline medium, leading to the formation of aldonic acids. If the reaction occurs with D-glucose, D-gluconic acid is obtained (Fig. 21).

$$R - CHO + I_2 + OH \rightarrow R - COOH + 2I- + Na+ + H_2O$$

Aldose (Aldehydic function) **Aldonic acid**

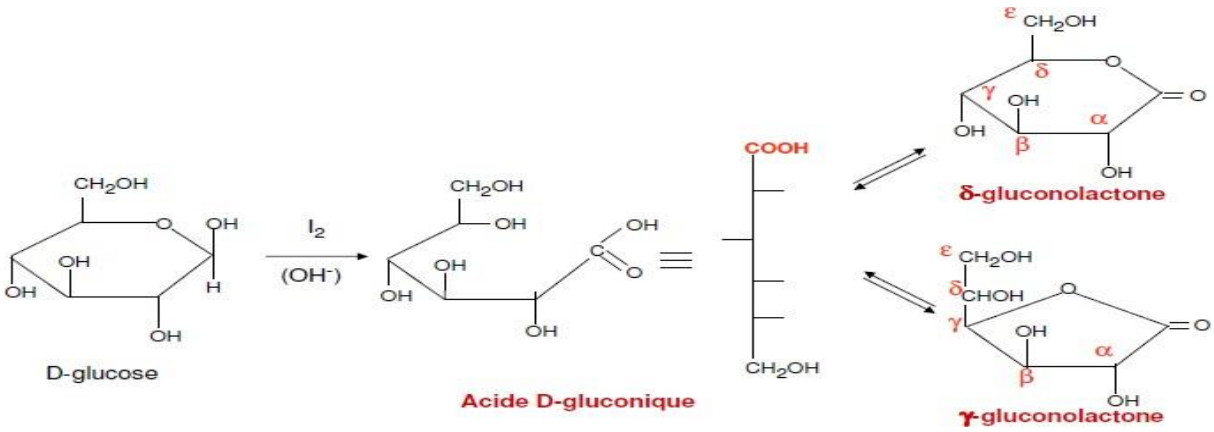


Fig. 21. Oxidation of an aldose in an alkaline medium and at low temperature.

When the solvent is subsequently evaporated, the aldonic acids condense into cyclic esters or lactones. Depending on the ring shape, a γ-lactone (furan) or δ-lactone (pyran) is obtained. To determine the name of the lactone formed, simply replace the suffix **-ose** of an aldose with the suffix **-onolactone**.

The **ketone** group is not oxidized by iodine in a basic medium.

II. 3. 7. 1. 2. 2. Oxidation by heavy metal salts

Reducing power of aldoses (Oxidation reaction of aldoses by Fehling's solution)

Some sugar molecules possess reducing power (providing electrons and protons H^+). In an alkaline environment, metallic salts (copper, iron, silver, mercury, etc.) are reduced by the pseudo-aldehyde group. When heated and in an alkaline environment, cupric oxide (blue) is reduced to insoluble cuprous oxide (brick red), while the aldose is oxidized to Aldonic acid (Fig.

22). To find the name of the product of mild oxidation, simply replace the suffix **-ose** with the suffix **-onic** or **-onate**. ***For example***: glucose yields gluconic acid, mannose yields mannonic acid, and galactose yields galactonic acid, etc.

$$\boldsymbol{R-CHO} + \boldsymbol{2Cu(OH)_2} \rightarrow \boldsymbol{R-COOH} + \boldsymbol{Cu_2O} + 2H_2O$$

Aldose — **Cupric oxide (Blue)** — **Aldonic acid** — **Cuprous oxide (Brick red)**

Fig. 22. Action of Fehling's solution with copper salts (when heated in the presence of a reducing sugar).

Ketones, which cannot undergo controlled oxidation, do not react with Fehling's solution (Fig. 23).

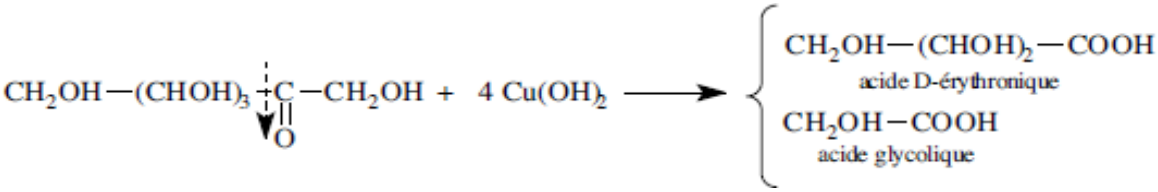


Fig. 23. Action of Fehling's solution in the presence of ketose.

II. 3. 7. 1. 2. 3. Oxidation by nitric acid (Advanced oxidation, in a hot acidic medium)

The strong oxidation of an aldose leads to the simultaneous attack of the primary alcohol and the aldehyde. This yields a dicarboxylic acid called **aldaric acid**. The name of an aldaric acid is obtained by replacing the suffix **-ose** with **-arate** or **-aric** in the name of the original aldose. For example, glucose yields glucaric acid (or glucarate, also called saccharic acid), galactose yields galactaric acid (or galactarate), and so on.

The same oxidation reaction causes the oxidative cleavage of the carbon skeleton of ketoses at the level of the ketone function and formation of a mixture of carboxylic acids (Fig. 24).

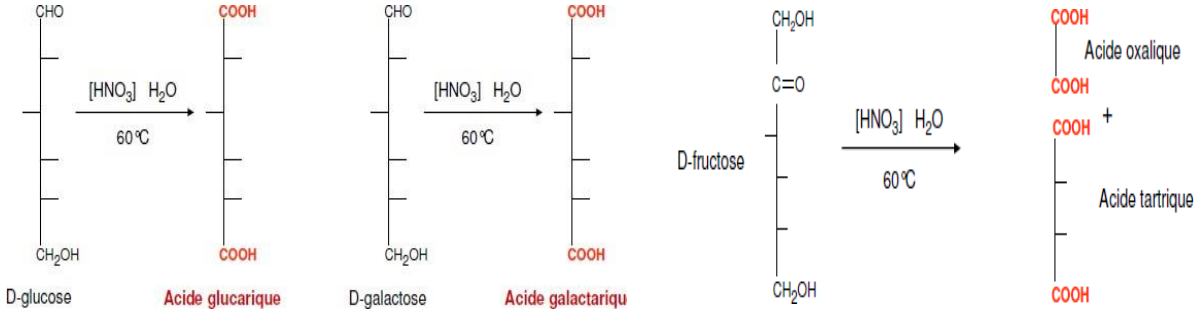
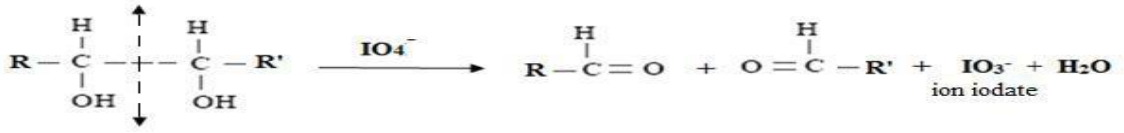
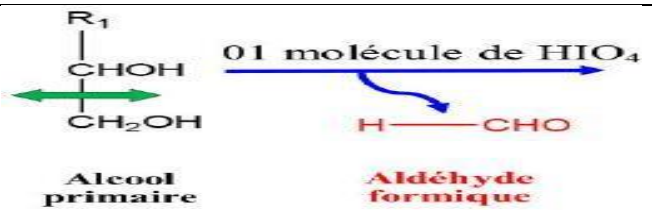
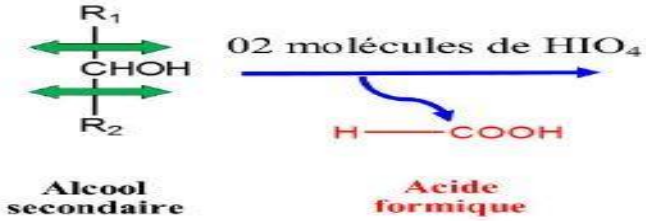


Fig. 24. Action of nitric acid on aldoses and ketoses.

II. 3. 7. 1. 2. 4. Oxidation by periodic acid (HIO_4)

Oxidation by periodic acid HIO_4 Method (Malaprade and Fleury method) is used to locate the oxidative bridge and determine the ring structure. It involves breaking the covalent bond between two adjacent carbons bearing a free glycol group. Secondary alcohol groups give either aldehydes or formic acid and the primary alcohol functions give formaldehyde (Table 4).

Table 4. Mode of cleavage of sugars by periodic acid HIO_4 and the reaction products released.

<table>
<tr><td colspan="2"> 
</td></tr>
<tr><td>Carbons bearing primary alcohol function are transformed into formaldehyde (formalin = HCHO).</td><td> 
</td></tr>
<tr><td>While the carbons bearing secondary alcohol functions are transformed into formic acid (HCOOH)</td><td> 
</td></tr>
</table>

The sugar is first methylated to protect the anomeric hydroxyl at C1 and then subjected to the action of HIO_4. By studying the reaction products and the amount of HIO_4 consumed, we can deduce the number, nature and position of the free hydroxyl groups (Fig. 25).

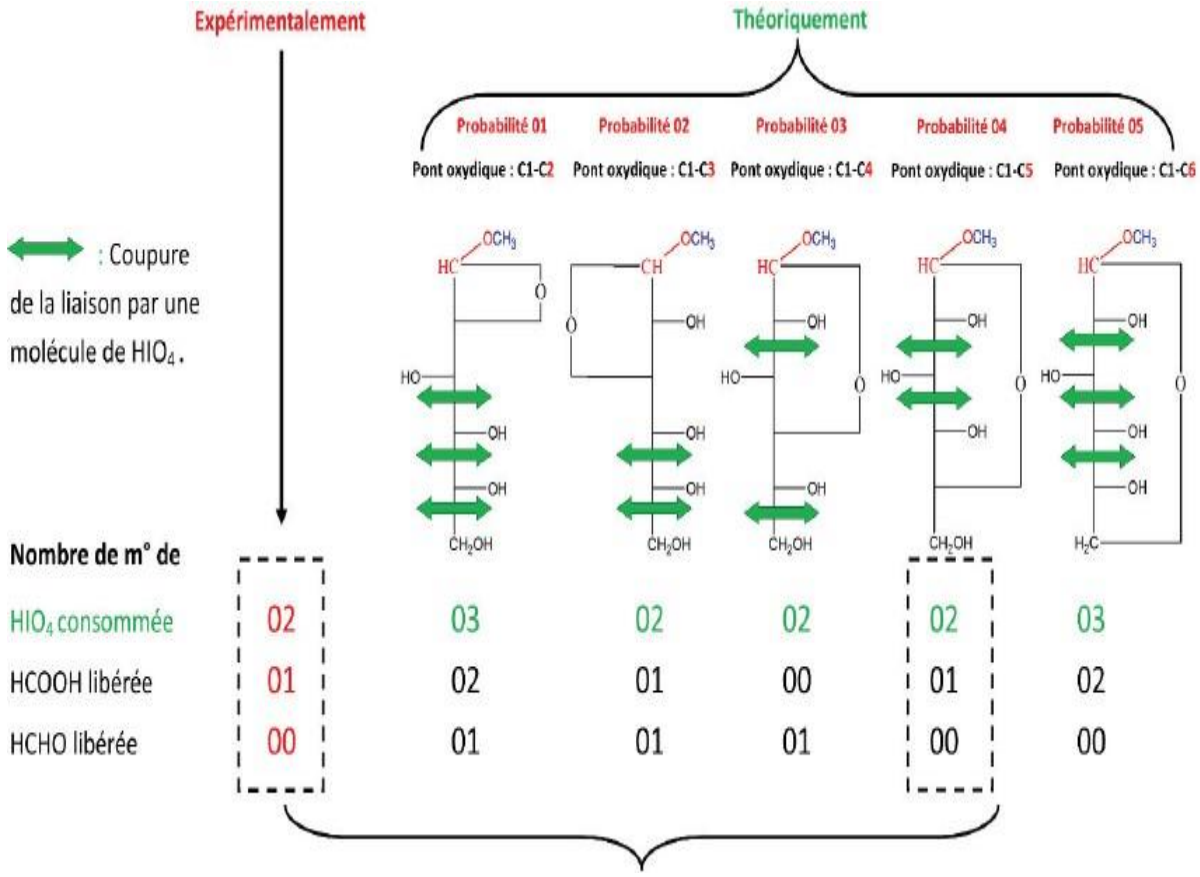


Fig. 25. Example of the action of periodic acid on the glucose molecule. These results allow us to conclude that the oxidation bridge is located between the C_1 and the C5.

II. 3. 7. 1. 3. Addition and Substitution Reactions

- Reaction with alcohols and phenols (addition): formation of glycosides

The action of alcohols leads to the formation of **methyloses** called **O-Heterosides** (Fig. 26).

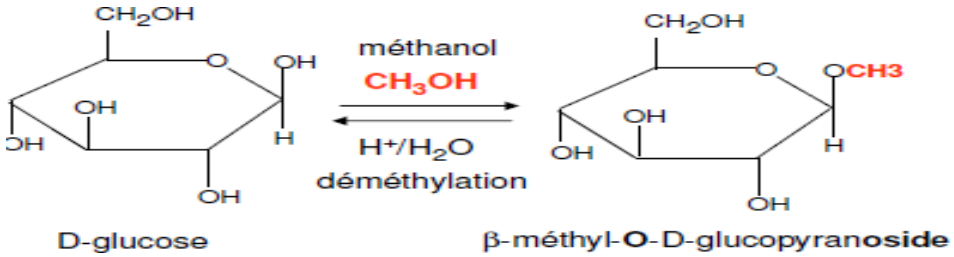


Fig. 26. Action of methanol on glucose.

An O-glycoside **has no reducing power** (it does not reduce metal oxides) and does not exhibit mutarotation.

– Action of amines (substitution)

Aldoses and ketoses condense with primary amines to give cyclic imines or **N-heterosides** (Fig. 27).

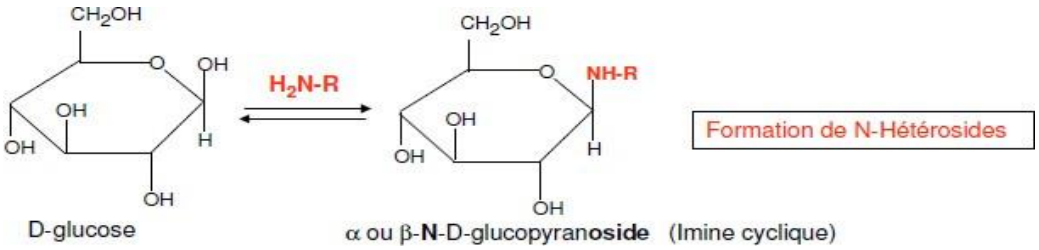


Fig. 27. Action of amines on sugars.

Imines formed by sugars tend towards an anomeric equilibrium (mutarotation), with α and β forms.

Cyclic imines or N-substituted glycosylamines, or N-glycosides, like O-glycosides, N-glycosides are components of many biological molecules, the best known of which are nucleosides and nucleotides, which make up nucleic acids.

-Action of thiols (substitution)

In acidic conditions, the aldehyde group of aldoses combines with thiols (R-SH) to form **S-heterosides** (Fig. 28). The ketone group of ketoses, however, does not combine.

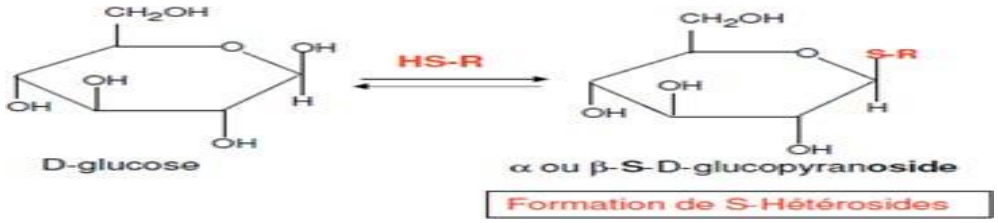


Fig. 28. Combination of the aldehyde group of aldoses with thiols and formation of an S-heteroside.

II. 3. 7. 2- Properties due to the alcohol function

II. 3. 7. 21 – Dehydration in an acidic medium

In a concentrated acidic medium and at high temperatures, sugars (from 5 C) are dehydrated into furfural or furfural derivatives (Fig. 29).

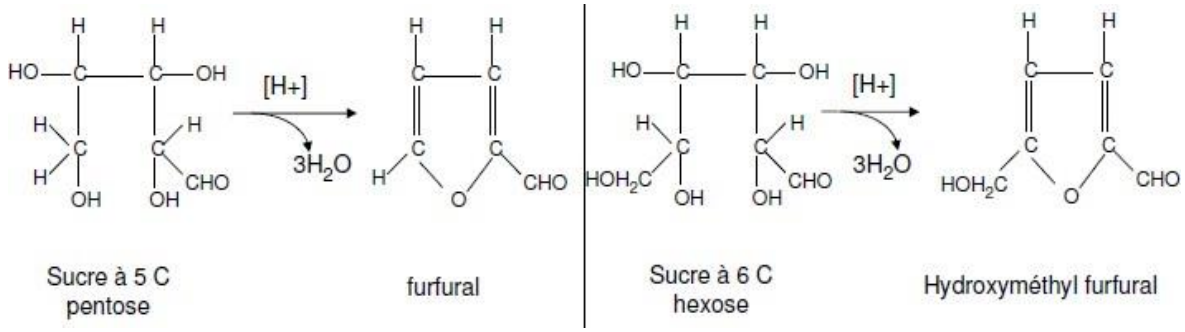
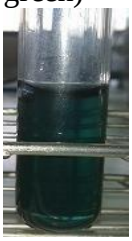
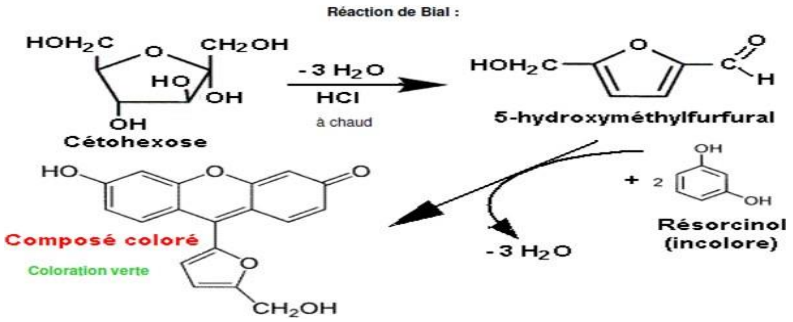
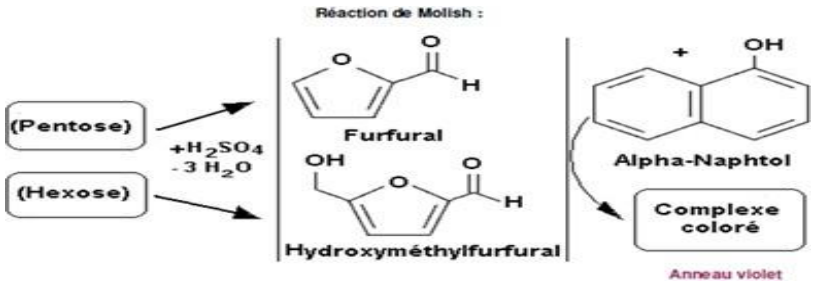
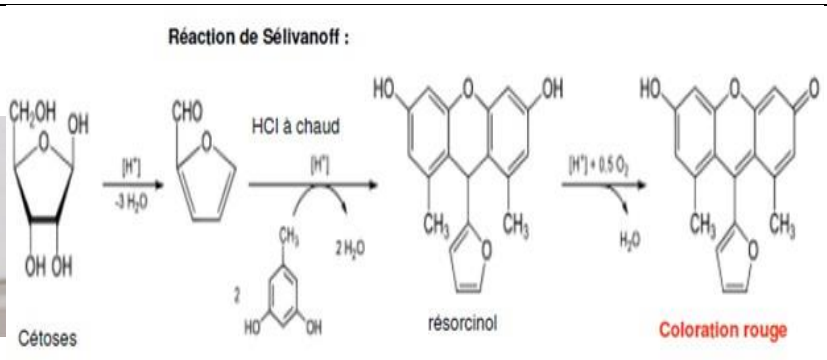


Fig. 29. Formation of a furfural and a furfural derivative by the action of a hot concentrated acid on a sugar.

Furfurals and their derivatives condense with phenols to give colored products used for the characterization and colorimetric determination of sugars (Table 5).

Table 5: Some characterization and colorimetric assay reactions of the obtained sugars.

Reaction	**Concerned oses**	**The reaction**
Bial's Reaction	Pentoses (Bottle green) 	
Molish's Reaction	All oses 	
Sélivanoff 's reaction	Ketoses (Cherry Red) 	

II. 3. 7. 2. 2. Formation of esters: Sugar esters exist in nature. Mono- and diphosphate sugars are essential in energy metabolism (Fig. 30).

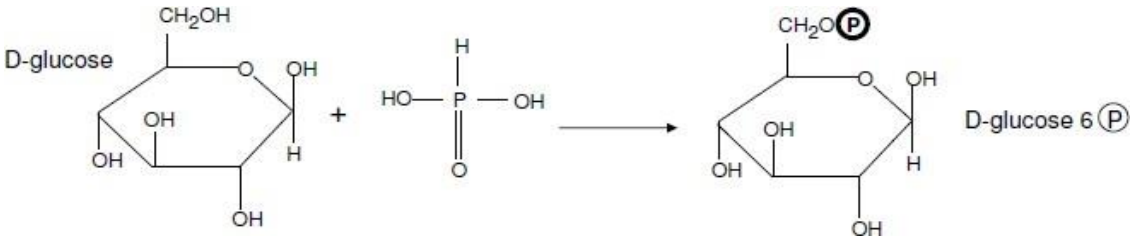


Fig. 30. Formation of oses's esters.

II. 3. 7. 2. 3. Formation of ethers: During a methylation reaction, a -CH_3 group is attached to an OH of a sugar to give ethers (RO-CH_3). In the laboratory, the methylation of sugars is carried out by methylating agents such as methyl iodide (ICH_3) with silver oxide (Ag_2O) or with dimethyl sulfate $(CH_3)_2SO_4$ in an alkaline medium (NaOH) (Fig. 31).

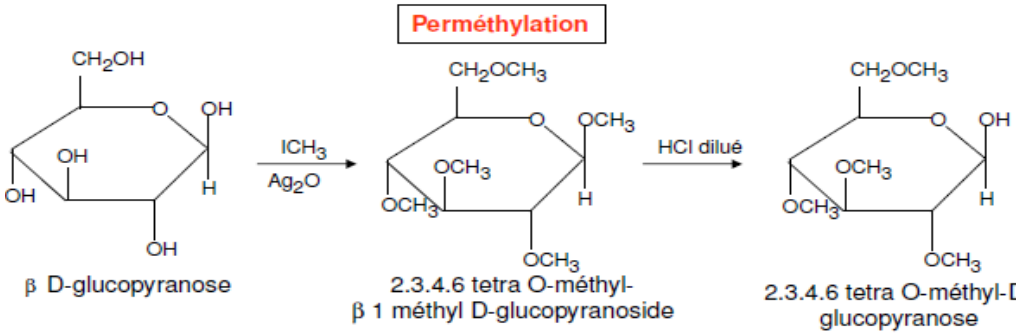


Fig. 31. Glucose methylation reaction.

Methylation can be **limited** (only the OH group of the hemiacetal is methylated) or **complete *(total, prolonged)***, also called ***permethylation***, where all the free OH groups of the sugar are methylated. Methyl ethers are used to determine the structure of rings and the linkages of holosides.

II. 3. 7. 2. 4. Oxidation of the primary alcohol function: By protecting the aldehyde group during oxidation, uronic acids oxidized only at the primary alcohol group in position C_6 are obtained. To determine the name of the uronic acid formed, simply replace the suffix **-ose** of an aldose with the suffix -**uronic**. For example, glucose yields glucuronic acid (Fig. 32), galactose yields galacturonic acid, and so on. Uronic acids are components of glycosaminoglycans, an essential constituent of the extracellular matrix.

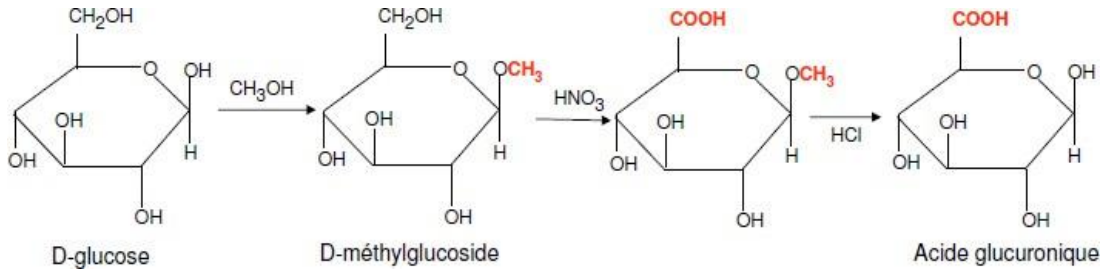


Fig. 32. Formation of glucuronic acid by oxidation of the primary alcohol function of glucose.

II. 3. 7. 3. Properties due to a neighboring alcohol group and carbonyl group

II. 3. 7. 3. 1. Action of phenylhydrazine (Osazone formation)

The sugar can react with one molecule of phenylhydrazine at room temperature, leading to the formation of a phenylhydrazone. At room temperature, the sugar can react with two molecules of phenylhydrazine to form one molecule of osazone (Fig. 33).

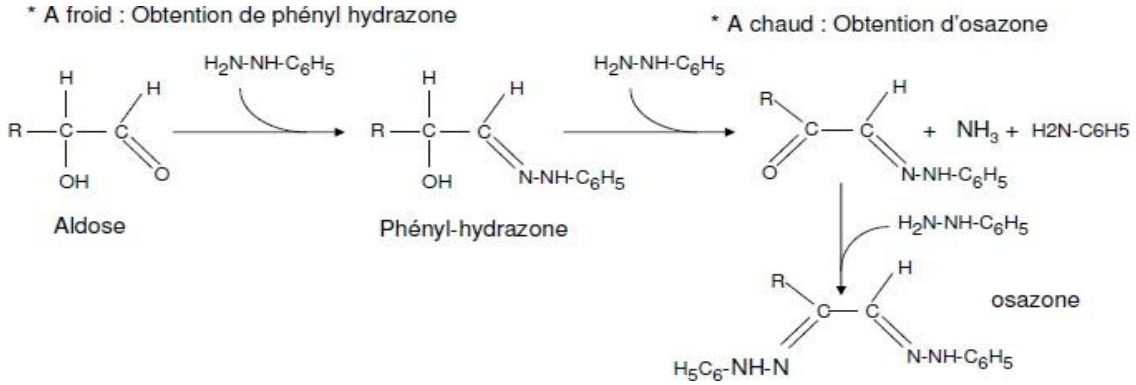


Fig. 33. Action of phenylhydrazine on a sugar.

Example: D-glucose reacts with one molecule of phenylhydrazine in cold conditions, leading to the formation of glucose phenylhydrazine. In hot conditions, D-glucose reacts with two molecules of phenylhydrazine to form a glucosazone (Fig. 34).

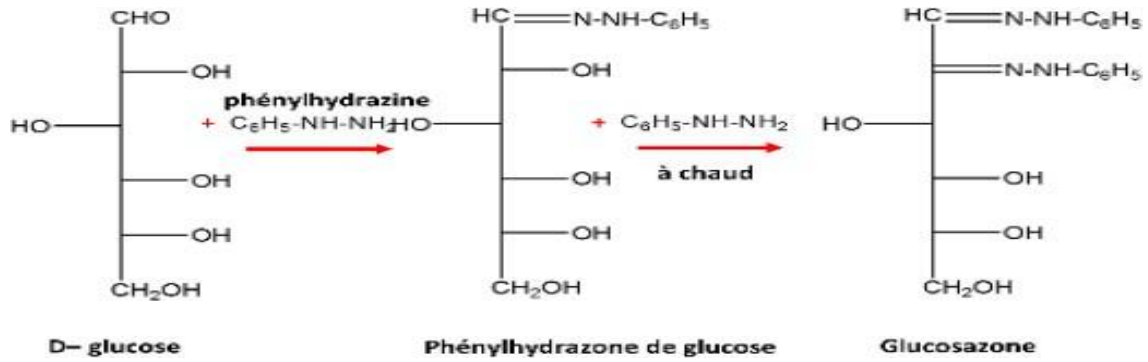


Fig. 34. The products of the reaction of D-glucose with a molecule of phenylhydrazine under hot and cold conditions.

When heated, sugar solutions mixed with phenylhydrazine precipitate. Observing the shape of the crystals using a microscope allows for the identification of these sugars in solution (Fig. 35).



Fig. 35. The different sugar crystals mixed with phenylhydrazine observed using a microscope.

II. 3. 7. 3. 2. Alkaline Isomerization

Ketoses, through a tautomerization process (enediol) that occurs in a basic medium, are in equilibrium with the corresponding aldose via a trans-enediol. The phenomenon is an interconversion (aldose -> ketose) (Fig. 36).

In trans-enediol, the carbon C_2 is no more asymmetric, it can undergo rearrangement to give a cis-enediol (epimeric for the OH group), which can then give an epimeric aldose. The D-glucose/D-mannose conversion is an **epimerization** (an inversion of the configuration of substituents on a single asymmetric carbon). Epimerization is an important biological reaction catalyzed by an enzyme.

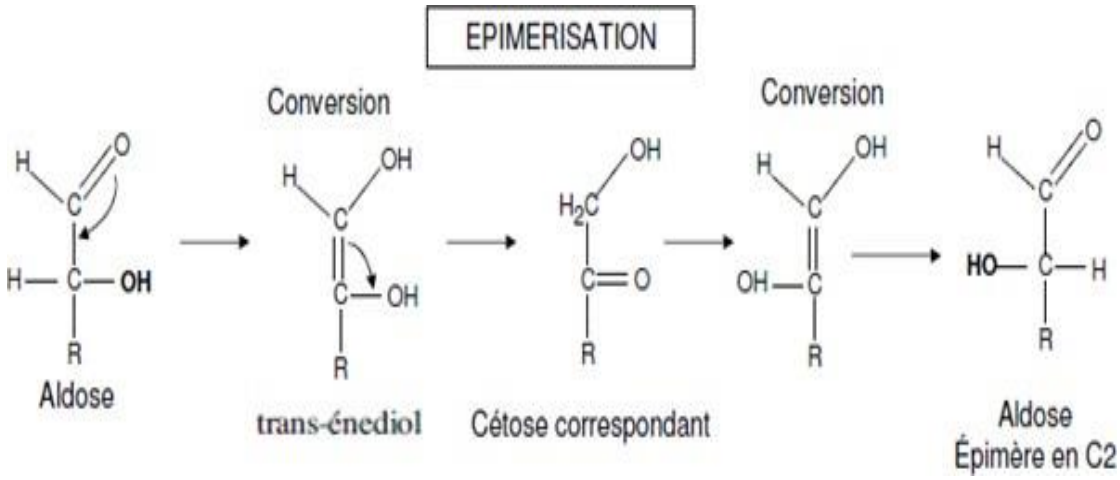


Fig. 36. Phenomenon of sugar interconversion.

II. 3. 8. Dare and deriveres of bones of the intereorganic

II. 3. 8. 1. Neutral sugars

II. 3. 8. 1. 1. Trioses

The D and L forms of glyceraldehyde occur naturally. The most important forms of the trioses are phosphorylated derivatives found in the early stages of glycolysis (oxidative catabolism): glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, obtained from the degradation of fructose 1,6-bisphosphate (Fig. 37). This reaction is catalyzed by the enzyme aldolase.

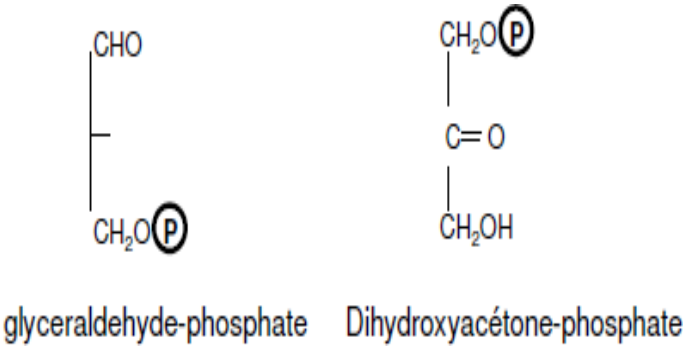


Fig. 37. The phosphorylated derivatives of glyceraldehyde.

II. 3. 8. 1. 2. Tetroses

The only tetrose of interest is the aldose D(-)erythrose. Its 4-phosphate ester is one of the many intermediates of photosynthesis and of a glucose degradation pathway branching off its aldonic oxidation product: phosphogluconic acid. It is also the precursor of aromatic amino acid biosynthesis by microorganisms.

II. 3. 8. 1. 3. Pentoses: They are classified according to the functions they perform in the cells Table 6.

II. 3. 8. 1. 4. Hexoses: The most important hexoses are: glucose, galactose, mannose, fructose (Table 6, Fig. 38).

II. 3. 8. 1. 5. Heptoses

They are important intermediates of carbohydrate metabolism in their phosphorylated form (pentose pathway).

Table 6: List of some oses that have functions in the body.

Pentosis	Location and function
D-xylose	It is found in the xylem of wood. It is also involved in the polysaccharides of animal extracellular matrices, or as a sugar of Glycan branching onto a protein (Fig. 38)
D and L-Arabinose	It is one of the few naturally occurring L-series sugars. They are found in all plants. D-Arabinose (Fig. 38) is the immediate precursor of D-glucose and D-mannose. It is not metabolized by the man and is eliminated directly in the urine.
D-ribose (and its derivative) The D-2-deoxyribose)	It is a component of nucleotides that can be used in the re-synthesis of the ribonucleic acids (RNA) and deoxyribonucleic acids (DNA), or as a coenzyme (Fig. 38).
D-ribulose	This ketopentose is found in the form of ribulose-1,5-bisphosphate, which is a fundamental element in the "pentose cycle" and reactions of photosynthesis (Fig. 38).
D-Ribulose and D-Xylulose	These ketopentoses are intermediates of carbohydrate metabolism in phosphorylated form (pentose cycle and photosynthesis) (Fig. 38).
D(+) glucose	It is the "fuel molecule" of the living world and thus the prototype for studies of the structure and properties of sugars. It is abundant in its free state in honey and fruits. It is water-soluble in biological fluids. In its polymerized form from α-D-glucopyranose, it constitutes the energy reserves (plant starch, animal glycogen) of higher organisms. The polymer formed from the β anomer yields a polysaccharide with specific physical and biological radically different from α polymers: cellulose.
D(+)galactose	The most widespread after glucose, it is a component of the Lactose from mammalian milk. It is found combined in certain oligosaccharides, heterosides and glycoproteins (Fig. 38).
D(+) mannose	It is not very abundant in its free state (orange peel), it is found in polymers such as mannans or in glycoproteins (Fig. 38).
D(-) fructose	It is found naturally in fruits and honey, to which it gives its consistency due to its difficulty in crystallizing. It can also be found in phosphorylated form (an important intermediate of carbohydrate metabolism) or condensed as a diholoside in sucrose or other glycosides (Fig. 38).

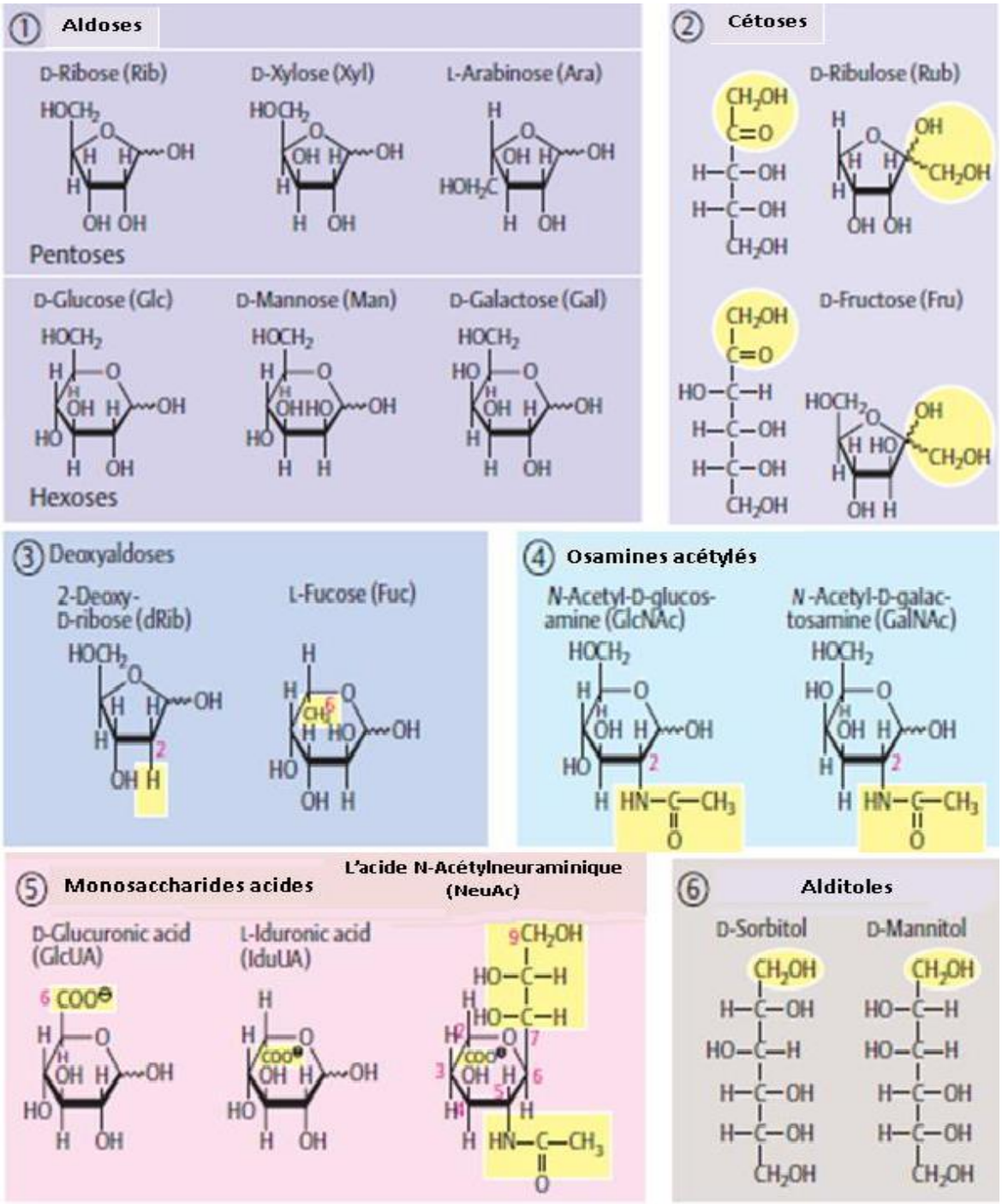


Fig. 38. Main monosaccharides and their derivatives found in nature.

II. 3. 8. 2. Some sugar derivatives

II. 3. 8. 2. 1. Dehydroxylated sugar derivatives

Dehydroxylation involves replacing a hydroxyl group with a hydrogen atom. The best-known example of a dehydroxylated sugar is ribose. Ribose cyclized to furanose can undergo dehydroxylation at the C_2 position, leading to the formation of deoxyribose, which is found in the structure of deoxyribonucleic acid (DNA).

Other examples of dehydroxylation exist in nature, such as:

- ***Fucose or 6-deoxy-L-galactose***present in the polysaccharides of insect, mammal and plant cells.

- ***Rhamnose or 6-deoxy-L-mannose***, present in plants as a heteroside and in the external membranes of certain bacteria.

- ***Quinovose is 6-deoxy-D-glucose***, present in plants and certain bacteria.

II. 3. 8. 2. 2. Osamines

These molecules are derived from sugars by replacing a hydroxyl group (usually the one on carbon 2) with an amine group (NH_2). The most important are hexosamines, derived from glucose or galactose by substitution at C_2. Hexosamines have the same properties as sugars (reducing properties, cyclic forms, etc.) and the properties of amines (basic: proton binding). Glucosamine, derived from glucose, and galactosamine, derived from galactose, are the two most studied osamines in the hexosamine family (Fig. 39). Their amine group is often acetylated to give N-acetylglucosamine or N-acetylgalactosamine.

They are present mainly in polymerized form, for example in chitin (skeleton of arthropods), in the making of murein (cell wall of bacteria) and in glycoproteins.

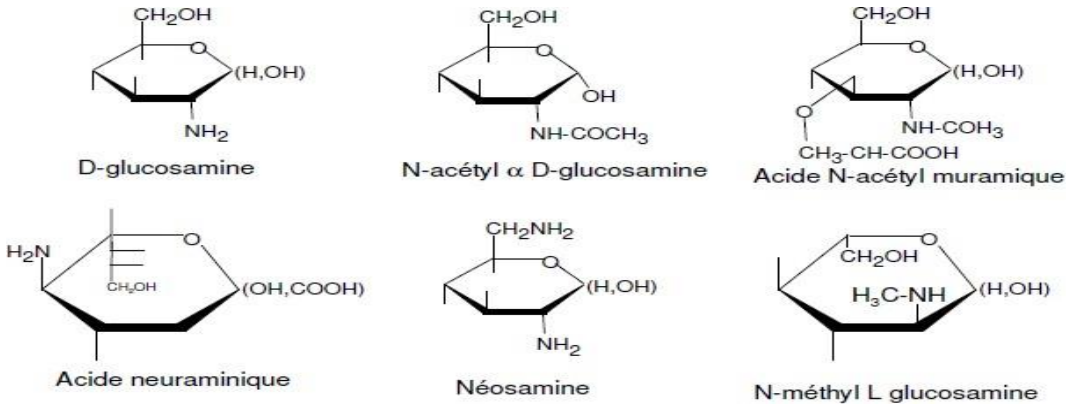


Fig. 39. Structures of some hexoamines.

II. 3. 8. 2. 3. Oses acid derivatives

a- α- Sialic acid

Sialic acid is a generic term for a family of over 50 derivatives of neuraminic acid, an acid sugar (ose) (carboxylic acid function in C1) with a skeleton of nine carbon atoms (Fig. 40).

The difference between the molecules constituting this family lies mainly in the number and position of the acetylations. The best known is N-acetylneuraminic acid (Neu5Ac or NANA), formed by condensation of pyruvic acid with D-mannosamine.

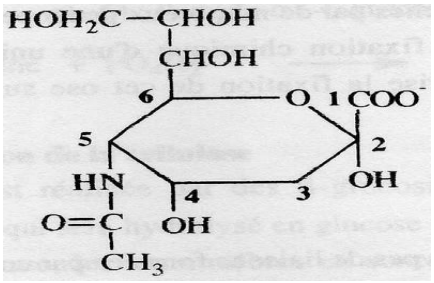


Fig. 40. Structure of sialic acid: NeuNAc N-acetylneuraminic acid.

These are the characteristic compounds of the glycoproteins and sphingolipids of cell membranes. The carbohydrate portion is directed towards the extracellular face of the membrane and plays an essential role in cell recognition phenomena.

b- L-ascorbic acid (or vitamin C)

Unlike most primates (including humans), most mammals are able to synthesize vitamin C (Fig. 41) in their liver or kidneys. It is therefore essential for humans, and its deficiency leads to scurvy (a connective tissue disease). Only the L-form of ascorbic acid constitutes vitamin C. Its structure is characterized by the presence of the enediol group (two OH groups attached to two adjacent carbons linked by a double bond). It is a lactone derived from 2-keto L-gulanic acid. Its reducing power allows it to be readily oxidized to dehydroascorbic acid.

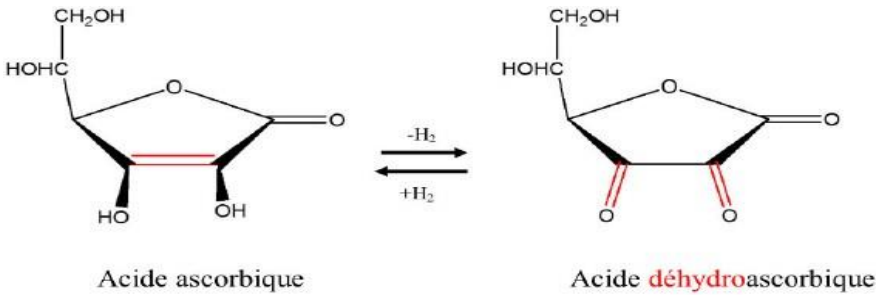


Fig. 41. Structure of vitamin C.

c- Uronic acids

These are sugar derivatives in which the primary alcohol group has been oxidized to a carboxylic acid group. These derivatives are found primarily in polymerized form in glycoconjugates (glycoproteins, mucopolysaccharides). Examples of these molecules include:

- glucuronic acid (Fig. 42) which can condense in the liver with many hydroxylated substances, thus promoting their solubility and urinary elimination; this is called detoxification by

glucuronidation (elimination of certain thyroid and sex hormones, bile pigments, certain drugs or toxins…),

- galacturonic acid (Fig. 42) which is a constituent of gums, mucilages and pectins of plants.

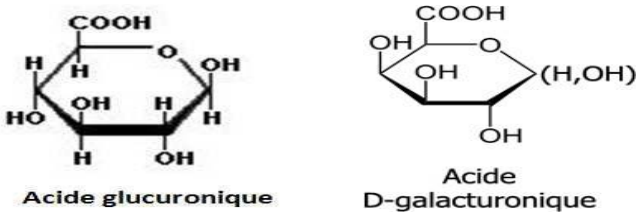


Fig. 42. Structure of the two acids glucuronic and galacturonic.

II. 3. 8. 2. 4. Polyols

They result from the reduction of the aldehyde or ketone group of a sugar to an alcohol group: this yields a polyol, a molecule in which each carbon atom bears an alcohol group. Among the most important are: glycerol (a constituent of glycerides and glycerophospholipids) obtained from glyceraldehyde, ribitol (obtained from ribose), sorbitol (obtained from glucose), galactitol (obtained from galactose), mannitol (obtained from mannose) ...

These polyols can be found in certain culture media used in bacteriology because they can serve as a source of carbon and energy for some bacteria. Some polyols (mannitol, sorbitol, xylitol, etc.) are also used as food additives to replace conventional sugar (sucrose). Some polyols are cyclic, such as inositol (a cyclic hexa-alcohol), which is a constituent of inositides (or phosphatidyl inositols), which are membrane-bound glycerophospholipids.

II. 4. Osides

Osides are polymers of sugars, among which we distinguish heterosides, whose hydrolysis releases sugars and non-carbohydrate compounds (aglycone), holosides, whose hydrolysis releases only sugars, and among these, oligosides and polyosides, the difference being in the number of monomers forming the polymer.

The plant kingdom abounds with various glycosides, whose aglycones possess pharmacodynamic properties. Nucleosides and nucleotides are glycosides.

II. 4. 1. Oligosides

Oligosides or oligoholosides are holosides that result from the condensation of 2 to 10 molecules of sugars or sugar derivatives.

II. 4. 1. 1. Glycosidic bond

The bonds that ensure the links between sugars or between sugars and aglycones are called glycosidic bonds. The glycosidic bond is formed between the reducing hydroxyl (semi-acetal OH) of a sugar located on the anomeric carbon (C1 for aldoses, C2 for ketoses), in the α or β position, with a hydroxyl of another sugar (Fig. 43).

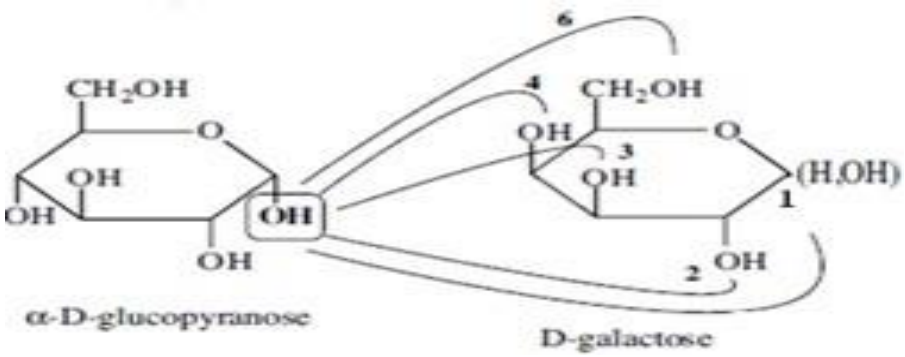


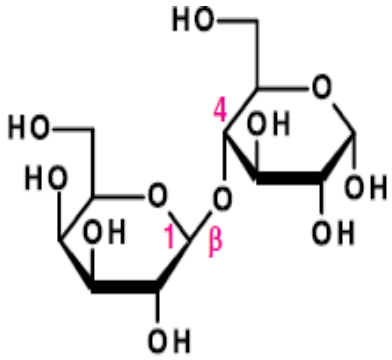
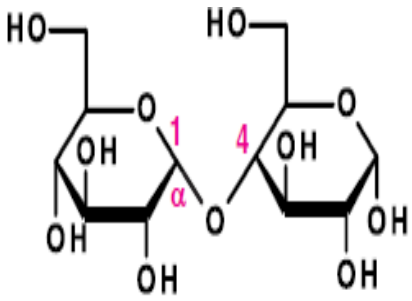
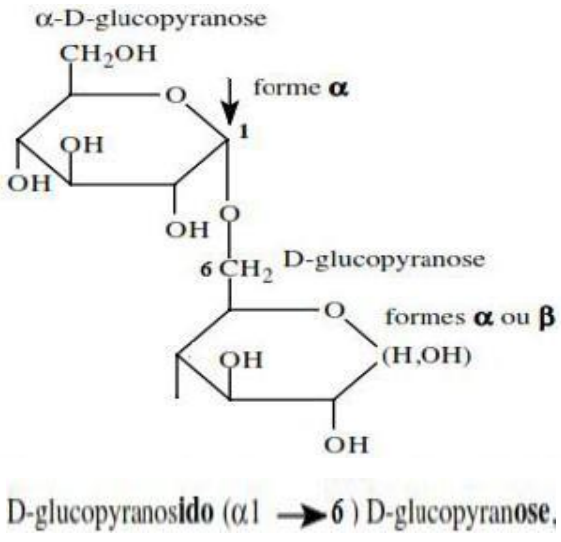
Fig. 43. The different possibilities for linking α D-glucopyranose via its hemiacetal –OH to α D-galactopyranose.

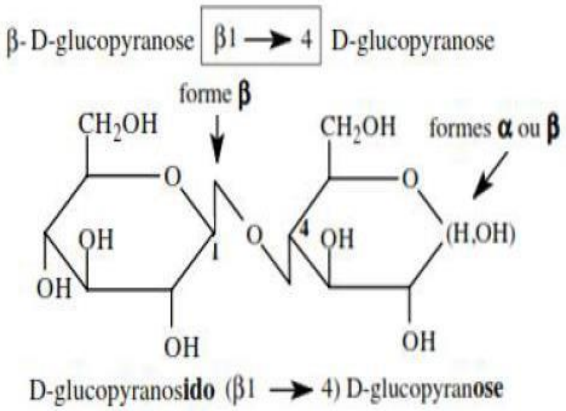
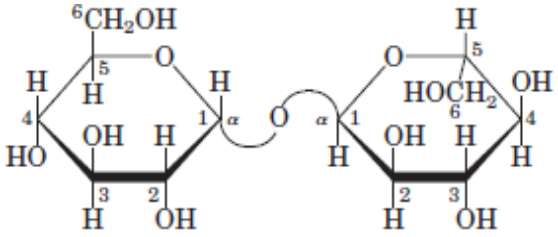
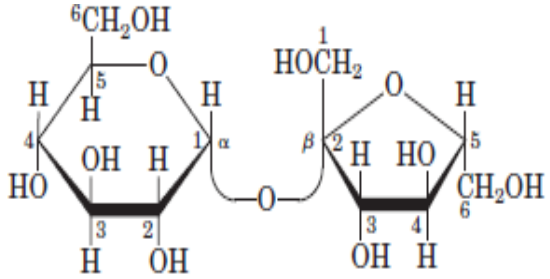
The glycosidic bond of a holoside is characterized by its anomeric structure, series, the types of sugars involved, and the shape of their rings (pyran or furan), starting with the sugar that is furthest to the left or top of the molecule. The name of the first sugar and those in intermediate positions ends with the suffix **-osido** or **-osyl**. The name of the last sugar ends in **-ose** if it is reducing, or **-oside** if it is not reducing. If the holoside is branched, a vertical line is added at the branch point. The number of the carbons of the OH groups involved in the glycosidic bond is indicated in parentheses for every two adjacent sugar names.

II. 4. 2. Diholosides

A disaccharide is formed by the interaction of two sugars through a glycosidic bond. If only one anomeric carbon is involved in the bond, the second anomeric carbon is free; this is called a ***reducing disaccharide*** (maltose, lactose). If both anomeric carbons are involved in the glycosidic bond, it is a disaccharide, and the disaccharide is ***non-reducing*** (Table 7) and does not undergo mutarotation (sucrose, trehalose).

Table 7: The main disaccharides.

Structure of the disaccharide	Composition and linkage	Comment
Lactose (reducing sugar)  (*O*-β-D-galactopyranosyl(1→4)-glucopyranose) Gal(β1→4)Glc	**β-D galactose** + α **ou** β **D-glucose** β **(1→4)**	It is the milk sugar of mammals. An intestinal lactase, anchored in the membrane of enterocytes, hydrolyzes it into glucose and galactose, which can then be absorbed. Lactose intolerance is linked to a lactase deficiency. Lactose is the substrate for lactic acid fermentation by lactobacilli, which is the basis of cheese fermentations.
Maltose (reducing sugar)  (*O*-α-D-glucopyranosyl(1→4)-α-D-glucopyranose)	**α-D-glucose** + α **ou** β **D-glucose** α **(1→4)**	It is not very abundant in its free state. It is obtained during the digestion of polysaccharides (starch) by the action of amylases. Under physiological conditions, it can be hydrolyzed into 2 glucose molecules by maltase.
Isomaltose (reducing sugar)  D-glucopyranosido (α1 → 6) D-glucopyranose,	**α-D-glucose** + **β-D-glucoseα** **(1→6)**	It is a product of the breakdown of starch and glycogen.

Cellobiose (reducing sugar)  	**β-D-glucose + α ou β D-glucose β(1→4)**	It is a product of cellulose degradation. Through hydrolysis, it yields 2 molecules of glucose. Not hydrolyzed in the body by maltase due to its binding
Trehalose (non-reducing sugar)  Trehalose α-D-glucopyranosyl α-D-glucopyranoside Glc(α1↔1α)Glc	**α-D-glucose + α-D-glucose α(1→1)**	It is a glycoside found in fungi, bacteria, and even in the hemolymph of insects. Many organizatio nsThey accumulate it in response to thermal shocks (cold) or desiccation.
Sucrose (non-reducing sugar)  Sucrose α-D-glucopyranosyl β-D-fructofuranoside Glc(α1↔2β)Fru	**α-D-glucose + β-D-fructose α(1→2)**	It is an edible sugar derived from sugar cane or sugar beet.

Chemical hydrolysis

The hydrolysis of glycosides can be carried out chemically. It is performed at acidic pH (NH_2Cl N/10) and under heat (60°C) for 1 hour. This hydrolysis has no specificity; all glycosidic bonds are broken, and the products obtained are the sugar units.

Enzymatic hydrolysis

The hydrolysis of glycosidic bonds is carried out by enzymatic hydrolysis catalysts (hydrolases) specific to glycosidic bonds (glycosidases). Specificity is such that a glycosidase

can act only on a single substrate (primary specificity) and on a single anomer and even a single type of bond (secondary specificity). For example, we have glycosidases, α- or β-glycosidases, α- or β-glucosidases, etc. Some examples can be cited:

Action of osidases (disaccharidases)

Under cellular conditions, the hydrolysis of the glycosidic bond is catalyzed by enzymes called osidases, which exhibit specificity for the type of bond. These enzymes hydrolyze only disaccharides and have no effect on higher-order polysaccharides. Some examples of disaccharides include:

Trehalase: an intestinal enzyme that is an α-glycosidase specific for (α1→α1) bonds.

Sucrase: an intestinal enzyme, α-glucosidase, that hydrolyzes the (α1→β2) bond of sucrose but also the (α1→4) bond of maltose.

Invertase: it is a β-fructosidase specific for the (α1→β2) linkage. It hydrolyzes sucrose but does not hydrolyze maltose.

Maltase: intestinal enzyme which is an α-glucosidase specific for the (α1→4) linkage of maltose and the (α1→β2) linkage of sucrose.

Isomaltase: intestinal enzyme which is an α-glycosidase specific for the (α1→ 6) linkage of isomaltose.

Lactase: an intestinal enzyme that is a β-galactosidase specific to the (β1→4) linkage of lactose. It does not hydrolyze cellobiose.

Cellobiase: a β-glucosidase specific to the (β1→4) linkage of cellobiose. It does not hydrolyze lactose.

II. 4. 3. Other oligosaccharides

Two trisaccharides are found in nature: raffinose and gentianose (Fig. 44). Raffinose, present in sugar beets, is eliminated during sugar refining. It is denoted Gal (α1→6) Sucrose or D-galactopyranosido (α1→6) D-glucopyranosido (α1→β2) D-fructofuranoside. Gentianose is present in gentian. It is denoted Glc (α1→6) Sucrose.

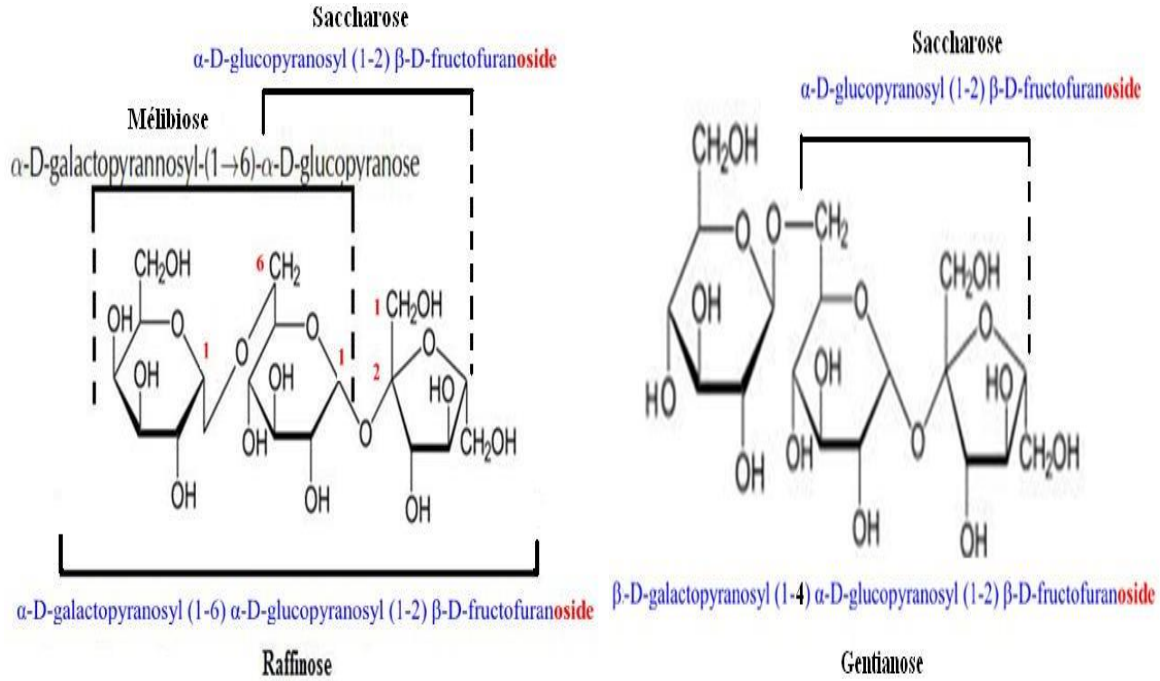


Fig. 44. Structure of raffinose and gentianose.

II. 4. 4. Polysaccharides

II. 4. 4. 1. Homopolysaccharides (homogeneous polysaccharides)

Also called homoglucans, they are composed of a single type of sugar or sugar derivatives (Table 8). They are formed by the repeated condensation of a sugar via glycosidic bonds exceeding 10 units, reaching several hundred or even thousands. Homopolysaccharides can be linear (amylose, cellulose, chitin) or branched (amylopectin, glycogen). They can be subdivided into two categories based on their functions:

Table 8: The main homopolysaccharides.

Name	Structure	Monomer	Connection	Kind
Amylose	linear	D-Glc*p*	α1→4	Glucane
Cellulose	linear	D-Glc*p*	β1→4	Glucane
Chitin	linear	D-GlcpN(Ac)*p*	β1→4	Chitosane
Amylopectin	branched	D-Glc*p*	α1→4	Glucane
Glycogen	branched	D-Glc*p*	α1→4	Glucane

II. 4. 4. 1. 1. Reserve polysaccharides

These are essentially glucosan (starch and glycogen) and a fructosan (inulin).

Starch

Starch is a polymer insoluble in cold water, although hydrophilic, with a water retention capacity of approximately 40%. Plants accumulate photosynthesized carbohydrates as a carbohydrate reserve (wheat, potatoes, rice, corn, etc.) in this condensed form. This homoglycan occurs as grains with two constituents: ***amylose***, which represents 15 to 30% of the starch, which is soluble in warm water and crystallizes upon cooling; and ***amylopectin***, which represents 70 to 95% of the starch, forms a viscous gel when heated.

Amyloidosis

Amylose (Fig. 45) is a perfectly repeating linear chain of 1000 to 4000 unbranched D-glucose monomers linked by a glycosidic bond (α1→4). Its molecular mass varies from 150,000 to 600,000.

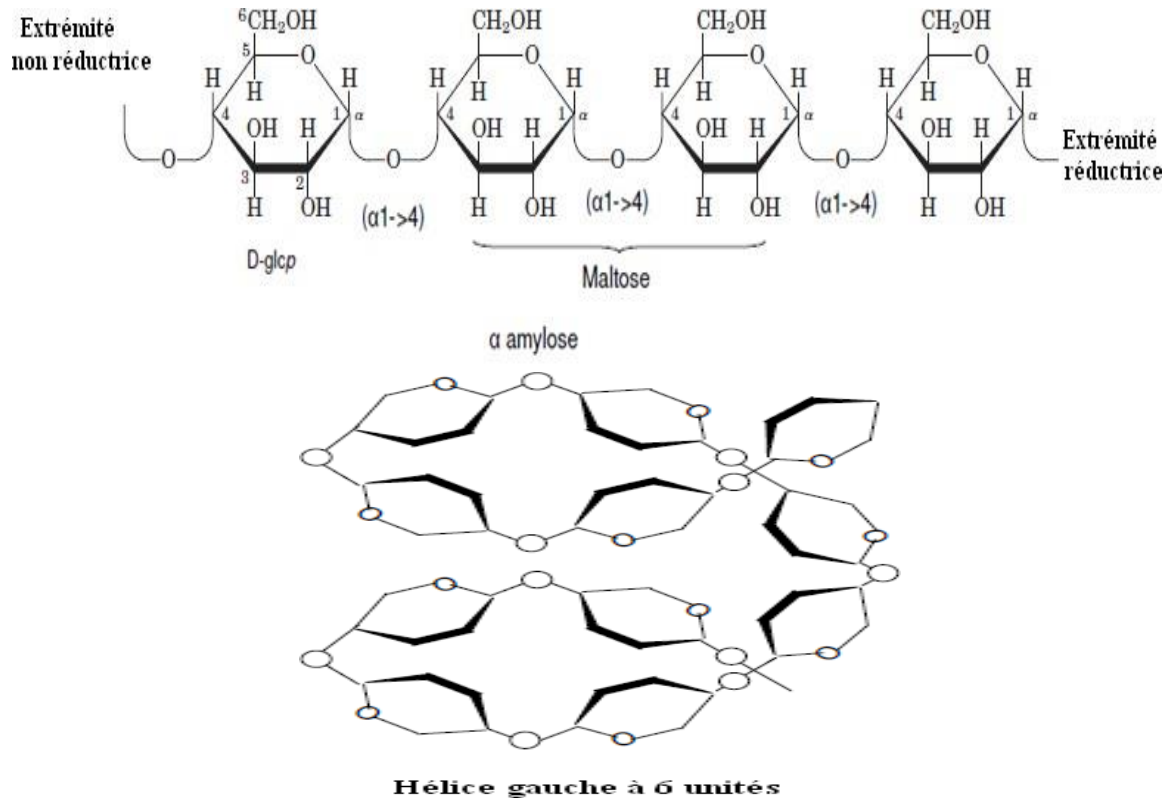


Fig. 45. Amylose structure.

X-ray analysis of the crystals reveals a left-handed helix structure by rotation around the glycosidic bond (α1→ 4) and maintained by a hydrogen bond between the hydroxyls at C2 of the first ring and C3 of the second ring, a helix with 6 glucoses per turn.

The action of amylase on amylose leads to the release of disaccharide maltose which can be split into two glucose molecules by acid hydrolysis or by maltase.

Amylopectin

Amylopectin (Fig. 46) is distinguished by a higher number of glucose molecules, but especially by its branched structure. On the main chain (α1→4), branch points, repeating approximately every 20 to 30 residues, are formed by an (α1→6) linkage where the anomeric carbon belongs to the branch. The entire molecule can contain more than 40,000 glucosidyl residues and reach molecular masses on the order of 1 million g/mol.

In contrast to the helical stretch of the amylose molecule, amylopectin takes on a compacted tree-like structure.

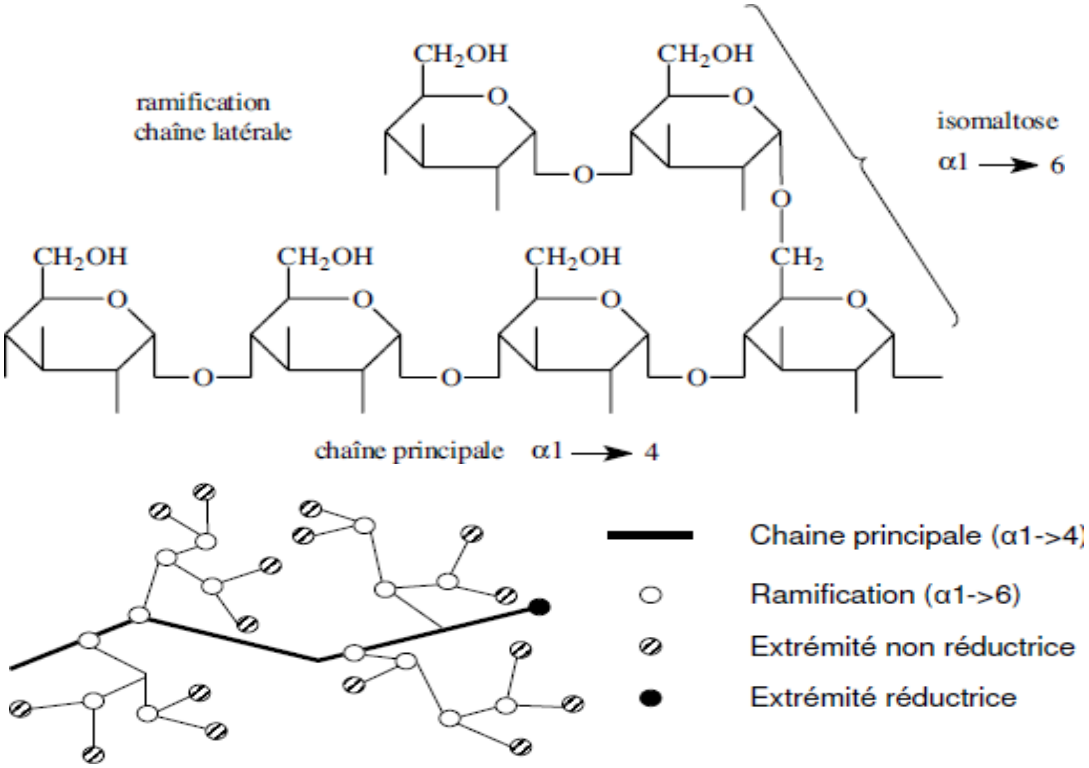


Fig. 46. Amylopectin structure.

Amylases can release maltose from amylopectin, as with amylose, specifically by cleaving the α(1→4) bonds. The α(1→6) bond is cleaved by another enzyme called: amylo-α-1,6-glucosidase (disconnecting enzyme).

Glycogen

This is the main form of carbohydrate energy reserve in animals (liver and muscles). It is a branched polymer of glucose (Fig. 47): the glucose residues are linked in linear chains by (α1→4) glycosidic bonds, with ramifications by branching between chains by (α1→6) bonds

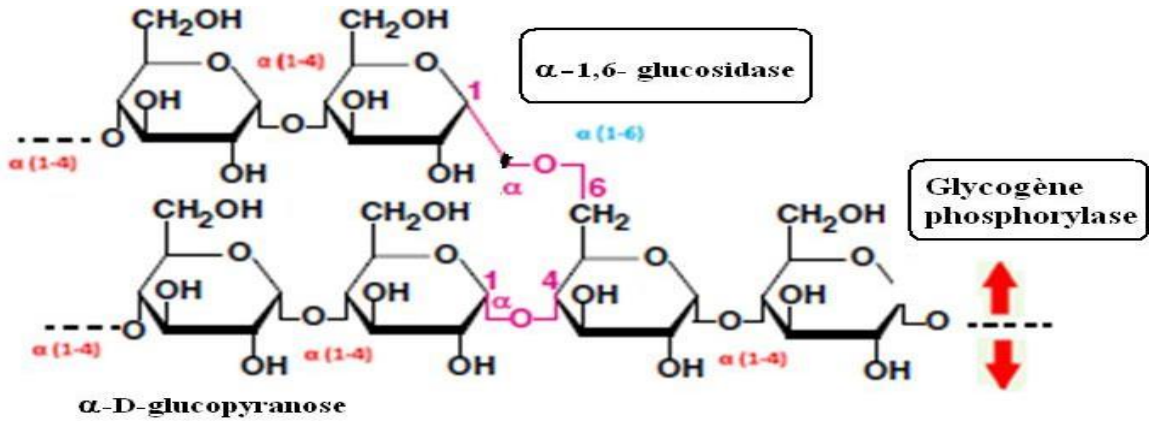


Fig. 47. Glycogen structure.

The structure of glycogen is similar to that of amylopectin, but with more frequent branching (branches occur every 8 to 12 residues, and even every 3 to 5 in the center of the molecule, and the average length of the branched chains is shorter), resulting in a more compact and denser appearance than amylopectin. Glycogen has an even higher molar mass, reaching up to 100 million g/mol.

Dietary glycogen is broken down in the same way as amylopectin. In the liver and muscle, the mechanism is different. A glycogen phosphorylase activated by hormones, glucagon in the liver, adrenaline in muscle, promotes the sequential breakdown of glycogen by releasing a residue from a non-reducing end, a phosphorylated residue. This sequential breakdown, to be complete, requires a "debranching" enzyme to hydrolyze the (α1→6) linkage: amylo α1-6 glucosidase (Fig. 47).

Inulin

Belonging to the fructan family, it is a storage compound, a polymer of β-D-fructofuranose with 30 to 100 units linked by (β2→1) bonds, found in certain plants: dahlias, artichokes, Jerusalem artichokes. It is the only known compound with a β configuration.

Dextranes

Bacterial and yeast reserves are α-D-glucose polymers linked by (α1→6) bonds, with occasional branches on C3 or C4. They are a component of dental plaque, a product of oral bacterial proliferation. They are used:

- as plasma substitutes in therapy
- as a phase for low-pressure liquid chromatography, by grafting ionized functional groups for ion exchangers.

II. 4. 4. 1. 2. Structural polysaccharides

Generally extracellular, they build the framework of the exoskeletons of algae, plants (cellulose), and animals (chitin carapace of arthropods). They are unbranched polymers of glucose or a derivative, and the link between units is a bond with the β anomer.

Cellulose

Present in certain bacteria, it is the major constituent of plant cell wall fibers. Cellulose (Fig. 48) represents half of the carbon available on earth, but it is not a source of glucose except for ruminants.

It is a linear polymer whose glycosidic linkage is of the (β1→4) type. This linkage is locked in a "head-to-tail" configuration stabilized by hydrogen bonds between the heterocyclic oxygen of one monomer and the OH function attached to the C3 of the following monomer.

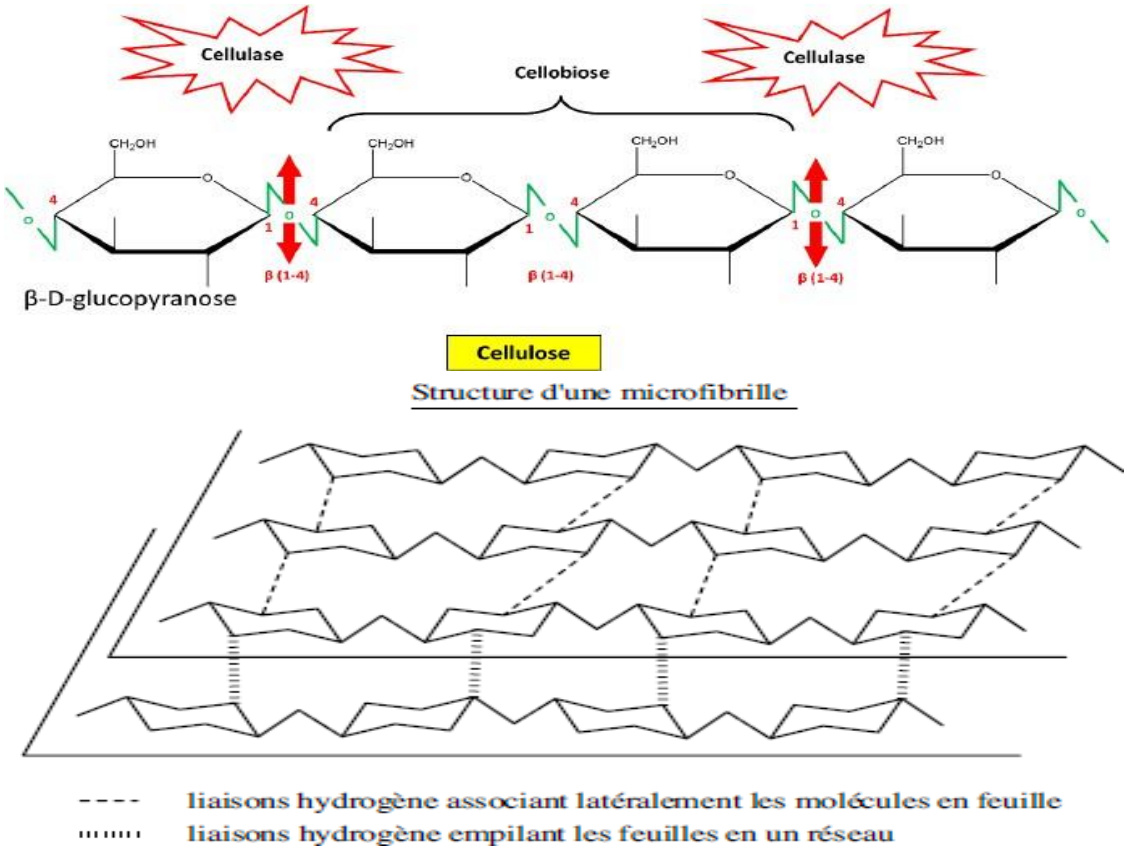


Fig. 48. Structure of cellulose.

These polymers organize themselves into sheets through hydrogen bonds between the different chains, which are laterally "glued" together. These sheets are stacked parallel to each other with a constant offset into microfibrils (from a few hundred to 2000 units and between 10 and 25 nm thick), a conformation always stabilized by hydrogen bonds between the units of the different sheets. These microfibrils associate into fibers or cross-laminated layers. The resulting structure exhibits remarkable mechanical strength and resistance to degradation. Cellulose is used in numerous products:

- Cotton contains approximately 95% cellulose
- Cellulose is used for making paper, papyrus.
- It is a support for adsorption chromatography and, by grafting ionized functional groups, for ion exchangers.

Cellulose degradation

This process is carried out by β-glucosidases, the cellulases. This hydrolysis leads to cellobiose, which is then hydrolyzed into glucose by the cellobiases. Snails have cellulases in abundance; mammals lack them and cannot assimilate grass, except for herbivores, which harbor saprophytic bacteria in their digestive tract that produce the necessary β-glucosidases.

Chitin Glc Nac (β1→ 4)

Chitin (Fig. 49) is an unbranched polymer of N-acetylglucosamine residues linked by (β1→4) bonds. It differs from cellulose only in that the glucose C2 is replaced by the acetylamine group. This polymer has the same structure as cellulose. This polymer constitutes the exoskeleton of invertebrates (crustaceans, mollusks, insects).

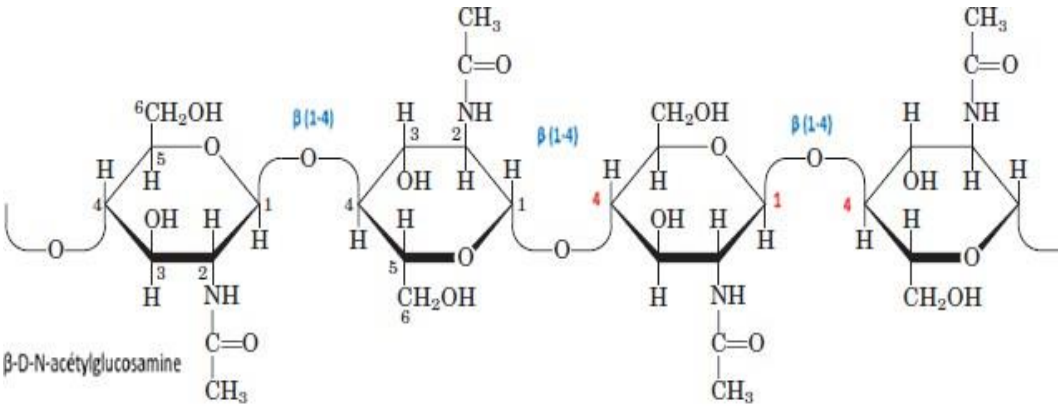


Fig. 49. Structure of chitin.

II. 4. 4. 2. Heterogeneous Polysaccharides

Also called heteropolyosides or heteropolysaccharides, they are made up of different types of sugars or sugar derivatives. They are mainly found as structural polymers, constituents of cell walls in plants and constituents of the extracellular matrix and cell coat in animals.

a – Glycosaminoglycans (GAG)

It is a polymer with a repeating motif made up of two sugars, one of which is an amino sugar, N-acetylglucosamine or N-acetylgalactosamine, and the other a sugar, glucuronic acid, iduronic acid, or galactose. The GAG chains can bind to a protein, leading to the formation of a proteoglycan.

Gums: The hydrophilic part of the secretions of "gum trees" such as acacias are highly branched galactoarabans (complex polymers made up of galactose and arabinofuranose).

Agar-agar or agar

Some red algae (Rhodophyceae) synthesize polysaccharides which they excrete into the culture medium, forming an agar-agar gel. This is a complex polysaccharide of D- and L-galactose with irregular sulfate content (hydrolysis yields Gal + GalNAc + SO_4^{--}). After extraction and purification, agarose (also called agar) is obtained.

This sulfated acid polysaccharide is of great interest due to its cross-linking and high water-retention capacity (it is very hydrophilic). It allows for the preparation of culture media in microbiology, as a support for electrophoresis (molecular biology) and immunodiffusion.

Alginates

Brown algae provide alginates, linear polyuronides made of a dioside consisting of two uronic acids (Fig. 51), β-D-mannuronic acid (β-D-ManUA) and α-L-guluronic acid (α-L-GulUA) linked by an (α1→4) bond.

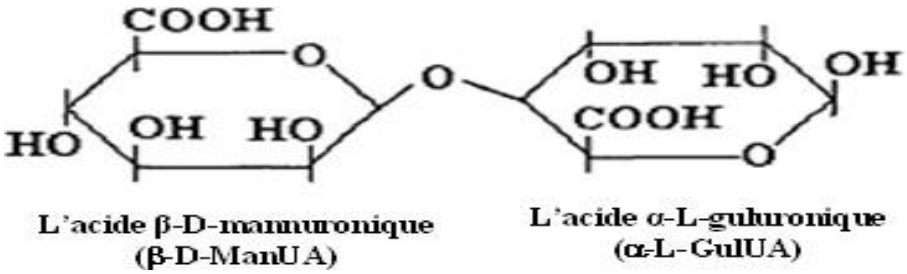


Fig. 50. Structure of alginates.

Carrageenans

Carrageenans, thickeners and gelling agents used in the food industry, are also extracted from red algae: these are linear polymers of diosidic units (1000 residues) of sulfated galactose (subunits called carrabiose) linked by a (β1→ 4) bond.

These carrabioses are linked together in the chain by (α1→3) bonds. Furthermore, the galactoses are either esterified with sulfuric acid or carry an oxygen bridge between carbons 3 and 6 (anhydrogalactose). The presence of sulfuric acid gives carrageenans a markedly acidic character. There are different types of carrabiose, including three main ones: kappa (k)carrabiose, iota (i)carrabiose, and lambda (l) carrabiose (Fig. 50).

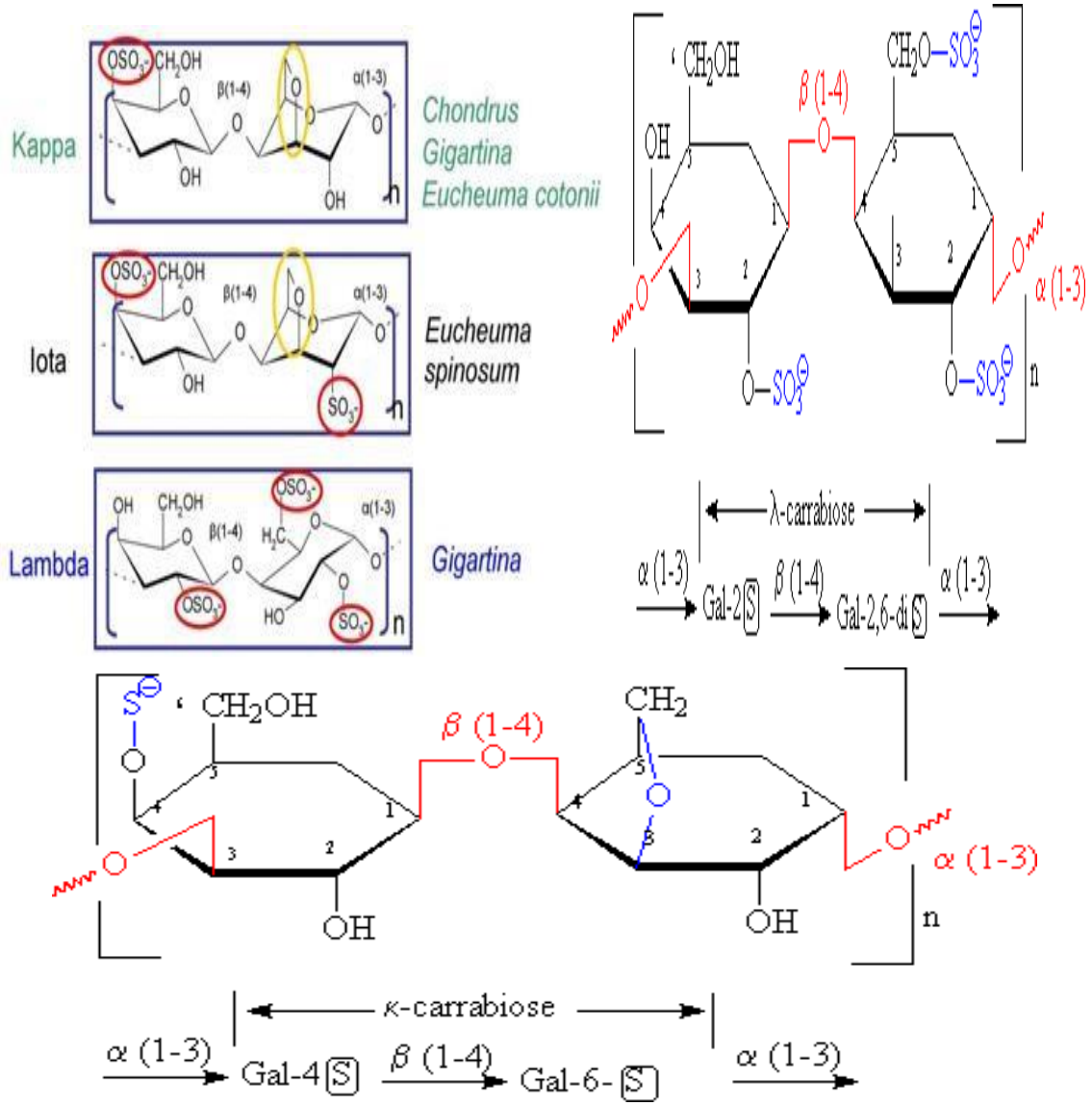


Fig. 51. Structure of the different types of carabidosis.

Bacterial polysaccharides

They are made up of sugars, osamines and uronic acids (Fig. 52). These are polysaccharides characteristic of the capsules, responsible for the virulence of the bacterium.

They possess certain specific sugars such as dideoxy 3-6 hexoses (e.g., Tyvelose) which serve to protect the bacteria from classical glycosidases. They contain muramic acid in their cell wall (a lactic acid residue linked by an ether bond to the OH group 3 of GlcNAc).

The glycosidic bonds of muramic acid are hydrolyzed by lysozyme. Lysozyme thus has a bacteriolytic effect by destroying the bacterial cell wall (it is found, for example, in egg white and milk).

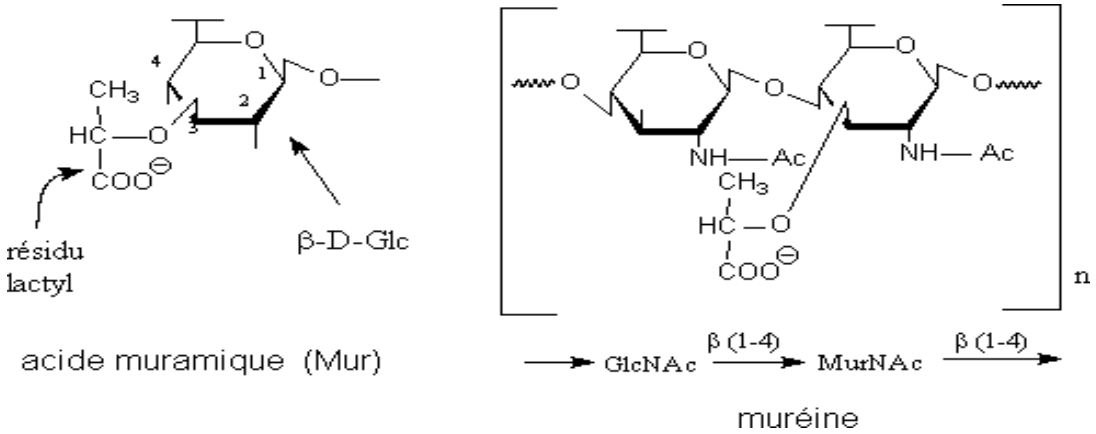


Fig. 52. Structure of bacterial polysaccharides.

II. 4. 4. 3. Heterosides

The semi-acetal group of a sugar can react with a non-carbohydrate compound. The resulting non-glycosidic compound is called an aglycone. This produces a glycoside, often referred to as a glycoconjugate.

a- Glycolipids

Lipids in the membranes of animal and bacterial cells carry oligosaccharide or polysaccharide chains: these are glycolipids. Glycolipids constitute a very heterogeneous group of glycoconjugates defined by the covalent bonding of various glycans to different lipid prosthetic groups. In higher organisms, three main types are distinguished: glycoglycerolipids, glycosphingolipids, and glycosyl phosphoinositides. They are primarily found in the membranes of eukaryotic cells, where their polar heads, composed of more or less complex oligosaccharides, appear on the outer surface.

b- Lipopolysaccharides

Lipopolysaccharides (LPS) are components of the outer membrane of Gram-negative bacteria. For example, the LPS of *Salmonella typhimurium* is composed of a glycolipid A lipid where six fatty acids are attached to two glucosamine residues, one of which is linked to a complex oligosaccharide. This complex oligosaccharide itself consists of a basal oligosaccharide and a specific O-chain, the chemical composition of which varies from one strain to another. Some LPS are toxic to humans and animals; they can therefore be responsible for the toxic shock syndrome observed during infections with Gram-negative bacteria.

c- Glycoproteins (GP)

These are proteins onto which short carbohydrate chains are grafted, the fraction of which generally varies from 1 to 20%. The glycosides are attached to the proteins by two types of bonds formed by condensation: the N-glycosidic bond (which is generally established between the N-acetylamine derivative of glucose and the amide function of asparagine) and the O-glycosidic bond is more diverse (established by the N-acetylamine derivative of galactose in mammals (mannose for yeasts…) and the alcohol function of serine or threonine).

d- Proteoglycans (PG)

These are often very long polysaccharides (glycosaminoglycans or GAGs) associated with a protein, remaining the majority (> 90%). They appear as soluble proteins or secreted into the extracellular matrix or as intrinsic transmembrane proteins (Fig. 53).

In matrix proteoglycans, chondroitin 4- or 6-sulfate is found in cartilage, tendons, ligaments, and the walls of the aorta; dermatan sulfate is present in the skin and certain blood vessels; keratin sulfate is found in certain horny structures: horn, claw, nail, but also in the cornea, cartilage, and bones (Fig. 53). Hyaluronates, with a molecular mass that can reach 10^7, never bound to proteins, give rise to the highly viscous solutions present in the vitreous humor of the eye and the synovial fluid of joints. Heparan sulfates constitute a very heterogeneous family; one of its members is heparin, an anticoagulant synthesized by mast cells and secreted into the circulating blood where it activates antithrombin III (Fig. 53).

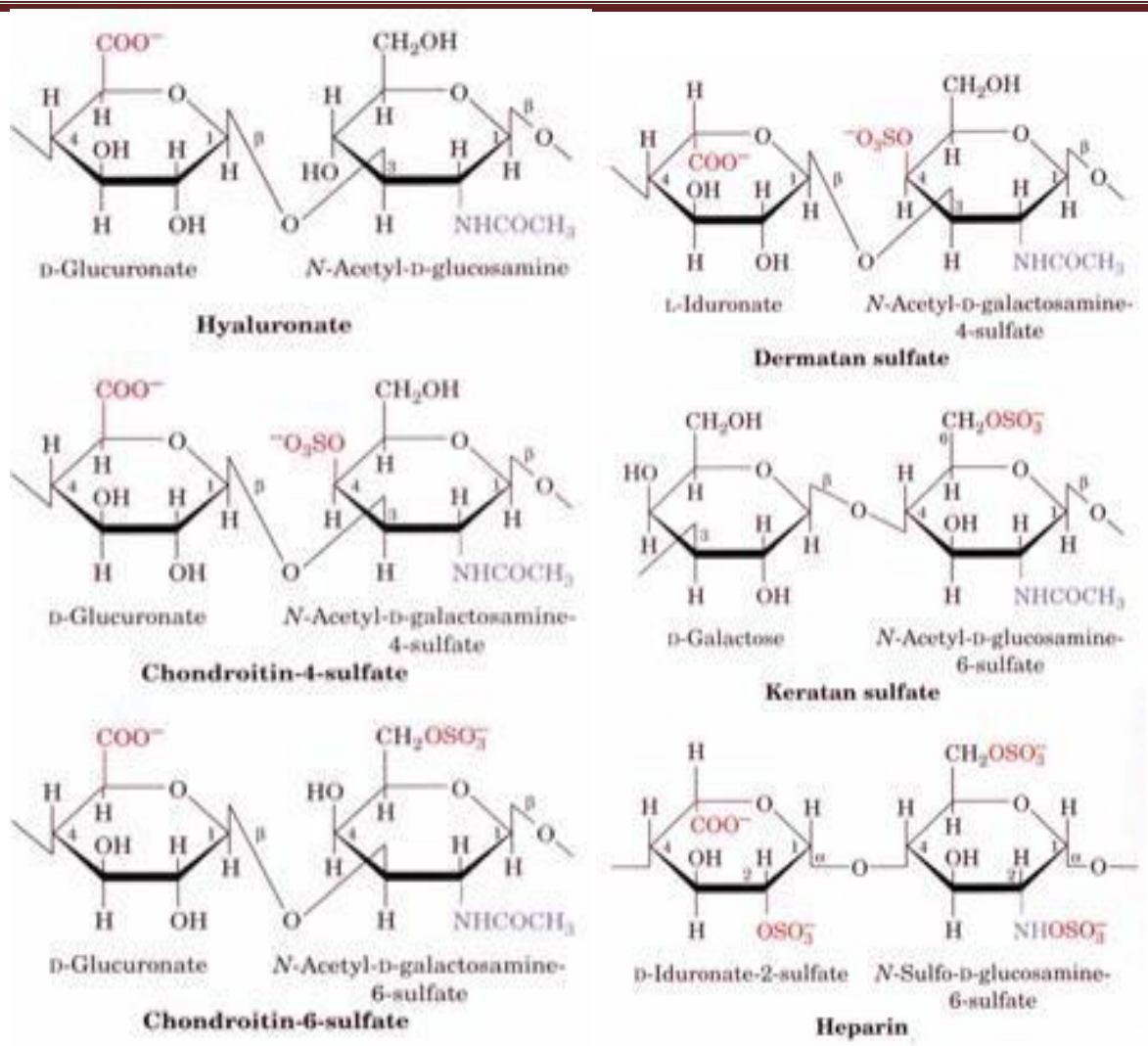


Fig. 53. Structure of certain proteoglycans.

e- Peptidoglycans

These are very large molecules, composed of the covalent association of proteins and carbohydrate polymers belonging to the glycosaminoglycan (GAG) family (Fig. 54). These result from the linear polycondensation of osamine and uronic acid units, which can be sulfated. The majority of these compounds are found in the extracellular matrix (connective tissue), in plasma membranes, and a few are intracellular.

Peptidoglycans form the cell wall of bacteria, giving them their shape and protecting them. The structure of murein, which constitutes the cell wall of *Staphylococcus aureus*, is a covalent association of:

- Polysaccharide: a repeating β-linked sequence of N-acetylglucosamine disaccharides, but one of the sugars (MurNAc) is substituted by condensation at the C3 alcohol group with lactic acid (muramic acid), and two oligopeptides: a tetrapeptide and a pentapeptide. The cross-linking is

formed by amide bonds: each MurNAc links its carboxyl group to the N-terminus of the tetrapeptide. The pentapeptide links the tetrapeptides of two chains by its N-terminus to the C-terminus of one tetrapeptide and by its C-terminus to the NH_2 group of lysine, the third amino acid of the other tetrapeptide.

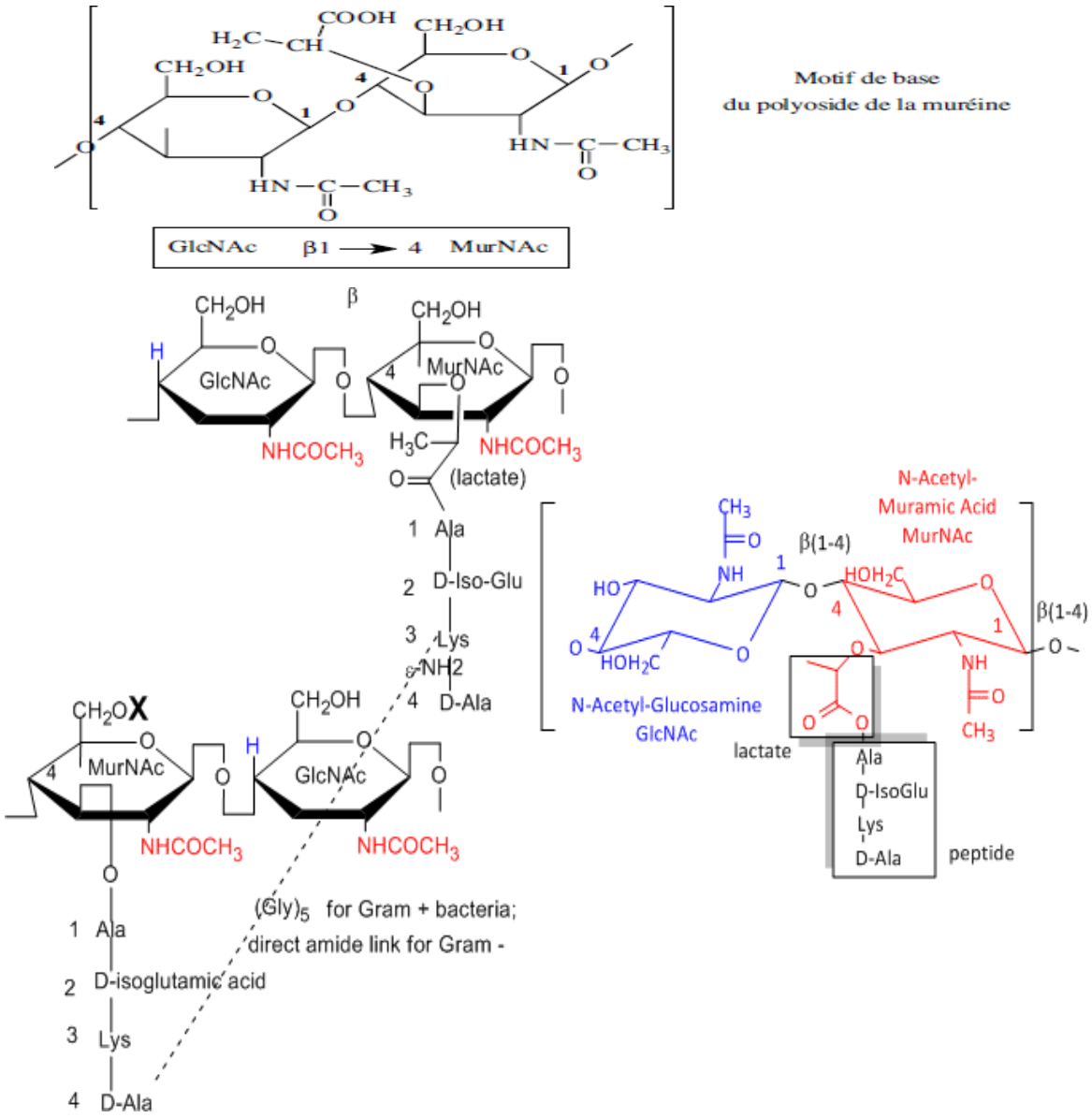
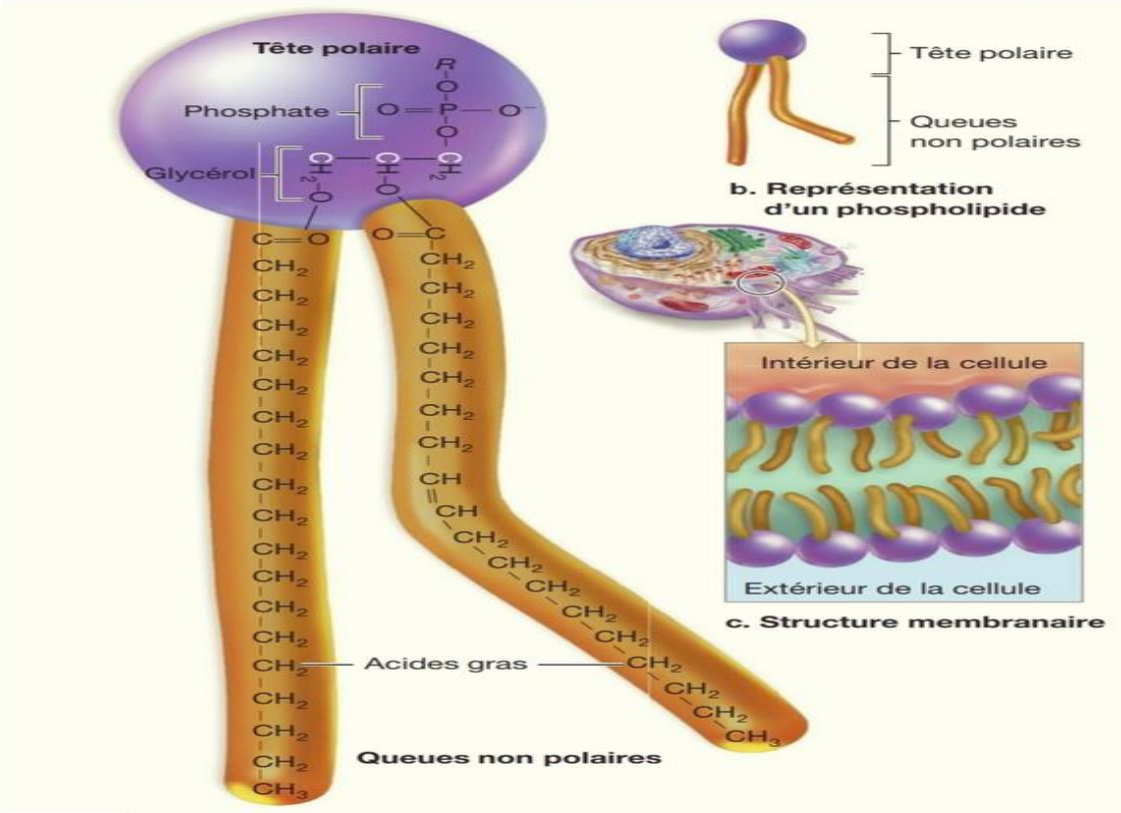


Fig. 54. Structure of peptidoglycan forming the cell wall of bacteria

CHAPTER III :

Lipids:

Structure and physicochemical properties



CHAPTER III
Lipids: Structure and physicochemical properties

III. 1. General presentation

Lipids (from the Greek lipos, fat) constitute a vast and heterogeneous group of substances of biological origin, which dissolve easily in organic solvents (methanol, chloroform, cyclohexane, ethyl ether, acetone…) but which are, on the contrary, poorly or not at all soluble in water (hydrophobic behavior).

Lipids can be classified according to their chemical nature and the role they play in the structure and function of organisms. We can thus distinguish: ***Structural lipids*** (phospholipids or sphingolipids), ***storage lipids*** (triglycerides), and ***lipids with biological activity*** (eicosaonoids: prostaglandins, prostacyclins, thromboxanes, leukotrienes).

III. 2. Classification

Lipids can be classified into three main categories (Fig. 55):

III. 2. 1. Simple lipids or homolipids

These lipids are made up of carbon, hydrogen and oxygen atoms. They are neutral, hydrolyzable lipids made up of fatty acids linked by an ester bond to an alcohol which can be of 3 types: a glycerol (in the case of a glyceride or glycerolipid), a sterol (in the case of a steride) or a fatty alcohol (in the case of a ceride).

III. 2. 2. Complex lipids or heterolipids

To the previous atomic composition is added phosphorus, nitrogen, sulfur, or a carbohydrate group (glycerophospholipids, sphingolipids, and glyceroglycolipids). These are polar hydrolyzable lipids that contain at least one fatty acid, which can be considered the basic element common to all lipids.

III. 2.3. Lipid-like compounds

These are non-hydrolyzable compounds. The absence of fatty acids in their structure means they are not true lipids. However, their hydrophobic (and fat-soluble) behavior leads to their classification as lipids. They include sterol derivatives and fat-soluble vitamins.

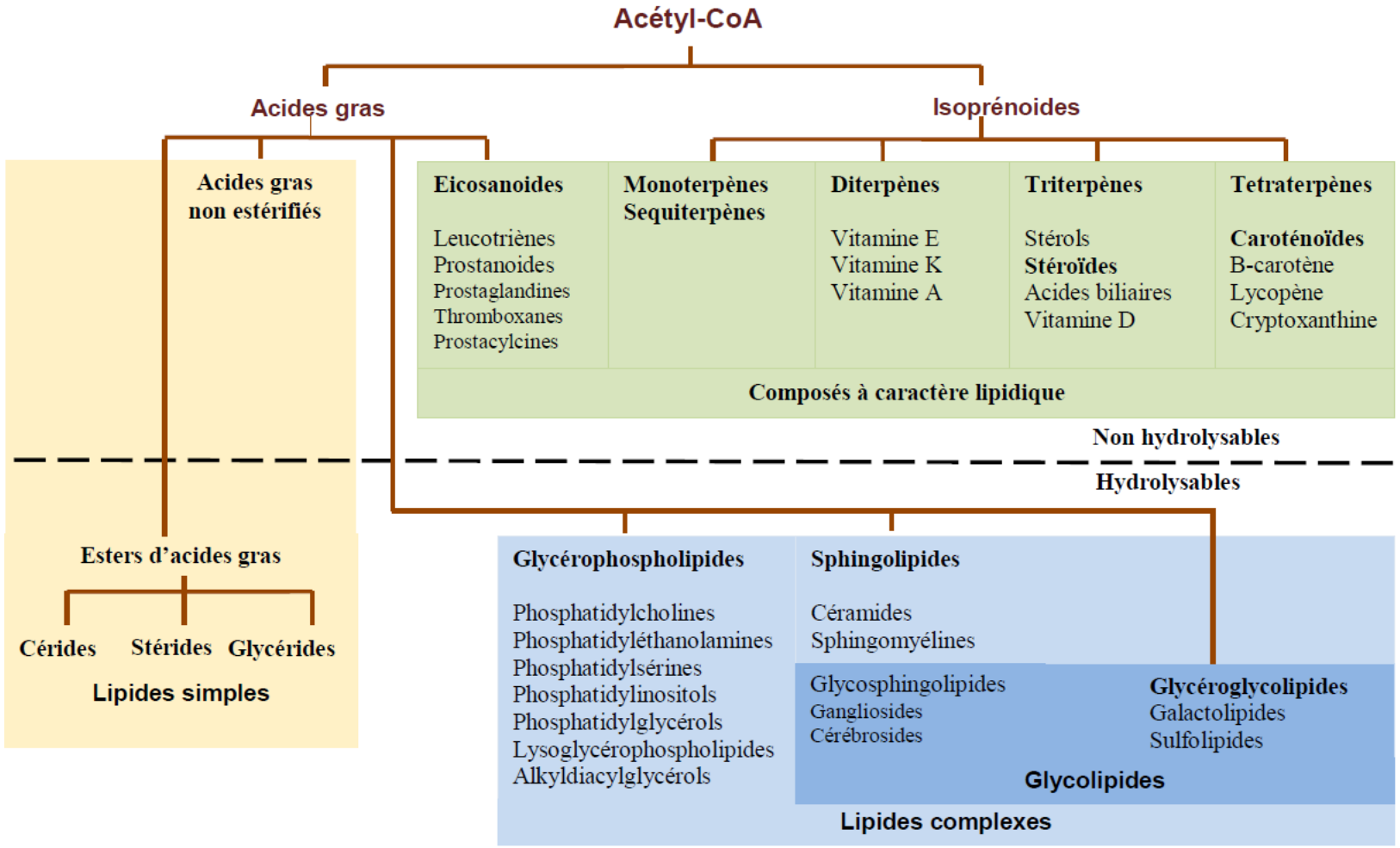


Fig. 55. Classification of lipids.

III. 3. Fatty acids

Fatty acids are carboxylic acids characterized by a repetition of methylene groups (-CH_2-) forming a long aliphatic chain (Fig. 56), generally composed of an even number of carbon atoms (from 4 to 36 carbon atoms). They are rarely found in their free state in nature and are primarily found in esterified form as major constituents of various lipids. Their structure includes: a carboxyl group (COOH) at one end, which gives them their acidic character. It dissociates at pH 7 and is hydrophilic; and a linear hydrocarbon chain with hydrophobic properties.

Carbons are identified by numbers: C1 is the carbon of the head carboxyl group, then we have C2, C3, etc., up to the terminal C of the methyl group. Another identification assigns the letter α to C2 and β to C3, the terminal C is denoted omega ω.

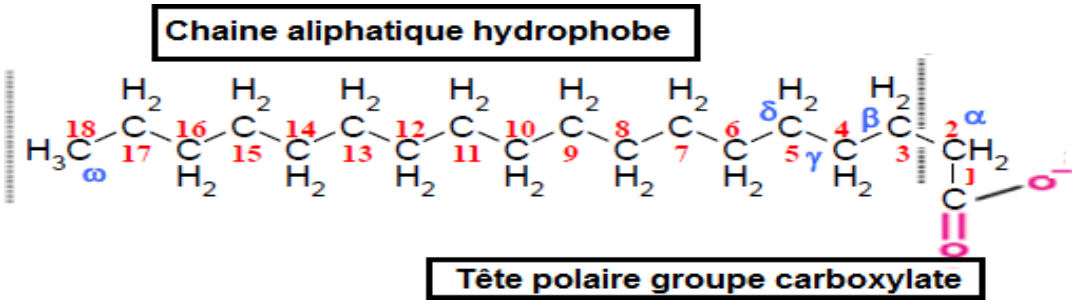


Fig. 56. Structure of a fatty acid.

The different fatty acids are distinguished on the one hand by the number of carbons, and on the other hand by the presence or absence of unsaturations (double bonds).

III. 3. 1. Classification of fatty acids

III. 3. 1. 1. Volatile fatty acids

These are special fatty acids because they are very short (2 to 4 carbons). As a result, they readily transition to a gaseous state, hence their generic name. This unique characteristic is further reinforced by their water solubility. Three are distinguished: acetic acid (C2:O), the common name for ethanoic acid; propionic acid (C3:O), also called propanoic acid; and butyric acid (C4:O), or butanoic acid.

These molecules can be produced by the intestinal flora (microbiota), and absorbed by the intestinal mucosa for energy purposes.

Butyric acid is a component of butter. Propionic acid is found in aged cheeses. Acetic acid is present in vinegar.

III. 3. 1. 2. Saturated fatty acids

These molecules have a general formula CH3 - $(CH_2)_n$ – COOH. Their aliphatic chains do not have a double bond, hence their generic name (Fig. 57). Therefore, only the carboxyl group has reactive properties.

Their symbol is represented by C followed by the number of carbons and 0 for the absence of a double bond since they are saturated. For example, palmitic acid, with 16 carbon atoms, is written C16:0.

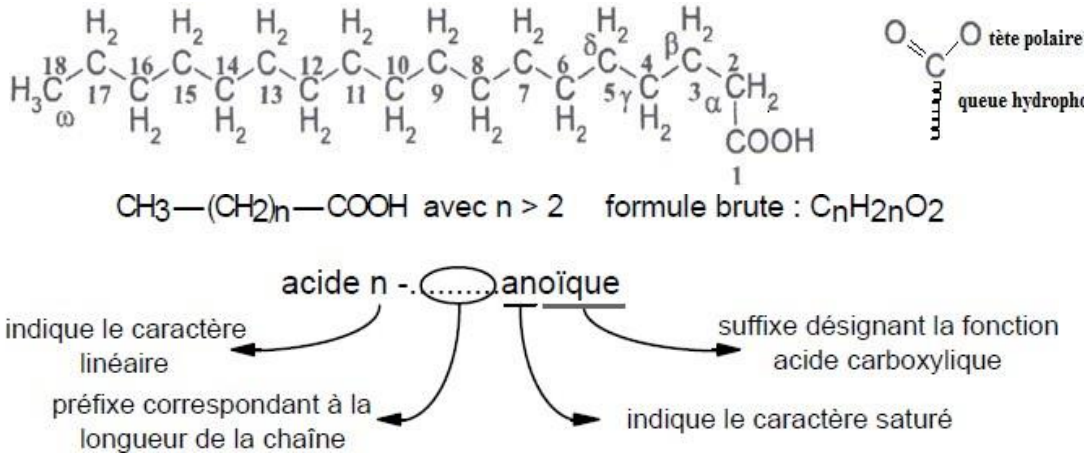


Fig. 57. Structure and nomenclature of a saturated fatty acid.

The most abundant are palmitic acid at 16C and stearic acid at 18C. For higher plants and animals, the most common fatty acids have 14 to 20 carbons, with a clear predominance of those with 16 or 18 carbons (Table 9).

Acids with fewer than 12 carbon atoms are found in mammalian milk and, of course, in butter. Fatty acids with more than 24 carbon atoms are primarily components of protective waxes produced by plants, bacteria, and insects.

III. 3. 1. 3. Unsaturated fatty acids

Their molecules contain one or more double bonds between two successive carbon atoms –HC=CH–. They are said to be monounsaturated or polyunsaturated. Their symbol (Fig. 58) includes the number of carbon atoms followed by the number of double bonds, their configuration (cis or trans), and the position of the double bonds is indicated, in superscript, by the letter delta Δ. Oleic acid is written C18:1Δ9. Example: oleic acid with the C18 structure has a double bond at position 9. The presence of these double bonds gives them particular physicochemical properties.

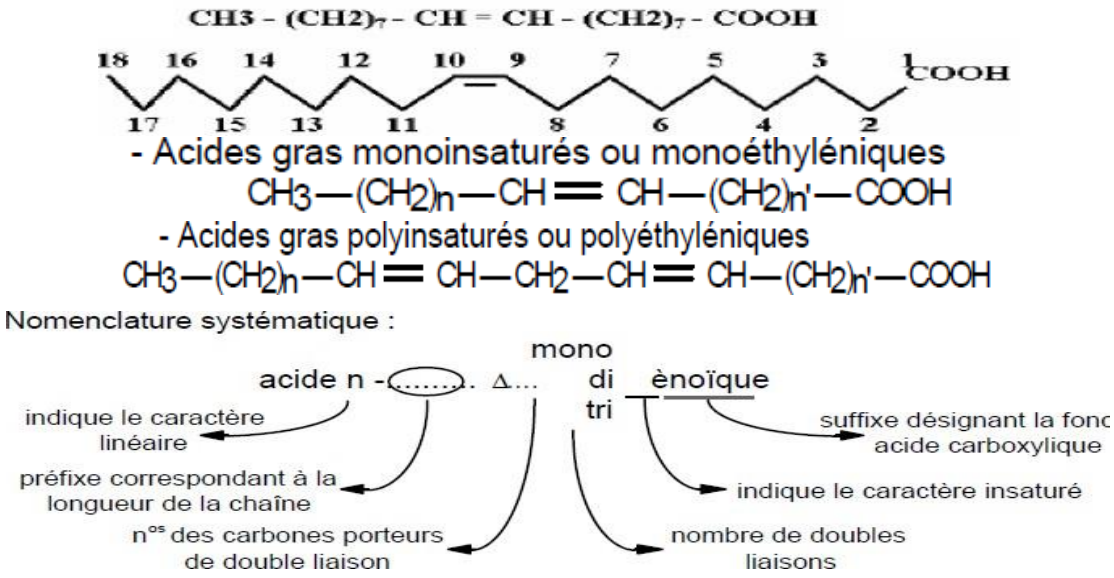


Fig. 58. Structure and nomenclature of an unsaturated fatty acid.

In these fatty acids, the double bonds are in a cis isomeric configuration where the H substituents are located on the same side (Fig. 59). This cis configuration causes the chain to bend at a flexure angle of 30°.

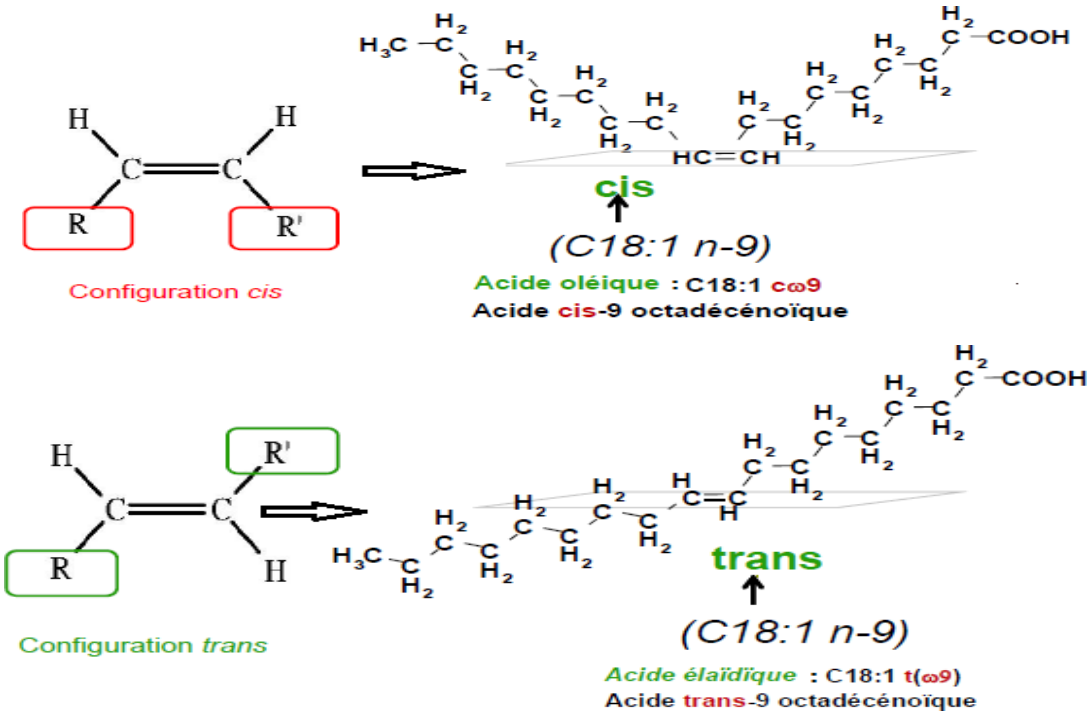


Fig. 59. The cis and trans configurations of a fatty acid.

In most monounsaturated fatty acids (Table 9), the double bond is located between carbons C9 and C10 (Δ9). Polyunsaturated fatty acids have several double bonds in their chains, always separated by one or more methylene groups: – CH=CH–CH2 – CH2–CH=CH–CH2–. The double bonds are in a cis configuration and

often in position C9, C12, C15 (Δ9, Δ12 and Δ15); arachidonic acid 20:4(Δ5,8,11,14), precursor of eicosanoid hormones, is a remarkable exception.

In addition to the systematic numbering, unsaturated fatty acids are classified in dietetics by series and not by the length of their chain (Fig. 60), the terminal methyl group is noted ω1. There are 4 main series: ω3, ω6, ω7 and ω9.

Either starting from carboxyl (1st carbon) : the symbol is: Δn

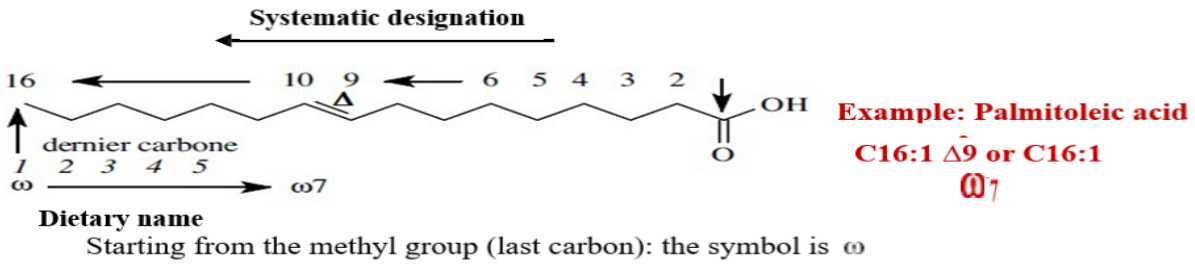


Fig. 60. The two possible names for a fatty acid.

Omega-3 fatty acids have a double bond on the third carbon atom from omega-1; thus, 18:3 omega-3 is alpha-linolenic acid. Omega-6 fatty acids have a double bond on the sixth carbon atom from the methyl group omega-1 (18:2 omega-6 is linoleic acid). Plants produce these fatty acids, but mammals, lacking the enzyme that allows the introduction of double bonds on carbon atoms beyond carbon C9, cannot synthesize these two fatty acids, known as "essential fatty acids." Humans must obtain omega-3 and omega-6 fatty acids from their diet. The importance of omega-3 and omega-6 fatty acids stems from the fact that they are precursors of prostaglandins and other essential molecules with biological activities.

Unsaturated fatty acids make up more than half of the fatty acids in plants and animals. Most unsaturated fatty acids have chain lengths of 16 to 20 carbons.

Polyunsaturated fatty acids can lose a hydrogen atom; this is called auto-oxidation. A lipid free radical, toxic to the cell, is formed, and when it reacts with O2, it forms a peroxide radical that is destructive to the cell. To neutralize these toxic radicals, the cell uses protective enzymes: superoxide dismutase and peroxidases. Vitamins C (ascorbic acid) and E (α-tocopherol) also scavenge these free radicals.

The most important families of fatty acids have as precursors oleic acid 18:1Δ9, linolenic acid 18:3Δ9,12,15, and linoleic acid 18:2Δ9,12. From these three compounds, the other most common fatty acids are formed in animals by a series of elongations and desaturations.

Table 9: Saturated and unsaturated fatty acids.

Relative Length	C NB	DB NB	Denomination	Common noun or usual	Abbreviated formula and series	
Short chain	4	0	n-Butanoic	Butyric acid	C4:0	Butter, rumen
	6	0	n-Hexanoic	A.C. Caproic	C6:0	Cow's milk
	8	0	n-Octanoic	Caprylic acid	C8:0	
	10	0	n-Decanoic	Capricorn	C10:0	
Medium chain	12	0	n- Dodecanoic	Ac laurique	C12:0	Laurel
	14	0	n-Tetradecanoic	Ac myristique	C14:0	Nutmeg
	16	0	n-Hexadecanoic	Palmitic acid	C16:0	Animal and vegetable fats (Oil of webbed)
	16	**1**	**Cis 9- Hexadecenoic**	**Palmitoleic acid**	**C16:1Δ9 or C16:1ω^{7}**	Animal fats (marine fish)
	18	0	n- Octadecanoic	Stearic acid	C18:0	Animal fats
	18	**1**	**Cis 9- Octadecenoic**	**Ac oleic**	**C18:1Δ9 or C18:1ω^{9}**	Olive oil
	18	**1**	**Cis 11- Octadecenoic**	**Ac vaccénique**	**C18:1Δ11 or C18:1ω^{7}**	Bacteria
	18	**2**	**Cis, cis 9, 12- Octadecadienoic**	**Linoleic acid**	**C18:2Δ9 C18:2ω^{6}**	Vegetable oils (sunflower, soybean, but)
	18	**3**	**All Cis 9, 12, 15 - Octadecatrienoic**	**Linolenic acid**	**C18:3Δ9 C18:3ω^{3}**	Vegetable oils (rape seed, soybeans)
Long chain	20	0	n-Eicosanoic	Peanut oil	C20:0	Peanut
	20	**4**	**All Cis 5, 8, 11, 14 Eicosatetraenoic**	**Arachidonic acid**	**C20: 4Δ5, 8, 11, 14 or C20: 4ω^{6}**	**Animals**
	20	**5**	**Tts Cis 5, 8, 11, 14, 17 Eicosapentaenoic**	**Ac EPA**	**C20 :4Δ$^{5, 8, 11, 14, 17}$or C20:5ω^{3}**	**Fish oils**
	22	0	n-Docosanoique	Ac behenic	C22:0	
	22	**0**	**Cis 13 Docosanoique**	**Erucic ace**	**C22 :1Δ13**	**Rape seed, Mustard**
	22	**0**	**All Cis 4, 7, 10, 13, 16, 19 Docosahexadecenoic**	**Cervical encephalopathy or DHA**	**C22 :6Δ4,7,10,13,16,19 C22:6 3**	Oily fish
	24	0	n-Tetracosanoic	Ac lignocérique	C24:0	Brain
	24	**1**	**Cis 15 Tetracosenoic**	**Nervous system ailment**	**C24:1Δ15 or C24:1ω^{9}**	Brain
	26	0	n- Hexacosanoic	Ac cerotic	C26:0	Plant waxes, bacteria, insects
	28	0	n- Octacosanoic	Montanique Academy	C28:0	
	30	0	n-Triacontanoic	Melisique acacia	C30:0	
	32	0	n- Dotriacontanoique	Ac lacerates	C32:0	

III. 3. 1. 4. Atypical fatty acids

III. 3. 1. 4. 1. Hydroxylated fatty acids

Plants are capable of synthesizing a series of hydroxylated fatty acids such as ricinoleic acid (12-hydroxy-9-cis-octadecenoic acid) 18:1ω^9(Fig. 61). Some of these hydroxylated fatty acids are used in the formation of cutin.

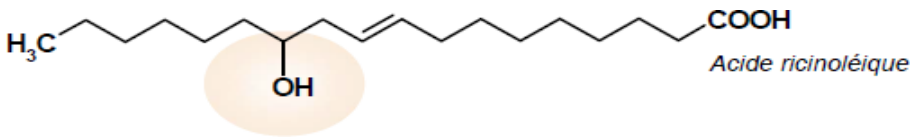


Fig. 61. Structure of ricinoleic acid: a hydroxylated fatty acid found in plants.

In mammals, other types of hydroxylated fatty acids are found. Some glycolipids contain large amounts of α-hydroxy acids (OH on carbon 2) at carbons 22, 23, 24 and 25.

III. 3. 1. 4. 2. Branched-chain fatty acids

The most common fatty acids have a branch (methyl, ethyl) on the carbon chain. An example is 15-methylhexadecenoic acid, which is particularly abundant in Gram-positive bacteria.

Derived from phytol, phytanic acid (3,7,11,15-methylhexadecanoic acid) is a constituent of certain bacterial lipids, particularly in halophilic and thermophilic bacteria (Fig. 62). This acid is normally catabolized in humans. Failure to degrade it leads to its accumulation in tissues (Refsum disease).

In other bacteria (mycobacteria, corynebacteria), mycolic acids are major components of the cell wall (Fig. 62). They are branched acids with very long chains. The main chain can have up to 70 carbons. It can be branched, hydroxylated, and may carry a ketone group or a propanic ring.

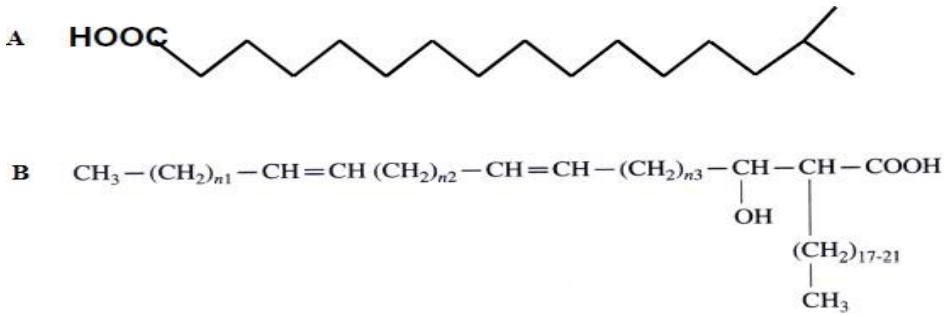


Fig. 62. Structure of phytanic acid (A) and mycolic acids (B).

III. 3.1.4.3. Prostaglandins and leukotrienes

Prostaglandins and leukotrienes (Fig. 63) are derived from 20-carbon polyunsaturated fatty acids.ω3 andω6 (hence the name eicosanoids) and more specifically arachidonic acid, under the action of cyclooxygenase (prostaglandins) and lipooxygenase (leukotrienes). In mammals, these are compounds with diverse hormonal actions.

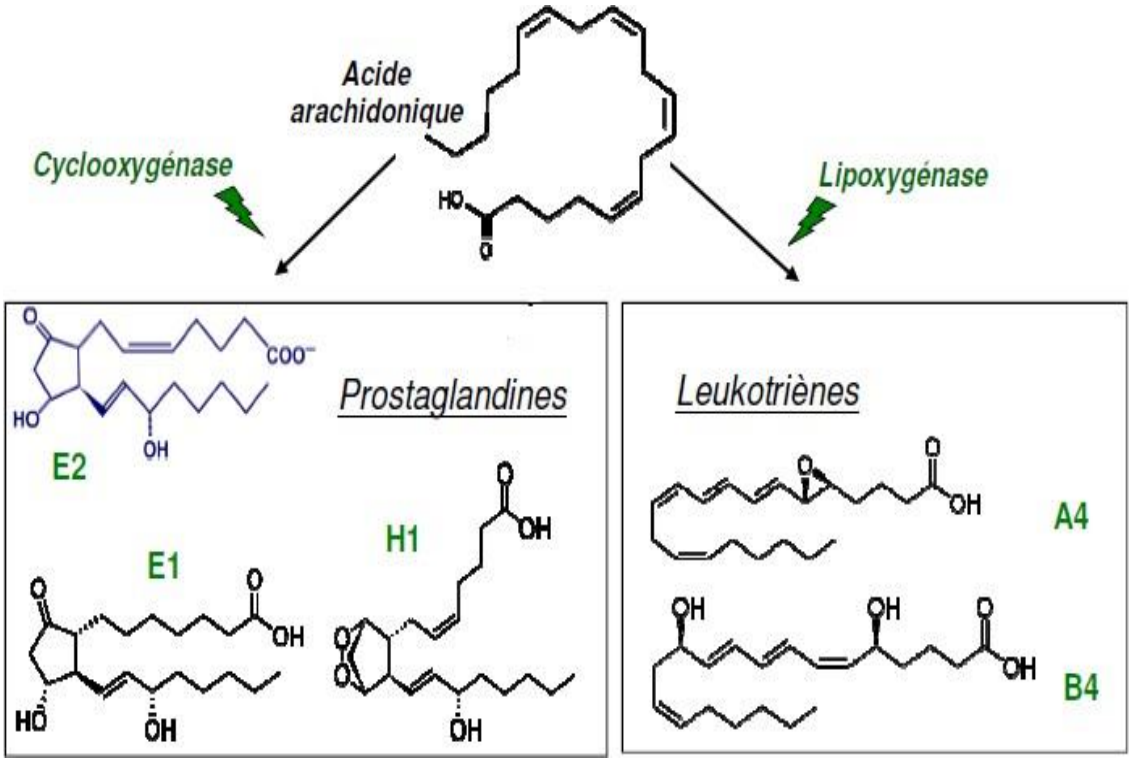


Fig. 63. Structure of prostaglandins and leukotrienes.

III.4. Physical properties

III.4.1. Melting point

The melting point is the temperature at which the fatty acid changes state, that is, goes from solid to liquid.

The longer and more saturated the carbon chain, the higher the melting point (for C < 10, fatty acids are liquid at room temperature; for C > 10, they are solid at room temperature). Indeed, the longer the aliphatic chain, the larger the alignment regions where Vander Waals forces are expressed, and therefore the more thermal energy is required to dissociate the molecules and make them transition to the liquid state (Fig. 64).

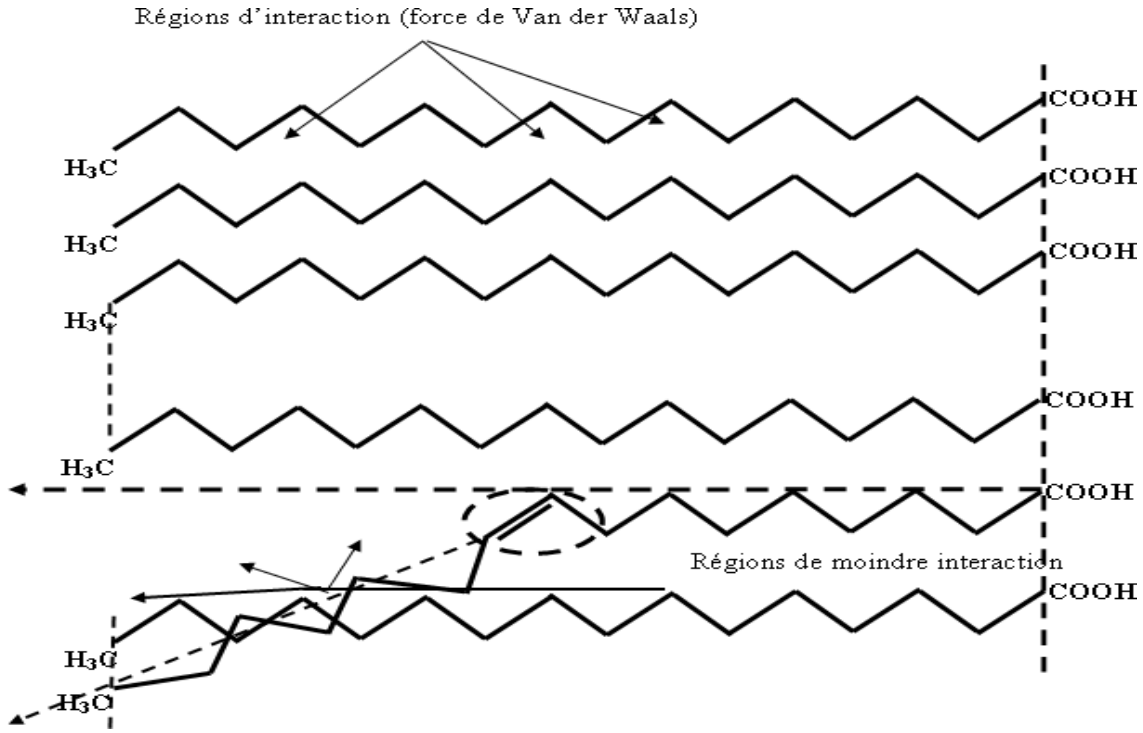


Fig. 64. Deflection of the aliphatic chain by the presence of a double bond.

Conversely, the melting point decreases with the degree of unsaturation of the carbon chain (Fig. 65). This helps explain why animal fats (rich in saturated fatty acids) are still solid at room temperature, while vegetable oils (rich in unsaturated fatty acids) are already liquid.

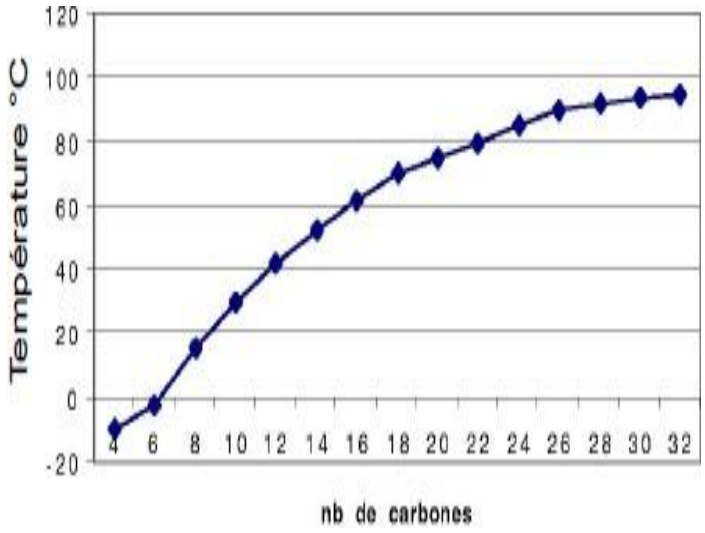


Fig. 65. Melting point of saturated fatty acids

Table 10: Effect of unsaturation on the melting point of certain fatty acids.

Fatty acid	**Melting point**
Butyric acid	-6.5°C
Palmitic acid	+63C°
Stearic acid	+70C°
Oleic acid	+13.5°C
Linoleic acid	-5°C
Elaidic Academy	+46°C
Linoleic acid	-9°C
Linolenic acid	-17°C
arachidonic acid	- 50°C

III.4.2. Density and solubility

Density

Fatty acids have a lower density than water (reference density), oil floats on the surface of water (or vinegar).

Solubility

The solvents used mainly for lipid extraction are alcohols (methanol, ethanol, isopropanol, n-butanol), acetone, acetonitrile, ethers (diethyl ether, isopropyl ether, dioxane, tetrahydrofuran), halogenated hydrocarbons (chloroform, dichloromethane), hydrocarbons (hexane, benzene, cyclohexane, isooctane) or mixtures thereof.

Short-chain fatty acids with up to four carbons are miscible with water (e.g., butyric acid has four carbons), but their solubility gradually decreases, becoming insoluble at concentrations of 10 carbons or more. Polar carboxyl groups are in contact with water. The overall behavior of the molecule depends on the size of the aliphatic chain. This implies that the water solubility of fatty acids decreases as the number of carbons increases.

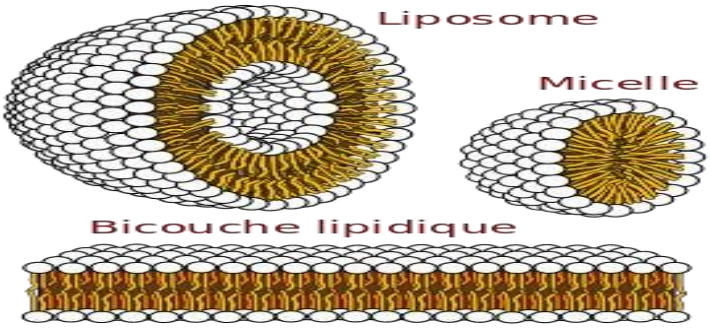


Fig. 66. Behavior of lipids in an aqueous environment.

Generally speaking, lipids are amphiphilic molecules: they have a polar (hydrophilic) head that attracts water and a nonpolar (hydrophobic) tail that repels water. This amphiphilic character is very pronounced in phospholipids, fatty acids in soaps, and sphingolipids. It is less pronounced in glycerides and sterols. This amphiphilic character determines the organization of lipids in water into: monolayers, bilayers (liposomes), or micelles (emulsions) (Fig. 66).

III.4. 3. Spectral properties

In their pure state, fatty acids are colorless. Conjugated double bonds give the fatty acid a characteristic spectrum in the UV range. The maximum absorption depends on the number of conjugated double bonds. This property allows for the quantification of unsaturated fatty acids. However, unsaturated fatty acids with malonic double bonds must first be treated with alcoholic potassium hydroxide at 180°C (heating in an alkaline medium) for one hour. This treatment

converts the fatty acid with the malonic double bond into its isomer with the conjugated double bond. This results in a characteristic UV spectrum between 316 and 332 nm.

III.5. Chemical properties of fatty acids

III.5.1. Properties due to the carboxylic function

III.5.1.1. Formation of salts with bases (saponification)

Fatty acids are weak acids (Fig. 67). Reacting with an alkali metal hydroxide yields an alkali salt of the fatty acid, or soap (solid salt with NaOH, resulting in hard soaps; liquid with KOH, resulting in soft soaps). Soaps are soluble in water and possess foaming, wetting, and emulsifying properties. Soap is a bipolar compound: a hydrophilic head (COO-) and a hydrophobic side chain (R). Soaps have detergent power due to their ability to reduce the adhesive forces between the soiling and the laundry, thus releasing the soiling and allowing it to pass into the aqueous phase.

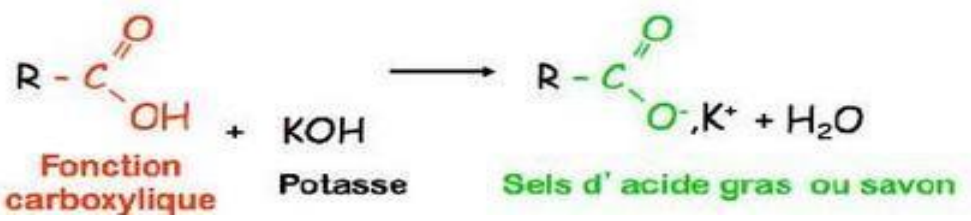


Fig. 67. Saponification reaction.

It is possible to use this property to calculate the saponification index, Is. The saponification index corresponds to the mass (in mg) of potassium hydroxide (KOH) required to saponify (neutralize) the fatty acids contained in 1g of lipid.

a. Ester formation

The carboxylic acid group is also characterized by its involvement in the esterification reaction (Fig. 68). This involves condensation with an alcohol group. Methyl esters are primarily produced (by the action of methanol, CH_3OH, in the presence of a catalyst), which allow for the fractionation of fatty acids by distillation or chromatography.

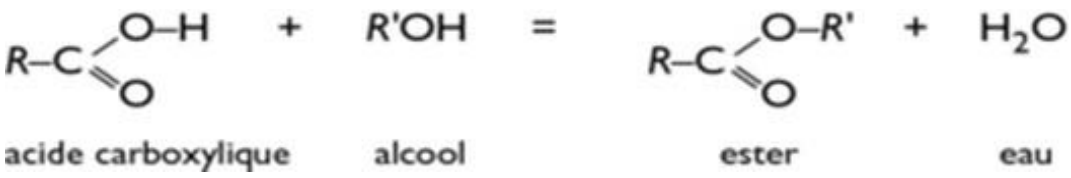


Fig. 68. The esterification reaction of lipids.

This is an important reaction for the body's use of fatty acids. The esterification reaction is important for the following reasons:

- It allows their storage (with glycerol, in the form of triglycerides), mainly in adipose tissue;
- It allows their metabolic use (β-oxidation) when it occurs with coenzyme A (noted CoA-SH). This forms an acyl-CoA which has a thioester bond. It is characterized by a high energy potential, enabling the initiation of enzymatic reactions using fatty acids (fatty acid activation reaction).
- For the analytical separation of fatty acids by gas chromatography or fractional distillation (distillation in several stages), they are transformed into methyl or ethyl esters. These esters have a boiling point 30 to 40°C lower than that of their non-esterified fatty acids.
- In the food and beverage industry, the esterification reaction is used to determine the fatty acid composition of vegetable oils and animal fats in order to detect fraudulent blends (mixtures with other oils of a different nature). The reverse esterification reaction is used for the production of alcohols from fats.

III.5.2. Properties due to the presence of double bonds

III.5.2.1. Addition reactions

When the carbon chain is saturated, it is very unreactive; it only expresses its hydrophobic behavior. Conversely, the presence of double bonds gives this region of the molecule a certain reactivity. Indeed, ethylenic bonds (unsaturations) can undergo an addition reaction.

a. Hydrogenation

This process is used to transform edible oils of unsaturated fatty acids into margarine. The latter is composed of saturated fatty acids that are solid at room temperature and, moreover, do not oxidize (Fig. 69). Under a pressure of 100 to 200 bars, at a temperature of 200 to 400 °C and in the presence of catalysts (platinum, nickel, zinc ...), unsaturated fatty acids fix hydrogen to give saturated fatty acids.

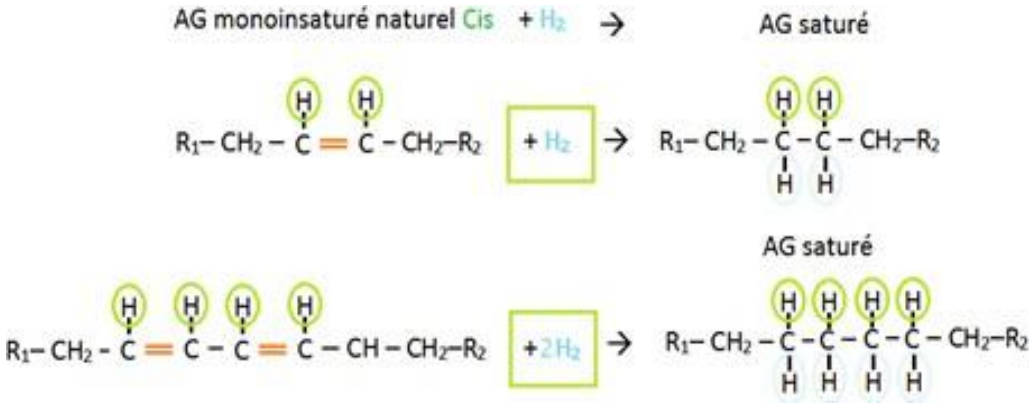


Fig. 69. Total hydrogenation of an unsaturated fatty acid.

During the industrial hydrogenation process, not all cis-configured unsaturated fatty acids become saturated (partial hydrogenation of an unsaturated fatty acid). Some mutate into trans-configured unsaturated fatty acids (Fig. 70). Trans unsaturated fatty acids are less digestible than natural cis-configured unsaturated fatty acids and are detrimental to health.

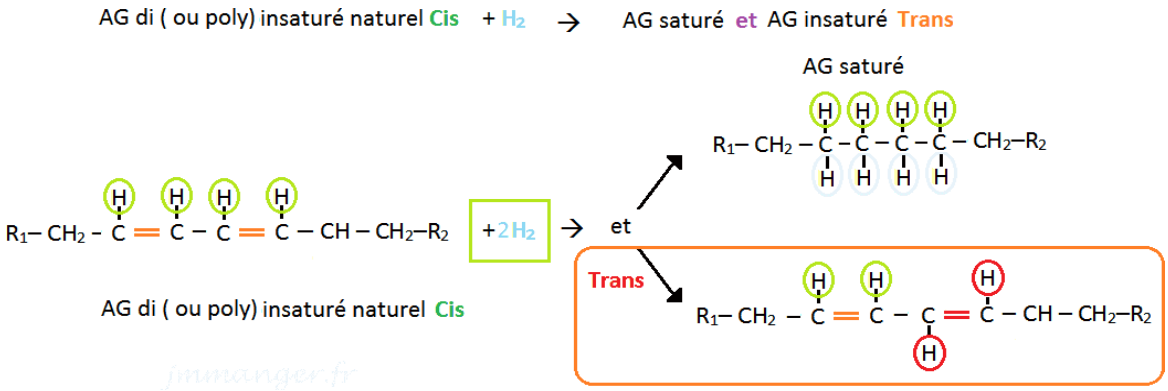


Fig. 70. Partial hydrogenation of an unsaturated fatty acid with production of a trans fatty acid.

b. Halogenation by Br or I2

The addition reaction can occur with halogens, such as iodine. The monounsaturated fatty acid is transformed by halogenation into a dihalogenated derivative (Fig. 71). This reaction allows the iodine value (Ii) to be defined.



Fig. 71. Fixation of iodine onto a double bond.

The iodine value corresponds to the mass of molecular iodine (in grams) bound to the double bonds per 100 grams of fat. It indicates the degree of unsaturation. This value is precisely defined for a given fatty acid. It increases with the number of unsaturates. It allows us to assess the purity of the fat used in a food preparation.

III. 5. 2. 2. Oxidation in the presence of KMnO4

An unsaturated fatty acid treated with a strong oxidant such as a concentrated KMnO4 solution, by cleaving the double bond, leads to the formation of a monoacid and one or more diacids (Fig. 72). The number of diacids is equivalent to the number of double bonds.

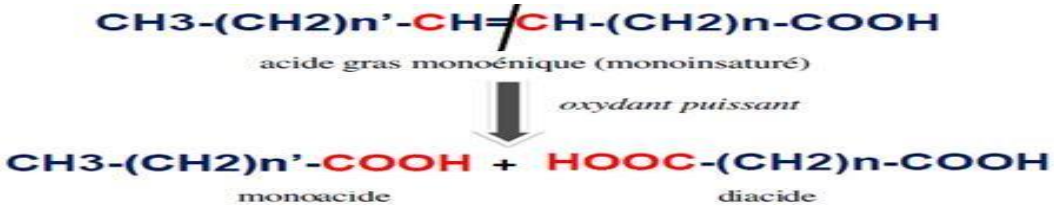


Fig. 72. Action of potassium permanganate on an unsaturated fatty acid.

The main factors determining lipid shelf life are oxidation reactions, which can be classified, according to their mechanism, as autooxidation, photooxidation, and enzymatic oxidation. The substrates for oxidation reactions are primarily unsaturated fatty acids. They generally oxidize more rapidly when they are free and more unsaturated. Saturated fatty acids only oxidize at temperatures above 60°C, while polyunsaturated fatty acids oxidize even during frozen food storage. The main problem posed by these reactions lies in the formation of volatile compounds with unpleasant odors (rancidity) and the formation of peroxides, which are carcinogenic molecules.

III. 6. Classes of lipids

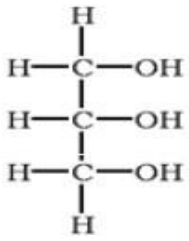
Lipids have been the subject of numerous classifications, but the most commonly used is that based on structure, where we distinguish: Simple lipids (homolipids) which include acylglycerols (or glycerides), cerides and sterides; Complex lipids (heterolipids) which include glycerophospholipids, glyceroglycolipids and sphingolipids which contain in their structure respectively phosphate, sulfate or carbohydrate groups.

III. 6. 1. Simple Lipids

Simple lipids are homolipids composed of C, H, O. They are esters of fatty acids, classified according to the alcohol: Acylglycerols (glycerides): esters of glycerol, cerides (fatty alcohol): esters of long-chain alcohols and sterides (polycyclic alcohol): esters of sterol.

III. 6. 1. 1. Glycerides

These are lipids whose structure is based on the esterification of fatty acids and a trialcohol: glycerol, which is a translucent, very viscous, odorless, non-toxic liquid with a sweet taste (Fig.73).



Glycérol (glycérine)
Propan-1,2,3-triol

Fig. 73. Structure of glycerol.

These simple lipids, also called glycerides or acylglycerols, are insoluble in water but soluble in acetone because they are highly nonpolar. Esterification with glycerol produces monoesters (monoacylglycerol = monoglyceride), diesters (diacylglycerol = diglyceride), and triesters (triacylglycerol = triglyceride). When the fatty acid molecules constituting the di- or

triester are identical, it is called homogeneous diacylglycerol or triacylglycerol. Otherwise, it is called mixed diacylglycerol or mixed triacylglycerol (Fig. 74).

Diacylglycerols are glycerol diesters. The fatty acid in position 2 is very often unsaturated. Diacylglycerols are formed during the digestion of triacylglycerols by lipases.

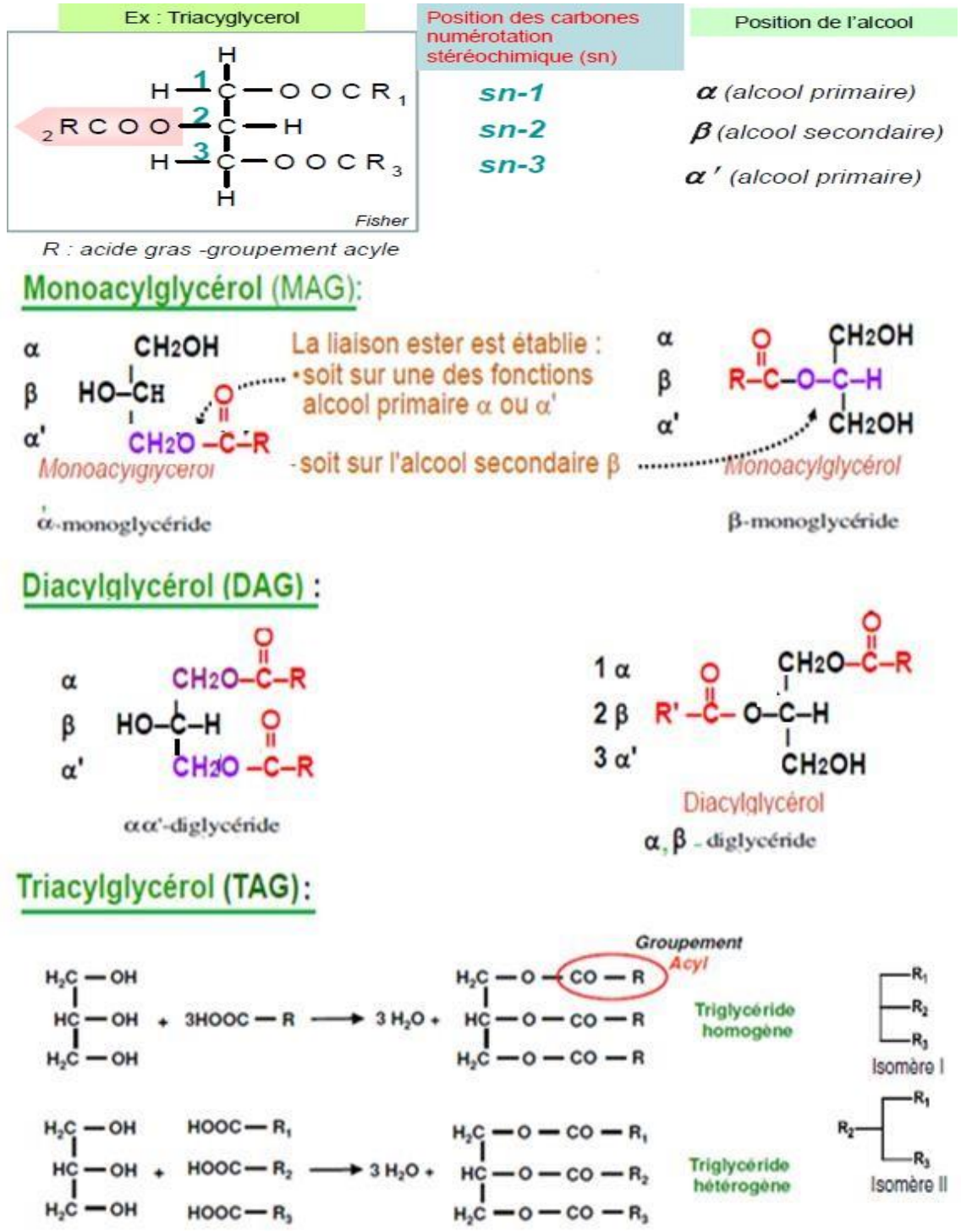


Fig. 74. Nomenclature of fatty acids linked to glycerol in the structure of glycerides.

In the cell, diacylglycerols can be produced from phospholipids, mainly phosphatidylinositides of the membrane, by the action of phospholipase C; the released diacylglycerol participates in the transmission of cellular signals by activating protein kinase C.

To indicate the position of fatty acids, the 3 carbon atoms of glycerol are numbered. The position of the fatty acid is indicated by the prefix sn (specific or stereospecifically numbered) or usually by the carbon number (Fig. 74).

Formerly known as triglycerides, triacylglycerols are neutral, hydrophobic lipids (because the hydroxyl or carboxylic acid groups are no longer free) with a density lower than that of water. They are storage lipids in our body (90% of adipose tissue) that are not found in cell membranes.

In triacylglycerols, the three alcohol groups of glycerol are esterified by fatty acids R1, R2, and R3. When the fatty acids at positions 1 and 2 are different, a center of asymmetry appears at C2, resulting in isomers I and II. The majority of natural triacylglycerols are isomers II, where the fatty acid at position 2 is most often unsaturated.

a- Nomenclature

The fatty acid involved in the ester bond becomes an "acyl" residue (Fig. 75). If it is palmitic acid, it becomes "palmityl", stearic acid becomes "stearyl", oleic acid becomes "oleyl"...

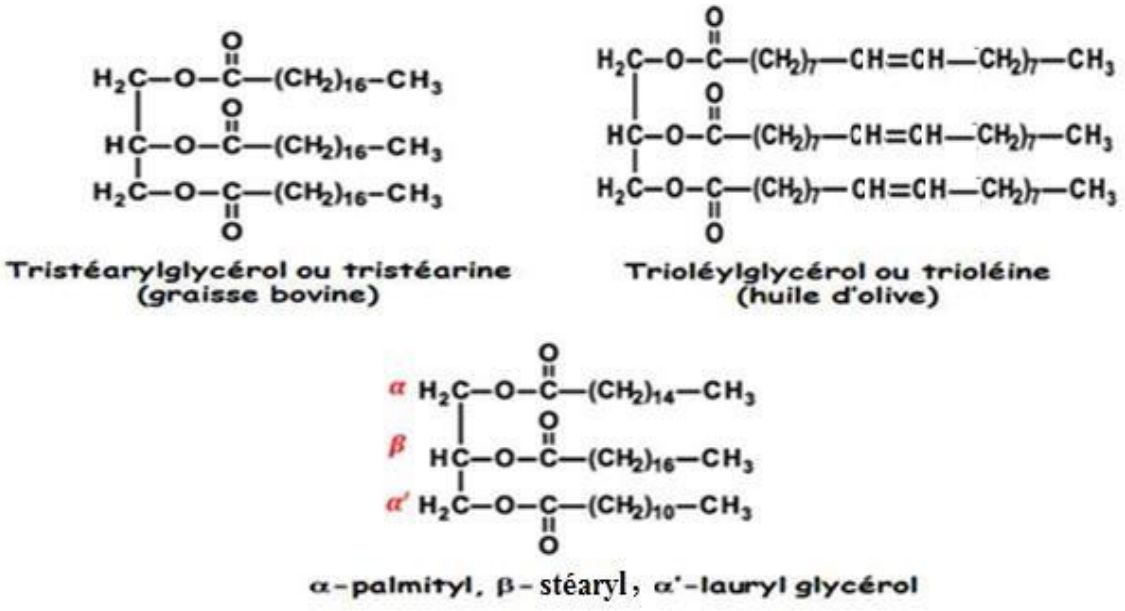


Fig. 75. Nomenclature of fatty acids in the structure of a glyceride.

Chemical hydrolysis

Acid treatment (5% H_2SO_4) of the triglyceride leads to the breaking (usually incomplete) of the ester bonds and the release of the constituents: fatty acids and glycerol (Fig. 76).

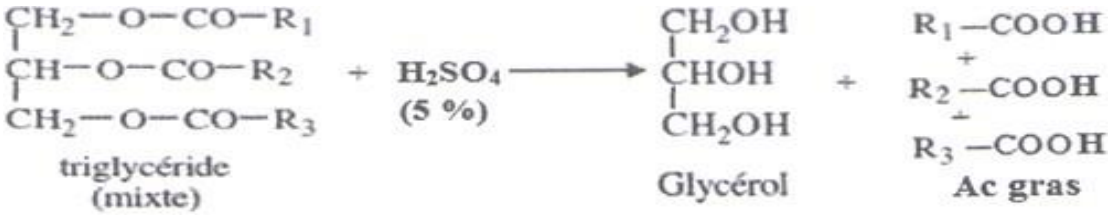


Fig. 76. Acid hydrolysis of a triglyceride.

b- Enzymatic hydrolysis

Pancreatic lipase hydrolyzes dietary TG into monoglycerides and two fatty acids which are absorbed by the intestine (Fig. 77).

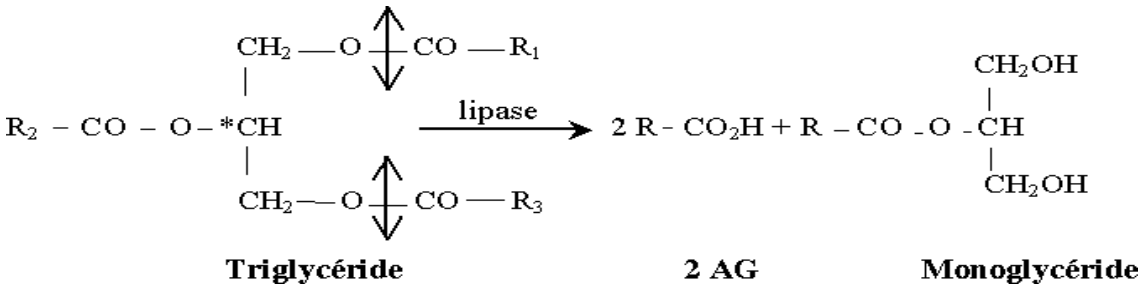


Fig. 77. Enzymatic hydrolysis of a triglyceride.

c- Saponification

The bases in alcoholic solution (sodium or potassium hydroxide) and when heated break the ester bonds of glycerides, releasing fatty acids in their sodium salt form (hard soaps) or potassium salt form (soft soaps). This is a rapid and complete reaction (Fig. 78).

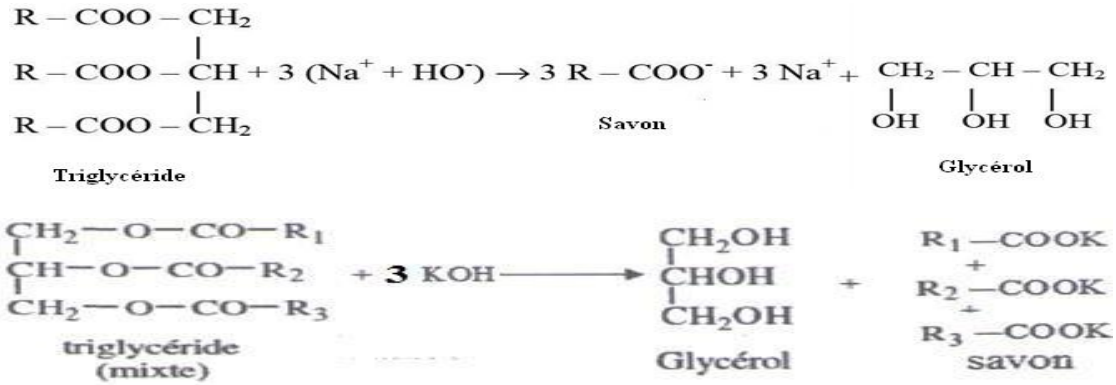


Fig. 78. The saponification of a triglyceride.

III. 6. 1. 2. Cerides

Cerides are monoesters of fatty acids (14 to 30 C) and long-chain aliphatic alcohols, which are generally primary alcohols with an even number of carbons (16 to 36 C), saturated and unbranched (Fig. 79). Examples of alcohols: cetyl alcohol C16:0 (present (in Cetaceans), ceryl alcohol C26:0 (present in waxes) and myricyl alcohol C30:0 (present in myricas, wax trees and myricin is bees wax).

They are chemically inert because they resist acids and most reagents and are difficult to saponify. They have high melting points, so they are solid at room temperature. They are very nonpolar but soluble in hot organic solvents.

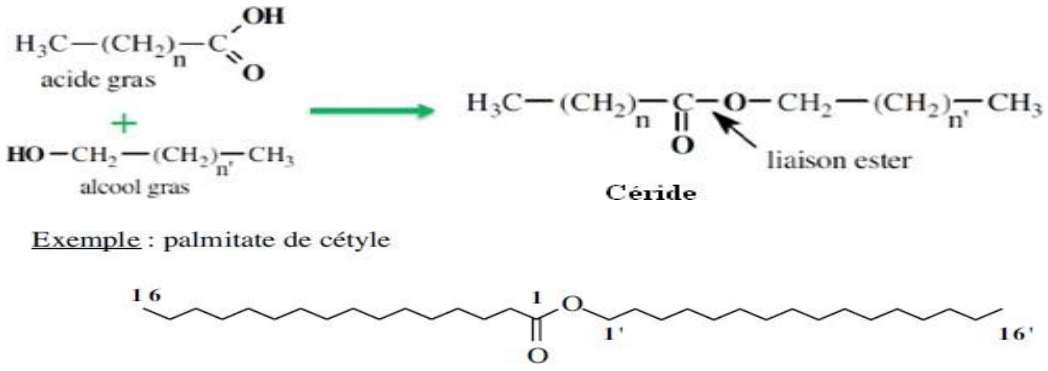


Fig. 79. Structure of a ceride.

They owe their generic name to the fact that they are the main constituents of animal waxes (sperm), vegetable waxes (leaf cuticles), and bacterial waxes (Koch's bacilli). Higher animals and humans cannot metabolize cerides. Beeswax, with its complex composition, is rich in cetyl palmitate. They primarily serve as protective coatings: waterproofing the feathers of waterfowl and the skin of marine animals, the cuticle of glossy leaves (holly, etc.), and the skin of fruits that prevents evaporation and inhibits the growth of mold and infestation by parasites.

III. 6. 1. 3. Steroids

These are esters of fatty acids and cyclic alcohols: sterols (Fig. 80). Sterols are alcohols derived from a quadricyclic alkane or sterane (cyclo-pentano-perhydro-phenantrenic), a structure with 17 carbon atoms and produced by the condensation of 4 rings (3 hexagonal rings denoted A, B and C, and one pentagonal ring denoted D).

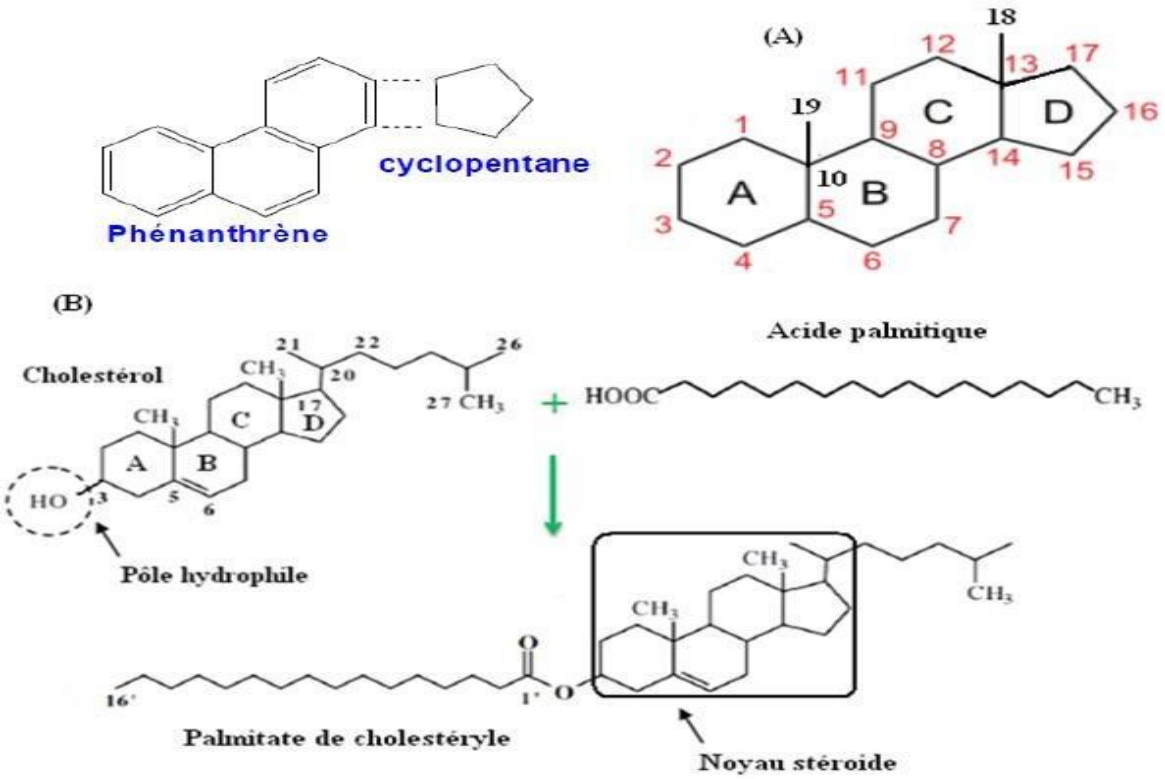


Fig. 80. (A) The sterane nucleus. (B) Structure of a steride: cholesteryl palmitate.

Sterane derivatives are simply called steranes and include, in particular, steroids and the sterols. Sterid is formed by esterification of a fatty acid on the alcohol function at position 3 of cholesterol.

III. 6. 2. Complex Lipids

These heterolipids contain, in addition to the elements C, H, O: phosphorus (P), nitrogen (N), sulfur (S) or a carbohydrate group. They are classified according to the molecule that binds the fatty acids: either glycerol, which is distinguished from acylglycerols by the heterogroup and which are subdivided into: glycerophospholipids and glyceroglycolipids, or a sphingoid base (amino diol), which defines the sphingolipids (Fig. 81).

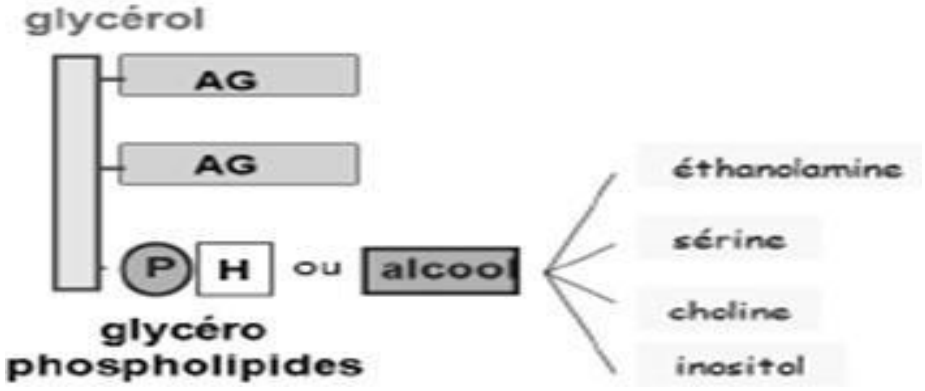


Fig. 81. General structure of a complex lipid.

III. 6. 2. 1. Glycerophospholipids

Also called phosphatides, these are the most numerous and abundant lipids. They are the main lipids in the membranes of eukaryotes and bacteria.

a- Phosphatidic acid (PA)

Phosphatidic acid (PA) is the basic building block of glycerophospholipids (Fig. 82). It is composed of glycerol, two fatty acids, and phosphoric acid (H3PO4). The two fatty acids have a long hydrocarbon chain (≥ 14 carbons), and the fatty acid in position 2 is often unsaturated. The acidity of the molecule arises from the two free mobile hydrogens of phosphoric acid. At blood pH (7.35–7.45), the two acidic groups are ionized. Phosphatidic acid is an intracellular second messenger.

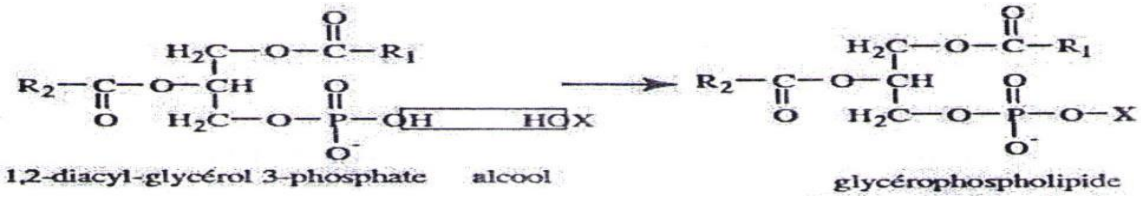


Fig. 82. Formation of a glycerophospholipid by association of phosphatidic acid and an alcohol.

Phosphoric acid compounds (PAs) rarely exist in nature; they are found in their derivatives, where an acidic group of phosphoric acid is esterified by an alcohol (amino or nitrogen-free). The alcohol involved in the esterification of phosphoric acid can be an amino alcohol (serine, ethanolamine, choline) or a nitrogen-free polyol (inositol, glycerol).

b- Phosphatidic acid derivatives

- **Nitrogenous glycerophospholipids**

Phosphoric acid is esterified by an amino alcohol which can be serine, its decarboxylation product, ethanolamine or choline (N-trimethyl derivative) (Fig. 83).

Phosphatidylserine represent 50 to 100% of the glycerophospholipids in the brain and have a marked acidic character.

Phosphatidylethanolamine (Cephalin) present in the brain are more acidic than lecithins.

Phosphatidylcholine (lecithin: Greek root: egg yolk): Lecithins are yellow, waxy substances that are insoluble in acetone. They are particularly abundant in the liver, brain, and egg yolk.

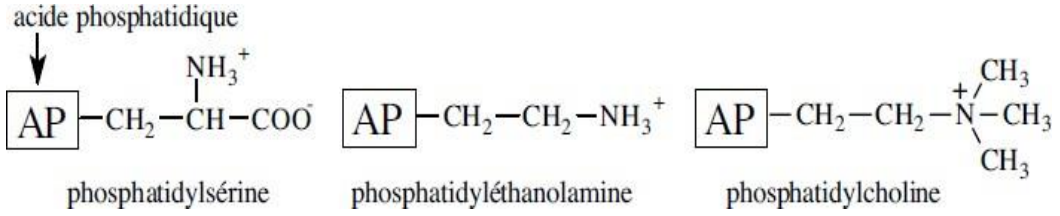


Fig. 83. Structure of the different types of nitrogenous glycerophospholipids.

➢ **Nitrogen-free glycerophospholipids**

Phosphoric acid is esterified by non-nitrogenous polyols (Fig. 84) such as glycerol, a stereoisomer of inositol (myo-inositol) or of its ester-phosphates (biphosphatidylglycerol: cardiolipin).

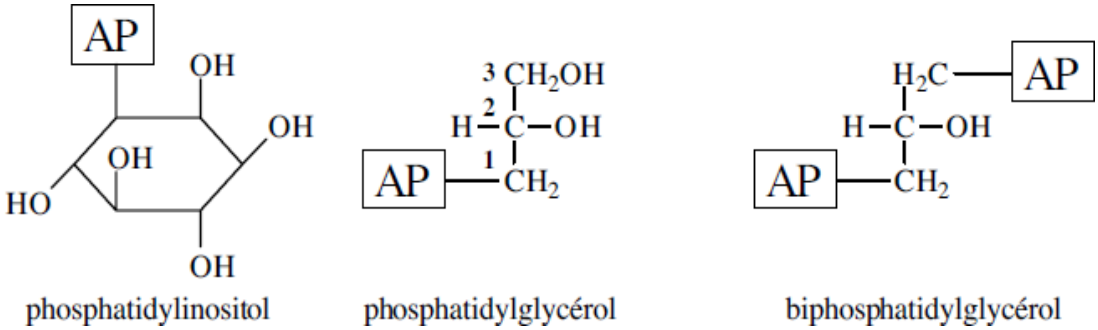


Fig. 84. Structure of the different types of non-nitrogenous glycerophospholipids.

PhosphatidylglycerolThey play a particularly important role in the dispersion of the pulmonary surfactant.

Phosphatidylinositol: present in animal tissues (brain), plant tissues (soybeans, peanuts, wheat), in bacteria (mainly mycobacteria) Biphosphatidylglycerol (cardiolipid: isolated from cardiac muscle): constituents of the mitochondrial membrane, isolated from bacteria and from beef heart.

c- Properties of glycerophospholipids

Overall, the phospholipid molecule is amphiphilic, possessing a nonpolar portion consisting of the hydrocarbon chains of fatty acids and a polar portion, the substituted phosphoglycerol. They therefore have properties identical to those of soaps (emulsifiers). They are amphoteric molecules because they possess both an acidic function provided by H_3PO_4 (pKa=1-3) and a basic function provided by the amino acid alcohol (serine, threonine) or by choline and ethanolamine.

The phosphoryl group of phospholipids is ionized and therefore carries a negative charge at physiological pH (7.35–7.45). The overall charge of each phospholipid depends on the nature of substituent X. If substituent X is neutral, the phospholipid molecule is negatively charged. If substituent X carries a positive charge, the phospholipid is electrically neutral.

d- Enzymatic hydrolysis of glycerophospholipids

Phospholipids can be hydrolyzed by the action of four phospholipases A1, A2, C and D, specific to the bonds (Fig. 85, Table 11). Phospholipases A1 (present in the pancreas, molds) and A2 (present in the venoms of snakes, bees, scorpions and pancreatic juice) lead to the

elimination of fatty acids located in positions 1 and 2 and to the formation of lysophospholipids.

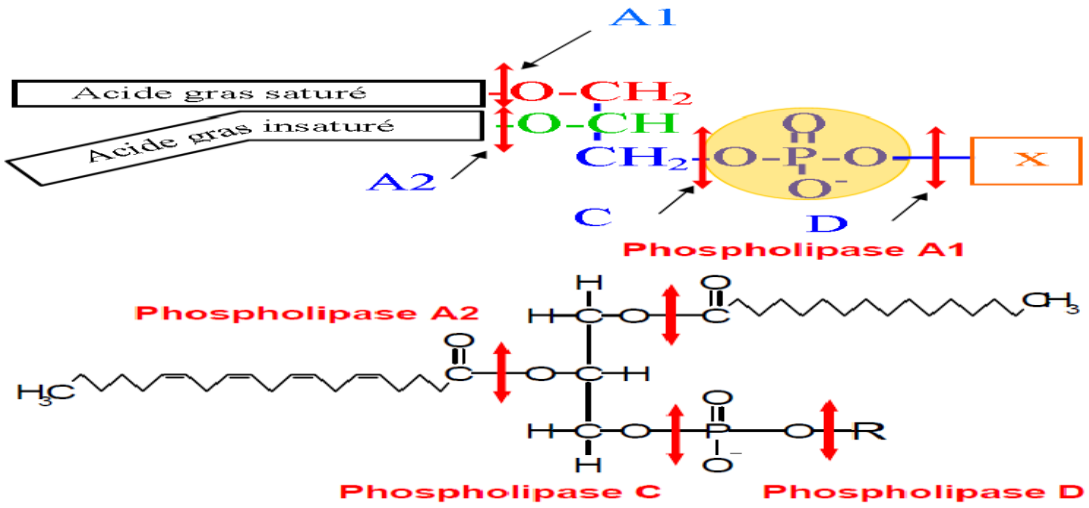


Fig. 85. Action of phospholipases A1, A2, C and D.

Table 11: Origin and site of action of the different types of phospholipases.

Enzyme	**Origin**	**Action site**	**Release**
Phospholipase A_1	Lysosome, membranes	Ester bond in C_1	Fatty acid in 1+ lysophosphatide
Phospholipase A_2	Snake venom, bees, scorpions	Ester bond in C_2	Fatty acid in 2 + lysophosphatide
Phospholipase C		Ester bond between phosphoric acid and glycerol	Phosphorylated alcohol + diglyceride
Phospholipase D		Ester bond between acid phosphoric and nitrogenous alcohol or not	Alcohol + phosphatidic acid

Phospholipase A2 releases unsaturated fatty acids that are used by the cell. It is involved in the biosynthesis of eicosanoids (prostaglandins and leukotrienes): the FA in position 2 is often arachidonic acid, which is the precursor of eicosanoids.

- Phospholipase C (present in bacteria) acts on the ester function linking glycerol and phosphate, releasing a diglyceride and a phosphoalcohol. It is the enzyme that generates DAGs and $IP_3$from phosphatidylinositol diphosphate (Fig. 86).

- Phospholipase D (present in plants) leads to the release of phosphatidic acid and an alcohol (Table 11).

- Phospholipase B is a hydrolase that releases the fatty acid esterifying the hydroxyl group of carbon 1 or carbon 2 of the glycerol in a phosphoglyceride to yield a lysophospholipid. As such, it combines the enzymatic activities of phospholipase A.$_1$and a phospholipase A_2(Table 11).

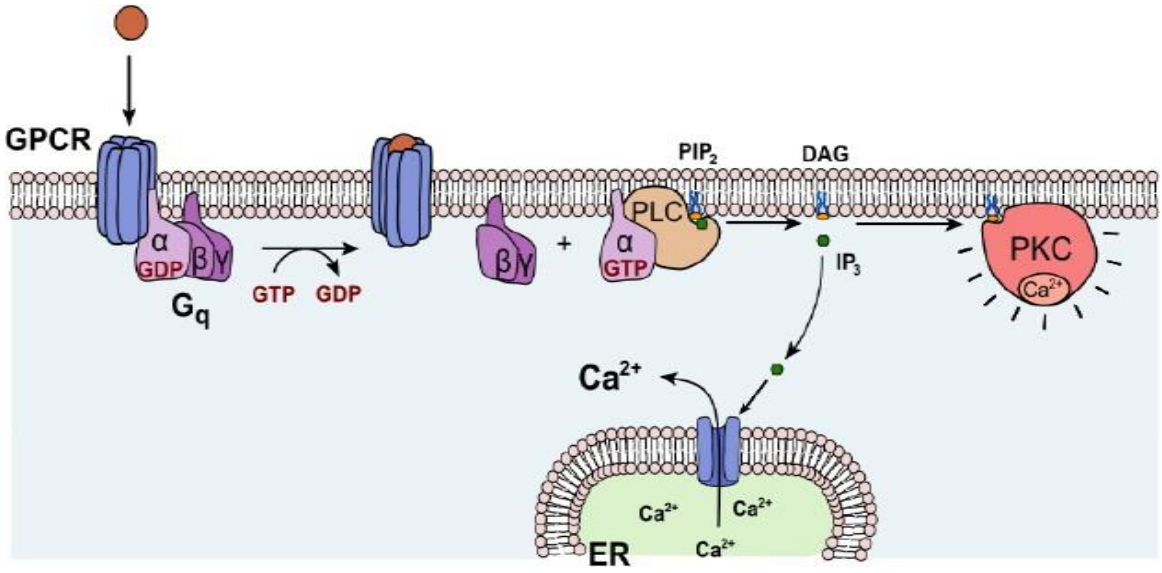


Fig. 86. Genesis of the DAG and the IP3 from phosphatidylinositol diphosphate by phospholipase C.

d. Etherglycerolipids (etherphospholipid)

It is a phosphatidyl choline or ethanolamine in which the fatty acid on sn-1 is replaced by a fatty alcohol linked to glycerol by an ether bond.

- **Plasmalogene**

Alcohol is unsaturated; it is present in the myelin sheaths of neurons, in the heart, muscle, and sperm (Fig. 87).

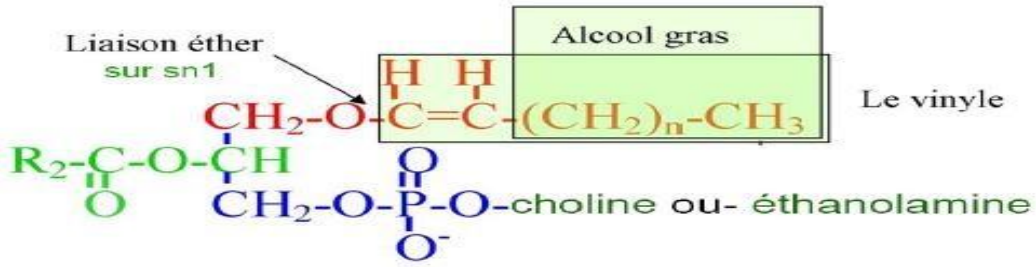


Fig. 87. Structure of an etherphospholipid: plasmalogen.

- PAF (Platelet-activating-factor)

The alcohol at position sn-1 is saturated. An acetyl group is attached at position sn-2. This is a mediator of the allergic and inflammatory reaction capable of inducing platelet aggregation (Fig. 88).

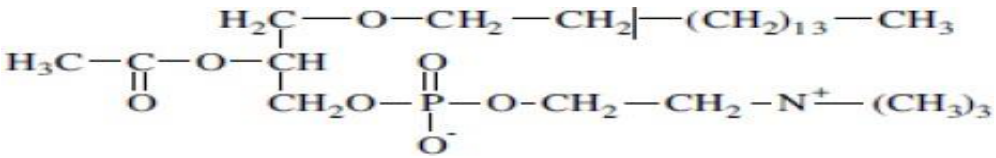


Fig. 88. Structure of the PAF (Platelet-activating-factor).

III. 6. 2. 2. Sphingolipids

These are complex lipids containing a sphingosine molecule or one of its derivatives (sphingosine base). Sphingosine is a long-chain (18C) amino dialcohol with two OH groups on C1 and C3, a primary amine group at C2, and a trans double bond (Fig. 89).

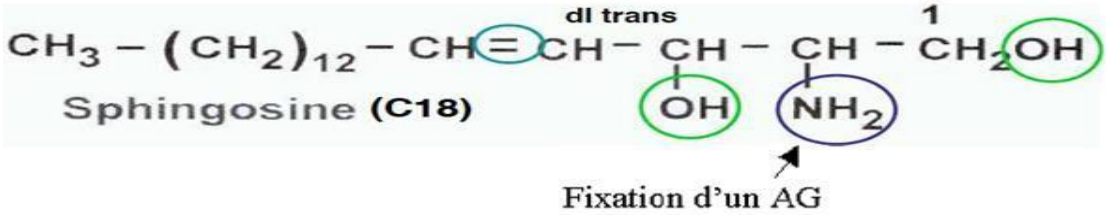


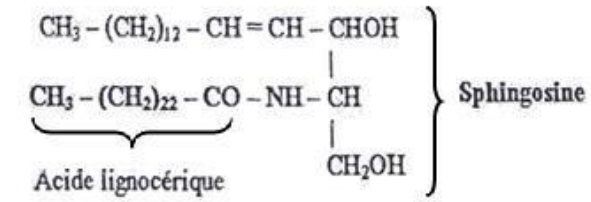
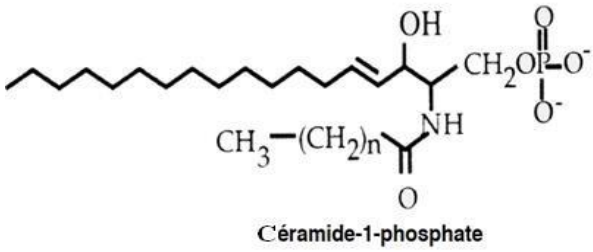
Fig. 89. Structure of sphingosine.

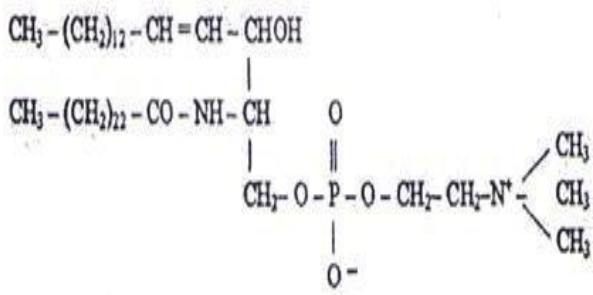
These are amides of sphingosine, formed by the bonding of the fatty acid's carboxyl group to the NH2 group of sphingosine. The attachment of a fatty acid to the amine group (amide bond: therefore, it is no longer an ester bond as in other lipids) yields a ceramide or acylsphingosine, which is the precursor molecule of lipids in this group.

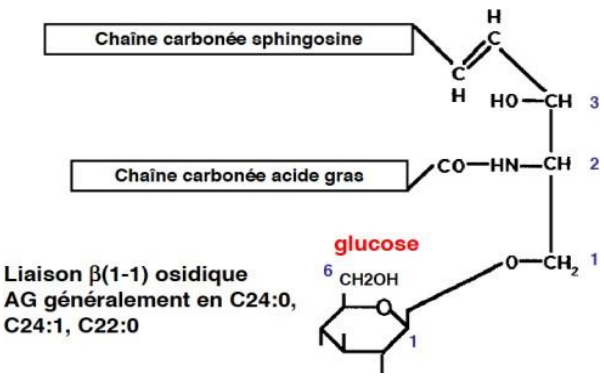
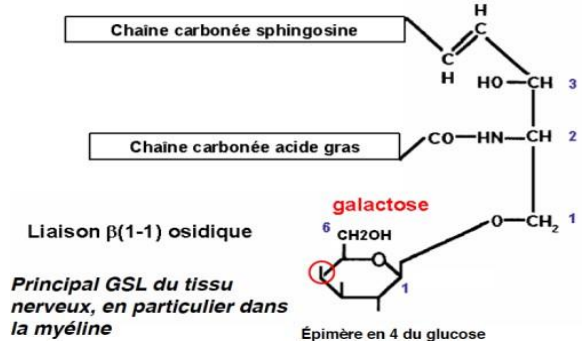
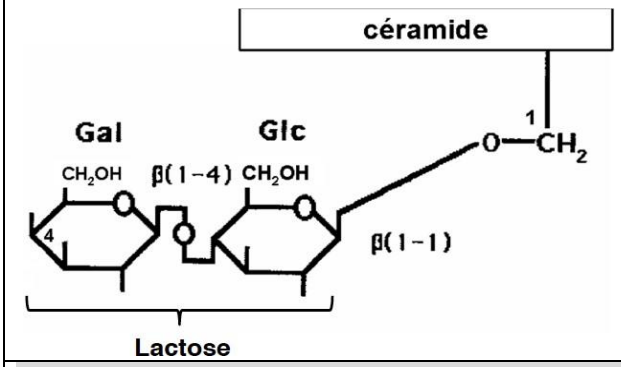
Free ceramides are present in small quantities in the cell because they are toxic and cause cell death by apoptosis. They do, however, play a role in the processes of cell differentiation and proliferation.

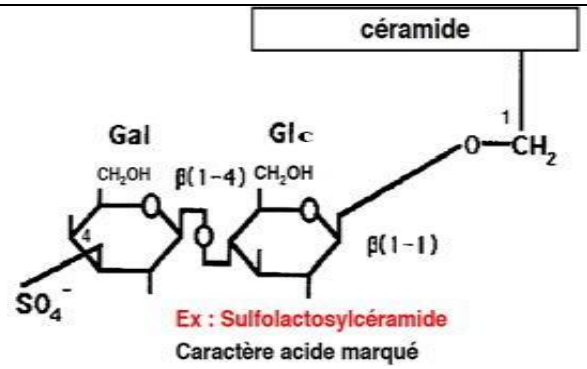
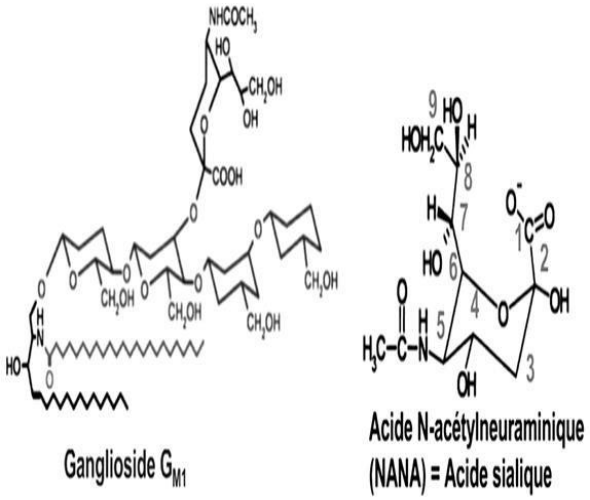
The fatty acid is primarily lignoceric acid ($C_{24}H_{48}O_2$) or stearic acid ($C_{18}H_{36}O_2$). Furthermore, the terminal OH of sphingosine can be bonded to another X group, which is phosphocholine or a sugar (or sugar derivative). Depending on the substituent, two groups of sphingolipids are defined: phosphosphingolipids and glycosphingolipids (Table 12).

Table 12: Main classes of sphingolipids.

<table>
<tr><th colspan="2">PHOSPHOSPHINGOLIPIDS</th></tr>
<tr><td>a- Acylsphingosine Or ceramide:
sphingosine + AG
 
</td><td>- The simplest of the sphingolipids
- AG is saturated and long-chain</td></tr>
<tr><td>b- Ceramide 1-phosphate
 

Céramide-1-phosphate</td><td>Synthesized from ceramide by a ceramide kinase: addition of a phosphate to the primary alcohol group
- Role in intracellular signaling
- Not abundant</td></tr>
<tr><td>c- Sphingomyelin:
sphingosine + AG + Phosphorylcholine
 
</td><td>- The most common fatty acid is lignoceric acid (C24:0)
- Found in nerve tissue (myelin sheaths) and in membranes
- Synthesized from ceramide 1-P by adding choline to phosphoric acid.
- 2nd most abundant phospholipid in plasma
- In significant quantities in the brain, cornea and myelin</td></tr>
<tr><th colspan="2">GLYCOSPHINGOLIPIDS</th></tr>
<tr><td colspan="2">The alcohol I function of ceramide attaches an oligosaccharide chain via a glycosidic bond.
- Present in all tissues and more particularly in nervous tissue and the brain with C24 fatty acids (lignoceric, cerebronic, nervonic acid)
- Present in the outer leaflet of the plasma membrane (the carbohydrate-containing part outside the cell). Depending on the substituents, we determine: Neutral and acidic glycosphingolipids</td></tr>
<tr><th colspan="2">neutral glycosphingolipids</th></tr>
<tr><td>Mono-glycosylceramides (cerebrosides)
-Glucosylceramide(glucosylcerebroside)</td><td></td></tr>
</table>

<table>
<tr>
<td> 
</td>
<td>Ceramide + glucose via a β1 bond→1. The General Assembly is often the C_{24}:0 or C_{24}:1Major glycolipid of extra-neural tissues (skin)
A deficiency of the enzyme that cleaves it leads to hepatosplenomegaly</td>
</tr>
<tr>
<td>Galactosylceramide (galactosylcerebroside):
Ceramide + galactose via a β1 bond →1
 
</td>
<td>It is the main glycosphingolipid of the nerve tissue, especially in the myelin</td>
</tr>
<tr>
<td>Oligo-glycosylceramides(Globosides: oligoside ≤ 6 residues)
Lactosylceramide (galactose +glucose)
Ceramide + lactose (β1→4) by β1 bonding→1
 
</td>
<td>Present in nerve tissue
More complex glycosphingolipids are formed by a succession of sugars: galactose, N-acetylglucosamine (GlcNac), N-acetylgalactosamine (GalNac) or mannose (man).
This is the case with blood groups: ABO surface proteins of red blood cells which are made of glycans linked to proteins (glycoproteins) or to membrane lipids (glycosphingolipids).</td>
</tr>
<tr>
<td colspan="2">acidic glycosphingolipids
A glycosidic residue carries a mineral (sulfuric) or organic (sialic) acid group.</td>
</tr>
<tr>
<td>Sulfoglycosphingolipids or sulfatides
SulfogalactosylceramideGalactosylceramide + sulfuric acid on galactose</td>
<td></td>
</tr>
</table>

	Present in the membranes of CNS cells (myelin) and kidneys Marked acidic character due to the sulfate group Synthesized by a sulfotransferase. Hydrolyzable by sulfatidases.
Sialoglycosphingolipids or gangliosides 	The R substituent$_1$is a glycan chainbranched with the same sugars as neutral glycolipids, but also N-acetylgalactosamine (GalNAc) and N-acetylglucosamine (GlcNAc) as well as sialic acid molecules (N-acetyl neuraminic acid or NeuNAc or NANA: N acetyl because it has an acetyl function on the amine, and acid because of the presence of a COOH group) attached to the oligosaccharide chain at its end or to an internal sugar. They are abundant in the lymph nodes, hence their name. They are present on the outer surface of the plasma membrane, e.g., blood group antigens. .

III. 7. Lipid-like compounds

Lipid-like compounds are natural compounds that do not contain fatty acids, but are related to them by their physical properties, particularly their solubility. They include: eicosanoids (derived from arachidonic acid) and isoprenoids (derived from isoprene).

III. 7. 1. Arachidonic acid derivatives (eicosanoids)

Eicosanoids (from the Greek eicos = twenty) are derived from 20C polyunsaturated fatty acids, the nature of which depends on the diet. Most often, it is arachidonic acid, usually released from phosphatidylcholine following the action of an enzyme called phospholipase A2 (Fig. 90).

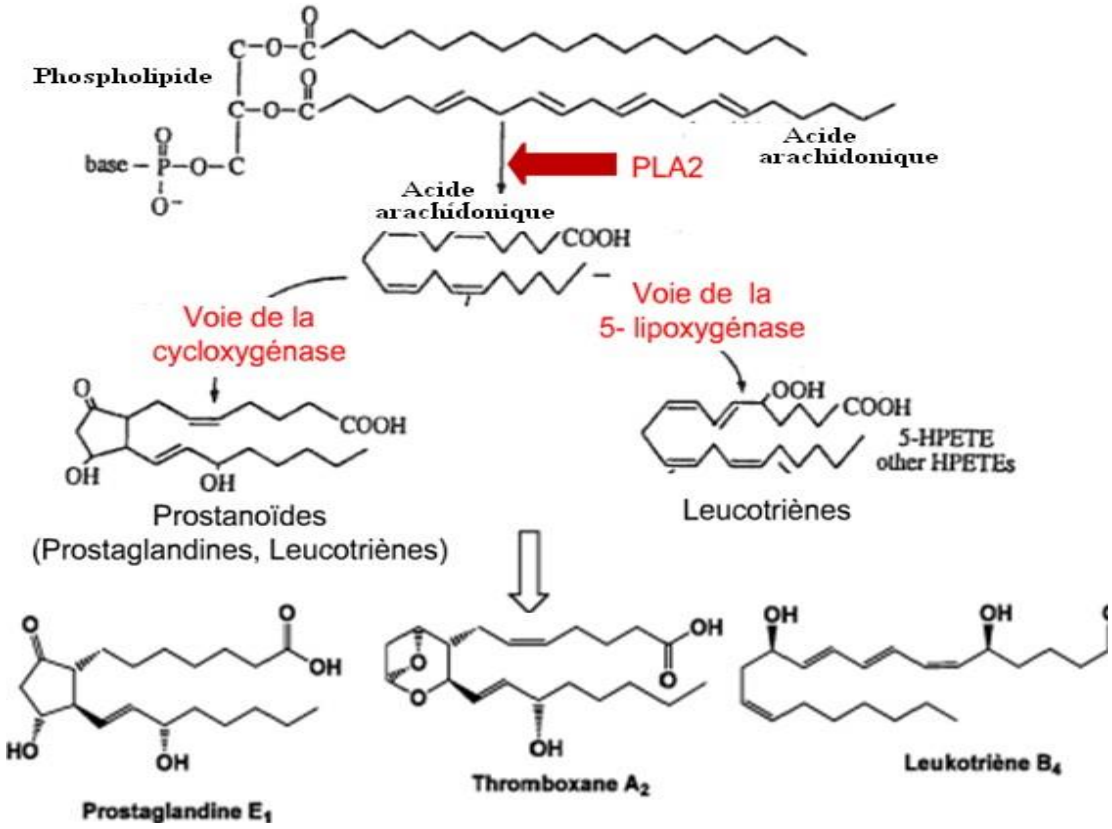


Fig. 90. Biosynthesis of eicosanoids (prostaglandins and leukotrienes): from arachidonic acid under the action of phospholipase A2.

Eicosanoids (prostaglandins, lipoxins, thromboxanes and leukotrienes) are small, highly diffusible molecules that act as local hormones and are involved in many physiological and pathological processes (Table 13).

Table 13: Main biological prostanoids effects.

Organs	Effects	Prostaglandins and Thrombanes
Vessels	Vasoconstriction Vasodilation	PGF_2TXA_2
Platelets	Pro-aggregant Anti-aggregant	TX PGE, PGI_2
Bronchi	Contraction Dilatation	PGF, TXA_2PGE, PGI_2
Intestine	Nausea, diarrhea	PGE, PGF
Stomach	Decrease of the gastric secretion	PGE, PGI_2
Uterus	Contractions	PGE, PGF
Kidney	Increase of renal blood flow	PGE, PGI_2

Inhibiting COX enzymes, which enable the synthesis of these arachidonic acid derivatives involved in pain and inflammation, therefore inhibits their production.

III. 7. 2. Isoprenoid lipids

The structure of these compounds is often a combination of isoprene units. Isoprene is a 5-carbon diethylenic hydrocarbon (Fig. 91). Several classes are distinguished: terpenoids, carotenoids (carotenes: red-orange pigments), xanthophylls (yellow pigments) and vitamin A (retinol), isoprene-chain quinones (vitamin E, vitamin K, ubiquinones and plastoquinones) and steroids (sterols, bile acids, steroid hormones and vitamin D).

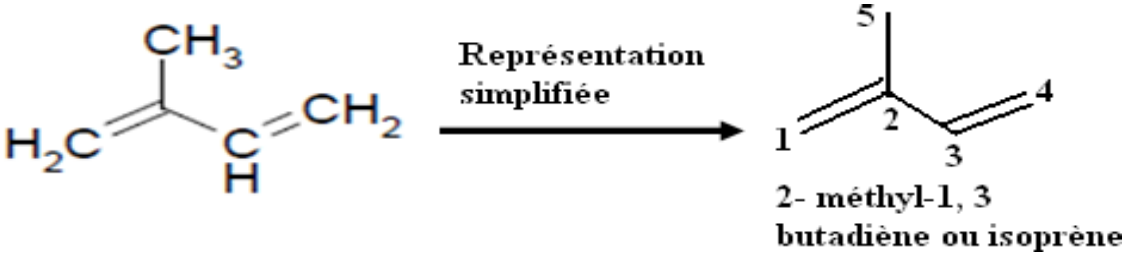


Fig. 91. Structure of the isoprene unit.

III. 7.2.1. Terpenes and terpenic compounds

A large number of natural compounds in the terpene family come from the polymerizations and rearrangements of the same precursor, isoprene.

Polymerization can occur either by a 4-1 or 4-4 condensation which causes the loss of a double bond at each unit (Fig. 92), the remaining double bonds are in trans conformation (structures stretched along the same axis):

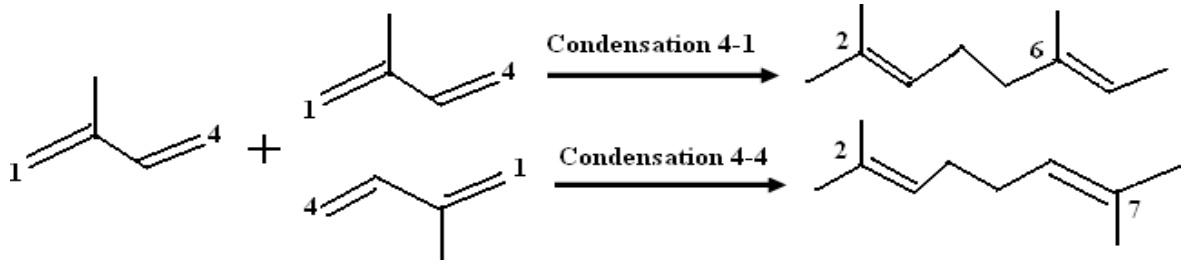


Fig. 92. Condensation of isoprene units: "Head-to-tail" or "head-to-head" bonding.

Depending on the number n (integer) of isoprene (5C) units, knowing that a terpene unit consists of two isoprene molecules, we can distinguish: monoterpenes (n = 2), diterpenes (n = 4), tetraterpenes (n = 8) (Table 14).

Table 14 : Classification of terpenes.

Class	Nb isoprene	Examples
Monoterpenes	2	Geraniol, camhor, menthol
Sesquiterpenes	3	farnesol
Diterpenes	4	Vitamins (A), E, K
Triterpenes	6	Steroids (cholesterol, vitamin D…)
Tetraterpenes	8 6-10 700-5000	Carotenoids Ubiquinone Rubber

They form the carotene and xanthophyll family in plants and are precursors of vitamin A (Fig. 93) in animals.

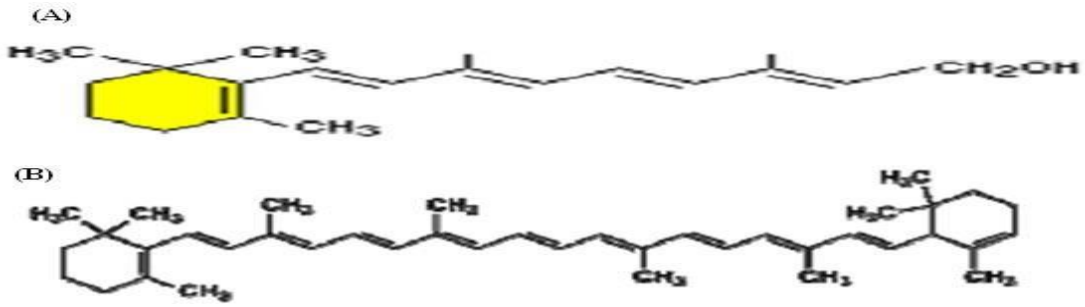


Fig. 93. (A) Retinol (one of the major forms of vitamin A, derived from the cleavage of ß - carotene. (B) Structure of theß- carotene.

III. 7.2.2. Sterols and steroid compounds

Steroids are a group of lipids derived from triterpenoids (lipids with 30 carbon atoms). They are characterized by a partially or fully hydrogenated hydrophobic sterane ring. Steroids differ from one another in the nature and position of the various groups attached to this ring, and in the possible presence and number of double bonds. Typically, carbons C10 and C13 are bonded to a methyl group (-CH3), and carbon C17 to an alkyl group (Table 15).

Natural steroids are divided into four series: sterols, bile acids and salts, hormonal steroids, and vitamin D and other derivatives.

Sterols

They have already been mentioned in the subgroup of sterides of simple lipids.

Cholesterol

It is a secondary, polycyclic, unsaturated monoalcohol with a hydroxyl group at C_3 (Fig. 94). It is the main sterol of animal origin, present in membrane structures.

Cholesterol is obtained from food and synthesized by the liver; it is transported in the blood by lipoproteins. It is a component of cell membranes (playing a role in fluidity)

and is used in the body for the synthesis of three groups of molecules: steroid hormones (cortisol, testosterone, etc.), vitamin D3, and bile acids.

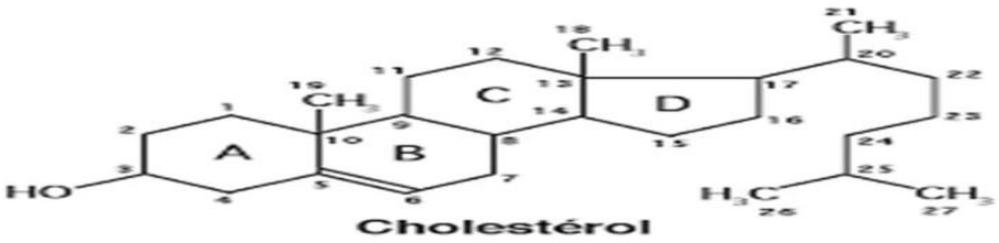


Fig. 94. Structure of cholesterol

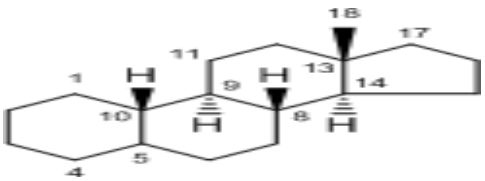
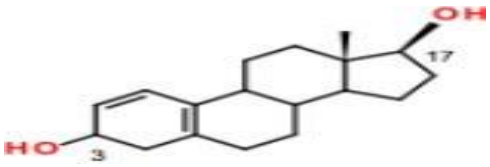
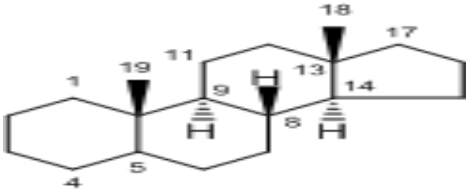
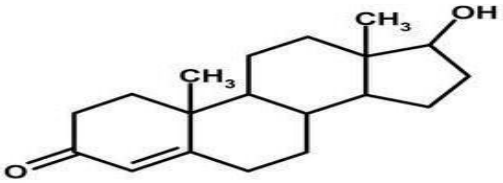
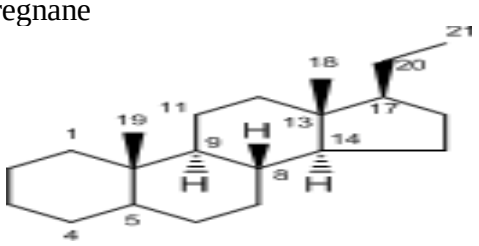
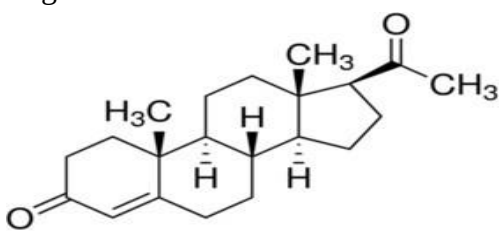
Hormonal steroids

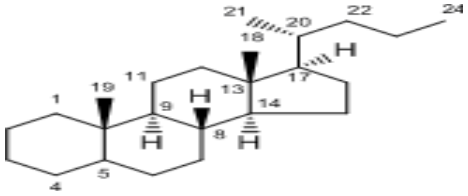
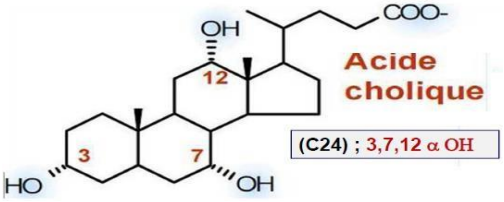
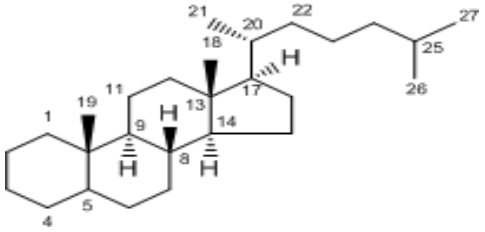
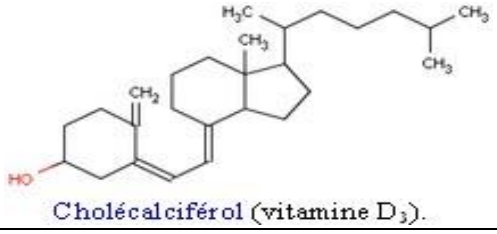
These molecules are present in animals and plants and are "informational" molecules, regulators of metabolism or cellular mediators.

Mammalian steroid hormones are the hormones:

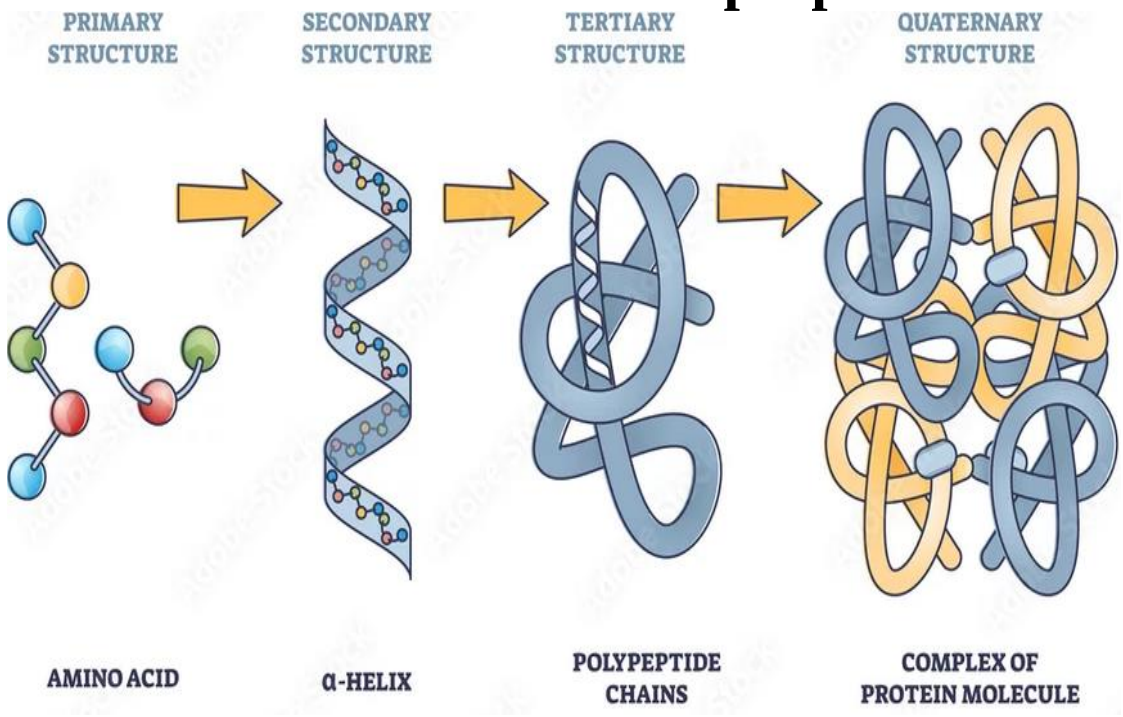
- ***sex glands and placenta***androgens, estrogens and progestogens.
- ***adrenal glands***Mineralocorticoids control mineral balance, while glucocorticoids control carbohydrate metabolism and the catabolism of stored lipids. They are all derived from cholesterol through side-chain cleavage, hydroxylation, or oxidation.

Table 15: The different types of steroid compounds.

Nuclei	C	Examples
Estrane 	18	Estradiol 
Androstane 	19	Testosterone 
Pregnane 	21	Progesterone 

Cholane 	24	Bile salts 
Cholestane	27	Cholesterol, Vitamin D
		

CHAPTER IV :
Amino acids, peptides, proteins.
Structure and main properties



CHAPTER IV

Amino acids, peptides, proteins. Structure and main properties

IV.1. Common amino acids of proteins

IV. 1. 1. General Structure

Amino acids, or amino acids (from the Greek "amin" *ammonia*, ammonia) are Carboxylic acids bearing amine functions. These are the basic structural units of proteins. The carbon bearing the acidic carboxylic function is said to be "0". The amine function is therefore α to the acid function, hence their name: α-amino acids or α-amino acids (Fig. 95). Usage in biochemistry has given them a common name, usually with the suffix "ine", recalling the origin.

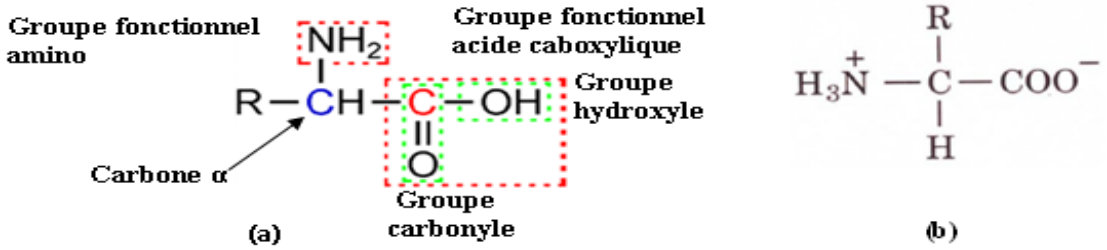


Fig. 95. General structure of an amino acid: (**a)**Theoretical structure: does not exist in reality. (b) Zwitterionic form: present at physiological pH values.

The 20 natural α-amino acids **proteinogens** (regardless of their origin, viral, bacterial, plant or animal) are characterized by their side chain; each α-amino acid has a name, initially abbreviated using a three-letter code and later a one-letter code, which allows us to write and compare protein sequences (Table 16).

IV. 1. 2. Classification

Proteinogenic amino acids can be classified:

IV. 1. 2. 1. According to the chemical nature of the R **side chain** which can be aliphatic or cyclic in:

a. Simple aliphatic amino acids

The R group consists solely of carbon and hydrogen atoms (Table 16). Glycine (Gly or G): also called glycine. Besides being a component of proteins, glycine participates in liver detoxification processes (conjugation reactions) and the formation of bile salts (sodium glycocholate). Alanine (Ala or A): is a very common amino acid in proteins; its R group is a methyl group. Valine (Val or V), leucine (Leu or L), and isoleucine (Ile or I) cannot be biosynthesized by the body. They are therefore essential amino acids.

b. Amino acid alcohols

There are two standard amino acids with an alcohol group on their side chain: serine (Ser or S) and threonine (Thr or T). Threonine is also one of the essential amino acids (Table 16). The alcohol group makes their side chain polar, and therefore hydrophilic. The primary alcohol function of serine can undergo esterification with phosphoric acid, forming phosphoserine present in phosphoproteins.

c. Sulfur-containing amino acids

Cysteine (Cys or C) has a thiol group. This makes the side chain polar and therefore hydrophilic. It is an important amino acid because it contributes to the stabilization of the tertiary structure of proteins through the formation of disulfide bonds. Methionine (Met or M) is one of the essential amino acids. Its R group is nonpolar and therefore hydrophobic (Table 16).

d. Dicarboxylic amino acids

These are aspartic acid (Asp or D) and glutamic acid (Glu or G) (Table 16). These two amino acids are very widespread in proteins. As free amino acids, they play an important role in nitrogen metabolism (transamination reactions, the urea cycle, amine group transport; glutamic acid also serves as a precursor for the formation of urea).γ-aminobutyric acid (GABA) mediator of the central nervous system.

e. Amino amide acids

Very similar to dicarboxylic amino acids, they nevertheless possess an amide group at the radical. Non-ionizable but polar; the side chain exhibits hydrophilic behavior. Asparagine (Asn or N) and glutamine (Gln or Q) also play an important role in nitrogen metabolism (Table 16).

f. Dibasic amino acids

These amino acids have a second amine group at the radical (Table 16). This group is ionized at physiological pH ($-NH3^{+}$) resulting in hydrophilic behavior.

Lysine (Lys or K) is one of the essential amino acids. It is found in collagen, where it has the unique characteristic of being hydroxylated (at the C5 position), thus forming hydroxylysine. Arginine (Arg or R) and histidine (His or H) are present in significant quantities in histones (basic proteins that enable DNA condensation in the nucleus). Arginine plays an important role in the urea cycle and participates in the formation of creatine (an energy reserve in the form of creatine phosphate in striated muscle).

g. Aromatic amino acids

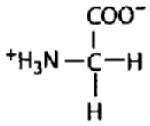
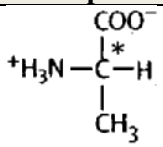
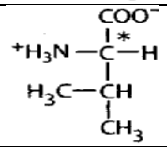
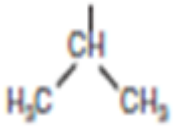
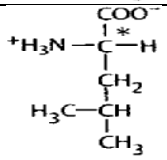
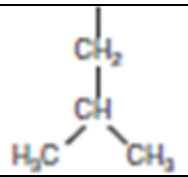
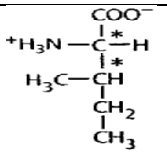
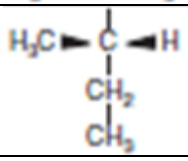
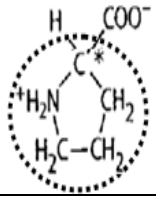
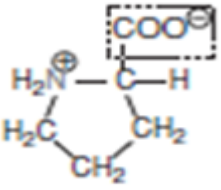
Phenylalanine (Phe or F) is one of the essential amino acids. As its name suggests, its structure is that of alanine substituted by a phenyl group, forming a hydrophobic radical.

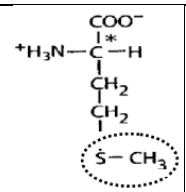
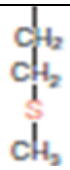
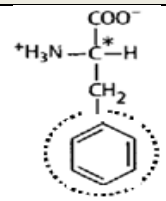
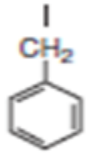
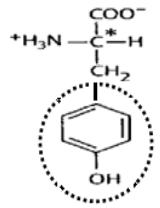
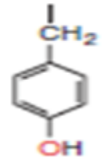
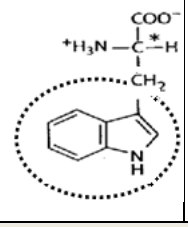
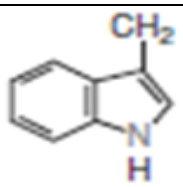
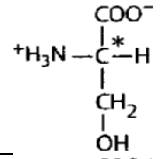
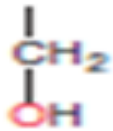
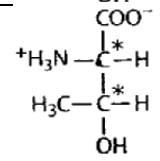
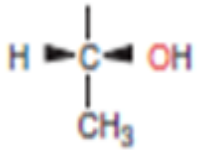
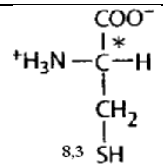
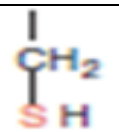
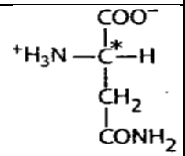
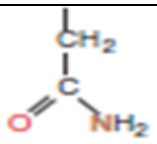
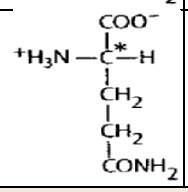
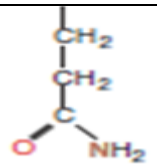
Tyrosine: obtained by hydroxylation of phenylalanine, its side chain becomes hydrophilic (Table 16). These two amino acids are important because they serve as precursors in the biosynthesis of catecholamines (adrenaline and noradrenaline). Tyrosine participates in the formation of thyroid hormones. Tryptophan (Trp or W) is an essential amino acid. It is the biosynthetic precursor of serotonin (a neurotransmitter in the central nervous system) and vitamin B3.

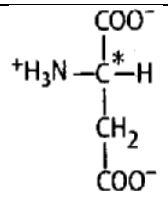
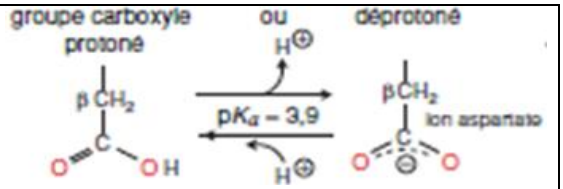
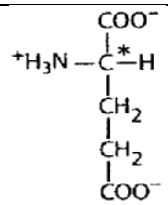
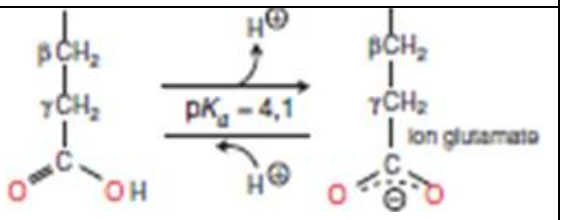
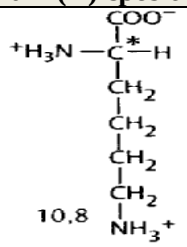
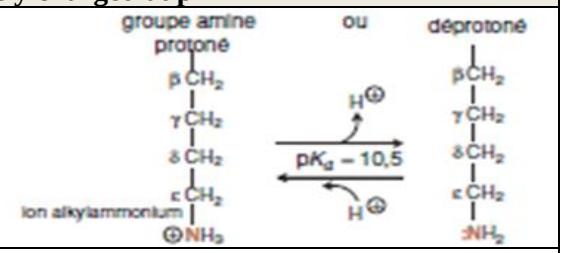
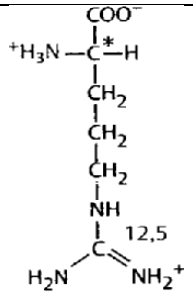
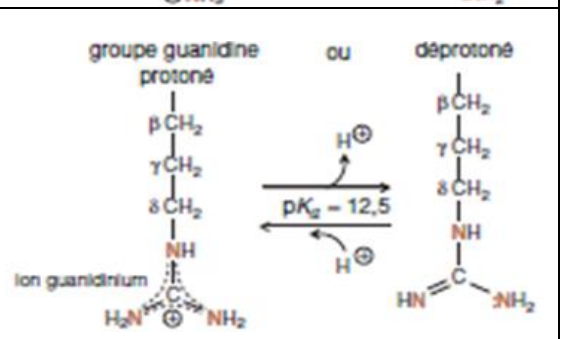
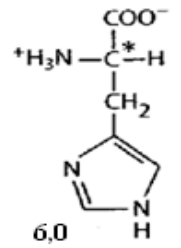
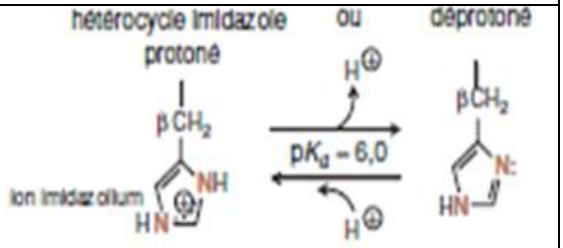
h. Heterocyclic amino acids

Proline (Pro or P) is an atypical amino acid because its side chain forms a pentagonal ring with the amine group (which becomes secondary) on the α carbon. Due to this cyclic structure, proline residues in a protein introduce a bend in the peptide chain. Like lysine, it has the unique characteristic of being hydroxylated within collagen (hydroxyproline).

Table 16: Chemical structure of the 20 proteinogenic amino acids.

Amino acid		**Formula**	**Side chain**
Name	**Abbreviations**		**Functional group**
Glycine	**Gly, G**		
Aliphatic apolar side chain (R)			
Alanine	**(Ala, A)**		
Valine	**(Val, V)**		
Leucine	**(Leu, L)**		
Isoleucine	**(Island, I)**		
Proline	**(Pro, P)**		

Methionine	**(Met, M)**		 **Thioether**
Aromatic side chain (R)			
Phenylalanine	**(Phe, F)**		 **Benzene ring**
Tyrosine	**(Tyr, Y)**		 **Pheol cycle**
Tryptophan	**(Try, W)**		 **Indol cycle**
Uncharged polar side chain (R) at pH 7			
Serine	**(Ser, S)**		
Threonine	**(Thr, T)**		
Cysteine	**(Cys, C)**		 **Thiol group**
Asparagine	**(Asn, N)**		
Glutamine	**(Gln, Q)**		
Lateral chain (R) cnegatively charged at pH 7			

Aspartate	**(Asp, D)**		
Glutamate	**(Glu, E)**		
Lateral chain (R) cpositively charged at pH 7			
Lysine	**(Lys, K)**		
Arginine	**(Arg, R)**		
Histidine	**(His, H)**		

IV.1.2.2. According to the polarity of the side chain

Depending on the nature of the lateral chain, three groups ofα-amino acids: nonpolar, neutral polar, and ionizable polar (Table 16). Nonpolar or hydrophobic amino acids. This category includes five amino acids with aliphatic side chains that often constitute the hydrophobic pockets of proteins (Gly, Ala, Val, Leu, Ile,) two with aromatic nuclei (Phe and Trp) one containing sulfur (Met) and the (Pro).

1. **AA polars, uncharged:** The side chains of these amino acids are more soluble in water, and therefore more hydrophilic, because they contain functional groups that form hydrogen bonds with water. This category includes: Ser, Thr, Cys, Tyr, Asn, and Gln.

2. **Negatively charged polar Aas:** The two amino acids whose side chains have a net negative charge at pH 7.0 are aspartic acid and glutamic acid.
3. **Polar amino acids are positively charged:** The amino acids in which the R side chains have a net positive charge at pH 7 are Lys, Arg, His.

IV.1.2.3. According to the essentiality of the amino acid

Some amino acids cannot be synthesized by the body and are therefore essential. In humans, there are eight essential amino acids provided by food (tryptophan, lysine, methionine, phenylalanine, threonine, valine, leucine and isoleucine). The histidine and the arginine, are called semi-essential because only infants need an exogenous supply (found in breast milk).cysteine, the glycine and the tyrosine are sometimes necessary for certain populations who are unable to synthesize them in sufficient quantities. For example, people with phenylketonuria must minimize the absorption of phenylalanine, but this amino acid is a precursor of tyrosine: the latter can then no longer be synthesized and becomes essential.

There are two mnemonic phrases to remember the 8amino acids essential for adults: The very lyrical Tristan makes Iseult meditate a lot, which gives: Leucine, Threonine, Lysine, Tryptophan, Phenylalanine, Valine, Methionine, and finally Isoleucine. The second The phrase is derived from the three-letter code for essential amino acids (excluding arginine): "Put it in the suitcase, he's making too much of a fuss."

IV. 1. 2. 4. According to whether they are gluconeogenic or ketogenic

Amino acids classified as gluconeogenic are compounds whose breakdown leads to the formation of metabolic intermediates involved in gluconeogenesis. The breakdown of certain amino acids leads to the formation of acetyl CoA or acetoacetyl CoA. These are classified as ketogenic (or ketone-forming) amino acids, meaning they contribute to the formation of ketone bodies (Table 17).

Table 17: Glucoformative and ketoforming amino acids.

Strict glucoformators aa	Strict ketogenic aa	Mixed aa
Gly, Ala, Ile, Trp, Phe, Arg, Gln, Asn, Thr, Asp, Glu, Cys, His, Pro	Leu, Lys	Ser, Met, Tyr, Val

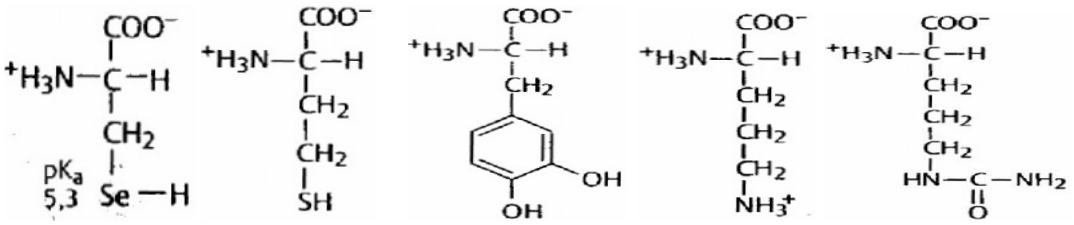
IV. 1. 2. 5. Specific amino acids

Besides proteinogenic amino acids, there are many other compounds of the same type in nature.

Selenocysteine is an analog of cysteine in which sulfur has been replaced by selenium, a more reactive trace element (Fig. 96). Besides its significantly lower pKa value compared to

the SH group of cysteine, the SeH group is essentially dissociated within the cell. Selenocysteine-containing proteins (such as glutathione peroxidase, an enzyme involved in the detoxification of lipid peroxides) generally have redox functions.

Homocysteine is an intermediate in the degradation of methionine. Its side chain is elongated by one CH2 group compared to cysteine (Fig. 96). Dopa (abbreviation 3,4-dihydroxyphenylalanine) is formed by the hydroxylation of tyrosine and is an intermediate in the biosynthesis of catecholamines and melanin. Ornithine, a basic amino acid, and citrulline, which is derived from it, are intermediates in the urea cycle. These compounds together form the class of non-proteinogenic amino acids (Fig. 96).



Selenocysteine **L-Homocysteine** **L-Dopa** **Ornithine** **Citrulline**
(Sec) **(Methionine metabolism)** **(Catecholamines Synthesis)** **(Urea Cycle)** **(Urea Cycle)**

Fig.96. Selenocysteine and certain non-proteinogenic amino acids.

IV. 1. 3. Properties of amino acids

IV. 1. 3. 1. Physical Properties

IV. 1. 3. 1. 1. Solubility

In their solid state, amino acids form crystals. They are fairly easily solvated by polar solvents: very soluble in water (colorless solution) and ethanol, but insoluble in nonpolar solvents such as benzene and ether.

Solubility decreases with the number of carbon atoms on the R group. Conversely, the presence of ionizable (NH_2, COOH) or hydrophilic (OH, SH) groups on the R group increases solubility. Amino acid solubility varies with pH and reaches a minimum corresponding to their pI (glycine, ala: highly soluble; leucine, tyrosine, cystine: very sparingly soluble and spontaneously crystallize in digestive or bacterial hydrolysates and in urine as soon as their concentration increases). These differences in solubility allow for the separation and identification of amino acids.

IV. 1. 3. 1. 2. Optical properties

a- Stereoisomerism

Apart from glycine, all standard amino acids have a central carbon (C_α) presenting four different substitutions (**asymmetrical carbon fiber**or chiral). This leads to optical isomerism, allowing the amino acid series to be defined.

When the models ofα-amino acids are arranged according to Fischer's convention, the carbon chain is vertical and viewed from its convex side; the –COO–, whose oxidation state is the highest, is positioned upwards, and the –NH3+ can then be located either to the left or to the right of the $C_\alpha$chiral; theα-amino acids then belong to the L series (from the Latin *laevus*, left side) or the D series (from the Latin *dexter*, right side), respectively (Fig. 97). Therefore, for eachα-amino acid, except for glycine where R = H, two stereoisomers (enantiomers), mirror images of each other; theα-amino acids of the proteins of all known living beings belong to the L series.

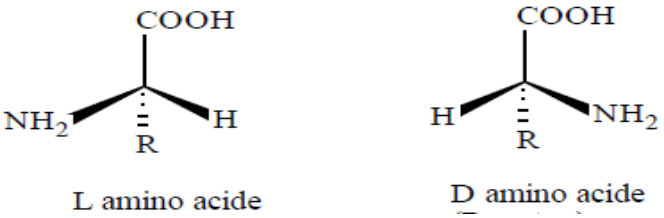


Fig. 97. Amino acid stereoisomers.

There are four amino acids that make up proteins and possess two asymmetric carbons. In this case, the number of stereoisomers is 2n, or 4. These isomers are then called diastereomers. This is the case for threonine, isoleucine, hydroxyproline, and hydroxylysine. These derivatives are called allo derivatives (Fig. 98).

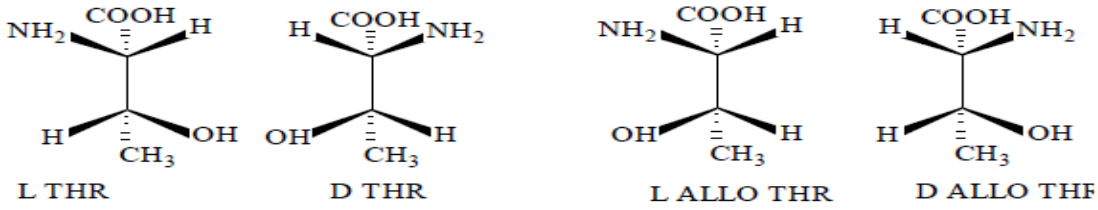


Fig. 98. Diastereomers of amino acids.

b- Spectral activities

The measurement of the amount of light absorbed by a molecule is called spectrophotometry. Amino acids do not absorb visible light (400 to 750 nm), so their solutions are colorless.

All amino acids exhibit significant absorption at wavelengths below 230 nm (Fig. 99). Aromatic amino acids (Phe, Tyr, and Trp) have the particularity of strongly absorbing

ultraviolet (UV) radiation (Trp 278–280 nm, Tyr 275 nm at acidic pH and 280 nm at pH > 10, Phe absorbs at 254 nm). This reactivity is due to the aromatic ring and the presence of delocalized electrons. This property allows their detection and quantification by spectrophotometry using theBeer-Lambert law:

$$A = \varepsilon_M . L.[C] \text{ and } A = \log (Io / I)$$

ε_M: molar absorption coefficient (molar-1.cm-1): ε_M= 5600 (TRP at 280 nm); ε_M = 1400 (TYR at 275 nm);ε_M = 200 (PHE at 257 nm).

Beer-Lambert's law shows that light absorption is proportional to molar concentration, provided that we work at the maximum absorption wavelength of the molecule or λmax and with a clear and sufficiently dilute solution.

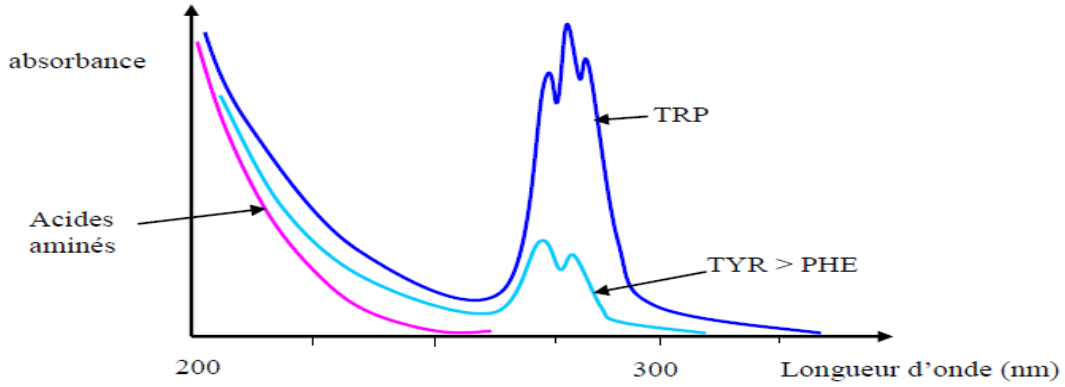


Fig. 99. Absorption spectra of amino acids in the ultraviolet.

d- Fluorescence

Some molecules, when excited by light incident at a certain length wavelengths where they absorb this radiation emit light of a longer wavelength: this is the phenomenon of fluorescence. This is the case for tryptophan and tyrosine, whose fluorescence allows the study of their immediate environment in proteins (analysis of three-dimensional structure or of the catalytic mechanism). Tryptophan, thanks to the structure of its indole nucleus, is fluorescent. Thus, when subjected to radiation at 278 nm, it emits fluorescence at 348 nm (Fig. 100).

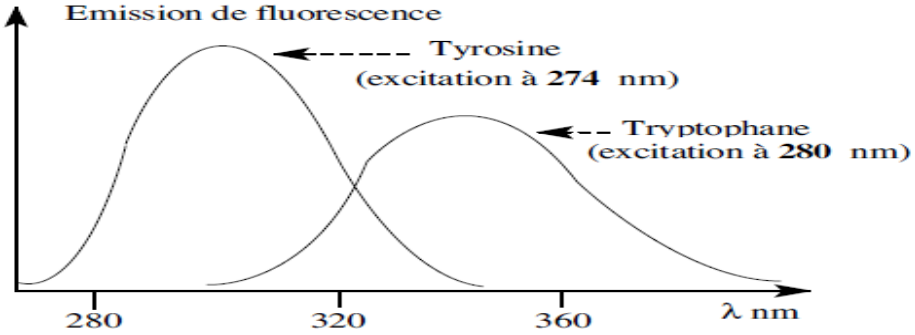


Fig. 100. Fluorescence of tryptophan (emission and excitation spectra).

IV. 1. 3. 2. Chemical properties and dosage

IV. 1. 3. 2. 1.Ionization, acid-base properties.

A crystalline amino acid, dissolved in water, behaves as a dipolar ion (Fig. 101). This amino acid possesses both acidic (proton donor) and basic (proton acceptor) character. They are amphoteric (ampholytes): the α-COOH group can donate a proton, and an anion is formed (R-COOH →R-COO- + H+). The α-amino group can bind a proton and a cation is formed ($R\text{-}NH_2 + H^+ \rightarrow R\text{-}NH3^+$).

In an acidic environment, in the presence of an excess of protons, the amino acid will be in its fully protonated form. As the proton concentration decreases (or the OH- concentration increases), the amino acid donates protons according to the following steps:

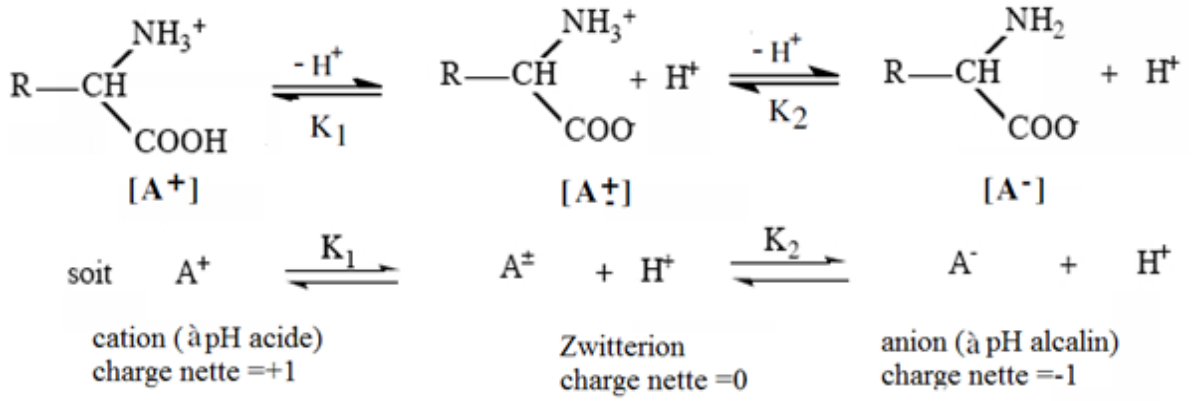


Fig. 101. Deprotonation equation of an amino acid.

A zwitterion (hybrid ion in German) is a molecule that carries both a positive and a negative charge. They can therefore serve as buffering agents.

The pH at which amino acid molecules are in the dipolar form and the net charge of the molecule is zero, corresponds to the isoionic or isoelectric point (pI or pHi) of the amino acid. At this pH, its solubility in water is minimal and it does not migrate in a direct current electric field (electrophoresis).

The two dissociation reactions of the carboxylic and amine groups correspond to equilibria governed by the law of mass action, so the proportions of ionized and non-ionized amino acids in solution depend on the concentration of H+ ions. Therefore, the two dissociation constants K1 and K2, corresponding to the two equilibria, can be written as follows:

$$K_1 = \frac{[R-COO^-][H^+]}{[R-COOH]} \qquad K_2 = \frac{[R-NH_2][H^+]}{[R-NH_3{}^+]}$$

Knowing the values of K1 and K2, it is possible, for each H+ ion concentration (at each pH), to calculate the percentage of ionized molecules. When the H+ ion concentration is equal to K1 or K2, there are as many ionized molecules as non-ionized molecules. The pK (-log K)

corresponds to the pH of half-dissociation; it indicates the ease with which this dissociation occurs and allows us to assess the strength of the acid and the base. To calculate the pK, we use the Henderson-Hasselbalch equation:

$$\mathbf{pK = pH + \log_{10} \frac{[Accepteur\ de\ protons]}{[Donneur\ de\ protons]}}$$

The pK values of the 20α-amino acids of proteins are given in Table 18.

Table 18 Characteristic constants of the different amino acids

Code	Abreviation	Amino acid	pK_a (α-COOH)	pK_b (α-NH3)	pK_R (side chain)	pI	Mass molar	(% protein) human beings)
A	**Ala**	**Alanine**	2.35	9.87	-	6.01	89	7.8
C	**Cys**	**Cysteine**	1.92	10.70	8.18	5.05	121	1.9
D	**Asp**	**A. aspartic**	1.99	9.90	3.90	2.85	133	5.3
E	**Glu**	**A. glutamic**	2.10	9.47	4.07	3.15	147	6.3
F	**Phe**	**Phenylalanine**	2.20	9.31	-	5.49	165	3.9
G	**Gly**	**Glycine**	2.35	9.78	-	6.06	75	7.2
H	**His**	**Histidine**	1.80	9.33	6.04	7.60	155	2.3
I	**lle**	**Isoleucine**	2.32	9.76	-	6.05	131	5.3
K	**Lys**	**Lysine**	2.16	9.06	10.54	9.60	146	5.9
I	**Leu**	**Leucine**	2.33	9.74	-	6.01	131	9.1
M	**Met**	**Methionine**	2.13	9.28	-	5.74	149	2.3
N	**Asn**	**Asparagine**	2.14	8.72	-	5.41	132	4.3
P	**Pro**	**Proline**	1.95	10.64	-	6.30	115	5.2
Q	**Gln**	**Glutarnin**	2.17	9.13	-	5.65	146	4.2
R	**Arg**	**Arginine**	1.82	8.99	12.48	10.76	174	5.1
S	**Ser**	**Serine**	2.19	9.21	-	5.68	105	6.8
T	**Thr**	**Threonine**	2.09	9.10	-	5.60	119	5.9
U	**Sec**	**Selenocysteine**	-	-	5.73	-	168	-
V	**Val**	**Valine**	2.39	9.41	-	6.00	117	6.6
W	**Trp**	**Tryptophan**	2.46	9.21	-	5.89	204	1.4
Y	**Tyr**	**Tyrosine**	2.20	9.74	10.46	5.64	181	3.2

Basic amino acid residues such as arginine, ornithine, citrulline, lysine, and histidine help combat acidosis by binding a proton. Conversely, aspartic acid or glutamic acid residues help combat alkalosis by releasing a proton. The buffering capacity of proteins (combating acidosis or maintaining acid-base balance) is due to these different constituent groups.

*IV. 1. 3. 2. 2.*Amino acid titration

The dissociation of the different polar groups of an amino acid can be easily studied by adding HCl or NaOH to the solution and measuring the pH after each addition. Titration curves, which will have different appearances depending on whether the amino acid is neutral, acidic, or basic, are obtained. These curves show the pH as a function of the amount of base or acid added and allow for the identification of the different pKa values of the amino acid. Knowing the pKa and pHi values, it is possible to plot the neutralization curve of an amino acid.

- **Ionization of an amino acidwith a side chain not containing an ionizable group**

In general, when the radical is not ionizable, the $pK_{\alpha COOH}$ is on the order of 2 and the $pK_{\alpha NH2}$ of the order of 10 (Figs. 102 and 103). The pI where the amino acid is amphoteric is calculated using the formula: $\mathbf{pH_i = \frac{(pK1+pK2)}{2}}$

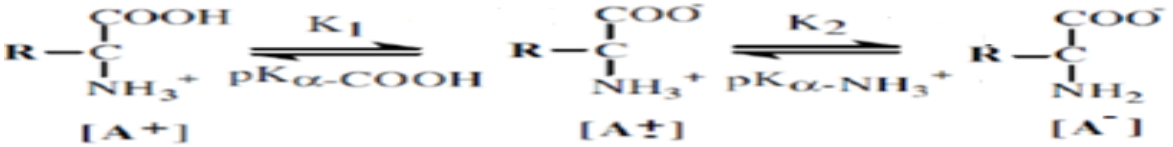


Fig. 102. Proton balance of a neutral amino acid.

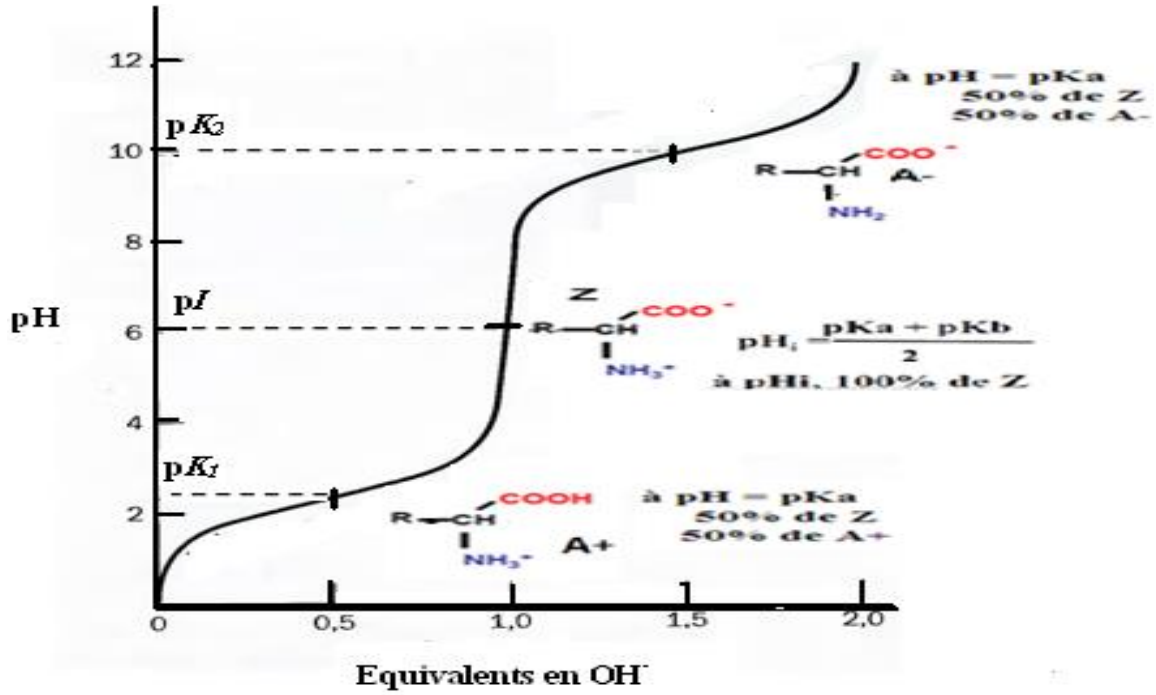


Fig. 103. Titration curve of a neutral amino acid (glycine) with sodium hydroxide.

It is observed that around the pK values, the pH changes little despite the constant addition of NaOH, due to the buffering effect of amino acids in solution (Fig. 103).

B- Ionization of an amino acid side chain containing an acidic function

These amino acids possess an additional ionizable group on their side chain. Similarly, a dissociation constant, denoted K_R, and a pKR (=-log K_R) are assigned to this group. When the amino acid is in its most protonated form, three functional groups are capable of releasing a proton (Figs. 104, 105). The order of release depends on the different pK values: from the lowest (more acidic) to the highest (less acidic).

For aspartate and glutamate, the pKαCOOH is around 4; the pHi varies from 2.7 to 3.2.

When the radical is ionizable, to calculate the pHi, it is necessary to use the pKs involved on either side of the amphoteric form, at the level of the dissociation equation.

$$\mathbf{pH_i = \frac{(pK1 + pK2)}{2}}$$

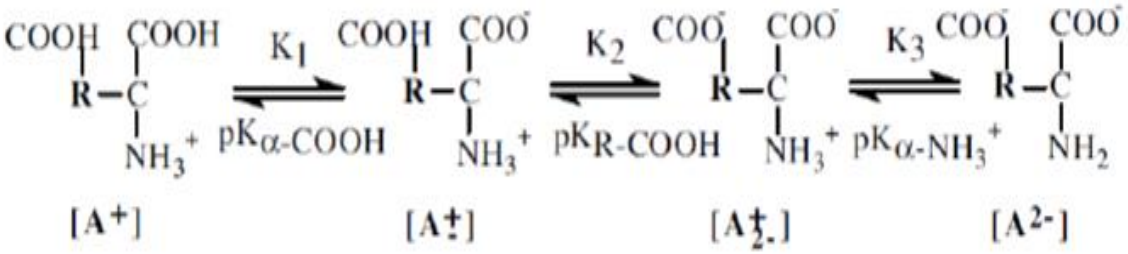


Fig. 104. Ionic state of an acidic amino acid as a function of pH.

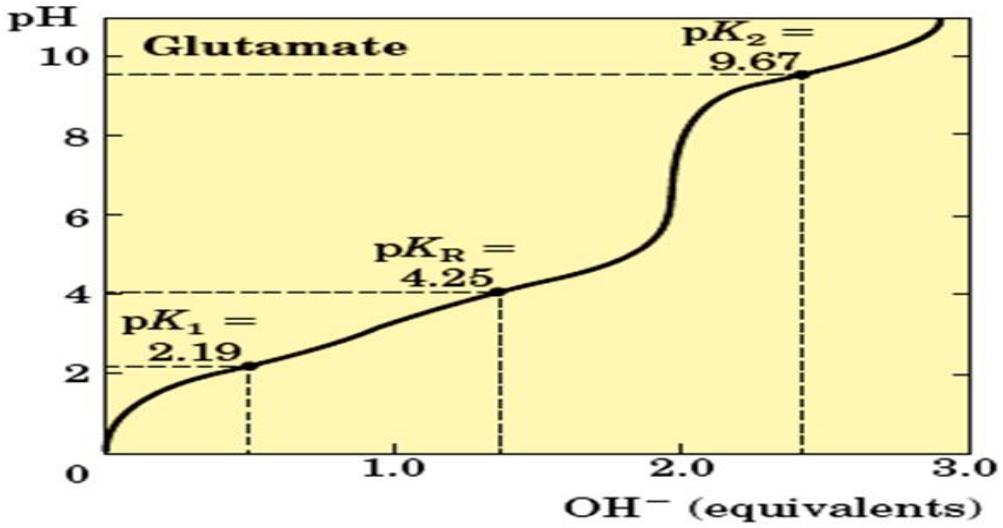


Fig. 105. Titration curve of an acidic amino acid (glutamic acid) with sodium

C- Ionization of an amino acid side chain with a base function

For basic amino acids (Figs. 106, 107), Le pHi is calculated by the formula:

$$\mathbf{pH_i = \frac{(pK2+pK3)}{2}}$$

For arginine and lysine, the pKαNH_2 is around 12 and the pHi ranges from 7.8 to 10.8.

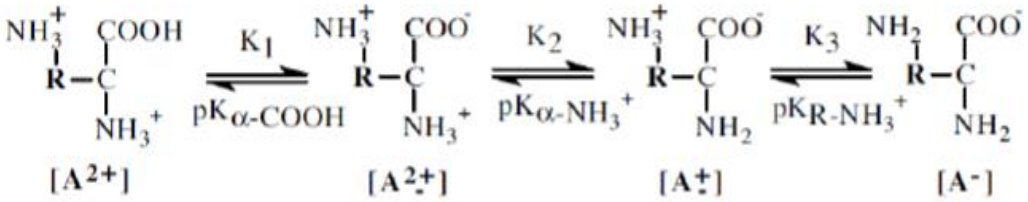


Fig. 106. Ionic state of a basic amino acid as a function of pH.

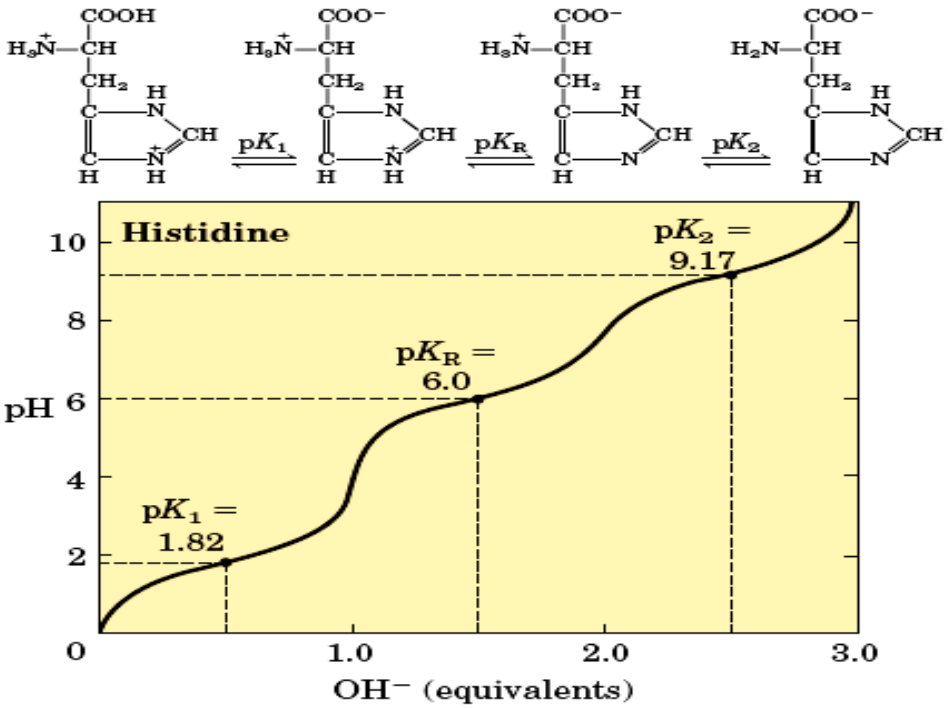
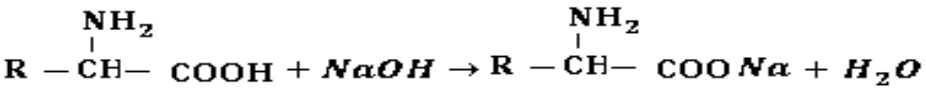


Fig. 107. Titration curve of a basic amino acid (histidine) with sodium hydroxide.

IV. 1. 3. 3. Reactions due to the presence of the carboxyl

A- Formation of salts

This property can be used to titrate amino acids. The carboxyl group of amino acids loses a proton when reacting with bases, thus forming salts which are named after the amino acid ending with the suffix **"ate"** of sodium (Fig. 108). Salt formation is generally done with sodium hydroxide (NaOH) or potassium hydroxide (KOH).

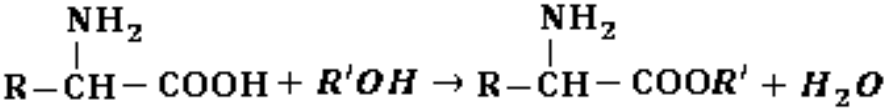


Amino acid **base** **salt**

Fig. 108. Reaction of an amino acid with a base.

B - Esterification

Esterification of the acid function by an alcohol takes place in a strongly acidic medium (Fig. 109). The obtained esters are volatile and can therefore be separated by fractional distillation or gas chromatography.

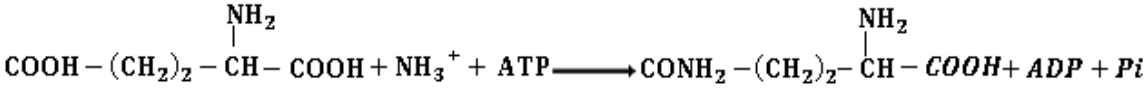


Amino acid **Alcohol** **Ester**

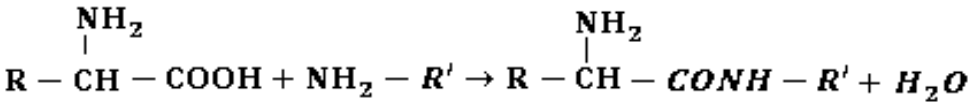
Fig. 109. Reaction of an amino acid with an alcohol.

C - Amidification

The carboxyl group of an amino acid can undergo amidation (Fig. 110), which allows the attachment of an ammonia molecule, an important reaction in urogenesis and ammoniogenesis. This reaction (catalyzed by glutamine synthetase) begins with glutamic acid.



Glutamic acid Ammonia Glutamine



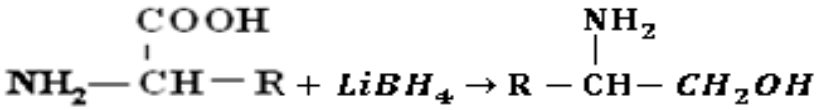
Amino acid Amine Amide Water

Fig. 110. Amidification reactions of an amino acid.

When the amino group is provided by another amino acid, the bond is called a peptide bond. If the bond involves a COOH or an NH_2 located in another position (β-COOH,γ-COOH,ε-NH_2), the bond is said to be peptide-like (e.g., the bond between glutamic acid and cysteine in glutathione).

D- Reduction

The acid function can be reduced to the alcohol function, in presence of $LiBH_4$ or $NaBH_4$ or enzymatically. The product obtained is an α-amino alcohol (Fig. 111). This reaction is used to identify amino acids.



Amino acid Amino alcohol

Fig. 111. Reduction reactions of an amino acid.

E- Decarboxylation

The carboxylic function (of the carbonα) can undergo a decarboxylation reaction leading to the formation of amines. This reaction is catalyzed by cellular decarboxylases and can give rise to bodies classified as biogenic because they possess considerable physiological activity (Table 19).

Table 19 : Main biogenic amines.

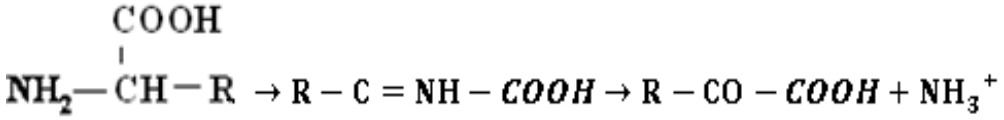
Aminated ac	Amine	Function
Aspartate	α-alanine β-alanine	In proteins Component of coenzyme A
Glutamate	γ-Amino-butyrate	Neurotransmitter (GABA) Mediator of the central nervous system
Cysteine	Cysteamine	Component of coenzyme A
Serine	Ethanolamine	Component of phospholipids
Histidine	Histamine	Mediator, Neurotransmitter
Threonine	Aminopropanol	Component of vitamin B12
Arginine	Agmatine	Neuromodulator
3,4-dihydroxy-Phenylalanine (DOPA)	Dopamine	Neurotransmitter
5-Hydroxytryptophan	Serotonin	Mediator, Neurotransmitter
Ornithine	Putrescine	Precursor of polyamines

IV. 1. 3. 4. Reactions due to the presence of the amine group

A- Deamination

Deamination is a reaction during which the amino acid loses its group.α-amino in the form of NH_3 (Fig. 112). It can be carried out chemically (nitrous acid HNO_2) or enzymatically (L-glutamate dehydrogenase and amino acid oxidase).

To maintain the intracellular reserve of the 20 amino acids used for protein synthesis, metabolism will involve deaminations with oxidation which will produce α ketone acids, the main source from which amino acids are synthesized.

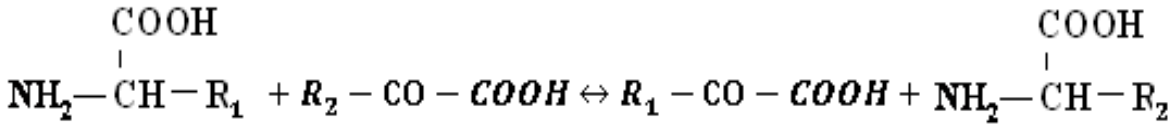


Amino acid **acid α-iminated** **Acid α-ketone**

Fig. 112. Deamination reactions of an amino acid.

B- Transaction

It refers to the reversible transfer of NH_2 from one amino acid to another.αketo acid; there is no release of NH_3. The enzymes that catalyze such reactions are called aminotransferases or transaminases (Fig. 113).



Acide aminé Acide α-cétonique 2 Acide α-cétoniquel Acide aminé 2

Fig. 113. Transamination reactions of an amino acid.

C- Reactions with aldehydes

Amino acids, in the presence of aldehydes, form imines called "Schiff bases" which are relatively unstable (Fig. 114). These Schiff bases very often appear as intermediates in enzymatic reactions involving amino acids as substrates. Proline, which contains a secondary amine group, does not react with aldehydes.

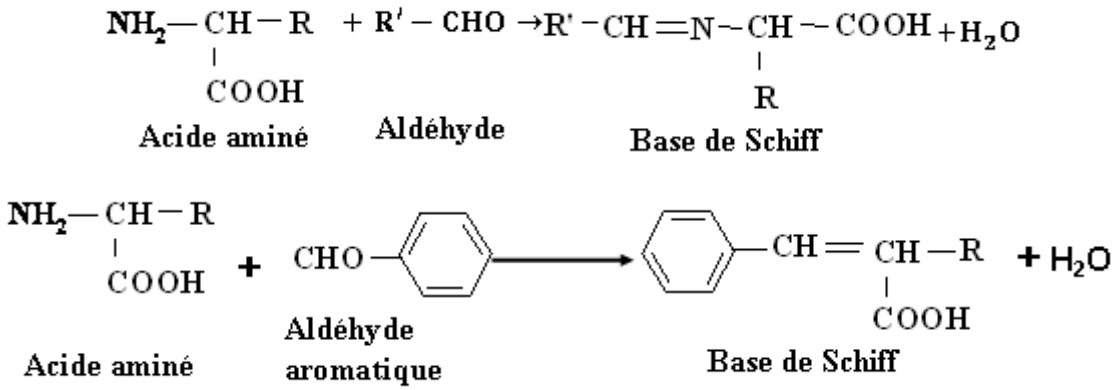


Fig. 114. Reaction of an aldehyde with an amino acid.

D- Substitution reactions

These reactions involve the substitution of a hydrogen of the NH2 function by an organic radical R', which can be an: alkylated (alkylation), aromatic (arylation), acyl (acylation) chain.

- **Alkylation (or methylation) reaction**

The best-known example is the methylation of glycine, which occurs in living organisms and in vitro (Fig. 115). Methylglycine is found in some plant proteins. The methylation of glycine to trimethylglycine, after reduction, leads to the formation of choline.

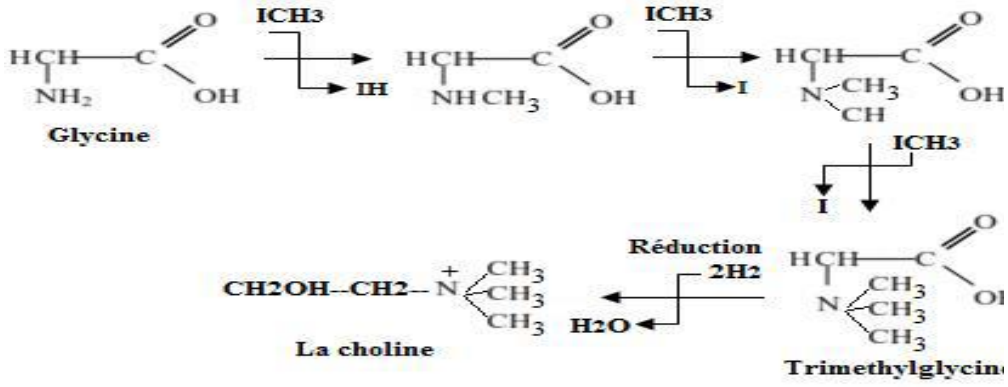
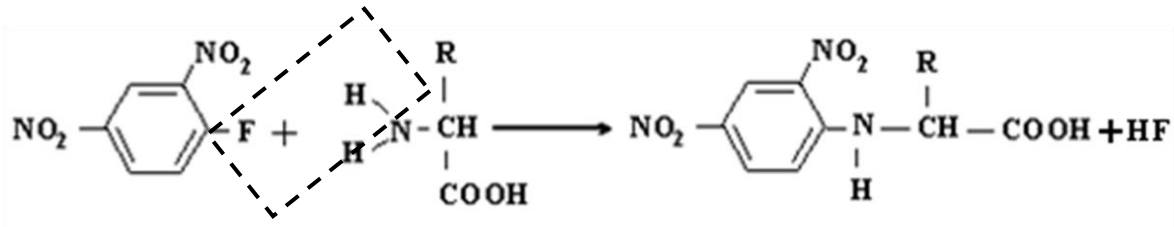


Fig. 115. Glycine methylation.

- Arylation reaction

Arylation is a reaction that replaces a hydrogen atom (H) in an NH_2 group with an aryl (aromatic) group. This reaction uses an aromatic derivative, the2, 4-dinitrofluorobenzene (DNFB) allowed Frederik SANGER in 1953 to establish the first primary structure of a protein: insulin (Nobel Prize in Chemistry 1958).

The dinitrobenzene ring combines with the free amine (Fig. 116). The protein is subsequently hydrolyzed in acidic or basic media. The amino acids are released except for those with a free NH2 group, which remain bound to the dinitrobenzene. DNP-aa is a yellow compound, easily identified by chromatography and quantified by spectrophotometry at 360 nm.



Sanger reagent **Amino acid** **DNP-aa**
(DNFB) (2,4- dinitrophenyl amino acid)

Fig. 116. Reaction of dinitrofluorobenzene chloride with an amino acid.

- Formation of N acylated derivatives

N-acylation is a reaction in which an acyl group is added to a molecule, this group being transferred from an acylant (Fig. 117). Acyl halides and anhydrides are widely used as acylizing agents.

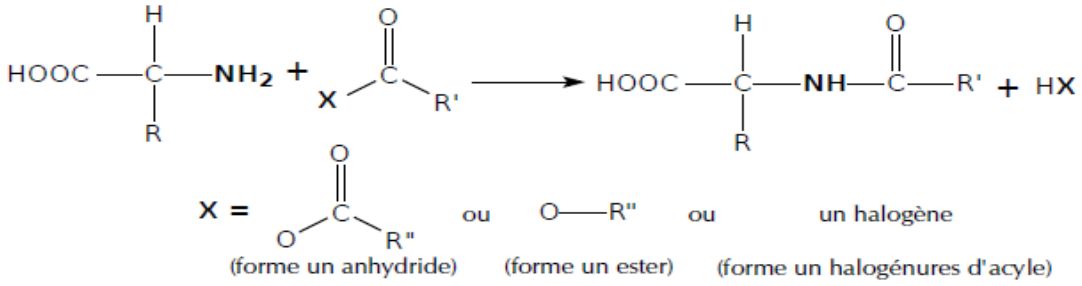
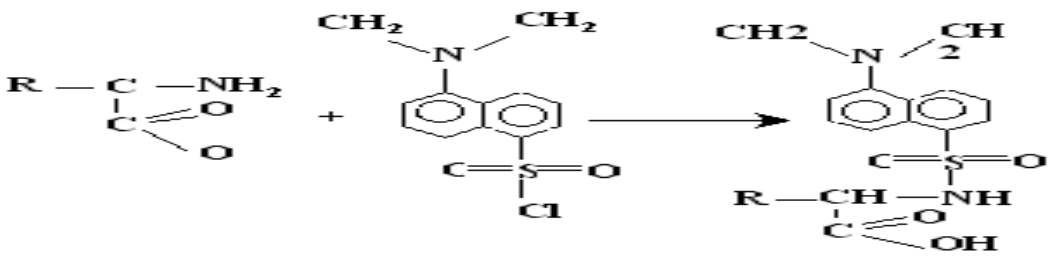


Fig. 117. N-acylation reaction.

The reaction with dansyl chloride (1-dimethyl-amino-naphthalene-5-sulfonyl) gives a more stable and fluorescent compound allowing high sensitivity in detection (Fig. 118).



Dansyl amino acid (Dansyl-amino acid)

Fig. 118. Reaction with dansyl chloride.

- **Carbamylation**

The so-called carbamylation reaction (Edman's method) with phenylisothiocyanate (PTC), at a basic pH of 9, gives derivatives (amino acid PTH) which absorb in the ultraviolet and are easily separable by chromatography (Fig. 119).

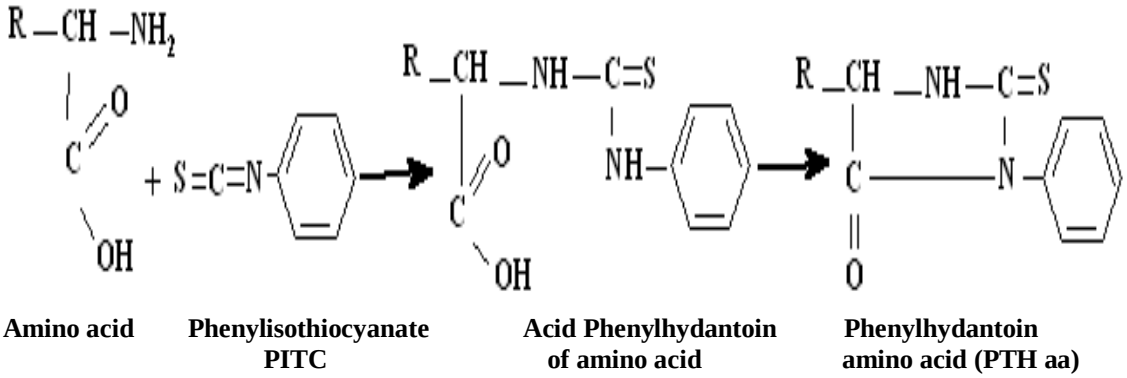


Fig. 119. Reaction of phenylisothicyanate with an amino acid.

- Substitution with formaldehyde (formalin)

This reaction allows the amine function to be blocked (Fig. 120). The amino acid is no longer amphoteric but is acidic. It can be titrated by alkalimetry in the presence of phenophthalein. This is the Sorensen formalin titration method.

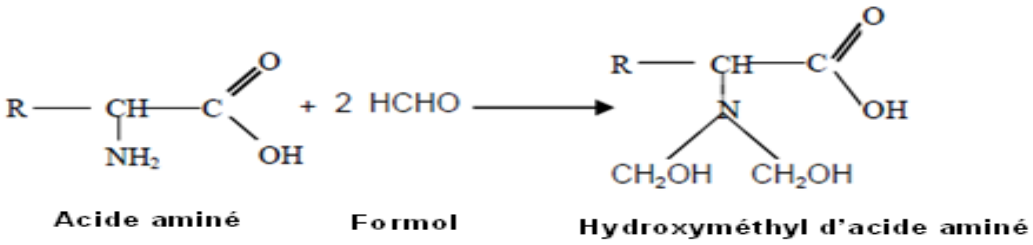


Fig. 120. Substitution reaction ofα-NH_2 of an amino acid by formaldehyde.

IV. 1. 3. 5. Reactions related to the COOH and NH_2 groups

Reaction with ninhydrin

Ninhydrin (2,2-dihydroxyindan-1,3-dione) is an **aromatic compound** and a powerful oxidant. By reacting with the primary amines, it generates a purple coloration (λ = 570nm) and with secondary amines (case of the proline) a yellow color (λ = 440nm). The reaction involves several steps (Fig. 121).

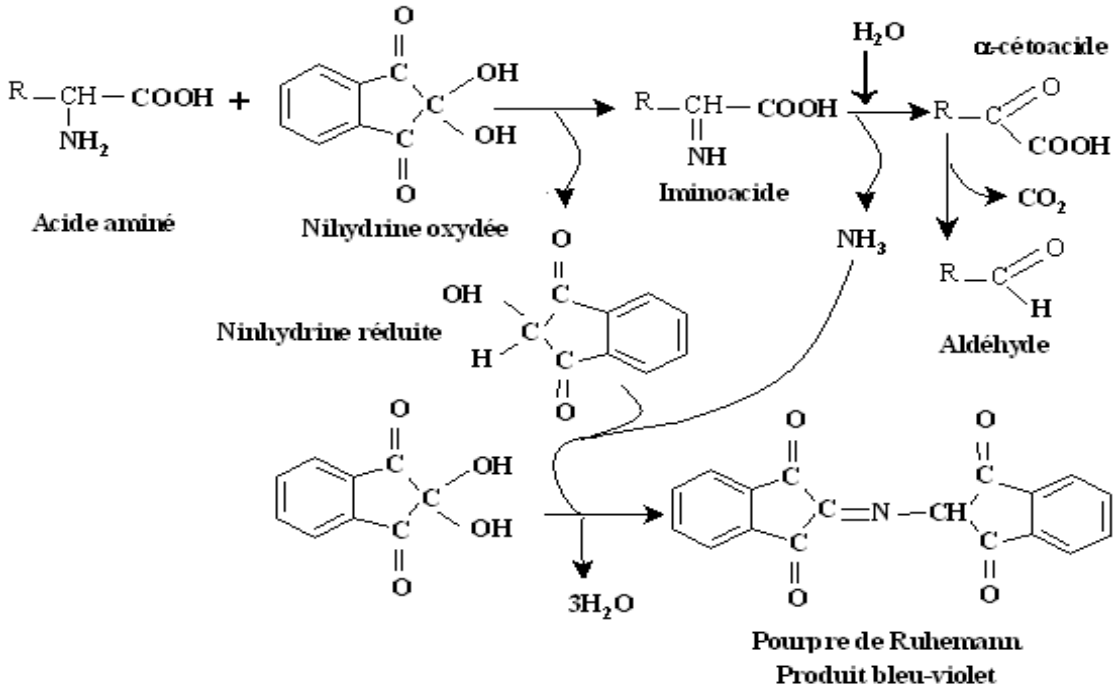


Fig. 121. Reaction of amino acids with ninhydrin.

Initially, the formation of reduced ninhydrin (hydrindantin) is observed, while the amino acid is transformed into an ***imino acid***; this then hydrolyzes intoα-ketoacid which is decarboxylated into an aldehyde having one less atom than the starting amino acid. Then, the ammonia (NH_3) generated in the first part of the reaction reacts with a molecule of reduced ninhydrin and a molecule of oxidized ninhydrin to give a product with a blue-violet color: Ruhemann purple (Fig. 121).

It should be noted that the coloration is not specific to amino acids. It occurs with other compounds having free amino groups: glucosamine, peptides, and proteins. This coloration can be used to quantify the amino acids after chromatography on a cation exchange column in an automated analyzer.

IV. 1. 3. 6. Reactions due toside chains

Carboxylic groups and amines: the lateral carboxylic groups of aspartic acid and glutamic acid and the amine groups of lysine have the same chemical reactivity as that of the groups carried by the central carbon.

- *Sakaguchi's reaction* (α-naphthol in the presence of hybobromite or sodium hypochlorite) et in a basic medium reacts with the guanidine group ($NH=C-NH_2$) of arginine to form a red-orange compound.

Imidazole group: the imidazole group of histidine allows certain histidine residues present in the active sites of enzymes to participate in proton transfer reactions.

Pauly's reaction (diazosulfanilic acid in alkaline medium): red color with histidine.

Aromatic amino acids

- Aromatic amino acids (Trp, Tyr, Phe) absorb light radiation in the UV and have different λmax values.

- *Phenol group:* The phenol group of tyrosine can be halogenated, giving monoiodo-tyrosine and diiodotyrosine respectively, which are precursors of thyroid hormones (Fig. 122).

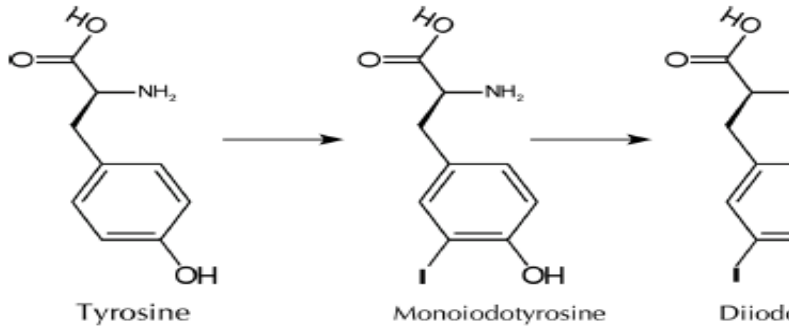


Fig. 122. Iodization of tyrosine.

- *Folin's reaction and Cioalteu* Phosphotungstomolybdic acid is reduced by tyrosine and tryptophan and forms various compounds colored in blue-violet.

- *Xantho-proprotein reaction* (concentrated nitric acid at boiling point). The aromatic rings form yellow nitrate derivatives during the nitration reactions.

- *Millon reaction*: Substituted phenolic (tyrosine) groups react with mercury (mercuric and mercurous nitrate in hot nitrous acidic medium) giving it a cherry-red color.

- *Uderfriend and Cooper's reaction* (α-nitroso-α-naphthol) giving a red color with tyrosine.

- Adamkiewich-Hopkins reaction (glyoxylic acid or commercial formaldehyde + concentrated sulfuric acid): Aldehydes react with the indole ring of tryptophan to form purple compounds.

Hydroxyl group: the alcohol functions of serine and threonine, as well as the phenol function of tyrosine, can be esterified, in particular by phosphoric acid.

Proline: It produces a distinctive color when combined with ninhydrin. It reacts with isatin to form an intense blue compound.

Sulfur-containing amino acids

- They react with lead acetate in an alkaline medium to form black lead sulfide.

- *Cysteine*: the thiol group of cysteine is very reactive; its oxidation in the presence of O_2 allows the formation of disulfide bridges (Fig. 123) which are found in proteins (intra-chain or inter-polypeptide cross-links). This reaction is catalyzed by the presence of metals.

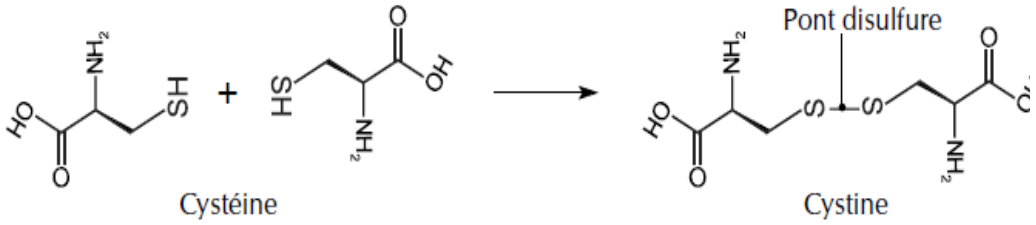


Fig. 123. Formation of a disulfide bridge.

Disulfide bonds can be broken by reducing agents; the most commonly used are : β-mercaptoethanol, DTT (dithiothreitol) and performic acid (irreversible).

- Sodium nitroprusside in dilute ammonia reacts with cysteine to give red derivatives.

- *Sullivan's reaction* (1,2-naphthoquinone-4-sulfonate sodium and sodium hydrosulfite) gives a red color with cysteine.

IV. 1. 4. Methods for the determination and analysis of amino acids

IV. 1. 4.1-*Total amino acid content*

These methods allow for the quantification of all amino acids, regardless of the nature of the R radical.

IV. 1. 4.1.1. Kjeldahl Method

Amino acids are completely mineralized by oxidation in a hot, concentrated sulfuric acid solution, in the presence of hydrogen peroxide, potassium sulfate and catalysts (Se, Cu, Hg). Ammonium sulfate is formed, a weak acid that can be titrated by acidimetry or colorimetry. The reactions involved are as follows (Fig. 124):

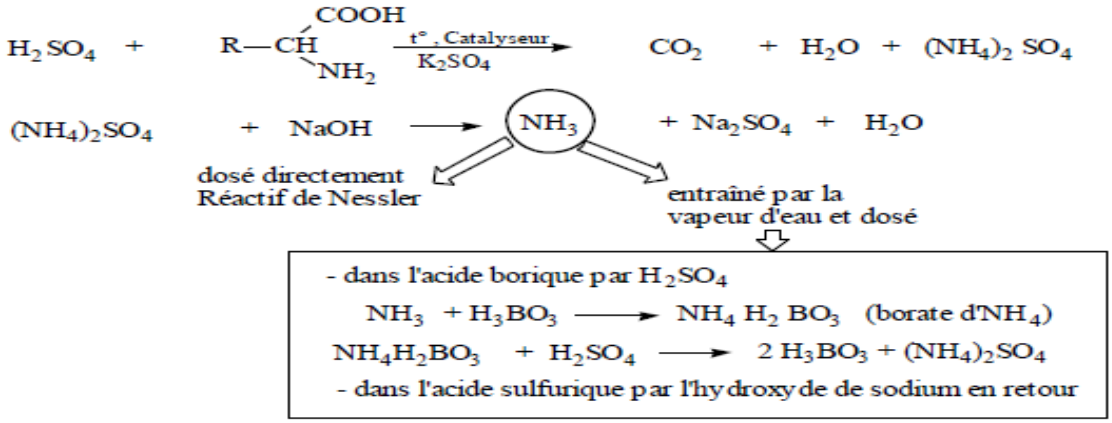


Fig. 124. Reactions involved in the method for determining total amino acids according to *Kjeldahl.*

This method is automated and mineralization at high temperatures shortens the analysis time to a few tens of minutes.

IV. 1. 4.1. 2. Sörensen's Method "Formaldehyde titration"

The reaction used is substitution with formaldehyde; the resulting compound is a tertiary amine, which is a weaker base (and therefore a stronger acid in its protonated form) than the original primary amine. Titration of the molecule is thus easier with a base in the presence of a colored indicator.

IV. 1. 4.1.3. Spectrophotometric assay with ninhydrin

Amino acids can be quantified spectrometrically after derivatization with ninhydrin because this reaction leads to the formation of a purple or yellow colored compound (proline), which absorbs strongly in the visible range.

IV. 1. 4. 2. Amino acid separation techniques

The amino acids that make up proteins are released by hydrolysis. A protein hydrolysate, obtained after boiling in prolonged (6N) hydrochloric acid (HCl), is a mixture of amino acids that must be identified and quantified.

has-Electrophoresis

This technique separates amino acids according to their electrical charge. An aqueous solution of a mixture of amino acids is placed on special paper (sheet of paper, sheet of cellulose acetate) or an electrophoresis gel (polyacrylamide, agarose). A high-voltage electric field is applied to this gel placed in a pH buffer solution fixed (Fig. 125).

Because of their different pH values, the amino acids possess distinct charges and migrate in different directions and at different speeds depending on the pH of the buffer system and the type of support. To establish their characteristic localization, samples of acids aminos witnesses are treated under the same conditions. To locate the amino acids on the support, it is dried, sprayed with a ninhydrin solution, and then heated. Blue spots indicate the presence of an amino acid on the support.

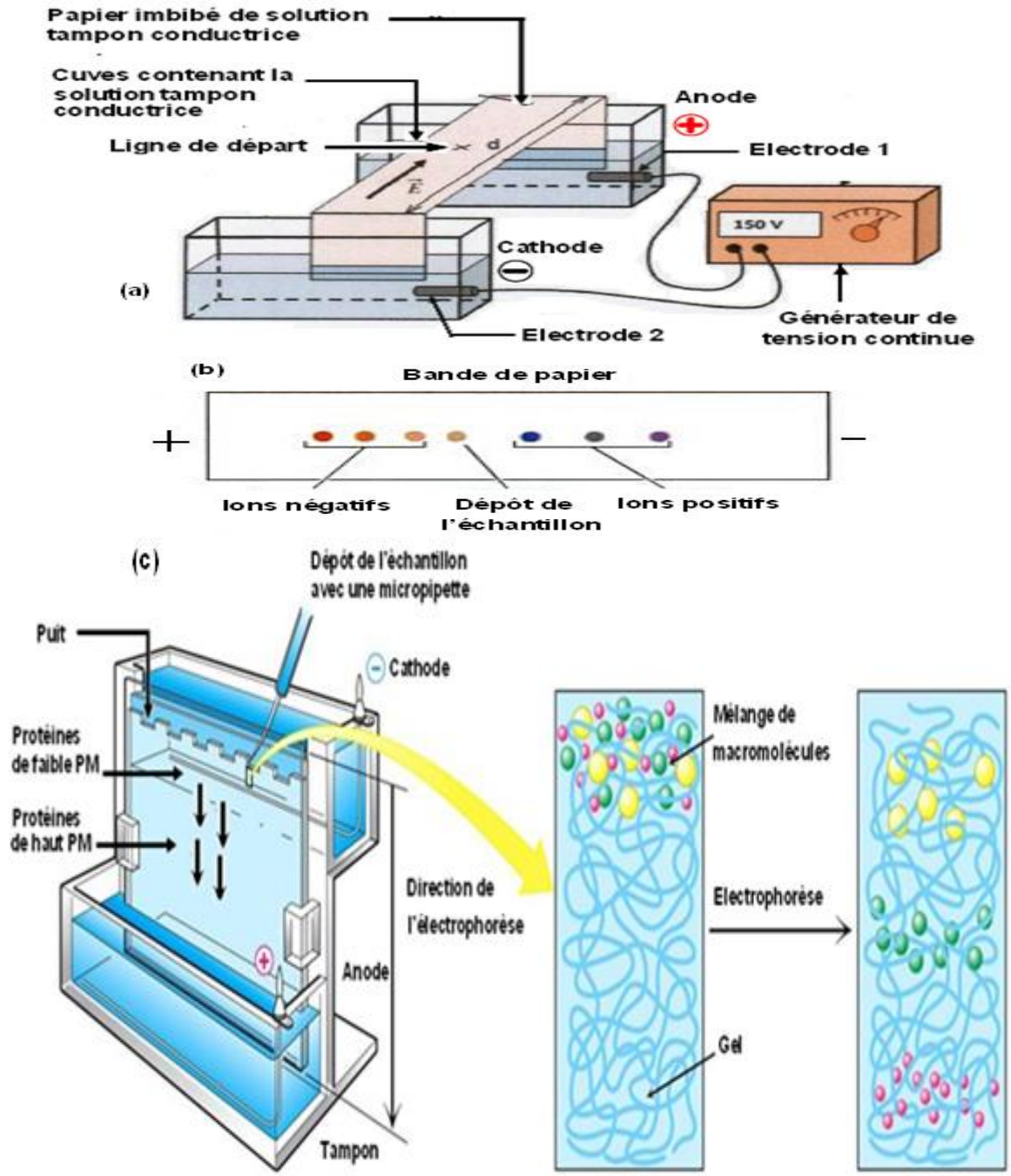


Fig. 125. The electrophoresis technique. (a) The horizontal paper electrophoresis apparatus. (b) Representation of the obtained electrophoretogram. (c) The vertical gel electrophoresis apparatus.

b-Chromatography

Chromatography is a physico-chemical analysis method that separates the constituents of a mixture by dragging a mobile phase (liquid or gas) along a stationary phase (solid or fixed liquid), thanks to the selective (re)partition of solutes between these two phases.

The factors involved in the separation of molecules between the fixed and mobile phases are: solubility in a liquid solvent, size (shape), polarity, electrical charge, and the presence of groups of atoms forming particular sites.

For amino acids, the techniques which can be implemented: paper chromatography, thin-layer chromatography, ion-exchange chromatography, gas chromatography.

- **Paper chromatography**

This is a partition chromatography technique where water (stationary phase) is retained on the paper and an organic solvent (mobile phase) rises by capillary action along the stationary phase, carrying amino acids with it to varying degrees depending on their polarity. This type of chromatography is performed in a hermetically sealed glass tank, the atmosphere of which is saturated by the mobile phase. The mixture to be analyzed is deposited in a small volume onto a starting point located near one end of the paper, which is then brought into contact with the mobile phase (Fig. 126).

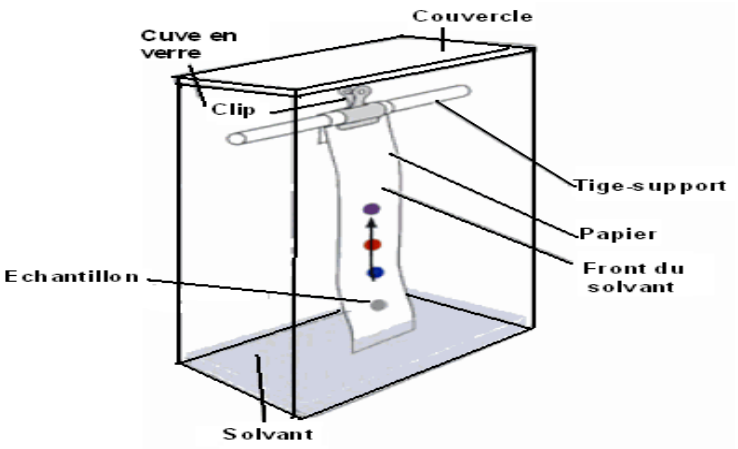


Fig. 126. Experimental setup for ascending paper chromatography.

The amino acids to be separated are chromatographed in the presence of standards. After migration and staining, the retention factor (Rf) of each unknown amino acid or standard is calculated: Rf = Migration distance of the compound / Migration distance of the solvent.

Rf is a characteristic of a compound in a given system for defined operating conditions. The more soluble the substances constituting the mixture are in this mobile phase, or the higher their Rf is, the more rapidly they migrate on the sheet.

Once the migration is complete, ninhydrin is sprayed onto the leaf. After heating, blue-violet spots appear in the areas where the amino acids are located.

Paper chromatography can be two-dimensional: the mixture to be analyzed is deposited at an angle, without a control, the migration in the 1st dimension is done with a first organic

solvent then the paper is turned a quarter turn and a new migration is done with another organic solvent of different polarity.

- **Thin-layer chromatography**

It is an adsorption chromatography with a polar stationary phase (cellulose or silica) spread in a thin layer on a rigid inert support (glass or metal foil) and a mobile phase which is a less polar organic solvent (Fig. 127).

The amino acids to be separated are chromatographed in the presence of standards. After migration and staining, the retention factor (Rf) of each unknown amino acid or standard is calculated. Thin-layer chromatography can also be two-dimensional.

After migration, the colorless spots must be revealed. This detection can be done by immersion in a potassium permanganate bath or by observation under UV (Ultraviolet) light if the silica plate contains a fluorescence indicator.

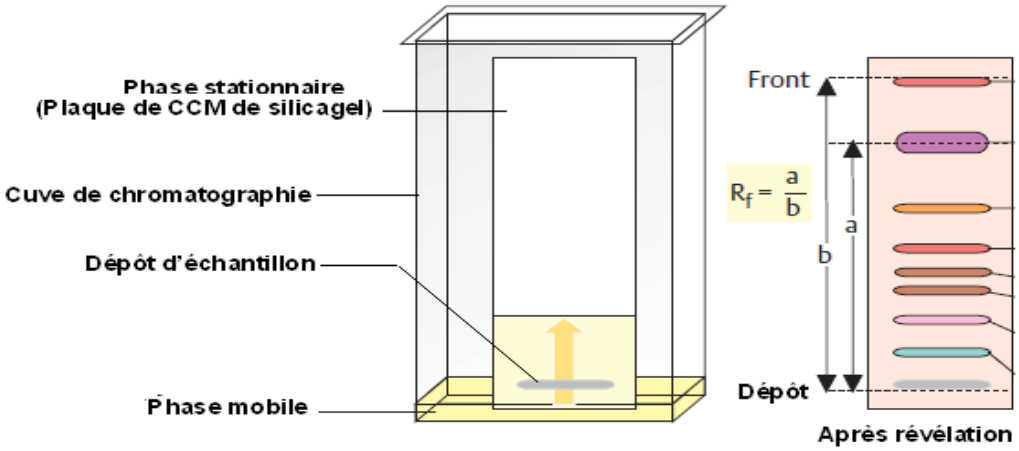


Fig. 127. Equipment for ascending thin-layer chromatography.

- **Ion-exchange chromatography**

It's a technic allowing a substance to be isolated electrically charged a mixture of molecules charged (liquid) molecules are separated. To do this, the mixture is passed over a stationary (solid) phase already bound to known ions, and these ions are replaced by the charged ions/molecules of the mixture to be separated. The stationary phase can be a resin in the form of beads. This resin is grafted with a charged radical (+ or -) (Fig. 128a). The resin is in a column, and the solution is passed through this column. The elution pH can change the charge of molecule M or B (in the case of an amino acid, for example), allowing only that molecule to be recovered (Fig. 128b).

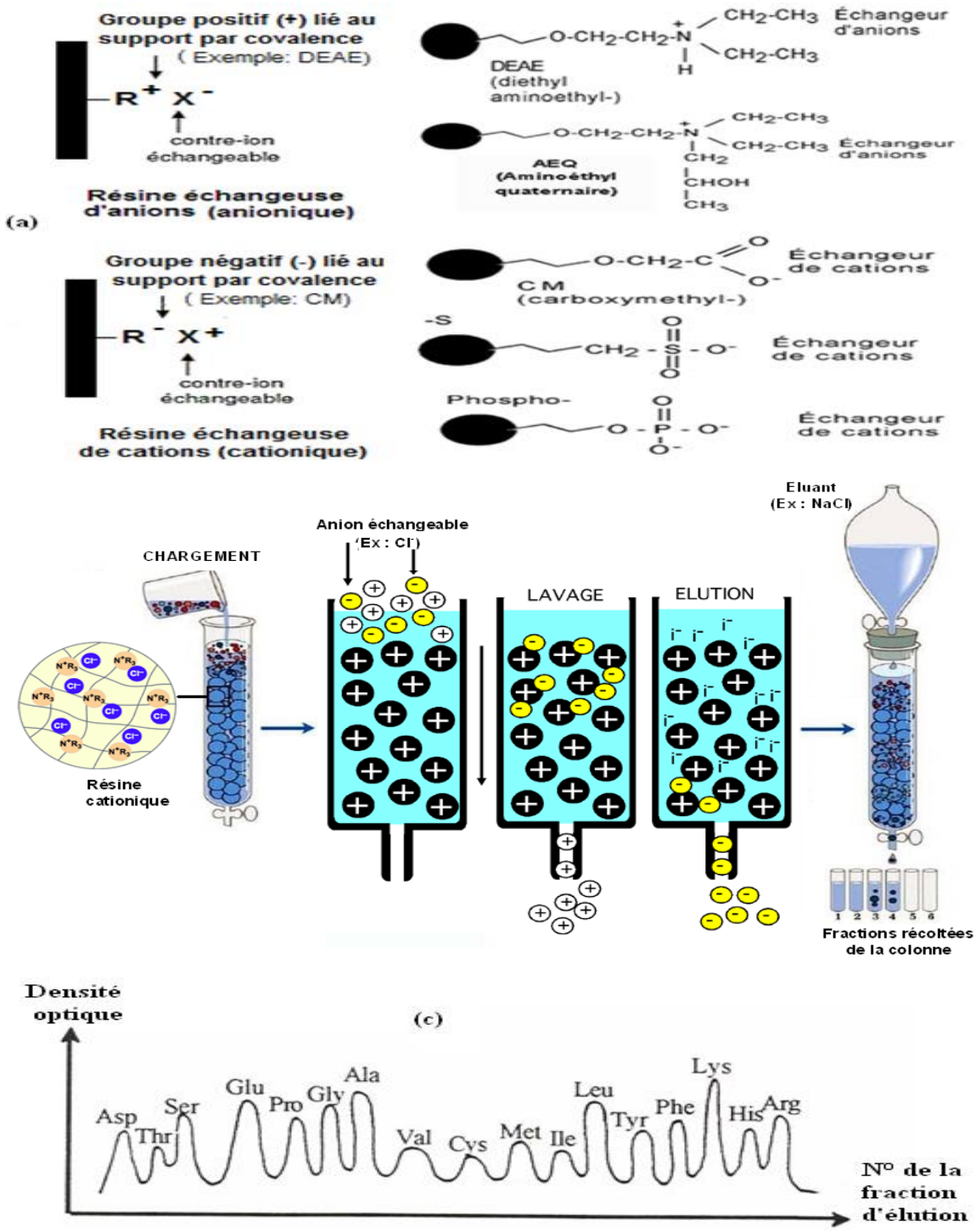


Fig. 128. (a) Charged groups grafted onto a synthetic resin of a chromatography column. (b) Device and principle of the ion-exchange chromatography. **(c)** Chromatogram of amino acids after ion-exchange chromatography.

The eluted amino acids are then collected at the column outlet, stained with ninhydrin, their absorbance is measured, and the chromatogram is presented as a graph showing peaks at the elute point of an amino acid (Fig. 128c).

IV. 2- THEPEPTIDES

IV. 2.1- Definition

Peptides are natural or synthetic compounds that result from the chaining of amino acids linked together by a covalent bond which is actually an amide bond formed by dehydration between the groupα-amine of an amino acid and the groupα-carboxyl of another amino acid. This bond is also called a peptide bond (Fig. 129). If the peptide bond involves a COOH or an NH_2 located in another position (β COOH, γ COOH, ε NH_2) it is called **peptidoid bond**.

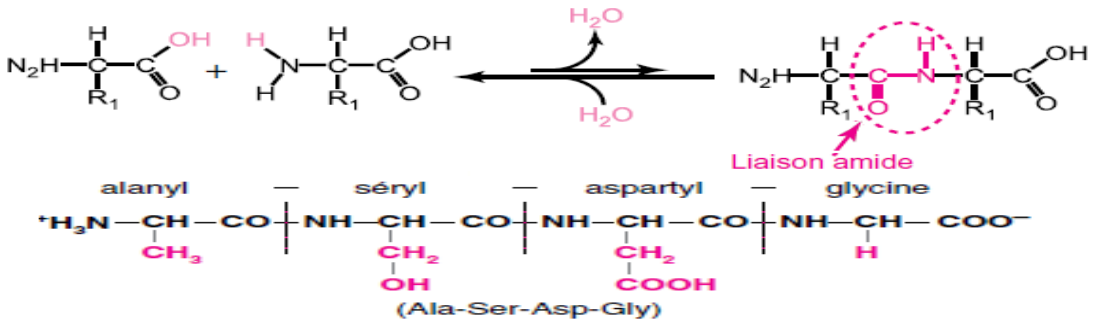


Fig. 129. Formation of the peptide bond.

The successive chain of amino acids linked together by peptide bonds constitutes the primary structure or sequence of the protein. The amino acids present in the peptide have thus lost the H from their NH_2 and the OH from their COOH: these are said to be "remnants" or "residues" of amino acids. It is named after the amino acid from which it is derived; with the addition of the suffix **-yl**: lysyl, tyrosyl, alanyl residues, etc. except at the C-terminus.

When there are two amino acid residues, it is a dipeptide; when there are three, it is a tripeptide. A peptide with 2 to 9 residues is called an oligopeptide, and one with more than 9 is called a polypeptide. Beyond 100 residues, it is no longer a polypeptide but a protein. The boundary between the two groups is arbitrarily set at an average molecular mass of 10,000. Since the average molecular mass of an amino acid residue is 100, a peptide with a molecular mass of 10,000 contains approximately 100 residues.

The resulting polypeptide chain has a polarity with a groupα-amine present at one end (N-terminal end) and the α carboxyl group is present at the other end (C-terminal end). By convention, an amino acid sequence is always written starting from the N-terminal residue. The peptide chain contains a repetitive part called the main chain or backbone and a variable part corresponding to the side chains of the amino acid residues.

The peptide bond is planar, rigid, and polar (Fig. 130); C, O, N, H, and the two Csαneighbors are in a plane where they constitute a peptide unit.

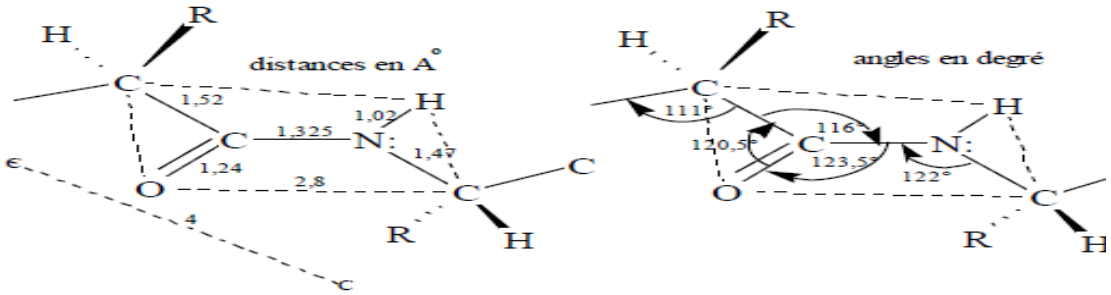


Fig. 130. Distances and angles of a peptide bond.

IV. 22. Properties of peptides

IV. 22.1. Physical properties

Solubility: The smaller the peptides are and the more hydrophilic amino acids they contain, the more soluble they are in water.

Light absorption: peptides absorb light in the ultraviolet at a wavelength of 280 nm if they contain an aromatic amino acid.

Acid-base properties

Numerous small peptides have been obtained in pure crystalline form. They have high melting points. None of the functionsα-carboxylic andα-amines combined in peptide bonds cannot ionize in the pH range of 0 to 14; the acid-base behavior of peptides is due to the function α-free amino group of the N-terminal amino acid, with the functionα-free carboxylic of the C-terminal amino acid and to those of the side chains capable of ionizing.

IV. 22.2. Biological peptides properties

Peptides participate in the structure of membranes as building blocks, and play important roles in biological activities.

IV. 22. 2.1. Peptides with a physico-chemical role

Glutathione (tripeptide γGlu-Cys-Gly: Glu is linked by the γ carbonyl group) plays a role central to cellular defense against dioxygen and its active derivatives (Fig. 1318). This is a very effective redox couple against peroxides:

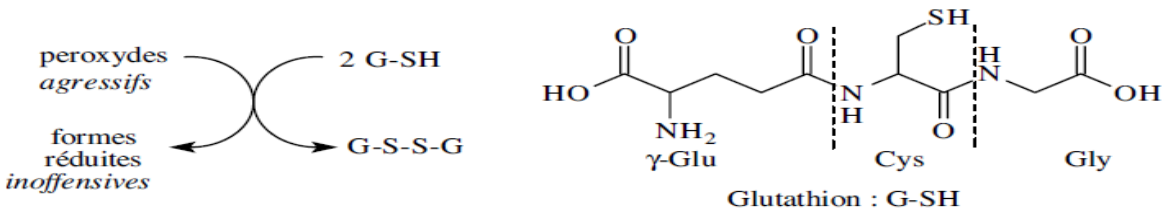


Fig. 131. Structure of glutathione.

Let's take as an example of a synthetic peptide widely used as a sweetener: aspartame. It is a dipeptide whose COOH end is esterified: aspartyl-phenylalanylmethyl ester

IV. 22. 2. 2. Peptides with mediating activity

This family of peptides is involved in communication functions: the endocrine system, nerve transmission and modulation, blood vessel motility, and inflammatory responses. Here are a few examples:

IV. 22. 2. 3. Hormonal peptides

The hypothalamus contains neurons with endocrine function that secrete two nonapeptides with hormonal activity:

- Oxytocin, which triggers contractions of smooth muscles (uterus for childbirth, mammary gland for milk ejection)
- Vasopressin: antidiuretic effect on the kidney (hypertensive action as a medication)

Other neurons produce activating (liberin) or inhibitory (inhibin) peptides of hormone secretion by the anterior lobe of the pituitary gland.

The anterior lobe of the pituitary gland biosynthesizes and secretes many peptide hormones: for example, ACTH (adrenocorticotropic hormone), a polypeptide of 39 amino acids.

The endocrine pancreas secretes:

- Insulin, a polypeptide made up of two chains (one of 21 and one of 30 amino acids), which regulates glucose metabolism (hypoglycemia)
- glucagon, a peptide of 29 amino acids, which causes an increase in blood glucose (hyperglycemia)
- The duodenal mucosa produces secretin, a peptide of 27 amino acids, which stimulates the exocrine pancreas during digestion

IV. 22. 2. 4. Neuropeptides

These are two families of molecules, ligands of brain receptors: the enkephalin family and the endorphin family. These molecules participate in pain control. They contain the same N-terminal tetrapeptide (Tyr-Gly-Gly-Phe).

Vasomotor peptides

Examples of vasoconstrictor peptides include:

- angiotensin II is an octapeptide
- endothelins, whose production is localized in lung tissue, are peptides of 21 amino acids, vasodilatory peptides:
- The atrium of the heart secretes a family of atrial natriuretic peptides (ANPs) which act on the kidneys by increasing the secretion of Na^+ ions and in diuresis (elimination)

water): in humans, α-ANP is a polypeptide of 28 amino acids.

- Bradykinin is a hypotensive agent released during an inflammatory reaction.

IV. 22. 2. 5. The molecules of inflammation

Tissue injury triggers a local defense reaction: this is the inflammatory reaction, the manifestations of which are pain, heat, redness and edema caused by vasodilatory molecules:

- the amines histamine and serotonin, decarboxylation products of histidine for histamine and of hydroxytryptophan for serotonin

- a family of peptides: the kinins, the prototype of which is bradykinin, which is a nonapeptide. Wasp venom contains a decapeptide: glycyl-bradykinin.

IV. 22.2.6. Antibiotic peptides

Some microorganisms synthesize peptides that inhibit protein synthesis, which serve as "weapons" for defense and colonization. These are cyclic peptides that incorporate D-amino acids or non-standard amino acids, properties that protect them from proteolytic degradation.

IV. 22.2.7. Bacterial peptides: Different strains of Bacillus can be isolated:

- the tyrocidine family, cyclic decapeptides including D-Phe and ornithine (5-carbon homolog of lysine)

- Bacitracin A is a 12-amino acid peptide, three of which belong to the D series (Glu, Asn, Phe) and contains ornithine in its structure.

IV. 22. 2. 8. Fungal peptides: The Penicillium mold produces penicillin, which is a dipeptide of two amino acid derivatives (Fig. 132):

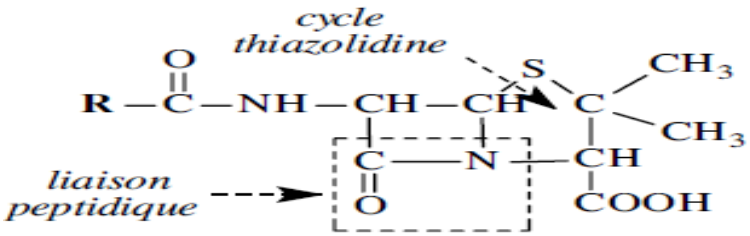


Fig. 132. Penicillin (R depends on the strain of Penicillium).

IV. 22. 2. 9. Immunomodulatory peptides

The micromycete fungus *Tolypocladium* secretes secondary metabolites, including a family of antifungal peptides called cyclosporines, which are immunosuppressants used in organ transplantation. They inhibit the activation of T lymphocytes without affecting other defense systems or the bone marrow.

Cyclosporine A is a cyclic decapeptide (11 amino acids) containing many

atypical amino acids: a D-alanine, 7 N-methylated amino acids: which results in 7 modified peptide bonds and a non-standard amino acid (in C9), specific to cyclosporines: 2-amino-3-hydroxyl-4-methyl-6octenoic acid.

IV. 3. 1. 1. 1.Study of peptide sequences

Determining the exact nature and proportions of each of the determining the constituent amino acids and the order of their sequence in a given peptide or protein requires, first and foremost, the cleavage of covalent bonds. The experimental approach used can be summarized as "divide and conquer".

Determining the crude composition of a peptide begins with its reduction, or oxidation, in order to eliminate disulfide bonds.

The Stein and Moore method. It comprises several steps:

– Acid hydrolysis (6 mol.L^{-1} HCl, 24h to 72h, 110°C) to cleave peptide bonds

– Separation of the released amino acids by ion chromatography

– Detection in the visible spectrum after ninhydrin derivatization

– Identification of amino acids by their retention time

– Quantification as a percentage by measuring the peak area

PTC amino acid method

– Acid hydrolysis

– Reaction with PTC phenylisothiocyanate.

– Reverse-phase adsorption separation by HPLC

– Detection in the UV

– Identification of amino acids by their retention time

– Quantification by measuring the peak area

- **Disulfide bridge cleavage**

In the case of multi-peptide molecules, the covalent structure of the peptides can exhibit disulfide bonds that can form between the chains. These disulfide bonds are involved between two cysteine residues. The dissociation of this bond can occur through theβ-mercaptoethanol or performic acid (Fig. 133).

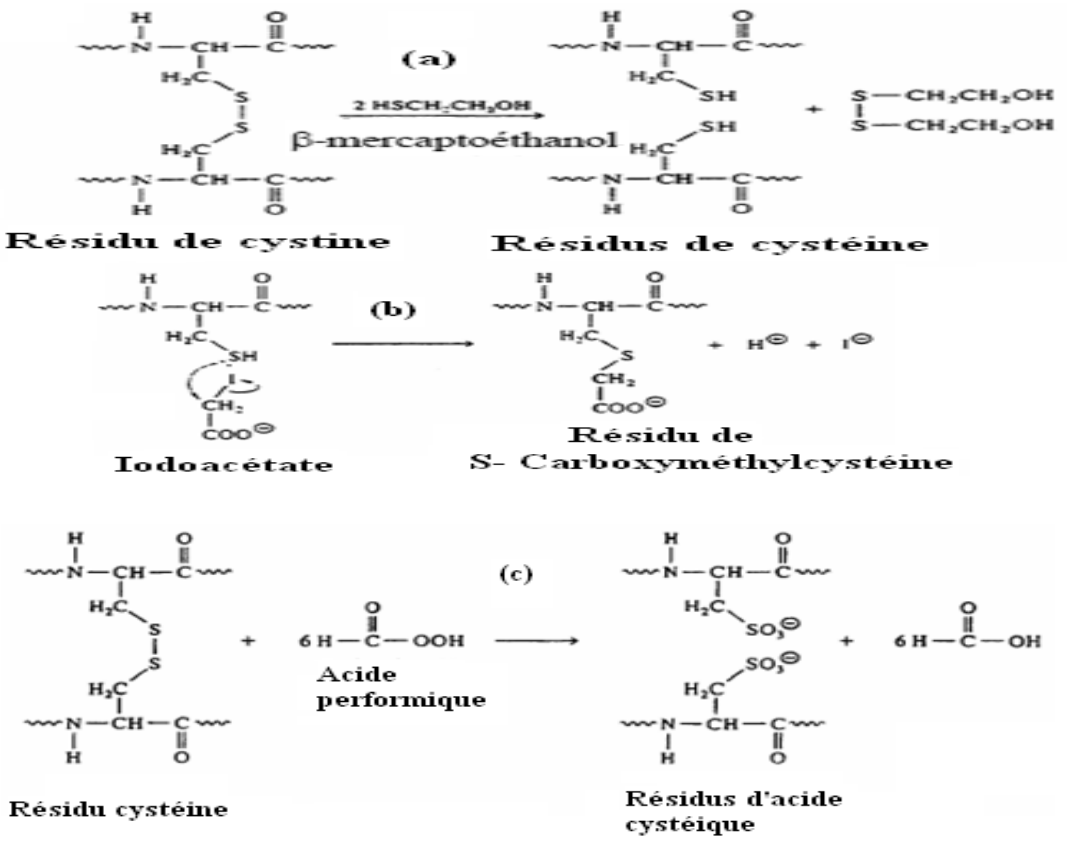


Fig. 133. Disulfide bridge splitting at β-mercaptoethanol and substitution by iodoacetate. (a) In the presence of β-mercaptoethanol, the protein exchanges its S-S groups with it: each cysteine is reduced to two cysteines and 2 molecules of β- mercaptoethanol are oxidized to disulfides. (b) The reduced protein is then treated with iodoacetate, an alkylating agent that converts free cysteine residues into S-cysteine. Thus substituted, the sulfhydryls are prevented from reoxidizing to S-S.(c) Performic acid (HCOOOH) breaks SS bridges in proteins and prevents their reformation by transforming the cystine residue into two cysteic acid residues.

- **Determination of amino acid composition**

A- Hydrolysis of peptide bonds

The breaking of peptide bonds, which allows amino acids to be obtained in their free state, is achieved by chemical or enzymatic action.

- **Acid hydrolysis**

The action of 6M hydrochloric acid (HCl) on a peptide, boiled for at least 24 hours, results in a hydrolysate containing the amino acids, with the following restrictions:

-Due to the sensitivity of the indole nucleus to acids, the Tryptophan is completely destroyed. Its dosage requires alkaline hydrolysis.

- the amides (Asn, Gln) are hydrolyzed into ammonia and corresponding acids (Asp, Glu)

- some amino acids (Tyr, Ser, Thr) can be partially destroyed (a shorter hydrolysis time solves the problem).

- Alkaline hydrolysis

It is done using 4 mol/L sodium hydroxide (NaOH) at a temperature of 110°C.
for approximately 4 to 8 hours. Total alkaline hydrolysis destroys serine, threonine, and cysteine.

The identification and quantification of the constituent amino acids will be carried out by chromatography on cation exchange resin after total hydrolysis of the peptide.

- Enzymatic hydrolysis

The hydrolysis of peptide bonds can be carried out by enzymes: peptidases or proteases (a mixture of endo and exopeptidases. This hydrolysis is slow and rarely complete. Enzymatic hydrolysis preserves tryptophan and dicarboxylic amino acid amides.

- **Analysis of terminal residues**

To determine the amino acid sequence, you must first identify the N- and C-terminal amino acids and their number in order to determine the structure of the peptide chain (linear, semi-cyclic, cyclic), then the order in which the other amino acids are linked.

HAS-Determination of the N-terminal amino acid

- **Chemical methods**

These methods involve treating the peptide with a suitable reagent to form a stable derivative characteristic of the C- or N-terminal functions. This derivative is isolated from the peptide by hydrolysis, then separated and quantified.

- Sanger method at DNFB

1-Fluoro-2,4-Dinitrobenzene (FDNB) reacts with free amino groups, and in particular the functionα-terminal amino acid to form a dinitrophenyl peptide (Fig. 134)Complete acid hydrolysis of the peptide chain releases dinitrophenyl compounds, including...α-dinitrophenyl-amino acid (DNPaa), corresponding to the N-terminal amino acid, can be extracted with ether, identified by paper chromatography, and quantified spectrophotometrically thanks to its yellow color. The detection of severalα-DNPaa is evidence of the existence of multiple peptide chains.

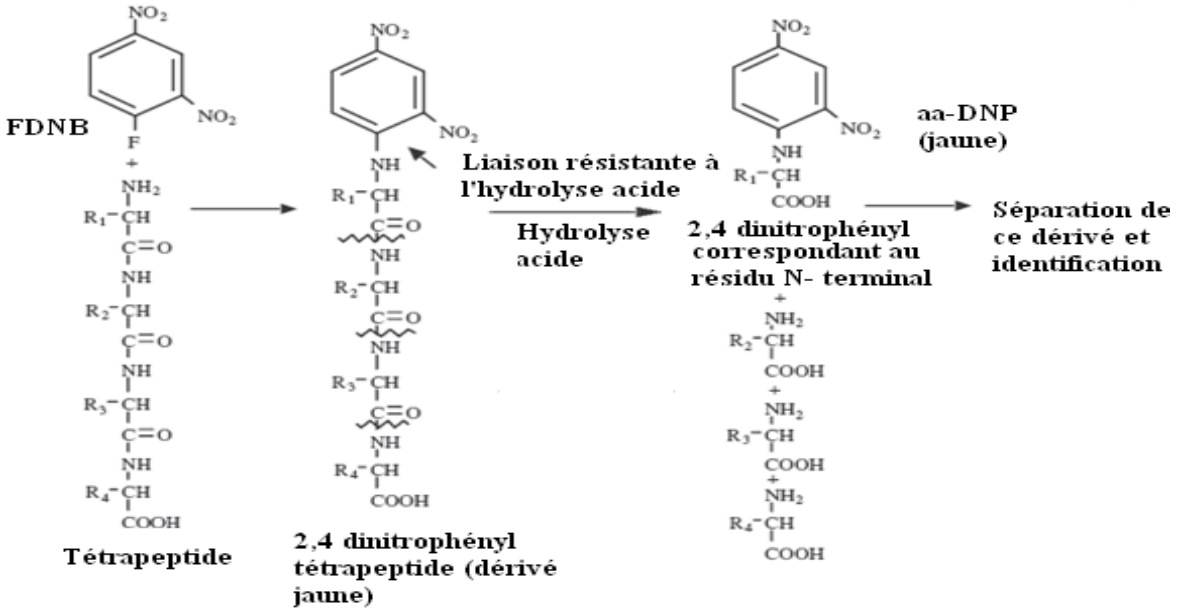


Fig. 134. Determination of the N-terminal amino acid by the Sanger's method.

- **Edman's recurrent method (using phenylisothiocyanate)**

Carbamylation with phenylisothiocyanate (PTC), at a basic pH of 9, gives derivatives that absorb in the ultraviolet and are easily separable by chromatography (Fig. 135).

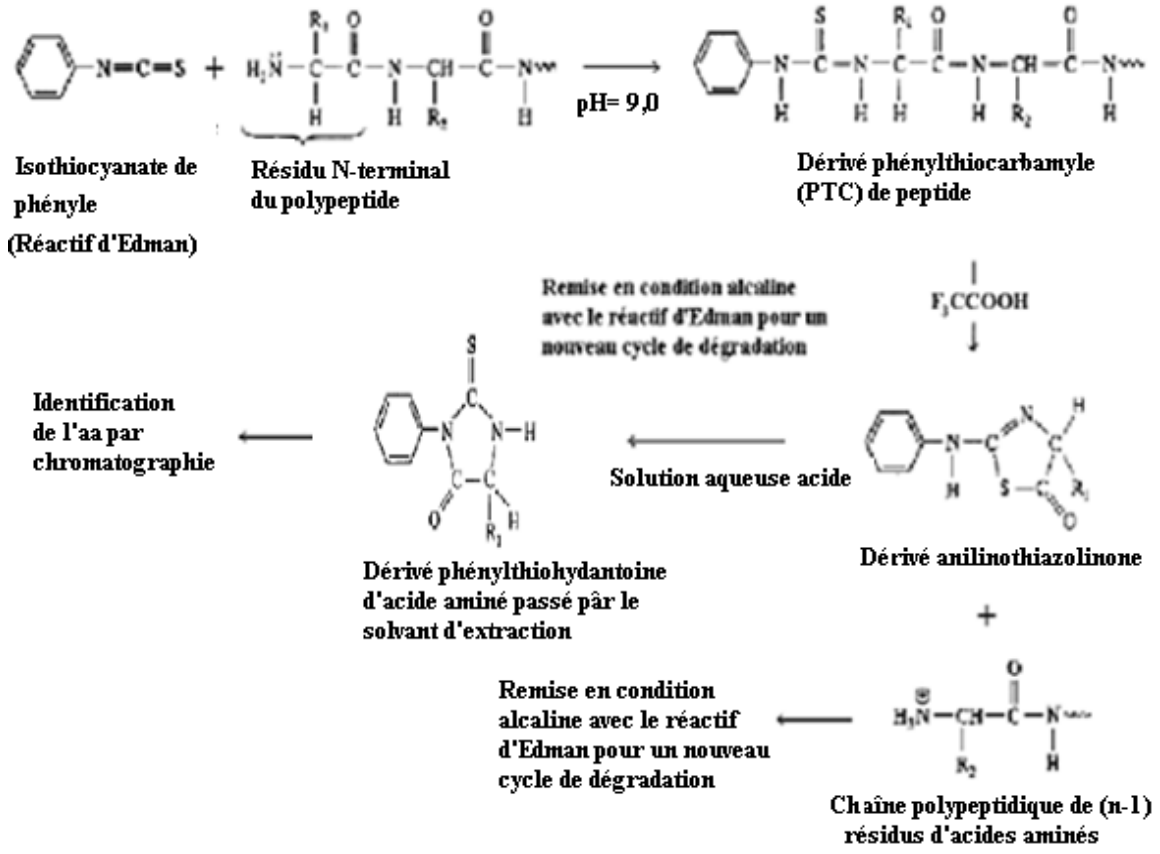


Fig. 135. Determination of the N-terminal amino acid by the Edman's method.

Furthermore, the reaction with the terminal amino acid of a protein releases the addition derivative and a protein missing its N-terminal amino acid: by iterating the process, the determination of the primary structure of the protein will be possible (recurrent Edman degradation).

In the case of an n-amino acid peptide, the PTC-peptide will undergo cyclization and cleavage at a slightly acidic pH, releasing an identifiable phenylthiohydantoin-amino acid (PTH-amino acid), which absorbs in the UV, and an (n-1)-amino acid peptide.

- **dansyl chloride method**

The chloride of 1-Dimethylaminonaphthalene-5-sulfonic acid (or dansyl chloride) reacts very readily with amino groups and leads to highly fluorescent sulfonamide derivatives after hydrolysis of the dansylpeptide (Fig. 136).

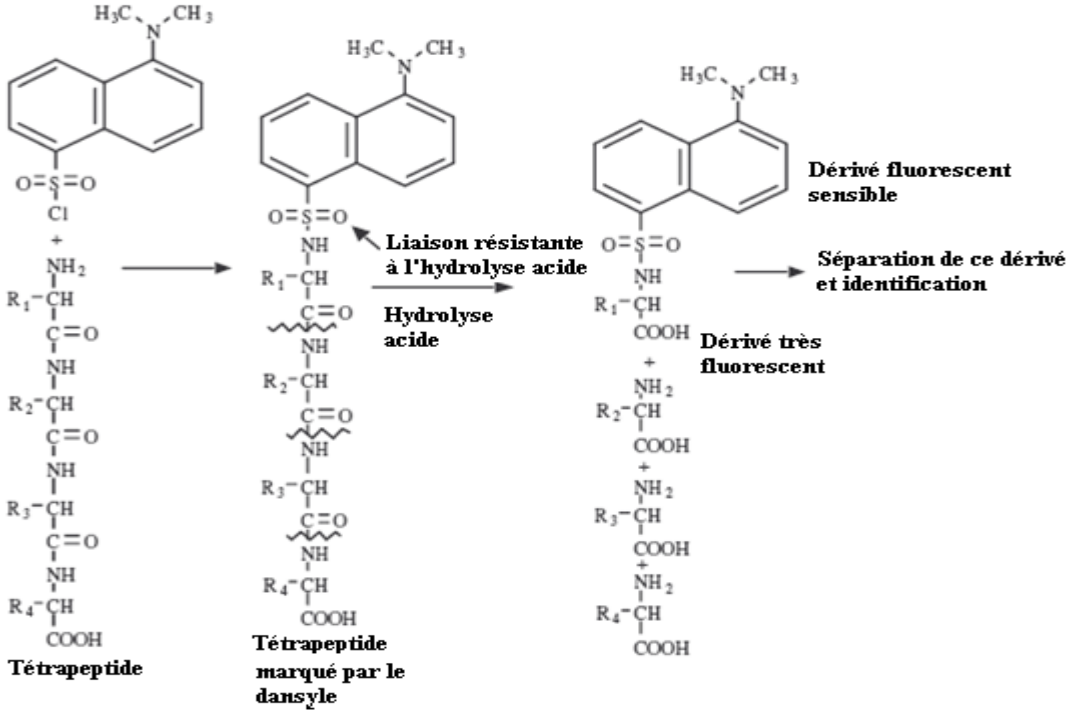


Fig. 136. Determination of the N-terminal amino acid by the dansyl chloride method.

- **Enzymatic methods**

The complete enzymatic hydrolysis of a peptide chain is a long and difficult process. Enzymes exhibit some specificity in attacking peptide bonds; they are primarily used for partial hydrolysis of peptide chains.

There are exopeptidases (Table 20) that specifically catalyze the release of terminal residues from a peptide chain: the presence of a functionα-amine or a functionα-free carboxylic acid is necessary for their activity.

The action of these enzymes is continuous; each released amino acid exposes a new terminal amino acid, which will in turn be detached. An aminopeptidase, called Leucine aminopeptidase (LAP), although not specific to this amino acid, detaches all N-terminal amino acids except proline, which will be released by a prolinase.

B- Identification of the C-terminal amino acid

- **Chemical methods**

Lithium borohydride and acid hydrolysis, the amino alcohol derivative is identifiable by chromatography (Fig. 137a).

Hydrazinolysis at 100°C allows the cleavage of peptide bonds with the formation of hydrazides, except at the C-terminal residue, which appears as an easily identifiable amino acid (Fig. 137b).

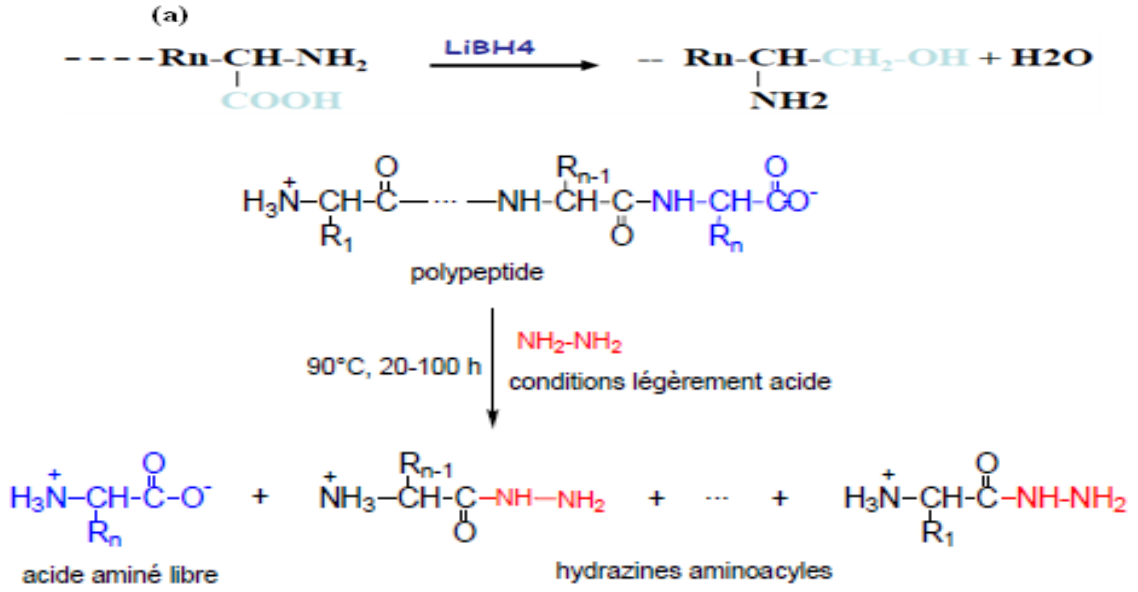


Fig. 137. Determination of the C-terminal amino acid by reduction (a) and by hydrazynolysis (b).

- **Enzymatic methods**

Carboxpeptidases A (3.4.12.2) or B (3.4.12.3) hydrolyze peptide bonds from the C-terminal residue. Carboxypeptidase A is a metalloenzyme of pancreatic juice that does not hydrolyze if the C-terminal amino acid is Arg, Lys, or Pro (it cleaves the C-terminal amino acid when it is acid or neutral).

Carboxypeptidase B acts on C-terminal Arg and Lys.

Carboxypeptidase C (3.4.12.1) detaches all C-terminal amino acids.

Proline carboxypeptidase (3.4.12.4) specifically detaches C-terminal proline.

The N-terminal (or C-terminal) amino acid is extracted and identified. Since the enzyme can continue its hydrolysis on the peptide with n-1 residues, several cycles can be performed in this way (Table 20).

Table 20: Examples of some exopeptidases with their specificities.

Kind	Name	Source	Special feature
Aminopeptidase	Leucine aminopeptidase	Pork kidney	-except for Pro
	Aminopeptidase M	Pork kidney	
	Aminopeptidase K	Mold	- Other than basic Stopped by Pro
Carboxypeptidase	Carboxypeptidase A	Beef pancreas	- Other than basic Stopped by Pro
	Carboxypeptidase B	Beef pancreas	
	Carboxypeptidase C	Lemon leaf	
	Carboxypeptidase P	Mold	-except for Ser and Gly

- Partial hydrolysis of intrachain peptide chains

If the peptide chain is too long, it is important to make cuts at specific points along the chain. Two methods can be used: chemical and enzymatic.

- **Chemical methods**

With cyanogen bromide

Cyanogen bromide cleavage (BrCN)of the peptide chain only acting on the carboxylic sidemethionine residues (Witkop and Gross method). A peptide containing 2 methionols (except for the terminal C) will give 3 cleavage peptides. A peptidyl homoserine lactone is produced by the action of BrCN on the peptide (Fig. 138).

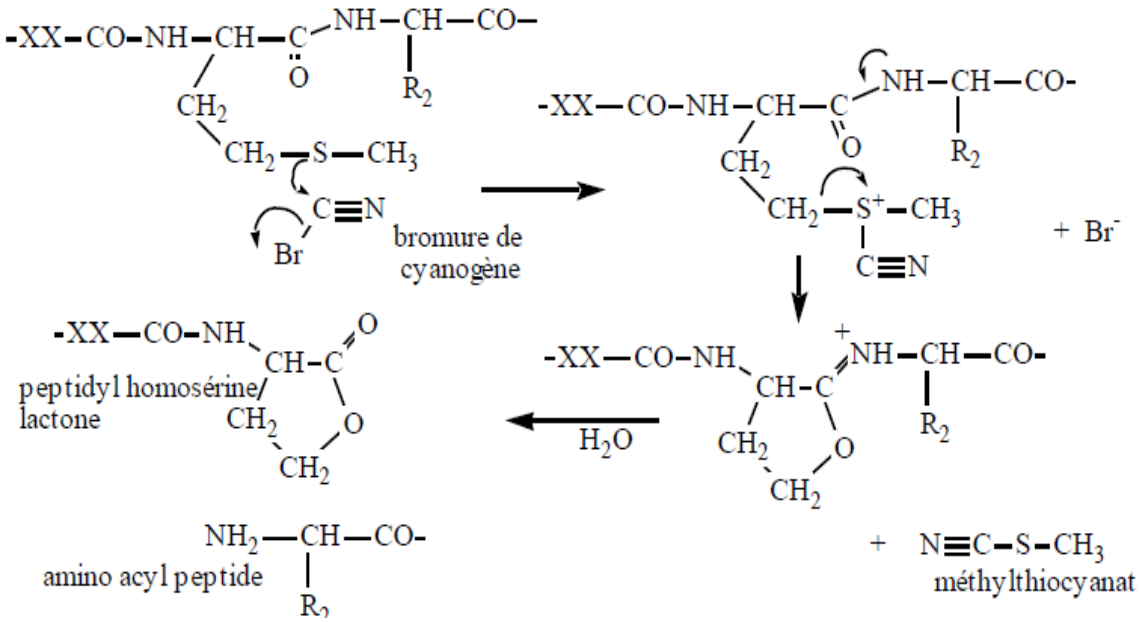


Fig. 138. Peptide chain cleavage site by cyanogen bromide (BrCN).

With 2 –nitro-5-thiocyanobenzoate (NTCB) hydrolyzes the peptide bond on the amine side of cysteine.

- **Enzymatic methods**

These are endopeptidases with narrow specificity of action that allow for very localized cleavage (Fig. 139). The enzyme hydrolyzes internal peptide bonds between two amino acids. The hydrolysis of a peptide by an endopeptidase will give several peptide fragments: if there are m cuts (m peptide bonds hydrolyzed), the peptide will be degraded into (m+1) peptide fragments.

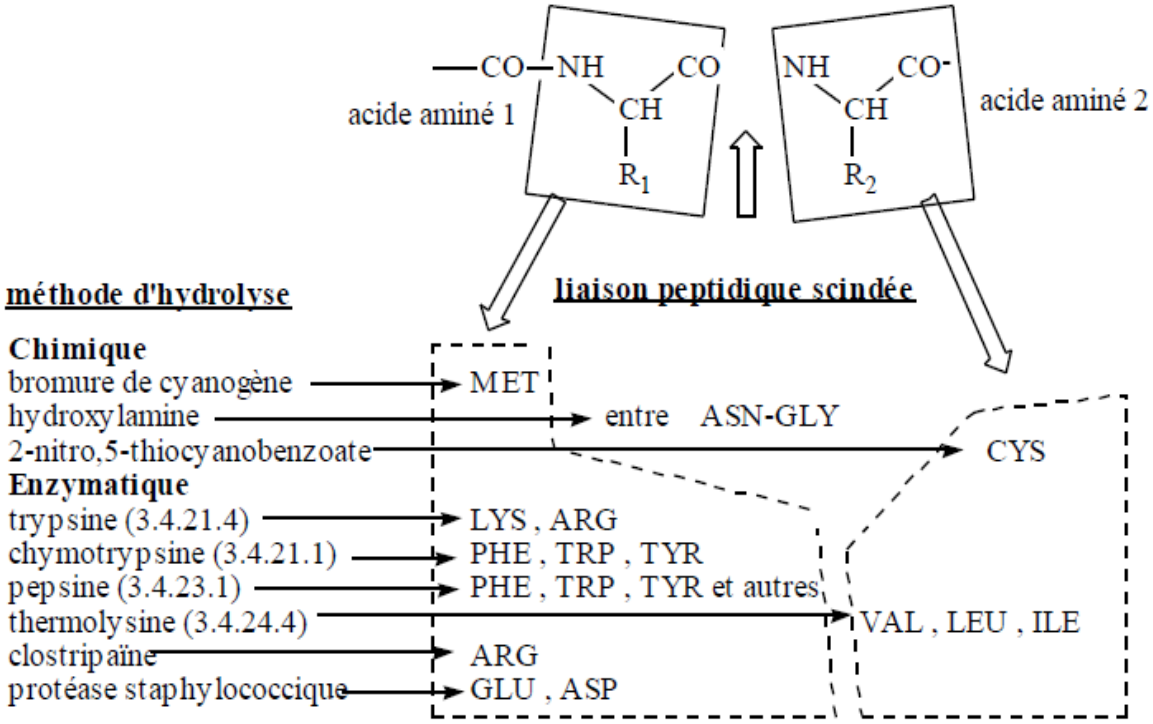


Fig. 139. Specific hydrolysis methods for peptide bonds.

Pepsin: gastric enzyme, specifically attacks peptide bonds which include the NH- of Phe, Tyr and Trp and to a lesser extent leu, Asp and Glu unless Pro is on the left.

Trypsin: pancreatic enzyme, specifically attacks peptide bonds that include the carboxyl of lysine or arginine unless Rn+1=Pro.

Chymotrypsin : also produced by the pancreas, attacks the bonds in which the carboxyl of an aromatic amino acid (tyrosine, tryptophan, phenylalanine) participates unless Rn+1=Pro.

Thermolysin: specific to hydrophosphorus amino acids (Ala, Val, Leu, Ile, Met) with a cleavage on the amino side of the peptide bond unless Rn-1=Pro.

Proteinase V8: specific to acidic amino acids with a cleavage on the carboxylic side after Asp, Glu unless Rn+1=Pro.

Clostripaine= specific to arginine with a cleavage on the carboxylic side.

Papain: Widely used in the food and chemical industries, it is particularly useful in butchery for tenderizing meat. It is also used in contact lens cleaning products and laundry detergents, where its action is to break down the proteins responsible for stains.

IV. 3. The proteins

Proteins (from the Greek prôtos, first) are polymers (macromolecules) composed of one or more chains of amino acids (polypeptide chains).

Depending on their composition, proteins are distinguished as follows: holoproteins, which are formed solely of polypeptide unit(s), and heteroproteins, to which a non-protein prosthetic group is attached. This group can consist of a carbohydrate chain, lipids, a metal ion, a nucleic acid, a coenzyme, heme, etc.

Depending on their shape, they are distinguished as follows: scleroproteins or fibrous proteins, elongated in shape, poorly soluble, very resistant, these are structural molecules; spheroproteins or globular proteins, compact in shape, soluble, fragile, these are molecules possessing an active biological function.

Proteins have very diverse functions:

- **the proteins of the structures**, which allow the cell to maintain its organization in space.
- **transport proteins**, which ensure the transfer of different molecules into and out of cells.
- **regulatory proteins**, which modulate the activity of other proteins (enzymes).
- **signaling proteins**, which capture external signals, and ensure their transmission within the cell or organism.
- **Motor proteins**.

IV. 3. 1.Three-dimensional structure of proteins

IV. 3. 1. 1. Primary structure of proteins

It corresponds to the linear sequence of acidsα-aminos linked together by a single peptide bond. This structure is in fact imposed by the genome and in turn conditions the secondary and tertiary structures of the protein. Furthermore, the functional role of the protein, which is linked to a characteristic sequence, depends on this structure; mutations in the genome can have more or less serious consequences: if the functional part of the protein is affected, a molecular disease will occur.

IV. 3. 1. 2. Secondary structure of proteins

Secondary structure characterizes the first degree of folding of the polypeptide chain. It describes the local folding of the main chain of a protein due to interactions between amino acid residues, via hydrogen bonds.

The existence of secondary structures arises from the fact that the energetically favorable folding of the peptide chain is limited, and only certain conformations are possible. The most common secondary structures are helices.αand the pagesβ.

IV. 3. 1. 2. 1. α**Helix**

This type of organization corresponds to a helical structure stabilized by intrachain hydrogen bonds, where the oxygen atom of the carbonyl group of each residue (n) interacts with the nitrogen atom of the amide group located four residues further along (n + 4) in the same sequence. The radicals of the residues are outside the helix, which minimizes steric bulk (Fig. 140).

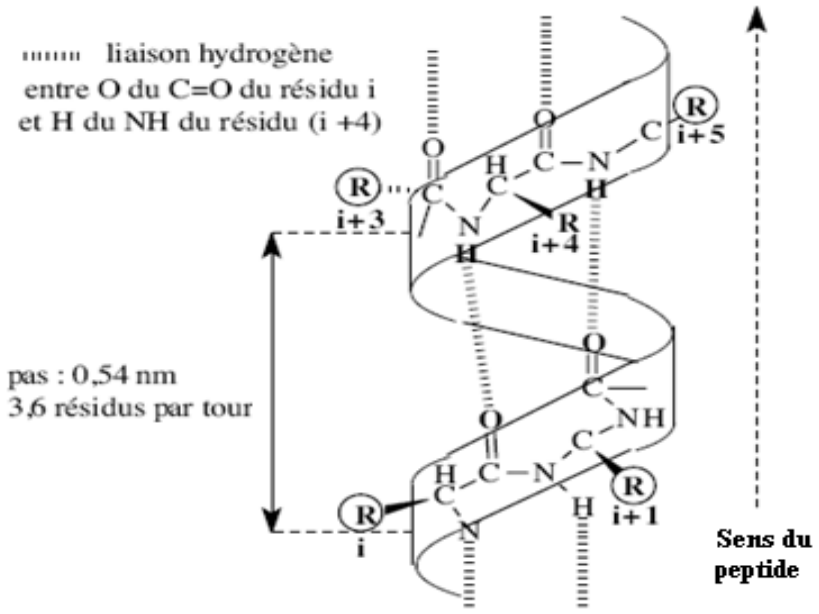


Fig. 140. Structure of a **right** αhelix.

One turn of the helix comprises 3.6 amino acid residues, spaced 0.15 nm apart from each other relative to the helix axis. The helix pitchαis therefore 0.54 nm (0.15 ×3.6). The propellers comprise a minimum of 5 residues and a maximum of 40 residues. The propellersαThey can be right-handed or left-handed, but steric constraints favor the right-handed helix.

This structure was predicted and named alpha helix by Linus Pauling and his collaborators in 1951 based on theoretical considerations, before being confirmed a few years later by structural biology.

Certain amino acids influence the stability of the helical structure. Leucine, tryptophan, and phenylalanine stabilize the helix through hydrophobic interactions. Conversely, valine, isoleucine, tyrosine, and neighboring diacid and dibasic residues destabilize it. The presence of proline in a polypeptide chain often leads to the disruption of the alpha helix structure.

IV. 3. 1. 2.2. β Sheet

The hydrogen bonds between the oxygen atoms (CO) and hydrogen atoms (NH_2) in a peptide bond are repeated not along a continuous portion of the peptide chain, but between different segments that may belong to the same chain or to different chains (Fig. 141).

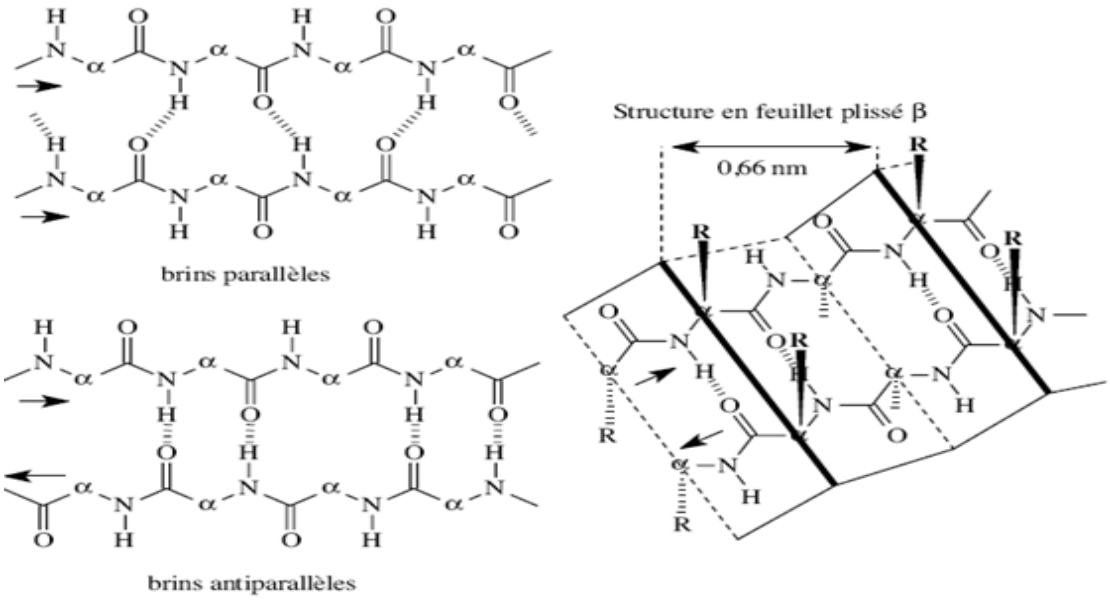


Fig. 141. Pleated β sheet structure.

This zigzag structure is a flat, stretched, and spread-out structure: a βsheet pleated. Often the destruction of hydrogen bonds in a αhelix by heating in an aqueous environment leads to the formation of sheets. The peptide orientation of the two adjacent strands can be identical or opposite; the sheets are then described as parallel or antiparallel, respectively. Antiparallel sheets are more stable, as the hydrogen bonds undergo less distortion. The side chains of the residues are distributed alternately on either side of the mid-plane.

This structure is found, for example, in fibrous proteins or scleroproteins. These are long, filament-like protein molecules found in tissues and structural elements such as muscles, bones, skin, and cell membranes. They perform structural functions and are composed of units with simple, repetitive secondary structures (alpha helix and beta sheet). Collagen and keratin are fibrous proteins with an alpha helix conformation, while silk fibroin has a beta-pleated sheet conformation.

IV. 3. 1. 3. Tertiary structure of proteins

The tertiary structure of a protein (Fig. 142) corresponds to the conformation adopted by secondary structure elements in space (three-dimensional or 3D structure). It depends not only on the amino acid sequence, but also on environmental conditions.

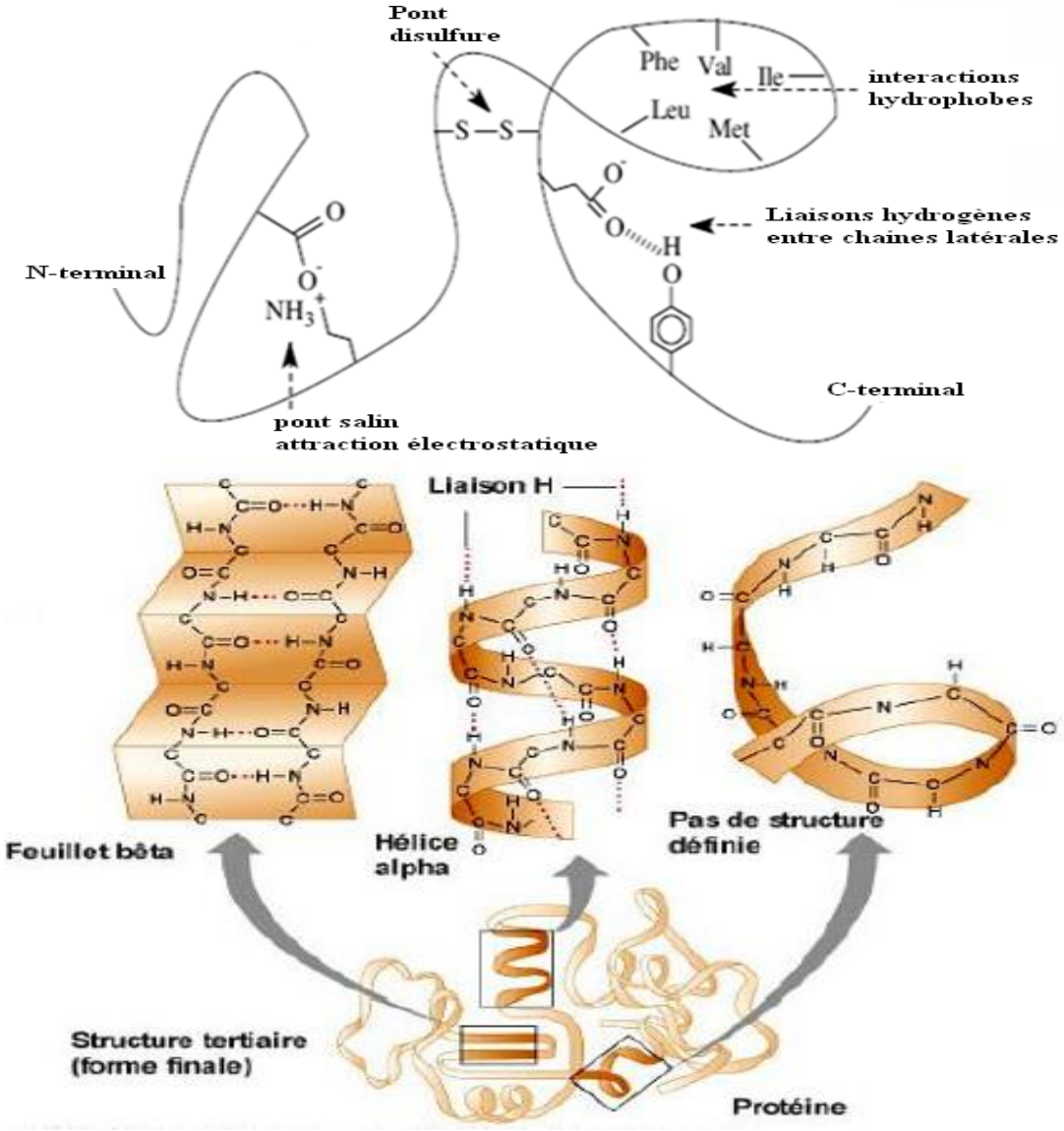


Fig. 142. The bonds or interactions between side chains of residues, involved in the tertiary structure of proteins.

This structure is maintained by interactions which can be of different natures: covalent interactions (disulfide bridges between cysteines); electrostatic interactions (ionic bonds, hydrogen bonds); Van Der Waals interactions; interactions with the solvent: in water, polar side chains may be exposed to the solvent, while nonpolar side chains will tend to "bury themselves" in hydrophobic pockets of the protein. The protein then appears in the form of a "coil".

IV. 3. 1. 4. Quaternary structure of proteins

The quaternary structure corresponds to the non-covalent association of several **subunits** identical or different proteins or monomers (Fig. 143). The protein structure resulting from this association is called an oligomer.

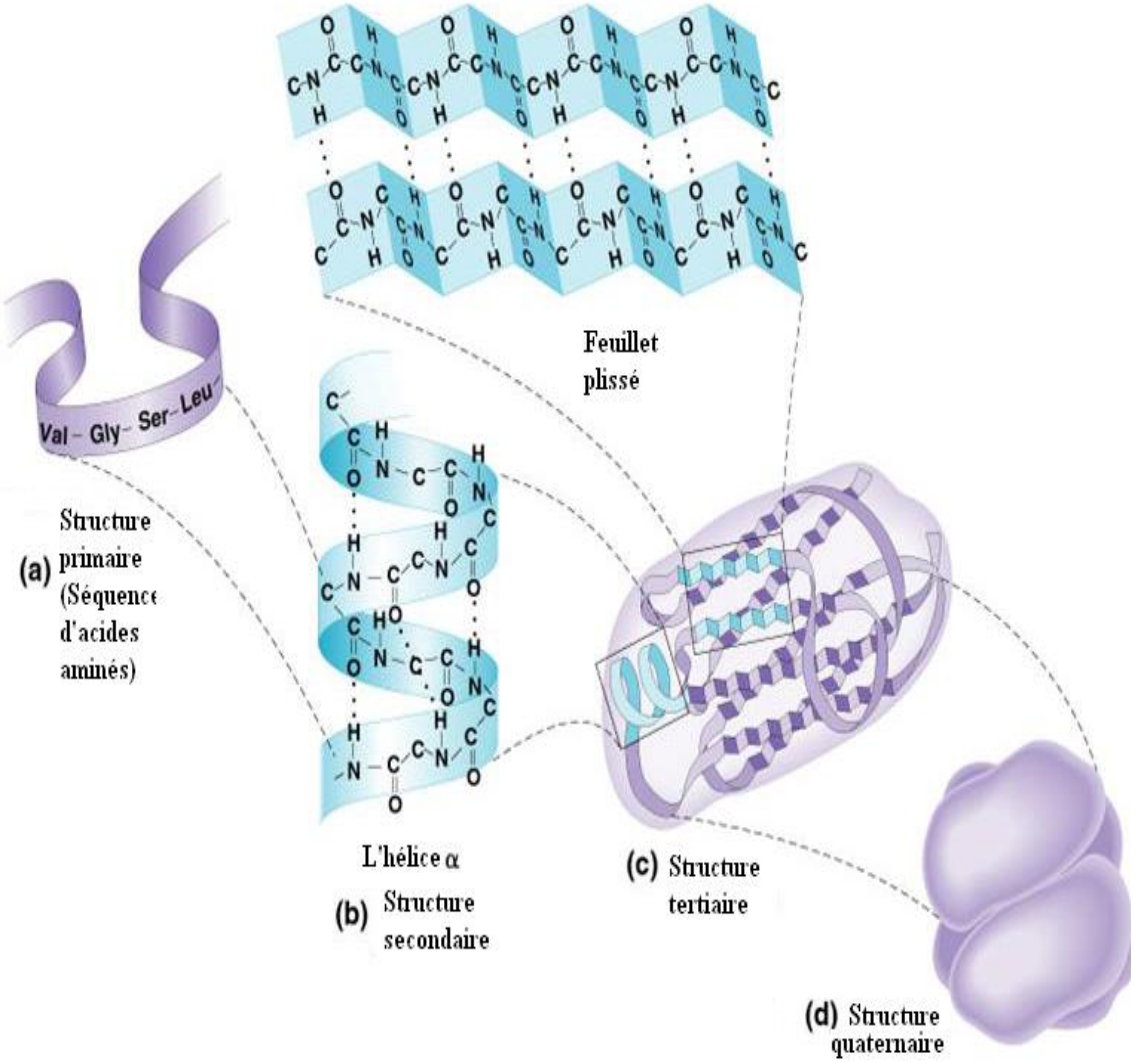


Fig. 143. The four levels of protein structure.

Oligomers consisting of two, three, four, or more subunits are called dimers, trimers, tetramers, etc. When the subunits of an oligomer are identical, the prefix homo- is used (e.g., homodimer). The prefix hetero- is used when the subunits of an oligomer are different (e.g., heterotetramer).

The association between subunits depends both on the complementarity of shape and especially on the weak interactions between the subunits: electrostatic bonds, hydrogen bonds, Van der Waals bonds, hydrophobic interactions.

IV. 3. 2. Protein denaturation

The maintenance of the secondary, tertiary, and quaternary structures of proteins is responsible for their biological activity; this is ensured by weak bonds. If these bonds are broken, the protein will lose its activity and certain properties: it is denatured.

Denaturation can be caused by a variety of physical or chemical agents: heat (the coagulation of ovalbumin in egg white is an example of denaturation), ultraviolet and ionizing radiation, pH changes, detergents, urea solutions, and organic solvents. Denaturation is sometimes irreversible, and sometimes reversible after removal of the denaturing agent.

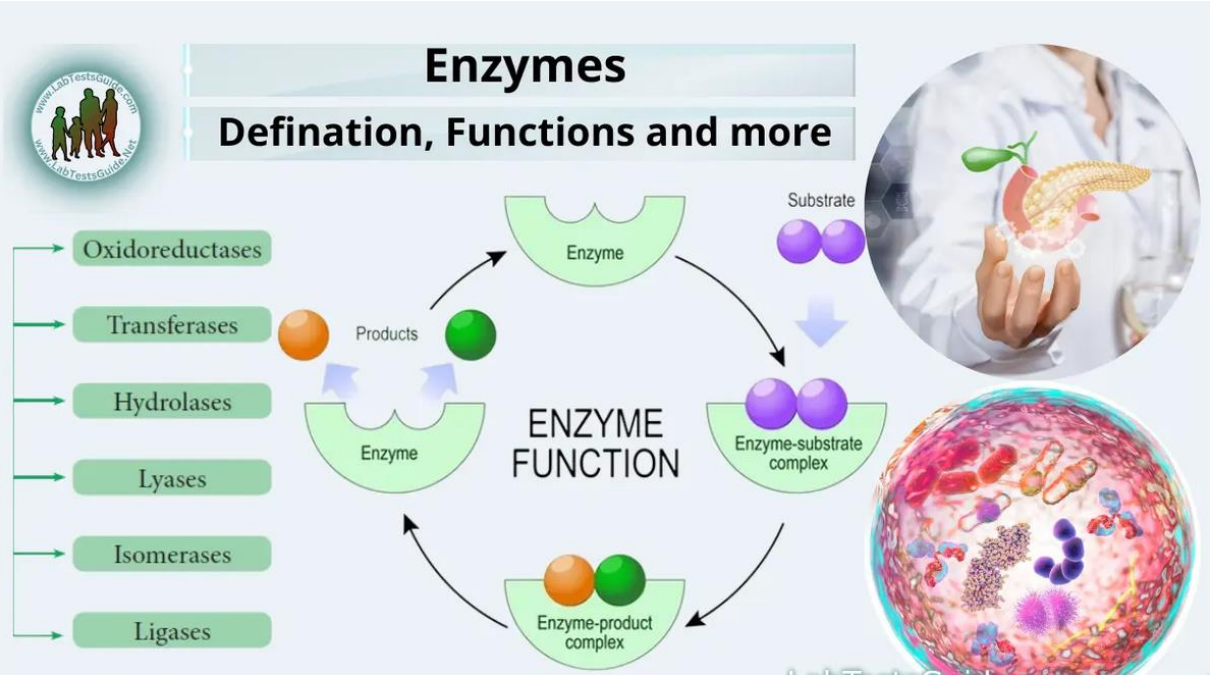
IV. 3. 3. Protein isolation, fractionation and purification

Obtaining a pure protein from a cell homogenate is generally a long and delicate task, as it often involves several hundred proteins with fairly similar chemical properties. Care must be taken not to denature the protein one wishes to isolate.

Several methods are frequently used, some of which include: dialysis to remove small molecules, precipitation by neutral salts (or salting out), precipitation at the isoelectric point, precipitation by organic solvents at low temperature, dextran gel filtration chromatography, electrophoresis, ultracentrifugation and crystallization.

CHAPTER V :

ENZYMES



CHAPTER V ENZYMES

V.1. Definition

An enzyme (sometimes used in the masculine form, the French Academy of Sciences suggests using the feminine form) is a protein specialized in catalyzing the chemical reactions of metabolism occurring within the cellular or extracellular environment. Enzymes act at low concentrations and are found intact at the end of the reaction.

V. 2. Nature and structure of enzymes

With the exception of ribozymes, which are RNAs with a catalytic function, enzymes are always proteins and therefore possess all of their physicochemical properties. There are two main categories of enzymes:

V. 2. 1. Purely protein enzymes (called apoenzyme): these are enzymes made up solely of amino acids.

V. 2. 2. Holoenzymes : Very often, enzymes consist of a protein (the apoenzyme) associated with a small chemical species of a different nature, a cofactor. The cofactor is thermostable, while the protein is thermolabile.

Cofactors are highly diverse. They can be metallic ions (Ca^{++}, Mg^{++}, Fe^{++}, Zn^{++}: the active site of alkaline phosphatase and carboxypeptidase), or coenzymes, organic molecules most often derived from vitamins (as in reactions catalyzed by dehydrogenases, which use proton and electron carriers as coenzymes). They possess a flavin ring, like FAD and FMN, or a nicotinamide ring, like NAD^+ and $NADP^+$. When the cofactor is tightly bound to the protein, it is called a prosthetic group. This is the case, for example, with iron-tetrapyrrole rings, integral parts of cytochromes. Conversely, nicotinic coenzymes (mobile coenzymes), NAD^+ and $NADP^+$, alternately switch between their oxidized and reduced states by moving from one enzyme to another.

V. 2. 3. Substrate: The substrate is the molecule bound to the active site and on which the enzyme will act. The interactions between enzymes and substrate involve non-covalent bonds and are spontaneous reactions that require no energy.

V. 2. 4. Ligands: Enzymes can interact with other molecules, which can be proteins or non-proteins; these molecules are called ligands. A protein denatured by heat is unable to bind its ligand.

V. 3. Concepts of Specificity

Enzymes catalyze biological reactions in which a substrate is transformed into a product. The overall reaction consists of two elementary reactions: the substrate first forms a complex with the enzyme, then this complex decomposes into product and enzyme (Fig. 144).

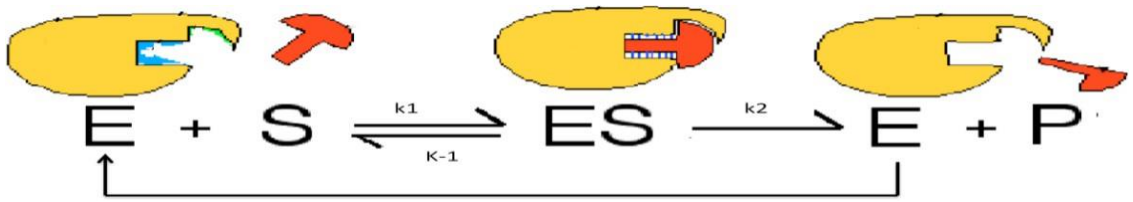


Fig. 144. The reaction of transformation of a substrate into a product catalyzed by an enzyme.
With k = velocity constant.
k1 = rate constant of formation of the ES complex
k-1 = dissociation rate constant of the ES complex
k2 = P formation rate constant "Turnover" number of catalytic cycles per second.

This dual action of substrate fixation and catalysis allows us to introduce the concept of reaction specificity and substrate specificity.

III. 3.1. Substrate specificity: Substrate specificity refers to the fact that an enzyme binds to and transforms a given substrate and not another. Substrate specificity varies; some enzymes have absolute specificity, transforming a single substrate into a single product (e.g., glucokinase, which only phosphorylates glucose).

Others have a broader specificity, transforming a class of substrate into as many products (Hexokinase: phosphorylates various hexoses, including glucose).

V. 3. 2. Reaction Specificity: Reaction specificity refers to the fact that an enzyme catalyzes a given chemical reaction and not another. An enzyme can thus open a metabolic pathway by directing the metabolism of a substrate towards a cascade of reactions, at the expense of other metabolic possibilities for that same substrate. Enzyme activity and specificity depend on the spatial conformation of the protein.

Kinases: They only catalyze phosphorylation reactions in the presence of ATP.

Decarboxylases: catalyze the decarboxylation of molecules containing a carboxyl group.

V. 3. 3. Regulation of catalytic activity

The activity of an enzyme is controlled by modulators (activators, inhibitors); this which allows the overall speed of metabolism to be adjusted to cellular needs:

Example: Phosphofructokinase is activated by AMP and inhibited by ATP. This modulation activates or inhibits glycolysis depending on whether the energy load is low or high.

V. 4. The active site

The function of enzymes is linked to the presence in their structure of a specific site called the active site. This is a privileged area, shaped like a cavity, located in the hydrophobic region of the protein, where the enzyme's catalytic power is selectively exerted. It is composed of a binding site and a catalytic site (Fig. 145):

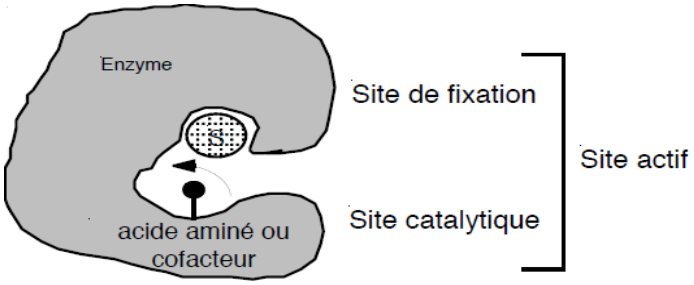


Fig. 145. Schematic representation of an enzyme.

- the binding site recognizes the shape complementarity with a specific substrate of the enzyme and determines the affinity and specificity of the reaction;

- The catalytic site allows the transformation of the substrate into the product and determines the rate of the reaction.

These two domains are made up of amino acids that are far apart in the primary structure but close together in the 3D structure. Denaturation of the enzyme leads to its inactivation.

It comprises 3 types of amino acids:

a- Contributing (collaborating) amino acids: They allow the enzyme protein to adopt a spatial conformation so that the ligand can fit into the protein.

b- Auxiliary amino acids: They ensure mobility in areas located near the active center

c- Contact amino acids: Site of the enzymatic reaction involving particular groups of these amino acids, which interact with one or more particular groups of the substrate.

V. 5. Nomenclature and classification

The name of the enzyme is formed from two parts: the first is the name of the substrate(s) and ends with the suffix **"ase"**, thus indicating both the substrate and the catalyzed reaction:

– *glucose-6 phosphatase:* the enzyme hydrolyzes the ester-phosphate bond at C_6 glucose;

– *glucokinase:* The enzyme transfers a phosphate group from ATP to glucose.

Since 1961, the International Union of Biochemistry has codified the nomenclature and classification of enzymes under a so-called official nomenclature. Enzymes are divided into six classes according to the type of reaction they catalyze, themselves subdivided into sub-

Classes that allow for a better definition of the function of each enzyme. All currently known enzymes are listed under a number consisting of four digits (Fig. 146, Table 21) separated by periods and preceded by EC (Enzyme Commission number): (EC: x1, x2, x3, x4). The meaning of the numbers is as follows:

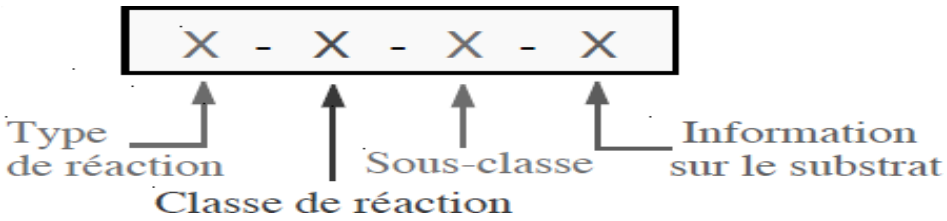


Fig. 146. Enzyme nomenclature

X_1: The first number, which can range from 1 to 6, indicates the type of reactions (6 classes) each containing 4 to 13 subclasses).

Types of catalyzed reactions

1 Oxidoreductases (catalyze redox reactions: transfer of electrons, hydrogen atoms or fixation of oxygen)

2 : Transferases (transfer of atoms or groups of atoms, a phosphate for example)

3 Hydrolases (Breaking of connections with consumption of H_2O)

4: Lyases (Breaking of bonds by modes other than hydrolysis, without consumption of H2O).

5: Isomerases (catalyze isomerization reactions in a molecule, a reaction that preserves the compound's molecular formula)

6: Ligases (catalyze the formation of covalent bonds between two molecules: CC, CO, CN, CS, CP, N-metal). These are synthetases that use the energy of XTPs.

X_2 :The second part defines the subclass (its mechanism of action). In the case of oxidoreductasesWe distinguish between dehydrogenases, monooxygenases and dioxygenases.

X_3 :The third number designates a sub-subclass: the nature of the molecule that serves as the acceptor when it comes to the transfer of electrons.

X_4 :The 4th number is an order number within the group and subgroup (enzyme characteristic).

Table 21: Examples of enzyme classification.

Lactate déshydrogénase E.C 1.2.1.22			
1. Oxydoréductase	**2.** Agit sur la fonction aldéhyde ou oxo du donneur d'électrons	**1.** L'accepteur d'électrons est NAD+ ou NADP+	**22.** Le substrat est le lactate
Hexokinase E.C 2.7.1.1			
2. Transférase	**7.** Le groupe transféré est un phosphate	**1.** L'accepteur est une fonction alcool	**1.** Le substrat est un hexose
Trypsine E.C 3.4.21.4			
3. Hydrolase	**4.** Agit sur des liaisons peptidiques	**21.** Serine endopeptidase	**4.** Clivage préférentiel : Arg, Lys

V. 6. Units of measurement of the catalytic activity of enzymes

V. 6. 1. International Unit of Activity (UI): One unit is the amount of enzyme transforming one µmol of substrate per minute under optimal assay conditions (IU = µ mol/min).

V. 6. 2. Katal: In medical practice, enzymatic activity is expressed in moles of substrate transformed per second. The usual unit is the nanokatal (one nanomole of substrate transformed per second (1 IU = 16.6 nanokatal)).

V. 6. 3. Specific Activity (SA): The number of substrate molecules transformed per minute and per milligram of enzyme. It measures the degree of purity of an enzyme preparation.

$$\frac{\mu \text{ mol/min}}{\text{mg de protéine}} = \frac{\text{UI}}{\text{mg de protéine}}$$

V. 6. 4. Specific Molecular Activity (SMA): The number of substrate molecules transformed per minute per enzyme molecule. This is a characteristic of the enzyme and can only be calculated if the enzyme preparation is pure.

$$\frac{\mu \text{ mol/min}}{\mu \text{ mol de protéine}} = \frac{\text{UI}}{\mu \text{ mol de protéine}}$$

V. 6. Enzyme kinetics

The purpose of enzyme kinetics is to identify and describe the mechanisms of biochemical reactions, catalyzed by enzymes, by studying their rate, that is to say their evolution over time.

V. 6. 1. Concept of "initial velocity"

As soon as an enzyme comes into contact with the substrate, product formation is observed over time (Fig. 147). In practice, the curve is considered to have a straight section, and the increase in product concentration d[P] is measured over a time interval dt. The results are considered acceptable as long as the product concentration remains below 10% of the initial substrate concentration. Strictly speaking, the tangent of this curve should be measured at the origin.

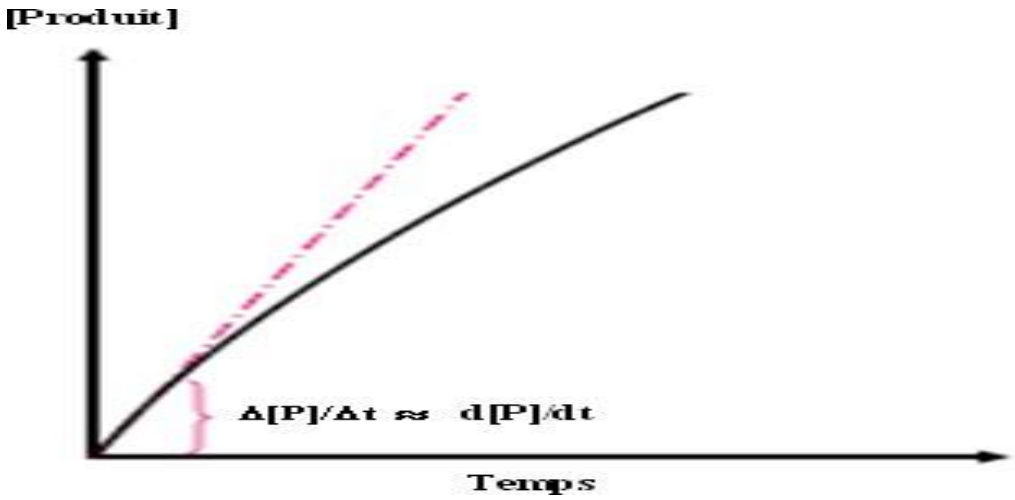


Fig. 147. Determining the initial rate of a reaction.

V. 6. 2. Influence of enzyme concentration on initial velocity

The measurement of the initial velocity in the presence of increasing concentrations of enzyme (a to d, Fig. 148A), for a constant substrate concentration, shows that this velocity is proportional to the enzyme concentration (Fig. 148B):

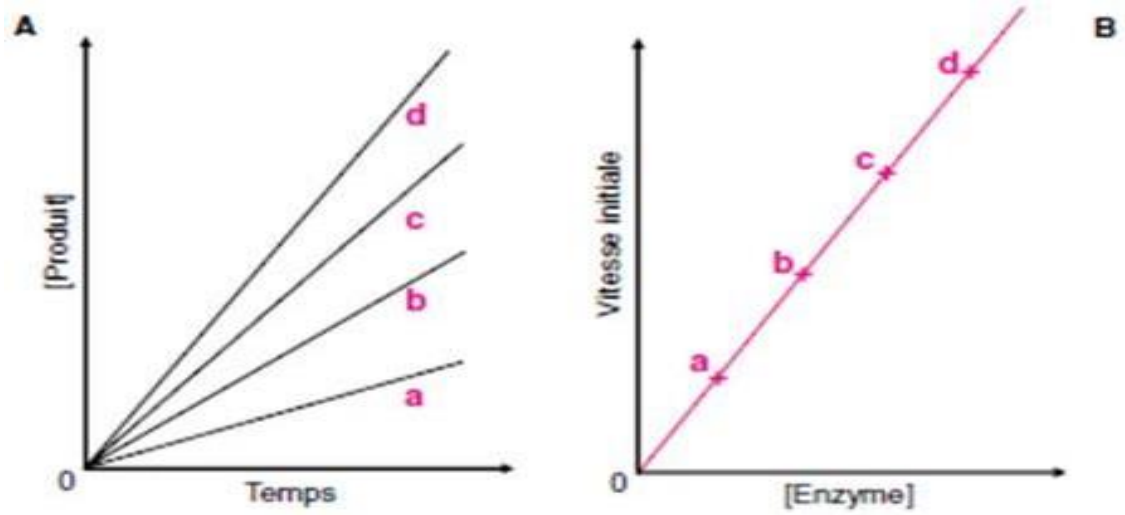


Fig. 148. Influence of enzyme concentration on initial velocity.

V. 6. 3. Influence of substrate concentration on initial velocity

The measurement of the initial velocity in the presence of a constant concentration of enzyme, but as a function of increasing concentrations of substrate (a to f, Fig. 149 C), shows that this velocity increases as a function of [S] (Fig. 149 D), but this velocity does not increase indefinitely; a limit is reached, the maximum velocity. Vmax: the maximum velocity is reached when all the enzyme molecules are in the ES form.

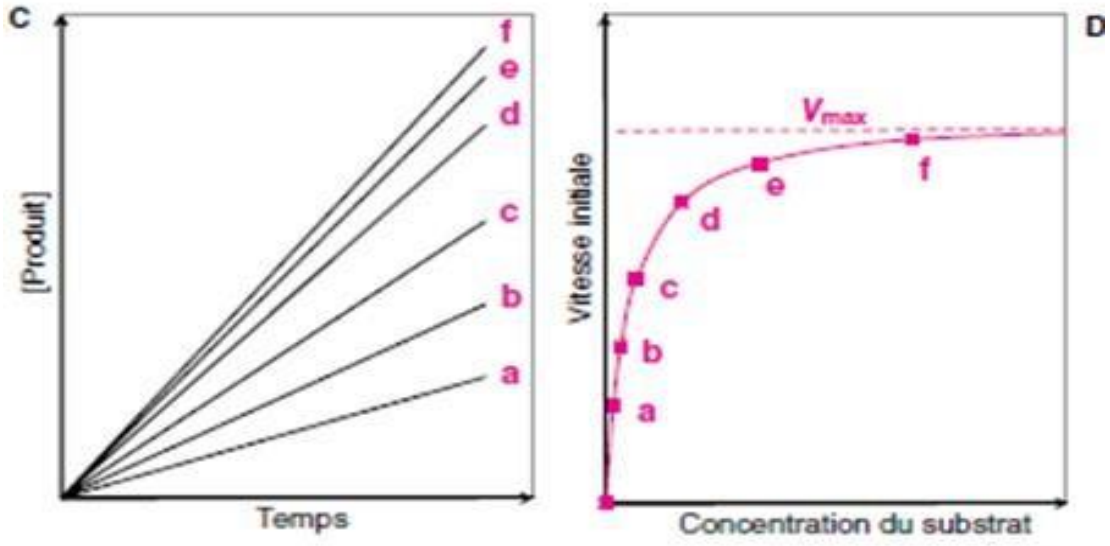


Fig. 149. Influence of substrate concentration on initial velocity.

V. 6. 4. Michaelis-Menten Model

The Michaelis-Menten equation describes the kinetics of a reaction catalyzed by an enzyme acting on a single substrate to give a product. It relates the reaction rate to the substrate concentration and constant parameters characteristic of the enzyme.

V. 6. 4. 1. Michaelis-Menten Equation

The enzymatic transformation of the substrate into the product requires the prior formation of the enzyme-substrate complex, ES, followed by its conversion into enzyme and product:

$$E + S \underset{K_2}{\overset{K_1}{\leftrightarrow}} ES \xrightarrow{K_3} E + P \quad (1)$$

In this diagram:

• K_1: rate constant of formation of the ES complex, dimension $mol.L^{-1}.s^{-1}$; the rate ofThe formation of the complex [ES] is: **V1 = K1 [E][S]**

• K_2: dissociation rate constant of the ES complex, dimension s^{-1}; the ratethe elimination of the [ES] complex is: **V = (K2 + K3) [ES]**

- K_{cat}: catalytic constant, rate constant that encompasses the formation of the EP complex and its dissociation into E + P; dimension s^{-1}.
- The product concentration is zero, since we are measuring the initial rate; therefore, we ignore any formation of EP complex from E + P. The reaction proceeds only towards product formation, hence the expression for the reaction rate: $V_0 = k_{cat}[[ES]$

Enzyme kinetics experiments provide relationships between the rate of reaction V, that is, the amount of substrate S that has disappeared or the amount of product P that has formed per unit of time during a reaction: $\boldsymbol{V = -\,d[S]/dt = d[P]/dt}$

The rate at which the products appear is: $\boldsymbol{dP/dt = V3 = k3.\,[ES]}$

The rate of substrate disappearance is: $\boldsymbol{-\,dS/dt = V1 - V2}$

During the stationary phase, the concentration of the enzyme-substrate complex [ES] is constant. Therefore, the rate of formation of this complex [ES] must be equal to the rate of dissociation: $V_1 - V_2 = V_3$ **so K1 [E][S] = (K2 + K3)[ES]**

The ratio of constants K_1K_2 **and** K_3 is also a constant, the latter being defined as the Michaelis constant (K_m). $\mathbf{K2 + K3 / K1 = [E].\,[S]\,/[ES] = Km}$

By calling $[E_T]$ the total enzyme concentration, the free enzyme concentration is equal has: $\mathbf{[E] = [ET] - [ES]}$ And $\mathbf{Km} = \dfrac{[E][S]}{[ES]} = \dfrac{([ET]-[ES])[S]}{[ES]}$

$$\mathbf{Km} + [S] = \frac{[ET][S]}{[[ES]} \Rightarrow [ES] = \frac{[ET][S]}{\mathbf{Km} + [S]}$$

V = rate of the enzymatic reaction = V_3

$$\boldsymbol{V = V3 = k3[[ES]]} \quad \text{So} \quad \mathbf{V} = \frac{\mathbf{k3}.[ET]\,.[S]}{\mathbf{Km} + [S]}$$

The rate of the reaction depends on the concentration: of total enzyme, of substrate and the Michaelis constant.

V. 6. 4. 2. Maximum speed V_{max}

At the saturating substrate concentration, V0 = Vmax; moreover, the entire enzyme is complexed and therefore [ES] = [ET] if we denote by [ET] the concentration of the total enzyme; we can write:

$\mathbf{Vmax = Kcat\,[ET]}$ and consequently: $\mathbf{V_i} = \dfrac{\mathbf{V_{max}}.[S]}{\mathbf{K_m} + [S]}$ This is the Michaelis equation and Menten with:

Vi: initial speed of the enzymatic reaction for a substrate concentration (in µMol/min);

V_{max}: Maximum speed measured for a saturating substrate concentration (in µM/min);

[S]: Substrate concentration (in mol/L);

K_m: Michaelis constant specific to the enzyme.

The curve that translates the variations of V as a function of [S] according to this equation is a branch of a hyperbola (Fig. 150).

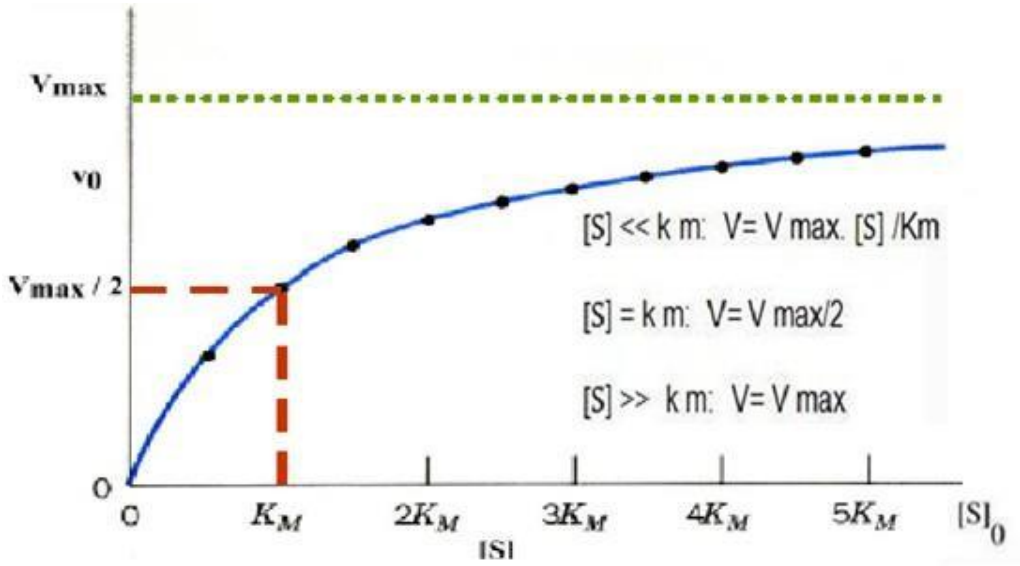


Fig. 150. Michaelis plot showing velocity as a function of substrate concentration.

V. 6. 4. 3. Physical meaning of the Michaelis constant KM

The Michaelis-Menten equation relates V0 to [S] and to the rate constants of the individual steps. KM, which is a characteristic constant of the enzyme known as the Michaelis constant, is defined by the ratio (k–1 + kcat)/k1. It corresponds to the substrate concentration at which the rate reaches half the maximum rate (V = Vmax/2). This is the substrate concentration that leads to a 50% saturation of the enzyme. It has the dimensions of a concentration(mole/liter).

KThe Km value represents the enzyme's affinity for its substrate. Affinity indicates how easily a substrate binds to an enzyme. Thus, the higher the Km value, the more the ES complex tends to dissociate, and therefore the lower the enzyme's affinity for the substrate. Km is therefore inversely proportional to the enzyme's affinity for the substrate (Table 22).

Table 22: K values$_m$ of certain enzymes.

ENZYME	SUBSTRATE	CONCENTRATION (µM)	K_M (µM)
Glucose phosphate isomerase	Glucose-6-phosphate	450	700
Aldolase	Fructose 1, 6-diphosphate	32	100
Trio phosphate isomerase	Glyceraldehyde-3-phosphate	3	460
Glyceraldehyde-3-phosphate dehydrogenase	Glyceraldehyde-3-phosphate	3	70
Phosphoglycerate kinase	3-phosphoglycerate	60	1200

V. 6. 4. 4. Physical meaning of the catalytic constant k_{cat}

The catalytic rate constant k_{Kcat} is the number of substrate molecules converted into product per enzyme molecule per unit of time, usually one second, under given experimental conditions, when the enzyme is fully saturated with the substrate. For this reason, Kcat is also called turnover.

The catalytic constant kcat is therefore a direct measure of an enzyme's catalytic activity: the larger kcat, the faster the catalytic events within the enzyme-substrate complex. The catalytic constant kcat is expressed in S–1, and its inverse has the dimensions of time: it is the time required for one enzyme molecule to transform one substrate molecule. The catalytic constant of most enzymes is between 10^2 and 10^4 S^{-1}.

Criterion Kcat/Km measures the specificity and efficiency of enzymes. The two constants K_M and Kcat therefore reflect the catalytic properties of an enzyme.

V. 6. 4. 5. Lineweaver and Burk Representation

There are several ways to rearrange the Michaelis-Menten equation that can transform it into a linear equation (Fig. 151). The best known is the double inverse representation according to Lineweaver-Burk. By taking the inverse of both sides

From the Michaelis-Menten equation, we obtain:

$$\frac{\mathbf{1}}{\boldsymbol{V}} = \frac{\boldsymbol{Km} + [\boldsymbol{S}]}{\boldsymbol{Vm} + [\boldsymbol{S}]} = \frac{\boldsymbol{Km}}{\boldsymbol{Vm}\,[\boldsymbol{S}]} + \frac{\mathbf{1}}{\boldsymbol{Vm}}$$

Which is of the type y = ax + b (equation of a line) in which:

1. $y = \frac{1}{V}$, 2. $a = \frac{K_m}{V_{max}}$, 3. $x = \frac{1}{[S]}$ And 4. $b = \frac{1}{V_{max}}$

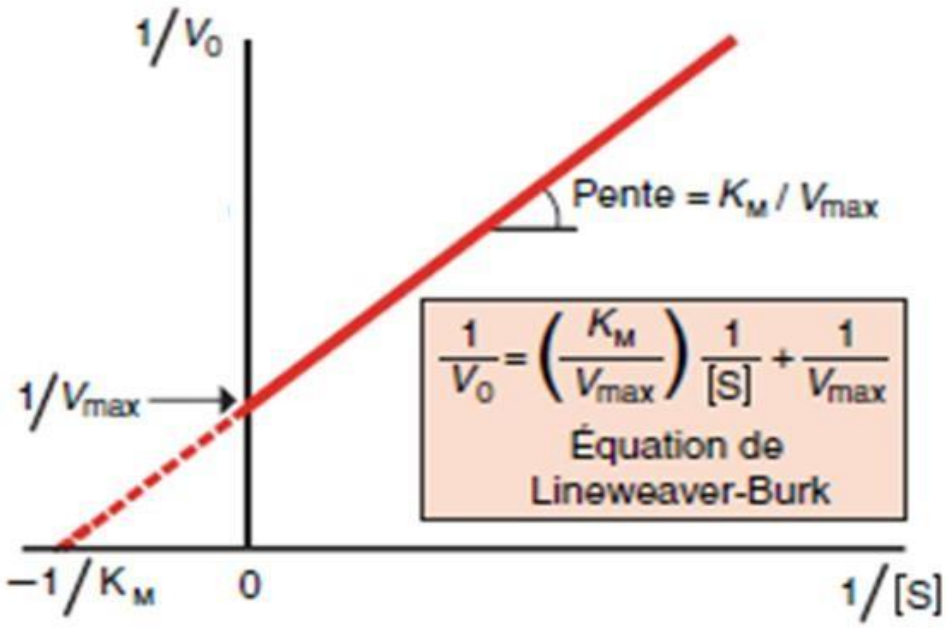


Fig. 151. Lineweaver-Burk representation.

In this form, we have a linear equation of the type y=ax+b, where the slope a=K**m/Vmax.**The intersection of the line representing the graph with the x-coordinate determines – 1/KM and its intersection with the ordinate 1/Vmax (ordinate at the origin).

V. 7. Effectors of enzymatic activity

V. 7. 1. Effects of physical agents

V. 7. 1. 1. Effects of temperature

Enzymatic activity is highest at the optimal temperature. This optimal temperature varies from one enzyme to another. Under optimal conditions, the temperature should generally be around 37 to 38 °C. These values are indicative of enzymes from warm-blooded animals, which is almost body temperature.

Conversely, if the temperature exceeds 60°C, the enzyme is denatured, the active site is altered, and the reaction cannot occur. If the temperature is below 5°C, the enzyme is inactivated because the molecular agitation caused by heat is limited: substrate molecules and enzymes have difficulty interacting. There are thermostable enzymes, such as DNA polymerase used in PCR. The effects on the enzymatic reaction result in two phases (Fig. 152):

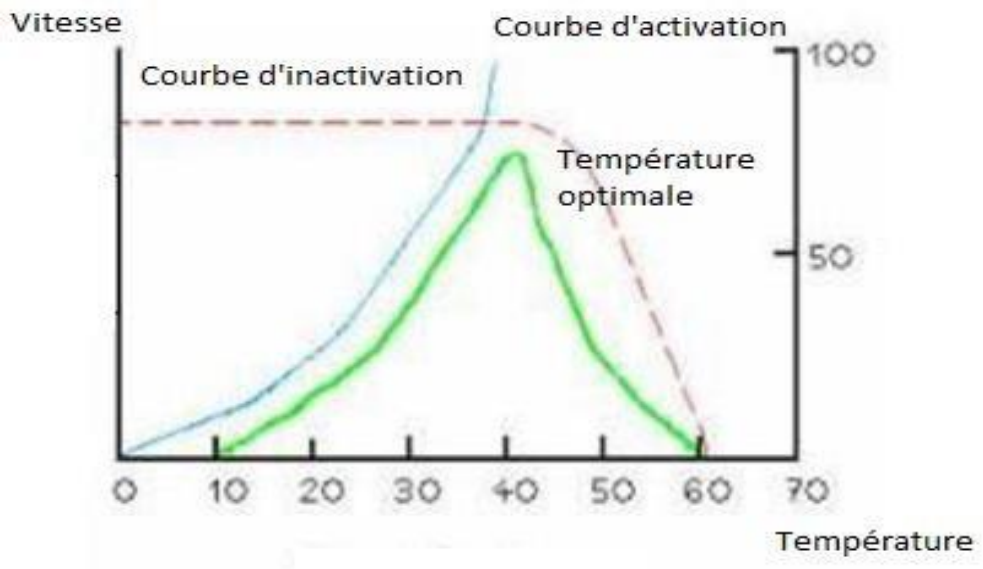


Fig. 152. Influence of temperature on the rate of an enzymatic reaction

V. 7. 1. 2. Effects of pH

Enzyme-substrate recognition and the resulting catalytic activity are highly pH-dependent. An enzyme possesses a large number of ionizable side chains and sometimes prosthetic groups intimately involved in its active site. Furthermore, the substrate often has ionized groups, and one or the other of the ionized forms may preferentially interact with the enzyme. This is why enzymes are generally only active within a limited pH range, and most have optimal catalytic activity at a specific pH (Fig. 153).

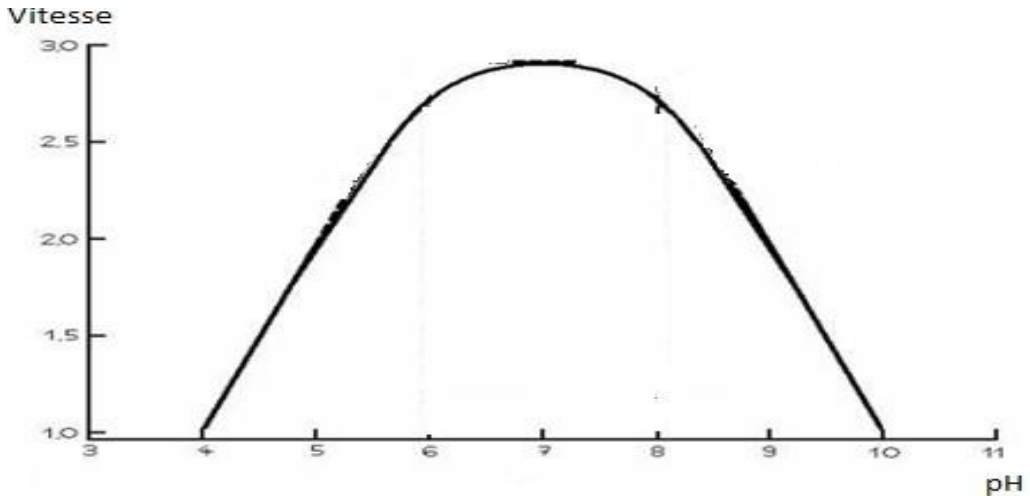


Fig. 153. Influence of pH on the rate of an enzymatic reaction

V. 7. 2. Enzyme inhibition

An enzyme inhibitor is a substance that binds to an enzyme and reduces its activity. An inhibitor can prevent the substrate from binding to the active site by binding to its

place, or cause a deformation of the enzyme which renders it inactive (allosteric inhibitor).

Enzyme inhibition plays a crucial role in controlling biological mechanisms, particularly in regulating metabolic pathways. Since inhibiting an enzyme can kill a pathogen or correct a metabolic imbalance, applications exist in numerous other fields: many drugs, pesticides, and insecticides are enzyme inhibitors. In enzymology, inhibitors are widely used to determine the mechanism of action of an enzyme.

Not all molecules that bind to an enzyme are inhibitors; enzyme activators also exist and increase the enzyme's activity. Inhibitions can be either reversible or irreversible.

V. 7. 2. 1. Competitive inhibitors

A competitive inhibitor generally has a structural similarity to the substrate, and both compete to bind to the same enzymatic site (isosteric effect). Binding of the competitive inhibitor precludes subsequent binding of the substrate, and vice versa. The EI complex formed by the association of the enzyme and the inhibitor is inactive (Fig. 154):

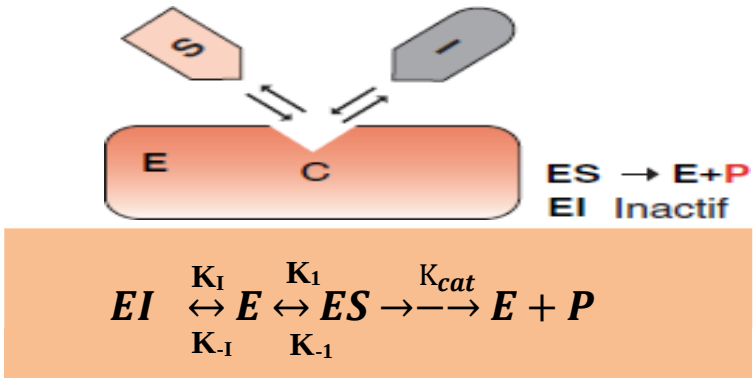


Fig. 154. Scheme and Michaelis-Menten equation for competitive inhibition.

Reversible binding of the inhibitor to the enzyme is characterized by the constant of K dissociation$_I$: $K_I = \frac{[\![E]\!][\![I]\!]}{[\![EI]\!]} = \frac{K_{-I}}{K_I}$

And the expression of the initial reaction rate as a function of substrate and inhibitor concentrations: $\frac{1}{V} = \frac{1}{Vm} + \frac{Km}{Vm}\left(1 + \frac{[I]}{KI}\right)\frac{1}{S}$

Compared to the classical Michaelis-Menten equation, the maximum reaction rate Vmax remains unchanged, but the enzyme's affinity for the substrate decreases (Km increases) because the latter cannot bind to the enzyme-inhibitor complex.

In the case of competitive inhibition, it is possible to reverse the inhibition by saturating the enzyme with substrate. The affinity of an inhibitor for an enzyme is given by the inhibition constant KI, which represents the inhibitor concentration at which half of the enzyme binding sites are occupied. Thus, the lower the KI, the greater the affinity of an inhibitor. This inhibition constant, expressed in moles per liter, also corresponds to the dissociation constant of the enzyme-inhibitor complex.

The curve v = f [S] is always an equilateral hyperbola admitting V = Vmax as a horizontal asymptote (Fig. 155). An initial velocity equal to half the maximum velocity is measured for a substrate concentration equal to a higher apparent Kmapp value.

that $K_m$measured in the absence of an inhibitor: $K_{mapp} = K_m\,(1 + \frac{[I]}{K_I})$

The equation for the Lineweaver-Burk double inverse representation is:

$$\frac{1}{V} = \frac{1}{V_m} + \frac{K_m}{V_m}\left(1 + \frac{[I]}{K_I}\right)\frac{1}{S}$$

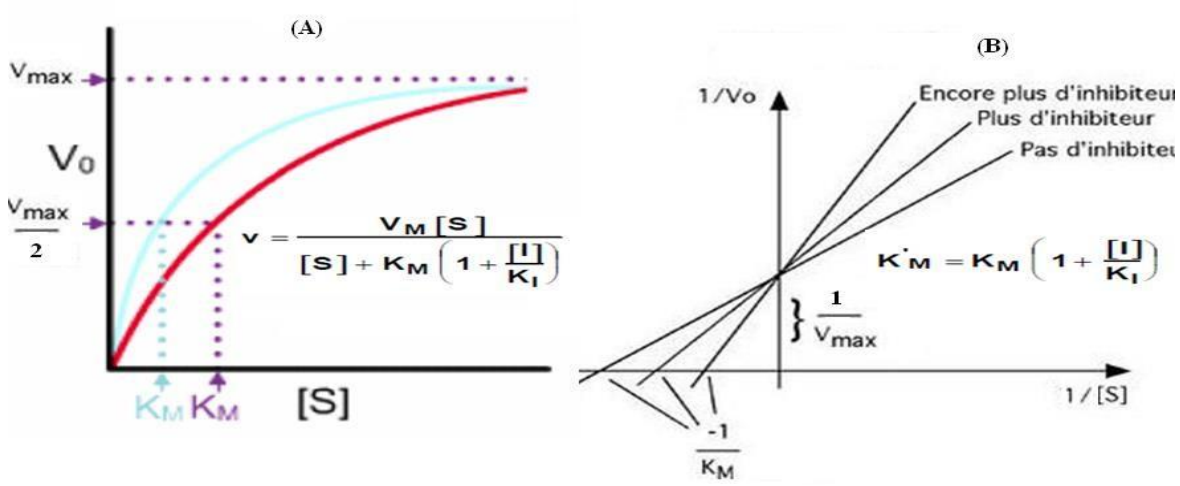


Fig. 155. Michaelis-Menten plot (A), Lineweaver-Burk plot (B) and determination of kinetic constants in case of competitive inhibition. The slope of the line is multiplied by a factor: 1 + [I]/Ki, Vmax remains unchanged. The Michaelis constant is increased: ES dissociation is favored, the enzyme's affinity for its substrate decreases.

Some competitive inhibitors

Malonic acid, an analog of succinic acid, is a competitive inhibitor of succinate dehydrogenase. Among antibiotics, chloramphenicol is a peptidyl transferase inhibitor, due to its similarity to the 3' end of aminoacyl-tRNAs.

Sulfanilamide, the first molecule in the sulfonamide class, inhibits folic acid formation due to its similarity to para-aminobenzoic acid. Glyphosate, used as a non-selective systemic herbicide (Roundup®), is an analog of phosphoenolpyruvate that inhibits the synthesis of aromatic amino acids in plants.

V. 7. 2. 2. Non-competitive inhibitors

A non-competitive inhibitor can bind simultaneously, and with the same affinity, to both the free enzyme and the substrate-bound enzyme (Fig. 156). However, the inhibitor and substrate do not compete for the same binding site: the substrate binds to the active site, and the inhibitor to a different binding site. The inhibitor induces a conformational change in the active site, which prevents the conversion of the substrate to a product but does not affect the recognition between the enzyme and the substrate. The reaction scheme is as follows:

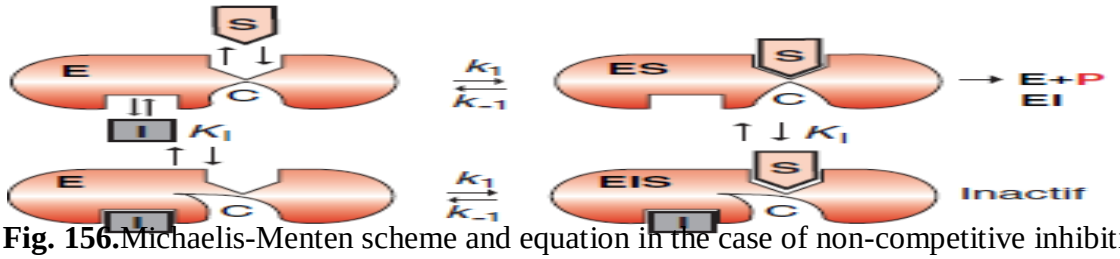


Fig. 156. Michaelis-Menten scheme and equation in the case of non-competitive inhibition.

With : $$KI = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[EIS]} = \frac{K_{-I}}{K_I}$$

The reaction scheme is represented by the initial rate equation as a function of substrate and inhibitor concentrations:

$$V = \frac{Vmax[S]}{[S]+Km} \times \frac{1}{1+\frac{[I]}{KI}}$$

According to this equation, regardless of the substrate concentration, the velocity is divided by a constant factor, hence the maximum apparent velocity: $$V_{max\ app} = \frac{Vmax}{1+\frac{[I]}{KI}}$$

The binding of a non-competitive inhibitor decreases the maximum reaction rate, but does not alter the enzyme's affinity for the substrate, as the latter binds equally well to the free enzyme and to the EI complex (Fig. 157). Enzymatic efficiency decreases, as some of the enzymes (those bound to the inhibitor) can no longer convert the substrate into product. This results in an apparent decrease in the amount of active enzymes.

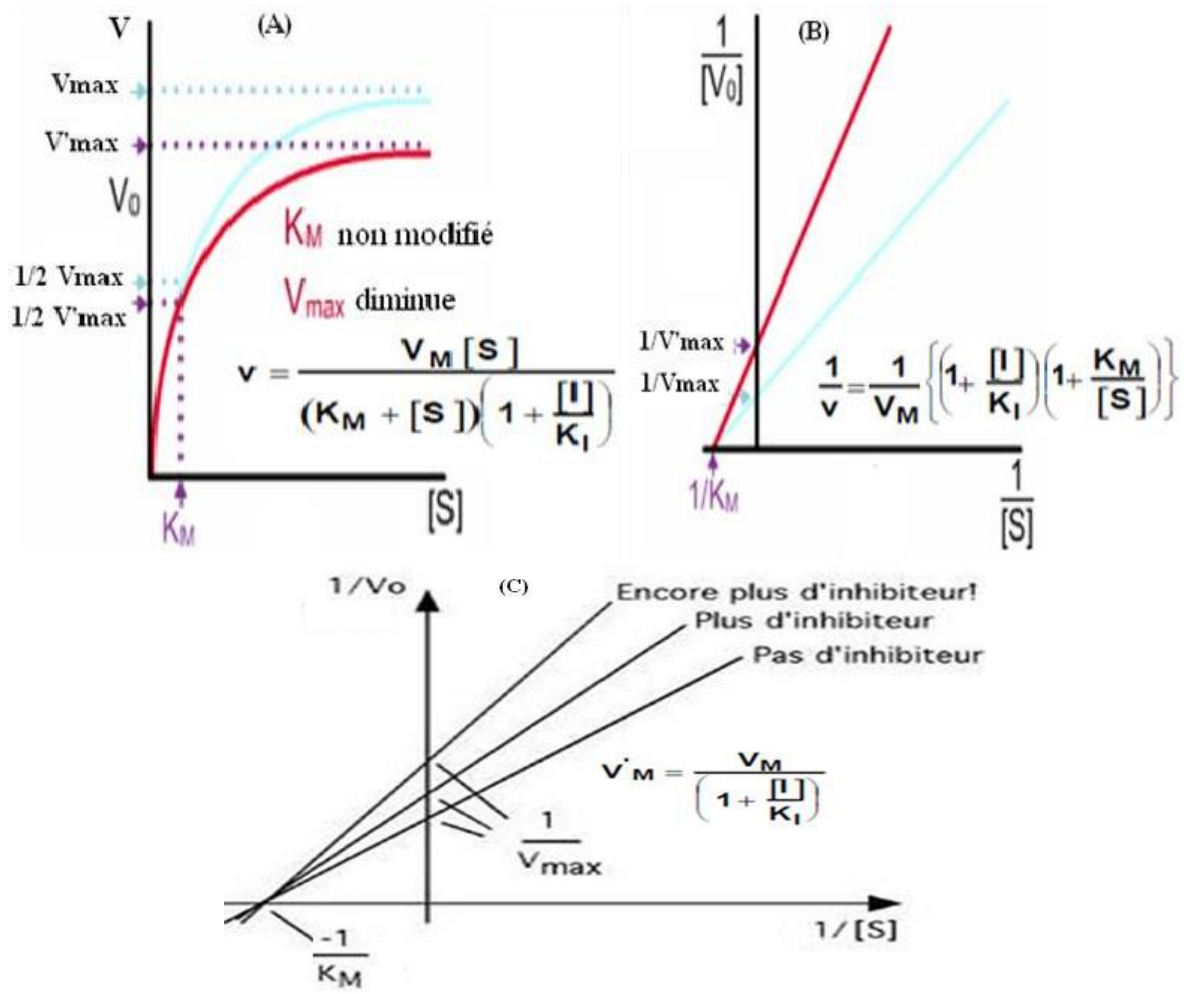


Fig. 157. Michaelis-Menten (A) and Lineweaver-Burk (B, C) plots and determination of kinetic constants in the case of non-competitive inhibition. Km unchanged, Vmax is reduced.

Non-competitive inhibition is reversible. It cannot be reversed by increasing the substrate concentration. However, dialyzing the solution containing the EI complex, or subjecting it to gel filtration, is sufficient to dissociate the complex, recover the free enzyme, and, in principle, its activity.

V. 7. 2. 3. Uncompetitive inhibitors

An anti-competitive or incompetitive inhibitor never binds to the free enzyme but only to the enzyme complexed with the substrate ES to form an inactive ternary complex ESI, thus preventing product formation (Fig. 158). Generally, substrate binding to the enzyme causes a conformational change in the enzyme, revealing a binding site for the inhibitor. The inhibitor, in turn, alters the conformation of the enzyme's active site, preventing the reaction. As its name suggests, an incompetitive inhibitor

does not compete with a substrate at its binding site. The system is described by the following reaction scheme:

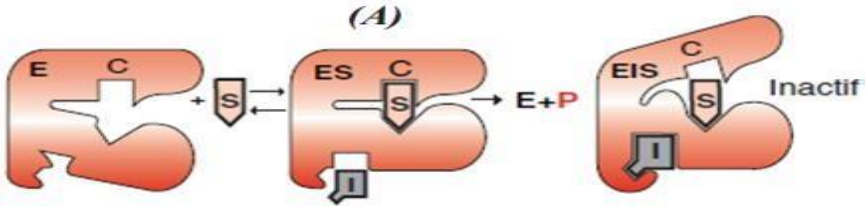


Fig. 158. Reaction pattern in case of uncompetitive inhibition.

The formation of this complex is governed by the dissociation constant: $KI = \frac{[ES][I]}{[ESI]} = \frac{K_I}{K_I}$

In steady state, this scheme leads to the velocity equation:

$$V = \frac{Vmax\,[S]}{[S]\,(1 + \frac{[I]}{KI}) + Km}$$

When we analyze this equation, we see that the maximum apparent speed and the K m values are obtained by dividing the values of Vmax and Km by the same factor:

$$V_{max\ app} = \frac{Vmax}{(1+\frac{[I]}{KI})} \quad ; \quad Km\ app = \frac{Km}{(1+\frac{[I]}{KI})}$$

The maximum reaction rate decreases, but the apparent affinity of the enzyme for the substrate increases (KM decreases). Indeed, the formation of the enzyme-substrate-inhibitor complex reduces the number of ES complexes and, according to the Law of Mass Action, promotes the binding of the substrate to the enzyme (Fig. 159).

The effects of an uncompetitive inhibitor are not observed at low substrate concentrations, as the enzyme is predominantly in its free form. Therefore, this type of inhibition cannot be overcome by increasing the substrate concentration.

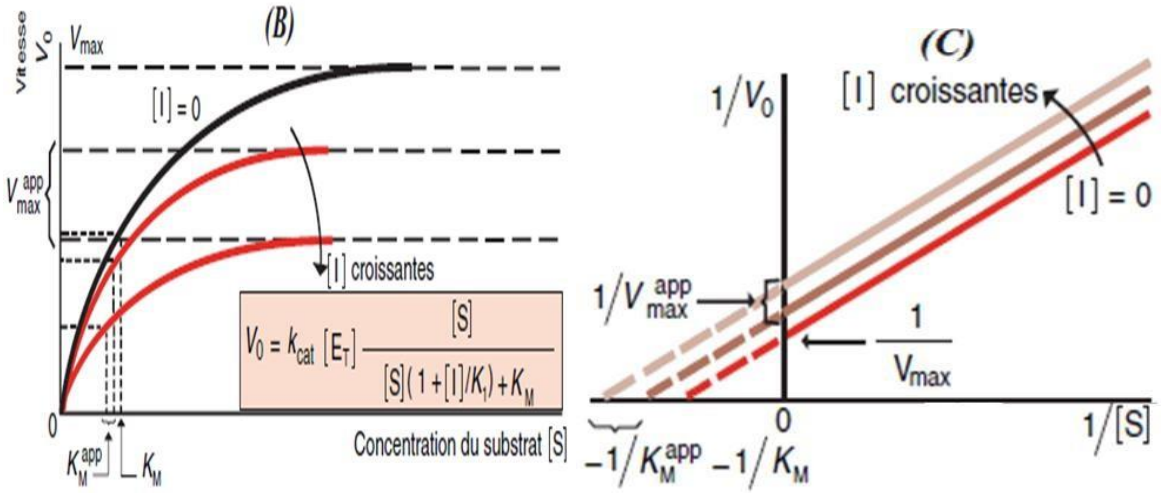


Fig. 159. Representation of Michaelis-Menten (B), of Lineweaver-Burk (C) And determination of kinetic constants in case of incompetitive inhibition.

V. 7. 2. 4. Examples of Anti-Competitive Inhibitors

The study of enzymes modifying a single substrate shows that anti-competitive inhibition is relatively uncommon. This inhibition is more frequently observed with enzymes that modify two substrates, where the binding of the first substrate necessarily precedes the binding of the second: a structural analog, competitive with the first substrate, can act as an anti-competitive inhibitor with respect to the second substrate.

The lithium ion (Li+) is an anti-competitive inhibitor of inositol-1-phosphatase. This inhibition could explain, at least in part, the action of lithium used to treat bipolar disorder.

V. 7. 2. 5. Irreversible inhibition

From a kinetic point of view, an irreversible inhibitor behaves like a non-competitive inhibitor: it decreases the maximum velocity without, however, altering the substrate's Km. The only difference lies in the stability of the enzyme-inhibitor complex.

V. 7. 2. 5. 1. Alkylating agents

These are reagents that allow groups to be covalently grafted onto the particularly reactive functions of the active sites. For example, iodoacetate inactivates papain, a papaya protease, by reacting with the thiolate function (cysteine residue) of the active site (Fig. 160):

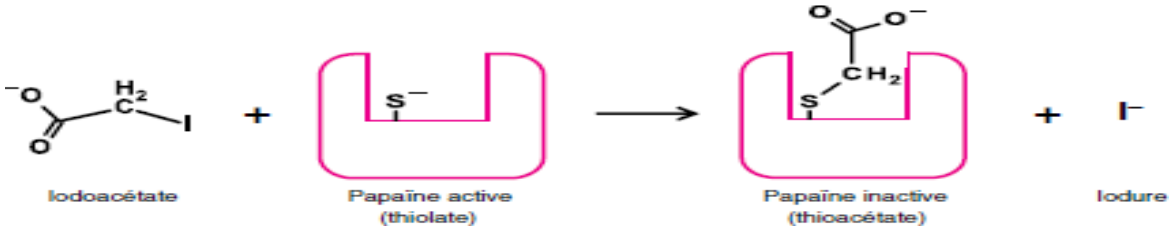


Fig. 160. Effects of iodoacetate on papain.

A similar reaction leads to the irreversible inactivation of proteases such as trypsin and chymotrypsin, and esterases such as acetylcholine esterase, whose active site contains a serine residue, by the action of parahydroxymercuribenzoate (pOHMB) or phenylmethylsulfonyl fluoride (PMSF). These two reagents are used in the laboratory to protect purified proteins from the potential action of serine proteases.

V. 7. 2. 5.2. Suicide inhibitors

Suicide inhibition is a mechanism where the inhibitor forms a stable complex with the enzyme, permanently inactivating it. The enzyme recognizes the inhibitor as its substrate and begins the process of modifying it. A step then occurs during this process, the modified inhibitor becomes highly reactive and binds very stably to the enzyme. The enzyme thus contributes to its own irreversible inactivation, hence the name "suicide" inhibition. The inhibitor can bind to

the enzyme's catalytic site or elsewhere. Several examples can be given:

- Aspirin inhibits the active cyclooxygenase that transforms arachidonic acid into a prostaglandin;
- Penicillin is a suicide inhibitor of transpeptidase involved in the synthesis of peptidoglycan, a component of bacterial cell walls. Its highly labile beta-lactam ring reacts with the enzyme's active site. It then binds to the enzyme at this site in a thermodynamically very stable manner.
- 5-Fluorouracil used in anti-cancer chemotherapy Inhibits thymidilate synthase; enzyme involved in the synthesis of thymine (DNA) leading to the cessation of tumor cell multiplication.
- Organophosphates, such as di-isopropylfluorophosphate (DIFP), are recognized as transition state analogs of serine proteases and esterases, but in addition they phosphorylate the target enzyme (Fig. 161):

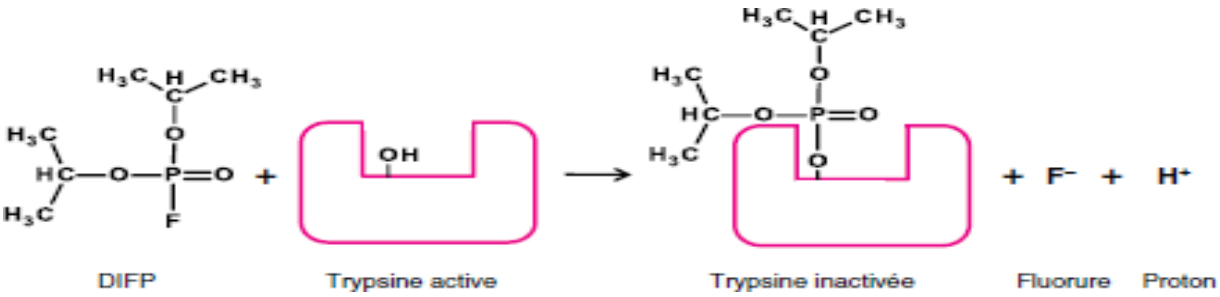


Fig. 161. Effects of di-isopropylfluorophosphate (DIFP) on trypsin activity.

V. 7. 2. 5. 3. Transition State Analogues

When they do not form a covalent bond with the active site, transition-state analogs behave as competitive inhibitors, as they bind reversibly to target enzymes. However, the inhibition of these enzymes results from the formation of an EI complex, followed by an isomerization step during which the protein changes conformation, leading to the terminal EI* complex:



The conformational steps leading to EI* complexes, and especially the dissociation of these complexes, are particularly slow. For example, the half-lives of EI* complexes are measured in tens of minutes, or even hours, whereas a conventional enzyme-inhibitor complex dissociates completely within a few milliseconds. Therefore, given the exceptionally high stability of EI* complexes, the inhibitions induced by transition-state analogs can be considered de facto irreversible.

V. 7. 2. 5. 5. Protein inhibitors

These molecules are part of the biological arsenal that regulates enzyme activity. The trypsin inhibitor, synthesized by the pancreas, is a 6 kDa protein that specifically inactivates trypsin (34 kDa). While inhibitor binding is reversible, the dissociation constant of the trypsin-inhibitor complex is 6×10^{-14} mol·L^{-1}. This complex is therefore very stable, and it is preferable to consider the tryptic inhibitor as irreversible.

V. 8. Allosteric enzymes

V. 8. 1. General Considerations

The regulation of enzyme activity occurs at two levels: by regulation of the quantity of enzyme by synthesis and/or degradation (long-term regulation) or by modification of the enzyme activities themselves (short-term regulation).

V. 8. 1. 1. Allostery

Allostery refers to a change in protein conformation under the effect of the binding of a substrate or effector molecule, resulting in the acquisition of particular properties (change in activity).

V. 8. 1. 2. Cooperativity effect

Cooperativity describes the fact that the binding of one molecule of an allosteric effector to an enzyme influences the binding of subsequent molecules. In the case of cooperativity in the presence of the substrate, if the allosteric effector is the substrate itself, this is called homotropic modulation. If the effector is different from the substrate, this is called heterotropic modulation. In positive cooperativity, the binding of one molecule of an effector increases the affinity for the same molecules, and vice versa in negative cooperativity.

V. 8. 2. Kinetics of allosteric enzymes

Some enzymes exhibit kinetics different from the Michaelis model. These are allosteric enzymes. Their kinetics do not follow a hyperbolic shape but a sigmoidal one (Fig. 162).

This difference in kinetics is explained by the structure of allosteric enzymes. These enzymes have a quaternary structure with several protomers (basic subunits) linked together by weak bonds and are therefore oligomeric. Each protomer has an active site capable of binding at least two molecules: the substrate and the allosteric effector (activator or inhibitor).

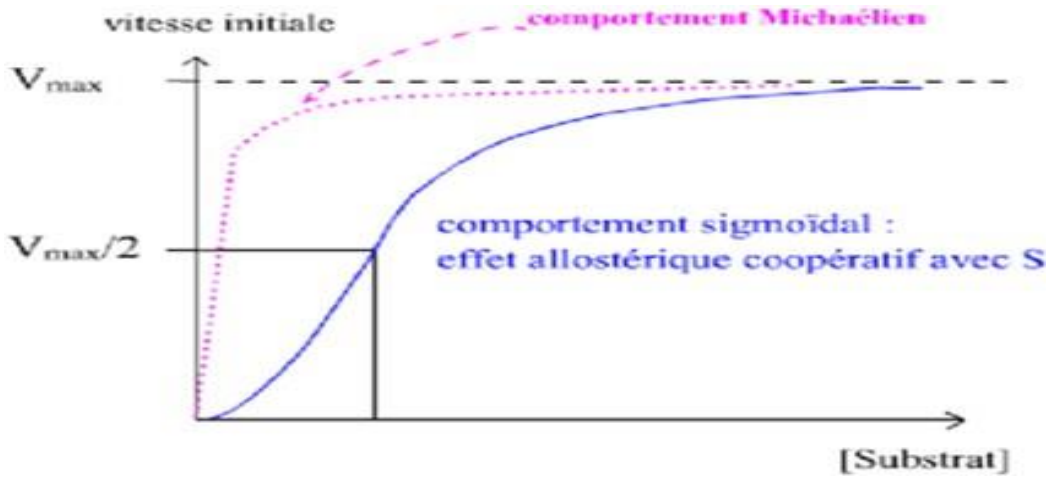


Fig. 162. Shape of the curves in the case of a Michaelis-Menten enzyme and an allosteric enzyme.

An allosteric enzyme therefore has several active sites, each consisting of a catalytic site for the substrate and a regulatory site for the allosteric effector.

Allosteric enzymes (allo = other, steria = form) exist in two forms (Fig. 2):

The first form, called the tense form (T), is inactive, with each protomer showing little affinity for the substrate. The second form, called the relaxed form (R), is active, with each protomer showing a high affinity for the substrate.



Fig. 163. The two forms R and T of allosteric enzymes.

The basic principle to remember is that any catalytic subunit binding the substrate undergoes a deformation (allosteric transition) which will be transmitted to the other subunits and therefore

modifies their affinity for the substrate. An inhibitory effector will favor the maintenance of the T form (inactive = without affinity for S) of the enzyme = negative allostery. An activating effector will favor the maintenance of the R form (active = with affinity for S) of the enzyme = positive allostery (Fig. 3).

Furthermore, allosteric effectors do not modify the Vmax of the enzyme but modify the enzyme's affinity for its substrate, i.e., the Km. The Km increases (affinity decreases) in the case of an allosteric inhibitor and decreases (affinity increases) in the case of an allosteric activator (Fig. 4).

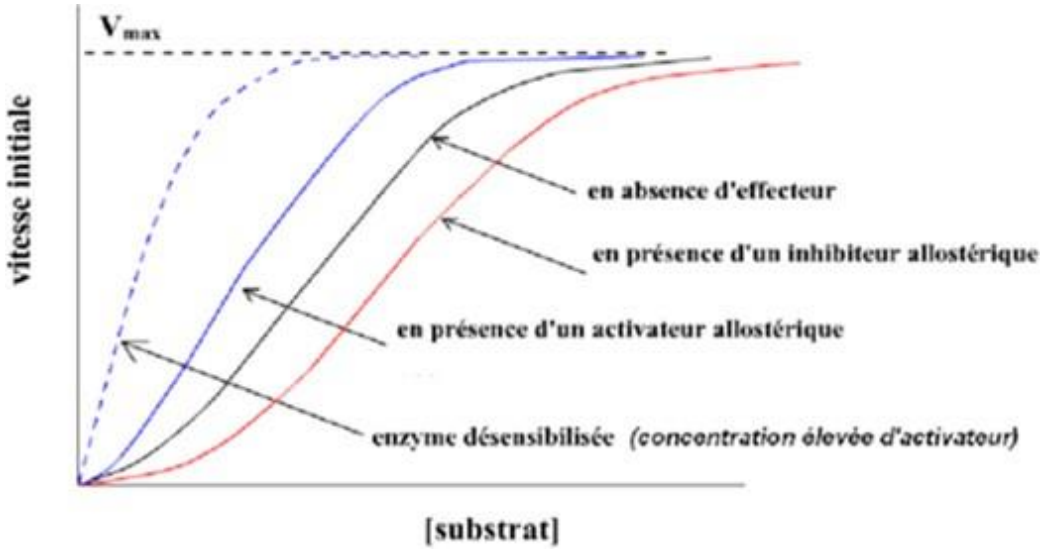


Fig. 164. Kinetics vi = f ([S]) for an allosteric enzyme, with cooperative substrate effect in the absence of effector and in the presence of an allosteric activator or inhibitor.

V. 8. 3. Physiological importance of allosteric enzymes

Allosteric enzymes are key enzymes in metabolic regulation.

The negative feedback effect: This is very common in metabolic pathways. The end product of the pathway is often an allosteric inhibitor of an enzyme catalyzing an initial step. The more the end product accumulates, the slower the initial reaction becomes (due to decreased affinity for one of the reaction reactants), and therefore less product will be formed (Fig. 4). This type of regulation prevents the accumulation of the end product, which can be toxic under certain circumstances, and prevents the organism from producing an excess of a molecule, which is energy-intensive.

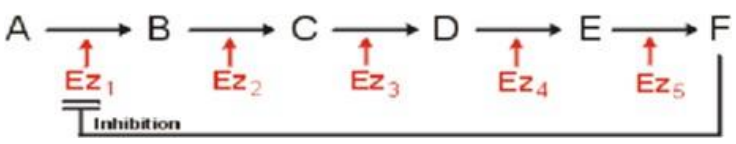


Fig. 165. The negative feedback effect.

For example, in glycolysis, phosphofructokinase-1 (PFK1) is an allosteric enzyme that converts fructose 6-phosphate to fructose 1,6-biphosphate in what can be considered the first step specific to this pathway. This enzyme reacts differently depending on the amount of ATP, which is the final "useful" product of glycolysis. At low concentrations, ATP binds to the enzyme's coenzyme site, effectively releasing the entire enzyme into its relaxed (active) form. At high concentrations, ATP binds to the enzyme's phosphate group.

The enzyme acts as an "inhibitor," effectively keeping the entire enzyme in its tense (inactive) state. However, ATP is a product of cellular respiration, a process that follows glycolysis and the Krebs cycle. Therefore, the feedback effect of ATP is to inhibit the pathway when present in sufficient concentration, thus preventing unnecessary glucose breakdown.

Another example is aspartate transcarbamylase ATCase (EC 2.1.3.2) in the pyrimidine biosynthesis pathway (Fig. 6). The enzyme catalyzes the first reaction in the biosynthesis pathway that leads to pyrimidine nucleotides.

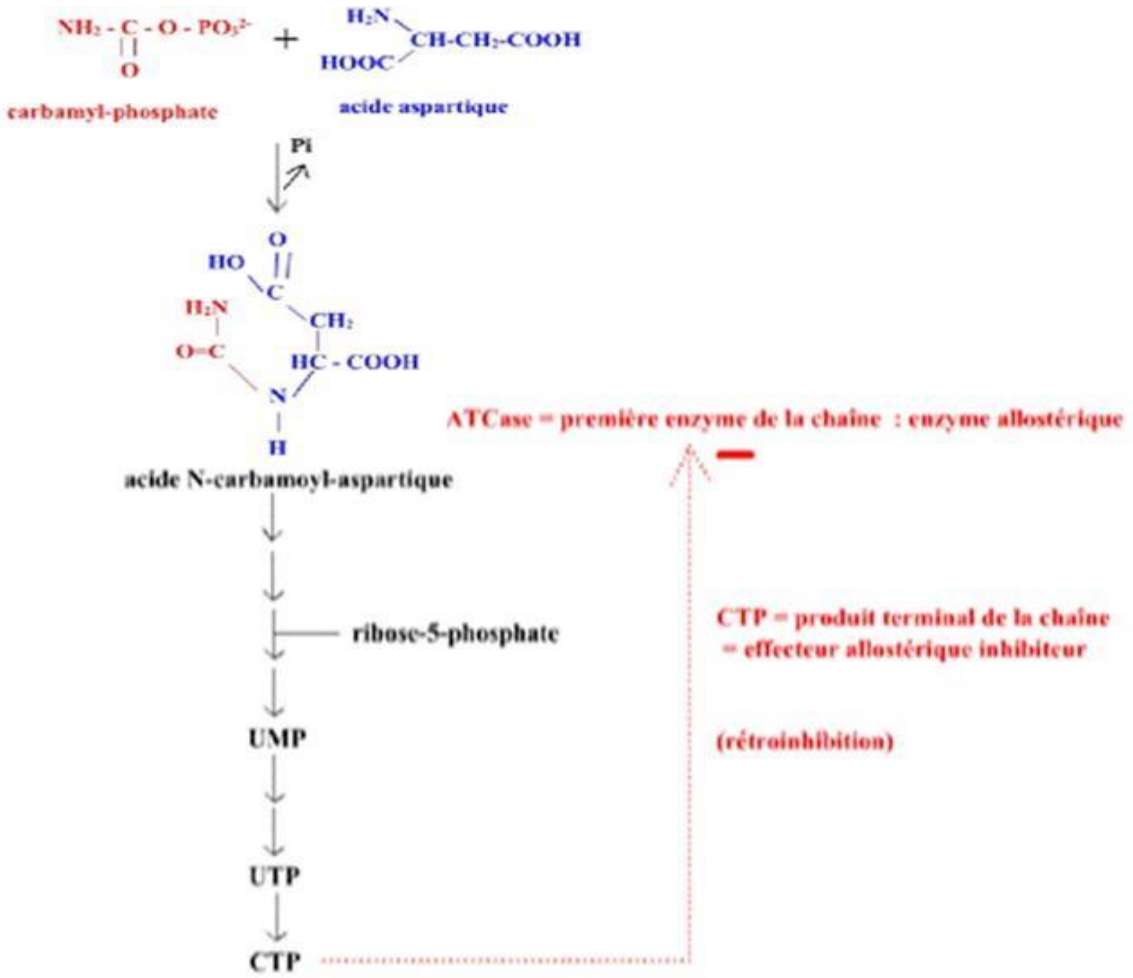


Fig. 166. The first reaction of the pyrimidine nucleotide biosynthesis pathway catalyzed by aspartate transcarbamylase ATCase.

This allosteric enzyme exhibits sigmoidal kinetics Vi = f([aspartate]) in the absence of an effector: a positive cooperative effect of the substrate aspartate; CTP is its allosteric inhibitor; ATP is its allosteric activator. CTP maintains the subunits in the T state (inhibition), whereas ATP would maintain them in the R state (stimulation).

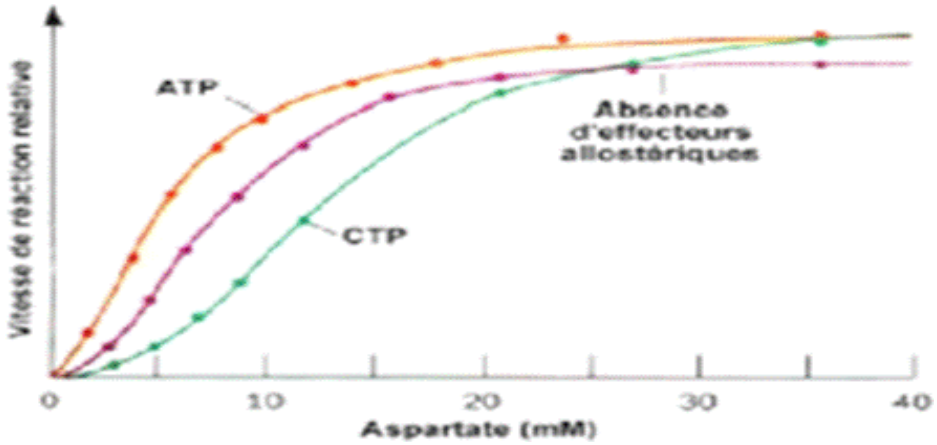
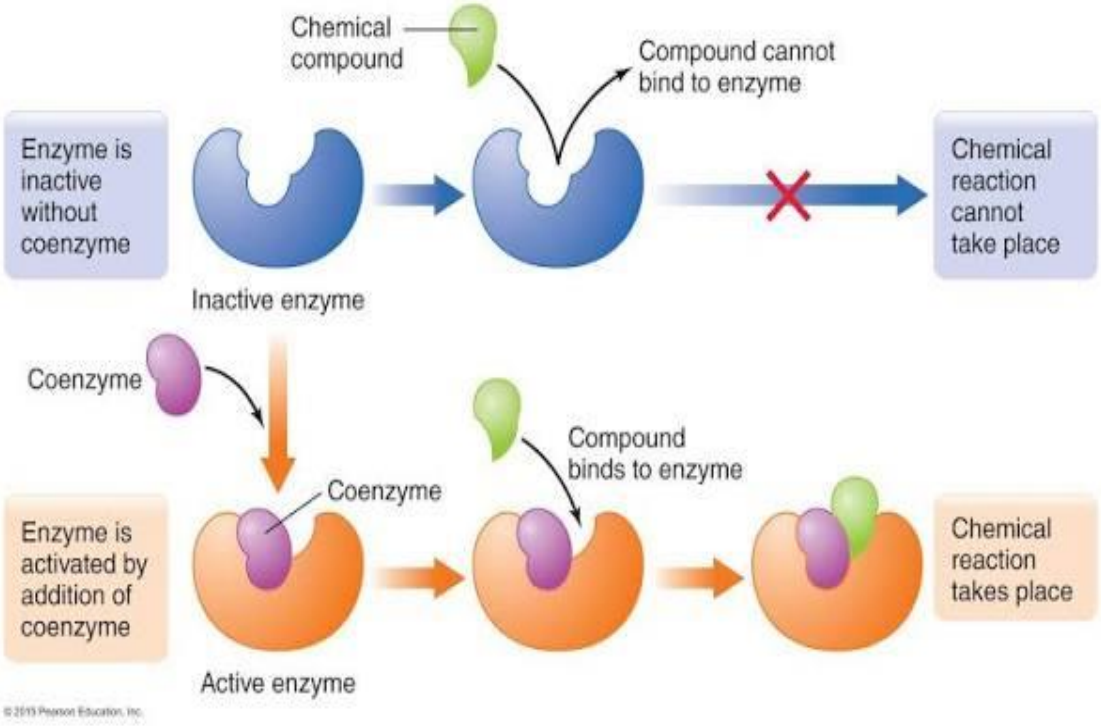


Fig. 167. Resultant kinetics of ATCase in the presence of ATP, CTP, and without allosteric effector.

CHAPTER VI :

VITAMINS AND COENZYMES



CHAPTER VI VITAMINS AND COENZYMES

VI.1- Vitamins

VI.1.1- Definition and nomenclature

Etymologically, "amines necessary for life," vitamins actually have varied structures and are not all amines. Unlike trace elements, they areThese are essential organic molecules with no energy value that the body is unable to synthesize (or does so in insufficient quantities). Therefore, they must be obtained through diet.Some vitamins are exceptions because there are other sources for them that can replace dietary intake: exposure of the skin to solar ultraviolet rays for vitamin D, synthesis from tryptophan for niacin, synthesis by the digestive microbial flora for vitamin K.

Daily vitamin requirements are only fractions of a microgram to a few milligrams. This is because most act as coenzymes or cofactors during enzymatic reactions.Some vitamins, such as vitamin C, participate directly in enzymatic reactions, but the vast majority of these molecules undergo transformations before finally being used as coenzymes.

Vitamins are separated into two groups according to their solubility (Table 23): water-soluble vitamins and fat-soluble vitamins.

Table 23: The different types of vitamins.

MOLECULE	ABBREVIATION	USUAL UNIT
WATER-SOLUBLE VITAMINS		
Thiamine	Vitamin B1	mg
Riboflavine	Vitamin B2	mg
Pantothenic acid	Vitamin B5	mg
Pyridoxine	Vitamin B6	mg
Niacin	Vitamin PP or B3	mg
Folic acid	Vitamin B9	µg
Cobalamin	Vitamin B12	µg
Ascorbic acid	Vitamin C	mg
Biotin	Vitamin H or B8	µg
FAT-SOLUBLE VITAMINS		
Retinol	Vitamin A	International unit (1 IU = 0.3 µg)
Calciferol	Vitamin D	International unit (1 IU = 0.025 µg)
Tocopherol	Vitamin E	International unit (1 IU = 1 mg acetate)
Phytomenadione phylloquinone	Vitamin K1	µg

A vitamin deficiency leads to significant problems if the deficiency is not corrected quickly (Table 24).

Table 24: The various disorders caused by a deficiency in certain types of vitamins.

Vitamin	Consequence of a deficit
Thiamine (B1)	Briberi
Nicotinic acid	Pellagra
Folic acid	Anemia
B12	Anemia, Methylmalonic acidosis
C (ascorbic acid)	Scurvy
HAS	Night blindness
D	Rickets, osteolacia
E	Inhibition of sperm production
K	Subcutaneous hemorrhage

VI.1.2 - Water-soluble vitamins

VI.1.2.1- B vitamins

VI.1.2.1- Vitamin B1

This was the first vitamin discovered, isolated from the bran of rice grains in 1913. Also called thiamine (or aneurin), it has a heterocyclic structure containing sulfur and nitrogen and comprises a pyrimidine ring and a thiazole ring (Fig. 168). Phosphorylation of thiamine leads to the coenzyme, thiamine diphosphate or TPP.

There is a possible endogenous source of this vitamin through the biosynthetic activity of the intestinal flora (microbiota), but this is by no means sufficient to meet the body's needs. Dietary intake therefore remains essential.

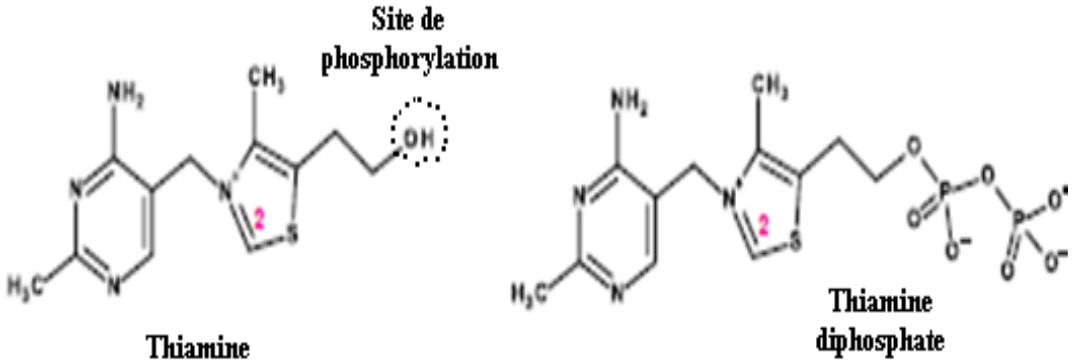


Fig. 168. Structure of thiamine.

In food, thiamine exists in two forms: free form, mainly in foods of plant origin (cereals, mushrooms...); phosphorylated form (thiamine pyrophosphate, TPP) mainly in foods of animal origin (liver, meats, dairy products...).

Thiamine is absorbed in the small intestine (duodenum and jejunum) by enterocytes. Once inside the enterocyte, TPP is dephosphorylated; therefore, thiamine passes into the

bloodstream exclusively in its free form. Its transport is primarily carried out by blood cells (red and white blood cells). It is then distributed to the cells that consume it (liver, heart, kidneys, and brain) where it is phosphorylated to TPP, which is its active coenzyme form. Vitamin B1 cannot be stored. It is eliminated via the kidneys in the form of metabolites (a degraded form that cannot benefit from a reabsorption mechanism).

Vitamin B1 deficiency is responsible for beriberi, which is characterized by dysfunctions of the nervous, circulatory and gastrointestinal systems.

VI.1.2.2- Vitamin B2 (Ribovlavin)

Vitamin B2 results from the condensation of a flavin (nitrogenous heterocycle with 3 nuclei) and an alcohol, ribitol, hence the name riboflavin (Fig. 169).

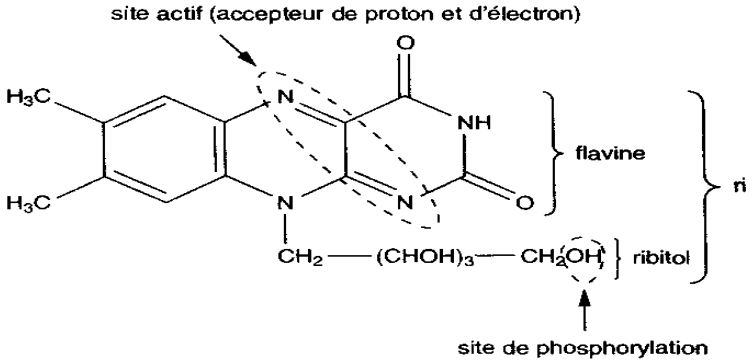


Fig. 169. Structure of riboflavin.

It is part of the composition of two metabolically important redox coenzymes: FMN (flavin mononucleotide) and FAD (flavin adenine dinuleotide) (Fig.170).

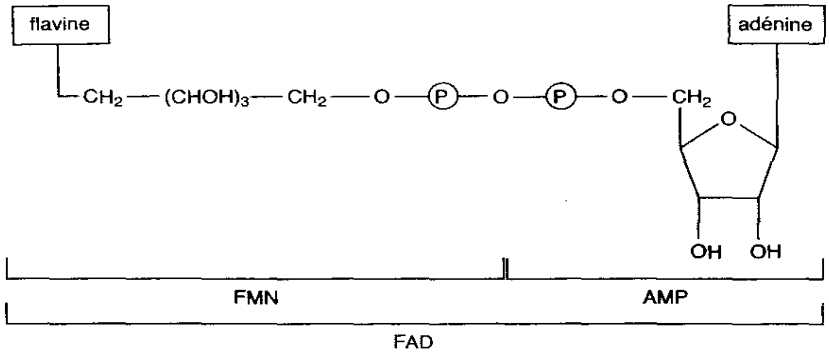


Fig. 170. Schematic structure of FMN and FAD.

Biosynthesis by the intestinal flora is possible but insufficient to meet the body's needs.

Vitamin B2 is present in foods of animal origin (liver, dairy products, fish) and plant origin (in more limited quantities: cereals and mushrooms).

It is found there in its free form, as FMN and FAD. Only the free form can be absorbed in the small intestine (primarily the duodenum and jejunum). The coenzyme forms are therefore

hydrolyzed. Through active transport, vitamin B2 passes into enterocytes where most of it is phosphorylated to FMN, then into the blood where transport (of B2 and FMN) is facilitated by albumin and red blood cells.

At the level of the consuming cells, vitamin B2 is transformed into FMN and especially into FAD, its main active form (approximately 80%) (Fig. 171). This biosynthesis of coenzymes is under the control of thyroid hormones.

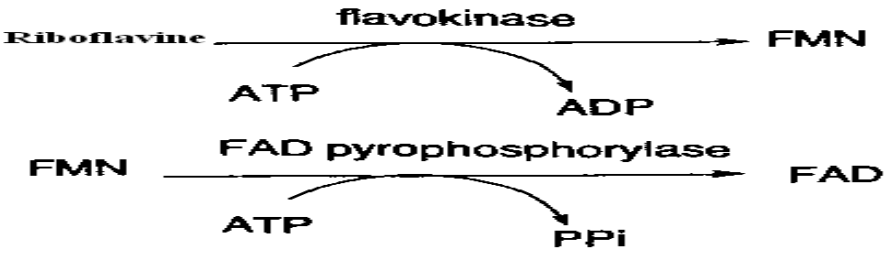


Fig. 171. Phosphorylation of riboflavin.

They are most frequently located in the liver, heart and kidneys, which can be considered a reserve allowing for autonomy of about 2 weeks.

Elimination occurs via the urinary tract, primarily in free form.

Vitamin B2 deficiency is relatively rare and causes non-specific symptoms (skin problems, nausea, vomiting…), often linked to other vitamin and protein deficiencies.

VI.1.2.3- Vitamin B3 (vitamin PP or niacin)

Vitamin B3 (Fg. 172) exists in two molecular forms: nicotinic acid and nicotinamide (the carboxylic function is replaced by an amide function).

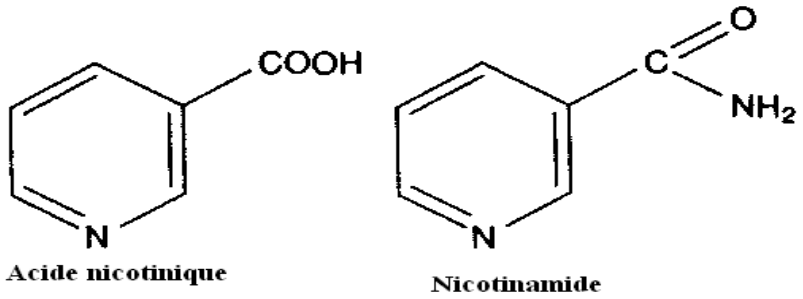


Fig. 172. Structure of vitamin B3.

Nicotinamide is a component of two redox coenzymes of great biochemical importance: NAD (nicotinamide adenine dinucleotide) and NADP+ (phosphorylated NAD) (Fig. 173).

In foods (liver, meats, wholemeal bread…), niacin is mainly present in the form of NAD and NADP (in cereals, it is also found in the form of nicotinyl ester, which is less easily assimilated).

Vitamin B3 can be produced endogenously. It is possible to synthesize it from tryptophan (Trp), which is, however, one of the essential amino acids. This possibility is nevertheless insufficient.

Thanks to intestinal juice, the coenzyme forms are hydrolyzed, allowing the absorption (via facilitated transport) of vitamin B3 by enterocytes throughout the small intestine. Tissue distribution (via the bloodstream) is facilitated by red blood cells. Once in the cell that uses it, vitamin B3 is converted into NAD (or NADP) in the cytosol and mitochondrial matrix (these coenzymes cannot cross biological membranes).

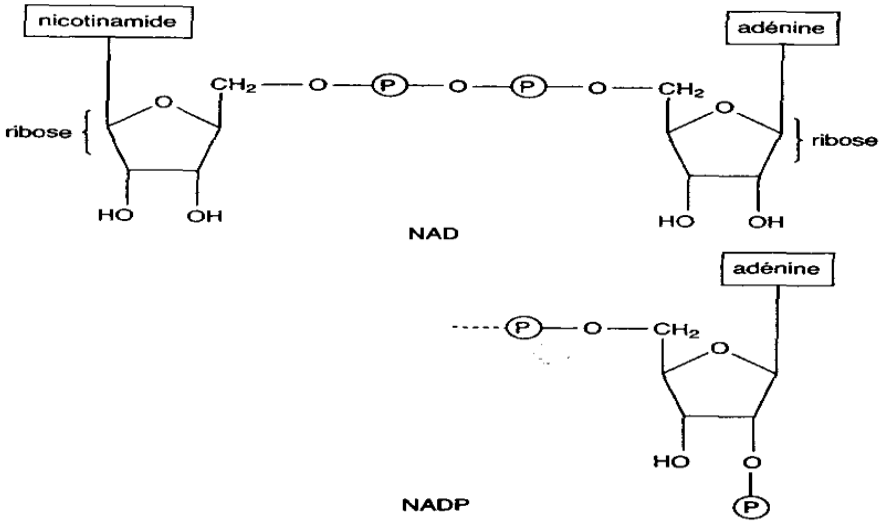


Fig. 173. Schematic structure of NAD and NADP.

The conversion of Trp to NAD is also intracellular and requires vitamin B6. It is estimated that 60 mg of Trp yields 1 mg of nicotinamide (sometimes noted 1 niacin equivalent). Even though the liver, muscles and kidneys are rich in vitamin B3, there is no significant storage.

Elimination occurs via the urinary tract, primarily in the form of metabolites.

A pronounced deficiency in vitamin B3 is relatively rare and leads to pellagra (hence the name vitamin PP, for pellagra preventive factor), which manifests itself through skin disorders, mucous membrane lesions, and psychological disturbances.

VI.1.2.4- Vitamin B5 (pantothenic acid)

This vitamin results from the condensation between pantoic acid and theβ-alanine (the amine function is attached to the carbonβ) thanks to an amide bond (Fig. 174).

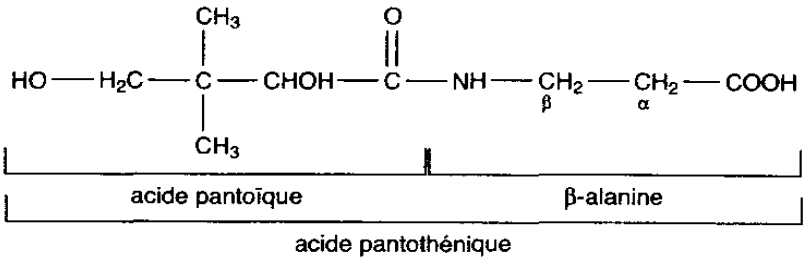


Fig. 174. Structure of vitamin B5.

It is part of the composition of a transfer coenzyme of great metabolic importance, coenzyme A, denoted CoA-SH.

Pantothenic acid (in a phosphorylated form) is also part of the composition of acyl synthase (at the level of the subunit noted ACP), an enzymatic complex that catalyzes the biosynthesis of fatty acids.

Its absorption in the small intestine requires hydrolysis of the coenzyme form. Through active transport, pantothenic acid is taken up by enterocytes and then passes into the bloodstream for tissue distribution in its free form. Vitamin B5 is converted to CoA-SH in the cytosol and mitochondrial matrix of the cells that use it. Here too, biosynthesis occurs in each compartment because CoA-SH cannot cross biological membranes. It cannot be stored.

Vitamin B5 is eliminated via the urinary tract in free form. Since vitamin B5 is widely present in food, deficiency is very rare and indicative of severe overall malnutrition.

VI.1.2.5- Vitamin B6 (pyridoxine)

The term vitamin B6 encompasses three vitamin forms: pyridoxine (pyridoxol), pyridoxal (an alcohol function is replaced by an aldehyde function) and pyridoxamine (the alcohol function is substituted by an amine function).

This vitamin acts as a transfer coenzyme in the form of pyridoxal phosphate (Fig. 175).

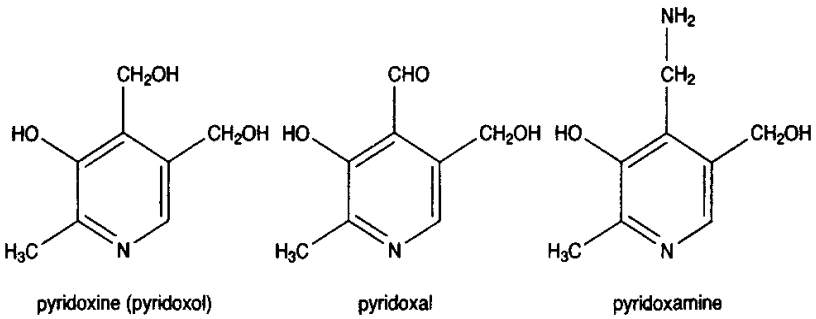


Fig. 175. Different forms of vitamin B6.

Vitamin B6 is present in various forms in food. However, it is predominantly found as pyridoxamine in plant-based foods (cereals, green vegetables, fruits, lentils, etc.), with lower

bioavailability than pyridoxal phosphate. This is the predominant form in foods of animal origin (liver, meat, fish, etc.).

Its absorption, primarily jejunal, is only possible in the dephosphorylated form. Vitamin B6 enters the bloodstream and is then transported to the liver, which converts the various forms to pyridoxal (a conversion requiring the presence of vitamin B2 in the form of FMN). It then returns to the bloodstream for distribution to the various tissues. Its transport in the blood is facilitated by albumin.

It is in the user cell that pyridoxal is phosphorylated to become an active coenzyme.

Its storage is very limited, with a small capacity in striated muscle. It is eliminated via the urinary tract in the form of metabolites (formed in the liver and kidneys).

VI.1.2.6- Vitamin B8 (biotin)

This vitamin consists of a double nitrogen and sulfur ring bearing a carboxylic function, which will allow its binding to the apoenzyme (Fig.176).

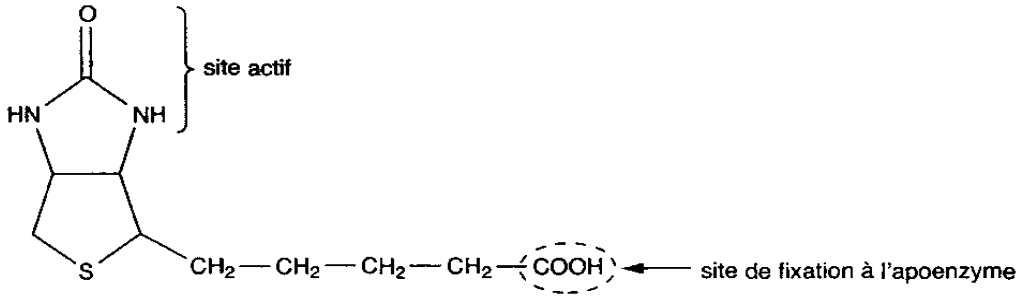


Fig. 176. Structure of vitamin B8.

In foods (liver, eggs, mushrooms, green vegetables, etc.), the vitamin is primarily bound to the apoenzyme by an amide bond (and not a peptide bond). Therefore, the action of a specific pancreatic enzyme, biotinidase, is required.

This vitamin can have an endogenous source. The intestinal flora is capable of biosynthesizing it, but dietary intake remains necessary.

Primarily absorbed in the jejunum, vitamin B8 then passes into the bloodstream where transport is facilitated by a specific plasma protein. Once in the cell that uses it, it binds to an apoenzyme (via a covalent bond) to act as a transfer coenzyme.

There is no possible storage and it is eliminated in free form via the urinary tract.

There is no spontaneous vitamin B8 deficiency in adults. It can exceptionally occur in children due to a genetic disease causing a biotinidase deficiency, leading to malabsorption of the vitamin, which can result in cutaneous and mucosal disorders and neuropsychiatric problems (psychomotor delays).

VI.1.2.7. Vitamin B9 (folic acid)

Vitamin B9 is a complex molecule composed of a pteridine nucleus, a para-aminobenzoic acid molecule and a glutamic acid (Glu) molecule.

Vitamin B9 gives rise to numerous derivatives grouped under the term folates. It can contain several Glu residues; this is then referred to as folic acid polyglutamate (Fig. 177).

Thanks to the action of digestive proteases, absorption occurs in the jejunum in the form of folic acid monoglutamate. Once in the bloodstream, via the portal vein, vitamin B9 reaches the liver where it is converted (by the attachment of four protons to the pteridine ring) into tetrahydrofolate, its active form. There is a storage capacity at this stage, allowing for a supply lasting approximately three months.

The active form can re-enter the bloodstream; its transport is then facilitated by albumin. Once in the cell, tetrahydrofolate is replaced by five Glu residues, forming an active coenzyme that participates in one-carbon radical transfer reactions. Elimination occurs via feces (through bile) and urine.

Folic acid deficiency results in problems such as anemia, asthenia, anorexia, and growth delays in children.

VI.1.2.8. Vitamin B12 (cobalamin)

Vitamin B12 is a macromolecule of such complexity that we will limit ourselves to its molecular formula: $C_{63}H_{88}O_{14}N_{14}PCo$. It is interesting to note the presence of a cobalt atom (located at the heart of the structure).

It is found exclusively in foods of animal origin (liver, meat, fish, eggs, etc.). It is then bound to dietary proteins.

Vitamin B12 deficiency is characterized by hematological (anemia), cutaneous and mucosal disorders and nervous disorders (loss of peripheral sensation).

In the gastric cavity, thanks to the combined action of hydrochloric acid and pepsin, vitamin B12 is released and bound by a specific glycoprotein: intrinsic factor, produced in the gastric mucosa by parietal cells (which also produce HCl). The intrinsic factor-vitamin B12 complex then passes into the small intestine. Absorption occurs in the ileum via a membrane receptor located in the apical membrane of enterocytes. Once bound to the receptor, the complex dissociates, allowing the absorption of cobalamin.

Once in the bloodstream, it binds to a transport protein (transcobalamin). A significant portion is taken up by the liver, which offers substantial storage capacity, as the body's autonomy is on the order of a year.

Its elimination occurs primarily via bile and feces, although an enterohepatic circulation (similar to that of bile salts) allows for the recycling of the molecule. The intestinal flora is capable of synthesizing vitamin B12, but it is not bioavailable.

Role as a coenzyme

From a biochemical standpoint, cobalamin acts as a transfer coenzyme in methylation and isomerization reactions. Thus, it plays a role in the catabolism of certain amino acids (Val, Ile, Met, Tyr) to succinyl-CoA.

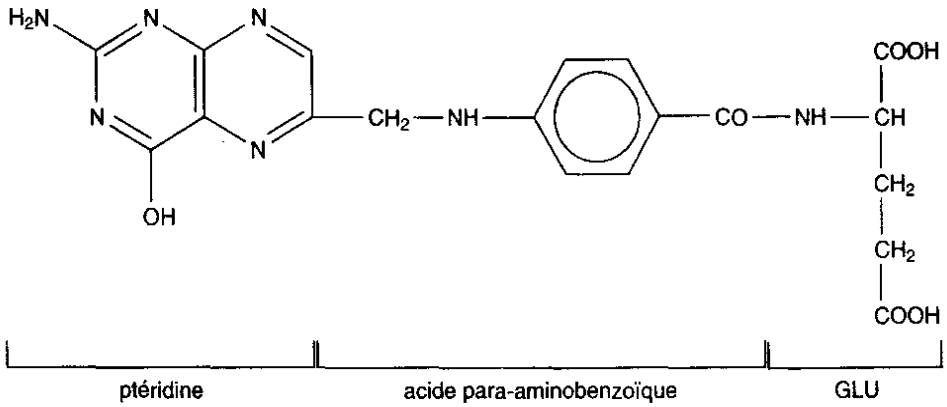


Fig. 177. Structure of folic acid.

It contributes to DNA biosynthesis by participating in the reduction of ribose to deoxyribose. It thereby promotes the formation and maturation of red blood cells.

VI.1.2.9. Vitamin C (ascorbic acid)

Ascorbic acid exists in two forms, oxidized and reduced, which give vitamin C reducing properties (Fig. 178).

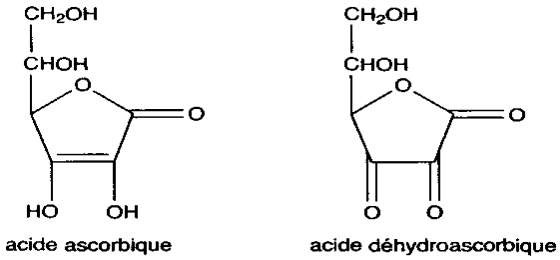


Fig. 178. Structure of vitamin C.

It is found in large quantities in plant-based foods (blackcurrants, kiwis, parsley, citrus fruits, strawberries, potatoes…), but it is also present in smaller quantities in some animal-based foods (liver, meat…).

It is absorbed via active transport, primarily in the ileum. Once in the bloodstream, vitamin C is distributed to the various tissues that consume it. It cannot be stored and is eliminated via the urine, with the possibility of tubular reabsorption.

Role as a coenzyme

Once inside the cell, vitamin C acts as a coenzyme in redox and hydroxylation reactions. Vitamin C is involved in:

- Collagen biosynthesis: this protein is produced by fibroblasts (connective tissue cells). Initially, biosynthesis generates procollagen. Its transformation into collagen requires vitamin C. It allows the hydroxylation of lysine and proline residues (formation of hydroxylysine and hydroxyproline), strengthening the resistance and plasticity qualities of collagen and, consequently, of connective tissue;
- of catecholamine biosynthesis: its precursor is phenylalanine or tyrosine. This biosynthesis requires hydroxylation reactions in which vitamin C is involved (Fig. 179);
- from the biosynthesis of carnitine (formed from lysine). This molecule allows the transport of acyl-CoA into the mitochondrial matrix;
- of the biosynthesis of bile salts from cholesterol (therefore, a hypocholesterolemic effect can be attributed to vitamin C);
- from the inhibition of the transformation of nitrites into nitrosamine, a molecule with carcinogenic effects, in the stomach. Nitrites come from food or from the transformation of nitrates taken from drinking water or vegetables;
- of cytochrome P450 activity, which plays an active role in liver detoxification processes;
- of iron absorption and tissue distribution by allowing the reduction of ferric ions (Fe3+) to ferrous ions (Fe2+). This iron can notably become part of the composition of hemoglobin. Thus, indirectly, vitamin C has an anti-anemic role;
- neutralizing free radicals, in synergy with vitamin E;
- of the immune response by increasing resistance to bacterial and viral infections. It would contribute to leukocyte mobility and increase the effectiveness of the humoral response (via immunoglobulins).

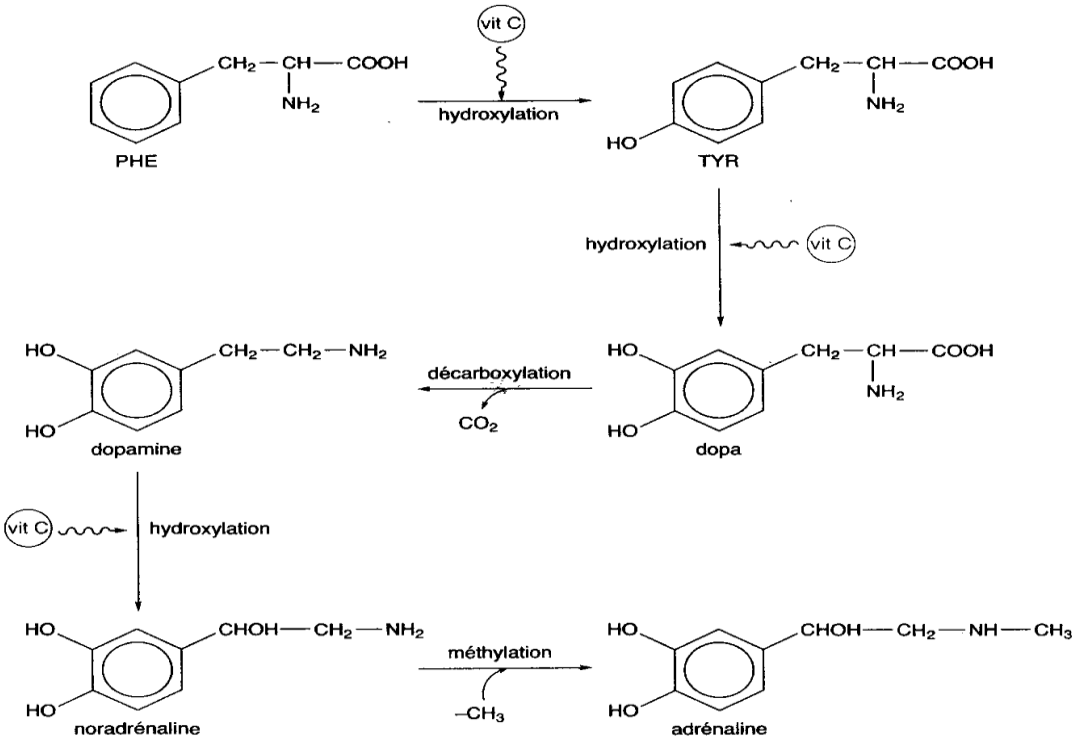


Fig. 179. Biosynthesis of catecholamines.

VI.1.2 - Fat-soluble vitamins

This family includes vitamins A, D, E, and K, which are soluble in oils and fats. These compounds share several characteristics: they are generally nonpolar, electrically neutral, and all derive from isoprene compounds.

VI.1.2.1- Vitamin A (retinol)

This molecule consists of a carbon ring and an aliphatic chain whose end is occupied by a primary alcohol function. It is therefore an alcohol (Fig. 180).

Retinol, vitamin A, is the precursor of two active molecules, retinal and retinoic acid.

Retinal, the aldehyde derived from retinol (ol: alcohol; al: aldehyde), is the prosthetic group of rhodopsins in the cone and rod cells of the retina. Retinoic acid is a growth factor for epithelial cells; moreover, it is a hormone essential for embryonic development.

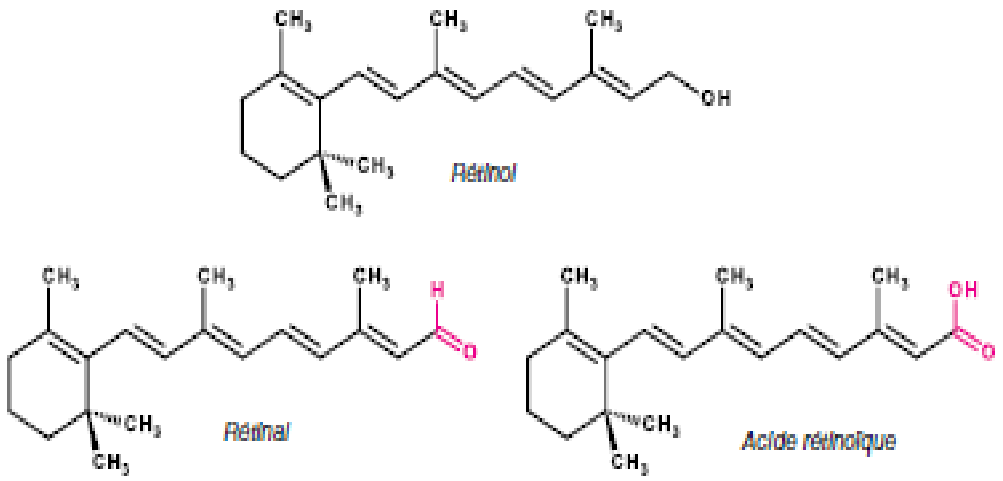


Fig. 180. Structure of the Retinol (vitamin A): the precursor of retinal and retinoic acid.

In foods of animal origin (fish liver oil, liver, butter, eggs, etc.), vitamin A is present in its esterified form, most often with palmitic acid. This is then referred to as palmitic retinol. Just like lipids, the combined action of bile salts and pancreatic lipase is necessary for the digestion and absorption of vitamin A in the small intestine by enterocytes.

In plant-based foods (carrots, spinach, melon, etc.), it is found in the form of provitamin A. This molecule belongs to the carotenoid family, of which it is the main representative; we are referring here toβ-carotene (Fig. 181).

After intestinal absorption, a significant portion of theβ-carotene is transformed in the enterocyte into retinol (the rest is transformed in the liver).

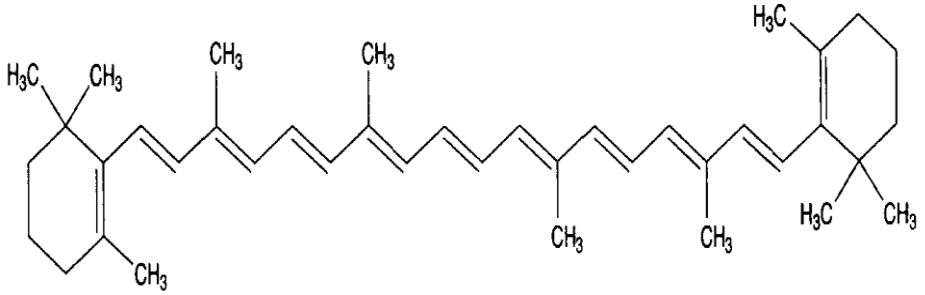


Fig. 181. Thereβ-carotene (provitamin A).

Vitamin A has an absorption efficiency of approximately 70%; it is only 50% for theβ-carotene due to its greater hydrophobicity. Once inside the enterocyte, retinol is re-esterified and incorporated into chylomicrons, which then pass into the lymph and subsequently into the blood. They are ultimately taken up by the liver. This organ ensures the storage of esterified retinol, allowing for several months of autonomy, which is exceptional for a vitamin.

Tissue distribution, via plasma, is ensured by a specific transport protein called RBP (retinol binding protein). This protein is produced by the liver and adipose tissue.

The main role of vitamin A relates to vision, particularly adaptation to darkness (this is called twilight vision).

It acts in the form of retinal (the primary alcohol function is replaced by an aldehyde function) in association with a protein: opsin. Together they form rhodopsin, which is a photosensitive chromoprotein specific to the retina (rod cells).

Vitamin A also has a physiological effect in the form of retinoic acid (the alcohol group is replaced by a carboxyl group). This molecule is involved in cell growth and differentiation, and in the wound healing process. In pregnant women, retinol plays an important role in fetal growth.

Provitamin A, on the other hand, has antioxidant properties, meaning that it contributes to the neutralization of free radicals.

Vitamin A is eliminated partly through feces (via bile) and partly through urine.

It is important to note that a vitamin A overdose is toxic. Such hypervitaminosis leads to digestive and neurological disorders. In pregnant women, it can increase the risk of fetal malformations.

VI.1.2.2- Vitamin D (calciferol)

This molecule belongs to the sterol family. It is present in small quantities in plant-based foods. Vitamin D2 (ergocalciferol, derived from a plant sterol: ergosterol) is then used as a supplement.

It is found mainly in foods of animal origin (oily fish, liver, egg yolk, etc.). More precisely, it is referred to as vitamin D3 (cholecalciferol) (Fig. 182).

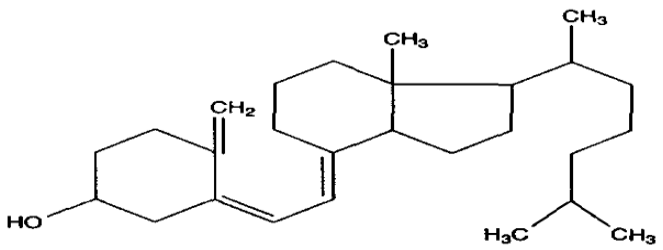


Fig. 182. Structure of vitamin D (cholecalciferol).

Vitamin D can be obtained through diet or synthesized from a cholesterol derivative, 7-dehydrocholesterol (Fig. 183). In the skin, this molecule undergoes photolysis by ultraviolet rays: the B ring is opened at the 9-10 bond. This inactive vitamin D3 (also called cholecalciferol) is transformed into calcitriol, the active vitamin, after two hydroxylations, first in the liver at C25 and then in the kidney at C1. The main role of vitamin D is to increase intestinal absorption of Ca2+, which is ultimately taken up by the bones.

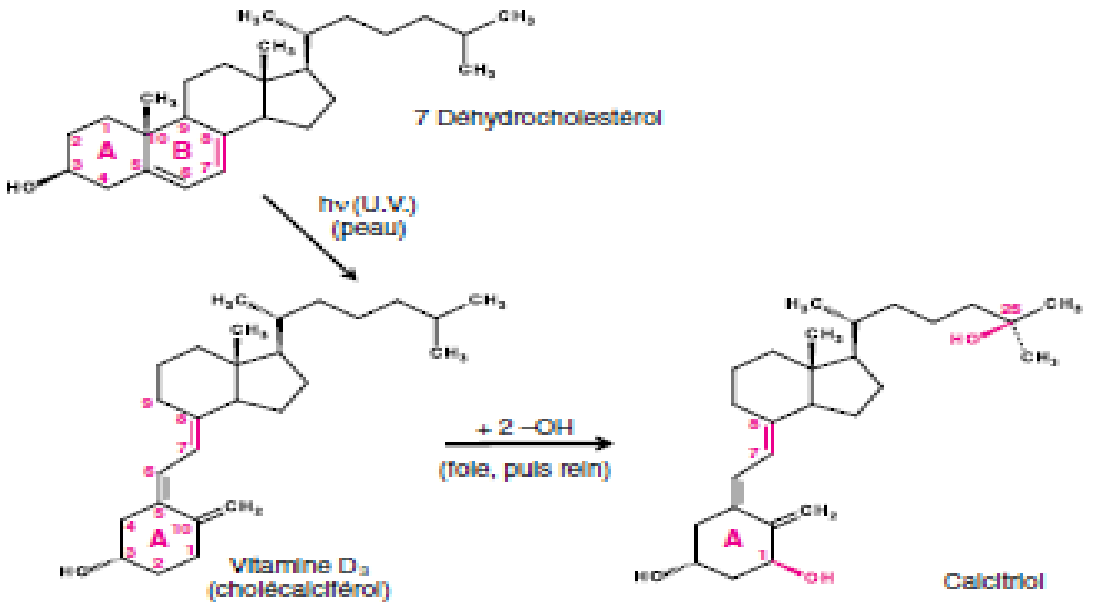


Fig. 183. Summary of the vitamin D from 7-dehydrocholesterol.

Vitamin D is derived from cholesterol. Initially, cholesterol undergoes a dehydrogenation reaction (which creates a second double bond in ring B). This yields 7-dehydrocholesterol. Under the influence of ultraviolet light (a photochemical reaction), ring B opens, resulting in vitamin D3.

The important characteristic is that the body is capable of carrying out this biosynthesis. It takes place in the dermis; this therefore constitutes an endogenous source, naturally dependent on sun exposure.

Like vitamin A, vitamin D is found in the liver. There, it undergoes a hydroxylation reaction (25(OH)D is formed because the new alcohol group is located on carbon 25). Then, thanks to a specific plasma transport protein (DBP), this molecule reaches the kidneys. There, it undergoes another hydroxylation reaction, resulting in the formation of calcitriol, the active form of vitamin D. Calcitriol is a hormonal factor that acts at three levels:

- At the intestinal level it stimulates the absorption of calcium and phosphorus by enterocytes (small intestine);
- at the level of bone tissue. Calcitriol acts on osteoclasts and especially on osteoblasts. Thus, it promotes bone tissue remodeling and the mineralization of growth cartilage.
- At the kidney level: it stimulates the tubular reabsorption of phosphorus and calcium. However, this action is less pronounced.

Calcitriol's overall role is to increase serum calcium and phosphate levels to facilitate bone tissue development. It acts synergistically with parathyroid hormone (produced by the

parathyroid glands), which also increases calcium levels, particularly as it stimulates renal hydroxylation, thus promoting calcitriol formation.

Calcitriol also stimulates placental transport of calcium and thus contributes to the mineralization of the fetal skeleton.

It also plays a role in the mammary glands by controlling the concentration of calcium in the milk.

Vitamin D is eliminated through feces (via bile). It is important to emphasize the risk of vitamin D overdose, which can lead to hypercalcemia. If repeated and severe, this can cause nervous and digestive disorders, as well as calcium deposits, particularly in the kidneys.

VI.1.2.3- Vitamin E (tocopherol)

This molecule is part of the phenols (aromatic ring with an alcohol function), also having a carbon side chain which enhances the hydrophobic behavior (Fig. 184).

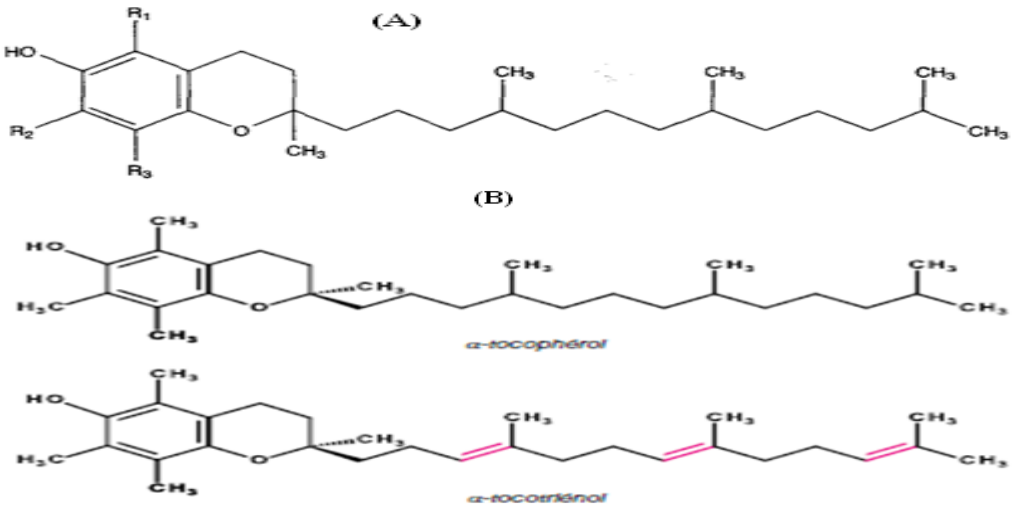


Fig. 184. Structure of vitamin E (A). Depending on R, R2, R3 (-H or -CH3), different forms of tocopherols are obtained (α,β,γ, δThe most common form being theα-tocopherol (R1 = R3 = -CH3) (B).

We need to talk about the vitamin E family, which includes the tocopherol and tocotrienol groups.α-Tocopherol, the most active vitamin in the first group, is an antioxidant; it inhibits the formation of reactive oxygen species (ROS) and prevents the peroxidation of polyunsaturated fatty acids.

Tocotrienols are molecules similar to tocopherols, from which they differ by the presence of three double bonds in the isoprene chain (hence their name, toco).*sorting*enols), which gives them increased solubility in non-polar environments and increases their protective power of polyunsaturated fatty acids.

This molecule is present in food in the form of tocopherol ester (cereal germ, sunflower oil, peanut oil…).

Its intestinal absorption (primarily in the jejunum) requires the action of bile salts and pancreatic lipase. Once inside the enterocyte, vitamin E is incorporated into chylomicrons and then passes into the lymph and bloodstream (via the thoracic vein). It is subsequently taken up by the liver and distributed to tissues via lipoproteins (LDL and HDL).

This vitamin can be stored in adipose tissue, the liver, and the adrenal glands.

Elimination occurs primarily through feces via bile secretion; a very small portion is eliminated through the urinary tract.

Vitamin E has an antioxidant role, meaning it can neutralize free radicals. Oxygen, while essential for life, is also responsible for the formation of molecules toxic to cells: free radicals. These are molecules with an odd number of electrons, which gives them a high reactivity, particularly towards membrane lipids (but also towards proteins and nucleic acids). Cytotoxicity is expressed, for example, at the level of unsaturated fatty acids. The presence of double bonds makes these molecules particularly sensitive to free radicals, which transform them into peroxides, thereby weakening plasma membranes.

Vitamin E's role is to stabilize cell membranes. This protection is particularly important for blood cells, platelets, and red blood cells (limiting hemolysis). Vitamin E's antioxidant action is also significant for LDL cholesterol. It limits its conversion into oxidized LDL (the most atherogenic lipoprotein).

Note that the action of vitamin E takes place in synergy with vitamin C, the latter participating in the regeneration of vitamin E following the neutralization of free radicals.

VI.1.2.4- Vitamin K

The vitamin K family includes chemically similar molecules that consist of a nucleus **naphthoquinone** and a polyisoprene chain (Fig. 185). The two main forms are:

- Vitamin K1 (phylloquinone), of plant origin (cabbage, spinach, parsley...);
- Vitamin K2 (menaquinone), especially of animal origin (liver, meat...).

Vitamin K is synthesized by plants. Furthermore, a unique characteristic of this vitamin is that it has an endogenous source, as the gut flora (microbiota) is capable of biosynthesizing it (in the form of vitamin K2). Through intestinal absorption, vitamin K is found in chylomicrons. It is then taken up by the liver, where there is a limited storage capacity. Distribution to the various tissues is carried out by LDL cholesterol.

It is eliminated through feces (via bile) and to a small extent through urine in the form of metabolites.

On a biochemical level, vitamin K acts as a cofactor for certain carboxylases. This notably concerns the carboxylation reactions of certain proteins at the level of the glutamic acid side chain, by increasing the protein's affinity for Ca2+ ions and thus inducing a biological effect.

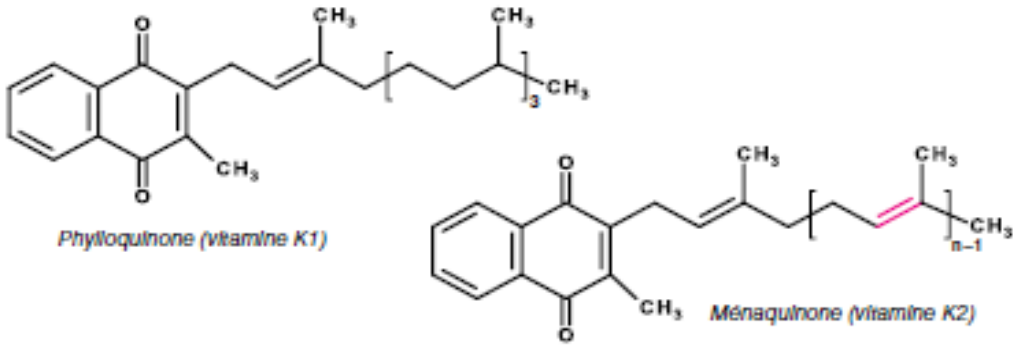


Fig. 185. Structure of vitamins K1 and K2.

Vitamin K deficiency causes bleeding, hence the name of the vitamin (K for the German koagulation).

Vitamin K is a precursor of γ-glutamyl carboxylase coenzymes, enzymes necessary for the post-translational modification of a group of proteins involved in blood clotting (prothrombin, coagulation factors VII, IX, and X). γ-carboxylglutamate residues allow these proteins to strongly complex with the Ca^{++} ion. Once these complexes are formed, these proteins bind to the membranes of blood platelets and endothelial cells and actively participate in the coagulation process.

The main physiological role of this vitamin is antihemorrhagic. It is essential for the functioning of certain coagulation factors (proteins undergoing carboxylation): prothrombin, proconvertin...

VI.2-Coenzymes

VI.2.1- Definition and properties

Coenzymes, or cofactors, are non-protein metabolites with a low molecular mass compared to that of enzymes. They have a simple structure and act at low concentrations. Like enzymes, they must be regenerated at the end of a reaction or sequence of reactions. They can be free or bound to the enzyme and are thermostable.

Coenzymes are introduced into the mammalian body through diet in the form of vitamins or growth factors.

Almost all known water-soluble and fat-soluble vitamins are involved in the structure of coenzymes except for vitamin C (water-soluble) and vitamin A (fat-soluble).

Coenzymes act as acceptors and transporters of radicals released during catalysis. They bind and transport:

- hydrogens and electrons in redox reactions.
- radicals other than hydrogen and electrons in other reactions.
- They transport the radical from compound A to compound B.

VI.22. Classification of coenzymes

The main coenzymes are listed in Table 25. They have diverse origins. Some coenzymes are synthesized from metabolic intermediates; these are mostly nucleotides or nucleotide derivatives. Other coenzymes are derived from vitamins.

VI.22.1 - Redox coenzymes

They participate in redox reactions by transporting hydrogen atoms in the form of electrons and protons (NAD+, FAD, etc.) or only electrons (cytochromes, etc.). They are found in all cellular redox reactions and in organized electron transport sequences such as respiration or photosynthesis. The enzymes that catalyze the reactions involving these coenzymes are dehydrogenases or reductases. These include: nicotinic or pyridine coenzymes, flavin coenzymes, quinonic coenzymes, metalloporphyrins, and iron-sulfur proteins.

Table 25: The main coenzymes.

Coenzyme	**Metabolic role**	**Vitamin origin**
Main activating coenzymes		
Flavin mononucleotide (FMN)	Redox reaction involving the transfer of one or two electrons	Riboflavine (Vitamin B2)
Flavin adenine dinucleotide (FAD)		
Thiamine pyrophosphate (TPP)	Aldehyde group transfer	Thiamine (Vitamin B1)
Pyridoxal phosphate (PLP)	Transfer of groups belonging to amino acids	Pyridoxine (Vitamin B6)
Biotin	Carboxylations and carboxyl transfer	Biotin (Vitamin H)
Adenosylcobalamin Methylcobalamin	Intramolecular rearrangements and methyl transfer	Cobalamin (Vitamin B12)
Lipoamide	Oxidation of a hydroxyalkyl group	Vitamin A
Cis-Retinal	Vision	Vitamin K
Vitamin K	Carboxylation of glutamate residues	
Main transport coenzymes		
Adenosine triphosphate	Methyl transfer	

(ATP)		
S-Adenosylmethionine	Sulfuryl transfer	
Nucleotidylated sugars	Transfer of carbohydrate groups	
Cytidylated alcohols	Alcohol transfer in lipid synthesis	
Nicotinamide adenine dinucleotide (NAD+) Nicotinamide adenine dinucleotide (NADP)	Redox reactions involving the transfer of two electrons	Niacin
Coenzyme A	Acyl transfer	Pantothenate
Tetrahydrofolate	Transfer of non-carbon groups	Folate
Ubiquinone	Electron transport	

HAS- Nicotinic or pyridine coenzymes

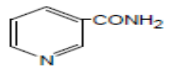
These coenzymes are derived from nicotinamide or vitamin PP and have a universal distribution since all cells contain them (Fig. 186). The two most represented types are: NAD+ (Nicotinamide Adenine Dinucleotide) and NADP+ (Nicotinamide Adenine Dinucleotide Phosphate).

Properties and structure

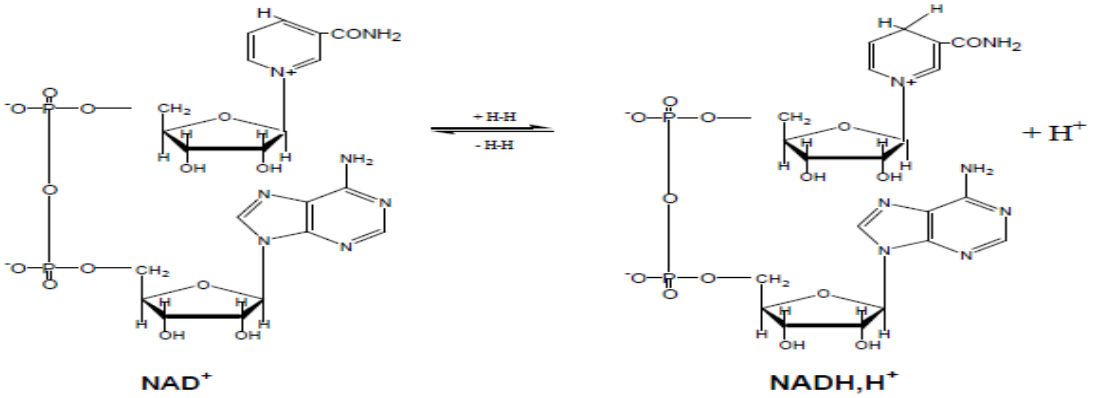
These are small, soluble molecules, found partly in a free state in mitochondria, chloroplasts, and the cytosol. They both participate in the redox reactions of alcohols to carboxylic acids and vice versa. The catalyzed reaction, starting from primary alcohols, is as follows:

$$R\text{-}CH_2OH + NAD(P)^+ \longleftrightarrow R\text{-}CHO + NAD(P)H,H^+$$

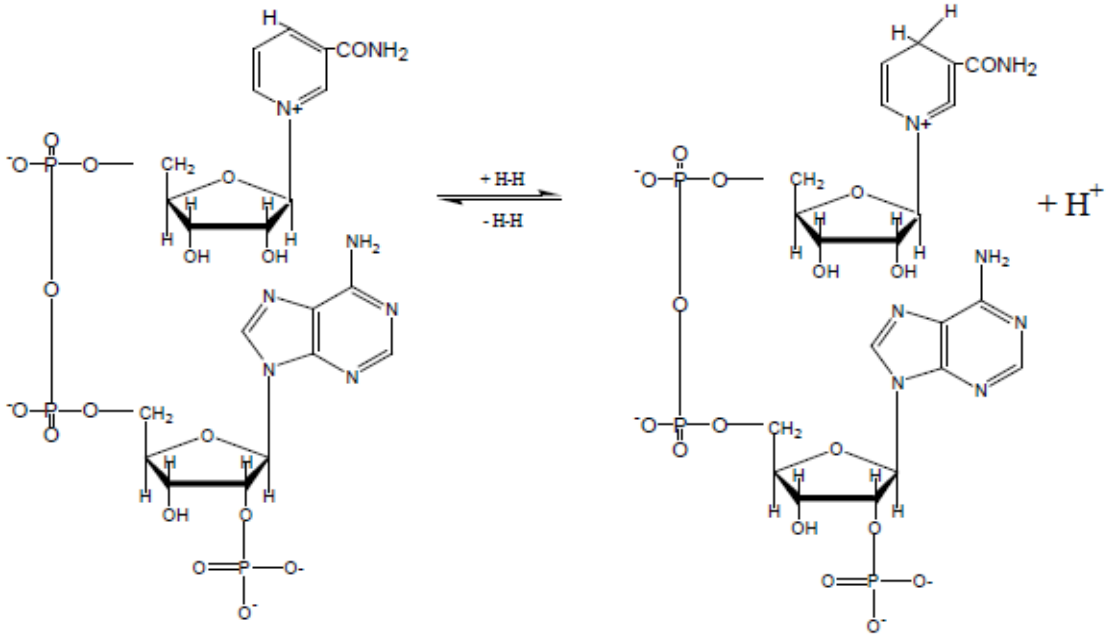
$$R\text{-}CHO + NAD(P)^+ + H_2O \longleftrightarrow R\text{-}COOH + NAD(P)H,H^+$$



Nicotinamide



NAD$^+$ **NADH,H$^+$**



NADP$^+$ **NADPH,H$^+$**

Fig. 186. Structure of the two nicotinic coenzymes derived from nicotinamide (Vit PP). The $NAD^+/NADH,H^+$ pair acts as an electron acceptor or donor in the redox reactions of catabolism. The $NADP^+/NADPH,H^+$ pair, in its reduced form, acts as an electron donor in biogenetic reactions.

NAD^+ generally occurs in oxidized form and takes charge of electrons and protons in catabolic dehydrogenations; in other words, NAD^+ is involved in degradation or catabolism systems leading to energy recovery.

$NADP^+$ often appears in its reduced form ($NADPH,H^+$) and is associated with dehydrogenases (reductases) in synthesis reactions. Within the cell, there are $NADH,H^+/NAD^+$ dehydrogenases and $NADPH,H^+/NADP^+$ dehydrogenases, although some cells accept both. These two pairs are rarely interchangeable because the products can be very different.

From a biochemical standpoint, niacin acts as a redox coenzyme. NAD (oxidized form) is involved in numerous important metabolic processes in association with dehydrogenases; it serves as a proton (2) and electron (2) acceptor in oxidation reactions.

- during glycolysis in association for example with glyceraldehyde 3-phosphate dehydrogenase;
- during the oxidative decarboxylation of pyruvate, to form acetylcoA;
- in the Krebs cycle, in association for example with isocitrate dehydrogenase;
- in the respiratory chain;
- in fatty acid catabolism, in association with theβ-OH acyl dehydrogenase;
- During lactic acid fermentation (in muscles during intense exercise), pyruvate is transformed into lactate. Here, it is $NADH,H^+$ (reduced form) that plays a role.

$NADP^+$, in association with dehydrogenases, plays a role in the pentose-phosphate pathway.
$NADPH,H^+$ serves as a coenzyme for reductases. It is involved in the biosynthesis of fatty acids and cholesterol. It also plays an important role in erythrocytes, in association with glutathione reductase.

B - Flavin coenzymes

Flavin coenzymes FMN and FAD are water-soluble, yellow compounds derived from vitamin B2 or riboflavin. They are linked to their apoenzyme by a covalent bond to form the holoenzyme structure known as flavoprotein (Fig. 187). We can distinguish:
- Flavin mononucleotide (FMN), which is the 5' phosphoric ester of riboflavin. It is membrane-bound and plays a role in electron transport in the respiratory chain;
- and the cytosolic flavin Adenine nucleotide (FAD), which has a 2nd nucleoside linked to the first by a pyrophosphate bond.

FMN and FAD are coenzymes of about fifty enzymes found in all living cells.

On a biochemical level, FMN and FAD play the role of redox coenzymes in association with dehydrogenases or oxidases. Coenzymes constitute the prosthetic groups of these

enzymes. The reactive part of FAD and FMN that participates in the redox reaction is the dimethyl-isoalloxazine ring of riboflavin (Figure 2).

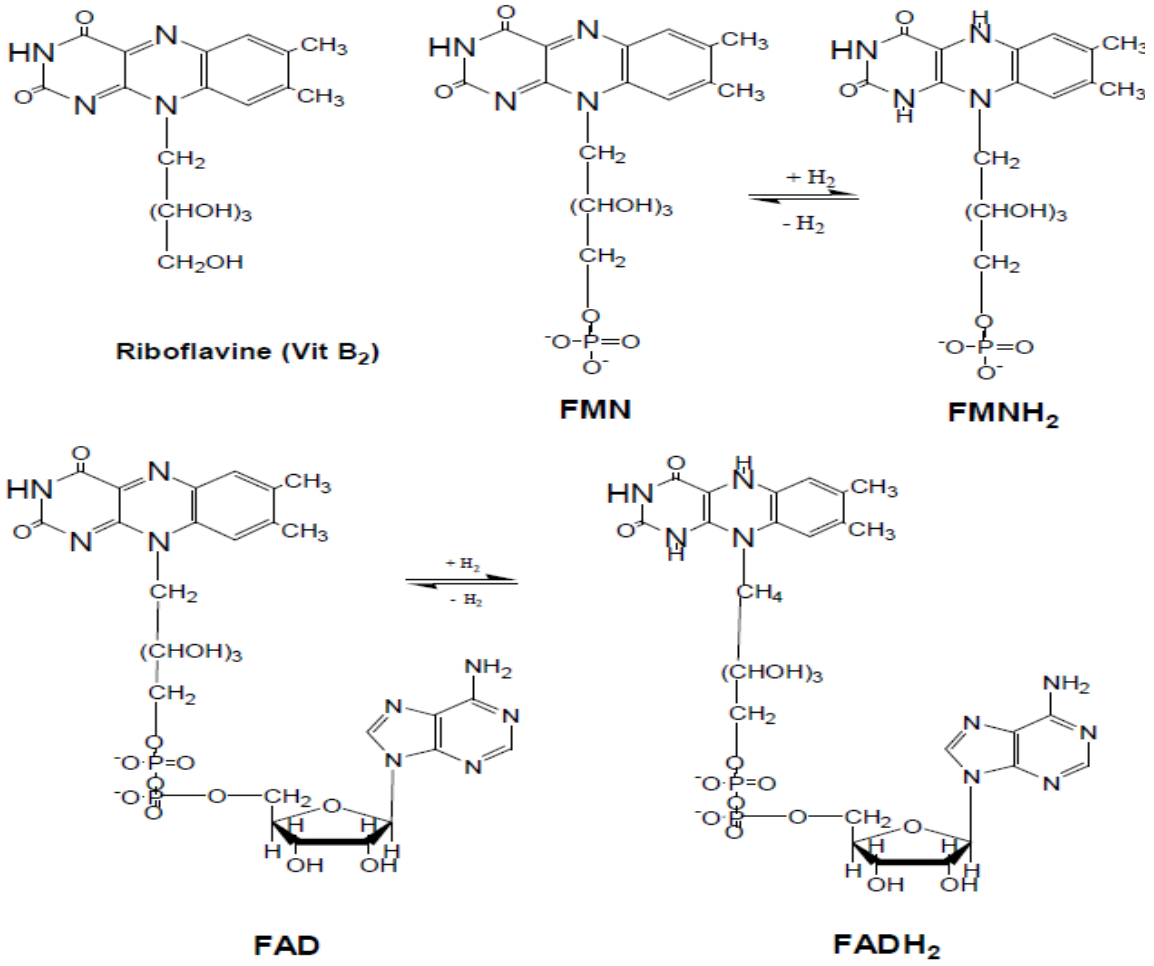


Fig. 187. Structure of the two flavin coenzymes derived from riboflavin (Vitamin B_2). The FMN/$FMNH_2$ pair is only involved in electron transport in the respiratory chain. The FAD/$FADH_2$ pair acts as an electron donor or acceptor in catabolic reactions.

FAD is involved in numerous metabolic processes:

- in the catabolism of fatty acids (β-oxidation), in association with acylCoA dehydrogenase;
- in the Krebs cycle, in association with succinate dehydrogenase allowing the oxidation of succinate to fumarate;
- in the respiratory chain (inner membrane of mitochondria);
- in the catabolism of amino acids by participating for example in the transformation of Leu, Ile into acetylCoA and of Val into succinylCoA;
- in the catabolism of purine bases, in association with xanthine oxidase, which contributes to the formation of uric acid.

The FMN intervenes at the level of the respiratory chain.

Mechanism of action

The isoalloxazine nucleus contains two conjugated double bonds capable of reversibly binding two hydrogen atoms. The oxidized form exhibits an absorption band at 450 nm, while the reduced form does not. These dehydrogenases can accept hydrogens from nicotinic coenzymes. A distinction is made between membrane flavoproteins, which are non-auto-oxidizable and have a complex structure, and cytoplasmic auto-oxidizable flavoproteins, which can transfer hydrogen directly to oxygen, forming hydrogen peroxide. For electron and proton transport, they sometimes require ions such as iron and molybdenum.

The main flavoproteins involved in the cell's energy metabolism are listed in the table below.

Table 26 : Main membrane flavoproteins.

Flavoproteins	Denomination	Coenzymes
NADH,H^+ -dehydrogenase	(FP1)	FMN
Succinate dehydrogenase	(FP2)	FAD
Dihydrolipoyl dehydrogenase		FAD
Acyl-CoA dehydrogenase	(FP3)	FAD
Glycerol 3-P-dehydrogenase	(FP4)	FAD

C - Quinonic coenzymes : ubiquinone and plastoquinone

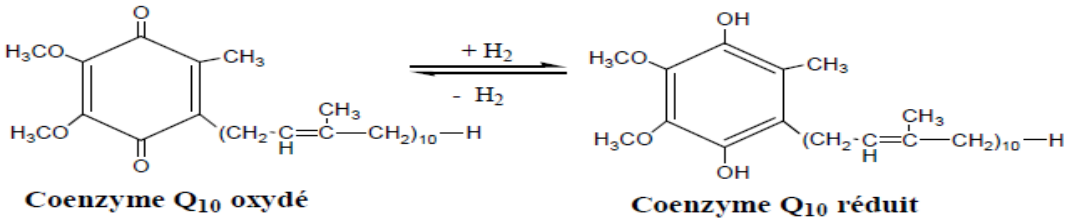
Ubiquinone, also called coenzyme Q10, is named for its very wide, ubiquitous distribution in plants, animals, and microorganisms (Latin ubique = present everywhere). 10 refers to the number of isoprene units (CH2-CH=C-(CH3)-CH2) present in the hydrophobic tail of the molecule (located in the membrane).

Ubiquinone is a lipid-soluble coenzyme that transports hydrogen from organic substrates to oxygen in the mitochondrial respiratory chain. It is not of vitamin origin and can be synthesized by all cells. It is not bound to a protein and can circulate freely in the phospholipid layer of the inner mitochondrial membrane.

Currently, several quinone coenzymes are known. They differ in the length of their side chain, which consists of n polymerized isoprene radicals. The number n is equal to 10 in ubiquinone, hence the name coenzyme Q10. In plants, there are quinones called plastoquinones that play a similar role in photosynthetic electron transport. $E°' = +0.1V$. Ubiquinone (oxidized form) has a characteristic absorption band between 270 and 290 nm, which disappears when the quinone is reduced.

Quinones possess two quinone functions which, by reduction, are transformed into dihydroquinone by acceptance of 2 electrons and 2 protons released by the substrate (Fig. 188).

Fig. 188 : Ubiquinone or Coenzyme Q10: Interconversion of the oxidized and reduced forms. This coenzyme acts as an electron acceptor in the respiratory chain.



D - Metalloporphyrins: cytochromes and peroxidases

Metalloporphyrins are true prosthetic groups linked to their respective apoenzymes by covalent bonds. Metalloporphyrins result from the union, in complex form, of an iron atom and a porphyrin (derived by substitution of the tetrapyrrole ring). They are found in cytochromes and peroxidases. They are classified as follows:

Cytochromes

Cytochromes are cellular pigments (chromos = color) detectable in the 300 to 600 nm range of the light spectrum. The term refers to a complex consisting of a protein and a prosthetic group (hemochrome) responsible for the characteristic absorption of light.

These chromoproteins are present in all cells, in the inner mitochondrial membrane, in the thylakoid membrane of chloroplasts, and in the cytoplasm. The ferroprotoporphyrin prosthetic group is the same (Fig. 189). Only the apoenzymes, which are firmly attached to them by covalent bonds, differ (with molecular masses ranging from 13,000 to 250,000) and give them different redox potentials. They play a role in the sequential transport of electrons through the change in iron valence. In the mitochondria of animal and plant cells and in chloroplasts, a large number of cytochromes have been isolated and identified, all functioning on the same principle. It is the iron of the prosthetic group that transports electrons by reversible conversion from ferric to ferrous iron (Fig. 189):

$$\text{Cyt b-Fe}^{+++} + e^- \longleftrightarrow \text{Cyt b-Fe}^{++}$$

During electron transport, the order of intervention of cytochromes is determined by their redox potential.

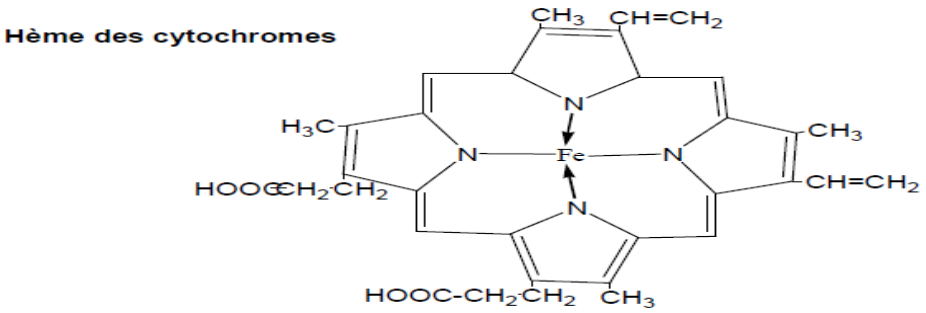


Fig.189. Structure of the heme of cytochromes. Cytochromes are present in all cells. They are involved in the transport of electrons in the respiratory chain and in photosynthesis by changing the valence of iron.

Catalases and peroxidases

These are also chromoproteins in which the iron remains in its ferric form and does not change its valence. They contain four hematoxylin and hexavalent ions and often copper. Catalases catalyze the decomposition of hydrogen peroxide: $H2O2 \rightarrow H2O + ½ O2$

Peroxidases oxidize certain substrates using a peroxide such as hydrogen peroxide.

Oxygenases

These metalloproteins catalyze the incorporation of an atom (monooxygenases) or a Oxygen molecules (dioxygenases) bind to a substrate. Some are cuproproteins and ferroproteins (non-heme iron). An example is tyrosine oxygenase and dopamine β-hydroxylase, which participates in the formation of norepinephrine in the adrenal medulla.

Iron-sulfur proteins

These iron-sulfur proteins are involved in electron transport sequences in both the respiratory chain and photosynthesis. The iron is non-heme and the sulfur is in sulfide form. Iron and sulfur are present in equimolar quantities. The apolipoprotein is linked to the iron via cysteines. The figure shows an example of the non-protein portion of these iron-sulfur proteins. These iron-sulfur proteins behave as subunits of a protein complex. Electron transport also occurs through a reversible change in iron valence from ferric to ferrous.

VI.22.2 - Coenzymes for the transport of one-carbon radicals

The one-carbon radicals that can be transported by their specific coenzymes are: CO_2, $-CH_3$, -CHO, $-CH_2OH$, etc. Among some coenzymes frequently encountered in this course on metabolism, we can distinguish:

A - CO_2 transport coenzyme

CO_2 is the most oxidized form of carbon. It is formed abundantly in the cell following the oxidation of the terminal carbons of an organic molecule, particularly under the action of dehydrogenases. The final reaction is a spontaneous decarboxylation reaction in organic acids with a ketone group at the β position (carbon 3). Decarboxylation is a common reaction in animal and plant cells. The reverse reaction, carboxylation, is less frequent and requires energy and a transporter. Examples include the carboxylation of pyruvate and acetyl-CoA, necessary for gluconeogenesis and fatty acid synthesis. A carboxylation reaction that plays a major role in the metabolism of chlorophyll-containing plants is that of ribulose-1,5-bisphosphate, which is responsible for the synthesis of carbohydrates from CO_2. Two coenzymes serve as transporters: biotin for small molecules and vitamin K for proteins.

Biotin or vitamin H

Biotin is a coenzyme that is joined to the apoenzyme by a covalent amide bond (between -COOH of the biotin side chain and $-NH_2$ of a lysine of the apoenzyme) to a lysine residue (Fig. 190). It is formed of 2 fused heterocycles, one containing 2 nitrogen atoms and the other one sulfur atom.

From a biochemical standpoint, biotin acts as a CO_2 transfer coenzyme for carboxylation reactions. Here are the three main examples involving biotin-dependent carboxylases:

- Pyruvate carboxylase is a mitochondrial enzyme that enables the transformation of pyruvate into oxaloacetate. This reaction is used for gluconeogenesis but also for the citrate-malate-pyruvate shuttle, required for the biosynthesis of fatty acids or cholesterol;
- Acetyl-CoA carboxylase is a cytosolic enzyme that catalyzes the first step in fatty acid biosynthesis;
- Propionyl-CoA carboxylase is a mitochondrial enzyme that converts propionyl-CoA to succinyl-CoA (via methylmalonyl-CoA), which can then enter the Krebs cycle for either energy production or gluconeogenesis. Propionyl-CoA is derived from the catabolism of odd-numbered fatty acids and certain amino acids (Val, Ile, Met, Thr).

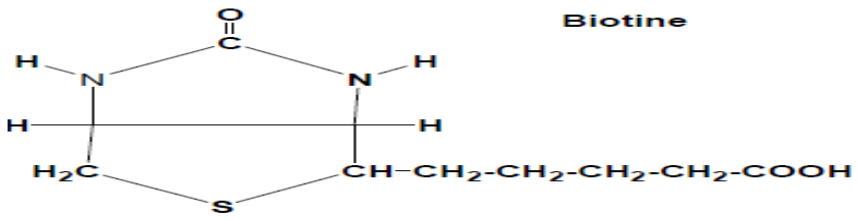


Fig. 190. Structure of biotin.

VI.22.3 - Coenzymes for transporting radicals other than CO_2

A - S-Adenosyl methionine

This coenzyme is derived from methionine, an essential amino acid obtained through diet. It is the active transport and binding form of the methyl group ($-CH_3$). It is formed from the reaction of ATP with methionine. This is the only reaction in which ATP loses its three phosphate groups (Fig. 191). It is a methyl donor in reactions catalyzed by methyltransferases, which attach methyl groups to suitable acceptors such as:

- nucleic acids
- proteins
- colamines to form cholines

- nicotinamide to form methylnicotinamide, the elimination form of this vitamin.

The loss of the methyl group transforms the cofactor into S-Adenosyl-Homocysteine.

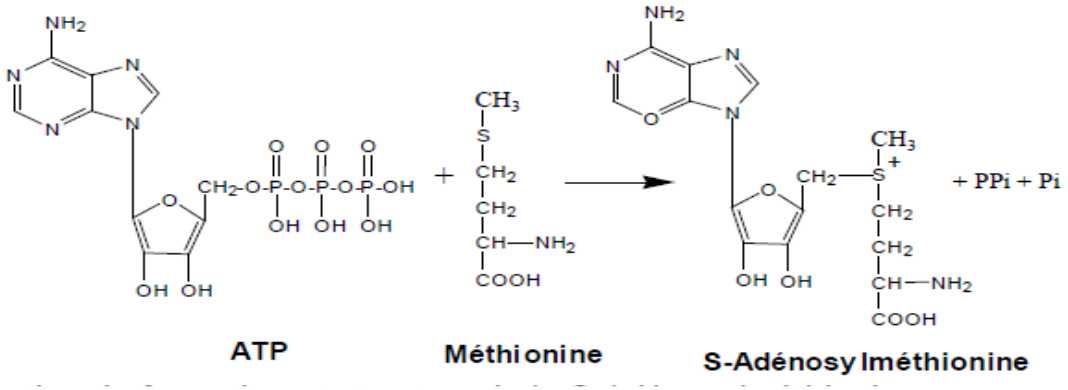


Fig. 191. Formation reaction and structure of S-Adenosylmethionine.

B - Folic acids (folates)

In fact, folic acids are numerous and are found mainly in leaves. They contain two nuclei that animals and some microorganisms cannot synthesize. These are the pterin nuclei and the p-aminobenzoic radical. Together with their apoenzyme, they constitute pteroproteins (Fig. 192).

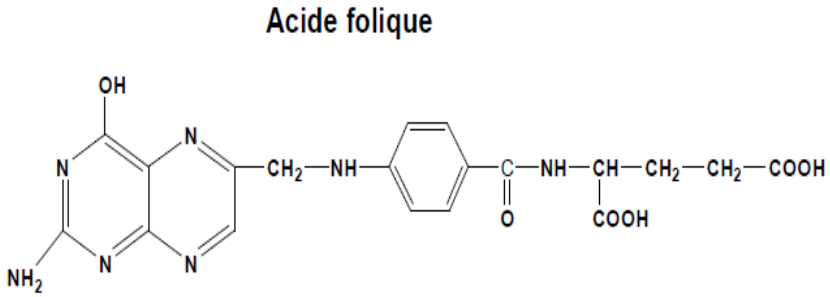


Fig. 193. Folic acid in its tetrahydrofolic form is a carrier of one-carbon radicals other than CO2.

Tetrahydrofolic acid (THF or FH_4) is the activated form and functions as a cofactor in many enzymatic systems that transfer radicals to a carbon other than CO2. The methyl group is transported to nitrogen 5, which can then be transferred to deoxyribouridine to form deoxythimidine. The coenzyme can also transport the formyl group to nitrogen N10, according to the formula above. The synthesis of the purine ring and nucleic acids depends on transformylation reactions (transport of –CHO).

Enzymes that function with folic acid coenzymes participate in amino acid metabolism, notably through interconversion reactions:

- catabolism of histidine, leading to the formation of glutamic acid;
- transformation of serine into glycine.

This coenzyme is also involved in the biosynthesis of proteins (during the initiation reaction) and nitrogenous bases (purines and pyrimidines), therefore of nucleic acids (DNA, RNA).

VI.22.4 - Coenzymes for transporting radicals with two or more carbon atoms

These are the coenzymes responsible for transporting radicals: acyl, aldehyde, carboxyl, etc. We will primarily study three important coenzymes given their involvement in the energy metabolism of carbohydrates. Their order of action in the transformation of pyruvate into acetyl~CoA will allow us to illustrate the concept of reaction sequence and that of the multi-enzymatic complex.

A - Thiamine pyrophosphate (TPP)

This coenzyme (prosthetic group) is derived from thiamine (vitamin B1). Thiamine is produced in a wide variety of bacteria and plants. It contains a pyrimidine ring and an imidazole ring (Fig. 193).

TPP acts as a transfer coenzyme. It serves as a coenzyme in decarboxylation reactions (thepyruvate decarboxylase, theα-ketoglutarate decarboxylase) acidsα-ketones, which take place in the mitochondrial matrix through enzymatic complexes. It is also the coenzyme of transketolase, an enzyme in the pentose phosphate pathway. During the reactions, the carbons of the ketone groups form a covalent bond with carbon 2 of the thiazole ring.

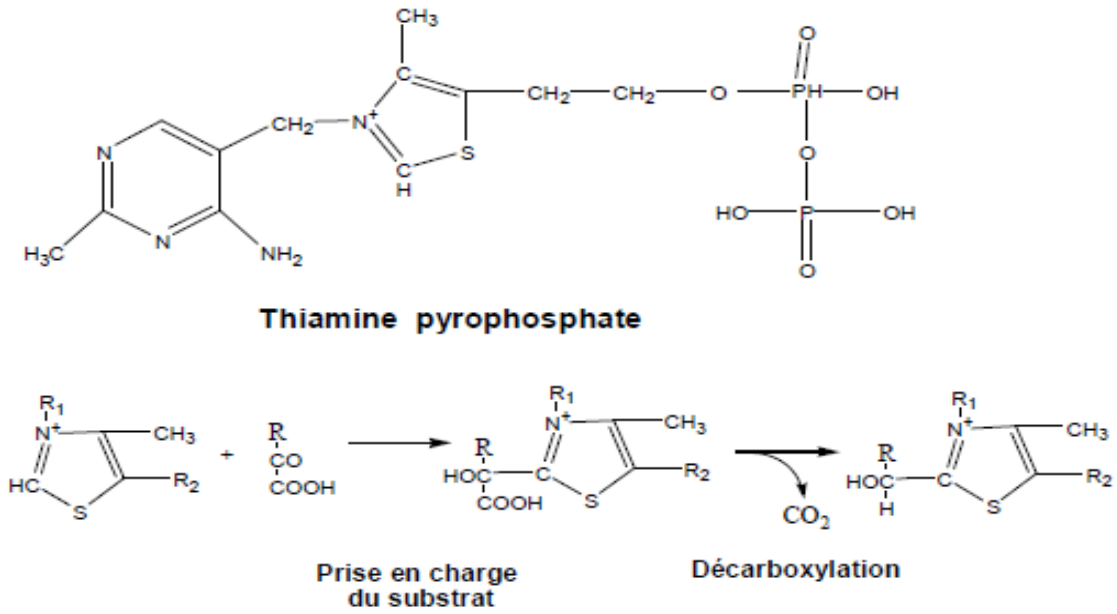


Fig. 193. Structure of thiamine pyrophosphate, a coenzyme derived from vitamin B1. Mechanism of action of dehydrogenase at the level of its coenzyme, a prosthetic group.

One important example is the transformation of pyruvate into acetyl-CoA by pyruvate dehydrogenase. It is possible to break down this reaction to appreciate the role played by TPP (Fig. 194).

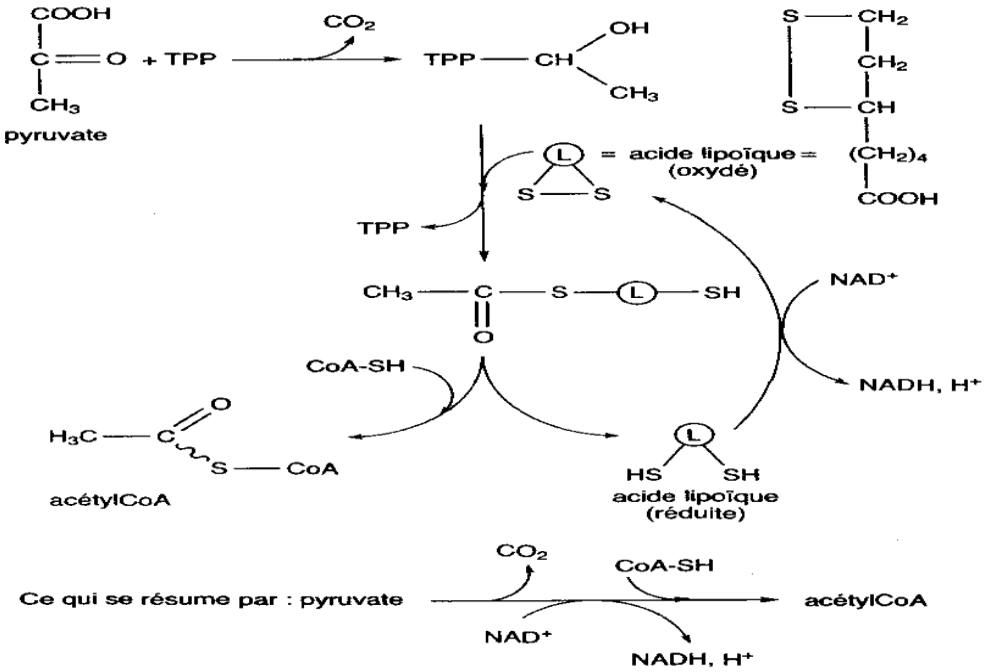
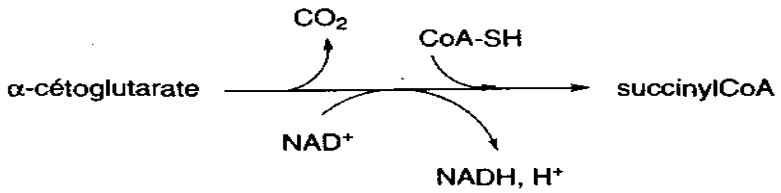


Fig. 194. Role of TPP in the oxidative decarboxylation of pyruvate.

This reaction involves three vitamin-derived coenzymes: TPP (vitamin B1); CoA-SH (vitamin B5) and NAD^+ (vitamin B3).

Similarly, TPP intervenes at the Krebs cycle level with theα-ketoglutarate dehydrogenase.



α-ketoglutarate dehydrogenase (+ TPP).

TPP acts as a coenzyme in the transketolization reactions of the pentose phosphate pathway. It also plays a role in the decarboxylation reactions of certain amino acids. For example, it contributes to the transformation of Leu and Ile into acetyl-CoA, and Val into succinyl-CoA.

B - Lipoic acid and lipoamide

Lipoic acid is an 8-carbon fatty acid with a disulfide bridge between carbons 6 and 8 (Figure 10). It is tightly bound to its apoenzyme. The resulting holoenzyme is called a lipoamide.

As previously mentioned, it acts as an acceptor of acyl radicals donated by thiamine pyrophosphate (Fig. 195). In the case of reactions starting from pyruvate or L'α-ketoglutarate, the transported radical is an acetyl or a succinyl. The component of the enzyme complex responsible for transporting these radicals to lipoate is the enzyme (E1) that initiates the sequence. Lipoate is not the final acceptor of acyl radicals. A second component (E2) of the complex, dihydrolipoyl acyltransferase, transfers the acyl radical to coenzyme A. Lipoate is reoxidized by component (E3), FAD-dependent dihydrolipoyl dehydrogenase, which ultimately transfers the electrons and protons removed from pyruvate or theα-ketoglutarate on NAD+.

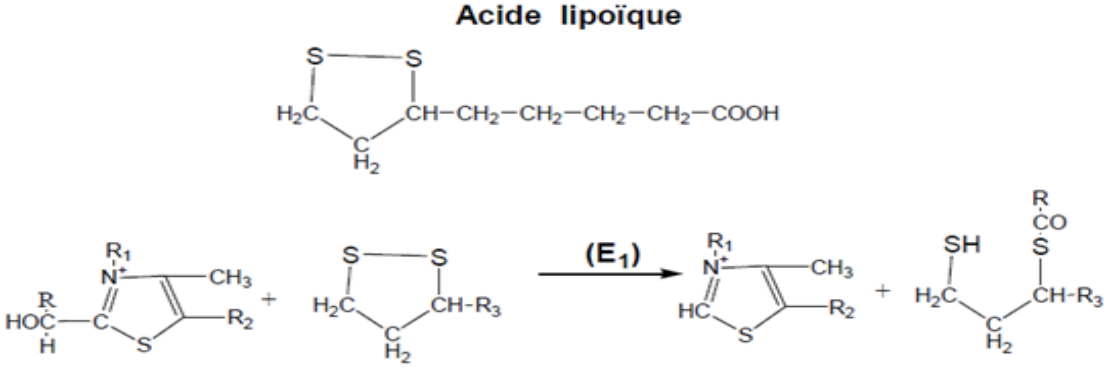


Fig. 196. Structure of lipoate and transfer reaction of radicals from thiamine pyrophosphate onto lipoate.

C - Coenzyme A (HSCoA)

This coenzyme is present in all cells. Its structure is similar to that of a dinucleotide (Fig. 197). It corresponds to the association of a pantothenic acid molecule which is the vitamin factor (not synthesized by animals) with adenosine 5'-diphosphateATP (with a particular structure since the third phosphate is separated from the other two) and a cysteamine or mercaptoethylamine (obtained by decarboxylation of cysteine). This thiol group can condense with a substrate, most often with a carboxyl group, leading to the formation of a thioester bond with a high energy potential (-CO~SCoA) making subsequent reactions thermodynamically possible (Fig. 197).

It acts as an activator of fatty acids in degradation reactions. Simultaneously, it plays the role of an acyl radical transporter (R-CO-). It is capable of making them soluble in the cytoplasm.

CoA-SH itself participates in numerous metabolic intermediates, of which the following are important examples:

- **acetyl-CoA,** a true metabolic crossroads, it is involved in most metabolic pathways (Krebs cycle, fatty acid metabolism, ketone body metabolism, catabolism of certain amino acids…);
- **malonylCoA** originates from the first step of fatty acid biosynthesis. More than a simple metabolic intermediate, it contributes to the regulation of fatty acid metabolism (allosteric regulation);
- **succinylCoA,** an important intermediate in the Krebs cycle, it also participates in the catabolism of ketone bodies, the biosynthesis of heme, and gluconeogenesis;
- **the HMG-CoA** is an intermediate in ketogenesis and cholesterol biosynthesis.

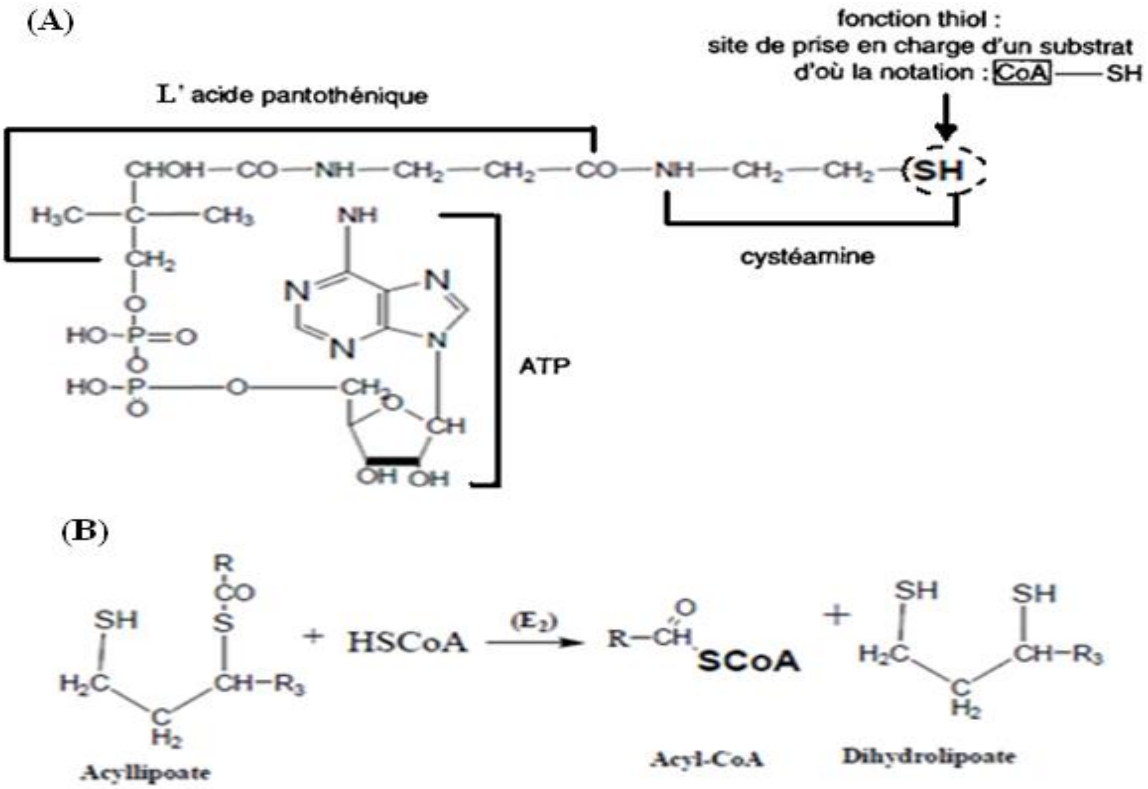


Fig. 197. (A) Structure of coenzyme A (CoA-SH). (B) Transfer reaction of acyl radicals from acyllipoate to coenzyme A

VI.2.5 - Coenzyme of aminotransferases: pyridoxal phosphate

It is a prosthetic group that is covalently linked to its apoenzyme, with which it forms an aromatic imine Schiff base. During catalysis, it also forms a Schiff base with the amino acid. Its structure is derived from vitamin B6, which includes pyridoxine, pyridoxamine, pyridoxal, and pyridoxal phosphate. The active coenzyme is pyridoxal phosphate (Fig. 198).

From a biochemical point of view, the Pyridoxal phosphate (PLP) is a coenzyme that acts as a coenzyme in many nitrogen metabolism reactions. It is common to all aminotransferases acts:

• in association with transaminases (Nitrogen metabolism);

• during the oxidative deamination reactions of amino acids, resulting in the formation of acidα-ketone and NH3

• with decarboxylases, allowing the formation of biogenic amines from an amino acid;

• during the transformation of tryptophan (Trp) into vitamin B3 or serotonin;

• during heme biosynthesis.

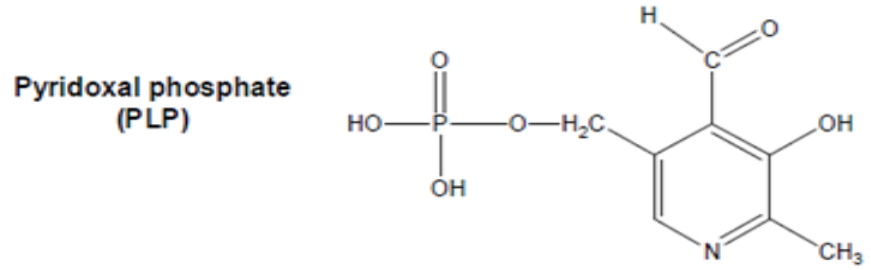
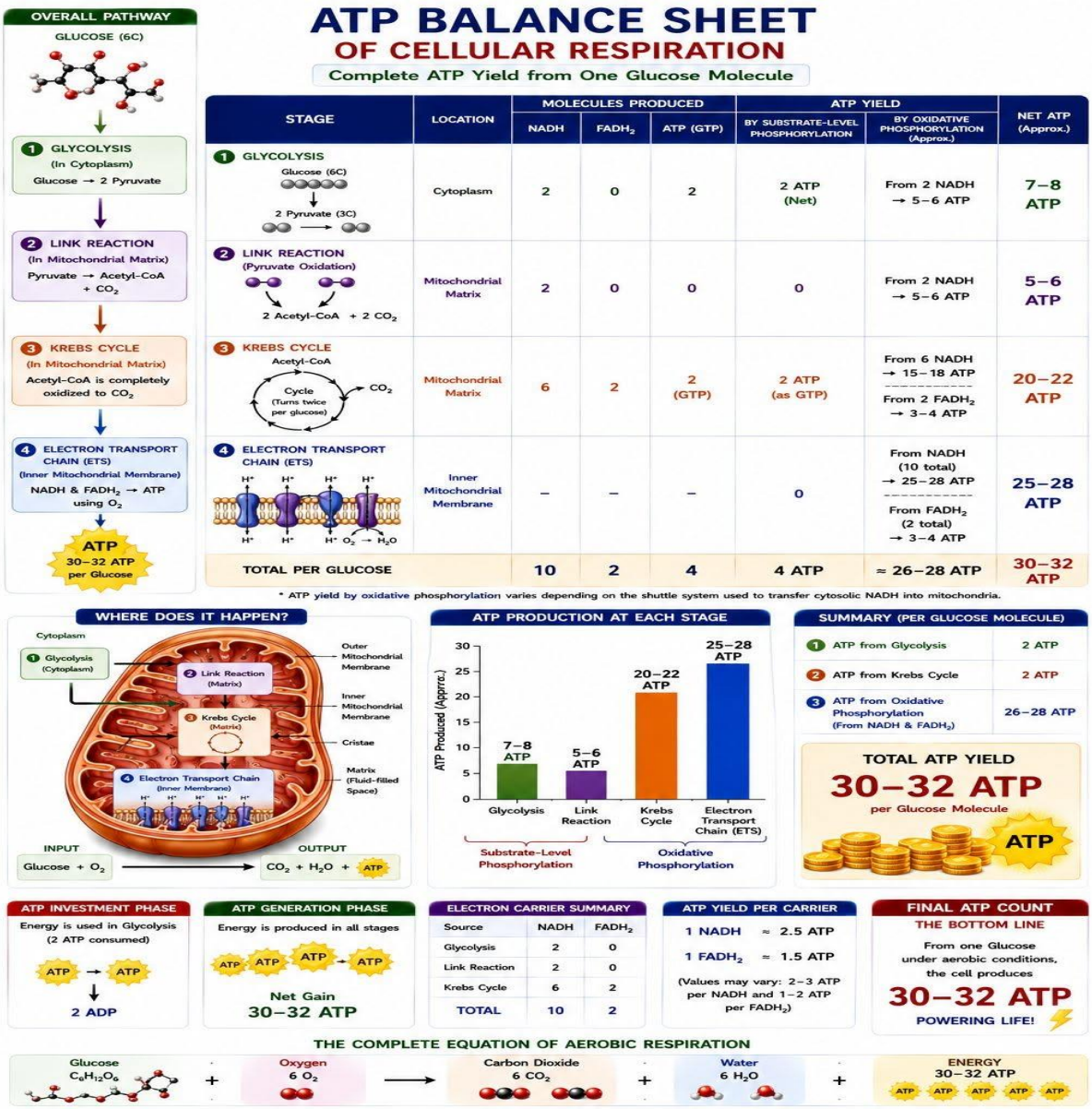


Fig. 198. Structure of pyridoxal phosphate (PLP), coenzyme group prosthetic aminotransferases.

CHAPTER VII :

ENERGY OF BIOCHEMICAL REACTIONS



CHAPTER VII ENERGY OF BIOCHEMICAL REACTIONS

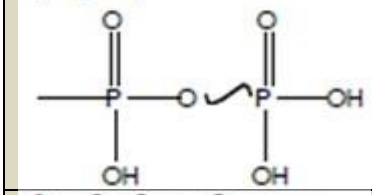
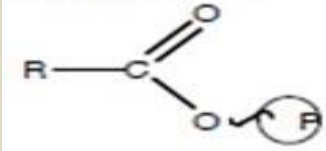
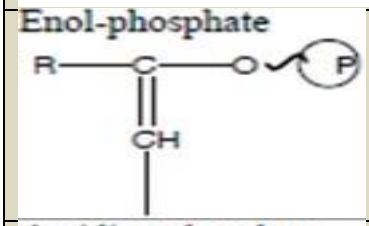
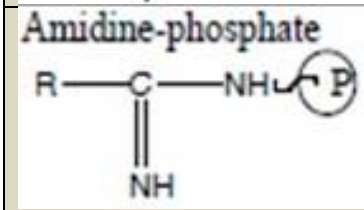
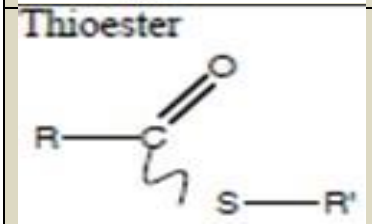
Bioenergetics studies energy exchanges in living organisms. At the heart of these transformations are the ATP/ADP or ATP/AMP pairs, which transfer energy from reactions that supply it to reactions that require it.

VII.1. Energy-rich compounds

VII.1.1. Definition

A compound which contains one or more bonds whose hydrolysis releases a large amount of energy (ΔG°' < - 21 kJ.mol-1) is called an energy-rich compound (Table 27) or a compound with a high hydrolysis potential.

Table 27: Other energy-rich compounds.

Connection type	Examples of compounds	Reaction	AG° (kJ.mol-1)
Pyrophosphate 	Nucleosides triphosphate	NTP + H_2O ⇔NDP+Pi	-30
	Nucleosides triphosphate	NDP + H_2O ⇔NMP+Pi	-30
Acyl-phosphate 	1.3 bisphosphoglycerate	1,3 biphosphoglycérate + H_2O ⇔ 3-phosphoglycérate + Pi	-42
Enol-phosphate 	Phosphoenolpyruvate te	Phosphoénolpyruvate + H_2O ⇔ pyruvate + Pi	-54
Amidine-phosphate 	Creatine phosphate	Créatine- phosphate + H_2O ⇔ créatine + Pi	-44
Thioester 	AcetylCoA	AcétylCoA + H_2O ⇔ CoASH + acétate	-37

VII.1.2. ATP

Adenosine Triphosphate (ATP) is the main compound with high energy potential. It has two high-energy bonds (Fig. 199).

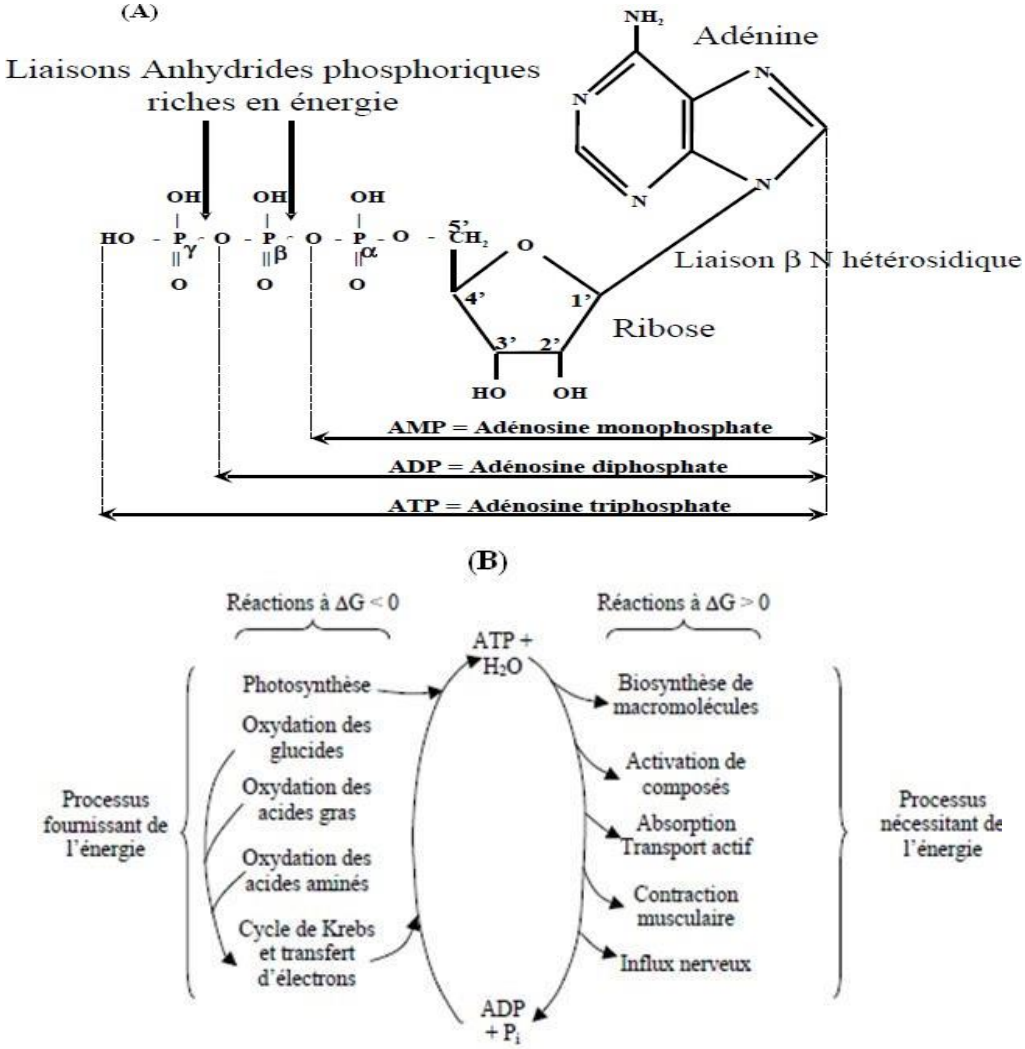


Fig. 199. (A) Structure of the ATP molecule. (B) Role of ATP in energy transfer.

ATP is hydrolyzed in several ways:

$ATP + H_2O \rightarrow ADP + Pi$ $\Delta G°' = -30\ kJ.mol^{-1}$

$ADP + H_2O \rightarrow AMP + Pi$ $\Delta G°' = -30\ kJ.mol^{-1}$

$ATP + H_2O \rightarrow AMP + PPi$ $\Delta G°' = -30\ kJ.mol^{-1}$

$AMP + H_2O \rightarrow adenosine + Pi$ $\Delta G°' = -10\ kJ.mol^{-1} > -21\ kJ.mol^{-1}$

AMP is recycled to ADP by adenylate kinase: **AMP + ATP → 2 ADP**

ADP is then phosphorylated into ATP during oxidative phosphorylation (respiration) or photosynthesis (photophosphorylation).

VII.2. Phosphorylant oxydations

VII.2.1. Generalities

The $FADH_2$ and NADH are reduced coenzymes produced in the mitochondrial matrix during various oxidation steps (Krebs cycle, fatty acid oxidation, etc.) and also in the cytoplasm during glycolysis. Since NADH molecules generated during glycolysis cannot enter the mitochondrial matrix, electrons are transferred there via two shuttle systems: the glycerol phosphate shuttle and the malate-aspartate shuttle.

Reduced coenzymes are reoxidized in the respiratory chain located in the inner mitochondrial membrane of eukaryotic cells. This reoxidation is accompanied by the formation of a proton gradient, which will be used for the formation of ATP, hence the name oxidative phosphorylation.

Therefore, oxidative phosphorylation is the process by which ADP is phosphorylated into ATP using the energy released by the oxidation of electron donors by the respiratory chain.

This process is common to almost all aerobic organisms, despite the fact that they feed on a wide variety of different nutrients that are oxidized through very diverse metabolic pathways. It allows cells to regenerate coenzymes reduced by catabolic reactions and to recover, in the form of ATP, the energy released by cellular respiration. Its energy efficiency is significantly better than that of glycolysis or anaerobic fermentation.

Oxidative phosphorylation is a vital metabolic function. However, it generates reactive oxygen species such as the superoxide anion $O2^{\bullet-}$ or hydrogen peroxide H_2O_2, which in turn produce harmful free radicals.

VII.2.2. The respiratory chain

The respiratory chain is part of oxidative phosphorylation. It catalyzes the stepwise transport of electrons from NADH or reduced ubiquinone (QH_2) to molecular oxygen.

The inner mitochondrial membrane (Fig. 200A), particularly the cristae, contains three respiratory enzyme complexes: NADH-Q oxidoreductase (Complex I), Q-cytochrome c oxidoreductase (Complex III), and cytochrome c oxidase (Complex IV), which pump protons from the matrix into the intermembrane space. These complexes are associated with two mobile transporters, ubiquinone (coenzyme Q) and cytochrome c, and together they constitute the mitochondrial respiratory chain (Fig. 200B).

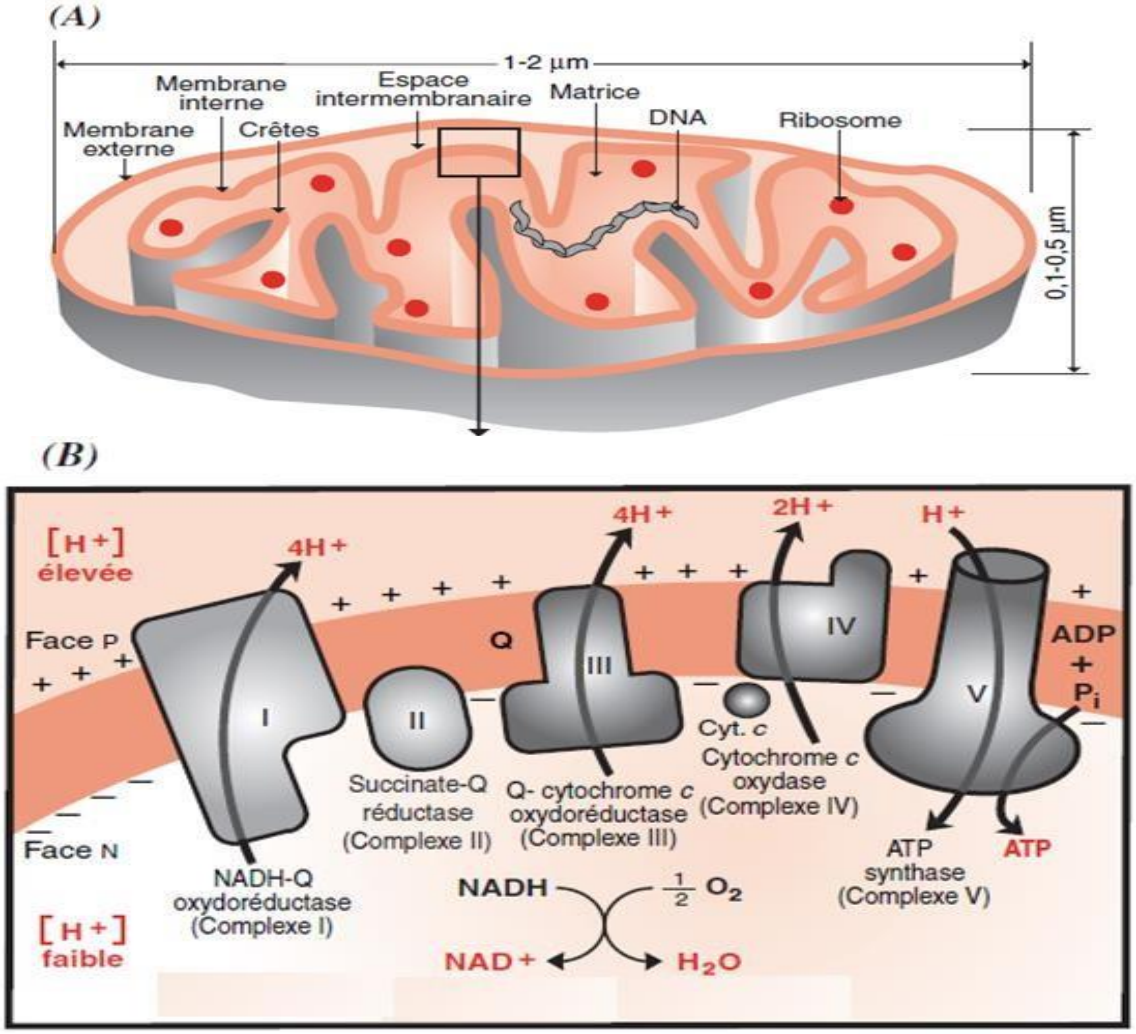


Fig. 200. (A) Structure of the mitochondrion. (B) The mitochondrial respiratory chain.

The inner membrane also contains succinate-Q reductase (or Complex II), which transports electrons from $FADH_2$ of succinate dehydrogenase in the citric acid cycle to ubiquinone but does not pump protons. Two other enzymes, acyl CoA dehydrogenase and glycerol phosphate dehydrogenase, similarly transfer high-potential electrons from $FADH_2$ to Q to form QH_2. Finally, the inner membrane possesses ATP synthase (or Complex V).

- **Complex I or NADH-ubiquinone oxidoreductase:** This complex transfers electrons from NADH to the coenzyme FMN. The electrons are then transferred to ubiquinone (CoQ) via iron-sulfur (Fe-S) proteins. This complex is specifically inhibited by rotenone (Table 28).
- **Complex II or succinate-ubiquinone reductase:** This complex possesses succinate dehydrogenase activity, which catalyzes the transfer of electrons from succinate to CoQ using FAD as a coenzyme. This complex is inhibited by malonate (Table 28).
- **Complex III or ubiquinol-cytochrome c reductase:** This complex transfers electrons from ubiquinol or reduced coenzyme Q ($CoQH_2$) to cytochrome c via thecytochromes b and c1. This complex is inhibited by antimycin A (Table 28).
- **Complex IV or cytochrome-c oxidase:** This complex transfers electrons from cytochrome c to oxygen via cytochromes a and a3. This complex is inhibited by cyanide, azide or carbon monoxide (Table 28).

Complexes I, III and IV are proton pumps, meaning that the transfer of electrons is accompanied by the transfer of protons from the matrix to the intermembrane space.

- Two mobile elements are also present. These are ubiquinone or coenzyme Q, a membrane lipid that can exist in oxidized or reduced form, and cytochrome c, a water-soluble protein located on the outer face of the inner mitochondrial membrane.

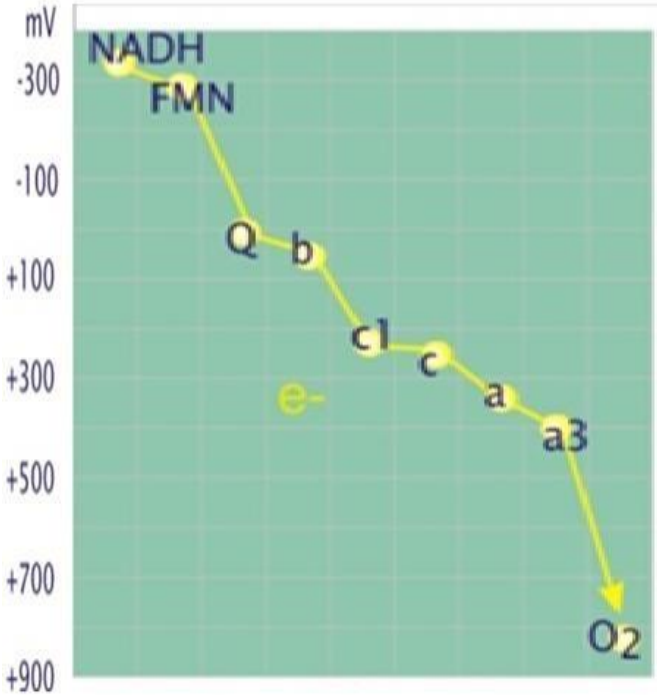
Table 28 below gives an idea of the size and heterogeneity of the respiratory chain complexes.

Table 28: Enzymatic functions and structural characteristics of respiratory chain complexes.

	Enzyme function	**Molecular weight (kDa)**	**Number of subunits**
Complex I	**NADH-quinone oxidoreductase**	**800**	**25**
Complex II	**Succinate-quinone oxidoreductase**	**140**	**4**
Complex III	**Quinol-cytochrome oxidoreductase**	**250**	**9-10**
Complex IV	**Cytochrome c oxidase**	**170**	**13**
Complex V	**ATP synthase**	**380**	**12-14**

I.2. 3. Functioning of the respiratory chain

The direction of electron flow along the transport chain is determined by the ability of the components to lose or gain electrons. For example, NAD^+ has a moderate affinity for electrons, so its reduced form, NADH ($+H^+$), can readily donate its two electrons. In contrast, molecular oxygen has a very high affinity for electrons and is therefore a very good oxidizing agent, readily capturing electrons from other molecules. The ability to "give away" or "take away" electrons is expressed by a parameter called the redox potential (symbol **Eò**), with NAD^+ having a potential of -320 mV and molecular oxygen a potential of +816 mV. Electrons move from molecules with a low redox potential to molecules with a high potential; and thus, in the case of the mitochondrial electron transport chain, from NADH to molecular oxygen (Fig. 201).



composant de la chaîne respiratoire		Eò (mV)
NADH		-320
complexe I	flavine mononucléotide	- 300
	ubiquinone	+002
complexe III	cytochrome b	+030
	cytochrome c1	+225
	cytochrome c	+235
complexe IV	cytochrome a	+310
	cytochrome a3	+385
O_2		+836

Fig. 201. The redox potentials of the components of the respiratory chain.

Reduced coenzymes (NADH and $FADH_2$) release their electrons at the level of the complexes I or II. At this level, hydrogen atoms are dissociated into protons and electrons. The released electrons are transferred to complex III by coenzyme Q, then to complex IV by cytochrome c. Electrons are transferred from systems with the lowest redox potentials (for NADH) to the highest (for dioxygen) and is accompanied by the formation of a proton gradient on either side of the membrane (Fig. 202).

The electrochemical gradient of H^+ protons generates a **proton-motive force** (a combination of the electrical potential difference and the proton gradient) between the two sides of the membrane, which is used for the synthesis of ATP from ADP and Pi. This synthesis is catalyzed by complex V of the respiratory chain, or the **ATP synthase-ATPase complex**. This mechanism was proposed by Peter Mitchell under the name of the **chemiosmotic theory**.

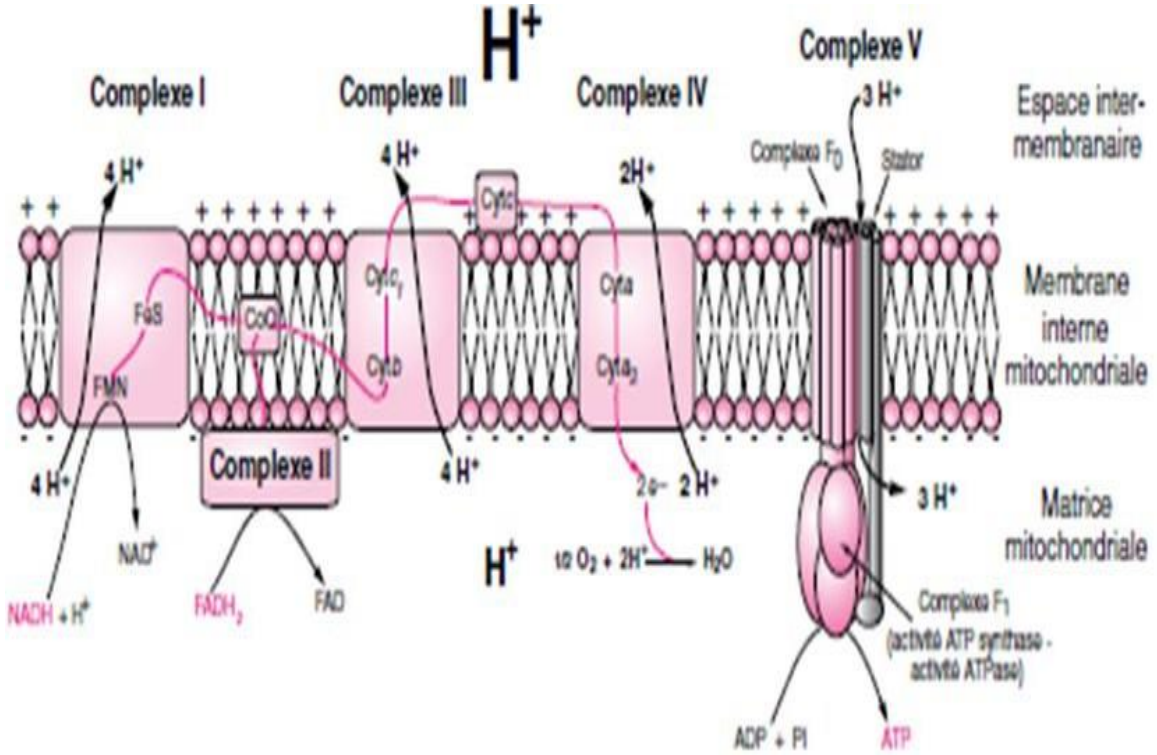


Fig. 202. Organization of the respiratory chain in the mitochondrial membrane illustrating the path of electrons (colored line) and the transport of protons.

ATP synthase: The enzyme that terminates oxidative phosphorylation is a large, mushroom-shaped protein complex. In mammals, this complex contains 16 subunits with a total mass of approximately 600 kDa. The membrane-bound region is called Fo-.

The "O" refers to oligomycin; it is not the number “0” and contains a ring of c subunits as well as the proton channel. The stalk and spheroidal head form the F1 region and are the site of ATP production. The spheroidal head at the end of the F1 region is composed of six proteins of two different types (three α subunits and three β subunits) while the stalk consists of a single protein, the γ subunit, which extends partially into the head. Both the α and β subunits bind to nucleotides, but only the β subunits catalyze ATP biosynthesis (Fig. 203).

It is present in all living things and functions in the same way in both eukaryotes and prokaryotes. It uses potential energy stored in the form of

The proton concentration gradient across a membrane is what drives the phosphorylation of ADP to ATP. The estimated number of protons required per ATP molecule synthesized varies between three and four depending on the source, with some experiments suggesting that cells may be able to adjust this ratio to adapt to changes in their environmental conditions.

ADP + phosphate + 4 $H^+_{intermembranes}$ ↔ 4 H^+_{matrix} + H_2O + ATP.

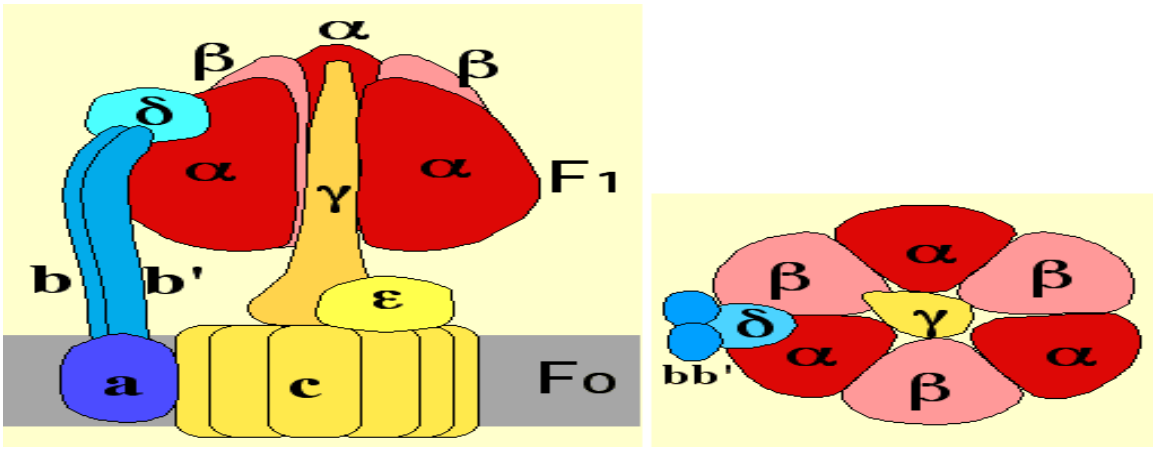


Fig. 203. Structure of ATP synthase. Side view (a β unit has been removed to visualize the central ϒ unit) and cross-sectional view.

The α and β subunits are homologous. An apical view relative to the membrane shows a ring-like (hexameric) arrangement in which the α and β subunits alternate. The crystal structure of F1 shows that the ϒ subunit forms a stalk within the ring formed by the α and β subunits. At the base of the ϒ subunit (on the Fo side), the associated δ and ε subunits are found. The three catalytic nucleotide-binding sites are located at the α-β interfaces, with a predominance of the β subunit. Each α subunit contains an ATP-binding site, but this site is inactive in catalysis (regulation). Adenylate nucleotides bind to the α and β subunits with Mg2+ (an essential cation). The δ subunit participates in the binding of the α and β subunit ring to the membrane domain of the enzyme. The Fo domain is a protein complex integrated into the membrane.

I.2. 4. Inhibitors

Several well-known drugs and toxins are inhibitors of oxidative phosphorylation (Table 29). Although each toxin inhibits only a single enzyme in the respiratory chain, inhibiting any one step in this chain blocks the entire process. For example, when ATP synthase is inhibited by oligomycin, protons can no longer re-enter the mitochondrial matrix through the inner membrane.

Proton pumps thus become unable to expel protons from the matrix when electrons flow through the respiratory chain because the proton concentration gradient across the membrane becomes too high. NADH is no longer oxidized to NAD^+, and the Krebs cycle, in turn, ceases to function due to insufficient NAD+ concentration.

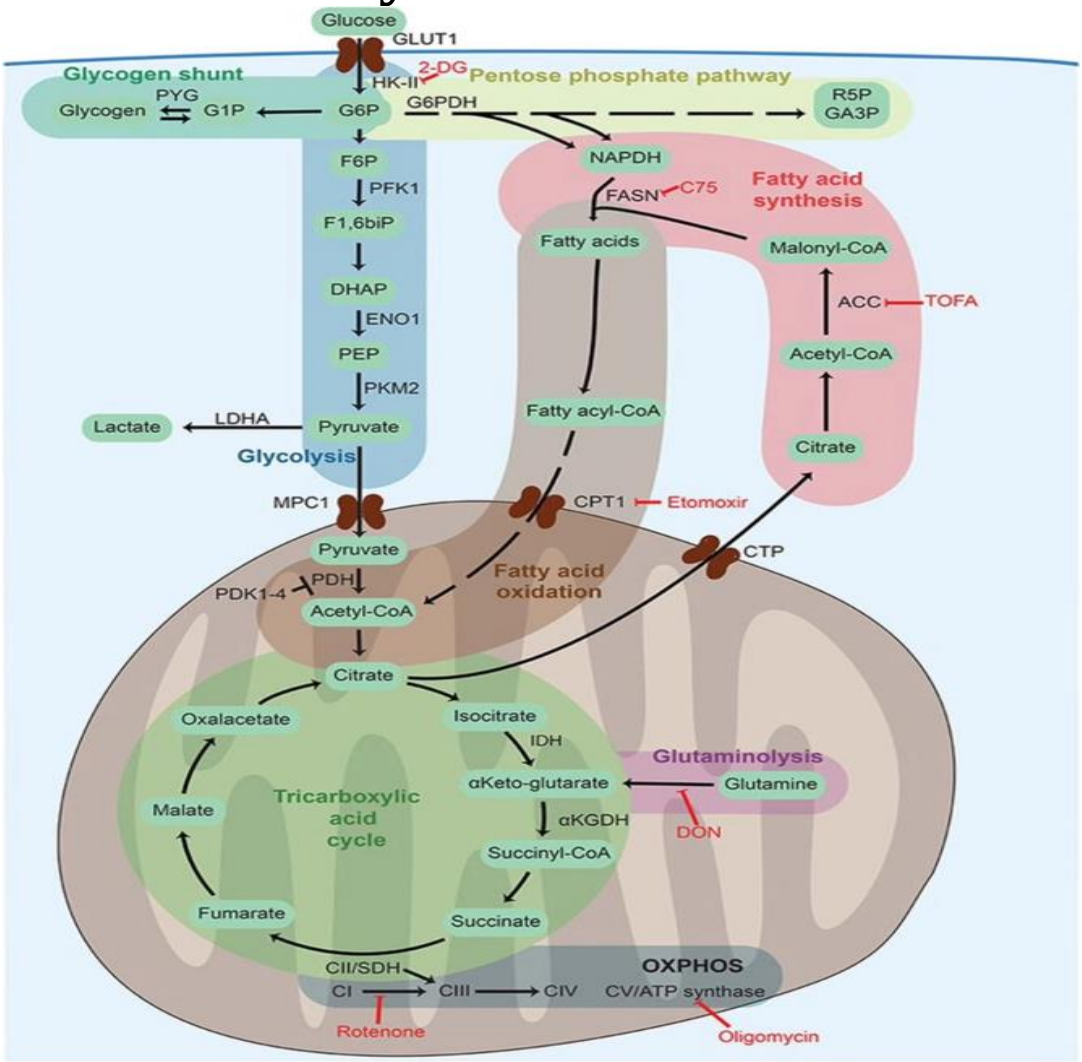
Table 29: Some inhibitors of oxidative phosphorylation and their modes of action.

Target	Compound	Use	Mode of action on the oxidative phosphorylation
Complex I	Rotenone	Pesticide	Blocks electron transfer from complex I to coenzyme Q10 by blocking the ubiquinone binding site.
Complex II	Oxaloacetate Malonate	Poisons	These are competitive inhibitors of succinate dehydrogenase (Complex II).
Complex III	Antimycin A	Pesticide	It binds to the Qi site of coenzyme Q-cytochrome c reductase, which blocks the oxidation of ubiquinone.
Complex IV	CN-, CO, N_{3-}, H_2S	Poisons	They inhibit the respiratory chain by binding more strongly than oxygen O2 to the Fe-Cu center of cytochrome c oxidase, thus preventing the reduction of oxygen.
Complex V	Oligomycin	Antibiotic	Inhibits ATP synthase by blocking proton flow through the F0 region.
Inner - membrane	Carbonylcyanide m-chlorophenylhydrazone 2, 4- Dinitrophenol	Weight-loss poisons	These are ionophores that break the concentration gradient, allowing protons to diffuse freely across the inner mitochondrial membrane.

However, not all inhibitors of oxidative phosphorylation (Table 29) are toxins. In brown adipose tissue, uncoupling proteins regulate proton diffusion across the inner mitochondrial membrane, thereby uncoupling the respiratory chain from ATP production. This uncoupled respiration produces heat instead of ATP, which plays a vital role in thermoregulation, particularly in hibernating animals, although these proteins may also have a more general function in the cellular stress response.

CHAPTER VIII :

Carbohydrate metabolism



CHAPTER VIII CARBOHYDRATE PATHWAY METABOLISM

VIII.1. Intermediate metabolism

Hundreds of chemical transformations take place within each cell, which can be broken down intosimple reactionswhich together constitute metabolism. The chemical compounds involved are called metabolites. The ordered and rapid succession of chemical reactions (metabolic pathway) is only possible thanks to the existence of specific enzymes. Most cells and organisms share a set of metabolic pathways (Fig. 204).

The polymers contained in food (proteins, polysaccharides, nucleic acids, and fats) cannot be directly used by organisms. Catabolic pathways break down these organic precursors into smaller monomers (amino acids, simple sugars, nucleobases, and fatty acids). The resulting "metabolite pool" is then further catabolicly transformed into energy production via oxidative phosphorylation, or used again for the synthesis of more complex molecules via anabolic metabolic pathways. Catabolic pathways provide reduced coenzymes that supply electrons to both oxidative phosphorylation (in the form of NADH) and anabolic pathways (in the form of NADPH).

In all cells, adenosine triphosphate (ATP) is the most important form of chemical energy storage. The pathways that ensure the synthesis, degradation, and transformation of important metabolites, as well as the conservation of energy, constitute intermediary metabolism.

VIII.1.1. Essential mechanisms of metabolic regulation

To adapt the synthesis and degradation of metabolites to physiological needs, the activities of all metabolic pathways are tightly controlled. The flow of metabolites in a metabolic pathway is primarily determined by the activities of the enzymes involved. To modulate this flow, it is sufficient to change the activity of the key enzyme that catalyzes the slowest step in that chain of reactions.

The control of intermediary metabolism occurs at multiple levels. All the regulatory mechanisms involved act on processes that influence the synthesis and/or activity of key enzymes.

VIII.1.1.1. Transcriptional control

At the genetic level, the transcription of genes encoding key enzymes is regulated. This regulation is ensured by regulatory proteins called transcription factors, which bind primarily to the promoter domain of the gene on the DNA. The activity of transcription factors is, in turn, regulated by metabolites and/or hormones. These processes take some time to manifest (from a few minutes to several hours).

VIII.1.1.2. Interconversion

Enzyme interconversion is significantly faster than transcriptional control. In this case, the enzyme is already at its site of action, but in an inactive form. Only when needed, and under the influence of a hormonal signal prolonged by a second messenger, will it be modified by an activating enzyme to produce the catalytically active form. If the metabolic pathway is no longer required, an inactivating enzyme converts the key enzyme back to its resting, inactive form.

In most cases, interconversion involves ATP-dependent phosphorylation of the enzyme by a protein kinase or dephosphorylation by a protein phosphatase. The dephosphorylated form of the key enzyme is generally the active form, but sometimes the reverse is true. Proteolytic activation and inactivation of enzymes is also a common regulatory mechanism.

VIII.1.1.3. Compartmentalization

Some pathways are blocked because the key enzyme is transferred to another subcellular compartment and held there by an inhibitor. When needed, it is returned to its site of action by a hormonal signal or a metabolite. The movement of glucokinase between the nucleus and the cytoplasm is an example of this.

VIII.1.1.4. Ligand modulation

The availability of precursors and coenzymes is important for metabolic flux in a pathway. If the coenzyme of a key enzyme is regenerated by a second, independent metabolic pathway, the rate of this second pathway can limit that of the first (example: respiratory control).

The fine control of an enzyme's activity is ensured by ligands (substrate, coenzyme, product) which modulate its activity as allosteric effectors.

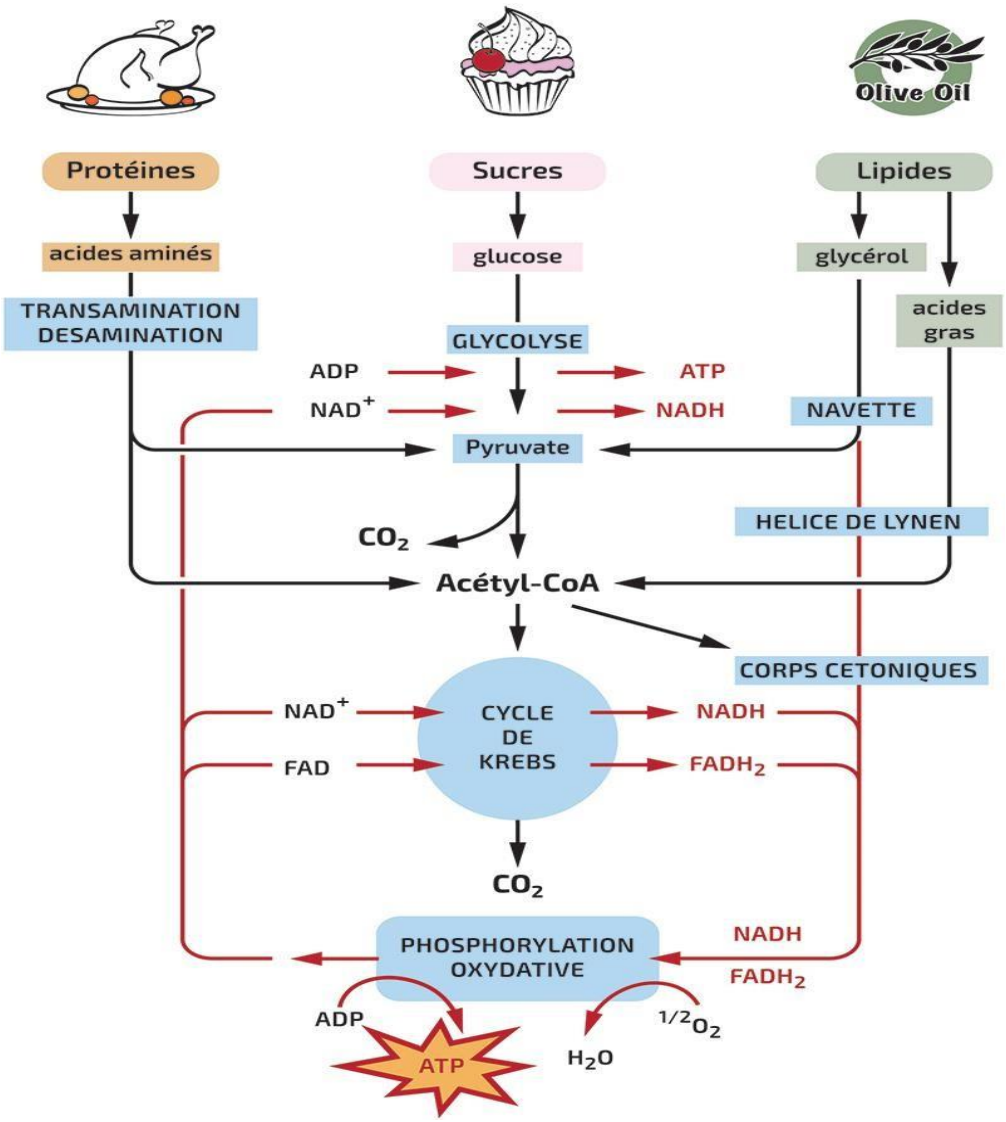


Fig. 204. Overview of intermediary metabolism.

Carbohydrates have different roles within the body: energy production or storage, synthesis of glycoproteins and macromolecules, synthesis of nucleotides (ribose and NADPH), metabolic interrelation.

VIII.2. GLUCOSE METABOLIC PATHWAYS

Glucose breakdown and resynthesis are the main energy pathways enabling and sustaining muscular exercise. However, glucose resynthesis can occur through multiple biochemical processes involving various substrates and/or metabolites (Fig. 205).

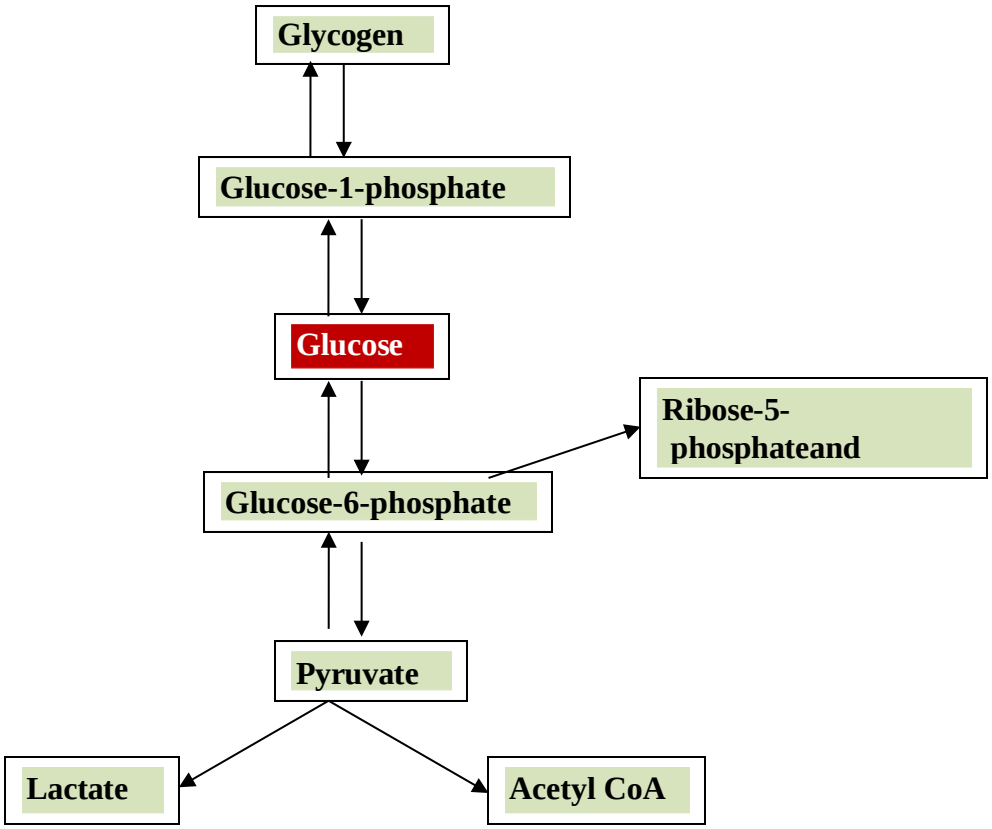


Fig. 205. Glucose metabolic pathways.

VIII.2.1. Glucose Absorption and Distribution

Glucose, a small water-soluble molecule, is transported in the blood in free form (Fig. 206). The blood level, or blood glucose, is relatively constant between 0.70 and 1.10 g/l.

• ***During the food situation*** **:** Glucose originates in the intestine. The rise in blood glucose triggers the secretion of insulin by the beta cells of the pancreas. The liver, the first tissue traversed by portal blood, absorbs 30 to 40% of the glucose. The remaining glucose is distributed among other tissues: brain, red blood cells, muscles, adipose tissue, etc. It is broken down into pyruvate via glycolysis and stored via glycogenesis in the liver and muscles.

• ***In a fasting situation:*** Blood glucose originates from the liver, from glycogenolysis and gluconeogenesis under the influence of high levels of glucagon secreted by the α cells of the pancreas.

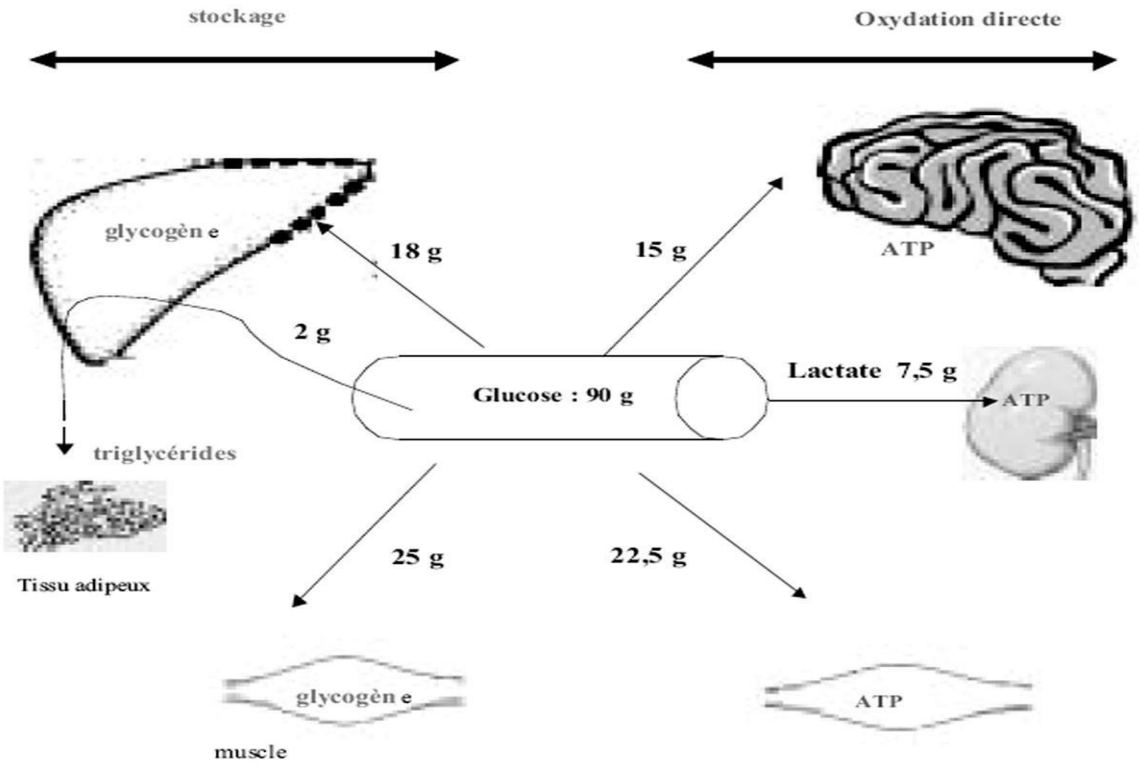


Fig. 206. Postprandial glucose metabolism.

Glucose cannot enter the cell by simple diffusion. Its entry is ensured by the following two mechanisms:

Easy transport: Glucose crosses the phospholipid and hydrophobic cell membrane via facilitated diffusion, using passive transporters. These transporters, called GLUTs (glucose transporters), are encoded by different genes and are listed in chronological order of their discovery. They are transmembrane glycoproteins. The binding of glucose to the extracellular side of the membrane causes a conformational change in the protein, which allows the sugar to move to the inner side where it is released. Currently, five known GLUTs (glucose transporters) are numbered 1 to 5.

These are proteins that have a certain resemblance in the first sequences but then exhibit sequences specific to their host membranes.

GLUT transporters are expressed to varying degrees depending on cell types and are distinguished by their Km for glucose.

This is why GLUT 1, abundant in red blood cells, has a Km close to 1mM, a concentration significantly lower than blood glucose (close to 5mM); they thus promote the penetration of glucose into the red blood cell (RBC) when blood glucose is low, during periods of fasting.

The predominant GLUT 2 in the liver and pancreas has a Km between 15 and 20mM, significantly higher than postprandial blood glucose; therefore, glucose diffuses very rapidly into the hepatocyte when the blood level is high, as is the case in the portal vein during feeding; conversely, glucose enters the hepatocyte only weakly when blood glucose is low, during fasting.

GLUT 3, which is abundant in the brain, possesses the same characteristics as GLUT 1.

The predominant GLUT 4 in body fat (adipose tissue) and muscles has a Km close to 5mM; the synthesis and affinity of GLUT 4 for glucose are regulated by insulin.

Active transport: This is an energy-consuming process. Glucose is transported against a concentration gradient, that is, from an environment with a low glucose concentration to the interior of the cell where the glucose concentration is higher. Glucose and Na+ are transported in the same direction and simultaneously across the membrane. This type of transport occurs in epithelial cells, the intestine, the kidney, etc.

VIII.2.2. GLYCOLYSIS

Most tissues have a minimal need for glucose, however for the brain and erythrocytes this need is important and even essential.

VIII.2.2.1. Definition

Glycolysis, or the Embden-Meyerhoff pathway, is a catabolic pathway present in virtually all organisms. It allows the breakdown, through progressive oxidation, of a 6-carbon glucose molecule into two 3-carbon pyruvate molecules, producing ATP and building blocks for the synthesis of cellular compounds. It is a series of chemical reactions catalyzed by enzymes located in the soluble fraction of the cytoplasm, or cytosol. Its primary role is therefore to participate in energy metabolism by enabling the biosynthesis of ATP.

It is not only the main pathway for glucose metabolism, but it is also the pathway for fructose and galactose present in food.

VIII.2.2.2. Glycolysis reactions

Glycolysis involves 10 individual steps with 3 isomerizations and 4 phosphate transfers (Fig. 207). The only redox reaction in the pathway occurs at step 6.

All intermediates of glycolysis between glucose and pyruvate are phosphorylated. Their phosphorylated group has three essential functions:

- It affects each intermediate of a negatively charged polar group, which makes it unable to cross the cell membrane, at pH 7, by simple diffusion.
- The phosphate group acts as a linking and recognition group for the formation of enzyme-substrate complexes.
- Finally, this phosphate group plays a role in the conservation of energy which will contribute to the formation of the 2 ATP molecules of glycolysis.

Glycolysis is divided into two main phases:

- **The first phase:** The ATP consumption phase is where a large number of metabolizable hexoses converge after their phosphorylation or phosphorolysis of polysaccharides at the expense of ATP. They are then all transformed into a common product, which is glyceraldehyde 3-P.
- **The second phase**: common to all hexoses, is characterized by a sequence of reactions that lead to the formation of pyruvate, 2 ATP and $NADH,H^+$, following the oxidation of a glyceraldehyde 3-P.

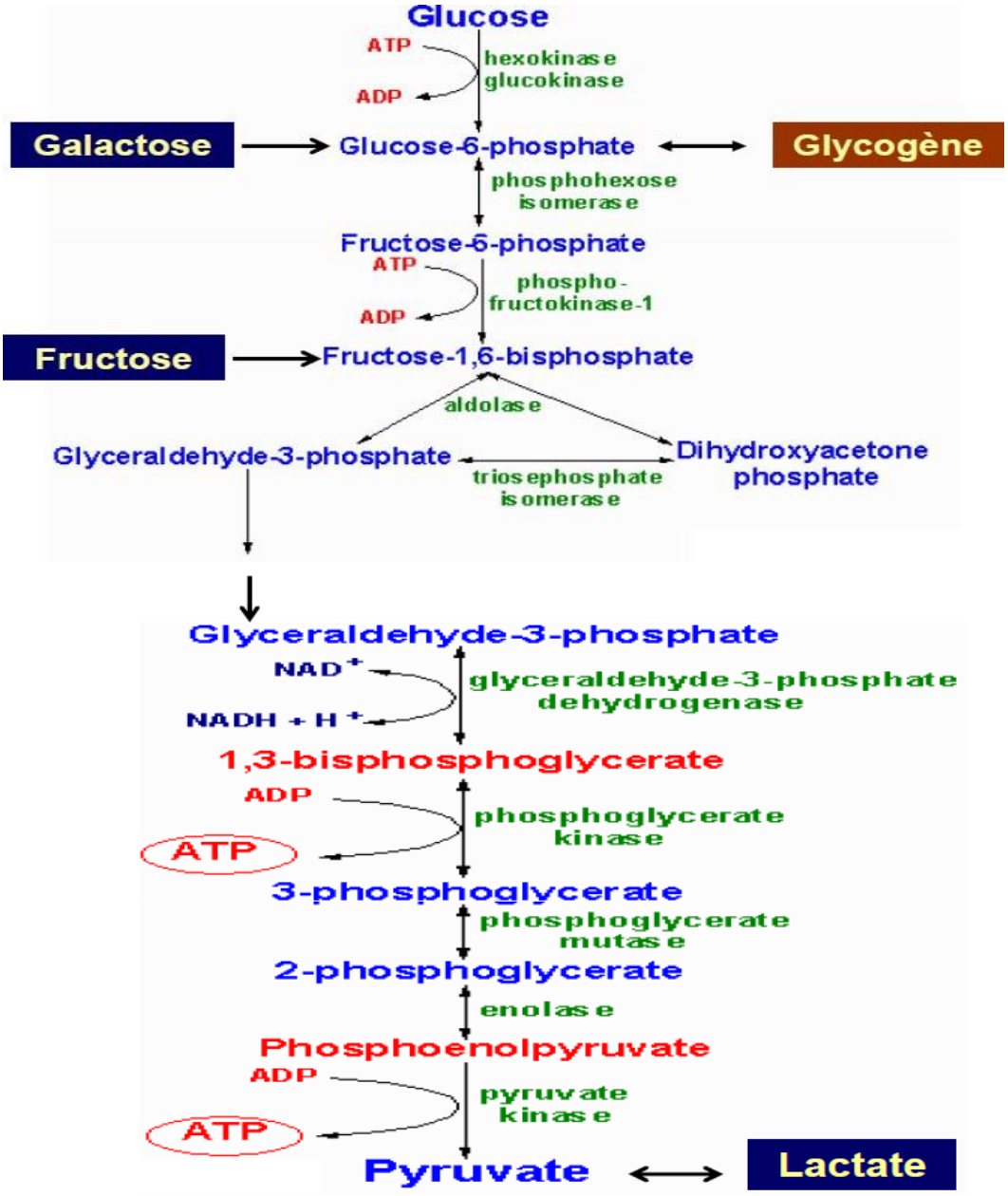


Fig. 207. Overview of glycolysis.

VIII.2.2.3. Enzymatic steps of the first phase

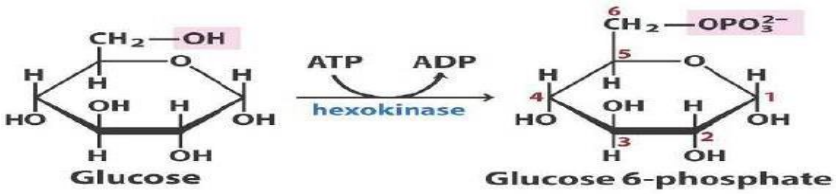
a. Phosphorylation of glucose by ATP

This is the first major step. It consumes one molecule of ATP (or one energy-rich phosphate bond). The irreversible reaction is catalyzed by hexokinase or glucokinase. Glucokinase is specific to glucose, while hexokinase, which is found in most cells, phosphorylates glucose as well as other hexoses.

such as fructose, galactose, glucosamine, etc. As in all phosphorylations

Magnesium^{++} is essential. The catalyzed reaction is as follows:

$$ATP + glucose \rightarrow ADP + Glucose\text{-}6\text{-}phosphate$$



b. *Isomerization of glucose 6-P to fructose 6-P*

This reaction is catalyzed by phosphoglucoisomerase (PGI). It is a reversible isomerization reaction. It is specific to these two compounds to the point that starting from one of them always leads to the same equilibrium.

$$Glucose - 6 - P \leftrightarrow Fructose\text{-}6\text{-}P$$



c. *Phosphorylation of fructose 6-P to fructose 1,6-BIS P*

The reaction is mediated by phosphofructokinase 1 (PFK1) or fructose-6-phosphate kinase as follows: $ATP + fructose\ 6 - P \rightarrow Fructose\text{-}1,6\text{-}bisP + ADP$

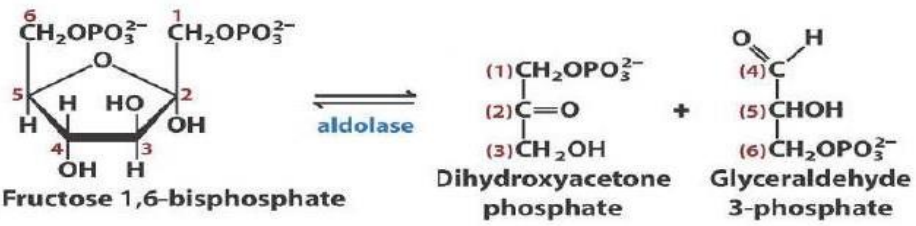


UTP can replace ATP. It is an allosteric or regulatory enzyme that is inhibited by high ATP concentrations. Mg2+ is essential. The reaction is completely irreversible.

d. *Fructose cleavage -1,6-bisphosphate*

The reversible reaction is catalyzed by fructose-1,6-bisphosphate aldolase (aldolase 1 or α). It is an aldehyde lyase.

$$Fructose\ 1,6\text{-}bisP \leftrightarrow 3\text{-}P\text{-}glyceraldehyde + 3\text{-}P\text{-}dihydroxyacetone$$



e. ***Triose phosphate interconversion***

Only 3-P-glyceraldehyde is degraded in the subsequent reactions of glycolysis. 3-P-Dihydroxyacetone is used after conversion to 3-P-glyceraldehyde. This reaction is catalyzed by a phosphotriose isomerase (redox isomerase). This reaction completes the first phase of glycolysis.

3-P glyceraldehyde ↔3-P dihydroxyacetone



At the end of this phase, all hexoses exhibit the following overall reaction:

Hexose + 2 ATP → 2 glyceraldehyde 3-P + 2 ADP

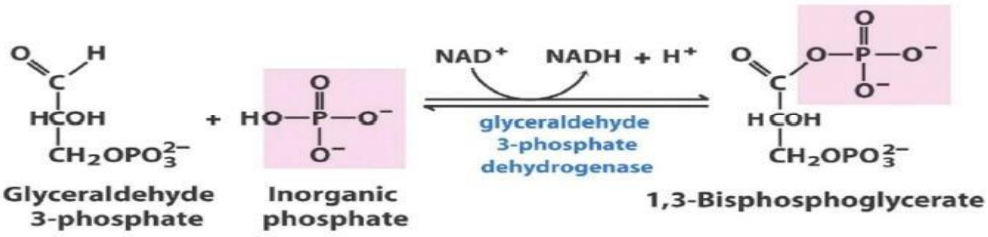
VIII.2.2.4. Enzymatic steps of the second phase

This second phase is the production of ATP and pyruvate. It contains the only redox reaction of glycolysis, which will lead to the formation of NADH,H+. The two glyceraldehydes obtained in the first phase will undergo a sequence of reactions leading to pyruvate. The sequence will be described using a 3-phosphate glyceraldehyde molecule.

a. ***Oxidation of 3-Pglyceraldehyde to 3-P glyceroyl-1-P***

The enzyme that catalyzes the reaction is 3-phosphoglyceraldehyde dehydrogenase. It requires the presence of mineral phosphate. The carboxyl group resulting from the oxidation of the aldehyde function is bonded to the phosphate by a high-energy bond. The product obtained is 3-phosphoglyceroyl-1-phosphate. The released electrons are taken up by NAD+. The reaction sequence is reversible and illustrated in the figure.

3-P glyceraldehyde + NAD+ + Pi ↔3-Pglyceroyl-P + NADH,H+

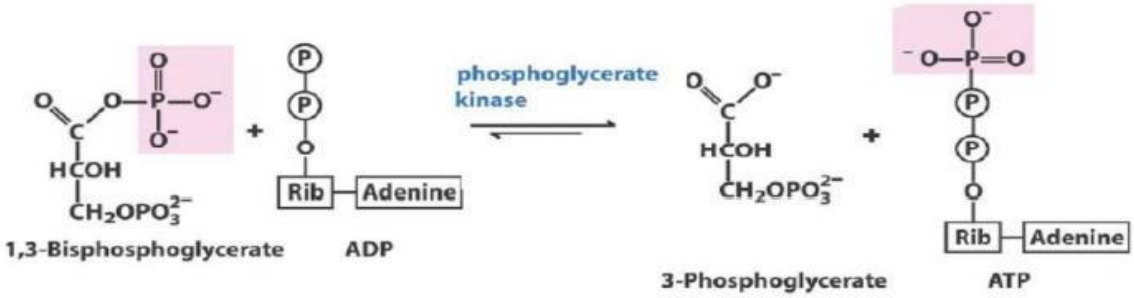


b. *Phosphate transfer to ADP – ATP synthesis*

It is catalyzed by 3-phosphoglycerate kinase (phosphotransferase). The reaction is **Reversible.** The reaction obtained is: 3-**P-glyceroyl-1-P + ADP ↔3-P-glycerate + ATP**

In summary, through the two enzymes (Multi-enzyme complex of 3-phosphoglyceride dehydrogenase) we have the resulting reaction which leads to the formation of ATP.

Glyceraldehyde 3−P + NAD + ADP + Pi → Glycerate 3−P + NADH, H + ATP



c. *Isomerization of 3-phosphoglycerate to 2-phosphoglycerate*

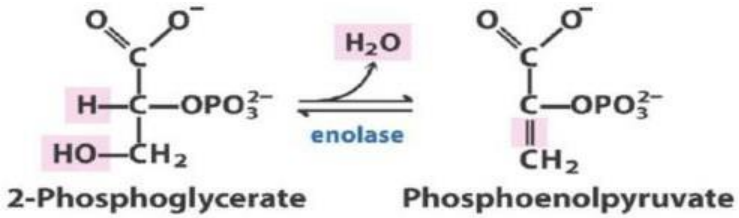
The phosphate is displaced from position 3 to position 2. The reaction is catalyzed by the **phosphoglycerate mutase** (Isomerization with intramolecular radical transfer); the Mg^{2+} is essential. The reaction is reversible: **Glycerate 3-P ↔ glycerate 2-P**



d. *Dehydration of 2-P glycerate to phosphoenolpyruvate*

The second reaction leads to the formation of an energy-rich phosphate bond. It is catalyzed by an enolase (hydratase). This elimination of water results in an electronic rearrangement within the molecule. This leads to the formation of phosphoenolpyruvate, the most energy-rich molecule produced by the cell.

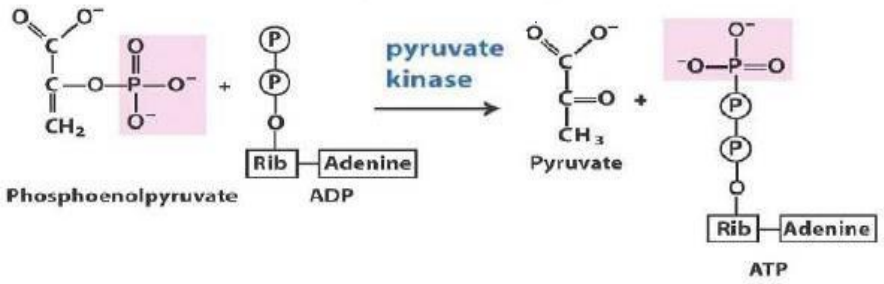
Glycerate 2 − P ↔ Phosphenolpyruvate + H2O



e. ***Phosphate transfer from phosphoenolpyruvate to ADP***

This reversible reaction is catalyzed by pyruvate kinase (phosphotransferase). The Mgor Mn is essential. The formation of pyruvate completes the sequence of reactions

From glycolysis: Phosphenolpyruvate + ADP → Pyruvate + ATP



VIII.2.2.5. Glycolysis balance (energy yield)

Glucose + ATP	→	Glucose 6-P + ADP
Glucose 6-P	⇌	Fructose 6-P
Fructose 6-P + ATP	→	Fructose 1,6 di-P + ADP
Fructose 1,6 di-P	⇌	2 Glyceraldéhyde 3-P
2 GA 3-P + 2 NAD^+ + 2 Pi	⇌	2 1,3-DPG + NADH + 2 H^+
2 1,3-DPG + 2 ADP	⇌	2 3-phosphoglycérate + 2 ATP
2 3-phosphoglycérate	⇌	2 2-phosphoglycérate
2 2-phosphoglycérate	⇌	2 phosphoénolpyruvate + 2 H_2O
2 phosphoénolpyruvate + 2 ADP	→	2 pyruvate + 2 ATP

Glucose + 2 NAD+ + 2 Pi + 2 ADP →
2 Pyruvate + 2 NADH + 2 ATP + 2 H_2O + 2 H^+

➢**2 ATP formés**

(Consumption of 2 ATP, production of 4 ATP; oxidation of reduced NAD will also produce ATP by oxidative phosphorylation).

VIII.2.2.6. Fate of pyruvate

At the end of the 10 enzymatic reactions of glycolysis, glucose yields 2 molecules of ATP, 2 molecules of NADH, H+, and 2 molecules of pyruvate in all cells. The fate of pyruvate will depend on the following conditions:

- The presence or absence of oxygen in the cell's environment
- The cell's energy status
- The enzymatic equipment that the cell will have to oxidize NADH, H^+ Pyruvate can then

- in the cytosol to be transformed into lactate or ethanol
- in the mitochondria be converted into oxaloacetate or be totally oxidized to CO_2.

- Aerobic glycolysis leads to the formation of 2 molecules of pyruvate, 2 ATP, 2 NADH, and H^+. This is an essential step in the formation of pyruvate and its mitochondrial conversion to acetyl-CoA, a key component of the Krebs cycle. Under these conditions, glucose undergoes complete oxidation to CO2 and H_2O.
- Anaerobic glycolysis also produces 2 molecules of pyruvate, 2 ATP, and 2 NADH, H^+.
- The following reactions depend on both the cell's enzymatic equipment and the availability of oxygen. They lead to the reoxidation of NADH, H^+ with the following final production:

• Either lactate (lactic fermentation): this is the system that provides ATP to cells that do not have mitochondria such as red blood cells and to cells in hypoxia such as striated muscles in rapid contraction.

• Either ethanol (alcoholic fermentation).

a. *REDUCTION OF PYRUVATE TO LACTATE*

When a cell lacks mitochondria (as in red blood cells), is deprived of oxygen (anaerobic conditions), or is under hypoxic conditions (rapidly contracting muscle tissue), pyruvate is reduced to lactate by NADH, a hydrogen ion produced during glycolysis. This reversible reaction, catalyzed by lactate dehydrogenase, regenerates NAD+.

$$\mathbf{CH_3\text{-}CO\text{-}COO^- + NADH, H^+} \longleftrightarrow \mathrm{CH_3\text{-}CHOH\text{-}COO^-} + \mathbf{NAD^+}$$

Pyruvate *Lactate*

Under the conditions mentioned, the overall reaction for the breakdown of glucose is:

$$\mathbf{Glucose + 2\ ADP + 2\ Pi} \longrightarrow 2 \text{ lactates} + 2 \text{ ATP}$$

Lactate is the end product of glucose breakdown. It diffuses into the extracellular space and constitutes a waste product. This waste product, released into the bloodstream in mammals, can be converted back into glucose in the liver.

b. *TRANSFORMATION OF PYRUVATE INTO ETHANOL*

This transformation of pyruvate into ethanol is found in yeasts that do not possess lactate dehydrogenase but instead possess a pyruvate decarboxylase (lyase).

Pyruvate is decarboxylated to acetaldehyde by pyruvate decarboxylase, whose coenzyme is thiamine pyrophosphate (TPP). This reaction is irreversible.

$$\mathbf{CH_3\text{-}CO\text{-}COO^-} \longrightarrow \mathbf{CH_3\text{-}CHO + CO_2}$$

Pyruvate *Acetaldehyde*

Acetaldehyde is reduced to alcohol or ethanol. The reaction is catalyzed by alcohol.

dehydrogenasewith consumption of NADH, H^+ formed in glycolysis and regeneration from NAD^+.

$$\mathbf{CH_3\text{-}CHO + NADH, H^+} \longleftrightarrow \mathbf{CH_3\text{-}CH_2OH + NAD^+}$$

Acetaldehyde *Ethanol*

When the reactions of glycolysis are continued by the transformation of pyruvate into ethanol, this is called alcoholic fermentation. The overall reaction for the breakdown of one glucose molecule is written: **Glucose + 2 ADP + 2 Pi → 2 Ethanol + 2 CO_2+ 2 ATP**

c. *CARBOXYLATION OF PYRUVATE TO OXALOACETATE*

Under aerobic conditions, pyruvate, once transported into the mitochondria, can be converted into oxaloacetate by pyruvate carboxylase, an allosteric ligase activated by acetyl-CoA. This enzyme uses biotin as a coenzyme.

$$\mathbf{CH_3\text{-}CO\text{-}COO^-} + \mathbf{CO_2} + \mathbf{ATP} \longrightarrow \mathbf{CH_3\text{-}CO\text{-}CH_2\text{-}COO^-} + \mathbf{ADP + Pi}$$

Pyruvate *Oxaloacetate*

The oxaloacetate formed is both a catalytic acceptor of the Krebs cycle and the intermediate metabolite through which pyruvate carbons are injected into gluconeogenesis.

d. *OXIDATION OF PYRUVATE TO CO2*

It takes place in the mitochondria. Pyruvate is transported into the mitochondria. It is first transformed by the multi-enzyme complex of pyruvate dehydrogenase into acetyl-CoA.

$$CH_3\text{-}CO\text{-}COO^- + HSCoA + NAD^+ \rightarrow CH_3\text{-}CO\sim SCoA + CO_2 + NADH,H^+$$

Pyruvate ***AcetylCoA***

The complete oxidation of acetyl-CoA is carried out in the Krebs cycle and is accompagned by the formation of 3 NADH, H^+, 1 FADH and 1 GTP.

We will see this in the Krebs cycle. The overall reaction for the oxidation of acetyl-CoA is:

$$CH\text{-}CO\sim SCoA + 3NAD^+ + FAD + GDP + Pi \rightarrow 2CO + HSCoA + 3NADH, H^+ + FADH2 + GTP$$

The set of the reduced coenzymes formed (cytosolic and mitochondrial $NADH,H^+$, $FADH_2$) are regenerated during the complete breakdown of glucose.
during their oxidation by the respiratory chain.

VIII.2.2.7. Glycolysis is a source of biosynthetic precursors.

A pathway is considered catabolic if it generates energy (ATP) and/or energy-rich reduced cofactors (NADH, H and $FADH_2$) and biosynthesis precursors (Table 30).

Glycolysis can be considered a catabolic pathway par excellence since it forms all three types of compounds. Among the metabolites of glycolysis, Some are important metabolic crossroads:
the G6P: leads to the synthesis of glycogen, pentose phosphates, glucosamine, constituents of glycoproteins.

- **the trios P:** are related to fructose, pentose P and glycerol 3 P.
- **pyruvate:** the final product of glycolysis, is in direct relation in the cytosol, with lactate and alanine, and in mitochondria with acetyl CoA and oxaloacetate.

The table below provides an overview of some biosynthetic precursors from glycolysis and their fate.

Table 30: Biosynthetic precursors generated by glycolysis.

Precursors	Formed products
Phosphodihydroxy acetone (PDHA)	- synthesis of glycerol-3-P (triglycerides and phospholipids).
3-Phosphoglycerate	- synthesis of serine (an important amino acid)
Pyruvate	- glucose (gluconeogenesis) - Acetyl-CoA: synthesis of fatty acids and lipids in animals and plants, synthesis of carbohydrates (molds and germinating oilseeds) - Ethanol (alcoholic fermentation). - Formation of oxaloacetate - Synthesis of alanine (transamination)
Phosphoenolpyruvate	-Synthesis of phenylalanine, tyrosine, tryptophan
Glucose-6-P	- Glycogen synthesis - Ribose synthesis - Synthesis of glucuronate (detoxification) and acidic polysaccharides or mucopolysaccharides) - vitamin C synthesis

VIII.2.2.8. Regulation of glycolysis

A/ Metabolic regulation:

Glycolysis provides both ATP, essential for meeting the energy needs of aerobic organisms, and biosynthetic precursors. The rate of glycolysis is established to satisfy these two needs. In metabolic pathways, irreversible reactions are often the control points. The three regulatory sites are located at the level of the three allosteric enzymes catalyzing the irreversible reactions of glycolysis: hexokinase, phosphofructokinase 1 (PFK 1), and pyruvate kinase.

First checkpoint:

The first step, glucose G6P, takes place under the action of hexokinase, which is an allosteric enzyme. Its activity is regulated by the concentration of its immediate product, G6P.

Second checkpoint:

The third stage F6P ⟶ F1,6 BiP takes place under the action of the allosteric enzyme PFK1, which is regulated as follows:

- Augmented by ADP or AMP:

When a cell is at a low energy level, the amounts of ADP and AMP are higher than normal, while ATP is scarce. Under these conditions, the enzyme is fully activated and exhibits a high affinity for its substrate, F6P.

- Inhibited by ATP, NADH, H^+, citrate:

• When the cell is at a high energy level, the ATP concentration is high, while the concentrations of AMP and ADP are low. In this case, ATP binds to a regulatory site on the enzyme to slow its activity. The enzyme now has a lower affinity for its substrate, and the reaction rate decreases.

• The enzyme is also inhibited by citrate, an intermediate in the citric acid cycle. High citrate levels mean that biosynthetic precursors are abundant, thus reducing the need for additional glucose to be broken down for this purpose. Citrate inhibits PFK by enhancing the inhibitory effect of ATP.

• NADH, H+It also inhibits PFK activity by potentiating the inhibitory effect of ATP. This inhibition will be lifted as soon as NADH, H+is reoxidized to NAD+

3thcheckpoint:

Located at the final stage of glycolysis PEP⟶PYRUVATE under the action of pyruvate kinase.

Pyruvate kinase, an allosteric enzyme, is activated by F 1,6 BIP and PEP. It is inhibited by ATP, citrate, and long-chain fatty acids.

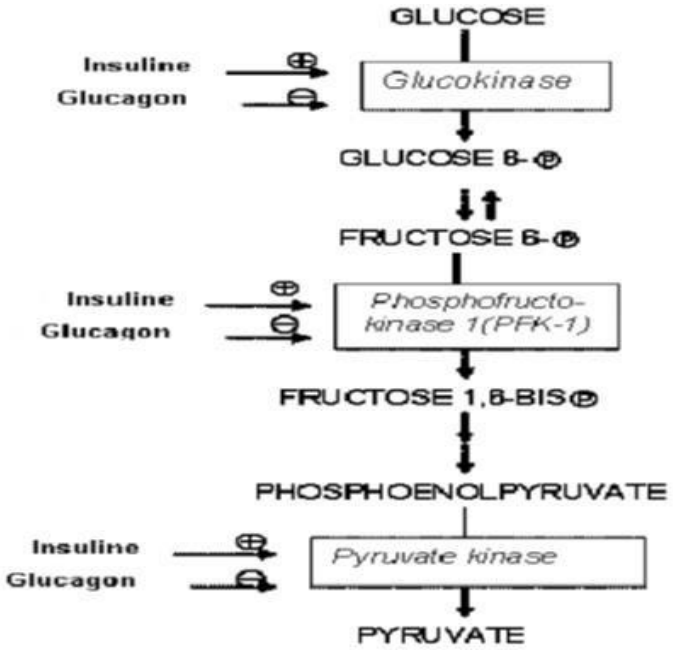


Fig. 208. Hormonal regulation of glycolysis.

Glycolysis is also regulated at the hormonal level (Fig. 208).

VIII.2.2.8. Entry of other carbohydrates into the glycolytic sequence (the different substrates of glycolysis)

All metabolizable carbohydrates are degraded via the glycolytic pathway. The second phase is common to all carbohydrates, whether polymerized or not. Each carbohydrate uses its own specific sequence of reactions, at the end of which it is converted into 3-P-glyceraldehyde. Carbohydrates degraded by the glycolytic pathway include: storage polysaccharides such as starch and glycogen, disaccharides, and monosaccharides other than glucose (Fig. 209).

VIII.2.2.9.1. Glycogen and starch

Glycogen and dietary starch, subjected to the action of hydrolytic enzymes in saliva, the pancreas, and the small intestine, are absorbed as free glucose, which is then phosphorylated to glucose 6-phosphate. The overall reaction can be written as:

Glycogen or starch (n glucose) + nH_2O + nATP ⟶ nGlucose 6-P + nADP

VIII.2.2.9.2. Disaccharides

Disaccharides ingested as sucrose, maltose, and lactose are first hydrolyzed into their constituents. Specific enzymes are secreted by the intestinal mucosa and remain bound to these components.

Sucrose + H_{20} ⟶ Glucose + fructose (β-fructosidase)

Maltose + H_{20} ⟶ 2 glucose (α-glucosidase)

Lactose + H_{20} ⟶ Glucose + galactose (□-galactosidase)

These sugars will enter glycolysis after phosphorylation according to the reactions described below:

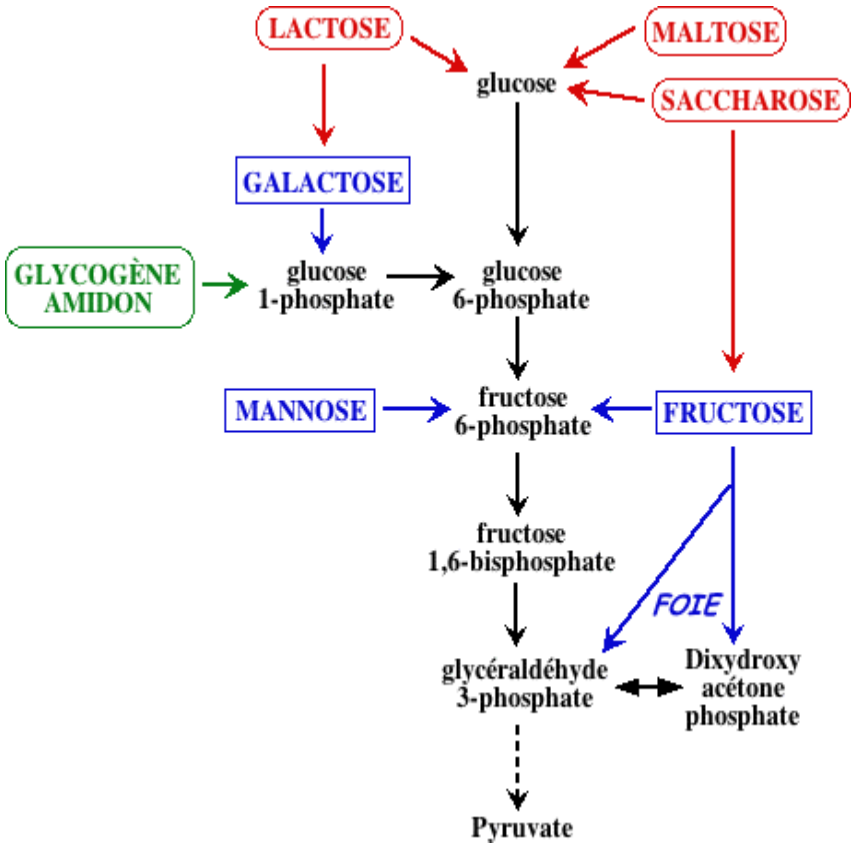


Fig. 209. The different substrates of glycolysis.

Dietary and endogenous polysaccharides:

- **dietary glycogen and starch**Salivary and pancreatic α-amylases, disaccharidases carried by the intestinal epithelium → D-glucose → blood
- Endogenous glycogen of hepatocytes and muscle cells: glycogen phosphorylase (α1→4), debranching enzyme (α1→6) and phosphoglucomutase:
Glycogen (n) + Pi → glucose 1-phosphate + glycogen (n-1) Glucose 1-phosphate ↔ glucose 6-phosphate

Dietary disaccharides:

Intestinal disaccharidases: dextrinase, maltase, lactase, sucrase and trehalase Food monosaccharides: Glucose, fructose, galactose and mannose.

Fructose:

In muscle and kidney: fructose + ATP → fructose 6-phosphate + ADP (hexokinase) In liver: fructose + ATP → fructose 1-phosphate + ADP (fructokinase)

VIII.2.2.9.3. Monosaccharides

- Fructose can be phosphorylated at carbon 6 by hexokinase, but the latter is saturated by glucose. It is primarily processed by hepatic fructokinase, which phosphorylates it at carbon 1. The sequence of reactions leading to glyceraldehyde 3-phosphate will involve, in order, fructokinase, fructose-1-phosphate aldolase (aldolase 2 or β), glyceraldehyde kinase, and phosphotriose isomerase (Fig. 210).

- Fructose + ATP ⟶ fructose 1-phosphate + ADP (fructokinase)
- Fructose 1-P ⟶ glyceraldehyde + 3-P dihydroxyacetone (aldolase)
- Glyceraldehyde + ATP ⟶ 3-P Glyceraldehyde + ADP (Glyceraldehyde kinase)
- 3-P dihydroxyacetone ⟷ Glyceraldehyde 3-P + 2ADP (Phosphotriose isomerase)

The overall reaction is:

- Fructose + 2 ATP ⟶ glyceraldehyde 3-P + 2ADP

Fructose 1-phosphate aldolase activity is low and is the limiting factor in fructose metabolism.

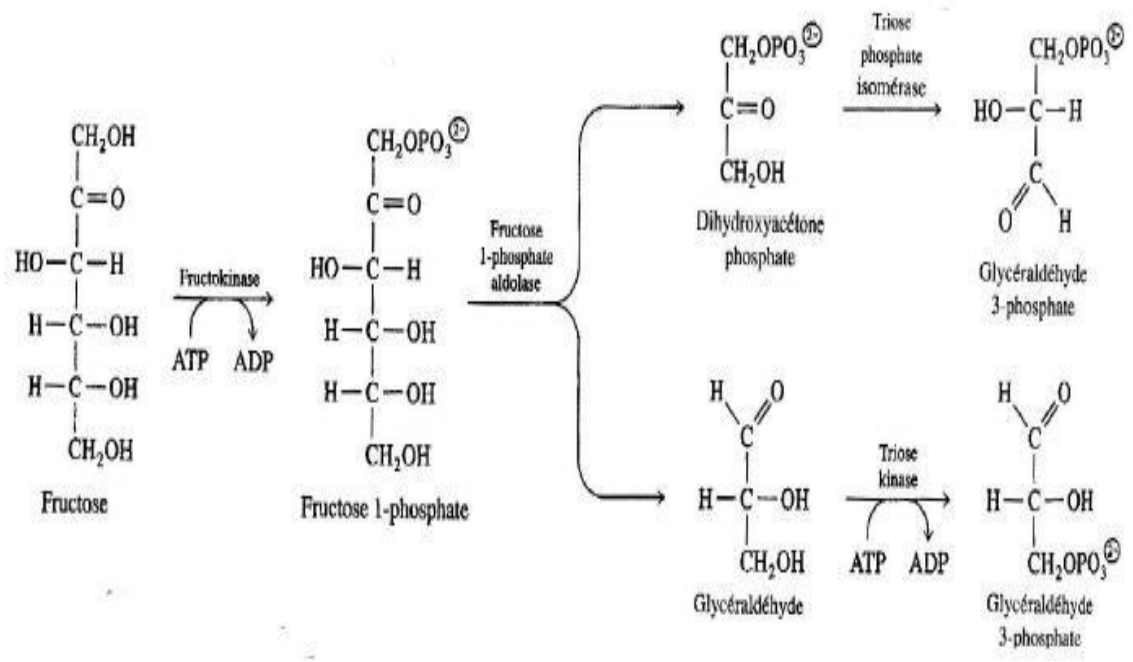


Fig. 210. Entry of fructose into glycolysis.

- Galactose can also be phosphorylated at carbon 6 by hexokinase, but for the same reasons as fructose, it is processed by hepatic galactokinase, which phosphorylates it at carbon 1. It is then committed to a secondary interconversion pathway.

galactose is converted into glucose for injection into glycolysis (Fig. 211). The sequence involves, in order, galactokinase, uridylyl transferase, UDP hexose 4'-epimerase and finally phosphoglucomutase (Fig. 13).

Galactose + ATP ⟶ Galactose 1-P + ADP (galactokinase)

Galactose 1-P + UDP glucose ⟷ Glucose 1-P + UDP Galactose (uridylyl transferase)

UDP Galactose ⟷ UDP glucose (UDP hexose 4'-epimerase)

Adding the last two reactions together gives:

Galactose 1-P ⟷ Glucose 1-P

Glucose 1-P is isomerized by phosphoglucomutase into glucose 6-P, the substrate of glycolysis.

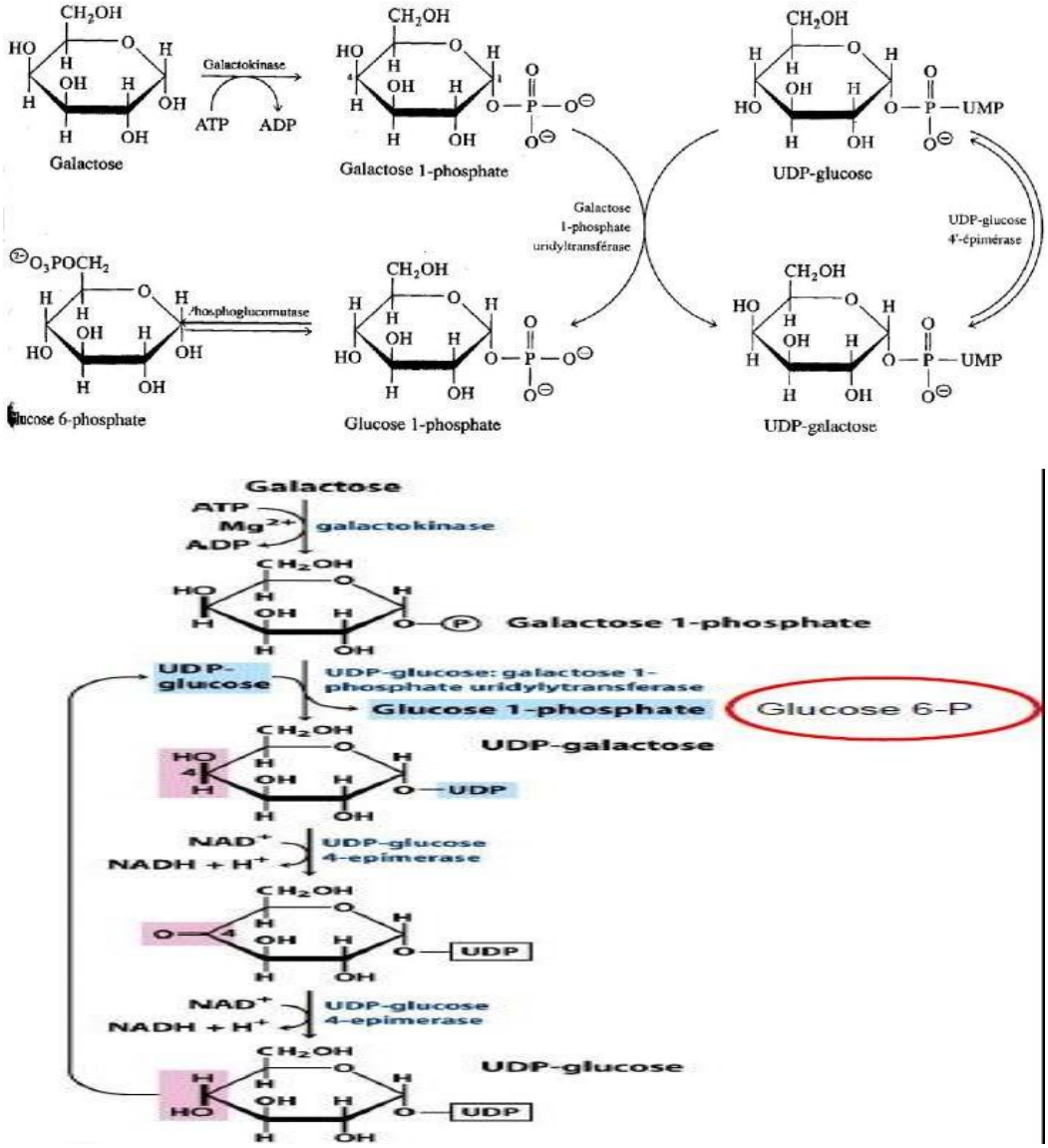


Fig. 211. Entry of galactose into glycolysis.

- Mannose enters glycolysis in two stages (Fig. 212):

Phosphorylation: Mannose is phosphorylated by hexokinase to mannose-6-phosphate by consuming one molecule of ATP.

IsomerizationMannose-6-phosphate is converted to fructose-6-phosphate by the enzyme phosphomannose isomerase. Fructose-6-phosphate then directly enters glycolysis at its second reversible stage (Fig. 14).

Mannose + ATP → 6-P mannose + ADP (hexokinase)

Mannose 6-P ↔ fructose 6-P (phosphomannose isomerase)

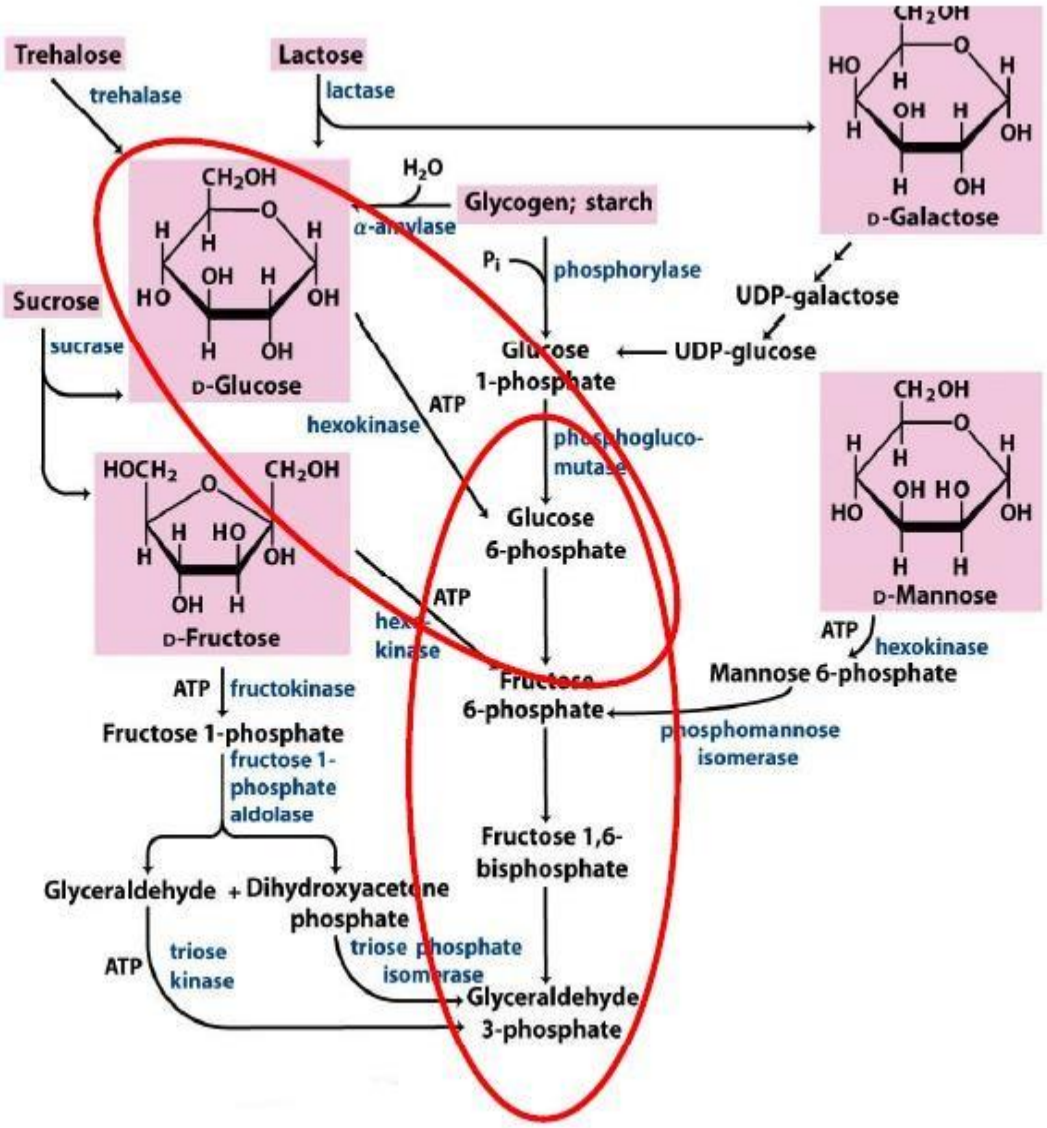


Fig. 212. Entry of mannose into glycolysis.

VIII.2.3. Citric acid cycle (tricarboxylic acid cycle or Krebs cycle)

Glycolysis and β-oxidation of fatty acids are the two preparatory processes for the release of energy from the bonds contained in carbohydrates and lipids (Fig. 213). Glycolysis leads to the formation of pyruvate, which can be converted into acetyl-CoA in the mitochondria. β-Oxidation of fatty acids with an even number of carbons directly yields acetyl-CoA, while that of fatty acids with an odd number of carbons yields acetyl-CoA and propionyl-CoA. Acetyl-CoA units, derived from pyruvate or from fatty acid degradation, are completely oxidized in the citrate cycle. This cycle is the final common pathway (Fig. 213) for the oxidation of energy molecules. It takes place entirely within the mitochondria. As a catabolic pathway, the tricarboxylic cycle provides energy (in small amounts in the form of GTP), energy-rich reduced cofactors (NADH, H+ and FADH2) and also precursors for biosynthesis.

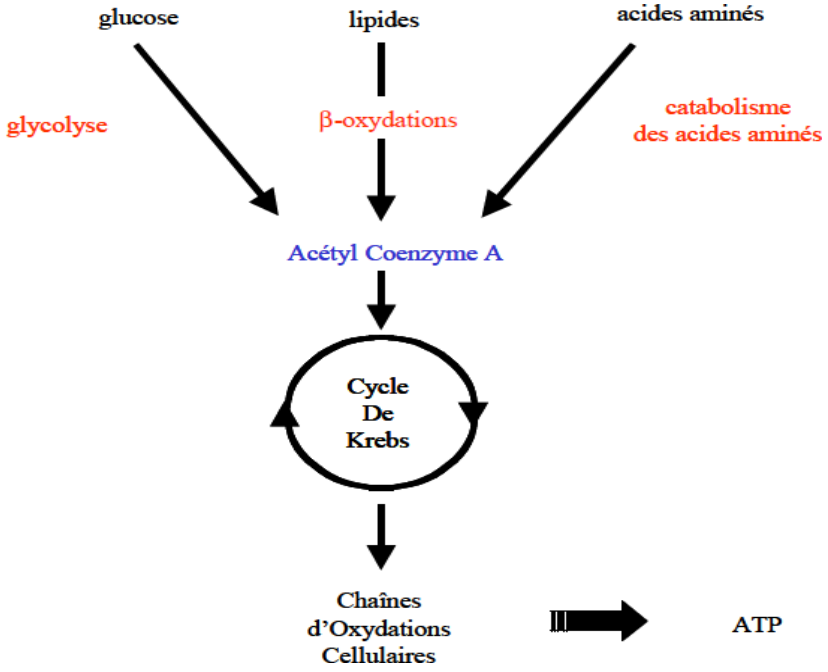


Fig. 213. The Krebs cycle: the final stage of food degradation.

VIII.2.3.1. Acetyl coenzyme A: entry point of the Krebs cycle.

Acetyl coenzyme A (Acetyl CoA) is formed from pyruvate, the end product of glycolysis, and coenzyme A (CoASH). The reaction can be written in simplified form as:

Pyruvate + CoASH + NAD^+ ⟶ **Acetyl CoA + CO_2+ NADH + H^+.**

In reality, this reaction takes place in several stages and involves a multi-enzymic complex: The pyruvate dehydrogenase complex.

VIII.2.3.2. The different stages of the Krebs cycle.

Once acetyl CoA is produced, it will be able to feed the Krebs cycle and its eight steps detailed below (Fig. 214).

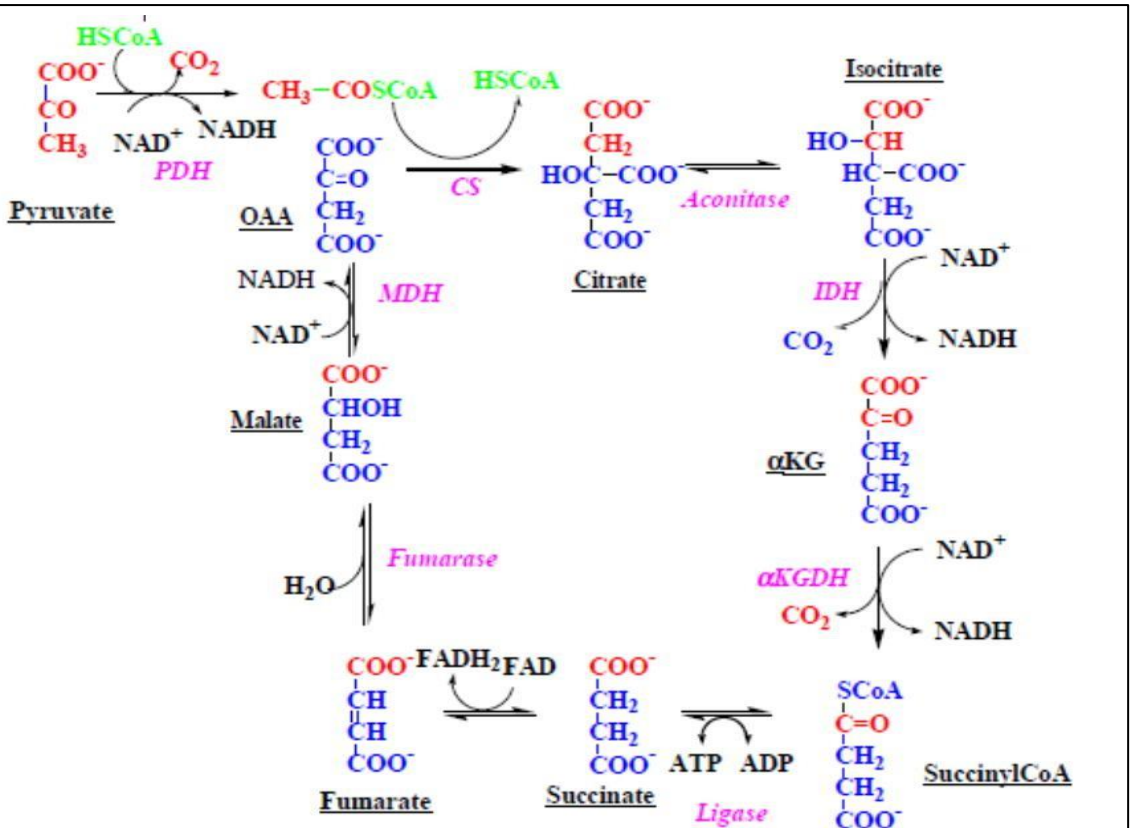
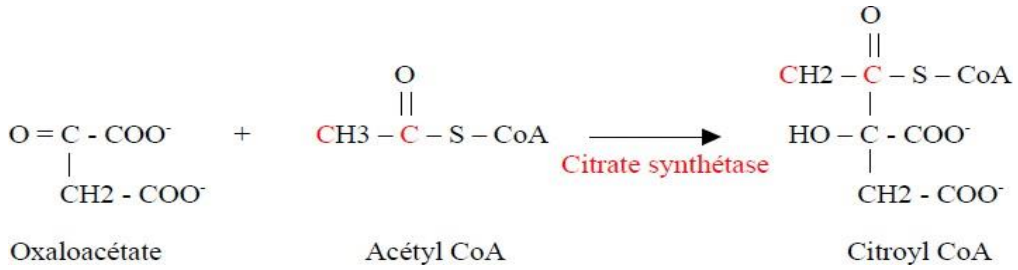
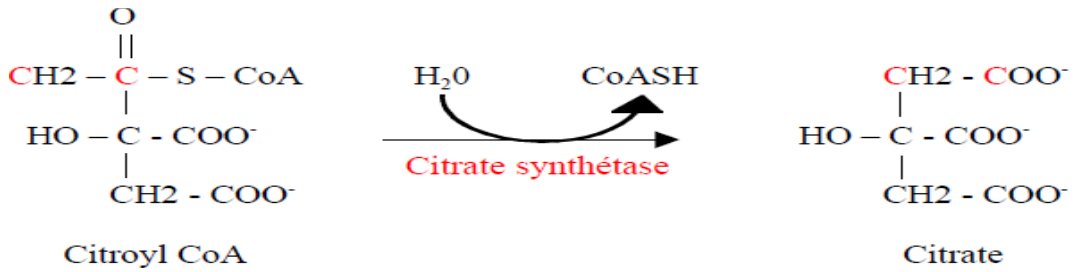


Fig. 214. Overview of the Krebs cycle.

A. *Condensation of acetyl CoA and oxaloacetate.*

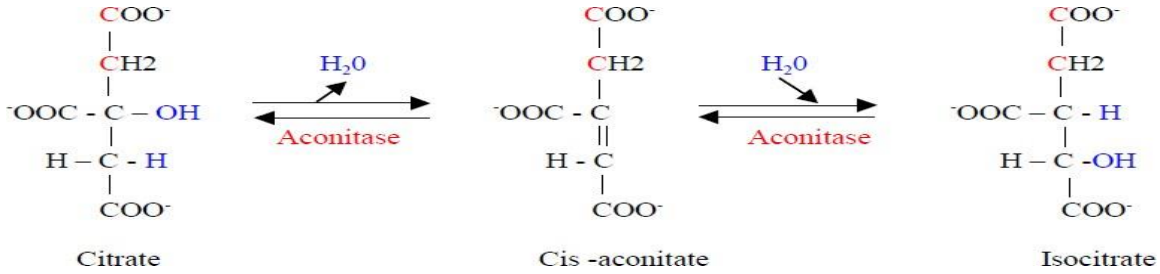
The condensation of acetyl CoA and oxaloacetate is catalyzed by citrate synthetase. This reaction is irreversible. Oxaloacetate first binds to the enzyme, which, by changing its conformation, allows the binding of acetyl CoA and the formation of an intermediate: citroyl CoA, which will then be hydrolyzed into citrate and coenzyme A.





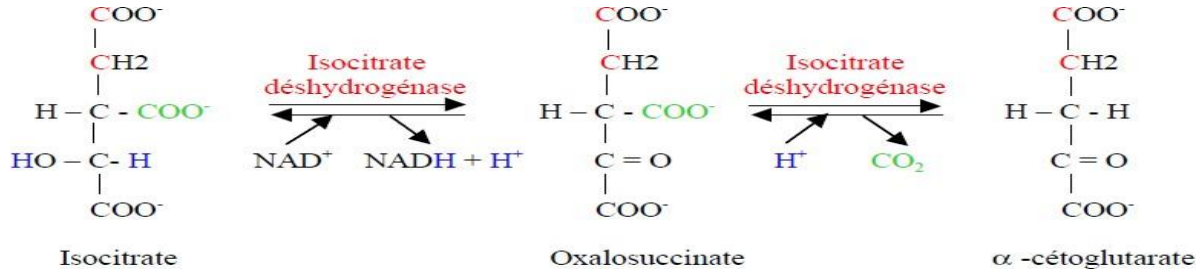
B. *Conversion of citrate to isocitrate.*

The reaction occurs in two steps with cis-aconitate as an intermediate. Both steps are catalyzed by aconitase.



C. *Oxidation and decarboxylation of isocitrate to α-ketoglutarate.*

Isocitrate is oxidized by isocitrate dehydrogenase to form oxalosuccinate and generate a molecule of NADH. Oxalosuccinate remains bound to the enzyme and loses a molecule of CO2 to form α-ketoglutarate (α-KG).



D. *Decarboxylation of α-ketoglutarate to succinyl coenzyme A.*

This reaction is catalyzed by the α-ketoglutarate dehydrogenase complex, whose structure and mechanism of action are very similar to those of pyruvate dehydrogenase, which converts pyruvate to acetyl-CoA. The reaction can be written in simplified form as: α-ketoglutarate + CoASH + NAD+ ⟶ succinyl CoA + CO_2 + NADH + H+

The complex contains three enzymes and their cofactors:

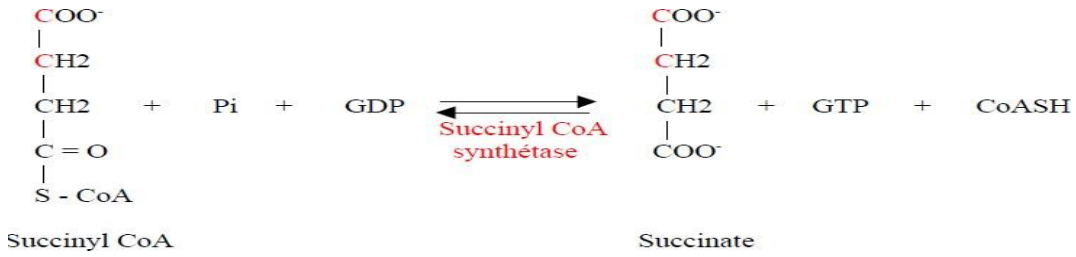
E'1: α-Ketoglutarate dehydrogenase + thiamine pyrophosphate (TPP). E'2: Dihydrolipoyl transsuccinylase + lipoamide.

E'3: Dihydrolipoyl dehydrogenase. + FAD. (identical to E3)

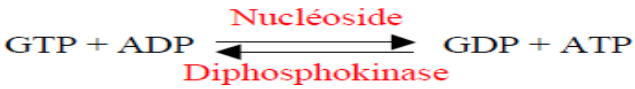
As with the pyruvate dehydrogenase complex, the α-ketoglutarate dehydrogenase complex is regulated by the ATP/ADP, NADH/NAD+ ratios, but also by the succinyl CoA/CoASH ratio.

E. *Formation of succinate.*

Succinate is produced by hydrolysis of the thioester bond between the succinyl residue and coenzyme A of succinyl CoA. Hydrolysis of this bond releases a large amount of energy, which is recovered by synthesizing a molecule of GTP through phosphorylation of a molecule of GDP. This reversible reaction is catalyzed by succinyl CoA synthetase.

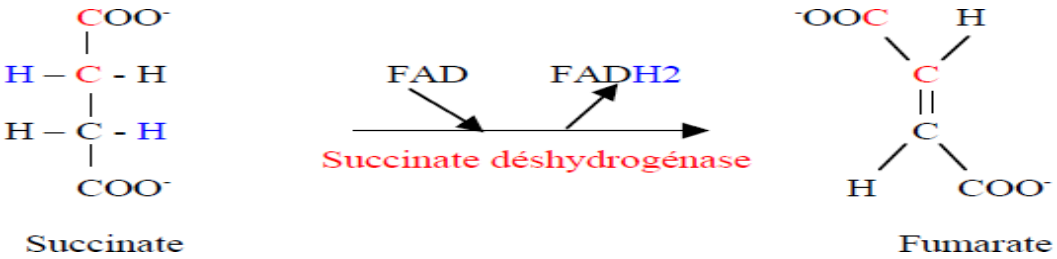


GTP indirectly constitutes an energy reserve since the phosphate it has just acquired can easily be transferred by a nucleoside diphosphokinase to an ADP molecule to form an ATP molecule.



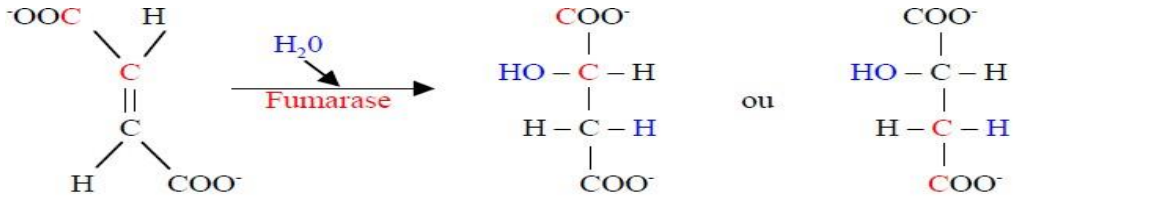
F. *Oxidation of succinate to fumarate.*

The reaction is catalyzed by succinate dehydrogenase, which, unlike the other enzymes in the cycle, is membrane-bound. In fact, it is an integral part of the inner mitochondrial membrane. Furthermore, unlike the dehydrogenases mentioned previously, succinate dehydrogenase uses FAD, not NAD^+, to transport hydrogen to the cellular oxidation chains. This is because, in the latter case, the reaction energy is insufficient to reduce NAD+.



G. *Hydration of fumarate into L-malate.*

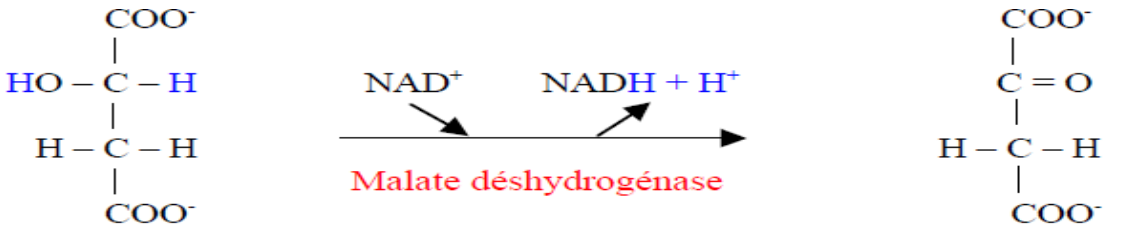
The reaction is catalyzed by fumarase. The H and OH atoms from a water molecule are added in trans to the fumarate, that is to say each on one side of the plane formed by the double bond.



Fumarate L-malate (Cf : remarque précédente)

H. *Oxidation of malate to oxaloacetate.*

The reaction is catalyzed by malate dehydrogenase, which uses NAD+ as a hydrogen carrier.



L-malate oxaloacétate

Once regenerated, oxaloacetate can condense to a molecule of acetyl CoA and thus begin a new cycle.

Summary of the Krebs cycle.

The Krebs cycle can be summarized by the following equation:

$$\text{Acétyl CoA} + 3\ NAD^+ + FAD + GDP + Pi + H_2O \downarrow$$
$$2\ CO_2 + 3\ NADH + 3H^+ + FADH_2 + GTP + CoASH$$

- Given that the reoxidation of NADH at the cellular oxidation chain level provides three ATP molecules and that of FADH22two were provided.
- Given that one molecule of GTP can easily enable the synthesis of one molecule of ATP.

The balance based on pyruvate:

Pyruvate + 4NAD+ + FAD + ADP + P⟶3 CO_2+ 4NADH + $FADH_2$+ ATP.

The balance based on acetyl CoA:

Pyruvate + 3NAD+ + FAD + ADP + P ⟶ $2CO_2$+ 3NADH + $FADH_2$+ ATP.

ATP balance taking into account the respiratory chain:

	From pyruvate		From acetyl-CoA	
	Regenerated coenzymes	**ATP formed**	**Regenerated coenzymes**	**ATP formed**
NADH	4	12	3	9
$FADH_2$	1	2	1	2
ATP	1	1	1	1
Total		15		12

The cycle therefore produces:

1 GTP donc 1 ATP
3 NADH donc 3 x 3 soit 9 ATP
1 $FADH_2$ donc 2 ATP

Bilan ➡ 12 ATP

If we take into account the NADH produced during the conversion of pyruvate to acetyl CoA, we arrive at a net result of 12 + 3 or 15 ATP molecules produced.

VIII.2.4. Pentose phosphate pathway: production of reducing power and ribose

Carbohydrates, via the glycolytic pathway, are the source of ATP and NADH, H^+. The electrons stored as NADH, H+ constitute the majority of metabolic energy. This energy is used for ATP production through oxidative phosphorylation.

Carbohydrates also initiate redox reactions, the released electrons and protons of which are stored as NADPH,H^+. The pool of NADPH,H^+ represents the reducing power that the cell needs for reducing biosynthetic reactions. NADPH,H^+ is formed in cells via the pentose phosphate pathway.

VIII.2.4.1. Definition and purpose of the pentose phosphate pathway

The pentose phosphate pathway is a glucose degradation pathway branched off from glycolysis, producing NADPH, H^+ and ribose-5-phosphate.

It starts at glucose-6-P and joins glycolysis at the level of fructose-6-P and 3-phosphoglyceraldehyde. It is a quantitatively minor pathway (less than 10% of glucose is broken down by this pathway).

This pentose phosphate pathway not only generates NADPH, H^+ but also pentoses, in particular ribose 5-P.

NADPH, H^+ is a reducing agent subsequently used for lipid biosynthesis, the reduction of glutathione.

Ribose-5-phosphate is an essential component of pyridine and flavin coenzymes, coenzyme A, ATP and nucleic acids (Ribose 5-P = component of: ATP, CoA, NAD^+, FAD, RNA, DNA).

The pentose phosphate pathway is fueled by glucose 6-P. It allows the interconversion of sugars with 3, 4, 5, 6, 7 carbons and it takes place in the cytosol (Fig. 215).

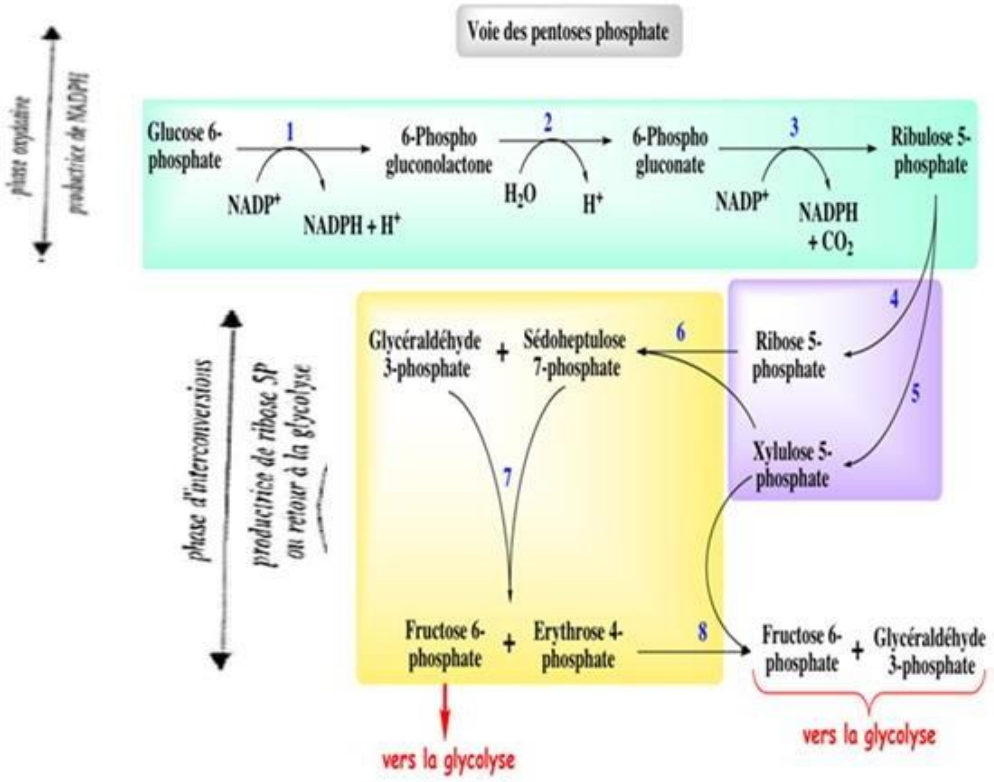


Fig. 215. Pentose phosphate pathway.

VIII.2.4.2. NADPH production, H^+: Reducing power

In this case, the cell primarily produces NADPH, H^+ from glucose. The sequence of enzymatic reactions is described in 3 phases.

Phase 1: Redox reactions

The oxidation reactions that occur during this phase are the sites of formation of reducing power in the form of NADPH, H^+. Electrons removed from glucose 6-P and gluconate 6-P are transferred to $NADP^+$ instead of NAD^+. They have no energy value but will be used in reducing redox reactions catalyzed by reductases.

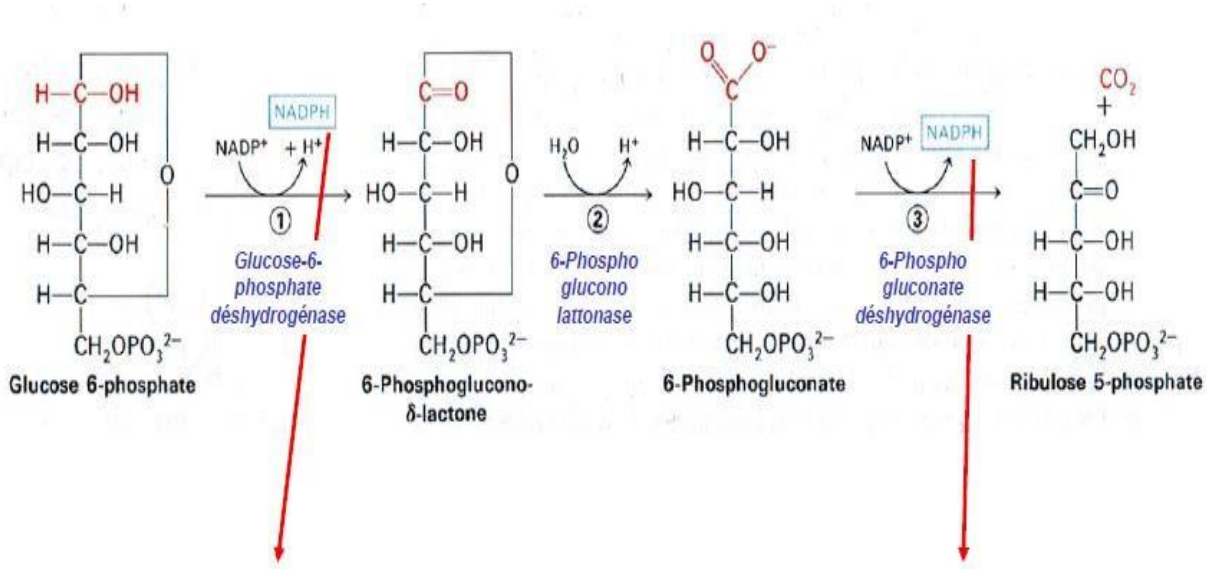
a - Dehydrogenation of glucose 6-P

In the presence of NADP+ as a cofactor and under the action of glucose-6-P dehydrogenase (G6PDH), glucose 6-P is oxidized to gluconate 6-P:

Glucose 6-P + NADP+ ⟶ gluconate-6-P + NADPH, H+

b– Decarboxylating dehydrogenation of 6-P gluconate

This reaction occurs through the action of gluconate-6-phosphate dehydrogenase, with NADP+ acting as a cofactor. It is the second redox reaction that leads to the oxidation of the alcohol group on carbon 3 to a ketone group. This ketone group destabilizes the terminal carboxyl group, resulting in decarboxylation and yielding ribulose 5-phosphate.



La phase 1 comprend la fonction principale de la voie: la synthèse de NADPH

PHASE 2: Isomerization of pentose phosphates

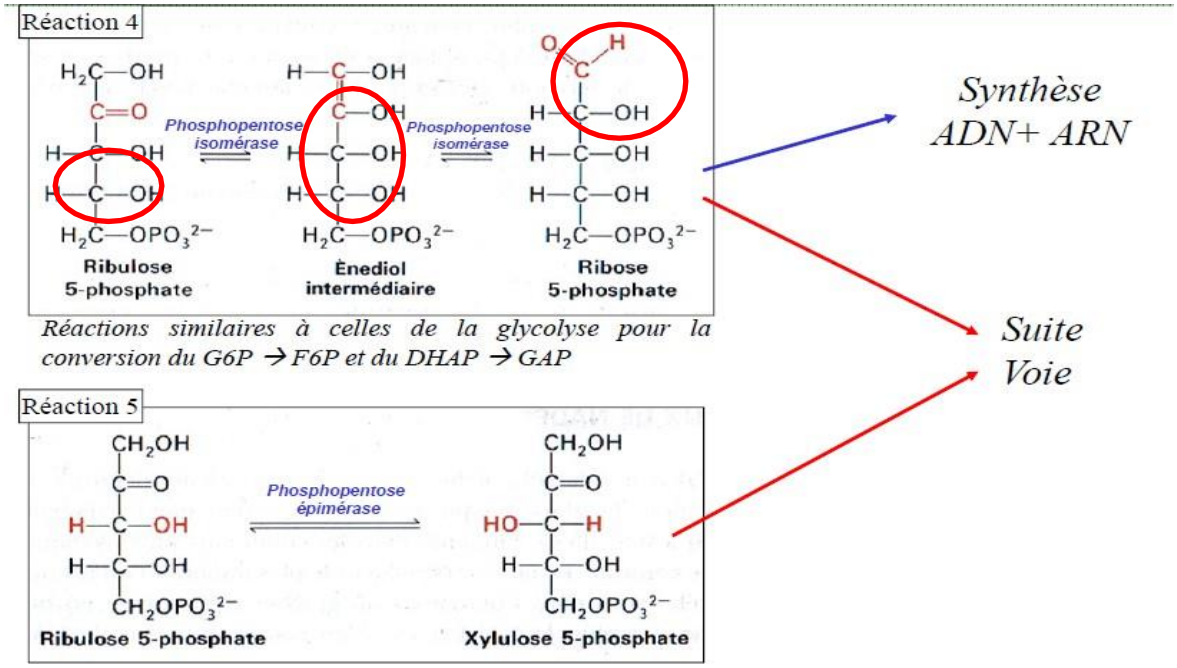
The two reactions in this phase prepare for the next one.

a - Epimerization of ribulose 5-P to xylulose 5-P

The reaction is catalyzed by a phosphopentose 3-epimerase. Two of the ribulose 5-P molecules are transformed into xylulose 5-P.

b - Isomerization of ribulose 5-P into Ribose 5-P

It is done under the action of ribose 5-phosphate isomerase.

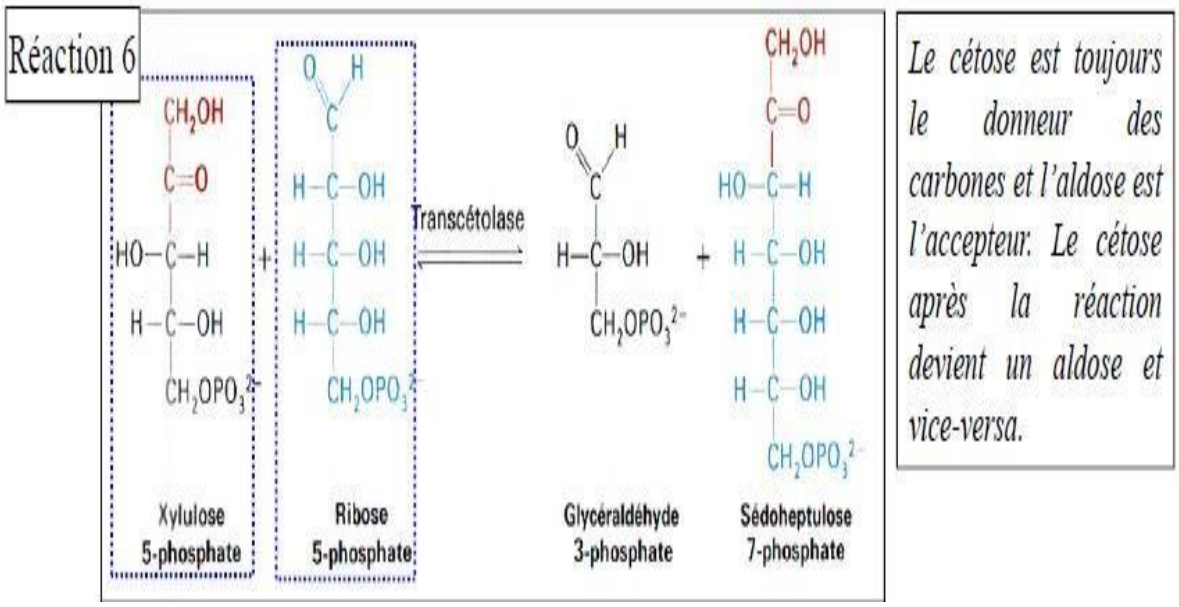


PHASE 3: Interconversion of phosphates

In phase 3, 3 reactions occur: 2 transketolation reactions and 1 transaldolization reaction, which, through the transfer of terminal carbons, allow the reformation of glucose 6-P via fructose 6-P. All these reactions are reversible.

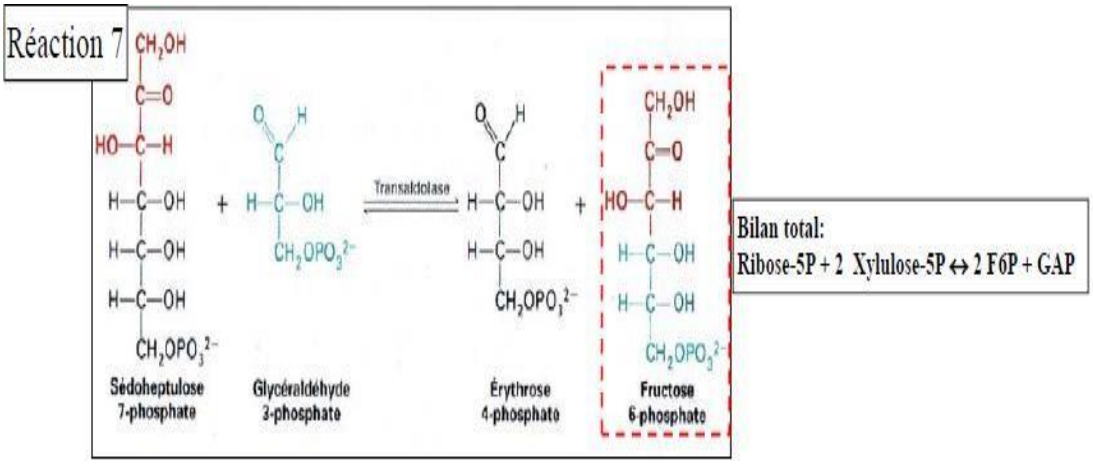
a - Formation of sedoheptulose 7-P by transcetolation

The reaction is catalyzed by transketolase and involves ribose-5-P and one of the xylulose 5-P. Two terminal carbons of xylulose 5-P are transferred to the end of ribose 5-P.



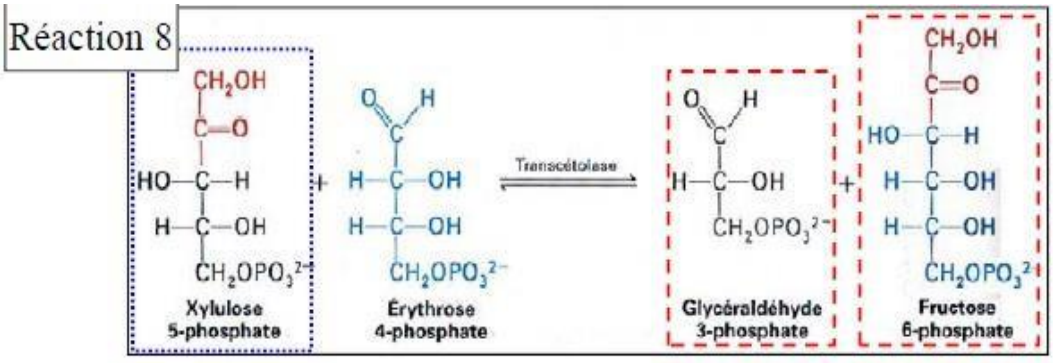
b - Formation of fructose 6-P by transaldolization

The products of the previous reaction become the substrates for transaldolase to form fructose 6-phosphate and erythrose 4-phosphate. Three terminal carbons of sedoheptulose 7-phosphate are transferred to glyceraldehyde 3-phosphate according to the reaction:



c - Formation of a second fructose 6-P by transcetolation

The first two carbons of the remaining xylulose 5-P are transferred to erythrose-4-P to form a second fructose 6-P with the release of glyceraldehyde-3-P. The reaction is catalyzed by transketolase.



d - Isomerization of the 2 fructose 6-P into glucose 6-P

2 fructose 6-Ⓟ ⟷ 2 glucose-6-Ⓟ

VIII.2.4.3. Road assessment

The overall equation for the sequence of reactions, geared towards the production of NADPH, H^+, is:

3 Glucose 6-Ⓟ + 6 $NADP^+$ → 2 Glucose 6-Ⓟ + Glycéraldéhyde 3-Ⓟ + 6 $NADPH,H^+$ + 3 CO_2. ou

glucose-6-Ⓟ + 6 $NADP^+$ ⟶ glycéraldéhyde 3-Ⓟ + 3 CO_2 + 6 $NADPH,H^+$

This means that with each cycle, one glucose molecule is oxidized and the following are formed: 3 CO2, 1 glyceraldehyde-3-

P and 6 NADPH, H^+. Knowing that glyceraldehyde is half of a glucose, we deduce that the complete oxidation of one glucose provides 12 NADPH, H^+, which gives.

$$\text{Glucose 6-Ⓟ} + 12\ NADP^+ \longrightarrow 6\ CO_2 + 12\ NADPH,H^+ + Pi$$

$NADP^+$ differs from NAD+ by the presence of an additional phosphate group on the C2' carbon of the ribose (Fig. 216). This small difference in its structure allows it to interact specifically with certain enzymes and play important roles. In the cytosol of hepatocytes, the NADP+/$NADPH,H^+$ ratio is 0.1, while that of $NAD^+/NADH,H^+$ is 1000, which gives NAD^+ a privileged role as an oxidant.

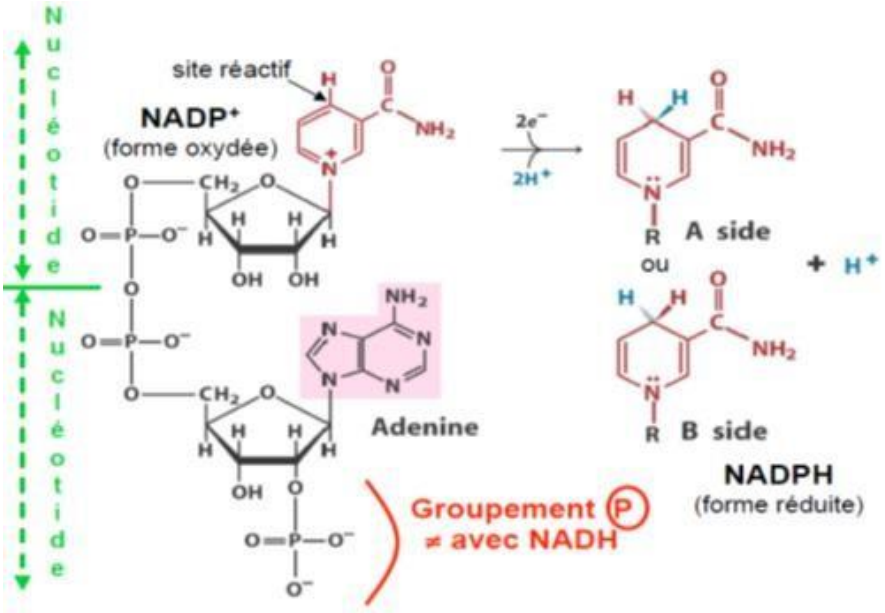


Fig. 216. Structure of Nicotinamide Adenine Dinucleotide Phosphate (NADP).

VIII.2.5. Glycogen metabolism

Animal organisms have developed a rapid mobilization process in the liver and striated muscles in response to immediate demand in the absence of dietary glucose. This process is glycogenolysis, or the breakdown of glycogen. While liver glycogen is mobilized to maintain blood glucose levels and supply peripheral tissues, glycogen stored in muscles is mobilized and used locally for muscle function.

Liver glycogen stores increase when animals are well-fed and can decrease during prolonged fasting until depletion. Muscle glycogen stores are little affected by prolonged fasting and can be replenished after activity that has used some of them. Whether in the liver or muscles, glycogen

is synthesized from glucose 6-phosphate as a precursor. Glycogen synthesis is the **glycogenesis**.

VIII.2.5.1. Glycogenolysis

A- Glycogen breakdown or glycogenolysis

The main enzyme in the breakdown of endogenous glycogen (hepatic and muscular) is glycogen phosphorylase, which releases glucose-1-phosphate and limiting dextrin. Two other enzymes, a glycosyltransferase and an α(1-6) glucosidase, are involved in the complete conversion of glycogen to glucose-6-phosphate. Only the liver can convert glucose-6-phosphate into glucose, which is then excreted into the blood (Fig. 217).

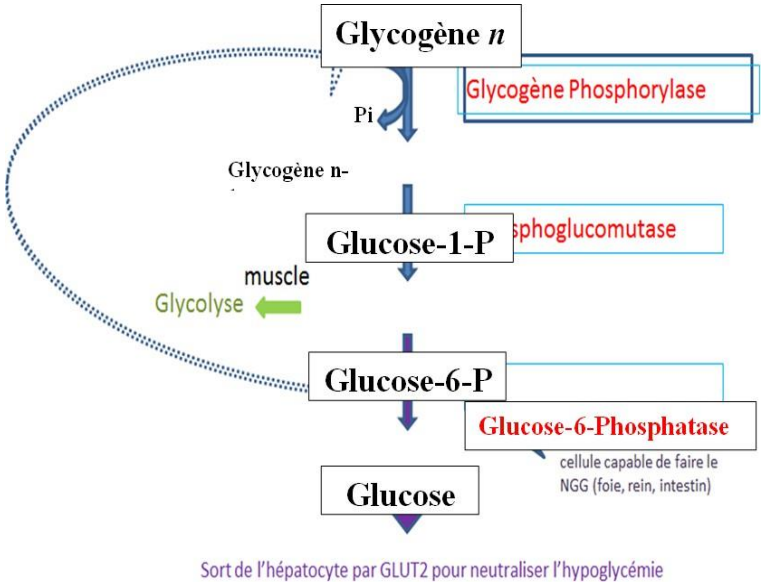


Fig. 217. Sequences of enzymatic reactions that allow the degradation of glycogen.

VIII.2.5.1.1. Sequences of enzymatic reactions

a - Phosphorolysis of glycogen

Phosphorolysis itself is catalyzed by glycogen phosphorylase. This enzyme cleaves the α(1-4) bond from the non-reducing end and attaches a phosphate group, supplied by ATP, to carbon 1 of the released glucose, yielding glucose 1-phosphate. Phosphorolysis is repeated sequentially on glycogen up to four glycolsyl residues on each chain before the α(1-6) bond. The residual structure, whose branching limits the action of phosphorylases, is called the limiting dextrin, which resists further action by phosphorylase (Fig. 218).

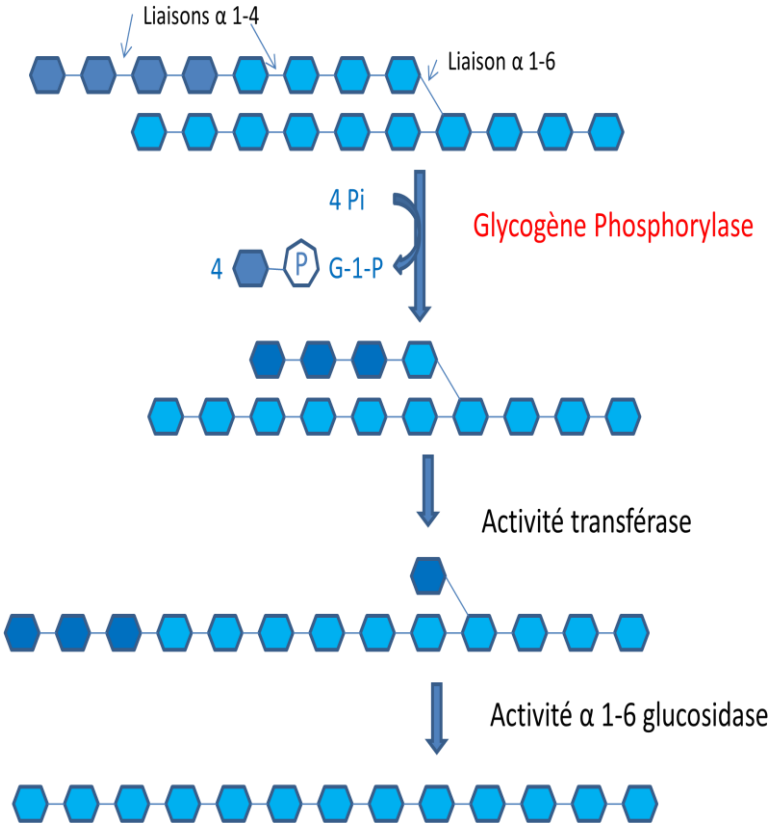


Fig. 218. Phosphorolysis of glycogen.

b - Glycosyl-4,4-transferase

Glucosyl-4,4-transferase acts on limit dextrin by removing an oligosyl group, composed of three glucose residues, from each limit dextrin chain. This oligosyl group is then used to extend another limit dextrin chain, thus allowing phosphorolysis to resume on that chain. After the action of this enzyme, a glucose molecule linked by an α(1-6) bond remains in place of the side chain.

c- α(1-6) Glucosidase

Finally, an α-glucosidase hydrolyzes the glucose residues linked by the α(1-6) bond and releases the glucose.

After the action of these three enzymes, glycogen releases primarily glucose 1-phosphate (via phosphorolysis) and a small amount of glucose (via hydrolysis). Glucose 1-phosphate is isomerized to glucose 6-phosphate by phosphoglucomutase. Glucose 6-phosphate can then enter glycolysis in the liver and muscle.

But the primary objective of hepatic glycogen breakdown is to maintain blood glucose levels.

To accomplish this, only the liver, after glycogen breakdown, possesses glucose 6-phosphatase, which hydrolyzes glucose 6-phosphate into glucose and excretes the latter into the blood stream. The two catalyzed reactions are as follows:

Glucose 1-Ⓟ ⟷ Glucose 6-Ⓟ *(Phosphoglucomutase)*

Glucose 6-Ⓟ + H_2O ⟶ Glucose + Pi *(Glucose 6-phosphatase)*

VIII.2.5.2. Glycogenesis

B – Glycogen synthesis

Glycogen synthesis aims to store, in the liver, some of the excess glucose following a diet rich in carbohydrates and proteins, and in the muscles to replenish glycogen stores, a portion of which has been used up by physical activity. Glycogen synthesis occurs primarily in the liver and muscles. The main enzyme is glycogen synthase. The precursor is glucose 6-phosphate (Fig. 219).

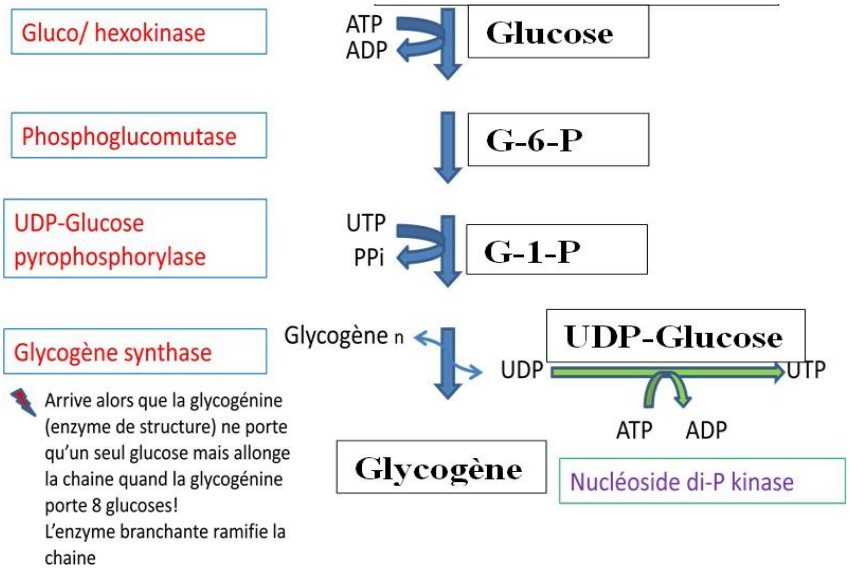


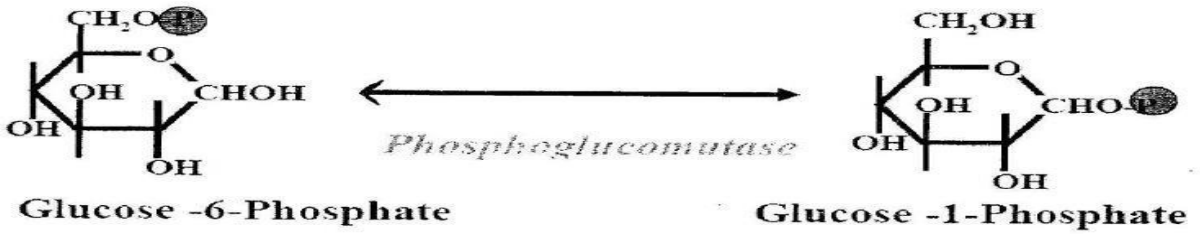
Fig. 219. Glycogenesis.

VIII.2.5.2.1. Sequences of enzymatic reactions

a - Isomerization of glucose 6Ⓟ in glucose 1-Ⓟ

The enzyme that catalyzes this reaction is phosphoglucomutase.

Glucose 6-Ⓟ ⟷ glucose 1-Ⓟ



Glucose -6-Phosphate Glucose -1-Phosphate

b - Transfer of the glucosyl residue onto UTP (formation of UDP glucose).

The donor of the glucose residue in the polymerization of glucose to glycogen is UDP-glucose (Fig. 220). Its formation is ensured by UDP-glucose pyrophosphorylase, which transfers the glucosyl group to UDP, releasing pyrophosphate (PPi). Hydrolysis of PPi by pyrophosphatase further promotes the reaction.

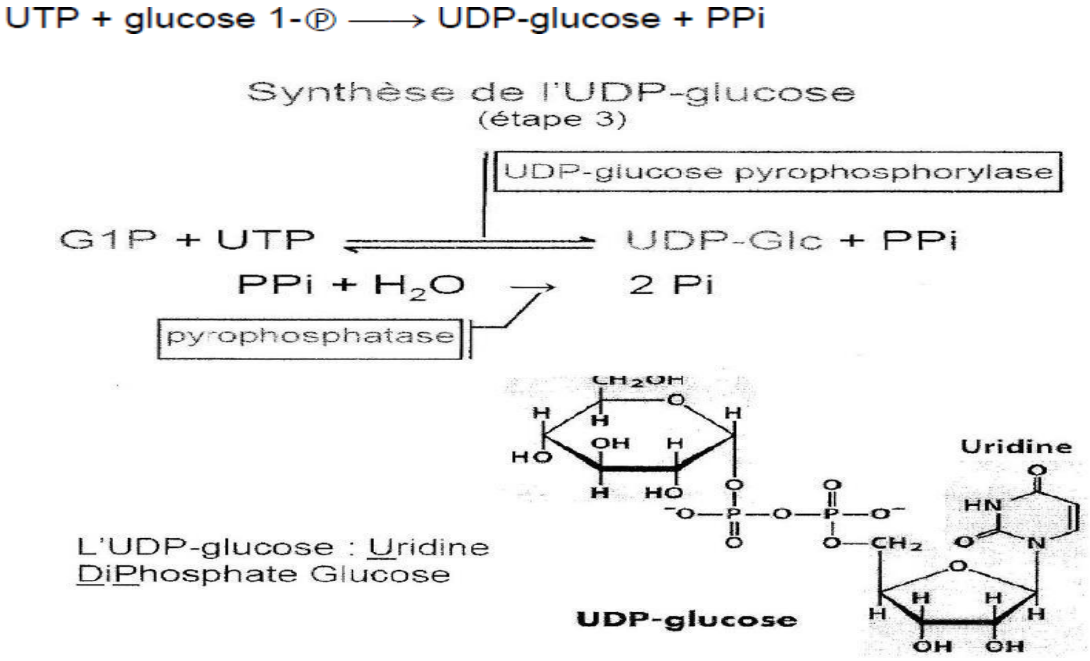


Fig. 220. Formation of glucose UDP.

c – Synthesis of a primer to initiate glycogen synthesis

Glycogen synthase, which is responsible for the formation of the α(1-4) linkage, is an elongation enzyme and cannot initiate de novo glycogen synthesis from glucose. A primer is required, which can be obtained in various ways:

- use of a glycogen fragment in the form of dextrin
- In the absence of this fragment, a specific cytoplasmic protein, glycogenin, intervenes. It possesses a tyrosine side chain which, thanks to its -OH group, acts as an acceptor for the first glucosyl residue from UDP glucose. Through its endogenous glucosyltransferase activity, glycogenin transfers a first glucose molecule from UDP glucose to the tyrosyl residue of its polypeptide chain. The formation of the first glycosidic bond is catalyzed by an initiating glycogen synthase. Glycogenin adds further glucose residues, linked by α(1-4) linkages, to complete the primer, which is approximately 8 glucose residues long (Fig. 221).

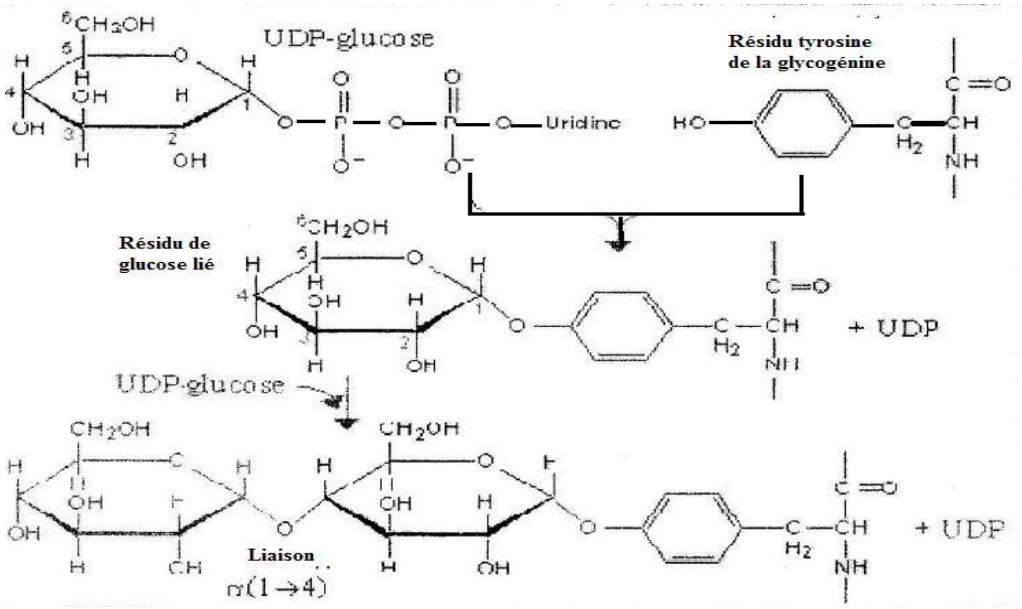


Fig. 221. Formation of UDP glucose.

d – Elongation of the chain by glycogen synthase

The elongation of the chain is ensured by glycogen synthase which transfers the glucosyl residue of UDP-glucose to the non-reducing end of the chain of the primer or elongating glycogen and sequentially makes the α(1-4) bond according to the reaction (Fig. 222):

$$\text{Glycogène (n glucose)} + \text{UDP-glucose} \longrightarrow \text{glycogène[(n+1)]glucose} + \text{UDP}$$

UDP is then converted back into UTP by a nucleoside diphosphate kinase in the presence of ATP according to the reaction:

$$\text{ATP} + \text{UDP} \longrightarrow \text{UTP} + \text{ADP}$$

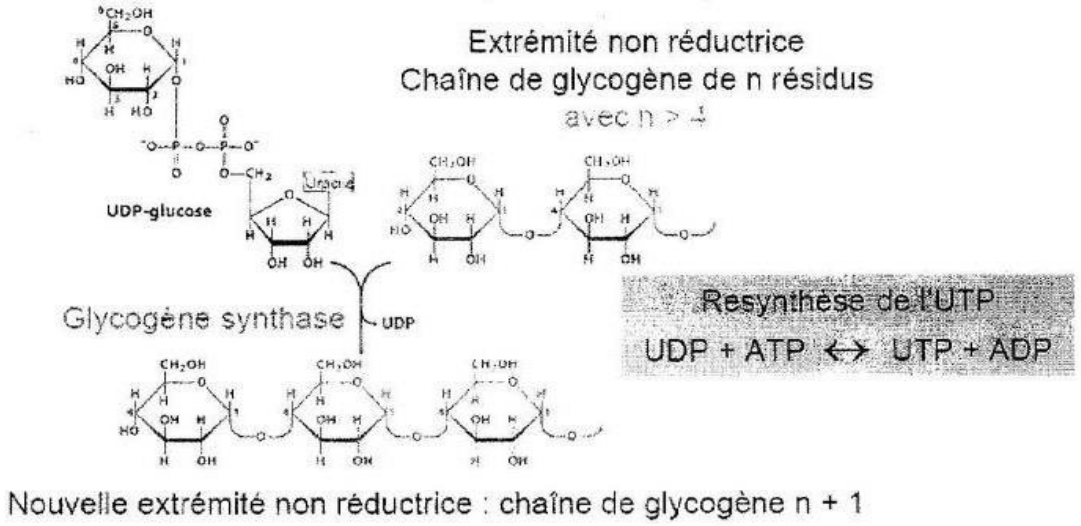


Fig. 222. Elongation of the glycogen chain by glycogen synthase.

e – Formation of lateral chains

At every 8 glucose residues on the linear chain synthesized by glycogen synthase, a branch forms, giving glycogen a highly branched structure. This increases the number of non-reducing ends, which are favorable to glycogen phosphorylase activity during the mobilization of glycogen reserves (Fig. 223). Branching is carried out by a branching enzyme: amylo (α-1,4 α-1,6) transglycosylase or glycosyl(4,6) transferase (Fig. 25). This enzyme takes an oligosaccharide of 5 to 8 glucose residues from the non-reducing end of the elongating chain and attaches it to a glucosyl residue on the main chain via an α(1-6) bond.

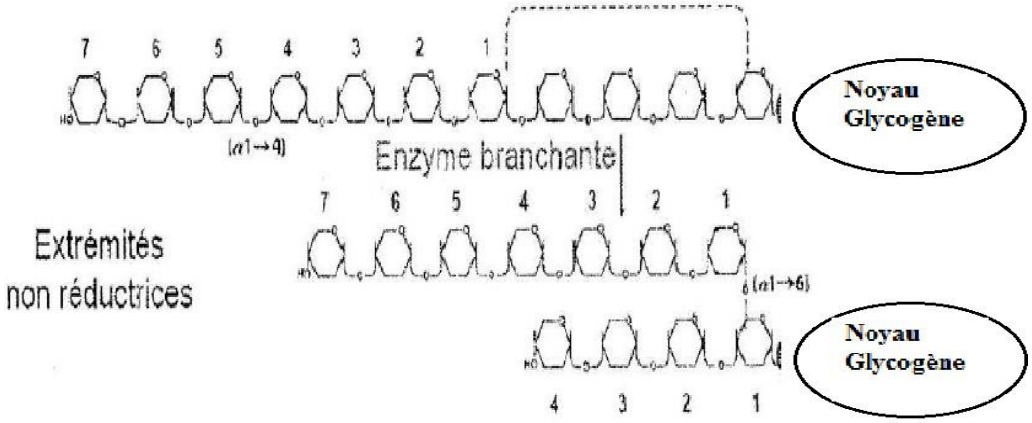
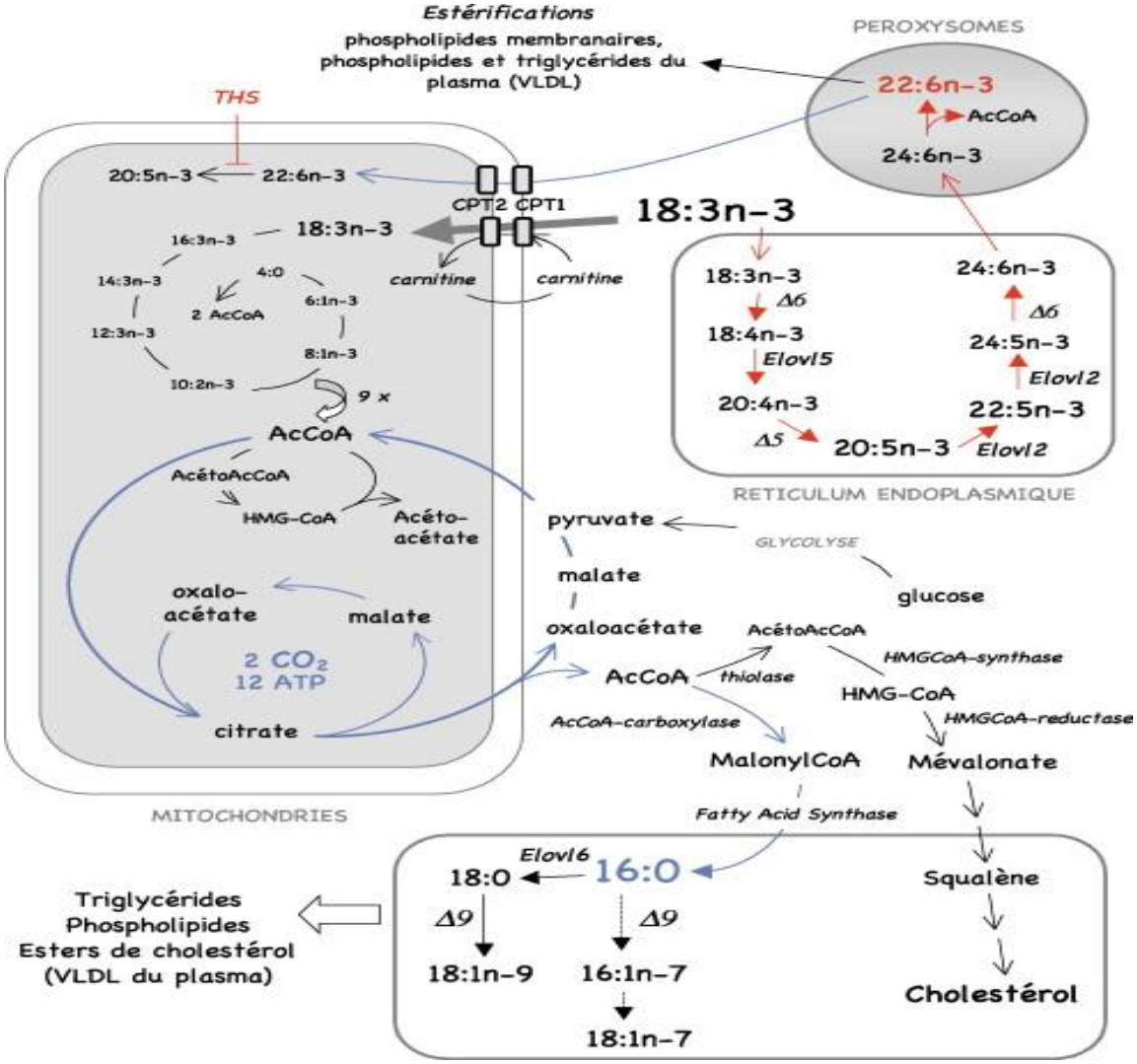


Fig. 223. Formation of glycogen chain branches.

CHAPTER IX :

FATTY ACID METABOLISM



CHAPTER IX FATTY ACID METABOLISM

Fatty acid (FA) metabolism encompasses both catabolism and synthesis. Fatty acids are quantitatively very important energy sources in both animals and plants. They are oxidized in numerous tissues (adipose tissue, liver, lungs, kidneys, heart, etc.). Cells can accumulate very large quantities of them in the form of triglycerides. In vertebrates, lipids provide approximately 40% of energy when they are on a normal diet. In fasting or hibernating animals and migratory birds, they constitute the sole source of energy. Triglycerides represent highly concentrated energy storage forms. These lipids are primarily stored in the cytoplasm of adipose cells, which are specialized in their synthesis. They are then transported by the blood to sites of use. The sequence of reactions in which they are involved begins with enzymatic hydrolysis by lipases, followed by a preparatory degradation called β-oxidation, with transformation of fatty acids into acetyl-CoA which then feeds the tricarboxylic cycle.

IX.1- DEGRADATION OF FATTY ACIDS (β-OXIDATION)

β-oxidation is the central reaction in fatty acid catabolism. Some organs, such as the liver and heart, meet more than 50% of their energy needs through fatty acid oxidation. The name β-oxidation comes from the fact that the reaction begins at the second carbon atom counting from the carboxyl group. This atom is called C-β (it is actually the third carbon in the chain).

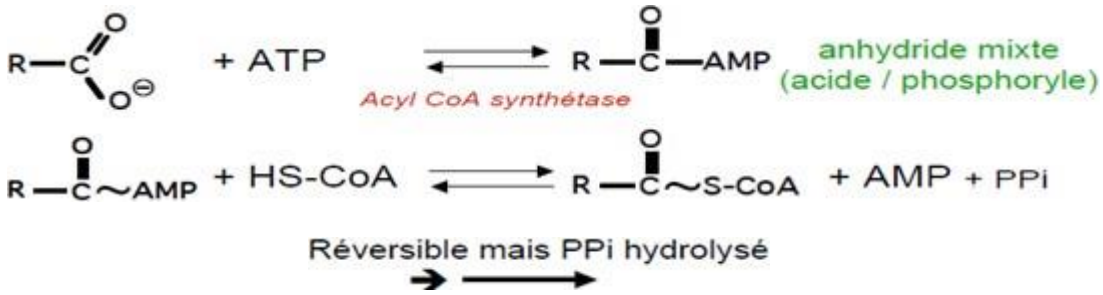
IX.1.1- β-oxidation of long-chain saturated fatty acids with an even number of carbon atoms

Long-chain fatty acids (with more than 10 carbon atoms) must first be activated to be oxidized. In the mitochondria, the acyl group is transferred to coenzyme A in the intermembrane space by an acyl-CoA synthetase, and then transported into the matrix by the acyl-carnitine shuttle across the inner mitochondrial membrane. Short-chain fatty acids (with 10 or fewer carbon atoms) can be transported directly into the matrix and activated there by a matrix acyl-CoA synthetase.

IX.1.1.1-Activation and entry of fatty acids into the mitochondria

a- Activation of fatty acids by coenzyme A

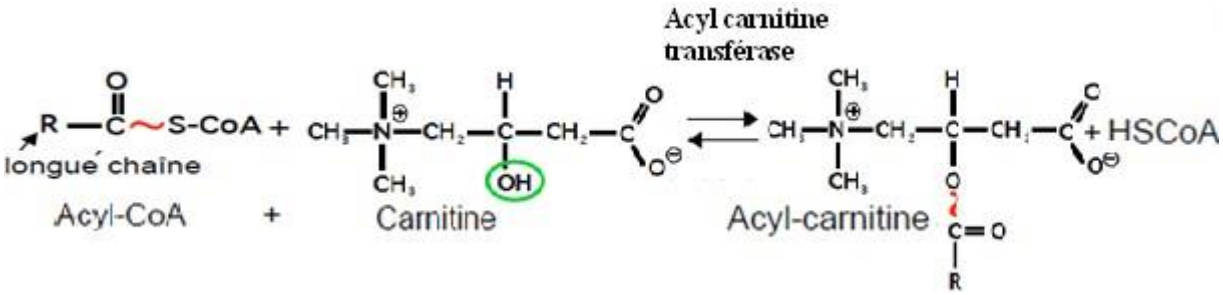
Fatty acids are activated by their binding to HSCoA. Activation is catalyzed by acyl-CoA synthetase (thiokinase). During the reaction, ATP undergoes cleavage, releasing pyrophosphate and AMP.



Pyrophosphate is hydrolyzed by pyrophosphatase to provide the additional energy needed for thioester bond formation. AMP is then rephosphorylated to ADP and subsequently to ATP by adenylate kinase.

b-Transfer onto carnitine

In the form of acyl-CoA, long-chain fatty acids cannot cross the inner mitochondrial membrane. Their passage is facilitated by carnitine, a widely distributed nitrogenous alcohol synthesized from lysine and methionine. The acyl group is supported by carnitine. The reaction is catalyzed by acyl-carnitine transferase 1 (located on the outer surface of the inner membrane). Acyl-carnitine and HSCoA are released into the intermembrane space.



b- Transfer via translocase

Acylcarnitine crosses the mitochondrial membrane through the action of an ***acylcarnitine translocase*** (Fig. 224). In the mitochondrial matrix, the acyl radical is retransferred to HSCoA. The reaction is catalyzed by acyl-carnitine transferase 2, located on the matrix side of the membrane.

internal. The reconstituted acyl-CoA thus becomes the substrate for the reactions that will take place in the mitochondrial matrix.

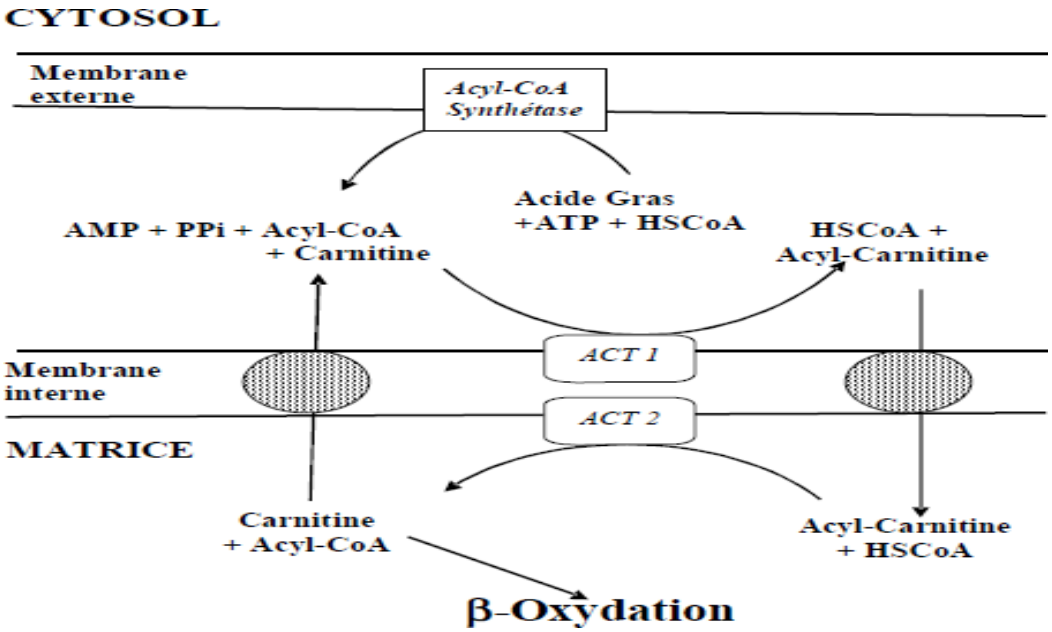


Fig. 224. Activation of long-chain fatty acids and transport of the acyl radical into the mitochondrial matrix by carnitine. ACT = Acyl-carnitine transferase.

IX.1.1.2- Stages of β-oxidation of fatty acids

The reaction sequence takes place in 4 steps, called turns. For a fatty acid with 2n carbons, (n-1) turns are required for its complete oxidation to n acetyl-CoA (Fig. 225).

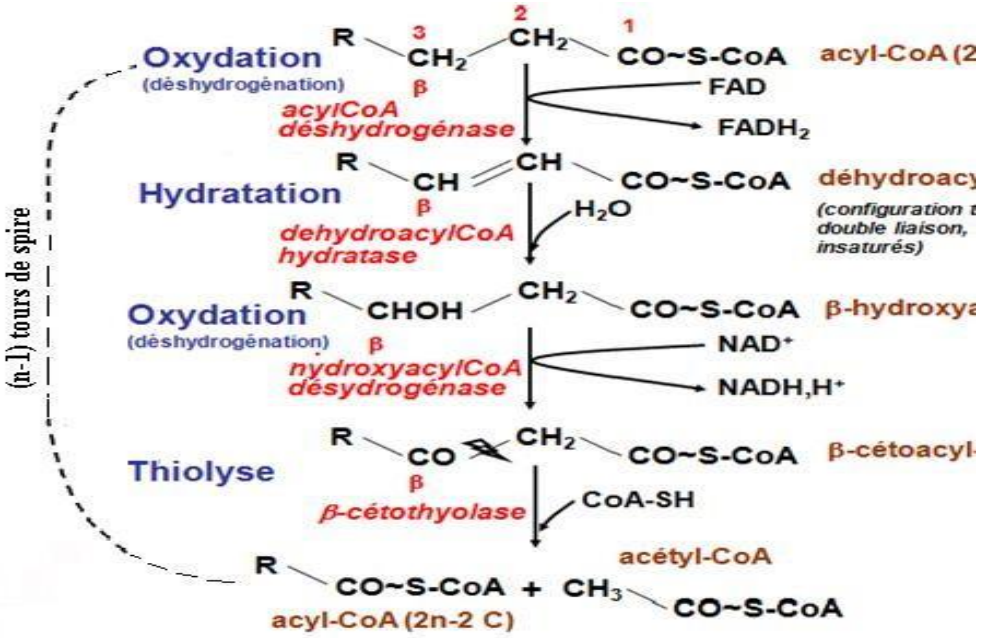
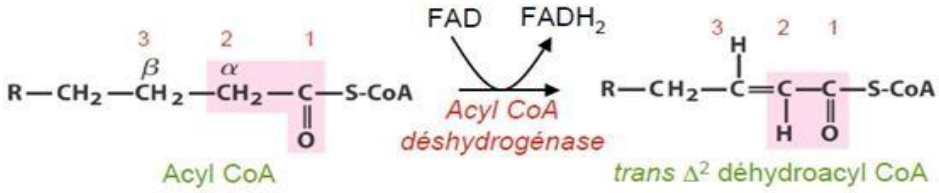


Fig. 225. Stages of β-oxidation of fatty acids.

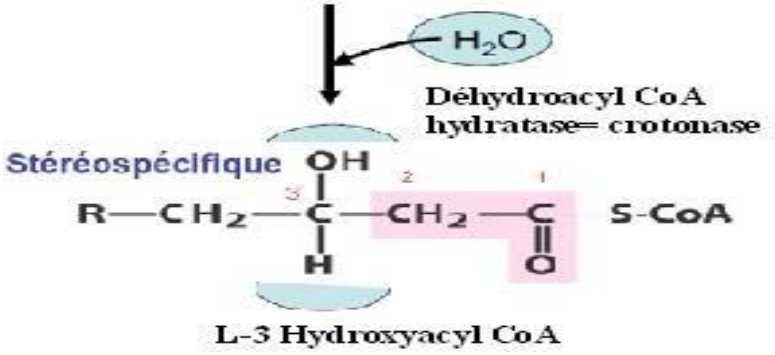
a- *First dehydrogenation of Acyl-CoA*

Between carbons 2 and 3 of acyl-CoA, dehydrogenation occurs, carried out by acyl-CoA dehydrogenase, a flavoprotein with FAD, which creates a double bond.



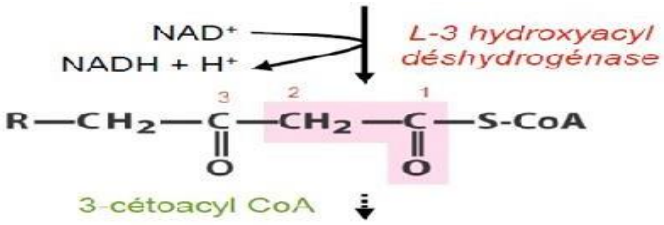
b- *Hydration of the double bond*

It is mediated by an enoyl-CoA hydratase. The product obtained is L 3-hydroxyacyl-CoA. The attachment of the OH radical is directed at carbon 3.



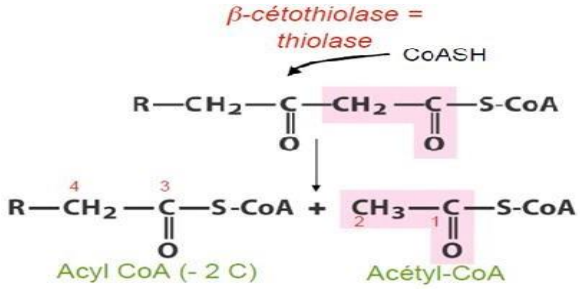
b- *Second dehydrogenation*

It concerns 3-hydroxyacyl-CoA. The hydrogen acceptor is NAD+. Oxidation of the alcohol group leads to a ketone group. The enzyme is 3-hydroxyacyl-CoA dehydrogenase, and the resulting compound is 3-ketoacyl-CoA.

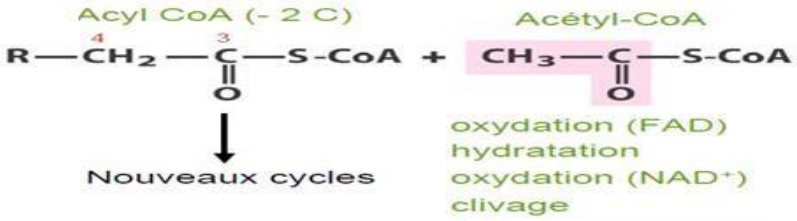


c- *Cleavage of fatty acids*

This is the final reaction in the sequence. The enzyme involved is β-ketothiolase (lyase). The sulfur atom of the thiol group in a CoA molecule attacks the carbon of the ketone function at the β position and causes the release of the acetyl group (thiolysis).
During thiolysis in the presence of HSCoA, an acetyl-CoA is released and an acyl-CoA is reformed, its chain being missing two carbons. This latter acyl-CoA will serve as a substrate for the next reaction.



At the end of each cycle, there is a release of acetyl-CoA and FADH2.2 and a NADH,H+ inside the matrix.



If we start with a fatty acid with 2n carbons, it takes (n-1) turns to achieve complete β-oxidation of the fatty acid with the release of n acetyl-CoA. In the case of a fatty acid with (2n+1) carbons, the β-oxidation of the acid leads to the release of (n-1) acetyl-CoA and one molecule of propionyl-CoA.

c- Summary

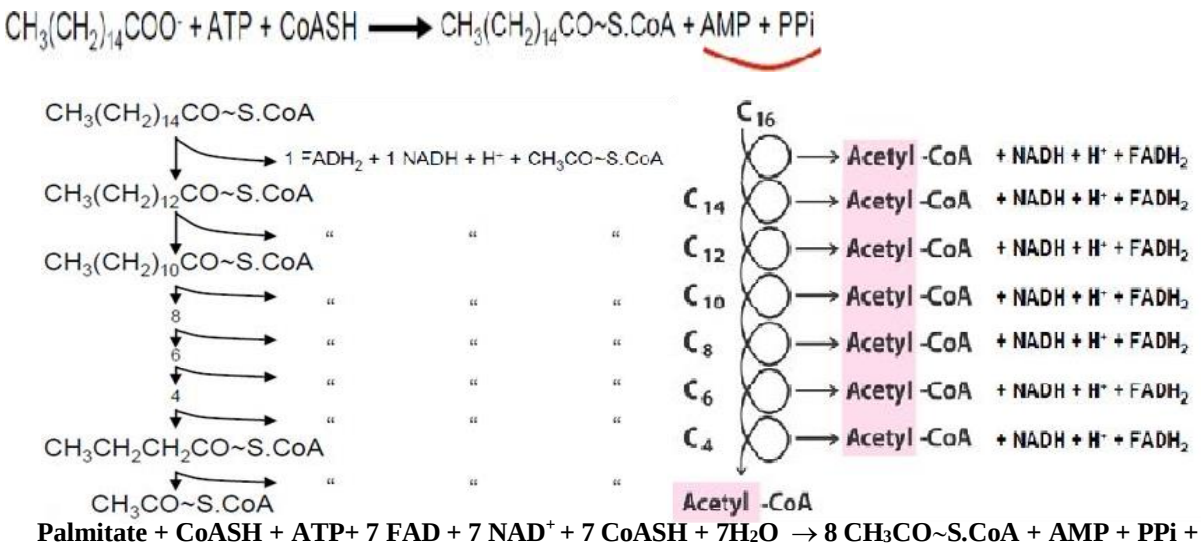
The overall picture of the degradation of a fatty acid by β-oxidation is summarized in Table 5.

Table 31: Summary of the degradation of a fatty acid by β-oxidation.

Number of carbons	**2n Carbons**	**(2n+1) Carbons**
Activation cost	2 phosphate bonds	2 phosphate bonds
Products of β-oxidation	(n-1) $FADH_2$	(n-1) $FADH_2$
	(n-1) $NADH,H^+$	(n-1) $NADH,H^+$
	n Acetyl-CoA	(n-1) Acetyl-CoA
		1 propionyl-CoA

Example

The complete oxidation of palmitate: $CH_3(CH_2)_{14}COO^-$



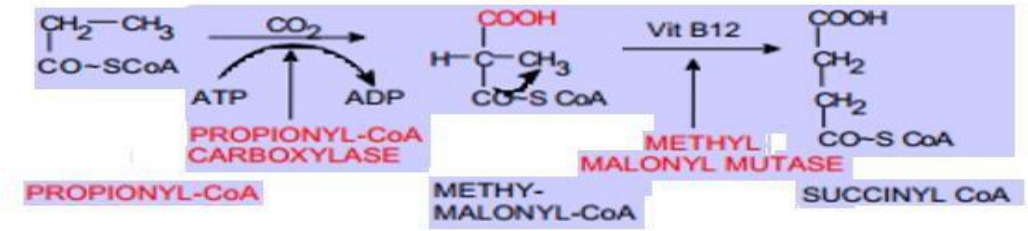
Palmitate + CoASH + ATP+ 7 FAD + 7 NAD^+ + 7 CoASH + 7H_2O → 8 CH_3CO~S.CoA + AMP + PPi + 7 $FADH_2$ + 7NADH,H^+

IX.1.2- Beta-oxidation of unsaturated fatty acids

While the oxidation of saturated fatty acids with an even number of carbon atoms proceeds without problems, saturated fatty acids with an odd number of carbon atoms and unsaturated fatty acids require additional enzymes.

***Fatty acids with an odd number of* carbons:** They are rare and found only in a few marine organisms and in plants. While they are not synthesized by the human body, they are nevertheless present in food and therefore must be metabolized.

In the case of these fatty acids, what remains after the last β-oxidation cycle is not acetyl-CoA (C2 compound), but propionyl-CoA (C3 compound). Propionyl-CoA undergoes three further reactions to finally yield succinyl-CoA, which can then enter the Krebs cycle (Fig. 226).



Unsaturated fatty acids: are degraded in the same way as saturated fatty acids after their activation and binding to coenzyme A. Unsaturated fatty acids contain double bonds of cis (cis-Δn) configuration.

Acyl-CoAs are degraded by the "usual" β-oxidation enzymes until they approach the double bond. There are 2 possibilities.

a. *Case of a double bond with even numbering*

For the breakdown of unsaturated fatty acids (with double bonds), two additional enzymes are needed: a reductase and an isomerase.

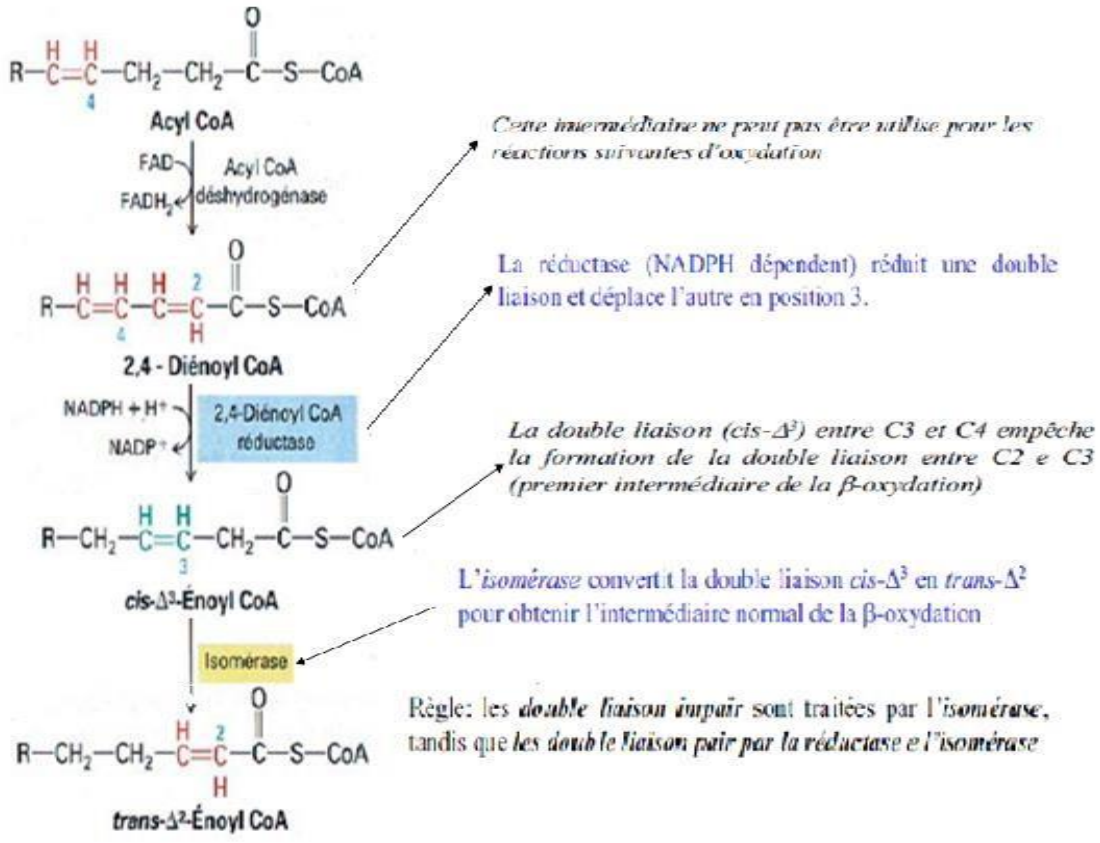
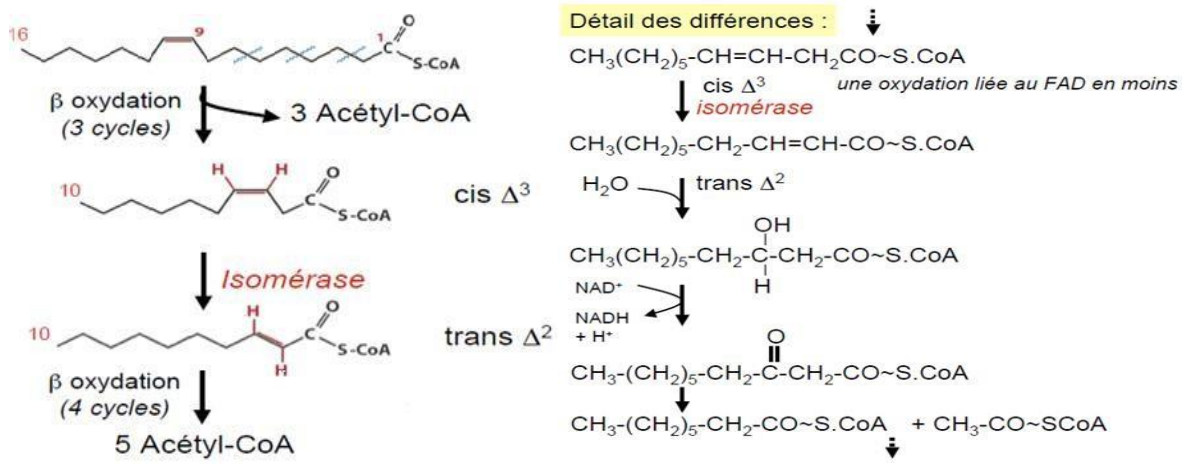


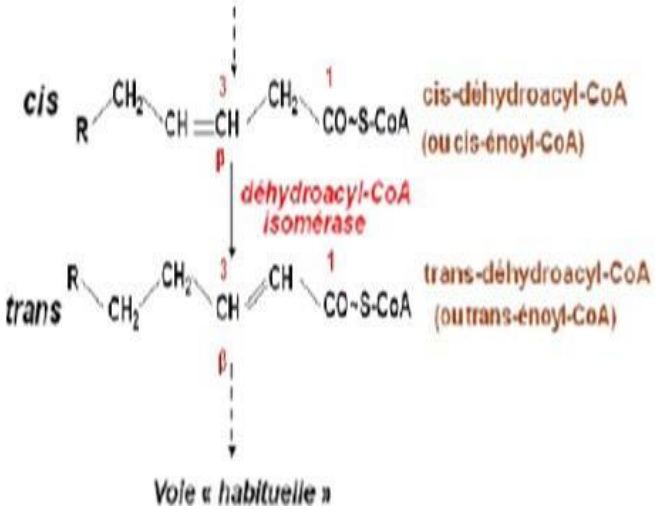
Fig. 226. Beta-oxidation of unsaturated fatty acids.

Example: Palmitoleate (Cis Δ^9 hexadécanoate)

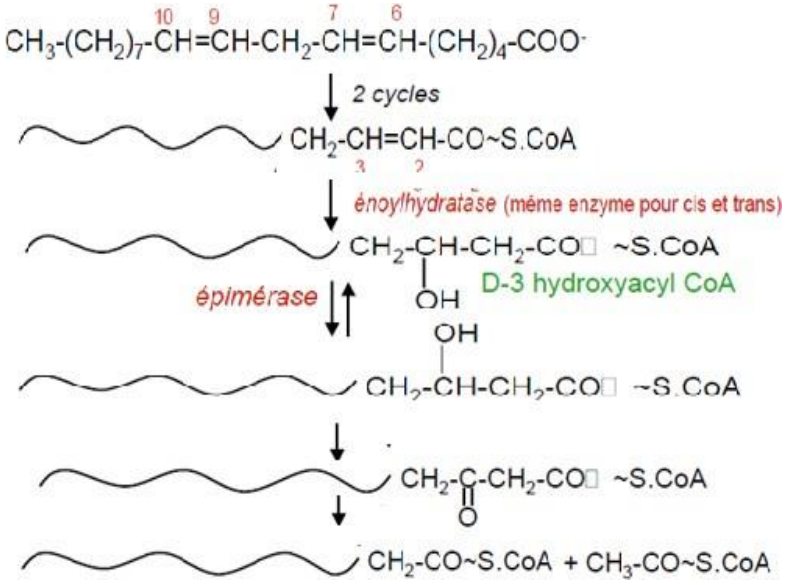


b. *Case of a double bond with odd numbers*

An isomerase displaces the double bond and at the same time transforms the cis isomerism into a trans one, which allows β-oxidation to proceed in the usual way.



c- Oxidation of polyunsaturated fatty acids Example: C18 Cis-⊗6,Cis-⊗9



Chemical breakdown of the degradation of an unsaturated fatty acid possessing x double bonds (CnΔx)

$$AG\ (Cn\Delta^{x}) + 2\ ATP + [n/2]\ HSCoA + ([n/2]-1-x)\ FAD + ([n/2]-1)\ NAD^{+} + ([n/2]-1)\ H_2O \rightarrow [n/2]\text{Acétyl CoA} + ([n/2]-1-x)\ FADH_2 + ([n/2]-1)NADH,H^{+} + 2\ AMP + 2\ Pi$$

Example :

$$\text{Oléate} + 2\ ATP + 9\ HSCoA + 7\ FAD + 8\ NAD^+ + 8\ H_2O \rightarrow$$
$$9\ \text{Acétyl CoA} + 7\ FADH_2 + 8\ NADH,H^+ + 2\ AMP + 2\ Pi$$

d- *Fate of glycerol, propionyl-CoA and acetyl-CoA*

Fate of glycerol

Glycerol resulting from the hydrolysis of triglycerides or phospholipids can be reused as a precursor for lipid or glucose synthesis (gluconeogenesis) or follow the glycolysis pathway. It undergoes the following sequence of reactions:

Glycerol phosphorylation

The reaction is catalyzed by glycerol kinase. The glycerol 3-phosphate formed can be collected for the

Lipid synthesis: $\text{Glycérol} + ATP \longrightarrow \text{glucérol 3-Ⓟ} + ADP$

Glycerol 3-P Dehydrogenation

It is catalyzed by glycerol-P dehydrogenase. 3-P-dihydroxyacetone is formed.

$$\text{Glycérol 3-Ⓟ} + NAD^+ \longleftrightarrow \text{3-Ⓟdihydroxyacétone} + NADH,H^+$$

Isomerization to glyceraldehyde 3-P

The enzyme involved is phosphotriose isomerase, which is found in glycolysis. Glyceraldehyde 3-P can follow the glycolysis pathway or the gluconeogenesis pathway.

$$\text{3-Ⓟdihydroxyacétone} \longleftrightarrow \text{glycéraldéhyde 3-Ⓟ}$$

Fate of propionyl-CoA

At the end of β-oxidation, a final residue is obtained, which is propionyl-CoA. This undergoes a sequence of reactions that transform it into succinyl-CoA.

Carboxylation and formation of 2-methyl malonyl-CoA

The reaction is catalyzed by propionyl-CoA carboxylase.

$$CH_3\text{-}CH_2\text{-}CO\sim SCoA + CO_2 + ATP \longrightarrow \underset{\displaystyle COOH\text{-}\overset{\displaystyle CH_3}{\overset{|}{C}}H\text{-}CO\sim SCoA}{} + ADP$$

Isomerization of 2-methyl malonyl-CoA

2-Methylmalonyl-CoA is converted to succinyl-CoA by 2-methylmalonyl-CoA carboxymutase, an intermediate of the Krebs cycle and capable of being converted into malate, a precursor of gluconeogenesis.

$$\underset{\displaystyle COOH\text{-}\overset{\displaystyle CH_3}{\overset{|}{C}}H\text{-}CO\sim SCoA}{} \longrightarrow HOOC\text{-}CH_2\text{-}CH_2\text{-}CO\sim SCoA$$

Fate of acetyl-CoA

Oxidation in the Krebs cycle

Acetyl-CoA is completely oxidized to CO_2. Following the overall reaction already seen:

$$\text{Acétyl-CoA} + 3\ NAD^+ + FAD + GDP + Pi \rightarrow 2\ CO_2 + HSCoA + 3\ NADH,H^+ + FADH_2 + GTP$$

IX.2- SYNTHESIS OF FATTY ACIDS

Fatty acid biosynthesis (or lipogenesis) is the main pathway for energy storage and is followed by the formation of triglycerides (TG). Depending on the body's energy supply, there is either ATP production from acetyl-CoA or fatty acid synthesis.

The pathway takes place in the cytosol of most cells; the liver and adipose tissue have a particularly high capacity for de novo fatty acid synthesis (90% of the process). Furthermore, lipogenesis can occur to a minimal extent in all tissues for the construction of cell membranes.

The main product of this cytosolic biosynthesis is palmitic acid, a 16-carbon saturated fatty acid (C16:0), in the form of palmitate. Stearic acid (C18:0), the next largest fatty acid, is also produced, but in smaller quantities.

Humans can only synthesize two monounsaturated fatty acids: palmitoleic acid (cis-9-hexadecenoic acid) and oleic acid (cis-9-octadecenoic acid), because their genome does not code for the Δ12 and Δ15 desaturase enzymes. These enzymes are essential for inserting double bonds beyond carbon 9 of the carbon chain. This explains why polyunsaturated fatty acids, such as linoleic and linolenic acids, are essential and serve as building blocks for other syntheses.

Fatty acids are bound to glycerol before being released into the bloodstream as lipoproteins. They are stored with dietary fatty acids in adipose tissue as triglycerides.

Fatty acid synthesis is not possible through the simple reversibility of β-oxidation. All biosynthesis, such as lipid synthesis, requires: energy supplied by ATP, reducing power provided in the form of NADPH, H^+ ions originating primarily from the functioning of the pentose phosphate pathway, and precursors; the only precursor for fatty acid synthesis is acetyl-CoA.

Acetyl-CoA comes from: β-oxidation of fatty acids (intra-mitochondrial), oxidation of pyruvate (mitochondrial) and oxidative degradation of so-called ketogenic amino acids.

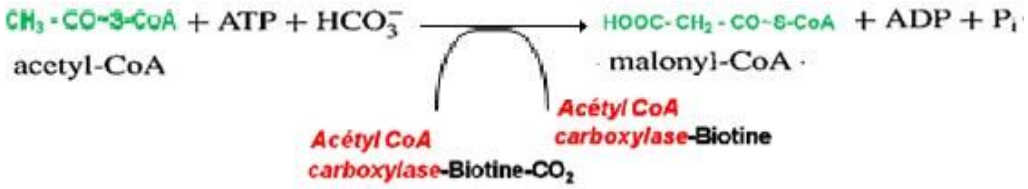
Acetyl-CoA, regardless of its origin, is formed in the mitochondria. To serve as a precursor in the cytosol for fatty acid synthesis, it must be transported from the mitochondrial matrix into the cytosol. Only the acetyl group is transported across the inner membrane by the citrate system.

IX.2.1. *Stages of fatty acid biosynthesis (lipogenesis)*

Fatty acid synthesis is made up of 3 phases from acetyl-CoA: Activation, elongation and termination.

IX.2.2. *Activation and formation of malonyl CoA*

Acetyl-CoA is activated prior to the actual synthesis of fatty acids. This reaction is also the key reaction in the entire chain of reactions. Malonyl CoA, the acetyl unit donor, is synthesized from acetyl CoA in a carboxylation reaction catalyzed by a biotin-coenzyme ACC acetyl-CoA carboxylase in the presence of ATP, according to the reaction scheme below.



This is the engagement reaction in the fatty acid biosynthesis pathway and the most important regulatory point (limiting step). All subsequent reactions take place within the Fatty Acid Synthase I multi-enzyme complex.

IX.2.3. *Elongation of the carbon chain of AG*

It takes place within the fatty acid synthase I (FAS I) complex. This complex is a homodimer in which each monomer consists of a polypeptide chain with seven enzymatic activities (acetyl-CoA-ACP transferase, malonyl-CoA-ACP transferase, β-ketoacyl-ACP synthase, β-ketoacyl-ACP reductase, Dβ-hydroxyacyl-ACP dehydratase, enoyl-ACP reductase, and palmityl thioesterase) linked to an acyl carrier protein called ACP (Acyl Carrier Protein). The intermediates of the pathway remain covalently linked to one of the two thiol groups (-SH) of the complex (Fig. 227).

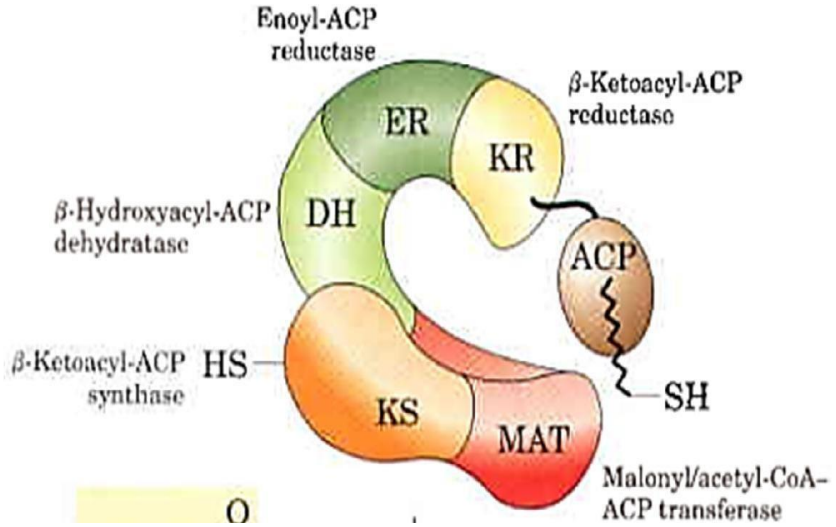


Fig. 227. Multi-enzyme complex of AGS I.

Elongation is carried out in several stages:

- Initial loading of acetyl and malonyl groups onto AGS I;
- Elongation of the carbon chain itself;
- Release of palmitic acid.

a. Loading the groups onto AGS I:

It occurs in three reactions (Fig. 228):

1. Transfer of the acetyl group from acetyl CoA to ACP catalyzed by acetyl CoAACP transferase:

$$\text{Acétyl-CoA + ACP-SH} \longrightarrow \text{acétyl-S-ACP + CoASH}$$

2. Transfer of this acetyl group from ACP to the -SH group of β-ketoacyl-ACP synthase (CS):

$$\text{Acétyl-S-ACP + CS-SH} \longrightarrow \text{acétyl-S-CS + ACP-SH}$$

The ACP is regenerated to capture the malonyl group.

3. Transfer of the malonyl group from malonyl CoA to ACP catalyzed by malonylCoA-ACP transferase:

$$\text{Malonyl-CoA + ACP-SH} \longrightarrow \text{malonyl-S-ACP + CoASH}$$

With the AGS I complex thus charged, and the acetyl and malonyl groups thus activated (by their bonds to the -SH groups of AGS I), the actual elongation process can begin.

b- Elongation of the carbon chain step properly speaking

This is a repeated sequence of four recurring reactions: Condensation/ Reduction/ Dehydration/ Reduction (Fig. 228).

1. **Reaction 1**: Condensation of acetyl and malonyl groups into acetoacetyl-ACP catalyzed by β-ketoacyl-ACP synthase known as condensation enzyme (CS or CE);
2. **Reaction 2**: Reduction of the carbonyl group at C3 of acetoacetyl-ACP to Dβ-hydroxybutyryl-ACP catalyzed by β-ketoacyl-ACP reductase with NADP coenzyme;
3. **Reaction 3**: C2-C3 dehydration of Dβ-hydroxybutyryl-ACP to produce Transδ2 butenoyl-ACP catalyzed by Dβ-hydroxyacyl-ACP dehydratase;
4. **Reaction 4**: Reduction of the double bond of Transδ2 butenoyl-ACP to produce butyryl-ACP catalyzed by Enoyl-ACP reductase with NADP coenzyme.

The synthesis of butyryl-ACP (4C) completes one turn of the fatty acid biosynthesis pathway. Next, the butyryl group from ACP is transferred to the -SH group of β-ketoacyl ACP synthase (CS), which initially attached the acetyl group. A malonyl group is also transferred from malonyl CoA to ACP. This initiates the following cycle of four reactions (condensation between the butyryl and malonyl groups, reduction, dehydration, reduction), lengthening the fatty acid chain by two additional carbons.

For reasons that are still very poorly understood, the elongation of the fatty chain stops at 16°C, so after 7 cycles palmityl-S-ACP is produced.

IX.2.1.3. Termination (Release of palmitic acid)

The elongation reactions are complete when the chain consists of 16 carbons. Palmitic acid, a saturated fatty acid with an even number of carbon atoms, is released from PCA by a hydrolysis reaction catalyzed by palmitic thioesterase:



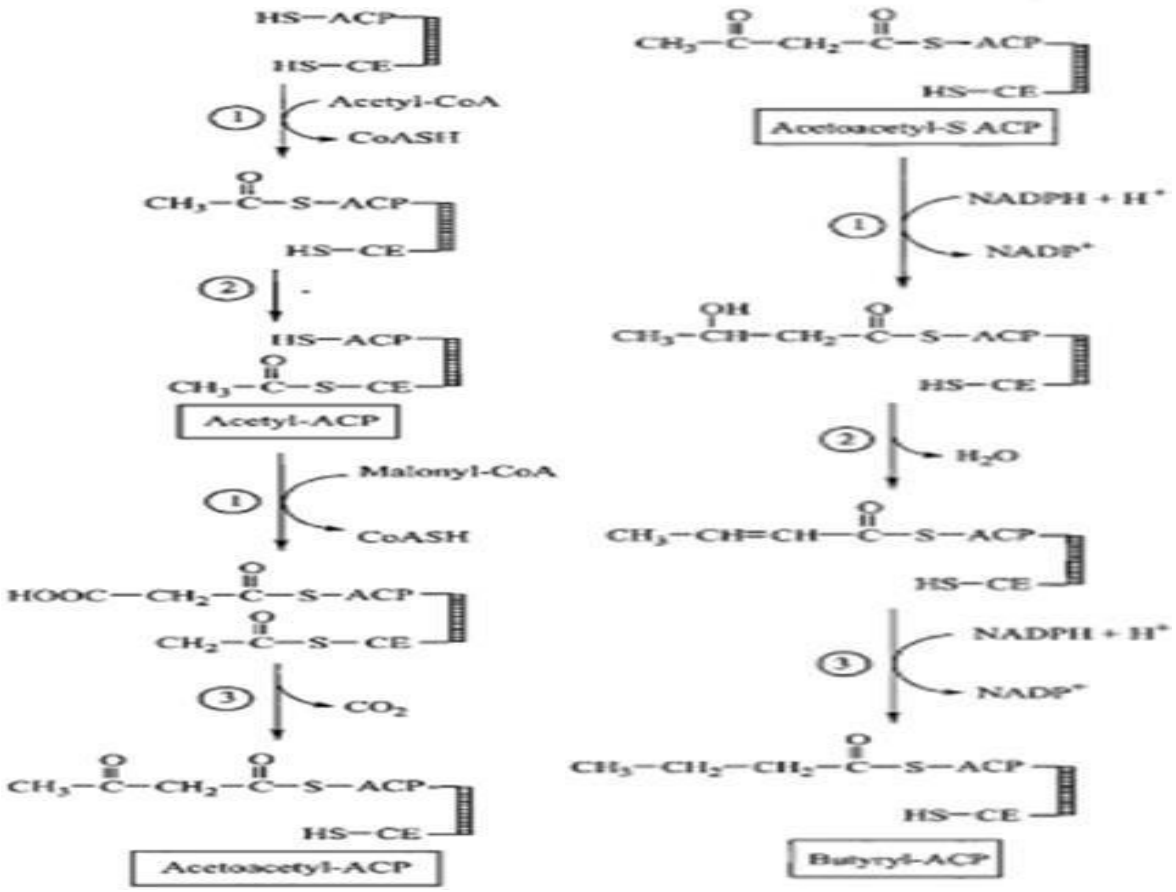


Fig. 228.(A) Loading of AGSI and condensation of the 2 initial substrates (acetyl and malonyl), (B) Reduction reaction, dehydration and reduction.

IX.2.2. Uses of palmitic acid

Palmitic acid produced by lipogenesis, once activated into palmitoyl CoA, will have two fates depending on the metabolic situation:

1. Elongation and/or desaturation;
2. Esterification in triglycerides.

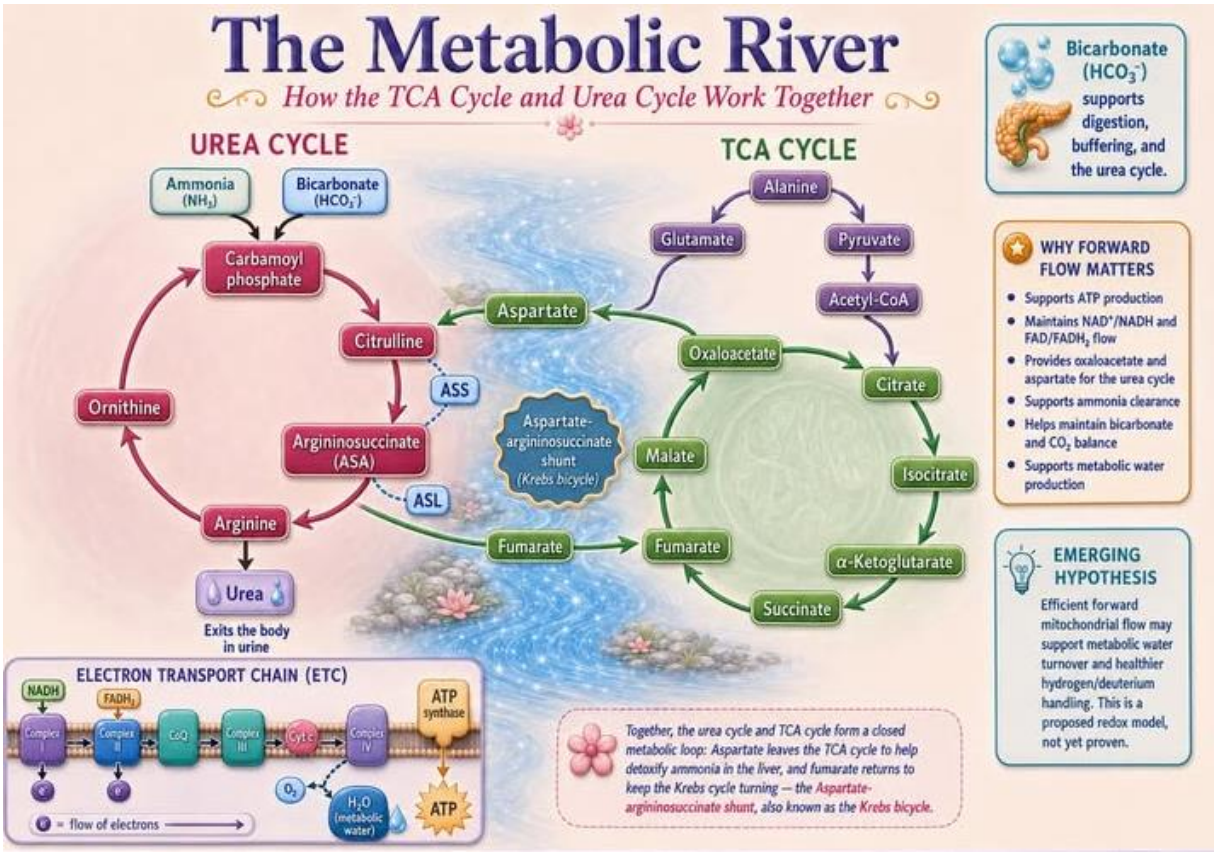
Summary of palmitic acid biosynthesis:

The metabolic balance of palmitic acid biosynthesis is as follows:

$$8CH_3\text{-}CO\text{-}S.CoA + 7\ ATP + 14\ NADPH, H^+ \xrightarrow[7\ tours]{} CH_3\text{-}(CH_2)_{14}\text{-}CO\text{-}S\text{-}ACP + 8\ CoA.SH + 7\ ADP + 7\ Pi + 7H_2O + 14\ NADP$$

CHAPTER X :

PROTEIN AND AMINO ACID METABOLISM



CHAPTER X

PROTEIN AND AMINO ACID METABOLISM

The human body contains thousands of different proteins, which play the role of "machines" in a multitude of physiological functions, ranging from oxygen transport (hemoglobin) to the catalysis of chemical reactions (enzymes), including motility (actomyosin), immunity (immunoglobulins), and endocrine function, since severalhormonesTheir transporters and receptors are proteins.

All of these proteins (10 kg in an adult man) undergo incessant remodeling (300 g/day in a young adult), and are finely regulated by hormones.

Amino acids, the building blocks of protein synthesis, are produced by the proteolysis of dietary or bodily proteins, and, for non-essential amino acids, by de novo endogenous synthesis. They are now considered not only as pieces of the gigantic puzzle of protein turnover, but also as metabolic regulators or "pharmaconutrients".

The turnover rate of tissue proteins is 1 to 2%. This means that 1 to 2% of tissue proteins are broken down into amino acids. 75% of the amino acids obtained are used to synthesize other proteins. The remainder is either broken down into waste products (urea) or converted into carbohydrates or ketone bodies. This means that the net daily protein loss is 30-40g. Nitrogen losses are 5-7g/day.

Only 20 amino acids are involved in the construction of proteins. This is a "plastic" role as opposed to the "energetic" role of carbohydrates and lipids (Fig. 229).

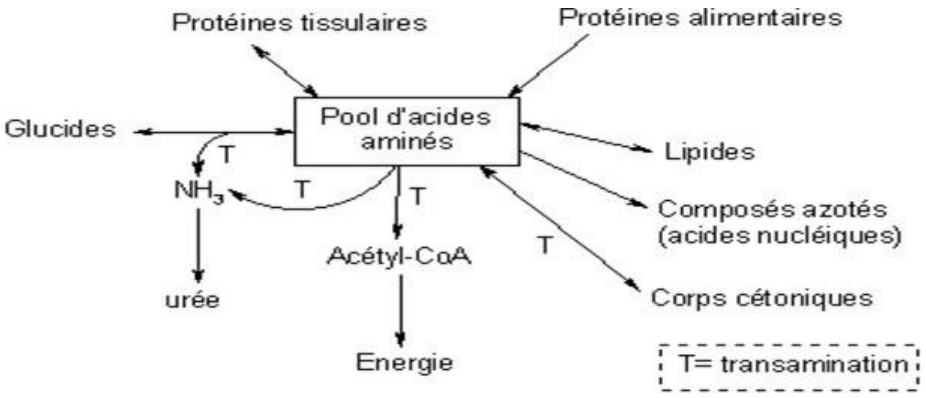


Fig. 229. General perception of protein metabolism.

X.1. Digestion of food proteins

The recommended daily protein intake is 60 to 90 g, which corresponds to 10 to 12 g of nitrogen. Proteins are broken down and transformed into their constituent amino acids by the action of gastric, pancreatic, and intestinal enzymes, which are of two types:

- **endopeptidases**: pepsin, trypsin, chymotrypsin
- **exopeptidases**amino and carboxypeptidases.

The released amino acids are absorbed by the brush border of enterocytes in the intestinal mucosa; they reach the liver via the portal vein. The liver also receives amino acids from peripheral metabolism.

X.1.1. Digestion in the stomach

The stomach secretes gastric juice containing hydrochloric acid and pepsinogen (a proenzyme). The hydrochloric acid, too dilute to perform hydrolytic action, acts as a bactericide and denatures proteins to allow for efficient attack by proteases. Pepsinogen is activated by cleavage of an inactivation amino acid sequence by hydrochloric acid or by autocatalysis by pepsin. Pepsin is an endopeptidase, stable in acidic conditions, that releases peptides and some amino acids (Fig. 230).

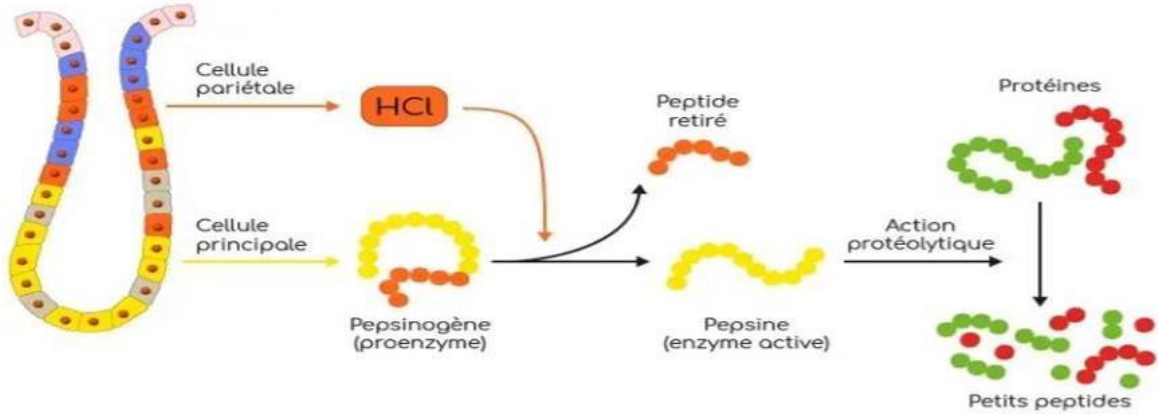


Fig. 230. Proteolytic digestion of dietary proteins by pepsin

X.1.2. Digestion by pancreatic enzymes

The polypeptides formed by pepsin upon entering the small intestine are broken down by pancreatic enzymes into oligopeptides and a few amino acids. The release and activation of these proenzymes are controlled by two hormones, cholecystokinin and secretin, found in the digestive tract. Enteropeptidase, or enterokinase, synthesized by intestinal mucosal cells and present on their surface, activates trypsin by cleaving trypsinogen to release a hexapeptide.

starting from the N-terminal end. Trypsin becomes an activator of all other pancreatic enzymes, which are also released as proenzymes (Fig. 331). Each pancreatic enzyme is specific to the hydrolyzed peptide bond.

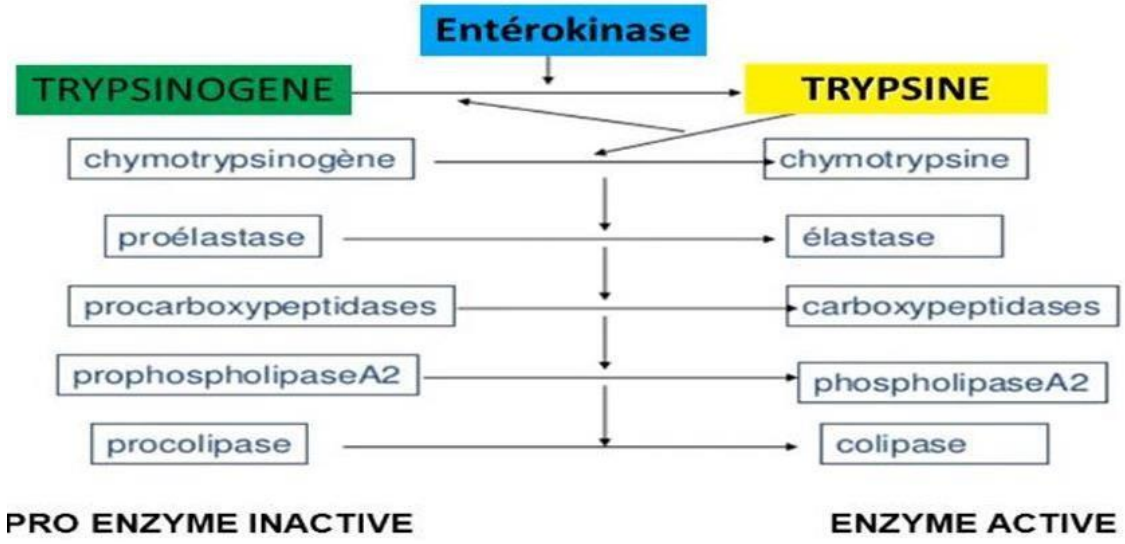


Fig. 331. Cascade activation of proteolytic enzymes by trypsin.

X.1.3. Digestion by enzymes in the small intestine

The intestinal mucosa produces aminopeptidases that hydrolyze oligopeptides and sequentially release free amino acids from the N-terminal end.

X.1.3.1. Absorption of free amino acids and dipeptides

At the end of digestion, the amino acids and dipeptides digested by the various digestive enzymes are absorbed in the small intestine. The dipeptides are hydrolyzed into amino acids in the cytosol of the intestinal cells. The amino acids are then excreted into the portal vein and transported to the liver, where they are metabolized or released into the bloodstream.

X.2. Fate of amino acids in the body

It is twofold. They participate in syntheses or are catabolized.

X.2.1. Degradation of amino acids

Also known as oxidative catabolism of amino acids, the irreversible degradation of amino acids should not be confused with proteolysis.

Unlike carbohydrates and lipids, excess amino acids cannot be stored; they are therefore rapidly degraded by transamination or oxidation to give an ammonium ion and a carbon skeleton.

The ammonium ion is eliminated by excretion or ureagenesis, or recycled for the synthesis of another amino acid. The carbon skeleton can also be reused to reform

the corresponding amino acid or serve as precursors either to the synthesis of carbohydrates (in the case of the skeletons of so-called glycoformative amino acids), or to the synthesis of fatty acids (in the case of the skeletons of so-called ketogenic amino acids).

X.2.2. Elimination of the amine group NH_2

The first step in amino acid catabolism is the removal of amino nitrogen. This leads to the production of a compound toxic to the central nervous system: ammonia (NH3), which is eliminated from the body in the form of:

- Urea: hepatic ureogenesis, major pathway (4/5 of nitrogen eliminated).
- From NH_{4+}: renal ammoniogenesis, minor pathway (1/5 of the nitrogen eliminated). The departure of the amino group involves 3 types of reactions:

A. The transamination

Transamination or aminotransfer is the general reaction of amino acid metabolism because it is involved in both their catabolism and their synthesis.

This process leads to the exchange of the α-amino group between an amino acid and an α-keto acid (Fig. 232). The enzymes that catalyze such reactions are called aminotransferases or transaminases. The catalyzed reaction is reversible. The cofactor involved is pyridoxal phosphate (the prosthetic group of all aminotransferases). It is derived from vitamin B6. In transamination reactions oriented toward the degradation of amino acids, the α-amino group acceptor is always α-ketoglutarate. This results in the formation of glutamate. All amino acids participate in the transamination reaction except lysine.

The goal is to collect the amine groups from the different amino acids in the form of a single one of them "L-glutamate" which channels them either towards biosynthetic pathways or excretion in the form of urea.

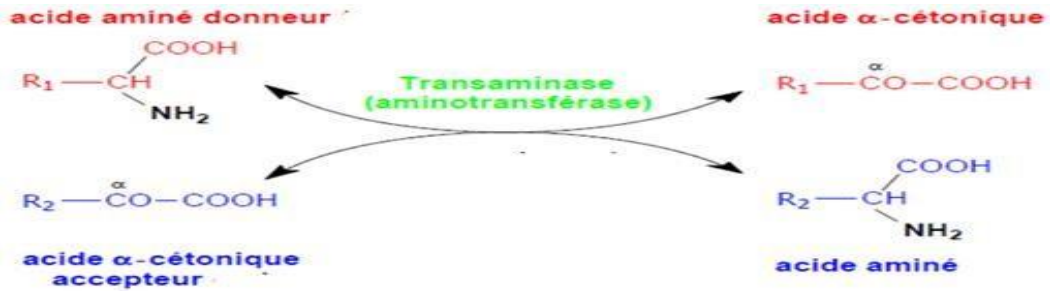
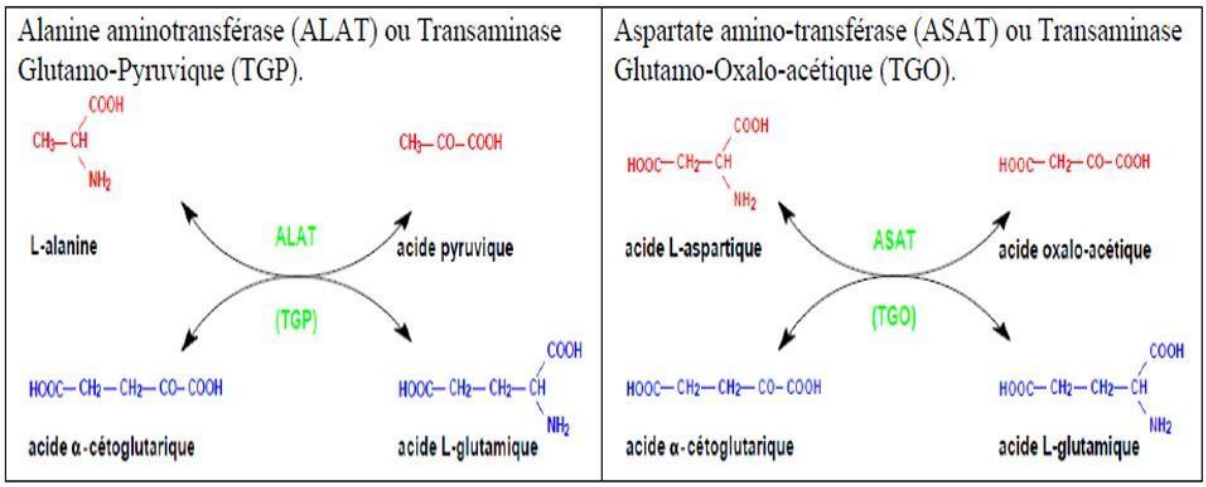


Fig. 232. General diagram of the reaction catalyzed by transaminases.

Among the transaminases, two are very important:



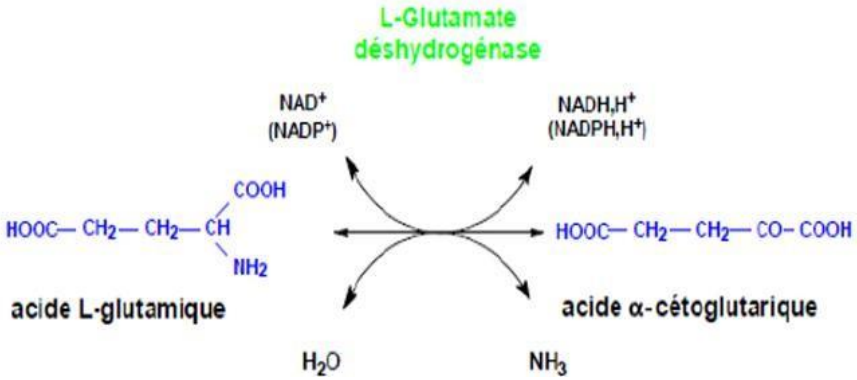
B. Oxidative deamination of L-glutamate

L-glutamate, produced by transamination reactions, is transported from the cytosol to the mitochondria to undergo oxidative deamination. Unlike transamination, which is a transfer reaction of the α-amino group, oxidative deamination releases it as free ammonia, with the formation of the corresponding α-keto acid skeleton. It is highly active in the liver and kidneys. Two types of enzymes are involved: glutamate dehydrogenase and amino acid oxidase.

In mammals, the ammonium ion, which is toxic to the cell, will be incorporated into the urea cycle and then excreted.

➢ **Glutamate dehydrogenase**

In amino acid catabolism, oxidative deamination is preceded by transamination. Glutamate dehydrogenase catalyzes a reversible reaction, preferentially using NAD+ in oxidative deamination (degradation pathway), and NADP+ in ammonia assimilation (synthesis pathway).



The direction depends on the relative concentrations of glutamate, α-ketoglutarate, and NH_4^+.3 and the ratio of reduced and oxidized forms of the coenzymes ($NADPH,H^+/NADP^+$ and $NADH,H^+/NAD^+$). For example, after a protein-rich meal resulting in a

With an increase in glutamate, the reaction shifts towards deamination and the formation of ammonia.

- **Amino acids oxidases**

This pathway is secondary to that of transamination. These enzymes use two types of flavoproteins as coenzymes: FAD or FMN.

- **L-amino acid oxidase**

It is a liver enzyme with FMN as a prosthetic group. It oxidizes amino acids by directly transferring electrons taken up by the coenzyme to molecular oxygen. This results in the formation of the corresponding α-keto acid, NH_3, and H_2O_2 (hydrogen peroxide), which is decomposed by catalases.

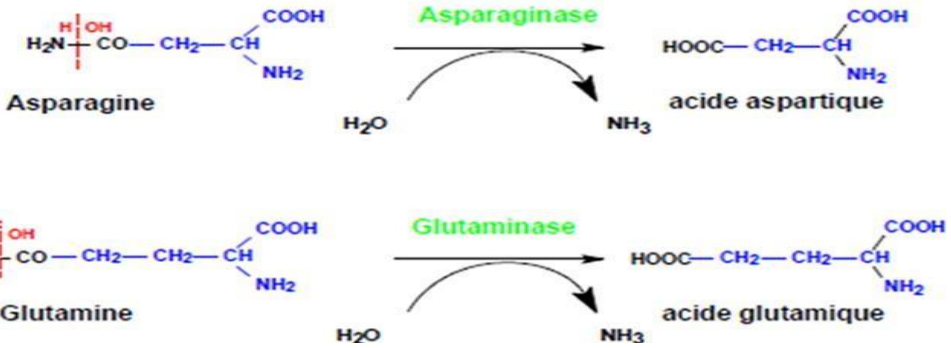


- **D-amino acid oxidase**

In the liver and kidneys, significant activity of this enzyme is found, which oxidizes D-amino acids (isomers of L-amino acids, not found in animals) originating from the hydrolysis of plant proteins and microbial cell membranes. These D-amino acids, which are not used by the animal organism, are therefore oxidized following the same principle as before, but by FAD-dependent D-amino acid oxidase, with the release of the corresponding α-keto acid, NH3, and the formation of H2O2 (hydrogen peroxide), which is decomposed by catalases.



C. Deamidation: concerns 2 amino acids (glutamine and asparagine) which contain an amide function carried by their side chain.



D. Transport of NH_3^+ groups from other tissues

- In the form of Glutamine

The NH_3^+ group will be transported as glutamine (Fig. 233), which can cross the membrane while glutamate cannot. Once in the liver, under the action of glutaminase in the mitochondria, the NH_3^+ group is released.

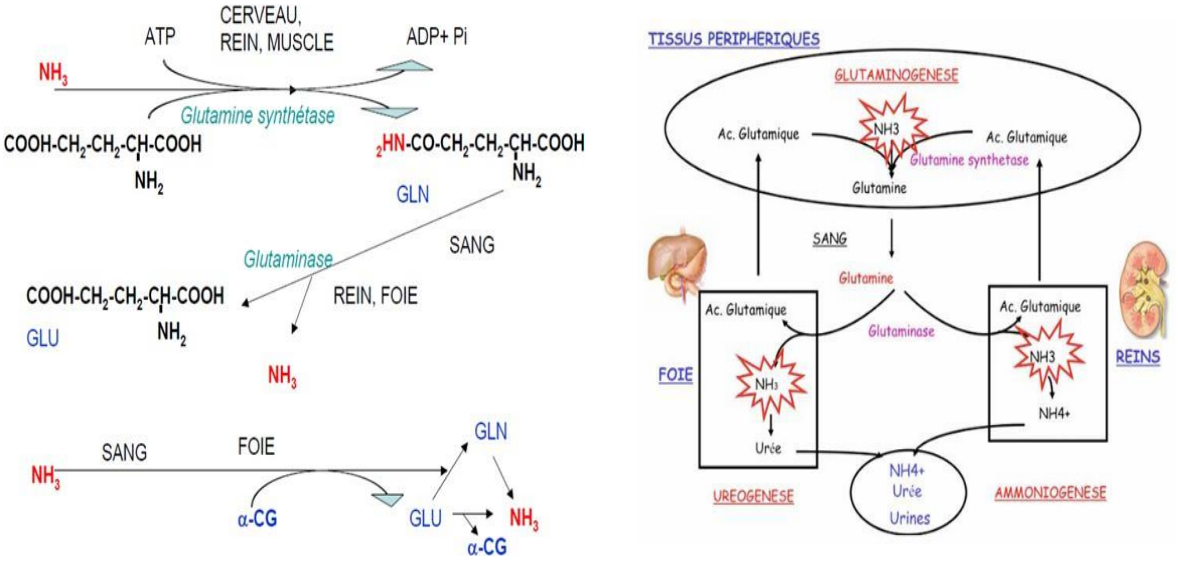


Fig. 233. General diagram illustrating the fate of ammonia from deamination and the reaction catalyzed by glutamine synthetase and glutaminase.

- In the form of Alanine

At the muscle level, there is a second form of NH group transport.2 is the Glucose Alanine cycle (Fig. 234). Alanine aminotransferase transfers the α amine group to pyruvate (product of glycolysis), the Alanine thus formed passes into the blood and returns to the liver where it undergoes transamination and provides its NH2 group to the liver and on the other hand the pyruvate which will be used for gluconeogenesis and therefore the burden of muscle gluconeogenesis is borne by the liver thus saving energy for the muscle.

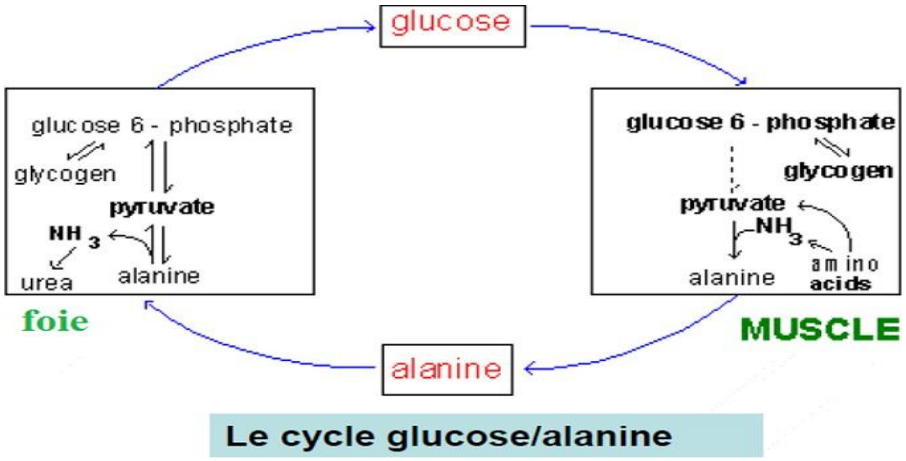
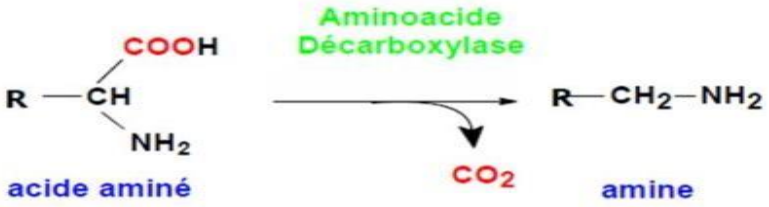


Fig. 234. The glucose/alanine cycle.

X.2.2.3. Elimination of the carboxyl group (COOH) or decarboxylation

Decarboxylation is a chemical reaction in which a molecule of carbon dioxide (CO_2) is removed.



The decarboxylation of amino acids is an irreversible reaction, catalyzed by pyridoxal coenzyme phosphate (PLP) decarboxylases giving rise to amines or polyamines (Table 32).

Table 32: Amines generated by decarboxylation of amino acids.

AA	AMINE	FONCTION
Trp	Sérotonine	Neuromédiateur
Glu	γ-amino butyrate	Neuromédiateur
His	Histamine	Neuromédiateur, médiateur immunitaire
Tyr	Dopamine, Noradrénaline, Adrénaline	Neuromédiateurs, hormone
Asp	ß-alanine	Composant du coenzyme A
Cys	Cystéamine	Composant du coenzyme A
Ser	Ethanolamine	Composant des phospholipides
Thr	Amino-propanol	Composant de la vitamine B12

X.2.2.4. Ammoniogenesis

In the kidney (Fig. 235), NH3 released from glutamine will associate with H^+ protons to form the ammonium ion NH_4^+ which will be eliminated in the urine.

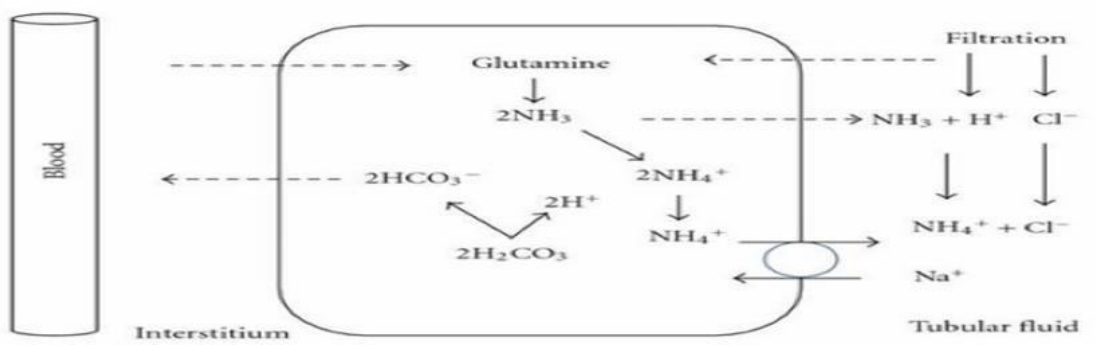


Fig. 235. Ammonigenesis.

X.2.2.5. Urea cycle or ureogenesis (ornithine cycle or Krebs-Henseleit cycle)

It was elucidated by Hans Krebs and Kurt Henseleit in 1932. The urea cycle is a cyclic hepatic metabolic pathway that eliminates excess nitrogen (excess ammonia) from the body, whether of endogenous or exogenous origin, by detoxifying ammonia (toxic) into urea. In mammals, this cycle takes place exclusively in the liver and involves five enzymatic reactions, two of which occur in the mitochondria and three in the cytoplasm (Fig. 236). It requires the presence of two transporters between the mitochondrial matrix and the cytosol:

- The citrulline-ornithine transporter.
- The glutamate–aspartate translocase.

The sequence of reactions that will occur includes a mitochondrial phase and a cytosolic phase.

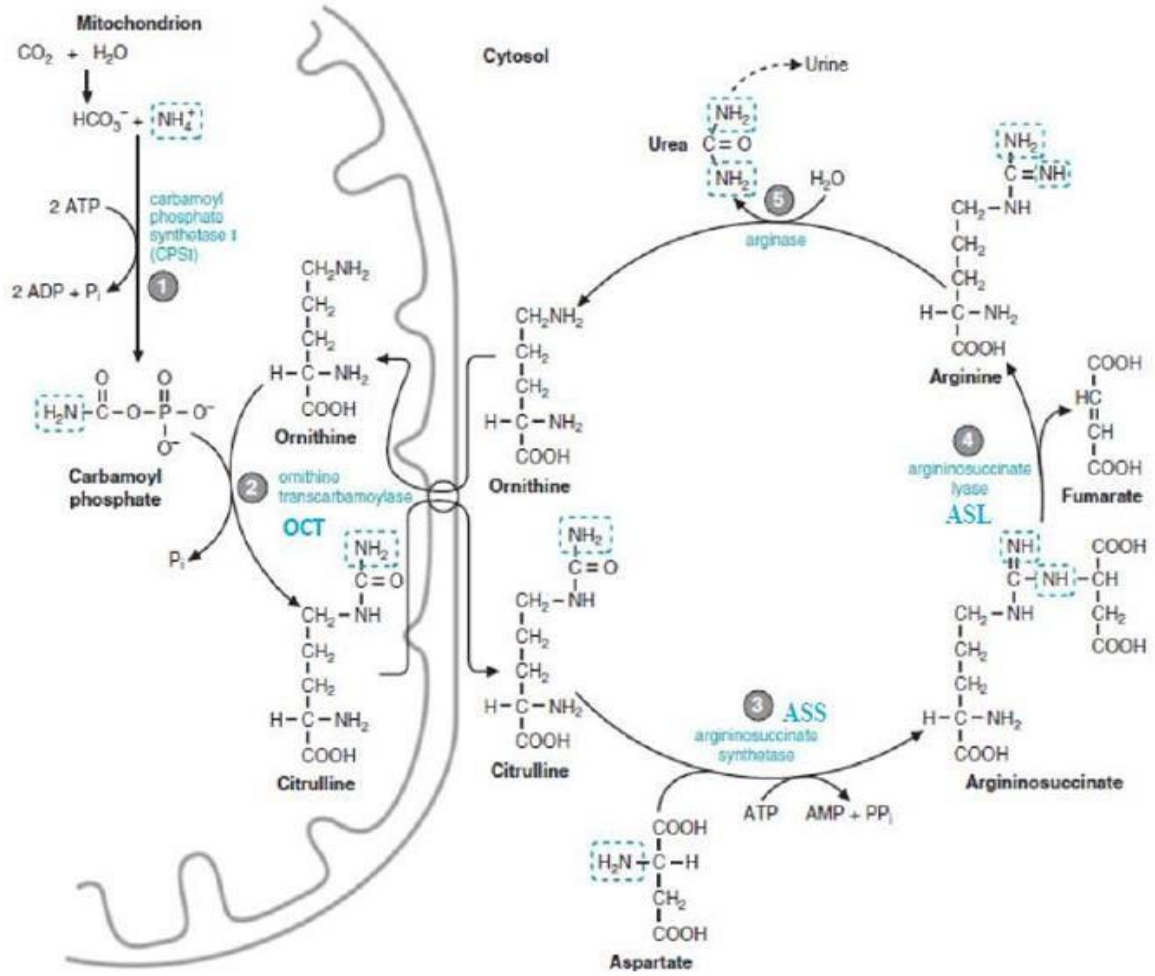


Fig. 236. Urea cycle (ornithine cycle or KREBS-HENSELEIT cycle).

A- MITOCHONDRIAL PHASE

1/ Formation of carbamoylphosphate

In mitochondria, carbamoyl phosphate synthetase (CPS-I) uses CO_2, NH_3 and 2 ATP as substrates to form carbamoyl phosphate. Two energy-rich phosphate bonds are consumed.

2/- Formation of citrulline

$$CO_2 + NH_3 + 2\ ATP \longrightarrow H_2N\text{-}C(=O)\text{-}O\text{-}\textcircled{P} + 2\ ADP + Pi$$

Once carbamoyl phosphate is formed, it is joined by ornithine transported from the cytosol. Under the action of ornithine carbamoyltransferase (transcarbamylase (OTC)), the carbamoyl group is transferred to ornithine to form citrulline.

Ornithine + carbamoyl phosphate ⟶ L-Citrulline + Pi

Citrulline moves from the mitochondria to the cytosol against an entry of ornithine via the citrulline-ornithine transporter.

B- CYTOSOLIC PHASE

3/. Formation of arginine-succinate

The citrulline obtained is transported into the cytosol. Under the action of arginine-succinate synthetase (ASS), citrulline condenses with aspartate to give arginine-succinate with the consumption of two energy-rich phosphate bonds of a single ATP molecule.

L-Citrulline + L-Aspartate + ATP ⟶ Arginino-succinate + AMP + PPi

4/Arginine formation

It is catalyzed by arginine-succinate lyase (ASL), which cleaves L-arginine into fumarate. Fumarate is transported into the mitochondria and taken up by the Krebs cycle, which oxidizes it to oxaloacetate. Oxaloacetate is then transaminated to aspartate by aspartate aminotransferase. This creates a link between the Krebs and urea cycles. Fumarate is then converted into glucose and CO_2.

Arginino-succimate ⟶ L-arginine + fumarate

5/Arginine hydrolysis

This is the final reaction of the cycle. It involves the hydrolytic cleavage (in the presence of H_2O) of arginine into urea and ornithine by cytosolic arginase. While urea is excreted in the urine, ornithine is transported into the mitochondria by the citrulline-ornithine transporter and enters a new cycle to react with carbamyl phosphate.

Arginine + H_2O ⟶ urea + ornithine

-Regulation of urea biosynthesis

Carbamoyl phosphate synthetase (CPS) is the key enzyme that regulates the urea cycle. Two isoforms of this enzyme are distinguished. The CPS I isoform (7) is mitochondrial and participates in the urea cycle. In contrast, the CPS II isoform is cytoplasmic and participates in pyrimidine synthesis. Furthermore, they differ in substrate choice: CPS I condenses ammonium and bicarbonate ions, while CPS II uses glutamine as its substrate. Physiologically relevant, urea biosynthesis is linked to the concentration of amino acids in the portal blood circulating to the liver. There is an approximately linear relationship between plasma nitrogen concentration and urea synthesis. CPS I reacts very rapidly to changes in concentration.

N-acetylglutamate is produced. If this signaling substance is lacking, CPS I is completely inactivated. Synthesizing N-acetylglutamate requires an increase in blood glutamate concentration, which is achieved through protein intake.

Table 33: Regulation of urea biosynthesis.

	Inhibée par	Activée par
Carbamoyl phosphate synthase I	Concentration plasmatique en azote aminé< 4,5 mg / 100 ml	Augmentation de la concentration plasmatique en azote aminé
	N-acétyl-glutamate ↓	N-acétyl-glutamate ↑

- **Cycle summary**

The summary of the cycle is written as follows:

NH_3 + CO2 + Aspartate + 3 ATP ⟶ Urea + Fumarate + 2 ADP + AMP + 2 Pi + PPi

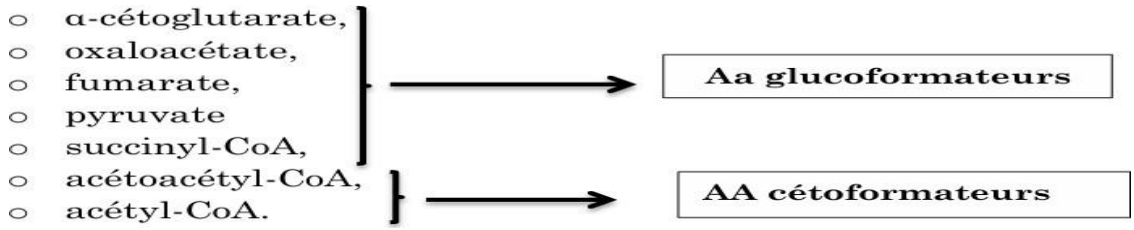
✓ During the formation of a urea molecule, 4 high-energy bonds were used (2 ATP into 2 ADP + 2 Pi, 1 ATP into AMP + PPi).

✓ When fumarate is converted to oxaloacetate (Krebs cycle) (to regenerate aspartate after transamination), the result is the formation of a molecule of NADH, H+ which corresponds to 3 ATP.

✓ In conclusion, the elimination of a free ammonium ion and the amine from aspartate in the form of a urea molecule consumes only one energy-rich phosphate bond.

X.2.2.6. Fate and catabolism of the carbon skeleton

After the release of the α-amino group as ammonia, the 20 amino acids found in proteins each release their corresponding α-keto acid (α-keto acid or carbon skeleton). The breakdown of these 20 carbon skeletons leads to the formation of seven compounds: α-ketoglutarate, oxaloacetate, fumarate, acetoacetyl-CoA, succinyl-CoA, pyruvate, and acetyl-CoA.



They enter intermediate metabolism for energy production or for the synthesis of carbohydrates or lipids. Depending on the fate of the carbon skeletons, amino acids are classified into three groups (Fig. 237):

a- Glucose-forming (glucogenic) amino acids

The breakdown of the carbon skeleton releases one of the following intermediates: α-ketoglutarate, oxaloacetate, fumarate, succinyl-CoA, and pyruvate. This class includes, among the non-essential amino acids: asparagine, aspartate, glutamate, glutamine, proline, alanine, glycine, serine, and cysteine; and among the essential amino acids: arginine, histidine, methionine, threonine, and valine.

- Oxaloacetate

This is the carbon skeleton of asparagine and aspartate. Asparagine is hydrolyzed by asparginase into aspartate and ammonia. Aspartate undergoes transamination as described above, releasing oxaloacetate and ammonia. Oxaloacetate can be recovered to form the corresponding amino acid or enter gluconeogenesis to form glucose.

- α-ketoglutarate

The breakdown of glutamine, glutamate, proline, arginine, and histidine leads to α-ketoglutarate.

- Glutamine is hydrolyzed into glutamate and ammonia by glutaminase. Glutamate is oxidized to α-ketoglutarate by transamination or oxidation in the presence of glutamate dehydrogenase.
- Proline is oxidized to D-pyrroline 5-carboxylate and then a second time to glutamate which is transamined to α-ketoglutarate.
- Arginine is hydrolyzed by arginase into ornithine and urea. Ornithine undergoes transamination, transforming it into γ-semialdehyde glutamate. The latter is then converted into α-ketoglutarate.
- Histidine undergoes a sequence of reactions that leads to the formation of N-formininoglutamate. The transfer of the forminin group to tetrahydrofolate releases glutamate, which is then oxidized to α-ketoglutarate.

α-Ketoglutarate, derived from all these amino acids, is oxidized in the Krebs cycle to malate, which is transported into the cytosol to serve as a precursor for gluconeogenesis.

- Pyruvate

The breakdown of alanine, cysteine, serine, glycine and threonine leads to pyruvate.

- Alanine is transamined to form pyruvate
- Cysteine undergoes desulfurization to alanine, which yields pyruvate.

• Serine can be converted into pyruvate through dehydration and transamination. But it is also the precursor of glycine.

• Glycine can be converted into serine or simply oxidized to $CO_{22}$and in NH_3

b- Ketogenic (or ketone) amino acids

The breakdown of the carbon skeleton yields acetyl-CoA or acetoacetyl CoA. Here we find 2 essential amino acids: leucine and lysine.

c- Amino acids that are both gluconeogenic and ketogenic: tyrosine (non-essential), phenylalanine, tryptophan and isoleucine (all 3 essential).

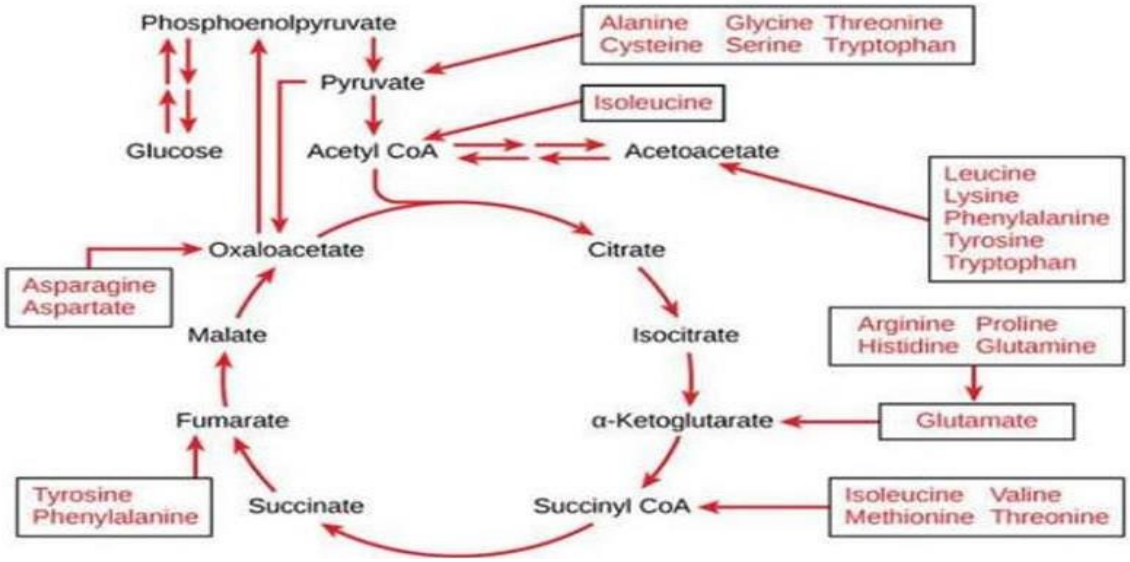


Fig. 237. Catabolism of carbon chains of amino acids.

X.3. Synthesis of amino acids

X.3.1. The two types of amino acids

The growth and development of living beings depend on their supply of 20 amino acids. All 20 are synthesized by higher plants and a few other organisms. Humans only synthesize 10 (non-essential amino acids), and the other ten (essential amino acids) must be obtained through diet in the form of plant or animal proteins (Table 34).

Table 34: Essential amino acids synthesized by the human body.

Synthesized acids	Essential acids
Alanine Glutamate glutamine Proline Aspartate Asparagine Glycine SerineTyrosine Cysteine	Isoleucine tryptophan Leucine Lysine Methionine Threonine Phenylalanine Valine Arginine Histidine

The precursors of amino acids constitute the α-keto acids directly usable for transamination or allow their synthesis. They are generated in degradation processes, the main ones being glycolysis and the Krebs cycle (Fig. 238).

Carbohydrates are the main sources of carbon, found in amino acids. A carbon skeleton can be the basis for the synthesis of several amino acids. These are then referred to as a family.

- α-ketoglutarate leads to the glutamate family: glutamate, glutamine, proline, arginine and lysine.
- Oxaloacetate gives rise to the aspartate family: aspartate, asparagine, methionine, threonine and isoleucine.
- Glycerate-3-phosphate leads to the serine family: serine, glycine, and cysteine.
- Pyruvate provides the alanine family: alanine, valine and leucine.
- Phosphoenolpyruvate and erythrose-4-phosphate are the starting point for phenylalanine, tyrosine and tryptophan.
- Ribose-5-phosphate is the precursor of histidine.

➢ Glutamate family

***1/ Glutamate and glutamine*:** Glutamate synthesis can occur through the action of:

- Glutamate dehydrogenase according to the reaction:

$$\alpha\text{-Cétoglutarate} + NH_4^+ + NADPH,H^+ \longrightarrow \text{glutamate} + NADP^+$$

- From an aminotransferase (transaminase)

Acide aminé + α-cétoglutarate ⟶ α-cétoacide + glutamate

Glutamine is synthesized under the action of glutamine synthetase.

$$\text{Glutamate} + NH_3 + ATP \longrightarrow \text{Glutamine} + ADP + Pi$$

***2/ Proline*:** It is formed from glutamate in two steps:

- The carboxyl group attached to carbon 5 is first reduced to an aldehyde. This requires energy supplied by ATP and is catalyzed by glutamyl phosphate dehydrogenase.



- The preceding semialdehyde glutamate undergoes cyclization followed by reduction to give proline.

$$\text{Semi aldehyde glutamate} + NADPH,H^+ \longrightarrow \text{Proline} + NADP^+$$

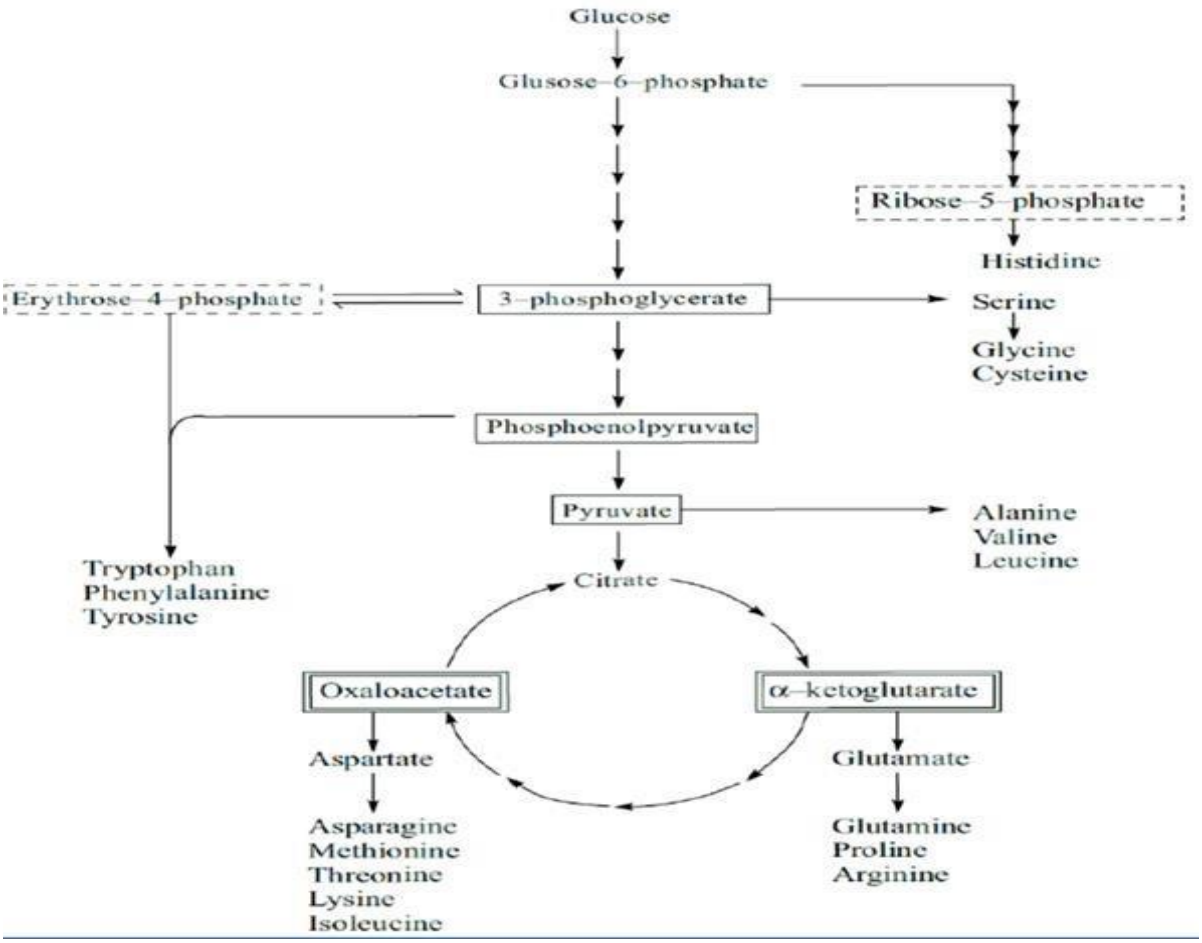


Fig. 238. Amino acid biosynthesis pathways

➢ Aspartate family

1/ Aspartate: Aspartate is formed by transamination of oxaloacetate. The group α-amino is donated by glutamate. The reaction is catalyzed by aspartate aminotransferase.

Oxaloacétate + glutamate ⟶ Aspartate + α-cétoglutarate

2/Asparagine: The synthesis is catalyzed by asparagine synthetase

Aspartate + glutamine + ATP ⟶ Asparagine + glutamate + AMP + Ppi

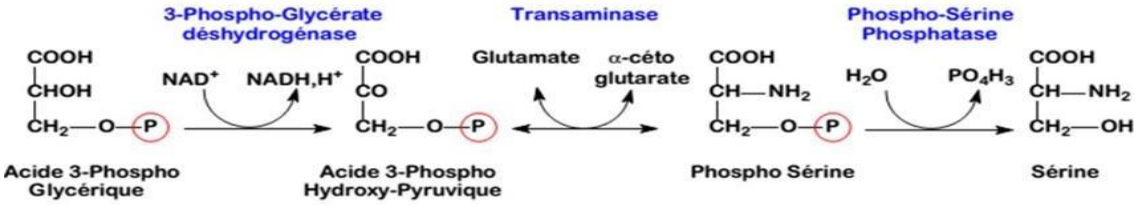
➢ Serine family

1/Serine: Serine is synthesized from 3-phosphoglycerate according to the sequence below:

- Dehydrogenation leads to 3-phospho-hydroxypyruvate, catalyzed by 3-phosphoglycerate dehydrogenase.

$PO\text{-}CH_2\text{-}CHOH\text{-}COO^- + NAD^+ \longrightarrow PO\text{-}CH_2\text{-}CO\text{-}COO^- + NADH,H^+$

- This is followed by transamination in the presence of glutamate by a phosphoserine transaminase. Under the action of a phosphatase, serine is obtained by hydrolysis of the phosphate group.



2/ Glycine: Glycine is synthesized from serine by the elimination of the CH_2OH radical. The reaction is catalyzed by serine hydroxymethyltransferase using tetrahydrofolate as a coenzyme.

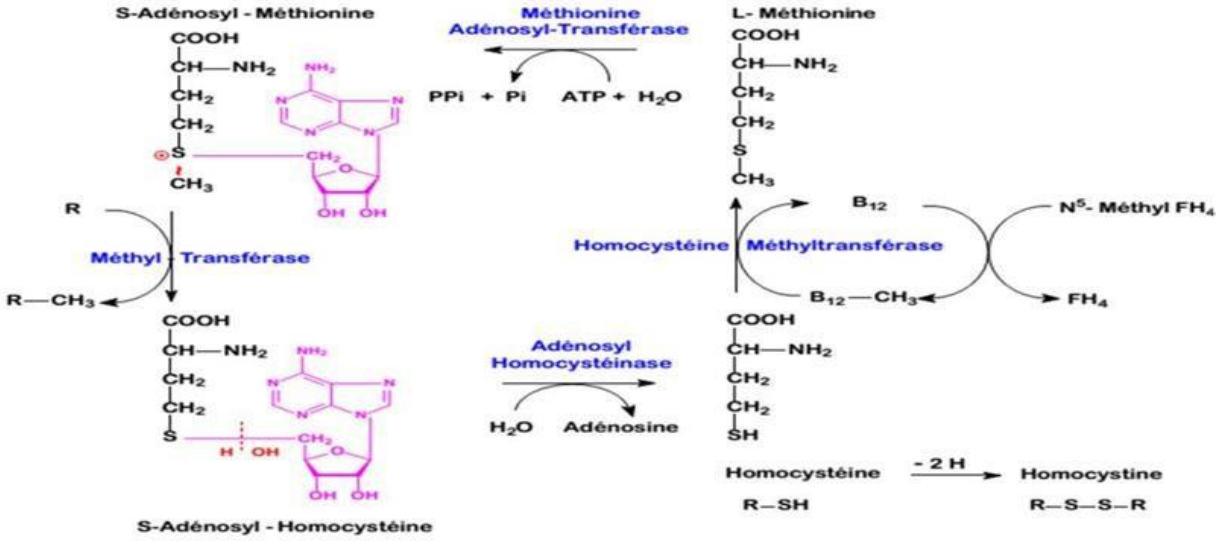


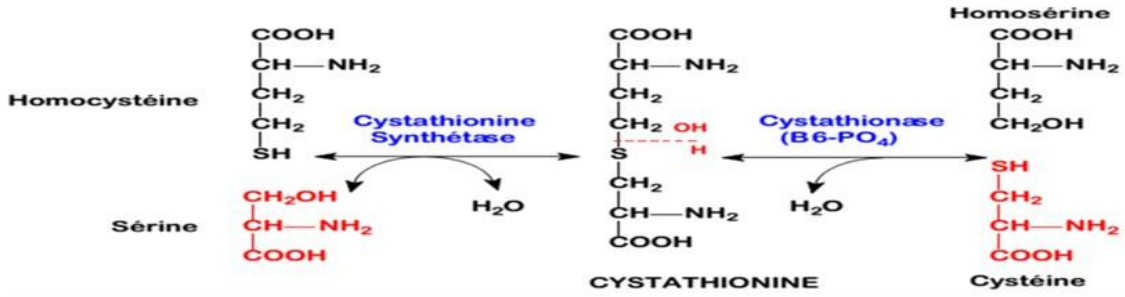
3/ Cysteine: Cysteine is formed from methionine by a sequence of complex reactions involving the formation of S-adenosylmethionine, the only case where ATP is deprived of these 3 phosphate groups in the same reaction:

ATP + Methionine + H_2O ⟶ S-adenosylmethionine + PPi + Pi

The sequence below leads to cysteine:

S-**adenosylmethionine** ⟶ **S-adenosyl-homocysteine** ⟶ **Cysteine**





- **Alanine family**

-Alanine: In this family, alanine is the only non-essential amino acid. It is obtained by transamination of pyruvate in the presence of glutamate. The reaction is catalyzed by glutamate pyruvate aminotransferase (GPAT).

- **Family of aromatic amino acids**:

-Tyrosine: This non-essential amino acid is unique in that it is derived from phenylalanine (an essential amino acid). It is considered a breakdown product of the latter. Tyrosine, or parahydroxy-phenylalanine, is formed during a reaction catalyzed by phenylalanine hydroxylase.

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