Ferhat Abbas University, Setif 1
Faculty of Natural and Life Sciences
Department of Basic Studies

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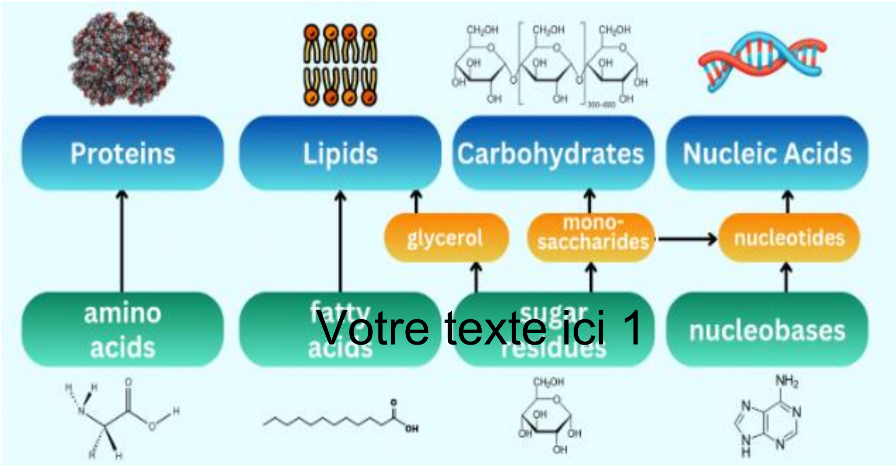
BIOCHEMISTRY



Dr. Madoui Soraya

Course intended for second-year

Common Core students

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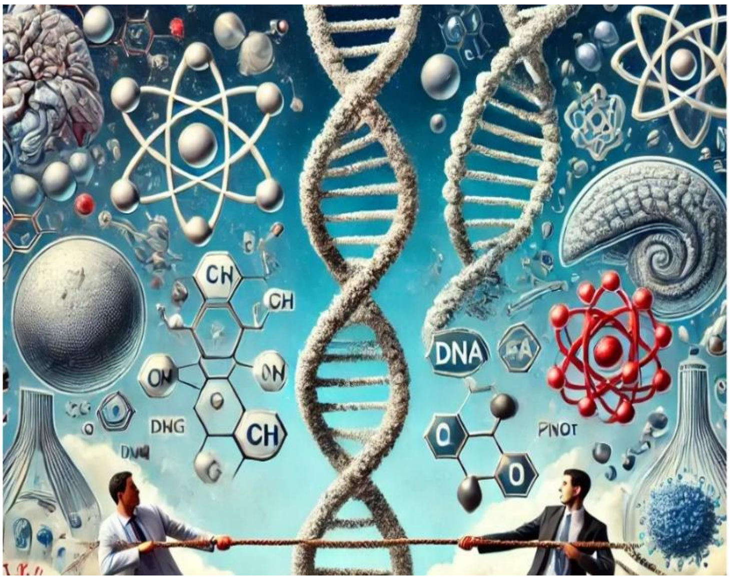
INTRODUCTION



Introduction

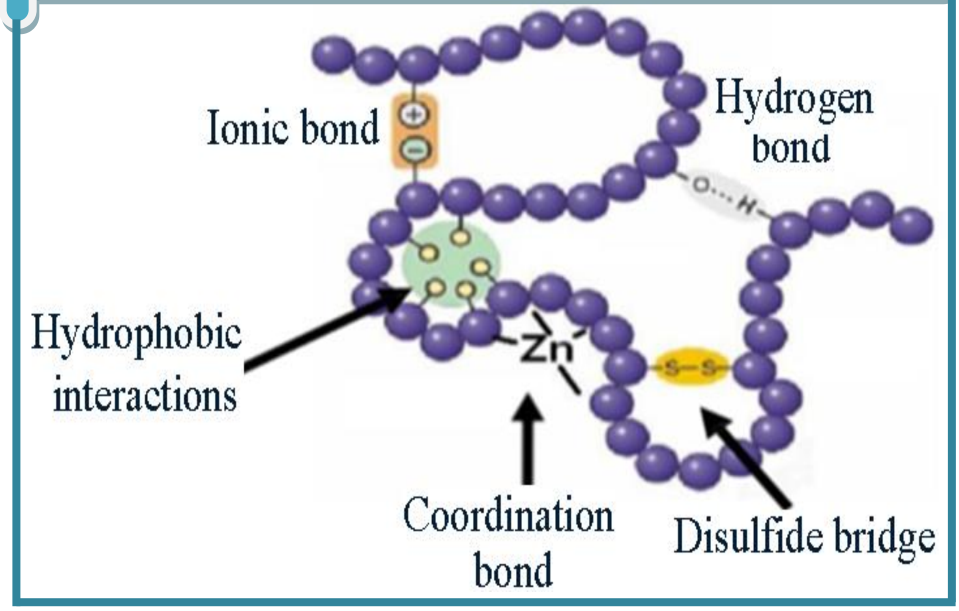
Biochemistry is the science that studies the structure and properties of the molecules that make up living organisms (such as carbohydrates, proteins, and lipids). It also examines the transformation reactions (metabolism) of these molecules, including degradation reactions (catabolism) and biosynthesis reactions (anabolism).

This structural biochemistry course is intended for second-year biology students. It aims to help them understand the structure and physicochemical properties of the three main components of living cells: proteins, carbohydrates, and lipids.

All life sciences require knowledge of biochemistry. The biochemistry of nucleic acids lies at the heart of genetics; conversely, genetic approaches have shed light on many areas of biochemistry. The field of physiology, which studies bodily functions, overlaps almost completely with that of biochemistry. Immunology relies heavily on biochemical techniques, and biochemists often employ immunological approaches. Pharmacology and pharmaceutical sciences are founded on solid biochemical and physiological principles; for instance, most drugs are metabolized through enzyme-catalyzed reactions. Poisons act on biochemical reactions or pathways, which are the main focus of toxicology. Biochemical approaches are increasingly used to investigate the fundamental aspects of pathology (the study of diseases), such as inflammation, cellular damage, and cancer. Many researchers in microbiology, animal biology, and plant biology rely almost exclusively on biochemical methods.

These interconnections are not surprising, since life as we know it depends on biochemical reactions and transformations. Indeed, the traditional boundaries between the life sciences are dissolving as biochemistry increasingly becomes their common language.

CHAPTRE I : CHEMICAL BONDS



I. General information

The role that chemistry plays in our lives is proportional to the ever-increasing growth of scientific knowledge and its applications. This process dates back to ancient times, and one of the great merits of historians of science is to reveal its evolution to us. Biology joins forces with physics in an attempt to elucidate phenomena observed on the atomic scale. Since the beginning of this century, biology has gradually erased what once separated it from physics, as it has come to be recognized that modeling in physico-chemical terms could likely explain the structures and processes that characterize living organisms. However, the description of complex atomic structures, macromolecules, is necessary to establish the link between the almost mineral world and the ever-changing living world.

The shape of these complex polyatomic structures depends on the chemical bonds that connect atoms to one another, belonging to a family of interactions collectively referred to as chemical bonds.

II. Definition of Molecules

A **molecule** is an assembly of two or more atoms. There are two types of molecules:

- **Homonuclear molecule:** formed by identical nuclei, e.g., H_2, O_3, S_6.

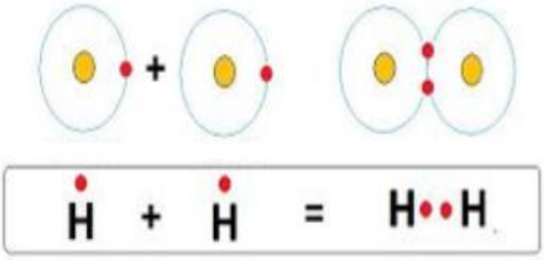


Fig. 1: Homonuclear molecule H–H

- **Heteronuclear molecule:** formed by different nuclei, e.g., H_2O, H_3PO_4. There exists an innumerable variety of molecules.

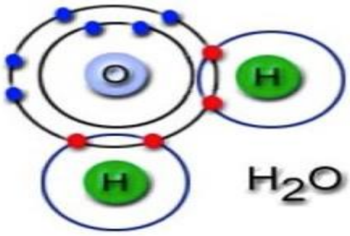
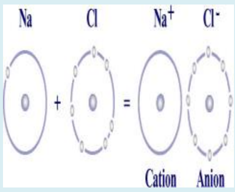
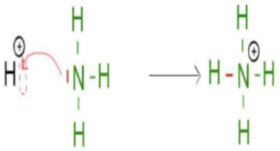
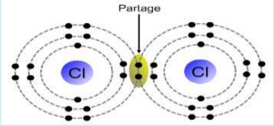
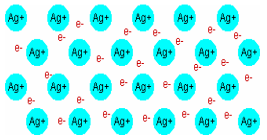
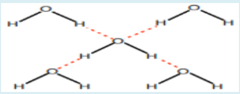
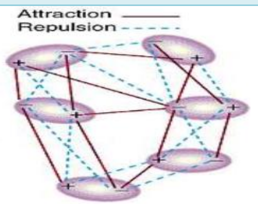
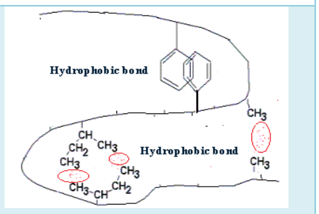


Fig. 2: Heteronuclear molecule

III. Classification of chemical bonds

Bonds are classified into two categories: **high-energy bonds** and **low-energy bonds** (Table I).

Table I: Classification of Chemical Bonds

Category	Type	Explanation	Example
Strong Bonds	Ionic Bond	Formed when electrons are transferred from one atom to another (usually an alkali metal donates its electron to another atom), leading to the formation of two oppositely charged ions. Since opposite charges attract, these ions tend to remain close together.	Sodium chloride (NaCl) 
	Coordinate or Dative Bond (Donor–Acceptor Bond)	A dative bond can form between a chemical species with an electron deficiency and another species possessing a lone electron pair. The first species gains, on average, one electron, decreasing its charge by one unit, while the second loses one electron, increasing its charge by one unit.	Ammonium ion (NH_4^+) 
	Covalent Bond	A covalent bond between two non-metal atoms A and B involves the sharing of two electrons. Each atom contributes one valence electron.	Dichlorine (Cl_2) 
	Metallic Bond	In metals, all valence electrons are shared collectively, forming a conduction band. This is why metals are capable of conducting electrical energy.	
Weak Bonds	Hydrogen Bond	Occurs between a hydrogen atom, already involved in a covalent bond and carrying a partial positive charge, and an acceptor atom, also engaged in a covalent bond and carrying a partial negative charge.	Hydrogen bonding in the organization of water dipoles. 
	Van der Waals Forces	A molecule with non-polar covalent bonds may have regions of slight negative and positive charge because electrons are not symmetrically distributed and may momentarily cluster in certain areas.	
	Hydrophobic Interaction	In large molecules, both hydrophilic (charged or polar regions, such as hydrogens bound to fluorine, oxygen, or nitrogen) and hydrophobic (non-polar regions) parts coexist.	

IV. Main biochemical functions

Chemical compounds that display similar chemical properties contain identical groupings of atoms (Table II). These groupings are referred to as functional groups, or simply functional groups.

Table II: Main types of functional groups

Fonction group	Formula	Biological role and Importance	Examples
Hydroxyl (Alcohol)	$-OH$	Makes the molecule polar; allows formation of hydrogen bonds (crucial for solubility in water and structural stability).	Carbohydrates (sugars, cellulose), amino acids (serine, threonine), steroids (cholesterol).
Carbonyl (Ketone)	$-C=O$	Reactive site. Ketoses are a form of sugar.	Carbohydrates (fructose, ribulose).
Carbonyl (Aldehyde)	$-CHO$	Reactive site. Aldoses are another form of sugar.	Carbohydrates (glucose, ribose in DNA/RNA).
Carboxyl (Carboxylic acid)	$-COOH$	Dissociates in solution into H^+ and $-COO^-$ (weak acid). Donates protons and gives the molecule a negative charge.	Amino acids (aspartic acid, glutamic acid), fatty acids.
Amino (Amine)	$-NH_2$	Proton (H^+) acceptor in solution (weak base). Becomes positively charged. Essential for peptide bond formation.	Amino acids (lysine), nitrogenous bases (adenine, cytosine) in DNA/RNA.
Phosphate	$-PO_4^{2-}$	Carries negative charges. Plays a key role in energy transfer and regulation.	ATP (adenosine triphosphate, the “energy currency”), ADP, DNA/RNA (phosphate backbone).
Sulfhydryl (Thiol)	$-SH$	Can form disulfide bridges ($-S-S-$) that stabilize the 3D structure of proteins.	Amino acids (cysteine), coenzymes (Coenzyme A).
Methyl	$-CH_3$	Nonpolar group. Involved in epigenetic regulation (DNA/histone methylation).	Modified DNA bases (methylcytosine), hormones (adrenaline).

V. Biological Importance of Functional Groups

Functional groups play a fundamental role in the chemistry of life. They determine the properties and behavior of biological molecules, influencing their reactivity, solubility, structure, and function. These characteristics underlie metabolic mechanisms, membrane formation, energy transfer, and cellular regulation. The five points summarized above illustrate these essential roles in the functioning of living systems.

1. **Determine Reactivity:** These groups define the reactive sites where most biochemical reactions occur (metabolism).
2. **Control Solubility and Interaction:**

- Polar or charged groups ($-OH$, $-COOH$, $-NH_2$, $-PO_4$) make molecules hydrosoluble.

- Nonpolar groups (such as the methyl group or long hydrocarbon chains) make molecules hydrophobic.
- This duality forms the basis of cell membrane structure (the lipid bilayer).

3. **Stabilize 3D Structures:** Hydrogen bonds (via $-OH$, $-NH_2$, $-C=O$) and disulfide bridges ($-S-S-$) are crucial for the structure of proteins and nucleic acids.
4. **Enable Energy Transfer:** The phosphate group is central to energy storage and transfer (ATP → ADP).
5. **Act as Regulatory Signals:** The addition or removal of groups such as phosphate (phosphorylation) or methyl (methylation) is a universal mechanism used to activate, deactivate, or regulate the activity of proteins and genes.

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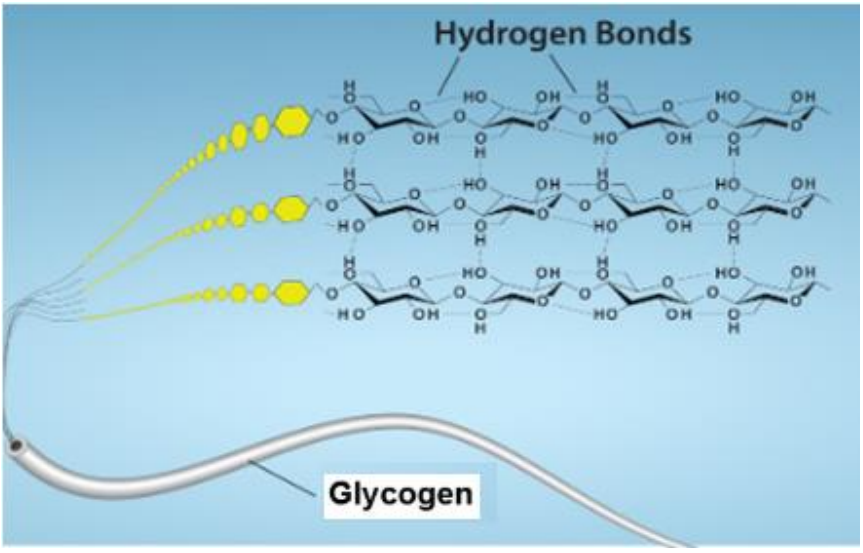
CHAPTRE II : CARBOHYDRATES



I. Definition and Biomedical Importance

Carbohydrates are organic molecules characterized by the presence of a carbon chain bearing hydroxyl groups "OH", and a carbonyl function; either aldehyde (-CHO) or ketone (C=O), and sometimes a carboxyl function (OH-C=O) or amine ($-NH_2$).

Carbohydrates are widespread in plants and animals, where they play important structural and metabolic roles. In plants, glucose is synthesized from carbon dioxide (CO_2) and water (H_2O) through photosynthesis; it is stored in the form of starch or used for the synthesis of cellulose in the cell walls of plants.

Animals can synthesize some carbohydrates from amino acids, but most animal carbohydrates come from plants. Glucose is the most important carbohydrate. Through the hydrolysis of starch and disaccharides from food, the majority of dietary carbohydrates are absorbed into the bloodstream in the form of glucose, and other sugars are converted into glucose in the liver. Glucose is the precursor for the synthesis of all other carbohydrates in the body, such as glycogen for storage, ribose and deoxyribose in nucleic acids, and galactose in the lactose of milk.

Diseases associated with carbohydrate metabolism include diabetes, galactosemia, glycogen storage diseases, and lactose intolerance.

II. Classification

Carbohydrates are subdivided according to their degree of polymerization:

II.1. Monosaccharides or Simple Sugars

Monosaccharides are molecules that contain both multiple hydroxyl (alcohol) groups and a reducing function, either aldehyde (-CHO) or ketone (C=O). The classification of monosaccharides is based on, first, the number of carbon atoms in their molecules (trioses [3C], tetroses [4C], pentoses [5C], hexoses [6C], heptoses [7C], octoses [8C], and nanoses [9C]), and second, on the nature of the reducing function (aldoses and ketoses) (Fig. 1). The combination of the number of carbons and the type of carbonyl function is possible; for example, aldotrioses, aldotetroses, ... ketotrioses, ketotetroses, and so on.

II.2. Osides or Complex Sugars (polysaccharides)

Polysaccharides are defined as substances whose hydrolysis releases one or more monosaccharides. There are two types (Fig.1):

- **Holosides,** whose hydrolysis releases only monosaccharides. Depending on the number of monosaccharide molecules released during hydrolysis, holosides are classified into **oligosides** (association of 2 to 10 monosaccharides via glycosidic bonds) and **polyosides** (polymers formed of 10 to several thousand monosaccharides). Polyosides are further classified into **homogeneous** or **heterogeneous** polyosides, depending on whether they are composed of a single type of monosaccharide or several types.
- **Heterosides,** whose hydrolysis releases, in addition to monosaccharides, non-carbohydrate substances or aglycones (proteins, lipids, nucleic acids, etc.).

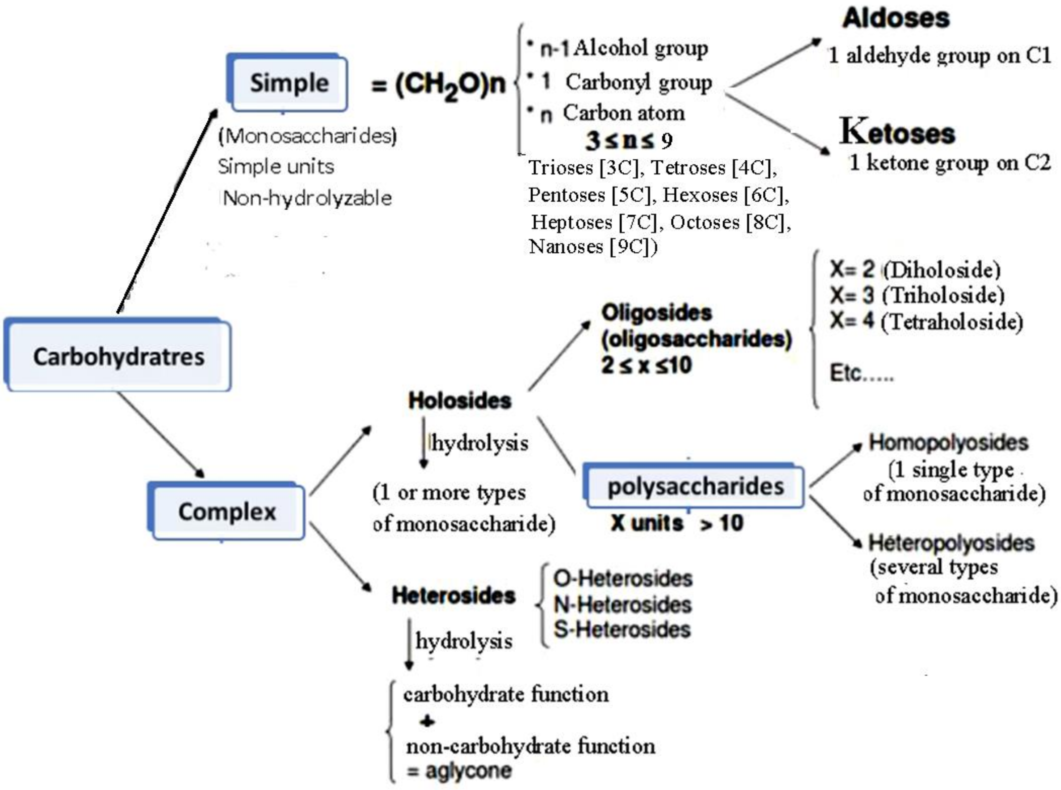


Fig. 1: Carbohydrate classification based on degree of polymerization

III.Monosaccharides

Monosaccharides are non-hydrolyzable molecules with the general formula ($C_nH_{2n}O_n$ / where n = the number of carbon atoms), most commonly containing 3 to 6 carbon atoms and a carbonyl group (aldehyde or ketone). The simplest monosaccharides have three carbon atoms: an aldotriose, **glyceraldehyde**, and a ketotriose, **dihydroxyacetone** (Fig.2).

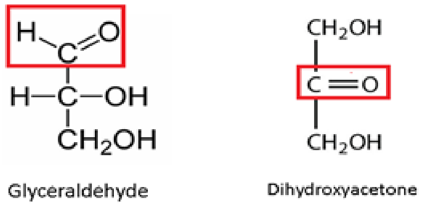


Fig. 2: Formulas of glyceraldehyde (aldotriose) and dihydroxyacetone (ketotriose).

In the Fischer projection, the carbon atoms of a monosaccharide are numbered in such a way that the most oxidized carbon (the carbon that carries the carbonyl group) is assigned the lowest number (Fig. 3). This projection clearly shows the asymmetric carbons present in the structure of monosaccharides.

The Fischer projection is useful for visualizing the configuration of the chiral centers, especially in distinguishing between different isomers of monosaccharides.

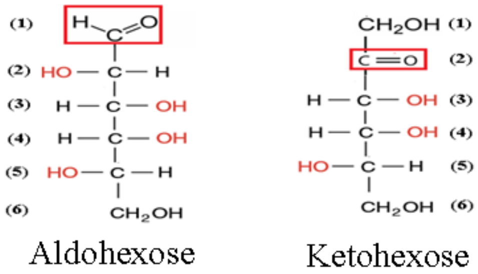


Fig. 3: Direction of numbering of carbon atoms in oses.

III.1. Asymmetric Carbon (Chiral)

A carbon atom is said to be asymmetric (C*) if it has four different substituents. The Fischer projection clearly highlights the asymmetric carbons present in the structure of monosaccharides. In the case of glyceraldehyde (Fig. 4), the carbon C_2, which has four different groups: CH_2OH, CHO, OH, and H, is referred to as an asymmetric or chiral carbon (C*) or a center of chirality.

This property is crucial for understanding the stereochemistry of sugars, as the arrangement of these groups around the chiral carbon can lead to different isomers with distinct biochemical properties.

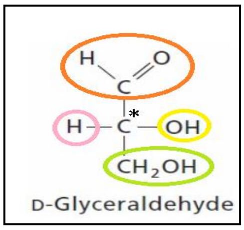


Fig. 4: Identification of an asymmetric (chiral) carbon.

If we consider the projection of the three-dimensional structure of glyceraldehyde, we see that there are two possibilities: the hydroxyl (OH) carried by the penultimate carbon (OH of C_{n-1}) is located to the right of the plane formed by the carbon chain (Fig. 5), we speak of the D configuration (D series); in the other case, the hydroxyl is located to the left of this plane, it is the L configuration (L series).

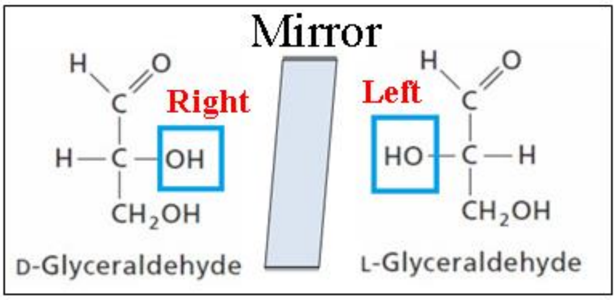


Fig. 5: Projections of the structures of the D and L configurations of glyceraldehyde.

III.2. Isomerism of Monosaccharides

III.2.1. Definition of Isomerism

Isomers are compounds that have the same molecular formula ($C_nH_{2n}O_n$ / where n = the number of carbon atoms) but different structural (stereochemical) formulas.

III.2.1.1. Functional Isomers

Functional isomers are two monosaccharides that have the same general formula (the same number of carbons) and belong to the same series, but differ in the nature of the carbonyl group; one is ketonic at C2 while the other is aldehydic at C1. An example of this is D-Glucose and D-Fructose (Fig. 6). This type of isomerism illustrates how small structural differences can lead to significant variations in the properties and functions of sugars.

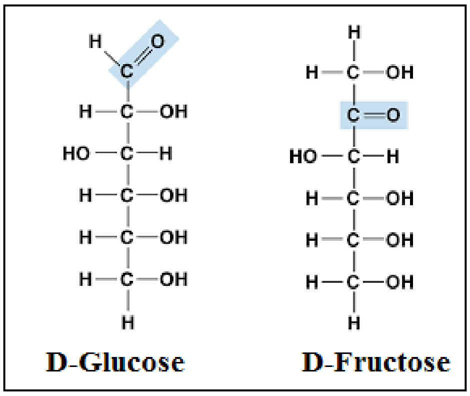


Fig. 6: Functional isomerism between Glucose and Fructose.

Monosaccharides can be differentiated by the spatial arrangement of their hydroxyl (OH) groups. This is referred to as stereoisomerism. This stereoisomerism is due to the presence of asymmetric carbons within the molecules. Different forms of isomerism are distinguished.

III.2.1.2. Epimerism

This occurs in two monosaccharides of the same series that differ only by the absolute configuration of a single asymmetric carbon (Fig. 7), such as between D-mannose and D-glucose (epimers at C2) or between D-glucose and D-galactose (epimers at C4).

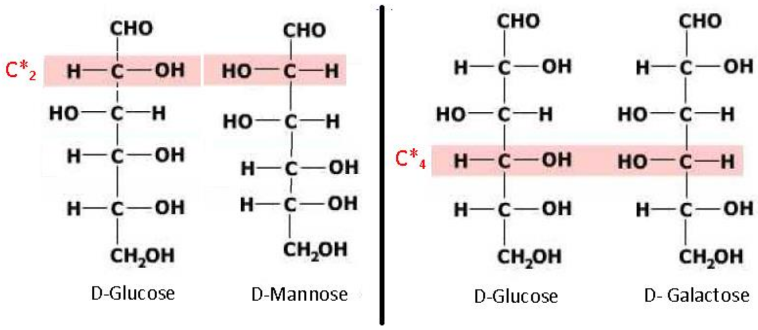
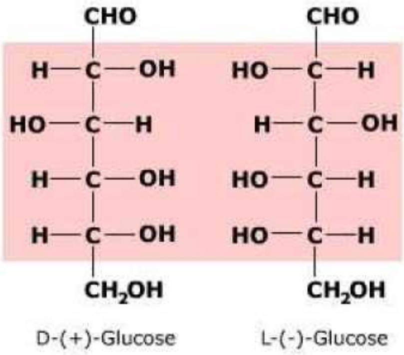


Fig. 7: Glucose epimerization

III.2.1.3. Enantiomerism

Also called optical isomers or optical antipodes, enantiomers are two monosaccharides that share the same name, one from the D series and the other from the L series. They differ in the configuration of all asymmetric carbons. Being images of each other with respect to a mirror, they are not superimposable. They are therefore symmetrical with respect to each other, with the plane of symmetry being the mirror, such as D-Glucose and L-Glucose (Fig. 8.A). The most concrete example of chirality is that of a right hand that cannot be superimposed on a left hand (Fig. 8.B). The chemical and physical properties of enantiomers are generally identical, except for one physical property: optical rotation.



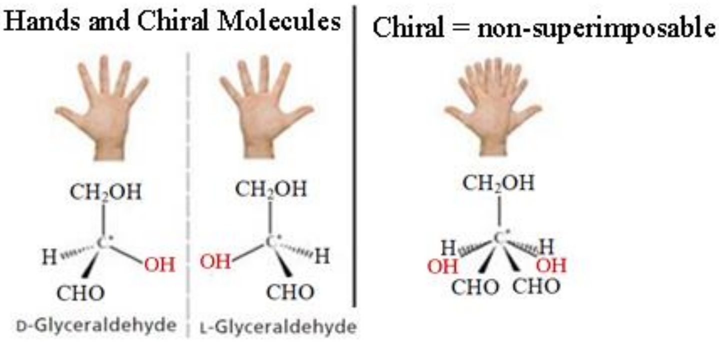


Fig. 8. A: Enantiomerization of glucose. **Fig. 8. B**: Enantiomerization of glyceraldehyde.

III.2.1.4. Diastereoisomerism

Diastereoisomers are two non-superimposable monosaccharides that are not mirror images of each other. They have opposite configurations at some asymmetric carbons but differ at fewer chiral centers than the total number of asymmetric carbons (Fig. 9).

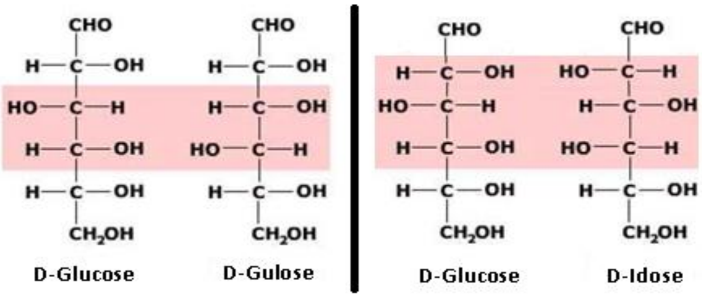


Fig. 9: Diastereoisomers of Carbohydrates (of Glucose)

III.2.2. General Rule for the Number of Isomers

- Generally, when a molecule has multiple chiral centers, for x chiral carbons (C*), there are 2^x different stereoisomers, with half in D configuration and the other half in L configuration.
- Specifically, for aldoses, the number of stereoisomers is 2^{n-2} . For ketoses, the number of stereoisomers is 2^{n-3} (where n is the number of carbon atoms in the chain).

Example: For an aldohexose (such as glucose) where n = 6, the total number of stereoisomers is 2^4=16 (8 from the D series and 8 from the L series).

III.2.3. Optical Activity of Monosaccharides

III.2.3.1. Rotatory Power

A substance is said to be optically active or possess rotatory power when it deviates the plane of polarized light by an angle α\alphaα (Fig. 10). The value of the angle of deviation of the plane of polarization is measured using a polarimeter. The rotatory power is related to the presence of one or more asymmetric carbon atoms within the molecule. All monosaccharides (except for dihydroxyacetone), being chiral molecules, exhibit some degree of rotatory power.

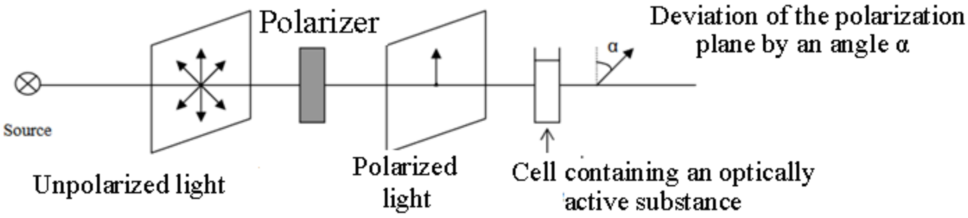


Fig. 10: Polarization of light at an angle α

Two types of optically active substances are distinguished:

- **Those that deviate the plane of polarized light by an angle α\alphaα to the right (clockwise):** By convention, the measured angle is positive, and the substance is said to be dextrorotatory (+).
- **Those that deviate the plane of polarized light by an angle α\alphaα to the left (counterclockwise):** By convention, the measured angle is negative, and the substance is said to be levorotatory (-).

Note:

- Configuration and rotatory power are independent. There is no link between the D or L form and the direction of deviation of the plane of polarized light. Examples: D-glucose is dextrorotatory, while D-fructose is levorotatory.
- The two enantiomers of the same monosaccharide have opposite rotatory powers: they are identical in absolute value, but one is dextrorotatory and the other is levorotatory. Example: D-glucose is dextrorotatory, and L-glucose is levorotatory.

III.2.3.2. Biot's Law

The property of rotatory power in monosaccharides allows for the polarimetric quantification of holosides in pure solution, thanks to Biot's law. This is a very rapid and straightforward method that is widely used, for example, in the sugar industry. The angle α\alphaα depends on the nature of the substance, its concentration, and the length of the optical path. It follows a law known as Biot's law:

$$[\alpha]^{20}_{D} = \frac{\alpha}{C * L}$$

Where:

- **$[\alpha]^{20}_{D}$:** Specific rotatory power of the optically active substance. This is a characteristic constant of the optically active substance that depends on the nature of the solute and solvent, temperature, and the wavelength at which the measurement is taken. "D" refers to the wavelength of the yellow line of sodium at 589.3 nm, and "20" refers to 20°C.
- **α:** Measured rotatory power using the polarimeter.
- **L:** Length of the tube containing the solution (dm).
- **C:** Concentration of the substance (g/ml).

a)- Additivity of Rotatory Powers

When a solution contains two optically active compounds, the angles of deviation of the plane of polarized light due to each optically active substance add together: the measured rotatory power is equal to the sum of the rotatory powers of each of the two substances.

$$\alpha_{\text{solution}} = \alpha_{\text{compound A}} + \alpha_{\text{compound B}}$$

$$\boldsymbol{\alpha}_{\textbf{solution}} = (\boldsymbol{[\alpha]^{20}_{D}}\ _{\text{compound A}} \text{ x } L \text{ x } C_{\text{compound A}}) + (\boldsymbol{[\alpha]^{20}_{D}}\ _{\text{compound B}} \text{ x } L \text{ x } C_{\text{compound B}}).$$

- **Special Case of Two Enantiomers**

Since the two enantiomers D and L of the same monosaccharide have opposite rotatory powers, an equimolar mixture of the two enantiomers is optically inactive (with zero rotatory power) and is called a racemic mixture.

Example: Equimolar mixture of the enantiomers D and L of glucose:

$\boldsymbol{\alpha}_{\textbf{mixture}} = \boldsymbol{\alpha}_{\textbf{D-glucose}} + \boldsymbol{\alpha}_{\textbf{L-glucose}}$

$\boldsymbol{\alpha}_{\textbf{mixture}} = (\boldsymbol{[\alpha]^{20}_{D}}\ _{\text{D-glucose}} \text{ x } L \text{ x } C_{\text{D-glucose}}) + (\boldsymbol{[\alpha]^{20}_{D}}\ _{\text{L-glucose}} \text{ x } L \text{ x } C_{\text{L-glucose}}).$

With : $C_{\text{D-glucose}}$ in solution = $C_{\text{L-glucose}}$ in solution **and** $\boldsymbol{[\alpha]^{20}_{D}}\ _{\text{D-glucose}} = \boldsymbol{-[\alpha]^{20}_{D}}\ _{\text{L-glucose}}$

$$\boldsymbol{\alpha}_{\textbf{mixture}} = \mathbf{0}$$

III.3. Chemical Lineage of Monosaccharides According to Fischer

The Kiliani-Fischer cyanohydrin synthesis consists of chemical reactions that allow for the synthesis of a monosaccharide with (n+1) carbons from a monosaccharide with (n) carbons (Fig. 11). The addition occurs at the end bearing the aldehyde group (C_1) in the case of aldoses, and at the ketone group (C_2) in the case of ketoses. This carbon is not asymmetric and exists in only one configuration.

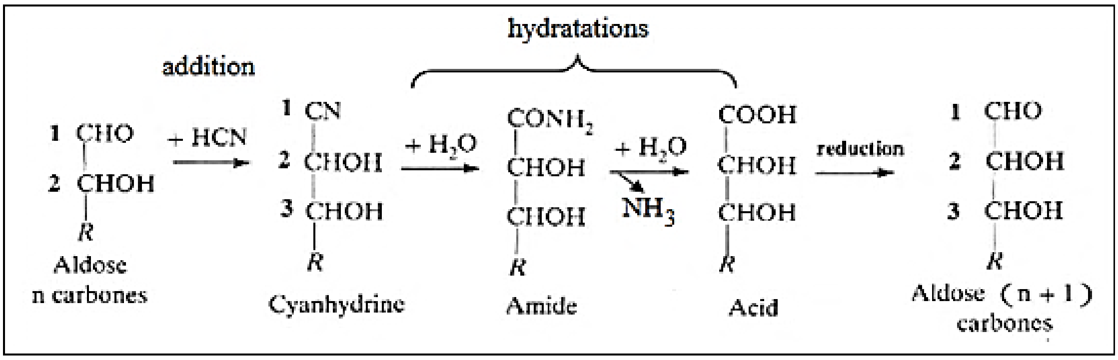


Fig. 11: Kiliani Fisher Hydrocyanic Synthesis

After the addition, C1 becomes positioned at C2, bearing a secondary alcohol group. Thus, each newly added carbon becomes a new center of chirality, with two possible relative orientations of the substituents. This creates a new pair of stereoisomers (Fig. 12, 13).

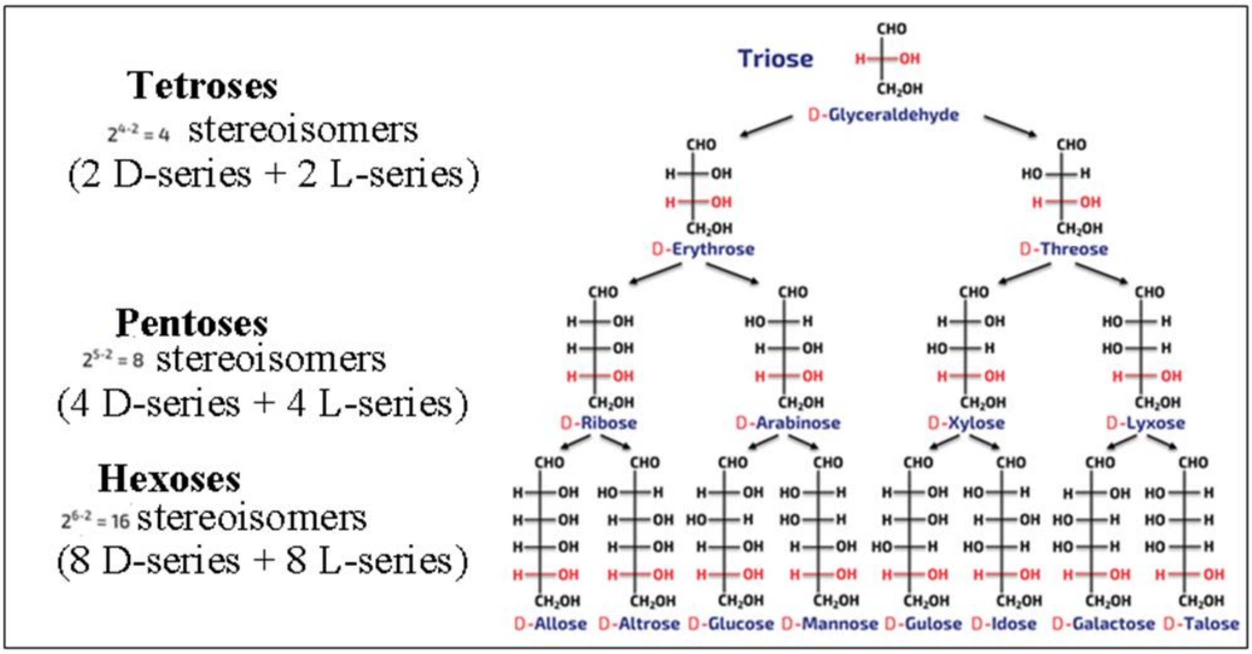


Fig. 12: Synthesis of Aldohexoses from an Aldotriose

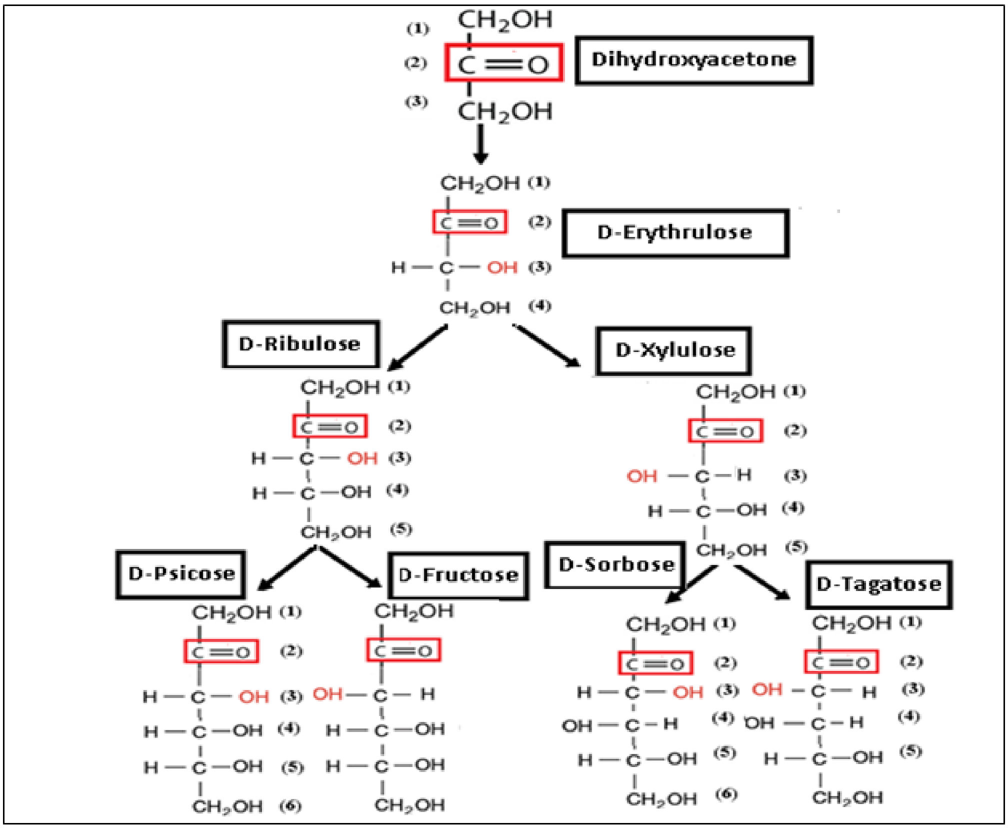


Fig. 13: synthesis of Ketohexoses from a Ketotriose.

III.4. Cyclic Structure of Monosaccharides

III.4.1. Objections to the Linear Structure of Monosaccharides

The linear or open-chain structure of monosaccharides does not account for all their properties when the number of atoms exceeds four. The following properties of monosaccharides can only be explained by their cyclic structure.

III.4.1.1. Formation of Acetal

A true aldehyde or ketone bonds with two alcohol molecules to eventually form an acetal (Fig. 14), whereas an aldose or ketose binds with only one alcohol molecule, resulting in a hemiacetal (Fig. 15).

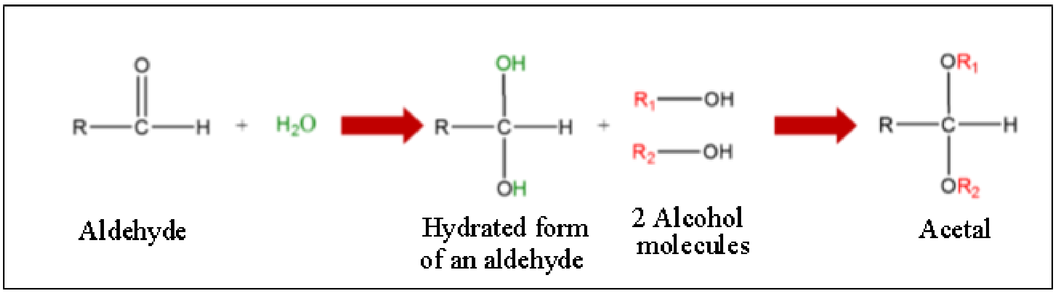


Fig.14 : Acetal form

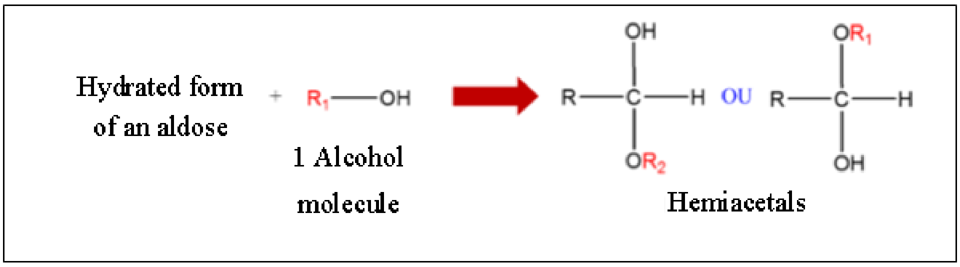


Fig. 15: Hemiacetal form

III.4.1.2. Methylation

Considering the linear structure of a hydrated aldohexose (e.g., glucose, n=6), the total methylation of its hydroxyl (OH) groups using methyl sulfate [$SO_4(CH_3)_2$] in the presence of sodium hydroxide or methyl iodide (ICH_3) with Ag_2O requires 7 methyl groups (–CH_3) and theoretically results in a **heptamethylated** molecule. However, experimentally, this hydrated aldohexose molecule can only bind with **5 methyl groups**, forming a pentamethylated molecule (Fig. 16).

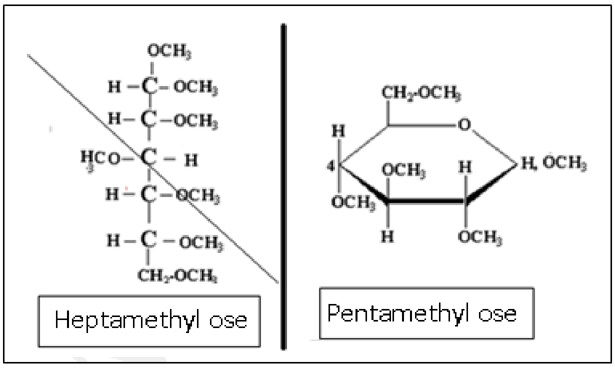


Fig. 16: Total methylation of the OH groups of a monosaccharide

III.4.2. Cyclization Mechanism

The Haworth perspective representation facilitates the depiction of various cyclic forms. It follows several conventions: the hydroxyl group attached to carbon 5 is positioned below the ring. A 90° rotation occurs around the bond between carbon 4 and carbon 5, so that the hydroxyl group on carbon 5 approaches the aldehyde group on carbon 1. As a result, carbon 6 undergoes a corresponding rotation and is positioned above the ring (Fig. 17).

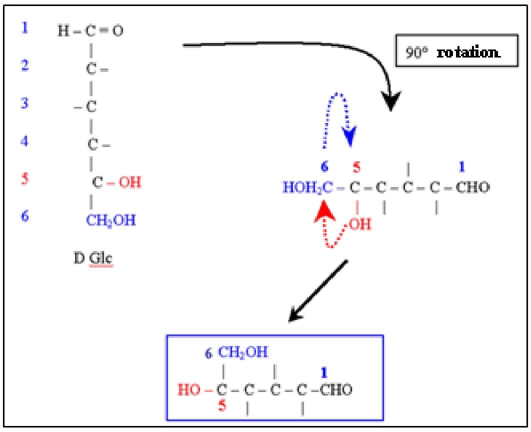


Fig. 17: Cyclization mechanism

From this point, one of the lone pairs of electrons on the oxygen atom can react on either side of the carbon atom, resulting in **α-D-glucopyranose** if the hydroxyl group attached to carbon 1 (the anomeric carbon) is positioned below the ring, and **β-D-glucopyranose** if it is positioned above the ring. This creates a new asymmetric carbon, and the two configurations produce two isomers called anomers (Fig. 18). The hydroxyl group on carbon 4 can also react, forming a five-membered ring, or **furan ring** (Fig. 19).

The same cyclization rule applies to **ketoses**; the difference lies in the site of intramolecular hemiacetalization, which occurs between C_2 and C5 to form a furan ring, or between C_2 and C_6 to form a pyran ring (Fig. 19).

The position of the hydroxyl groups is determined by their location in the Fischer projection: -OH groups found on the right in the Fischer representation will be positioned below the plane of the ring, while -OH groups found on the left will be positioned above the plane of the ring (Fig. 19).

The position of the primary alcohol function relative to the plane of the ring determines the series: **D-series** for -CH_2OH above the plane, and **L-series** for -CH_2OH below the plane.

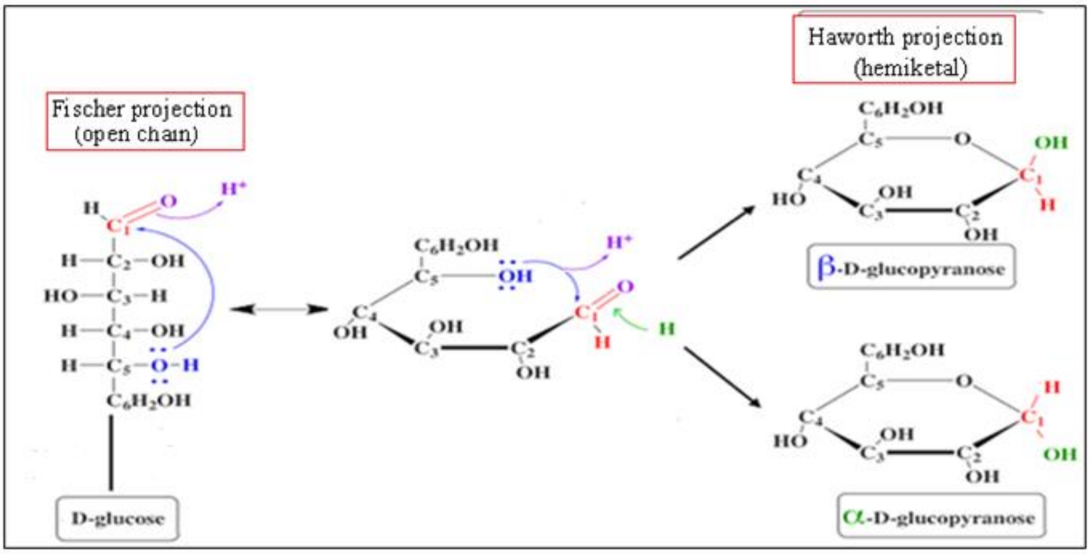


Fig. 18: Mechanism of formation of anomers

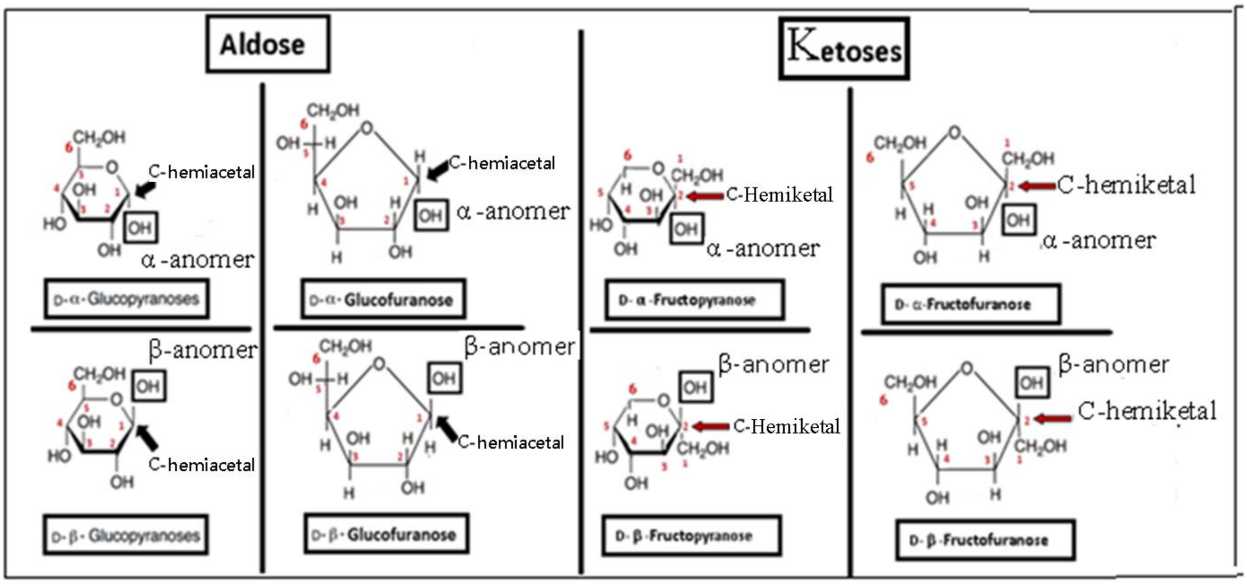


Fig. 19: Pyranic and furanic forms of oses

In the simplified representation, the C and H are not noted, and only the OH groups are represented, sometimes just by vertical lines. The following table shows the difference between the linear form and the cyclic form (Table I).

Table I: Difference between the linear form and the cyclic form.

Lineair form	Cyclic form
- OH on the right - OH on the left	-OH below (at the bottom) of the ring -OH above (at the top) of the ring
Aldehyde or ketone reducing function	Hemiacetal reducing function
Non-asymmetric reducing carbon	Asymmetric reducing carbon -Hemiacetal OH below: α -Hemiacetal OH above: β
Penultimate carbon in the chain -D-series: -OH on the right -L-series: -OH on the left	Primary alcohol determines the series -D-series: –CH_2OH above the plane -L-series: –CH_2OH below the plane

III.4.3. Mutarotation

a) Phenomenon of mutarotation

- Starting from the hydrolysis of starch under specific conditions, a solution of pure α-D-glucose ($[\alpha]^{D}_{20}$ = +112.2°) is obtained, whose optical rotation is not stable and decreases over time.
- Starting from the hydrolysis of cellulose under specific conditions, a solution of pure β-D-glucose ($[\alpha]^{D}_{20}$ = +18.7°) is obtained, whose optical rotation is not stable and increases over time.
- When linear crystallized glucose is dissolved in water, it leads to the cyclization of glucose, forming both α and β anomers in equivalent proportions. It is observed that the optical rotation of this freshly prepared solution decreases (Fig. 20).

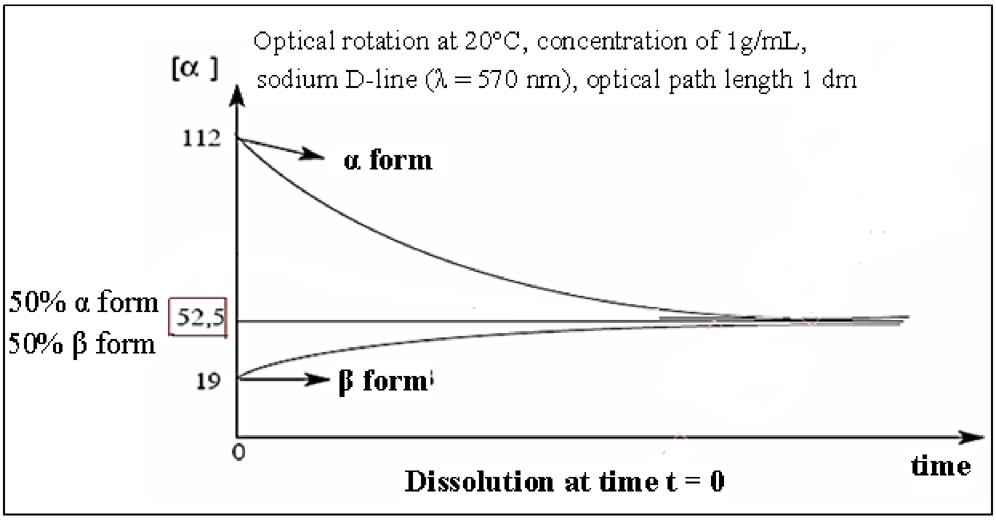


Fig. 20: Mutarotation phenomenon

This change in the optical rotation of a sugar solution is called the phenomenon of mutarotation. This variation in optical rotation accompanies the conversion of the α anomer into the β anomer until equilibrium is reached between these two forms. The optical rotation value of a sugar is not fixed immediately; it becomes stable after a certain period. This phenomenon is related to the existence of two isomeric forms, the α or β anomer.

***Exemple :**

$$\textbf{Time = 0} \Rightarrow \left.\begin{array}{l}\text{Optical rotation (OR) of } \alpha\text{-D glucose} = +112° \\ \text{Optical rotation (OR) of } \beta\text{-D glucose} = +18.7°\end{array}\right\} \xrightarrow{\textbf{Time = n}} \text{Optical rotation (OR) of glucose } (\alpha, \beta) = +52.7°$$

III.5. Physicochemical properties of sugars

III.5.1. Physical properties

1. Some sugars (fructose) or glycosides (sucrose) have a sweet taste.
2. Sugars are highly soluble in water due to their numerous hydroxyl groups; they are slightly soluble in methanol or ethanol (forming crystals) and insoluble in ether.
3. Aldoses are reducing sugars due to their hemiacetal (pseudo-aldehydic) function. Ketoses are much less reducing.
4. Except for dihydroxyacetone, all other sugars have optical activity that allows their identification using a polarimeter.
5. Sugars absorb infrared radiation but do not absorb UV or visible spectrum radiation. This is why they usually appear as white crystals.
6. Heat can lead to the degradation of reducing sugars. The result of sugar degradation is the formation of aromatic compounds (after sugar condensation and complex polymer formation) and browning, accompanied by the characteristic smell of caramel.

III.5.2. Chemical properties

III.5.2.1. Oxidation

a) Mild oxidation

Iodine (I_2) or bromine (Br_2) in a slightly alkaline and cold environment specifically oxidizes the aldehyde group of aldoses into a carboxylic acid group.

$$\text{R-CHO} + I_2 + 2OH^- \longrightarrow \text{R-COOH} + 2I^- + H_2O$$

The aldose is thus transformed into aldonic acids:

- Glucose gives **gluconic acid** (Fig. 21)

- Mannose gives **mannonic acid**
- Galactose gives **galactonic acid**

- **Ketoses are not involved in this reaction.**

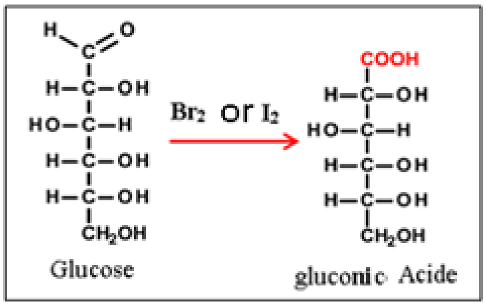


Fig. 21: Mild oxidation of glucose.

Note

Gluconic acid is unstable, undergoing internal cyclization to form an intermediate compound: Gluconolactone (Fig. 22).

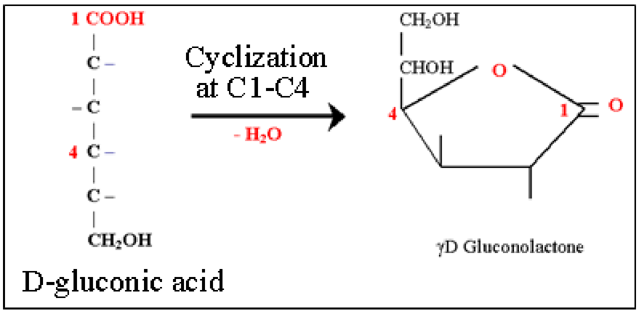


Fig.22: Transformation of glucose into gluconolactone.

b)- Oxidation by Heavy Metal Salts

During the oxidation reaction of aldoses with Fehling's solution, when heated in an alkaline medium, the aldose is oxidized to aldonic acid, reducing cupric oxide (blue) to insoluble cuprous oxide (brick red) (Fig. 23).

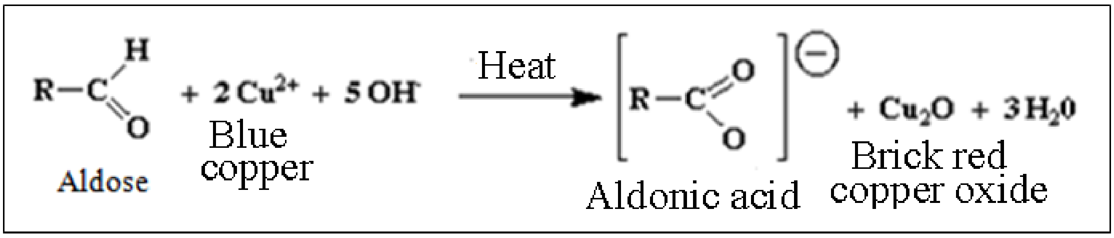


Fig. 23: Reaction of Fehling's solution

C)- Strong Oxidation

Strong oxidation with nitric acid (HNO_3) when heated yields aldaric acids, which are dicarboxylic acids possessing a carboxylic function on carbon 1 and another on carbon 6 (Fig. 24).

- Glucose yields glucaric acid.
- Galactose yields galactaric acid (mucic acid). This acid has a plane of symmetry, making it inactive on polarized light.

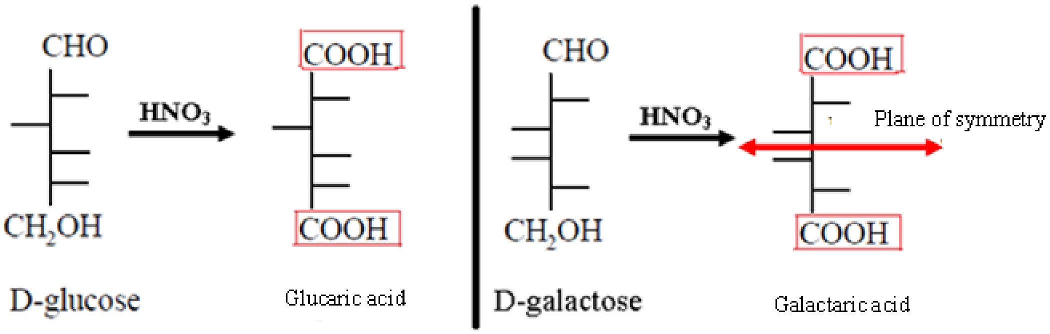


Fig.24: Strong oxidation of aldoses

Ketoses are degraded under these conditions. The chain is broken at the ketone function, resulting in the formation of a mixture of carboxylic acids (Fig. 25).

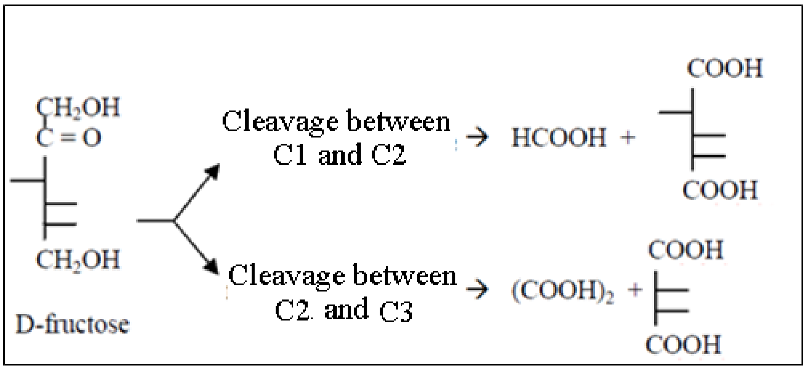


Fig. 25: Strong oxidation of ketoses

Note

If the aldehyde function is protected during oxidation, uronic acids are obtained that are oxidized only at the primary alcohol function (C6): glucose yields glucuronic acid, and galactose yields galacturonic acid (Fig. 26). These are components of glycosaminoglycans, and their biological role is essential in hepatic detoxification.

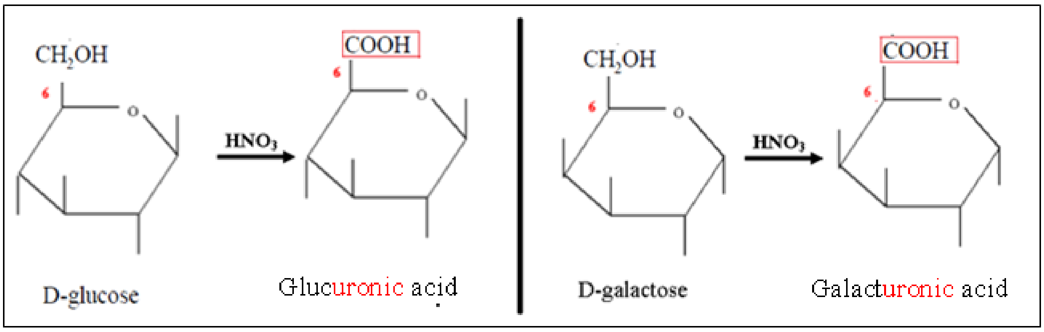


Fig. 26: Uronic acids

d)- Oxidation by Periodic Acid (HIO_4)

- **Function of Periodic Acid**

  Periodic acid can cleave the covalent bond between two carbons that have two adjacent free OH groups.

- The carbons bearing primary alcohol functions are oxidized to **formic aldehydes** (HCHO), as shown in figure 27.

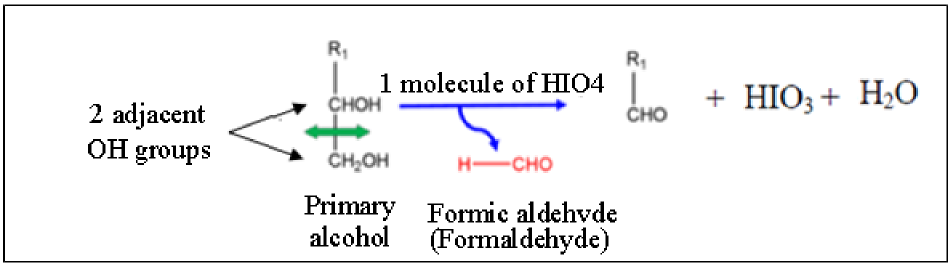


Fig. 27 : Formation of formaldehyde

- The carbons bearing secondary alcohol functions are converted into **formic acids** (HCOOH) when they undergo two oxidations (Fig. 28).

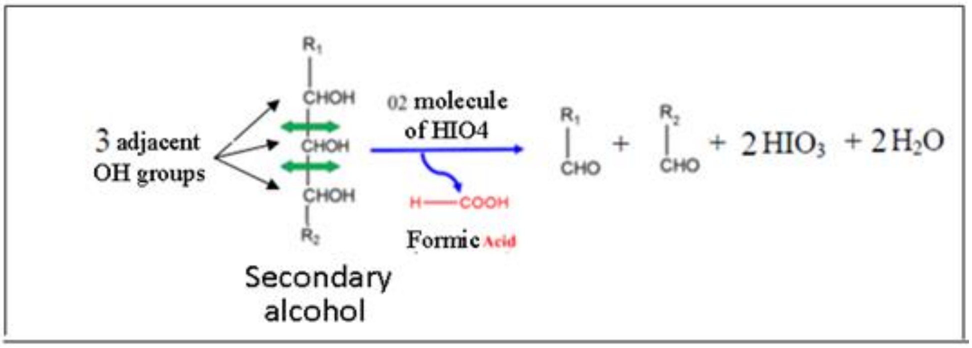


Fig. 28: Formation of formic acid

2)- Application of Periodic Oxidation to the Determination of the Cyclic Structure

Periodic acid can be used to determine the cyclic structure of sugars. Protection of the aldehyde or ketone function through methylation is necessary before using periodic acid.

- **Pyranic form**

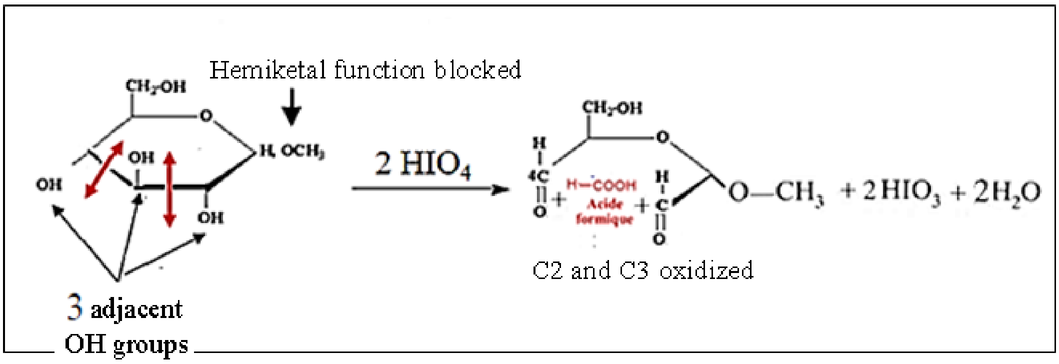


Fig. 29: Formation of formic acid from the pyranose form

- **Furan form**

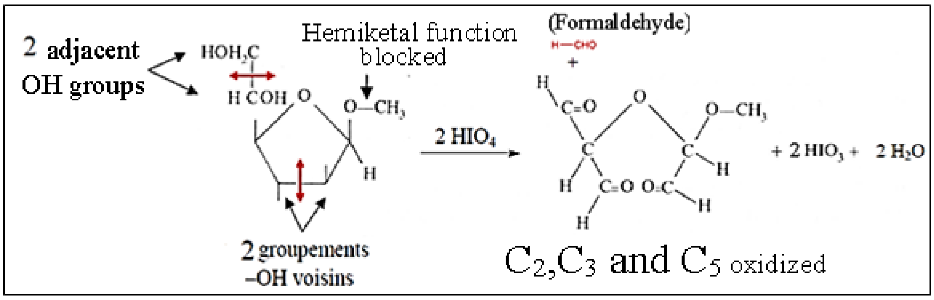


Fig. 30: Formation of formic aldehyde from the furanose form.

III.5.2.2. Reduction

Reduction reactions occur through catalytic hydrogenation using an alkaline borohydride such as $LiBH_4$ or $NaBH_4$. The aldehyde or ketone function is reduced to an alcohol, resulting in a **polyol** (Fig. 31).

- Glucose ⟶ Glucitol (or Sorbitol)
- Galactose ⟶ Galactitol (or Dulcitol)
- Mannose ⟶ Mannitol
- Ribose ⟶ Ribitol

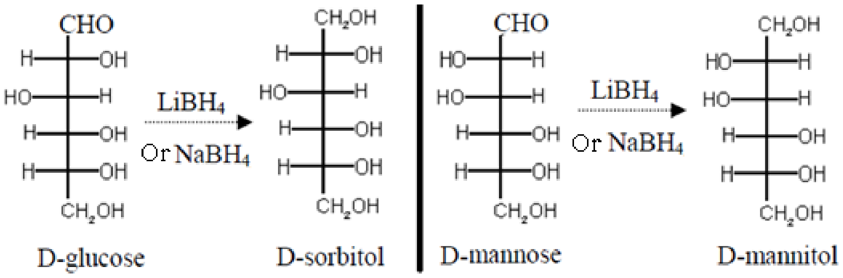


Fig. 31: Reduction by the action of an alkaline borohydride

Regarding ketoses, two polyol epimers are obtained at C_2.

- Fructose yields **two polyols** because the reduction of C=O leads to the formation of an asymmetric carbon (C*): Sorbitol (OH on C_2 is on the right) and Mannitol (OH on C_2 is on the left).

III.5.2.3. Dehydration

When heated in the presence of concentrated strong acid, sugars undergo dehydration and are transformed into furfurals or their derivatives. Pentoses dehydrate to form furfural, while hexoses form hydroxymethyl furfural. Furfurals and their derivatives can react with molecules containing phenol to form colored products characteristic of the sugar from which they derive, with the intensity allowing for quantification (Fig. 32).

- **Molish Reaction**: This reaction allows for the characterization of sugars with 5 or more carbons using α-naphthol in sulfuric acid and under heat. The product of the reaction is colored violet.
- **Bial Reaction**: This reaction allows for the characterization of pentoses using orcinol in the presence of hydrochloric acid and under heat. The product of the reaction is colored green.
- **Seliwanoff Reaction**: This reaction allows for the characterization of ketoses (which dehydrate more rapidly than aldoses) using resorcinol in the presence of hydrochloric acid and under heat. The product of the reaction is colored red.

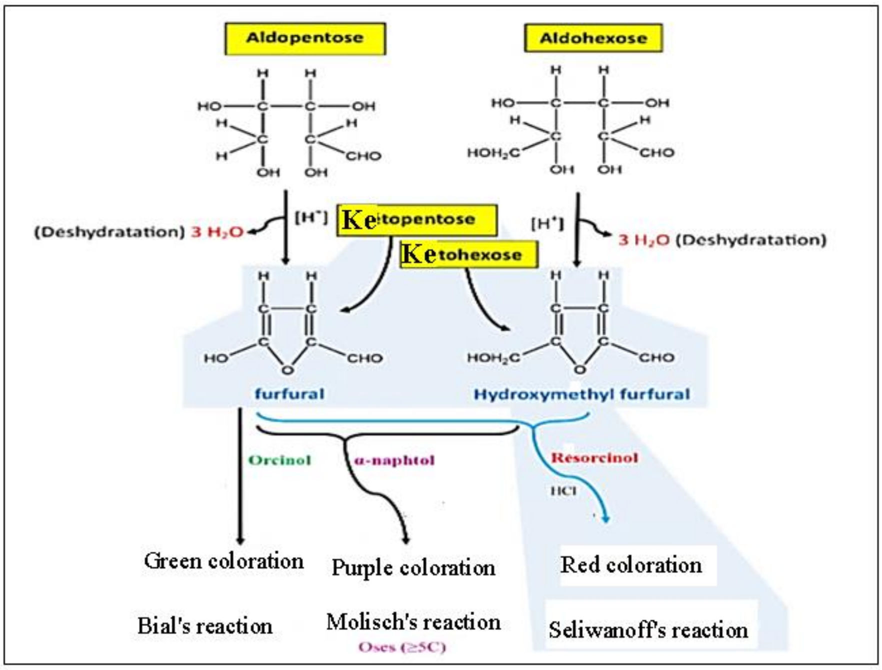


Fig. 32: Dehydration of monosaccharides

III.5.2.4. Methylation

The methylation of sugars is an etherification reaction leading to the addition of a methyl group to a hydroxyl group using methyl iodide (ICH_3) with Ag_2O or dimethyl sulfate [$SO_4(CH_3)_2$] in the presence of sodium hydroxide.

Permethylation is a reaction that allows for the methylation of all hydroxyl groups of a sugar (Fig. 33). This type of reaction can affect the anomeric carbon by forming an acetal, which has different properties compared to ethers. One of the differing properties is that acetals can be hydrolyzed in acidic conditions, leading to the release of the methyl group.

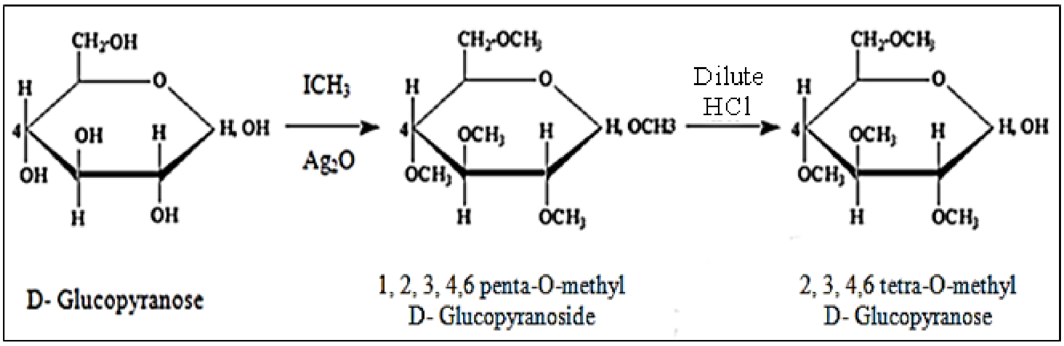


Fig. 33: Methylation of glucose

Note

Methylation is an important technique with two main applications:

a) Determination of the structure of rings.

b) Determination of the sequence in polysaccharides.

III.5.2.5. Esterification

Sugars can be esterified at the level of primary or secondary alcohols by phosphoric acid (H_3PO_4) to form phosphoric esters. Three types of esterification lead to the formation of three very important energy substrates in the cell: esterification at C_1 resulting in the formation of glucose-1-phosphate, esterification at C_6 yielding glucose-6-phosphate, or a bi-esterification at C_1 and C_6 leading to the formation of glucose-1,6-bisphosphate (Fig. 34).

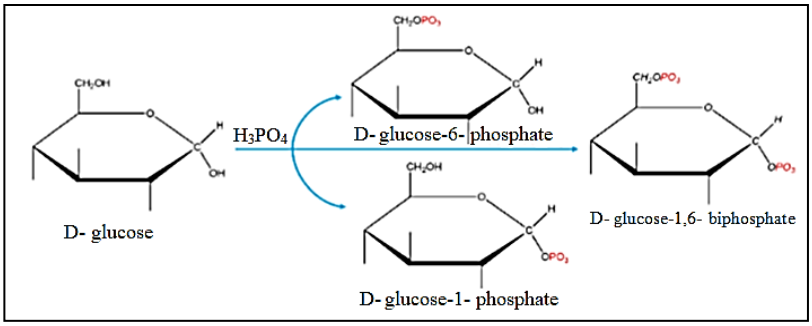


Fig. 34: Esterification of glucose.

III.5.2.6. Action of Phenylhydrazine

The sugar can react with a molecule of phenylhydrazine at cold temperatures, leading to the formation of a phenylhydrazone. When heated, the sugar can react with two molecules of phenylhydrazine to form a molecule of osazone (Fig. 35).

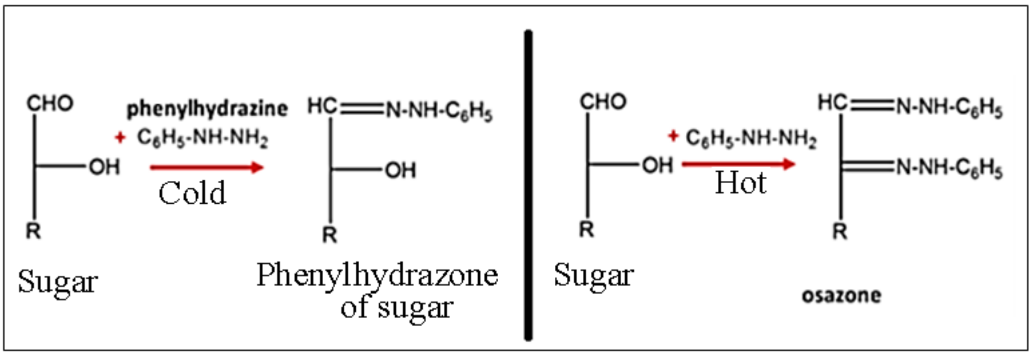


Fig. 35: Action of phenylhydrazine on sugars.

III.6. Biological Sugar Derivatives

III.6.1. Deoxy Sugars

These are molecules that correspond to a sugar in which a hydroxyl group is replaced by a hydrogen atom (often at positions 2 or 6).

- **Deoxyribose (D-2-deoxyribose)**: present in all cells as a component of deoxyribonucleotides, which are monomers of DNA.
- **6-deoxyhexoses**, also known as methyl-pentoses.
- **Fucose (6-deoxy-L-galactose)** found in polysaccharides from the cells of insects, mammals, and plants.
- **Rhamnose (6-deoxy-L-mannose)** present in plants as an heteroside and in the outer membranes of certain bacteria.
- **Quinovose (6-deoxy-D-glucose)** found in plants and certain bacteria.
- **Di-3,6-deoxy-hexoses** that are components of bacterial cell walls.
- **Di-2',3'-deoxy-nucleotides** used for DNA sequencing.

III.6.2. Osamines

They result from the replacement of a hydroxyl group with an amine function (Fig. 36). The amine function is often acetylated [N-acetyl D-glucosamine (a component of peptidoglycan) and N-acetyl D-mannosamine-6-phosphate (a precursor of sialic acids)]. These osamines are found in many complex carbohydrates such as glycoproteins and sphingolipids.

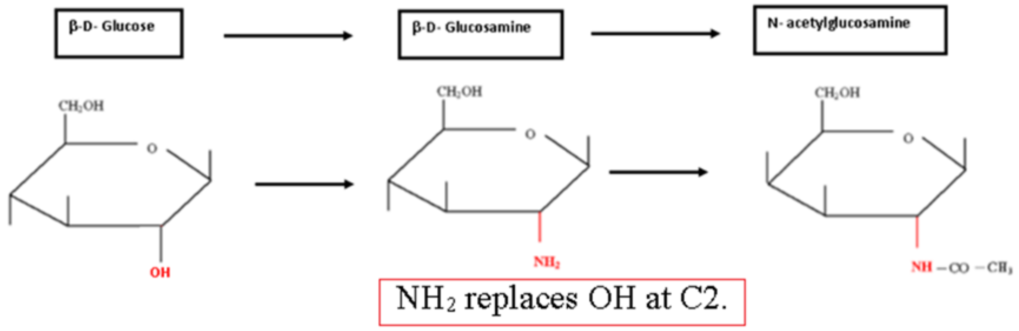
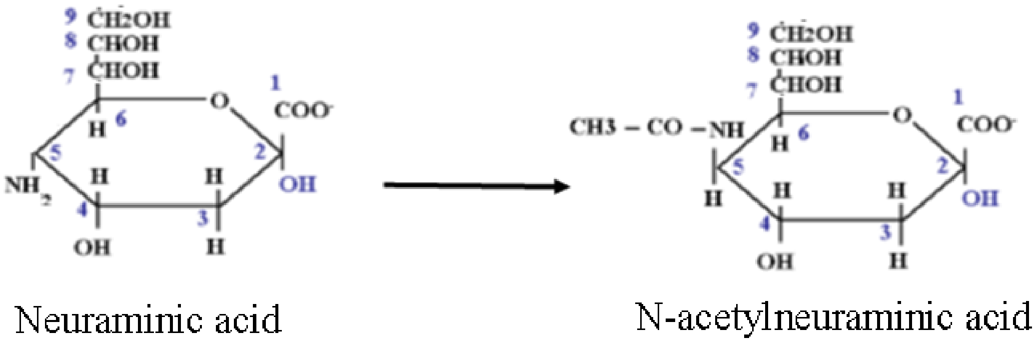


Fig. 36: Osamines

III.6.3. Sialic Acids [N-Acetylneuraminic Acid (NANA)]

The sialic acid family includes several molecules all derived from neuraminic acid (Fig. 37). The difference between the molecules in this family mainly lies in the number and position of acetylations. The most well-known is N-acetylneuraminic acid (NANA), formed by the condensation of pyruvic acid with D-mannosamine. These are components of glycoproteins and glycolipids in the membranes of living cells (primarily animals, but also to a lesser extent in plants, bacteria, and fungi).



Neuraminic acid N-acetylneuraminic acid

Fig. 37: Sialic acids

III.6.4. L-Ascorbic Acid (Vitamin C)

Vitamin C is a lactone derived from 2-keto-L-gulonic acid (Fig. 38). Its structure is characterized by the presence of the enediol function (two OH groups attached to two neighboring carbons linked by a double bond). Its reducing power allows it to participate in cellular redox reactions. It serves as a coenzyme for prolyl hydroxylase, which is involved in the synthesis of hydroxyproline. It also plays a role in steroid synthesis. A deficiency leads to abnormalities in collagen synthesis.

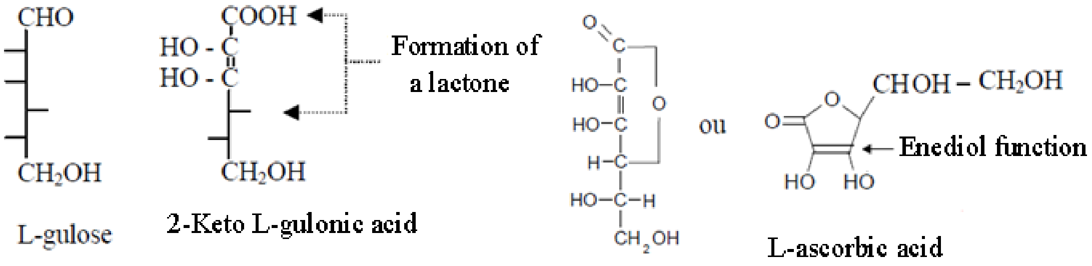


Fig. 38: L-ascorbic acid (vitamin C)**.**

IV. Osides

Osides are polymers that yield two or more monosaccharide molecules upon hydrolysis. These monosaccharides can be identical or different. We distinguish between heterosides, whose hydrolysis releases monosaccharides and non-carbohydrate compounds (aglycone = lipids, proteins, nucleic acids, etc.), and holosides, whose hydrolysis releases only monosaccharides. Holosides can be oligosides or polysides, depending on the number of monomers forming the polymer. The bond between two monosaccharide units is called a glycosidic bond.

IV.1. Glycosidic Bond or Osidic Bond

IV.1.1. Definition

A glycosidic bond is an ether bond (C-O-C) resulting from the condensation (loss of a water molecule) between the reducing hydroxyl of the anomeric carbon (C_1 for aldoses and C_2 for ketoses) and the hydroxyl of another sugar.

IV.1.2. Types of Glycosidic Bonds

Two types of bonds can form between two sugars, determining whether the resulting disaccharide is reducing or non-reducing.

IV.1.2.1. Osido-sugar Bond (Reducing Disaccharide)

One of the sugars must be engaged by the -OH group of its hemiacetal function, while the other sugar is linked by the -OH of one of its alcohol functions, either primary (C_6) or secondary (C_2, C_3, C_4), thus retaining its free hemiacetal function. The resulting disaccharide is reducing (Fig. 39. A). It exhibits mutarotation (reducing property: when the glycosidic bond does not engage the hemiacetal function of the last sugar, allowing the anomeric carbon to exist in both anomeric forms, α and β).

IV.1.2.2. Osido-Osides Linkage (Non-Reducing Disaccharide)

In this type of linkage, the disaccharide is formed by the condensation of 2 sugars connected via their hemiacetal OH groups (Fig. 39.B). The resulting disaccharide is non-reducing, and thus does not exhibit mutarotation because there is no free hemiacetal function (the anomeric carbon can only exist in a single anomeric form, either α or β).

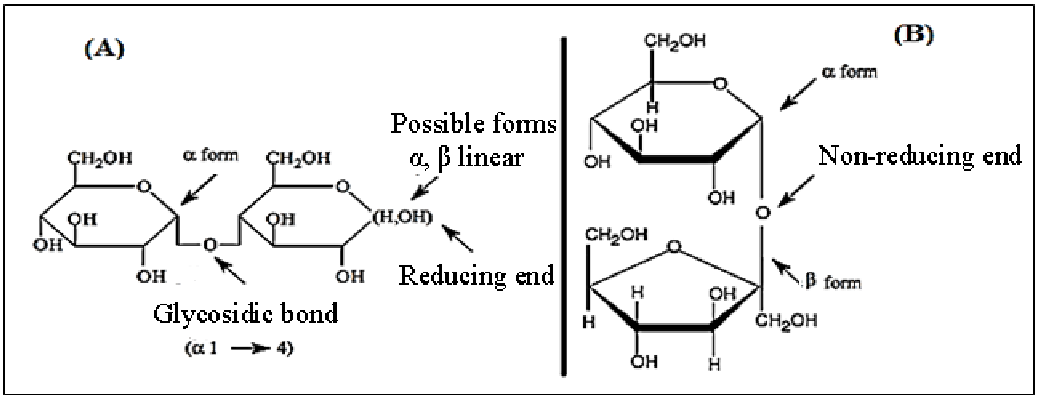


Fig. 39: Introduction to Reducing and Non-Reducing Bonding

IV.1.3. Nomenclature

The glycosidic bond is defined not only by the sugars involved, but also by the anomer of the sugar that participates with its hemiacetal function and by the number of the atom of the other sugar. Generally, the name will be:

- The 1st sugar [the series - the anomer - the name of the sugar + the cyclic form of the sugar (pyranose or furanose) + the suffix -osyl or -osido (the sugar engaging its anomeric carbon in the glycosidic bond)].
- The glycosidic bond (specify the number of the carbon involved in this bond).
- The 2nd sugar [the series - the anomer - the name of the sugar + the cyclic form of the sugar + the suffix -oside (if the sugar engages its anomeric carbon in the glycosidic bond) or the suffix -ose (if the sugar does not engage its anomeric carbon in the glycosidic bond)].

* Examples

The names of the two sugars from figure 39:

- **Structure A:** D-α-Glucopyrano**syl** (1-4) D-α-Glucopyran**ose** / D-α-Glucopyran**osido** (1-4) D-α-Glucopyran**ose** (reducing).
- **Structure B:** D-α-Glucopyrano**syl** (1-2) D-β-Fructofurano**side** / D-α-Glucopyran**osido** (1-2) D-β-Fructofurano**side** (non-reducing).

IV.1.4. Hydrolysis of Glycosidic Bonds

The hydrolysis of osides can be performed through two pathways:

IV.1.4.1. Chemical Pathway

Catalyzed by protons (H^+), hydrolysis occurs at acidic pH (0.1 M HCl) and at high temperature (60°C) for 1 hour. This hydrolysis has no specificity, and all glycosidic bonds are broken, resulting in the formation of sugar units.

IV.1.4.2. Enzymatic Pathway

The hydrolysis of glycosidic bonds is carried out by enzymatic catalysts called hydrolases, which are specific to glycosidic bonds (glycosidases). The specificity of these enzymes means that glycosidases can only act on a single substrate, such as α or β-glycosidases (e.g., α-glucosidases / β-galactosidases, etc.).

IV.1.5. Methods for Studying Glycosidic Bonds

When studying an oside, it is essential to first determine the nature of its constituent sugars. To do this, osides are hydrolyzed in an acidic medium to break all glycosidic bonds, and then the sugars are separated using chromatographic techniques, identified, and quantified individually. In the case of heterosides, it is also necessary to determine the nature of the aglycone. Finally, the type of bond between the constituent sugars must be established. Several analytical methods can be used for this purpose:

IV.1.5.1. Methylation of Free Hydroxyls

In this experiment, a methylating agent such as ICH_3 is reacted with an oside (only the free –OH groups will form a methoxy ether), and then the experiment is continued with a controlled acid hydrolysis to break the glycosidic bonds (Fig. 40). The resulting products are then separated, identified, and quantified, which allows for the deduction of the position of the –OH groups involved in the glycosidic bond(s).

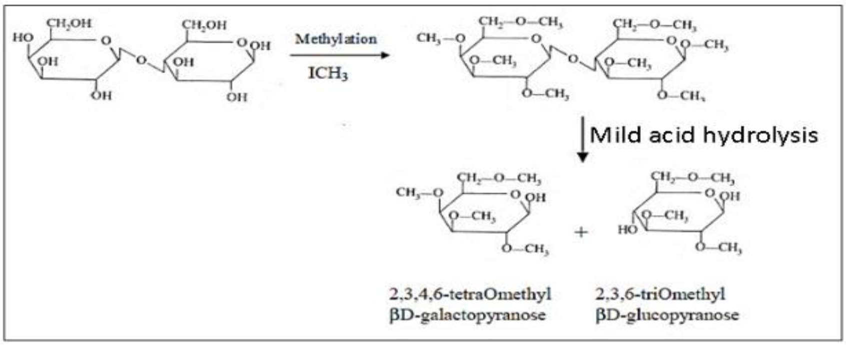


Fig. 40: Methylation method.

IV.1.5.2. Oxidation by Periodic Acid

When a hydroxyl group is involved in a glycosidic bond, it is no longer free and cannot be oxidized by periodic acid. This modifies the periodic oxidation sites on the molecule compared to the results obtained for the same sugar in its free form.

IV.1.5.3. Action of Osidases

Osidases exhibit stereospecificity concerning the α or β position.

IV. 2. Types of Osides

Osides are distinguished into Holosides and Hétérosides.

IV.2.1. Holosides

They are divided into two types according to their size: oligosides and polyosides.

IV.2.1.1. Oligosides

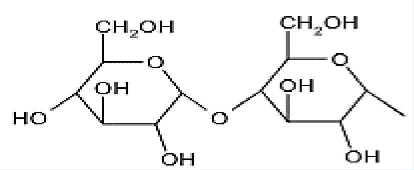
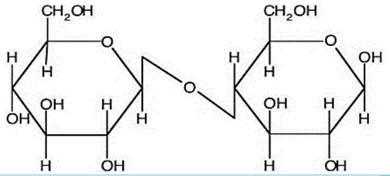
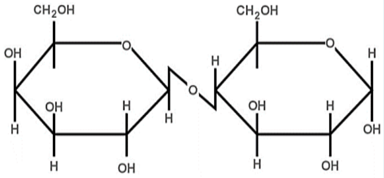
Oligosides are holosides that result from the condensation of 2 to 10 sugar molecules.

a) **Diholosides**

Diholosides are two sugars linked together by a glycosidic bond. Depending on the type of linkage between the two sugars, the diholoside can be non-reducing or reducing.

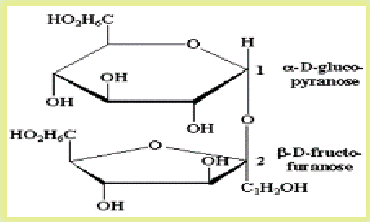
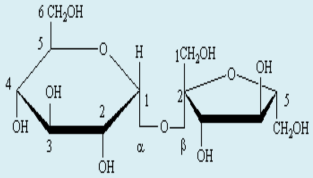
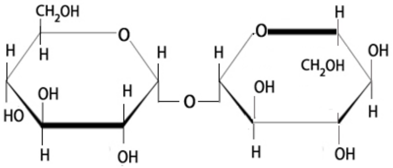
Reducing Diholosides (Glycosidic Bond between Sugars): There is condensation of a hemiacetal function of one sugar with an alcoholic function of a second sugar via a glycosidic bond. Therefore, the diholoside retains a free hemiacetal -OH group responsible for the reducing power of the molecule (which exists in two anomeric forms). The main reducing diholosides are presented in Table II.

Table II: Main reducing diholosides

Common name	Official nomenclature	Structure
Maltose	α-D-glucopyranosyl (1-4) D-glucopyranose	
Cellobiose	ß-D-glucopyranosyl (1-4) D-glucopyranose	
Lactose (milk sugar)	ß-D-galactopyranosyl (1-4) D glucopyranose	

- Non-reducing disaccharides (glycosidic bond): The hemiacetal function of each sugar is condensed through a glycosidic bond. The main non-reducing disaccharides are shown in Table III.

Table III : Main non-reducing diholosides

Common name	Official nomenclature	Structure
Sucrose	α-D-glucopyranosyl (1-2) ß-D- fructofuranoside	 
Trehalose	-D-glucopyranosyl (1-1) α-D-glucopyranoside	

b)- Other Oligosaccharides

There are a few oligosaccharides of biological interest:

1. **Triholosides**

   Triholosides are derived from sucrose.

   - **Gentianose** (found in the gentian plant)

     β-D-glucopyranosyl-(1,6)-α-D-glucopyranosyl-(1,2)-β-D-fructofuranoside
   - **Raffinose** (found in beets)

     α-D-galactopyranosyl-(1,6)-α-D-glucopyranosyl-(1,2)-β-D-fructofuranoside
2. **α-Galactosides**

   α-Galactosides are reserve oligosaccharides in plants, consisting of one or more α-D-galactopyranosyl residues linked by α(1-6) to the glucose residue of sucrose (Fig. 41). The carbohydrates in the seeds and flours of legumes (peas, beans, soybeans) all contain α-galactosides in varying proportions. These substances are indigestible for humans. These carbohydrates are thermostable and are not degraded by the digestive enzymes of higher animals (which lack α-galactosidase). As a result, these carbohydrates are not absorbed in the small intestine, and when they reach the colon, they are fermented by microorganisms, which can lead to intestinal disorders (flatulence and diarrhea).

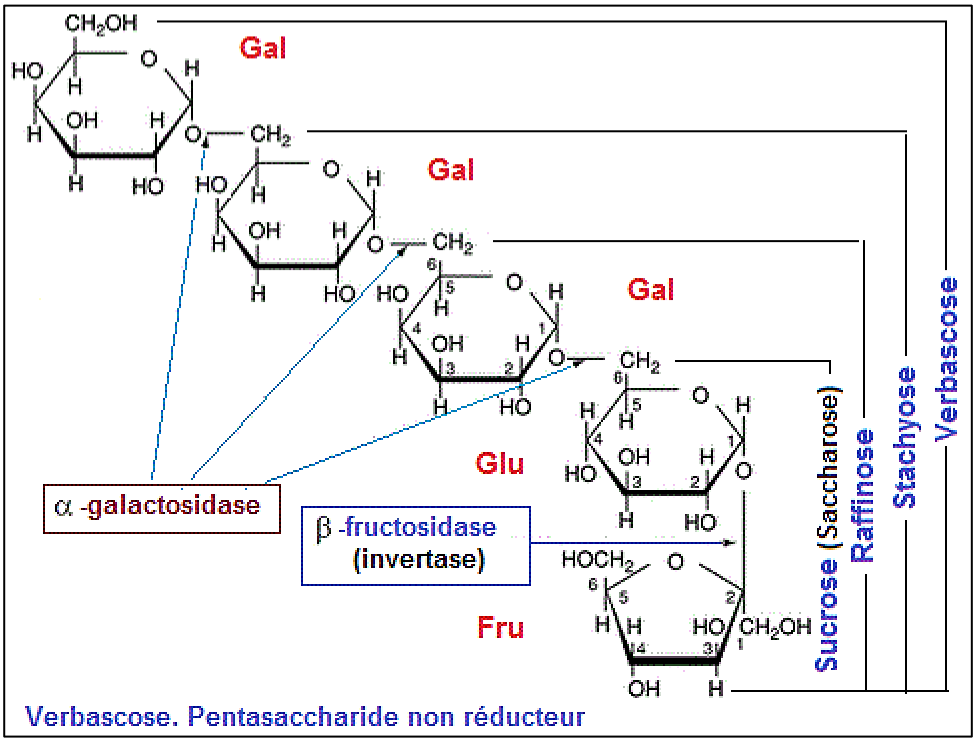


Fig. 41: α-galactosides

IV.2.1.2. Polyholosides

These are polymers with very high molar mass, resulting from the condensation of a large number of sugar molecules. In nature, they serve two main functions:

- They act as a form of carbohydrate energy storage (e.g., starch, glycogen).
- They play a structural role in certain cells (e.g., cellulose, chitin).

a) **Homopolyosides**

Homogeneous polysaccharides are composed of a single type of sugar or sugar derivative (Table IV).

Table IV : Homogeneous polysaccharides

Name and role	Structure	Degrading enzymes
Starch **(Plant reserve polysaccharide)**	Starch is composed of two polysaccharides: ➢ **Amylose:** Amylose accounts for 15% to 30% of the starch mass. It is a linear polymer of glucose residues (ranging from 500 to 20,000 residues, with a molar mass of 10^5 to 10^6 Da) linked by α-(1,4)-D-glucosidic bonds. The branching occurs through α-(1,6)-D-glucosidic bonds, which make up only about 1%. ➢ **Amylopectin:** Amylopectin accounts for 70% to 85% of the starch mass. It differs from amylose in that it is a branched polymer.	**1)- Amylases:** There are two types: **a- α-amylases (endoamylase)** cut the α-1,4 bonds inside the chains by forming small oligosides (3 to 8 residues) that can contain 1 or 2 branch points since they do not cut the α-1,6 bonds. **b- β-amylases (exoamylase)** cut every other bond from the non-reducing end, thus releasing maltosyl units. During hydrolysis, they cause an inversion on C1 of maltose and it is the β form that is released. 2) **Debranching enzymes:** they cut the α-1,6 bonds of the branch points.
Glycogen **(Reserve polysaccharide in animals)**	**Glycogen** is very similar to amylopectin: it consists of glucose chains linked by α-(1,4) bonds with branches formed by α-(1,6) bonds. However, the chains in glycogen are much shorter, and the glycogen molecule is more densely packed, with a molecular mass that can reach up to 100 million Da.	**1. α and β amylases** **2. Debranching enzyme** **3. Phosphorylases:** they attack the chains from the non-reducing ends, through phosphorolysis of α-1,4 bonds, releasing D-glucose-1-phosphate.
Cellulose **(Support polysaccharide (cell walls of plant cells)**	It consists of long linear chains of glucose linked by β-(1,4) D-glucosidic bonds. The repeating unit is cellobiose.	- Humans are unable to digest cellulose because they lack enzymes that act on β-glucosidic bonds. Its hydrolysis by acid produces cellobiose.
Dextrans **(Reserve polysaccharide in bacteria and yeast.)**	These polysaccharides are made up of α-glucose units (1-6) with occasional branching at C3 or C4.	These bonds are non-hydrolyzable in the human body.
Inulin **(family of fructans)** **Reserve polysaccharide in some plants**	It is a polymer of fructose residues (30 to 100 residues) linked by glycosidic bonds (β2-1). [β−D−fructofuranosyl(2−1)]n[β-D-fructofuranosyl (2-1)]_n[β−D−fructofuranosyl(2−1)]n	Inulin is not digested by intestinal enzymes. It reaches the colon, where it is utilized by the gut flora, which metabolizes it, releasing significant amounts of carbon dioxide, hydrogen, and/or methane.
Arabans **Responsible for gel formation.**	Formed from α-arabinofuranose units linked by 1-5 bonds, they are found in pectins, which are present in fruits."	Cytases are enzymes found in the seeds of certain plants, particularly cereals.".
Xylans **They play a bridging role between cellulose fibers.**	Formed from units of xylopyranose, they are found in the lignified tissues of certain plants.	Cytases
Chitin **A supportive polysaccharide found in the exoskeleton of invertebrates (crustaceans, insects)."**	It differs from cellulose by the C2 of glucose, where the hydroxyl group is replaced by an acetamide group. It is a polymer of N-acetyl-D-glucosamine residues linked by glycosidic bonds (β2-1). → [N-acetyl-β-D-fructofuranosyl (2-1)]n.	Chitinases

b)- Heterogeneous Polysaccharides

Heterogeneous polysaccharides (or mixed or heteroglycans) are formed from several types of sugars or sugar derivatives (often limited to two types):

- **Gums**: The hydrophilic part of the secretions from 'gum trees,' such as acacias, are highly branched galactoan arabans.
- **Agar-agar** or gelatin: Extracted from red algae and widely used in microbiology for gel cultures, it is a complex polysaccharide of irregularly sulfated D- and L-galactose. From these algae, we also extract:
    - **Carrageenans**: Extracted from algae used as thickeners and gelling agents in the food industry, they are linear polymers of sulfated galactose disaccharide units (carrabiose) linked by a (β1 → 4) bond.
    - **Alginates**: Linear polyuronides made of two uronic acids, β-D-mannuronic acid and α-L-guluronic acid, linked by a (β1 → 4) bond.

IV.2.2. Heterosides

This term encompasses molecules resulting from the covalent association of carbohydrates with other types of molecules, often referred to as glyconjugates:

- **Lipids**: The membrane lipids of animal or bacterial cells carry oligo- or polysaccharide chains; these are called glycolipids.
- **Proteins**: Regarding the association of proteins with carbohydrates, we distinguish:
    - **Proteoglycans**: Long polysaccharides (glycosaminoglycans or GAGs) associated with a protein, where the carbohydrate component remains predominant (> 90%).
    - **Glycoproteins**: These are proteins to which short carbohydrate chains are attached.
    - **Peptidoglycans**: Networks of polysaccharides linked by numerous small peptides.
    - **Glycated proteins**: Products of the chemical attachment of a glucose unit to hemoglobin (a metalloprotein). Insulin-dependent hyperglycemia promotes the binding of this sugar to plasma proteins (a marker of diabetes).
- **Nucleic Acids**: These are N-heterosides. The carbohydrate component is either D-ribose or deoxy-D-ribose. The aglycone is a base from the pyrimidine or purine group. They are of great importance in biology [(ribonucliec acids (RNA) and deoxyribonucleic acids (DNA)].

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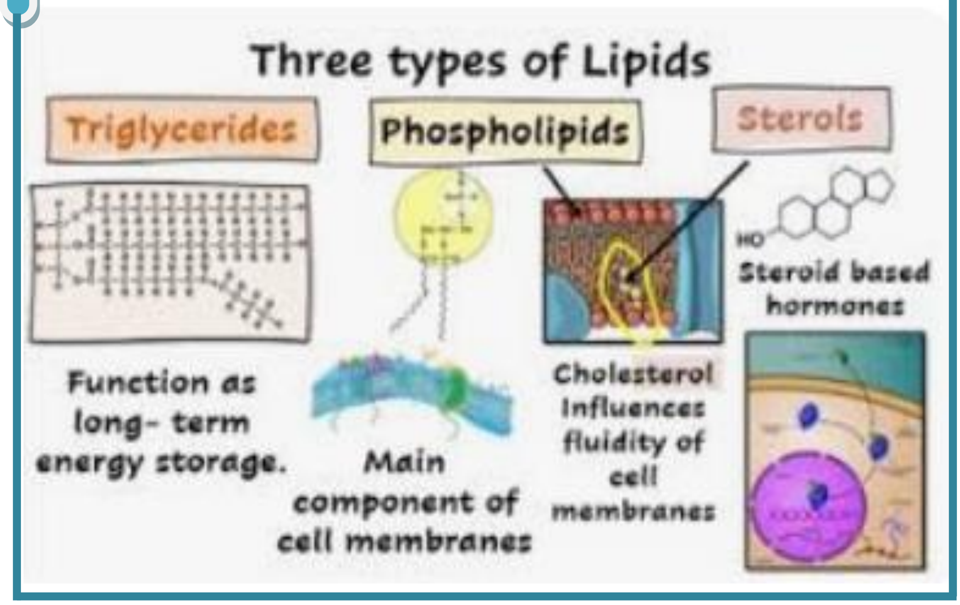
CHAPTRE III : LIPIDS



I. Definition and Biomedical Importance

Lipids form a **heterogeneous** group of compounds that includes fats, oils, steroids, and waxes. They are more closely related by their physical properties than by their chemical ones. Their key characteristic is being relatively **insoluble** in water but **soluble** in nonpolar organic solvents (such as benzene, ether, cyclohexane, isobutanol/ethanol, chloroform, etc.).

Lipids are important components of the diet, not only because of their **high energy value** but also due to the **fat-soluble vitamins** and essential fatty acids found in the fats of natural foods.

Combinations of **lipids and proteins (lipoproteins)** are involved in transporting lipids in the bloodstream. Similarly, **lipid-carbohydrate** combinations **(glycolipids)** are important and widely distributed, expressed on cell membranes, and play key roles in cell recognition and signaling. For instance, the glycolipids present on red blood cell membranes form the antigens that determine blood groups.

Understanding lipid biochemistry is crucial for several biomedical fields, including obesity, diabetes, atherosclerosis, and the study of polyunsaturated fatty acids in nutrition and health.

II. Classification

The most commonly used classification is as follows:

- **True lipids**: These result from the condensation of fatty acids (FAs) with alcohols through an ester or amide bond and are subdivided into:
    - **Simple lipids**: Composed of three elements (C, H, O), they are neutral and classified according to the alcohol that esterifies the fatty acid:
        - **Glycerolipids**: The alcohol is glycerol.
        - **Cerides**: The alcohols are long-chain (fatty) alcohols.
        - **Sterides**: The alcohol is a sterol (polycyclic).
    - **Complex lipids**: In addition to the elements in simple lipids, they contain phosphorus, nitrogen, sulfur, or sugars.
        - **Phospholipids**: Lipids containing, in addition to FAs and alcohol, a phosphoric acid residue, e.g., glycerophospholipids and sphingophospholipids.
        - **Glycolipids**: Lipids containing, in addition to FAs and alcohol, a sugar.
- **Lipid-like compounds (lipoids)**
    - **Isoprenoids**: Derivatives of isoprene units, including the terpene compounds group and sterol derivatives.

- **Eicosanoids**: Mediators derived from fatty acids.

- **Associations of lipids and conjugated lipids**: These lipids participate in supramolecular non-covalent complexes that include proteins. In some cases, proteins may have a covalently bound lipid fraction.

III. Fatty Acids

III.1. Definition of fatty acids

Fatty acids (FAs) are organic carboxylic acids with the general formula R-COOH, where the R group is an aliphatic hydrocarbon chain. Fatty acids contain an **even** number of carbon atoms, typically ranging from 4 to 32, which imparts their hydrophobic (fatty) nature. The linear chain of these carbon atoms can be either **saturated** (absence of double bonds) or **unsaturated** (presence of one or more double bonds).

III.2. Types of Fatty Acids

III.2.1. Saturated Fatty Acids (SFAs)

Fatty acids can be synthesized from acetic acid (CH_3-COOH), which is considered the first member of a series where –CH_2– units are progressively added between the terminal CH_3– and –COOH groups (Fig. 1). They follow the general formula $\mathbf{C_nH_{2n}O_2}$ or CH_3-$(CH_2)_n$-COOH.

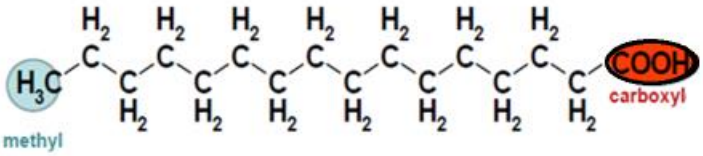


Fig. 1: Structure of Saturated Fatty Acid

The most important fatty acids are listed in Table I. The symbol for saturated fatty acids is $\mathbf{C_n:0}$ (0 indicates that the chain is saturated), and the **common name** refers to its origin. The **systematic name** of a saturated fatty acid is determined as follows: **n-[nC]** anoic acid

- **n**: Linear characteristic
- **[nC]**: Chain length (number of carbons)
- **ano**: The chain is saturated
- **ic**: Suffix designating the carboxylic acid function.

For higher plants and animals, the most common fatty acids have between 14 and 20 carbons, with a clear predominance of those with **16 or 18** carbons. Fatty acids with fewer than 12 carbons are found in mammalian milk and, of course, in butter. Fatty acids with more than 24 carbons are primarily components of protective waxes produced by plants, bacteria, and insects.

Table I: Saturated fatty acids.

Relative Length	Nc	Systematic Name	Common Name	Origin
Court Chain	4	n- butanoic acid	Butyric	Butter, Goat milk
	6	n- hexanoic acid	Caproic	
	8	n- octanoic acid	Caprylic	
	10	n- decanoic acid	Capric	
Medium Chain	12	n- dodecanoic acid	Lauric	Oil, Animal and vegetable fats
	14	n- tetradecanoic acid	Myristic	
	16	n- hexadecanoic acid	Palmitic	
	18	n- octadecanoic acid	Stearic	
Long Chain	20	n- icosanoic acid	Arachidic	Seeds
	22	n- docosanoic acid	Behenic	
	24	n- tétracosanoic acid	Lignoceric	
	26	n- hexacosanoic acid	Cerotic	Plant waxes, Bacteria, Insects
	28	n- octacosanoic acid	Montanic	
	30	n- triacontanoic acid	Melissic	
	32	n- dotriacontanoic acid	Laceroic	

III.2.2. Unsaturated fatty acids

They represent more than half of the fatty acids in plants and animals. They possess one double bond: **monounsaturated acids (monoenic)**, or multiple double bonds: they are **polyunsaturated acids (polyenic)**. They conform to the general formula $C_nH_{2n-2x}O_2$ (where **x** is the number of double bonds) or $CH_3\text{-}(CH_2)_x\text{-}CH{=}CH\text{-}(CH_2)_y\text{-}COOH$. Most unsaturated fatty acids have chain lengths of 16 to 20 carbons. Generally, multiple double bonds **are not conjugated** but separated by a **methylene** group, and the double bonds are in the ***cis*** configuration (Fig. 2).

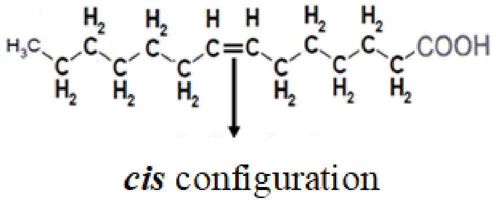


Fig. 2: *Cis* Configuration of the double bonds

Mammals require polyunsaturated fatty acids, but the majority cannot be synthesized. These are referred to as **essential fatty acids** and must be included in our diet. In unsaturated fatty acids, two numbering systems coexist: one **systematic** and the other used in **dietetics**, which groups unsaturated fatty acids into series.

- **Systematic numbering:** The position of the first double bond is expressed starting from the carboxyl group (the **first** carbon), and the symbol used is delta: Δ (Fig. 3). The nomenclature is **Cn : m Δ $^{(p, p',..)}$.**

  - **Cn** : number of carbons
  - **m** Δ : number of double bonds
  - **(p, p',…)** : positions of the double bonds in standard numbering.

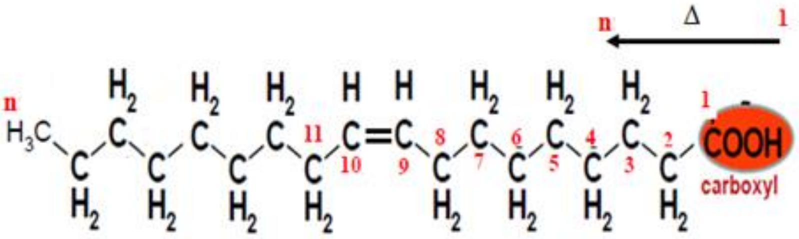


Fig. 3: Systematic numbering of unsaturated fatty acids

- **Numbering used in dietetics:** The position of the double bond is expressed starting from the methyl group (the first carbon). The symbol is in the form ω **n**, where **n** is the position of the first double bond (Fig. 4). There are four main **series**: ω 3, ω 6, ω 7, and ω9 (along with other secondary series, such as ω 4 and ω 5.

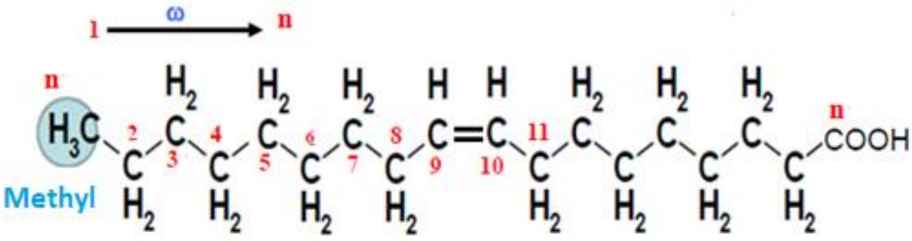


Fig. 4: Numbering used in dietetics for unsaturated fatty acids.

The most important unsaturated fatty acids are listed in Table II. The common name refers to its origin. **The systematic** name of the unsaturated fatty acid is determined as follows: Cis-**x-[nC]-z- enoic**

- **x**: Position of the double bonds.
- **[nC]**: Chain length (number of carbons).
- Z: Number of double bonds.
- **eno**: The chain is unsaturated.
- **ic**: Suffix designating the carboxylic acid function.

Table II : Unsaturated fatty acids

nC	Systematic Name	Common Name	Symbol		Origin
			Δ	ω	
16	cis-9- hexadecenoic	Palmitoleic	$C16:1\ \Delta^{9}$	$C16:1\ \omega^{7}$	Very widespread.
18	cis-9- octadecenoic	Oleic	$C18:1\ \Delta^{9}$	$C18:1\ \omega^{9}$	
	cis-9-12-octadecadienoic	Linoleic	$C18:2\ \Delta^{9,12}$	$C18:2\ \omega^{6,9}$	Seeds
	cis-9-12-15-octadecatrienoic	Linolenic	$C18:3\ \Delta^{9,12,15}$	$C18:3\ \omega^{3,6,9}$	
20	cis-5-8-11-14-icosatetraenoic	Arachidonic	$C20:4\Delta^{5,8,11,14}$	$C20:4\omega^{6,9,12,15}$	Animals
	cis-5-8-11-14-17- icosapentaenoic	Eicosapentaenoic	$C20:5\Delta^{5,8,11,14,17}$	$C20:5\omega^{3,6,9,12,15}$	Fish oils
24	cis-15-tetracosenoic	Nervonic	$C24:1\ \Delta^{15}$	$C24:1\ \omega^{9}$	Brain

The essential fatty acids for the body are: **linoleic acid** (referred to as essential because it is the precursor of fatty acids in the omega-6 family) and **linolenic acid**. It is linoleic acid that gives rise to arachidonic acid in the body. In the absence of linoleic acid in the diet, **arachidonic acid** becomes **essential**.

III.2.3. Atypical fatty acids

Fatty acids with an **odd** number of carbons are found in animal fats or in microbial lipids. There are also fatty acids with modifications to the carbon chain involving unsaturation or that have undergone substitutions or cyclizations in the plant, microbial, or animal kingdoms. Here are a few examples:

a)- Acid with an odd number of carbon atoms

Undecylenic acid is a monounsaturated fatty acid with the molecular formula $C_{11}H_{20}O_2$. It is a colorless oil primarily used for treating fungal skin infections, but it is also a precursor in the manufacture of many pharmaceuticals, personal care products, cosmetics, and fragrances.

b)- Particular Unsaturations

- ***Trans* configuration:** Very rare, it is found in bacteria of the rumen microflora in the stomachs of ruminants, in trans-vaccenic acid ($\mathbf{C_{18}:1\ \Delta^{11}}$), the trans isomer of oleic acid (elaidic acid).
- **Abnormal positions:** The monounsaturated acid $\mathbf{C_{22}:1\ \Delta^{13}}$ (erucic acid) from rapeseed.
- **Conjugated double bonds:** These exist in plant fatty acids, particularly in highly unsaturated acetylenic derivatives; certain plants produce fatty acids with conjugated triple bonds, such as erythrogenic acid $\mathbf{C_{18}:3\ \Delta^{9,11,13}}$.

c)- Substitutions

- **Hydroxylation:** These substitutions are present in the fatty acids of the brain (cerebronic acid **C_{24}:0**), in certain microbes, and in vegetable oils or waxes. Castor seeds contain a hydroxyl group at position **C_2.** The carbon bearing the hydroxyl group then becomes an asymmetric carbon.
- **Branching:** This often occurs through methylation. The fat that ducks coat their feathers with contains fatty acids with **C_{10}** or **C_{11}** that are tetramethylated at positions 2, 4, 6, and 8. The highly resistant waxy walls of mycobacteria consist of polymethylated fatty acids (mycoceric acid from Koch's bacillus: **C_{28}** tetramethylated at positions 2, 4, 6, and 8).

d)- Cyclizations

Prostaglandins (PG), thromboxanes (TX), and leukotrienes are biological mediators; they are cyclopentenoid fatty acids of the eicosanoid family (C20), as shown in figure 5, and are derived from arachidonic acid. There are three classes of prostaglandins (1, 2, and 3) based on the number of double bonds outside the cycle.

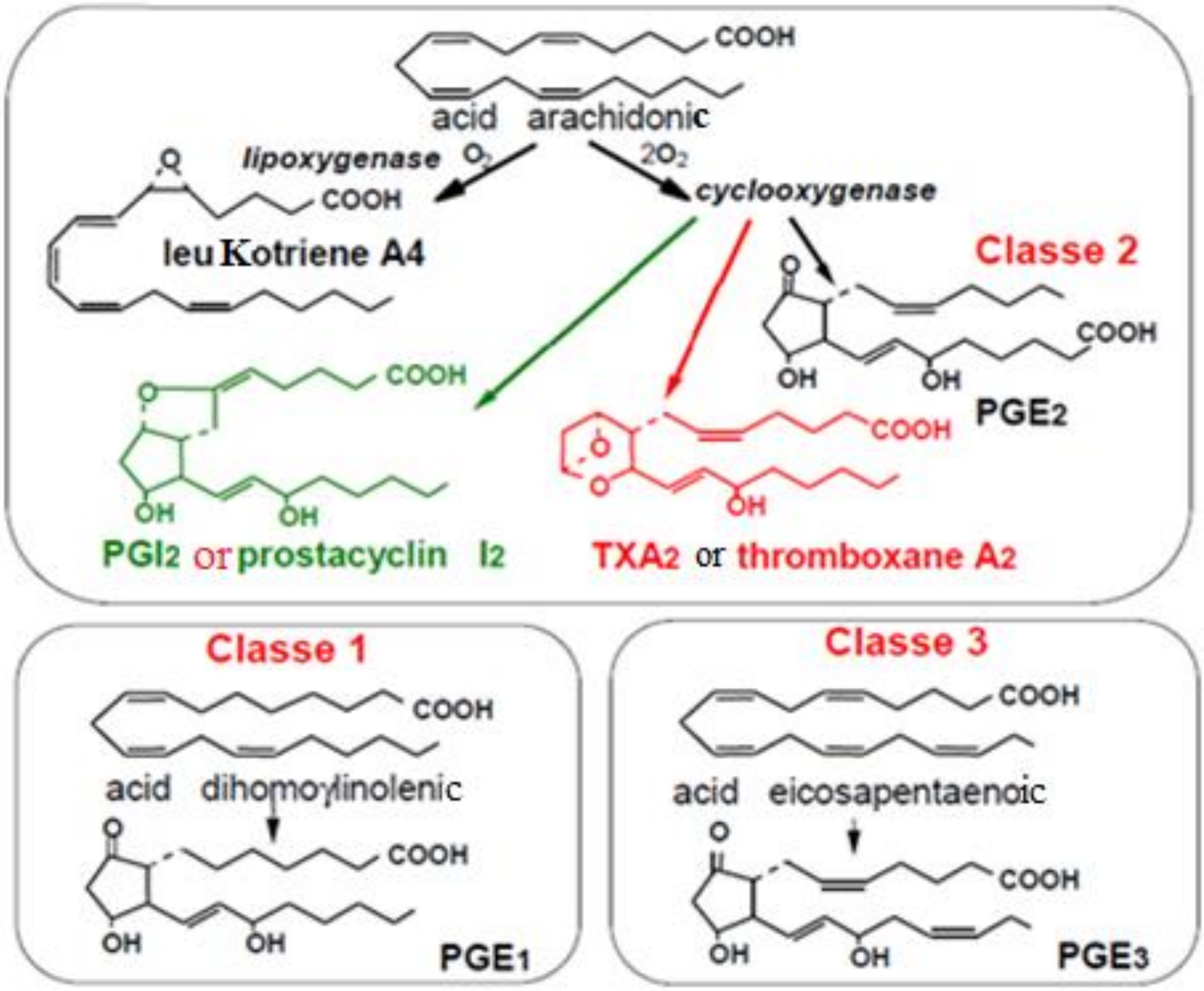


Fig.5 : Cyclic fatty acids

III.3. Physicochemical properties of fatty acids

III.3.1. Physical properties

III.3.1.1. Solubility

Fatty acids are **amphiphilic or amphipathic** compounds, meaning they have dual polarity: hydrophobicity due to the carbon chain (-R) and hydrophilicity due to the carboxyl group (-COOH). Negatively charged carboxyl groups are in contact with the aqueous phase, while the non-polar hydrocarbon chains are oriented inward, resulting in the formation of a micelle (Fig. 6).

The hydrophobicity of the aliphatic chain increases with the number of carbons; only short-chain fatty acids (C_4, C_6) are soluble in water. Fatty acids are soluble in non-polar organic solvents such as benzene, chloroform, etc. Double bonds decrease the non-polar character.

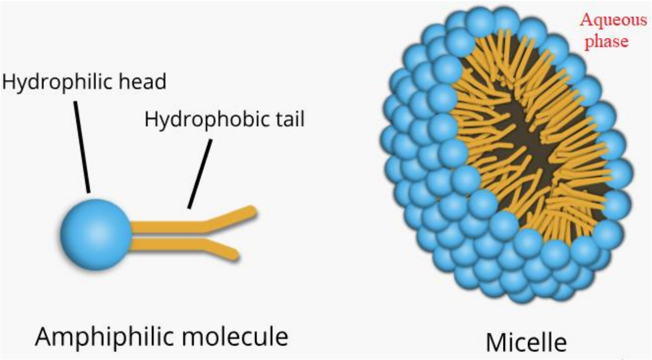


Fig. 6: Position of the carboxyl group and the carbon chain of a fatty acid (FA).

III.3.1.2. Melting Point

The melting point is the temperature at which a fatty acid exists in a **liquid** state. It varies according to two parameters: the **number of carbons** (C) and the **degree of unsaturation** (number of double bonds).

- The **higher** the number of **carbons**, the **higher** the melting point.
- The melting point **decreases** with an increasing **degree of unsaturation.**

For example:

- Stearic acid (C_{18}:0) has a melting point of +69.60 °C.
- Oleic acid (C_{18}:1 Δ^9) has a melting point of +16 °C.

III.3.1.3. Density

The density of fatty acids is low, causing oil to float on water.

III.3.2. Chemical Properties

These properties depend on the presence of the -COOH group and the possible presence of double bonds.

III.3.2.1. Properties Due to the Presence of -COOH

a)- Neutralization by Bases (Saponification)

A fatty acid subjected to the action of a base (NaOH or KOH) produces an **alkaline salt of the fatty acid** (soap).

$$\text{CH}_3\text{--------------COO}^- + \text{NaOH} \longrightarrow \text{CH}_3\text{----------- COONa} + \text{H}_2\text{O}$$

Soaps are fully ionizable due to the carboxylic function. They dissociate into Na^+ + $R\text{-}COO^-$. These molecules, referred to as amphiphiles or amphipathic, lower the surface tension of water, which accounts for their properties.

- **Saponification Index (SI):** It is the amount in **mg** of **KOH** required to saponify **1 g** of fat. It allows for the determination of the **molecular weight** (MW) of the fat.

$$S_I\ (\text{mg}) \longrightarrow 1\text{g (fat)}$$

$$\textbf{MW}\ \text{of KOH (56 g/ mol)} \longrightarrow \text{MW (fat)}$$

b)-Esterification

It explains the formation of more complex lipids. There are enzymes that facilitate this reaction.

$$\text{R-COOH} + \text{R'-OH} \longrightarrow \text{R-(C=O)-O-R'} + \text{H}_2\text{O}$$

Fatty acid Alcohol Ester

c)-Amidificatin

Amidification with amines (NH_2) allows for the formation of sphingolipids.

$$\text{R-COOH} + \text{R'-NH}_2 \longrightarrow \text{R'-NH-CO-R} + \text{H}_2\text{O}$$

FA Amine Amide Bond

III.3.2.2. Properties Due to the Presence of Double Bonds

a)- Hydrogenation

This process is used to transform edible oils of unsaturated fatty acids into margarine, which is composed of saturated fatty acids that are solid at room temperature and, moreover, do not oxidize.

$$\text{----CH}_2\text{-CH=CH-CH}_2\text{---} + \text{H}_2 \text{ ----------> ---CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{---}$$

b)- Halogenation

It is a routine procedure for evaluating the degree of unsaturation of a fatty acid by the addition of iodine under specific conditions.

$$\text{—CH=CH—} + I_2 \longrightarrow \text{—}\underset{\substack{| \\ I}}{\text{CH}}\text{-}\underset{\substack{| \\ I}}{\text{CH}}\text{—}$$

- **Iodine Index (II):** It is the amount in **grams** of **iodine** required to neutralize **100 g** of **fat**. It allows for the determination of the **degree of unsaturation**.

$$I_I\ (g) \longrightarrow 100\ g\ (fat)$$

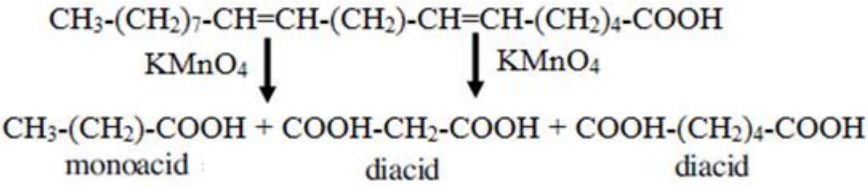
$$N * MW\ of\ I_2\ (127*2=254\ g/\ mol) \longrightarrow MW\ (fat)$$

Knowing that N: number of double bonds.

c)- Oxidation in the Presence of $KMnO_4$ (Powerful Oxidant)

Oxidation leads to the formation of a monoacid and one or more diacids. The number of diacids is equivalent to the number of double bonds.

For example, the oxidation of linoleic acid (C_{18}:2 $\Delta^{9,\,12}$) by potassium permanganate ($KMnO_4$).



IV. True Lipids

IV.1. Simple Lipids

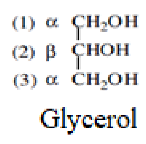
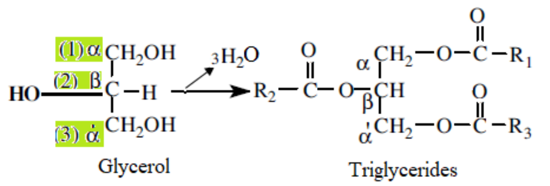
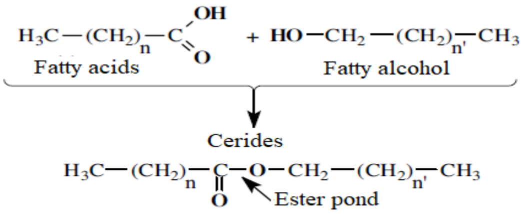
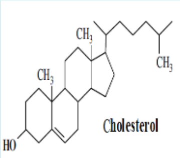
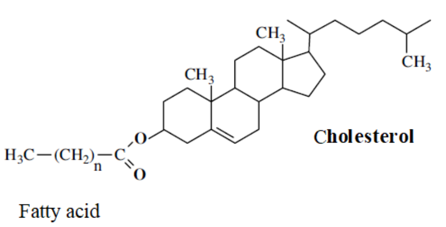
Simple lipids are ternary compounds (C, H, O). They are esters of fatty acids that are classified based on the type of alcohol (Table III), and they are neutral.

Most eukaryotes store neutral lipids in oily inclusions in the cytosol, such as in oilseed plant seeds and the adipose tissue of mammals. This energy reserve offers advantages over carbohydrates:

- Their catabolism through oxidation results in the production of energy that is twice as high.
- Being neutral and highly hydrophobic, their storage occurs in a compact physical form without water.

- Acylglycerols serve as a long-term reserve lasting several months, whereas glycogen is a rapid source of glucose that can be depleted within a day. This is particularly important for migratory birds, hibernating animals, and polar species, as well as for water storage in camels (fat hump), which is produced during catabolism.

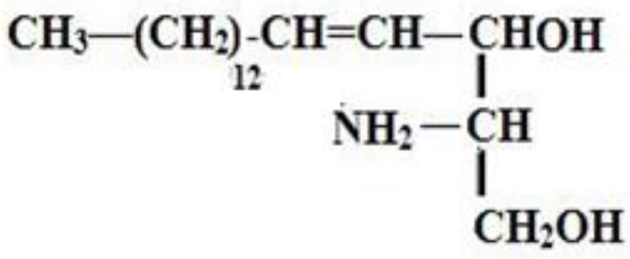
Table III : Simple lipids

Classes	Alcohol	Definition & structure	Nomenclature
Glyceride	Glycerol 	**Glycerides** are esters formed from **glycerol**. Depending on the number of -OH groups of glycerol that are esterified, they are classified as mono-, di-, or triglycerides. When the fatty acid molecules constituting the triester are identical, they are referred to as **homogeneous** triacylglycerols; otherwise, they are called **mixed** triacylglycerols. 	We add the radical "**oyl**" or "**yl**" for the acid and then the ending "**glycerol**. **Ex**: TriPalmitoyl Glycerol=Tripalmitine **Ex**: 1,3 dipalmityl, 2 oleyl glycerol.
Ceride	Aliphatic Alcohols $HO-(CH_2)_{n'}-CH_3$ Fatty alcohol	**Cerides** are monoesters of fatty acids and long-chain **aliphatic alcohols**, which are generally primary alcohols with an even number of carbons, saturated, and unbranched. They derive their generic name from the fact that they are the main constituents of animal, plant, and bacterial waxes. 	**Ex**: **Beeswax:** Palmitic acid + Cetyl alcohol (C_{16}) → Cetyl Palmitate
Sterides	**sterols** 	They result from the esterification of fatty acids by sterols (polycyclic alcohols). These alcohols derive from the steroid nucleus, which is produced from the condensation of four cycles, with the hydroxyl group being a secondary alcohol function always located at the same position (C3). The most representative example is **cholesterol**. 	**Ex** : Cholesteryl Palmitate

IV.2. Complex lipids

These heterolipids contain phosphate, sulfate, or carbohydrate groups. They are classified based on the molecule that binds the fatty acids:

- Either glycerol (Table IV), which is distinguished from acylglycerols by the heterogroup and is subdivided into:
    - Glycerophospholipids
    - Glyceroglycolipids
- Or a sphingoid base (Table V), which defines sphingolipids. The latter are amides of sphingosine (Fig. 7) that form through the linkage of the carboxyl group of the fatty acid to the NH_2 group of sphingosine. The attachment of a fatty acid to the amino group results in a ceramide, which is the precursor molecule for the lipids in this group.



$$CH_3-(CH_2)_{12}-CH=CH-CHOH-CH(NH_2)-CH_2OH$$

Fig.7 : Structure of sphingisine

Table IV: Complex lipids based on glycerol

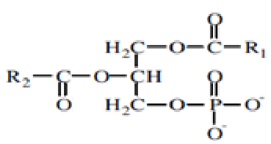
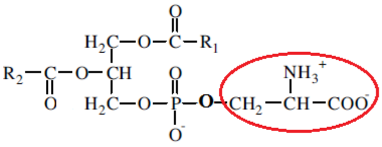
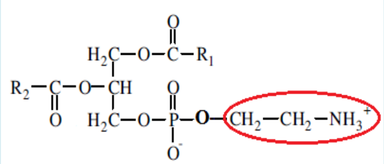
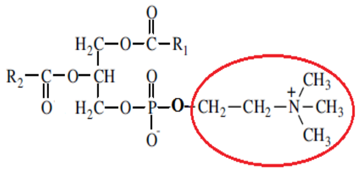
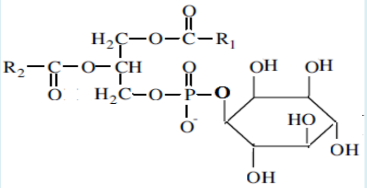
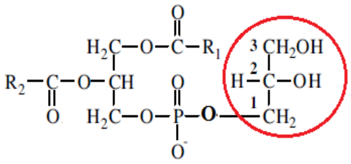
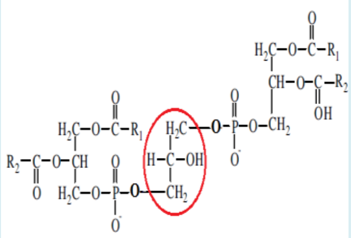
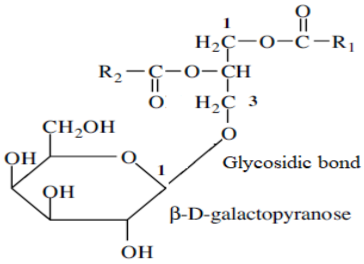
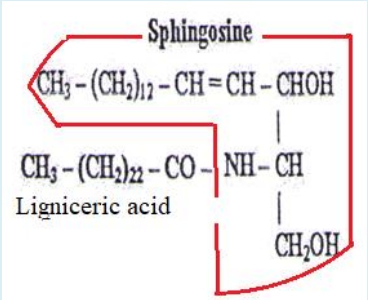
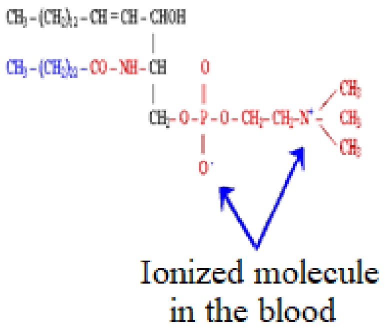
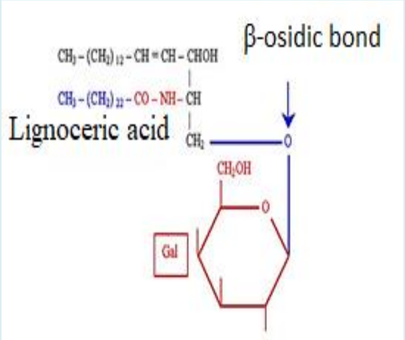
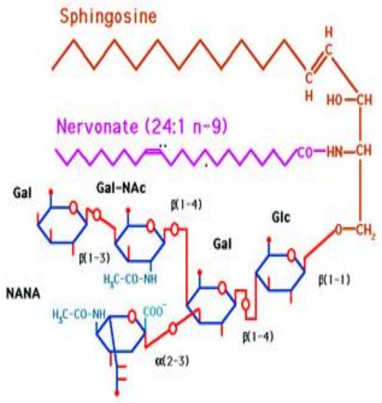
Classes	Derivatives of the class	Esterification of the acid function	Structure	Common name
Glycerophos-pholipides	Phosphatidics Acids	/		/
	Nitrogenous glycerophospholipids	phosphatidyl serine		/
		Phosphatidyl ethanolamine		Cephalins
		phosphatidyl choline		Lecithins
	Non-nitrogenous glycerophospholipids	phosphatidyl inositol		Inositides
		phosphatidyl glycerol		//
		Biphosphatidyl glycerol (cardiolipin)		Cardiolipins
Glycero glycolipides	/	/		1, 2-diacyl- β-D-galactopyranosyl-glycerol

Table V: Complex lipids based on sphingosine

Classes	Name	Lipid component	Structure
Acyl-Sphingosines	Ceramide	Sphingosine + fatty acid	
Sphingo-Phospholipide	Sphingo-myeline	Sphingosine + fatty acid + Phosphorylcholine	
Sphingo-Sidolipides	Cerebrosides	Sphingosine + fatty acid + sugar These sugars can be Gal (galactosylceramide) or Glu (glucosylceramide)	
	Gangliosides oligosylceramide	Sphingosine + fatty acid + chain of several sugars and sugar derivatives (**NANA**: N-acetylneuraminic acid, **GalNAc**: N-acetylgalactosamine)	

IV.3. Properties of glycerophospholipids

- They are **amphipathic** molecules (amphiphiles) because they have two poles: one hydrophobic due to the fatty acids and the other hydrophilic due to the phosphoric ester.
- They are **amphoteric** molecules as they possess both an acidic function from H_3PO_4 and a basic function from the alcohol amino acids (serine, threonine) or from choline and ethanolamine.
- **Enzymatic hydrolysis** is carried out by specific phospholipases for the different ester bonds; there are **four specific phospholipases** (Fig.8):
  - **PLA_1** for the ester bond at carbon 1
  - **PLA_2** for carbon 2
  - **PLC** and **PLD** for the ester bonds with phosphoric acid.

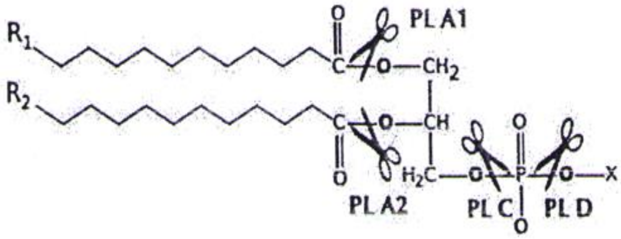


Fig. 8: Effect of phospholipases

- **Saponification:** Under the action of potassium hydroxide (KOH), glycerol is released and fatty acid salts are formed: soaps.
- **Saponification index (S_I):** It is the amount in mg of **KOH** required to saponify 1 g of fat. It helps determine the **molecular weight (MW)** of the fat substance.

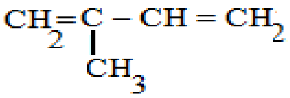
$$S_I\ (mg) \longrightarrow 1g\ (fate)$$
$$n * 56g/mol \longrightarrow MW\ (fate)$$

Knowing that n: number of KOH molecules used.

V. Unsaponifiable lipids

Unsaponifiable lipids include isoprenoid lipids, which all derive from a common precursor: isoprene (Fig. 9). Two subgroups are distinguished (Table VI):

- Steroids from animal cells, which derive from a polycyclic nucleus, sterane (Fig. 10).
- Terpenes from plants.



$CH_2{=}C(CH_3){-}CH{=}CH_2$

Fig. 9: Structure of isoprene

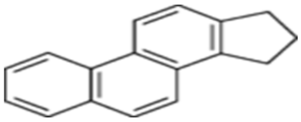


Fig. 10: Cyclopentanophenanthrene

Tableau VI : Insaponifiable lipids

Classes	Sub-classes	Example	Properties
Steroids	Sterols	Cholesterol $C_{27}H_{45}OH$	-Cholesterol is the precursor of all steroids. -It is synthesized in the liver and endocrine glands (adrenal cortex, ovaries, testes) and is also obtained from the diet. -It is part of cell membranes and is transported in the blood in two forms: • 1/3 One-third in free form • 2/3 Two-thirds in esterified form.
	Steroid hormones	Estrogens (C18)	Responsible for various sexual characteristics in females and are synthesized in the ovaries and placenta.
		Testosterone (C19)	Responsible for male sexual characteristics, secreted in the testes.
		Progesterone (C21)	Responsible for implantation and gestation, secreted by the corpus luteum, placenta, and adrenal cortex.
		Glucocorticoids (cortisol, cortisone)	Stimulate protein catabolism and gluconeogenesis in the liver and oppose the inflammatory response.
		Mineralocorticoids (aldosterone)	Allow for the reabsorption of sodium, chloride, and water in the kidneys.
	Bile acids	///	Present in bile, bile acids are derived from the catabolism of cholesterol in the liver. They are conjugated with glycine and taurine (which comes from the oxidation and decarboxylation of cysteine). Released into the digestive tract, they facilitate fat digestion by promoting emulsions during digestion. Additionally, they activate lipases.
	Vitamin D (calciferols)	Vitamin **D_2** (plant origin).	Vitamin D (ergocalciferol) plays a role in the absorption of calcium and phosphorus by the intestines.
		Vitamin **D3** (animal origin).	Vitamin D (cholecalciferol) is a fat-soluble vitamin synthesized in the human body from a derivative of cholesterol under the action of UV radiation from sunlight.
Terpenes	Vitamin	Vitamin **A**	In the human body, vitamin A occurs as retinol, retinal, and retinoic acid. It plays an essential role in bone growth, cell differentiation, and the synthesis of visual pigments in the retina.
		Vitamin **E** Tocopherol	Is recognized primarily as an antioxidant, slowing down skin aging. It is found in large quantities in vegetable oils.
		Vitamin **K**	There are two types of vitamin K: - **Vitamin K1** (phylloquinone, from a plant synthesis) - **Vitamin K2** (menaquinone, from a bacterial synthesis), which have different structures and actions. K2 is involved in the synthesis of blood coagulation factors and the binding of calcium in the bones.
	Ubiquinone	(CoE Q10)	Present in all living organisms, ubiquinone acts as an electron transporter in the respiratory chain.
	Pigments	Xanthophylls	Yellow pigments found in algae.
		Carotenes	Yellow and red pigments found in carrots.
		Lycopenes	Red pigments found in tomatoes.

VI. Lipoproteins

Lipoproteins are responsible for the transport of lipids in a soluble form within the plasma. They are spherical macromolecular complexes consisting of a hydrophobic core and a peripheral monolayer. They differ from each other by:

- The importance and nature of their protein component.
- The composition of their lipid fraction: the larger the lipid core diameter, the more they contain apolar lipids, and the lower their density.

The different classes of lipoproteins are:

- **VLDL (Very Low Density Lipoproteins)**: Endogenous triglycerides.
- **IDL (Intermediate Density Lipoproteins)**: Provide triglycerides.
- **LDL (Low Density Lipoproteins)**: Supply cholesterol to tissues that cannot synthesize enough for their needs. LDLs transport cholesterol, either free or esterified, in the blood and throughout the body to cells. LDLs are produced by the liver from very low density lipoproteins (VLDL).
- **HDL (High Density Lipoproteins)**: Provide phospholipids.
- **Chylomicrons**: Triglycerides (during postprandial periods), transport dietary lipids. Chylomicrons, formed in the intestinal cells from dietary lipids, are very large lipid droplets, rich in triglycerides (about 90%). Upon exiting intestinal cells, triglycerides will provide fatty acids.

A deficiency in the uptake of LDL by target tissue cells increases cholesterol levels in blood vessels, leading to deposition and resulting in atherosclerosis. For this reason, LDL is often referred to as "bad cholesterol," in contrast to HDL, which is known as "good cholesterol."

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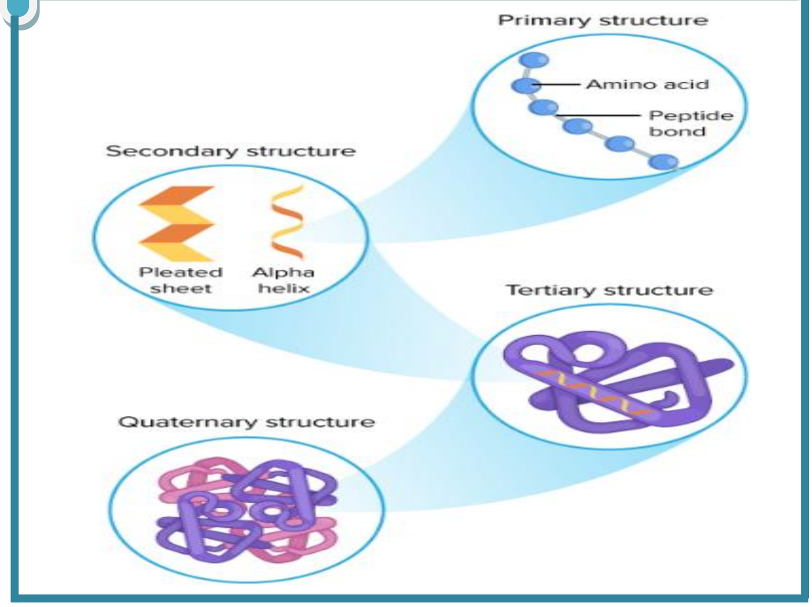
CHAPTRE IV : PROTEIN



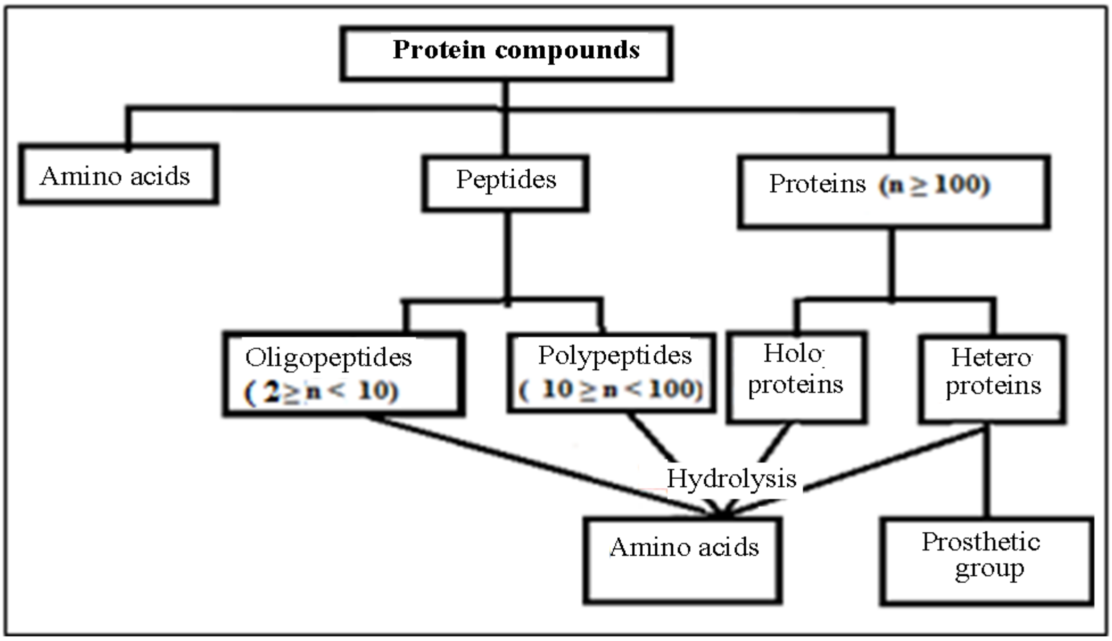
I. Generalities

The term protein comes from a Greek word meaning "**of primary importance**" or "**in first place**." Indeed, proteins are extremely important components of living cells; they generally represent **more than half** of the dry weight of cells. Alongside **structural proteins** (such as protein fibers, the cytoskeleton, coordinated movement: muscles, sperm cells), there are proteins with a crucial **biological role**, particularly:

- Transport and storage: hemoglobin
- Transport of substances across the cell membrane
- Forming messages: hormones, neurotransmitters
- Catalyzing chemical reactions: enzymes
- Identity of an organism and its defense: MHC and antibodies
- Regulation of biosynthetic and metabolic machinery: activators or repressors
- Proteins can be harmful: toxins and viral proteins.

All proteins contain the four elements: C, H, O, and N; many contain sulfur, and some contain phosphorus. They are macromolecules, formed by the condensation of a large number of units called **amino acids**. Two major groups of proteins are distinguished:

- **Holoproteins**: composed only of chains of amino acids.
- **Heteroproteins (conjugated proteins)**: which, in addition to one or more chains of amino acids, contain a prosthetic group of very diverse nature.



PART I: AMINO ACIDS

I. Definition

An amino acid (AA) is an organic compound and forms the **structural unit** of proteins. It contains a **carboxylic acid** group (-COOH) and **generally** a **primary amine** group (-NH_2), both of which are attached to the **alpha (α) carbon**, hence the term **α amino aci**ds. The alpha carbon is, in turn, bonded to a **hydrogen** atom on one side and a **side chain** R (radical) on the other (Fig. 1).

Note: Except for proline, which has a **secondary amine group.**

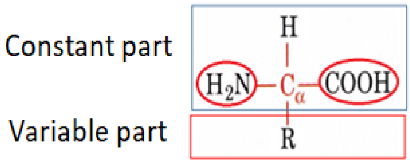


Fig. 1: General formula of an amino acid

There are more than 300 amino acids in nature, which are found either in their free form within the cell or bound to other molecules. These are distinguished into:

- 22 amino acids that make up natural proteins, also known as **standard amino acids**.
- 22 amino acids differ from each other by the structure of their **side chain or radical (R).**
- 22 amino acids are represented either by a **three-letter code** (usually the first three letters of their name) starting with a capital letter (the most commonly used nomenclature): For example, glycine (Gly), alanine (Ala), leucine (Leu), glutamine (Gln), etc., or by a one-letter code (Table I).

II. Classification of the 20 Natural Amino Acids

The 20 natural amino acids are classified into several groups, based on the nature of the functional groups present in the side chain (R).

II.1. Based on the linear or cyclic structure of R

- **Aliphatic amino acids** (non-cyclic R)
- **Aromatic amino acids** (benzene-type cyclic R)
- **Heterocyclic amino acids** (cyclic R containing an atom other than carbon in the ring)

II.2. Based on the polarity and the ability of the side chain to carry an electric charge

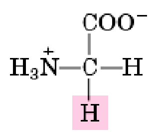
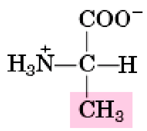
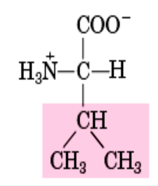
The different groups are presented in Table I.

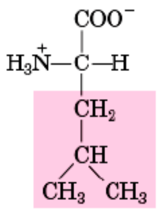
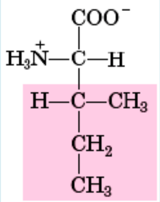
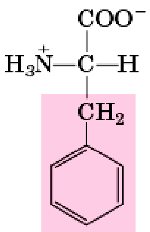
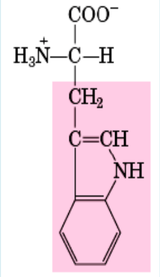
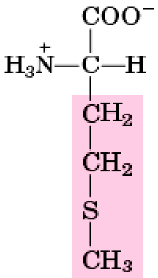
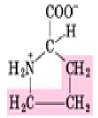
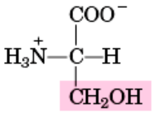
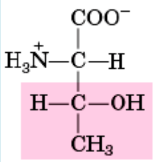
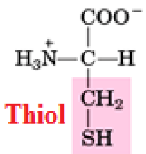
- **Non-polar (hydrophobic) amino acids**: Gly, Ala, Val, Leu, Ile, Pro, Met, Trp, Phe.
- **Polar (hydrophilic) amino acids**:
  - **Neutral (uncharged) amino acids**: Cys, Asn, Gln, Ser, Thr, Tyr.
  - **Acidic amino acids (potential negative charge)**: Glu, Asp.
  - **Basic amino acids (potential positive charge)**: Lys, Arg, His.

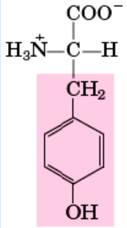
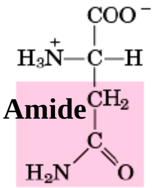
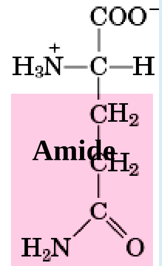
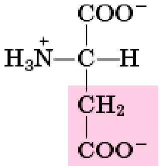
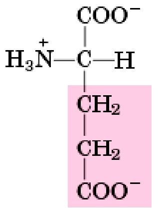
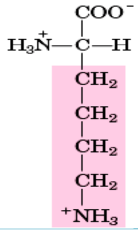
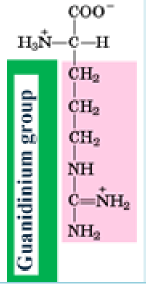
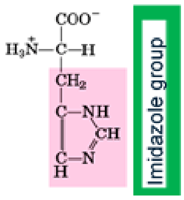
II.3. Based on the presence or absence of a functional group

- **Aliphatic amino acids** (no functional group in R): Gly, Ala, Val, Leu, Ile.
- **Aromatic amino acids** (R is an aromatic ring): Trp, Phe, Tyr.
- **Acidic amino acids** (presence of a carboxyl group): Glu, Asp.
- **Amide amino acids** (derived from the previous ones by amidation): Asn, Gln.
- **Hydroxyl-containing amino acids** (presence of an alcohol or phenol group): Ser, Thr.
- **Sulfur-containing amino acids** (presence of a thiol or thioether group): Met, Cys.
- **Basic amino acids** (presence of a nitrogen group, amine, or similar): His, Arg, Lys.
- **Amino acid with a secondary amine function**: Pro.

Table I: Classification of the 20 Amino Acids Based on Polarity and the Ability of the Side Chain to Carry an Electric Charge

Polarity	Electric Charge	Name	Symbol	Structure	pk_1	pk_2	pk_3	pH_i
		Glycine/ Glycocolle	Gly [G]		2.4	9.8	/	6.0
		Alanine	Ala [A]		2.4	9.9	/	6.0
		Valine	Val [V]		2.2	9.7	/	6.0

Non-polar AA = Hydrophobic	Uncharged	Leucine	Leu [L]		2.3	9.7	/	6.0
		Isoleucine	Ile [I]		2.3	9.8	/	6.1
		Phenylalanine	Phe [F]		2.2	9.2	/	5.5
		Tryptophane	Trp /Try [W]		2.4	9.4	/	5.9
Non-polar AA = Hydrophobic		Methionine	Met [M]		2.1	9.3	/	5.8
		Proline	Pro [P]	 Secondary amine	2.0	10.6	/	6.3
	Neutral (Uncharged)	Serine	Ser [S]		2.2	9.2	/	5.7
		Threonine	Thr [T]		2.1	9.1	/	6.5
		Cysteine	Cys [C]		1.9	10.8	8.3	5.0

Polar AA = Hydrophilic		Tyrosine	Tyr [Y]		2.2	9.1	10.1	7.4
		Asparagine	Asn [N]		2.1	8.8	/	5.4
		Glutamine	Gln [Q]		2.2	9.1	/	5.7
	Acidic Negative charge	Aspartic acid (Aspartate)	Asp [D]		2.1	9.9	3.9	3.0
		Glutamic acid (Glutamate)	Glu [E]		2.1	9.5	4.1	3.2
	Basic Positive charge	Lysine	Lys [K]		2.2	9.2	10.8	9.8
		Arginine	Arg [R]		1.8	9.0	12.5	10.8
		Histidine	His [H]		1.8	9.3	6.0	7.6

- **Amino acids (AA)** contain both an **amine** group and a **carboxylic acid** group, both of which can ionize in an aqueous environment. The ionization of an amino acid depends on the pH of the medium.

  - A **pKa** is characterized for each of the acidic-basic functions (Fig.2): • A carboxyl group, whose pKa value is generally between **1.8 and 2.4** (denoted **pKa** or **pK1**): COOH <=> COO^- + H^+

• An amine group, whose **pKb** value is generally between **8.8 and 11** (denoted **pKb** or **pK2**): $\mathbf{NH_3^+}$ <=> NH_2 + H^+

• Another acidic or basic function in the side chain (R) of certain amino acids, with the **pK** denoted as **pKR** or **pK3**.

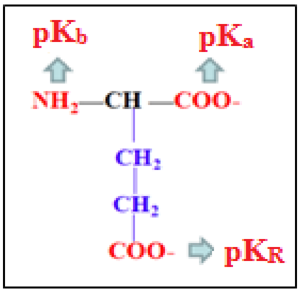


Fig. 2: Position of pK in the Structure of Amino Acids

Essential Amino Acids

Some of these amino acids are not synthesized by human cells and must be obtained through the diet. They are referred to as **essential** amino acids. There are **9 essential amino acids** in children and **8 essential amino acids** in adults. These include: **Histidine** (in children), Valine, Leucine, Isoleucine, Lysine, Methionine, Threonine, Tryptophane et Phenylalanine.

Other Amino Acids

- **The 21st amino acid (Selenocysteine)**

The 21st amino acid, **selenocysteine**, was discovered in 1986. It is found in some proteins, including certain oxidoreductases. As its name suggests, a **selenium atom** replaces the sulfur atom in its structural analogue, **cysteine** (Fig. 3). The **pK3** of selenocysteine is 5.2. Like the other 20 amino acids, selenocysteine is specified by a single three-letter codon [Sec] and a one-letter code [U].

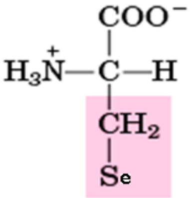


Fig. 3: General formula of Selenocysteine

- **The 22nd amino acid (Pyrrolysine)**

The 22nd amino acid, **pyrrolysine**, was discovered in 2002 (Fig. 4). It has been identified only in **methanogenic archaea** (a group of archaebacteria), within enzymes specific to their unique metabolism. Pyrrolysine is specified by a single three-letter codon [Pyl] and a one-letter code [O].

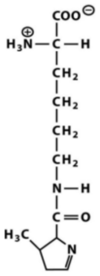


Fig. 4: General formula Pyrrolysine

Free Amino Acids

Many other amino acids exist in their free form and play important metabolic roles. For example, ornithine and citrulline (Fig. 5) are involved in ureogenesis a cycle of biochemical reactions in various animals that produces urea from ammonia.

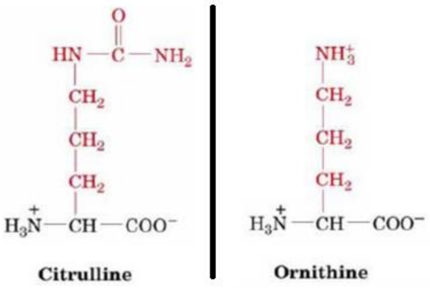


Fig. 5: Structures of free Amino Acids

Post-translationally Modified Amino Acids

Proteins can undergo enzymatic modifications on some of the side chains of the amino acid residues that make them up. These modifications occur **only after translation** and are known as **post-translational modifications**. Modified amino acids play various important roles in the structure and function of proteins. For example (Fig.6):

- **Hydroxyproline**: This is proline modified by the addition of a hydroxyl group.
- **Thyroid hormones**: These are responsible for regulating the metabolism of the body. They are called **T3 (Triiodothyronine)** and **T4 (Thyroxine)**. These hormones are modifications of **tyrosine** that have additional iodine atoms attached.

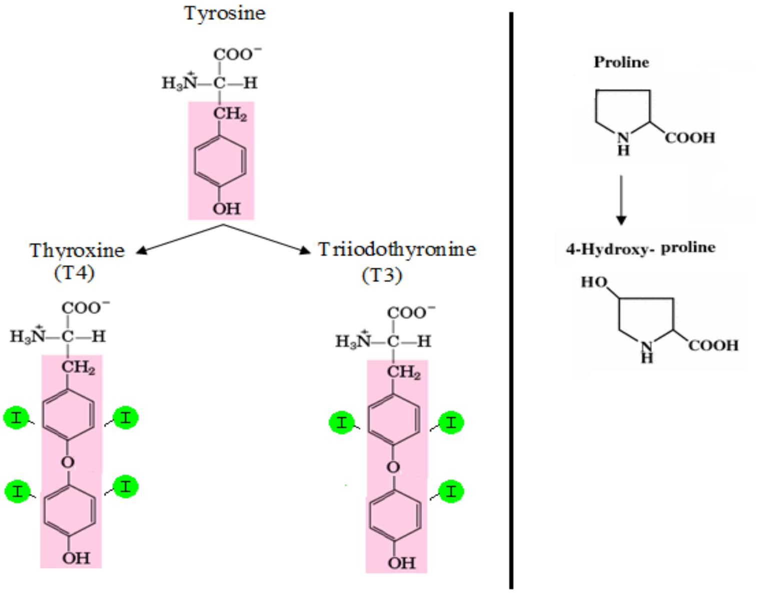


Fig. 6: Post-translationally Modified Amino Acids

III. Properties of Amino Acids

III.1. Physical Properties of Amino Acids

III.1.1. Solubility

Amino acids in their solid form are generally **crystals**. They have varying solubility in **water** and **organic solvents** depending on the nature of the side chain (R group):

The charged functional groups of amino acids confer good solubility in **polar solvents** like **water and ethanol**, whereas they are insoluble in non-polar solvents like benzene, hexane, or ether. Solubility in water **decreases** with the number of carbon atoms in the R group; conversely, the presence of ionizable groups (NH_2, COOH) or hydrophilic groups (OH, SH) on the R group **increases** solubility. The solubility of amino acids also varies with pH, reaching a minimum at their pHi.

III.1.2. Melting Point

Amino acids have a high melting point, above 200°C, which requires a significant amount of energy to break the ionic bonds.

III.1.3. Ultraviolet Light Absorption

All amino acids absorb **ultraviolet** radiation at wavelengths shorter than **230 nm**. **Aromatic** amino acids **absorb UV** light between **250 and 280 nm** (Phe at 257.4 nm; Tyr at 274.6 nm; Trp at 279.8 nm) (Fig. 7).

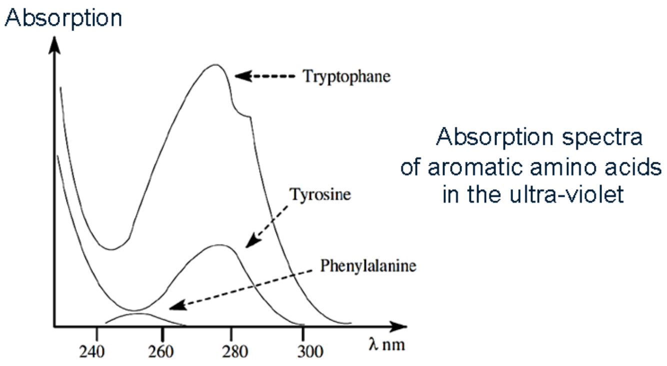


Fig. 7: UV Absorption Spectrum of Aromatic Amino Acids.

III.1.4. Stereochemistry

All amino acids, except **glycocolle (Glycine)**, have at least one carbon with **four different** substituents, called the **asymmetric** C* (or **chiral**) carbon, which is the **α-carbon.** Therefore, the amino acid can exist in two **different forms**, one being the mirror image of the other. The two **stereoisomers** are called **enantiomers** or **optical isomers** (one from the D series and the other from the L series).

According to Fischer's representation:

- In Fischer's projection, the carboxyl group –COOH is always placed at the top of the chiral carbon (C*), and the R group (here, $–CH_3$) is placed at the bottom.
- The NH_2 group and the hydrogen (H) are positioned to the left or right.
- The enantiomer where the **$–NH_2$** group is on the **right** belongs to the **D series**.
- The enantiomer where the **$–NH_2$** group is on the **left** belongs to the **L series**.

- Most natural amino acids are from the L series. According to Fischer, amino acids from the D series are written with the **amino group** (NH_2) on **the right**, and those from the **L series** with the NH_2 on the **left** (Fig. 8).

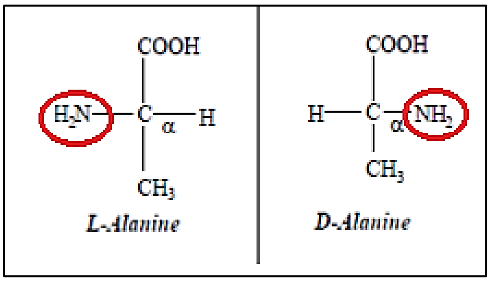


Fig. 8: Structure of Optical Isomers, Example of Alanine

Note: Most natural amino acids are from the **L series**, whereas various bacterial peptides, including those that form the cell wall or certain peptides with antibiotic activity, contain amino acids from the **D series**.

Some amino acids possess an additional asymmetric carbon in the side chain, which increases the possibilities of isomerism. For example, for **Threonine and Isoleucine**, there are four isomers: two isomers from the D series and two from the L series, with the prefix "**allo**" applied to the isomer not found in proteins (Fig. 9).

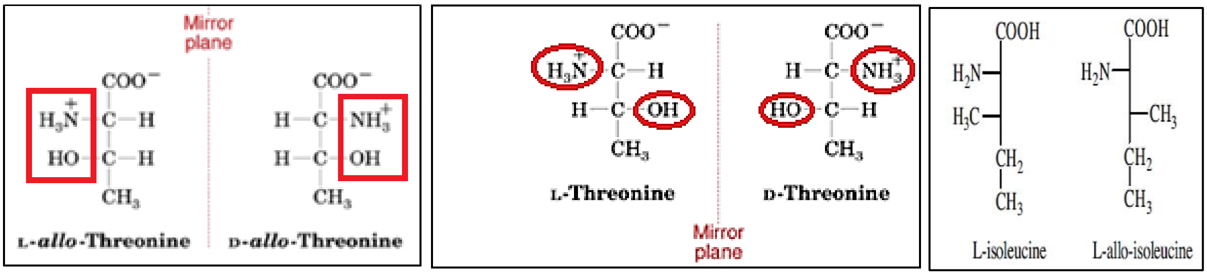


Fig. 9: Structure of the Isomers of Threonine and Isoleucine.

III.1.5. Optical Properties or Rotatory Power

In solution, the **enantiomeric** forms of a molecule containing a chiral carbon (C*) exhibit different optical properties. This is referred to as the **rotatory power**. It is the ability of enantiomers to rotate polarized light (Fig. 10). If the light beam is rotated **clockwise** (to the **right**), the enantiomer is called dextrorotatory, denoted (+).

If, on the other hand, it rotates the **light counterclockwise** (to the **left**), it is called **levorotatory**, denoted (-). All amino acids, **except glycine**, have rotatory power.

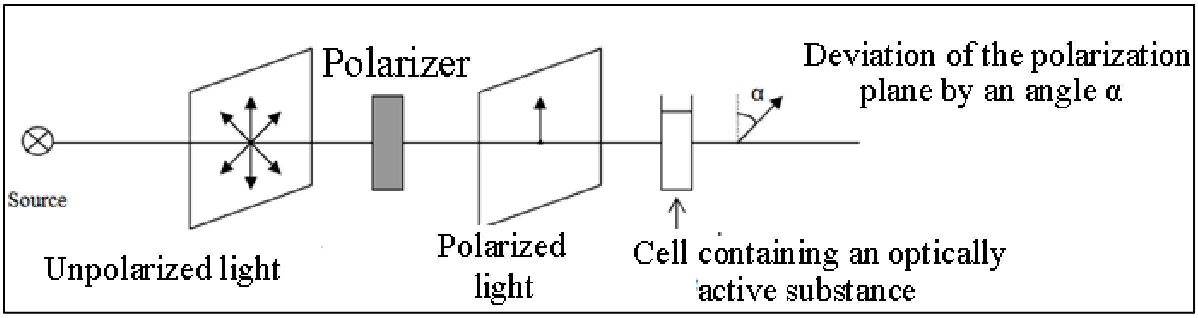


Fig. 10: Polarization of light at an angle α

The optical activity of organic compounds is measured using a polarimeter and according to **Biot's** law:

$$[\alpha]_{D}^{20} = \frac{\alpha}{C * L}$$

Where:

- **$[\alpha]^{20}_{D}$:** Specific rotatory power of the optically active substance. This is a characteristic **constant** of the optically active substance that depends on the nature of the solute and solvent, temperature, and the wavelength at which the measurement is taken. "D" refers to the wavelength of the yellow line of sodium at 589.3 nm, and "20" refers to 20°C.
- **α:** Measured rotatory power using the polarimeter.
- **L:** Length of the tube containing the solution (dm).
- **C:** Concentration of the substance (g/ml).

III.2. Chemical Properties

III.2.1. Acid-Base Properties of Amino Acids

An essential property because it determines the behavior of amino acids in aqueous solution depending on the pH of the solution.

a) **Amphoteric Character**

An amino acid can behave both as an acid and a base because all amino acids have at least two **ionizable** groups. They are called **amphoteric** and exist in different ionized forms depending on the **pH**.

- **Acidic pH (excess H^+):** The amine group (**NH_2**) behaves as a weak base and accepts a proton (H+) (becomes NH_3^+) (Fig.11).
- **Alkaline or basic pH (excess OH^-):** The carboxyl group (**COOH**) behaves as a weak acid and donates a proton (H+) (becomes COO^-) (Fig. 11).

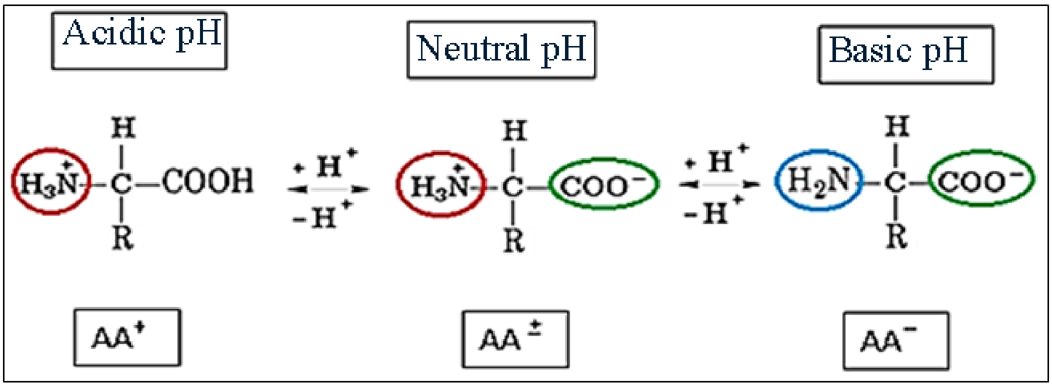


Fig. 11: Equilibrium reaction between the acidic and basic forms of an Amino Acid

b)- Concepts of pK_a, pK_b, pK_R

- **COOH group: pK_a**

  An acid is a compound that donates one or more protons (H+), while a base is a compound that accepts one or more protons (H+).

$$-COOH <=> [-COO^-] + [H^+]$$

This equilibrium is governed by a **dissociation constant K_a** :

$K_a = [-COO^-] \times [H^+] / [-COOH]$

We define: $pK_a = - \log K_a$
It is known that: $pH = - \log [H^+]$
When the concentration of the acid form (AH or -COOH) **is equal** to the concentration of its base (A^- or $–COO^-$), the proton concentration (H+) is equal to K.

If $[-COO^-] = [-COOH] <=> Ka = [H^+] <=> pH = pK_a$

The **pK_a** corresponds to the pH value at which **50%** of the molecules have a dissociated group and 50% have a non-dissociated group (the pH of **half-dissociation** of the **α-COOH** group). Therefore, when pH = pK_a, there is as much base as conjugate acid.

- **Amine $-NH_2$ group: pk_b**

  This functional group has a basic behavior: capable of accepting a proton.

$$NH_2 + H^+ \leftrightarrow NH_3^+$$

- The dissociation equilibrium can be quantified with:

$k_2 = [-NH_2] [H^+] / [NH_3^+]$:

- Similarly: $pk_b = -\log [-NH_2] [H^+] / [NH_3^{\ +}]$.

For: $pH = Pk_b \rightarrow - \log [H+] = -\log [NH_2] [H^+]/ [NH_3^+] \rightarrow [NH_3^+]/[NH_2] = 1$

Thus: $[NH_3^+] =[NH_2]$

- Therefore, when pH = pK_b, the concentration of the dissociated form is equal to that of the non-dissociated form.

- The higher the pKa, the stronger the basic behavior of the function. The **pK_b** corresponds to the pH value at which **50%** of the molecules have a dissociated group and 50% have a non-dissociated group (the pH of **half-dissociation** of the **α-NH_3^+** group).

- **Radical R** : pK_R

The pK_R is defined for ionizable R groups.

c) Isoelectric Point (pHi)

The isoelectric pH (pHi) is the pH at which the amino acid is completely ionized and carries as many positive charges as negative charges ($NH_3^+ = COO^-$). The amino acid is called a **dipolar ion** or **zwitterion**, and its net charge is 0.

The pHi is calculated by averaging the pK values around the zwitterionic form.

$$\mathbf{pHi} = \frac{\mathbf{pKa\ (carboxyl\ group) + pKb(amine\ group)}}{\mathbf{2}}$$

Note:

It is important to write the ionization equation in order to calculate the pHi of an amino acid (Fig. 12, 13 and 14). First, start by writing the most protonated form, then write the equations according to the increasing pH scale; the chronological order of dissociation is given by the pK values, and finally, evaluate the overall charges of the molecule to calculate the pHi.

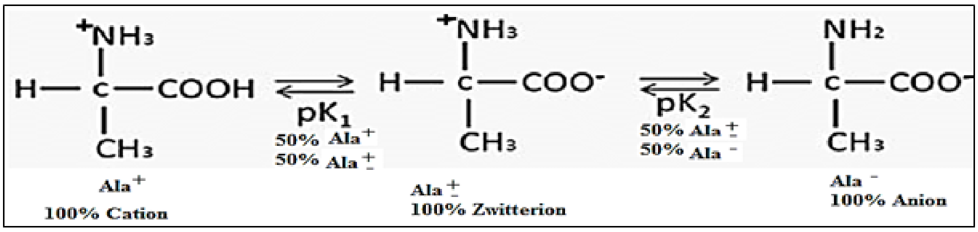


Fig. 12: Ionization equation of a neutral amino acid (Ala).

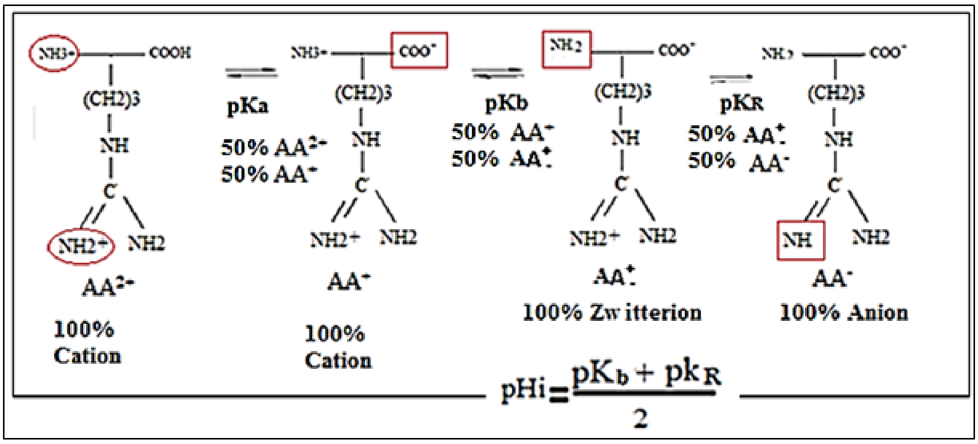


Fig.13: Ionization equation of a basic amino acid (Arg).

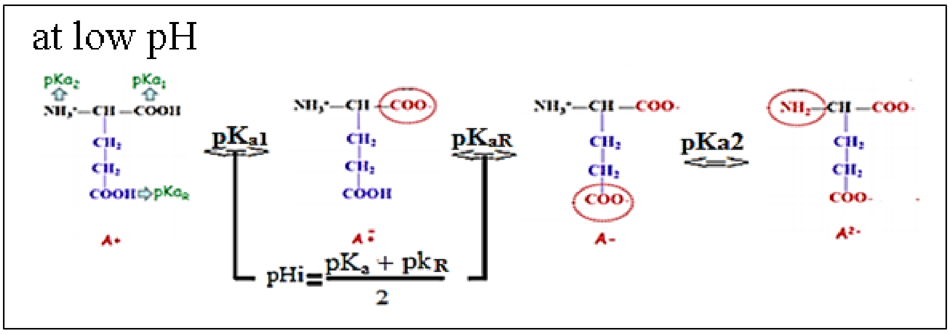


Fig. 14: Ionization equation of a acidic amino acid (Glu).

d)- Titration Curve

To monitor the ionization state of the amino acid (AA) as a function of pH, a **titration curve** is made. Each AA has its own titration curve and **pHi.**

It is possible to measure AAs using a pH meter in aqueous solution to study all the functions present.

Initially, it is essential to make the solution very acidic by adding a **strong acid**. The AA is then **positively charged** (A^+ or A_2^+). Then, a concentrated **sodium hydroxide** (NaOH) solution is gradually added. Each addition is followed by measuring the pH.

There are **2** buffer regions (**inflection points**) for AAs with a **non-ionizable side chain** (Fig. 15). There are **3 inflection points** for AAs with an **ionizable side chain** (Fig. 16, 17). There are **7 AAs** with an ionizable side chain:

- Carboxyl group: **Asp and Glu**
- Amine group: **Lys**
- Guanidyl group: **Arg**
- Imidazole ring: **His**
- Thiol group: **Cys**
- Phenol group: **Tyr**
- **Serine and Threonine** are ionizable, but only at pH = 13: therefore, they are never in an ionized form in our cells.

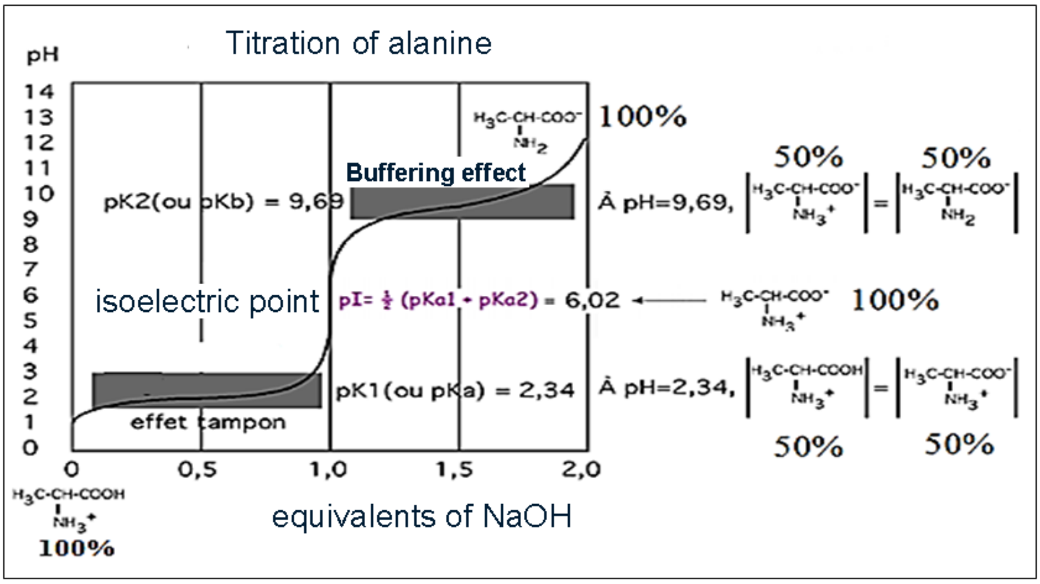


Fig. 15: Titration Curve of an Amino Acid with a Non-Ionizable Side Chain

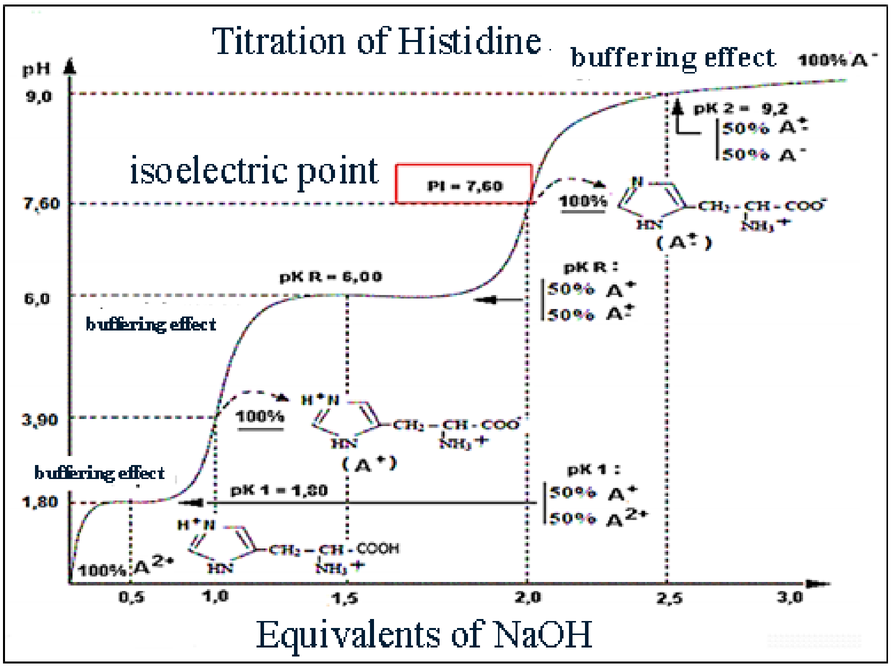


Fig. 16: Titration curve of a basic amino acid (His).

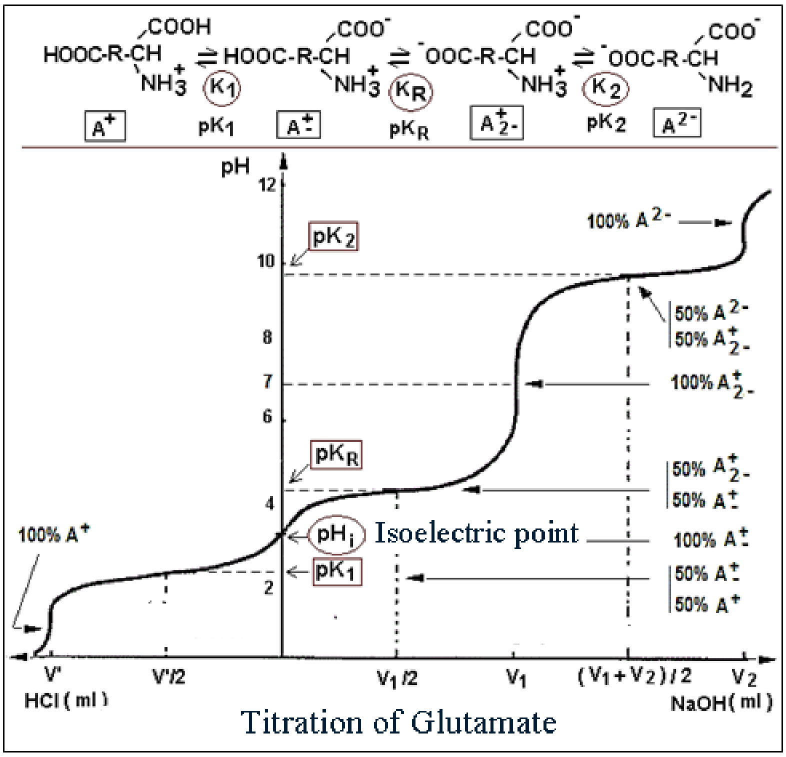


Fig. 17: Titration curve of an acidic amino acid (Glu).

This experimental titration curve can be expressed mathematically by the **ANDERSON-HASSELBACH** equation:

$AH \longrightarrow A^{-} + H+$

The ability of a weak acid to donate protons is measured by the **constant K**:

$k = [A^{-}] [H+] / [AH]$

AH : Acidic form

A^- : Basic form

$[H^+] = K [AH] / [A^-]$

Thus:

$- \log [H^+] = - \log K - \log [AH] / [A^-]$

$- \log [H^+] = - \log K + \log [A^-]/ [AH]$

Knowing that: $-\log [H^+] = pH$ et $-\log k = pk$

Thus :

$$\boxed{\mathbf{pH = Pk + \log [A^-] / [AH]}}$$

III.2.2. Poperties Resulting from the Carboxyl Group

a)-Decarboxylation Reaction

The decarboxylation reaction occurs through a chemical or enzymatic process and results in the formation of an amine (Fig. 18).

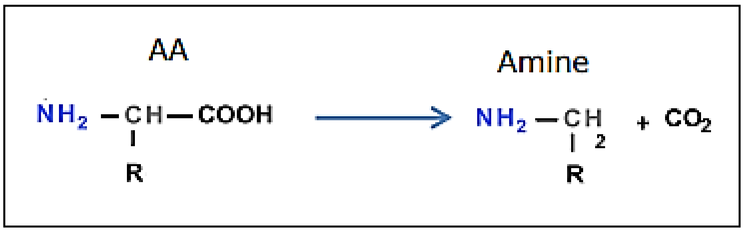


Fig. 18: Decarboxylation reaction

It plays an important role and leads to the disappearance of the amino acid and the formation of an amine. This reaction is catalyzed by cellular decarboxylases and can result in the formation of compounds with significant biological activity (Table II).

Table II: Main Biogenic Amine

Amino Acids	Amine/Derivative	Localization or Role
Serine	Ethanolamine	Found in phosphatides (precursor of choline)
Histidine	Histamine	Vasodilator involved in allergy or inflammation reactions
Glutamic acid	Gamma-Aminobutyric Acid (GABA)	**Neurotransmitter:** Mediator of the central nervous system
Aspartic acid	α-Alanine β-Alanine	Found in proteins Involved in Coenzyme A
Cysteine	Mercaptoethylamine	Part of the composition of Coenzyme A
3,4-Dihydroxy-phenylalanine (DOPA)	Dopamine (Epinephrine)	Precursor of adrenaline (hormone)
5-Hydroxy-tryptophan	Serotonin	Vasoconstrictor tissue hormone

b)- Esterification by an alcohol

A reaction used to protect the -COOH group during peptide synthesis (Fig.19).

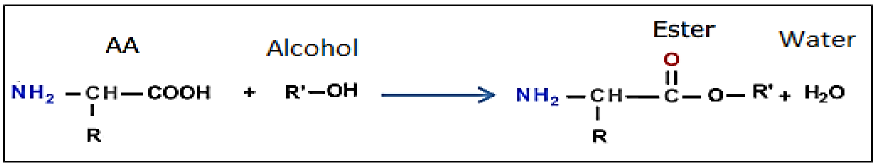


Fig.19: Esterification of AA

c)-Formation of salt

Amino acids react with **bases** to form **salts** (Fig. 20). This property can be used to titrate amino acids. The name of the salt formed is obtained by adding **"ate"** to the name of the amino acid.

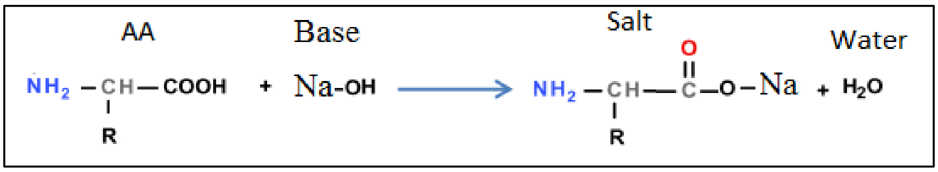


Fig. 20: Formation of salt

III.2.3. Resulting from the Amino Group

a)-Deamination

A reaction in which the amino acid loses its group in the form of NH_3 (Fig. 21). Deamination and transamination are two very important reactions in the metabolism of nitrogen-containing compounds.

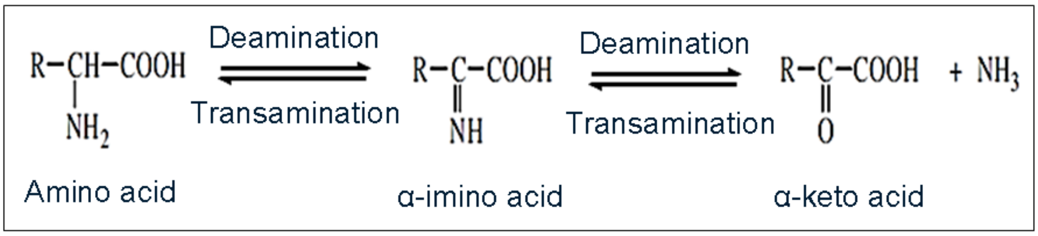


Fig. 21: Deamination of AA

b)- **Action of Nitrous Acid**

Nitrous acid reacts with the **amino group (–NH_2)** of amino acids, resulting in the **release of nitrogen gas (N_2)**. This reaction forms the basis of the **Van Slyke method** for the quantitative determination of amino acids, which relies on measuring the volume of nitrogen evolved **(Fig. 22).**

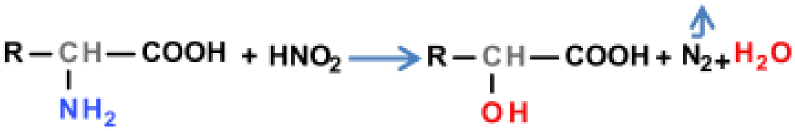


Fig. 22: Formation of a hydroxy acid with liberation of nitrogen gas

c)-Formation of an Imine (Schiff Base)

The formation of an imine is a reaction involving an **aldehyde**, through the addition to the carbonyl group (Fig. 23). Schiff bases often appear as intermediates in enzymatic reactions that involve amino acids as substrates.

Note that **proline**, which contains a **secondary amine function**, does **not react with aldehydes**.

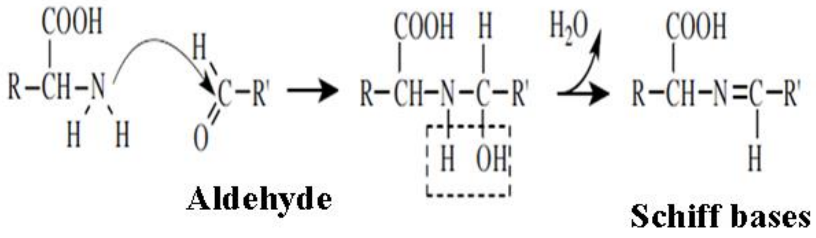


Fig. 23: Formation of an imine (Schiff base)

d)-Action of 1-fluoro-2,4-dinitrobenzene (FDNB)

FDNB readily reacts with **amino groups** (Fig. 24) to form an **N-2,4-dinitrophenyl (DNP) derivative**. This **yellow compound** can be easily identified by **chromatography** and quantitatively determined by spectrophotometry at 360 nm. This reaction enabled Frederick Sanger (1953) to establish the first primary structure of a protein: insulin.

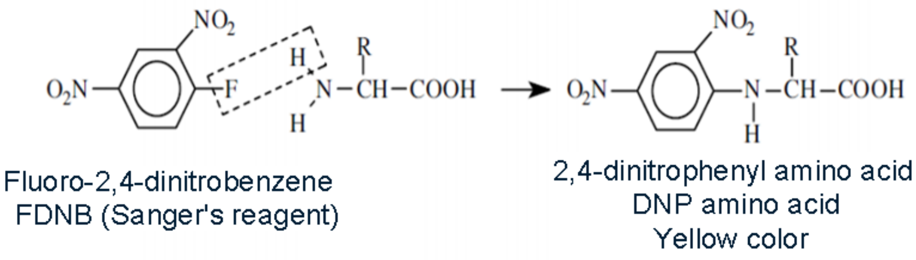


Fig. 24: Sanger Reaction

e)-Action of Phenylisothiocyanate (Edman Reaction)

Carbamylation with phenylisothiocyanate (PITC), under basic conditions (pH ≈ 9), produces a phenylthiohydantoin–amino acid (PTH–amino acid) derivative. This compound absorbs in the UV range and can be easily separated by chromatography (Fig. 25).

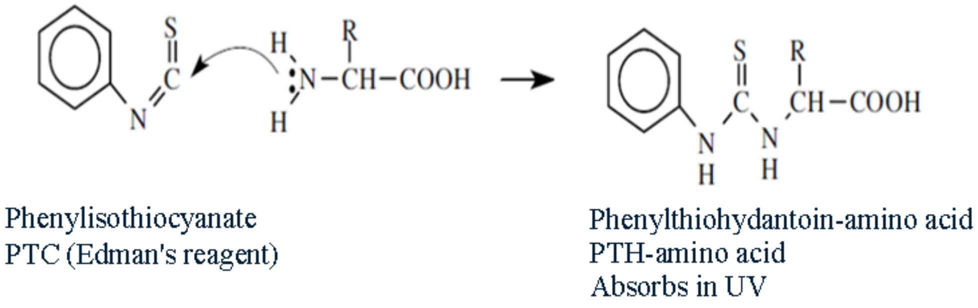


Fig. 25: Edman Reaction

f)- Dansylation

The action of dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride) produces a stable and fluorescent DNS–amino acid (Fig. 26).

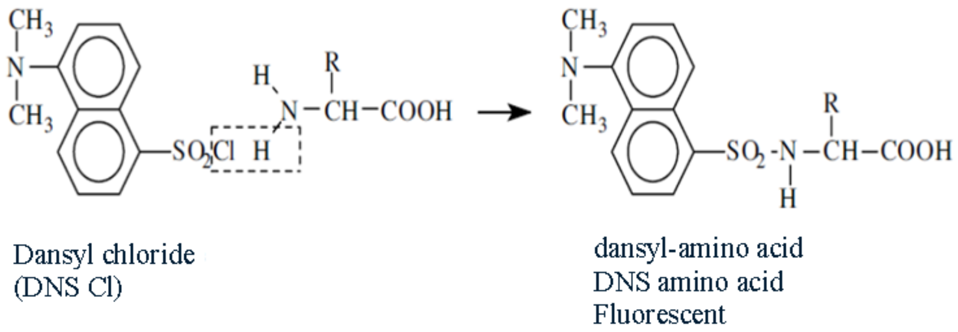


Fig. 26: Dansyl chloride reaction

III.2.4. Properties Resulting from the Amino and Carboxyl Groups

a)-Reaction with Ninhydrin

The reaction of ninhydrin with amino acids occurs in several steps. First, ninhydrin is reduced, while the amino acid is converted into an imino acid. The imino acid then undergoes hydrolysis to form an α-keto acid, which is subsequently decarboxylated to yield an aldehyde containing one carbon atom less than the original amino acid (Fig. 27).

The ammonia (NH_3) released in this process reacts with one molecule of reduced ninhydrin and one molecule of oxidized ninhydrin, producing a **blue–violet** compound with primary amines, and a **yellow** compound with secondary amines.

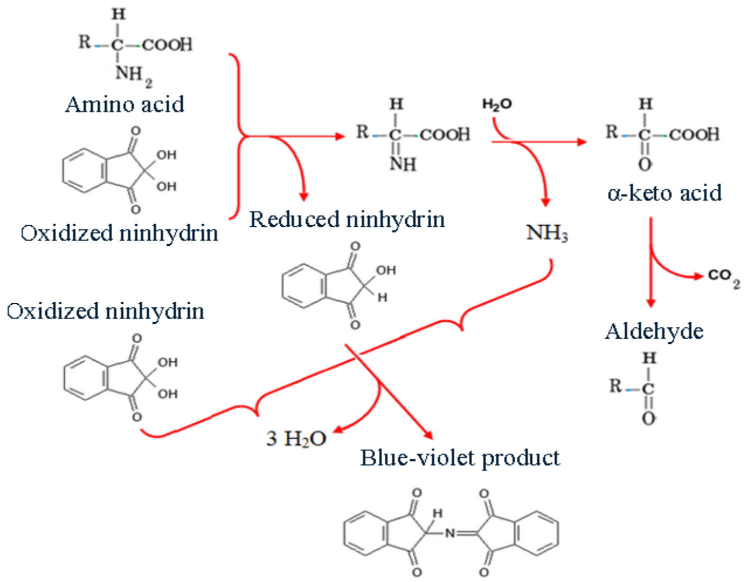


Fig. 27: Ninhydrin reaction of amino acids

b)- Formation of amide

A substituted amide bond or peptide bond (-CO-NH-) can form between two amino acids, with the α-COOH group of one amino acid being linked to the α-NH₂ group of the other amino acid (Fig. 28).

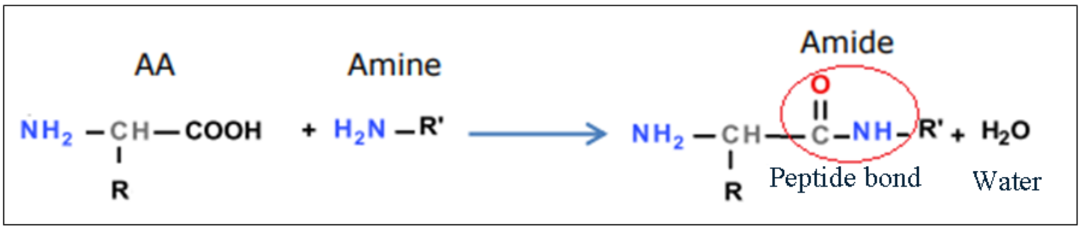


Fig. 28: peptide bond

If the bond involves a **COOH** or **NH₂** group located in the **side chain** (β-COOH, γ-COOH, ε-NH₂), it is referred to as a **peptidoid bond** or **pseudopeptide bond** (Fig. 29).

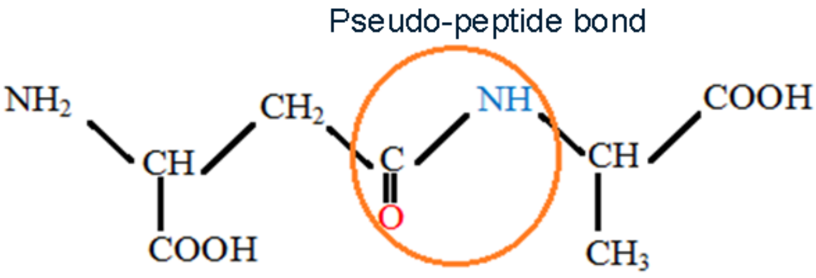


Fig. 29: Peptidoid or pseudopeptide bond

III.2.5. Properties Resulting from the Side-Chain Functional Groups

a) Oxidation of the Thiol Group

Oxidation of SH (cysteine); formation of disulfide bonds (Fig. 30). Cysteine can be oxidized to cystine. The oxidation of cysteine contributes to maintaining the three-dimensional structure of proteins.

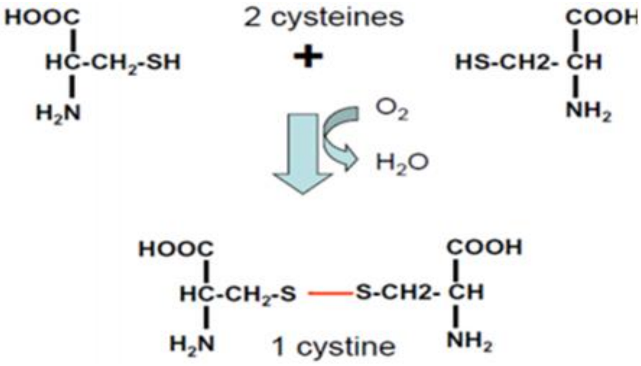


Fig. 30: Formation of disulfide bond.

b)-Formation of a Phosphate Ester

Three amino acids can undergo phosphorylation: serine and threonine, which contain alcohol functional groups, and tyrosine, which has a phenolic function. These groups can be esterified, particularly by phosphoric acid, leading to the formation of a phosphate ester (Fig. 31).

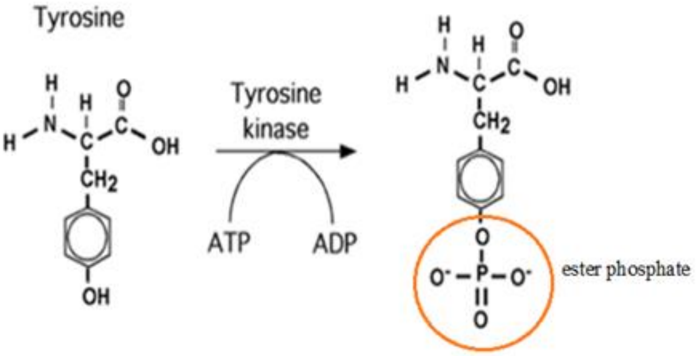


Fig. 31: Phosphorylation of tyrosine by the enzyme tyrosine kinase

c)- O-Glycosylation

Condensation of an amino acid bearing a hydroxyl side chain with a sugar molecule (Fig. 32).

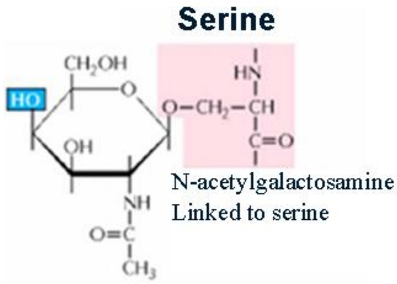


Fig. 32: O-Glycosylation

d)- Amide Functions (N-Glycosylation)

Condensation of an amino acid with an **amide side chain** (*asparagine*, *glutamine*) with a **sugar molecule** (Fig. 33).

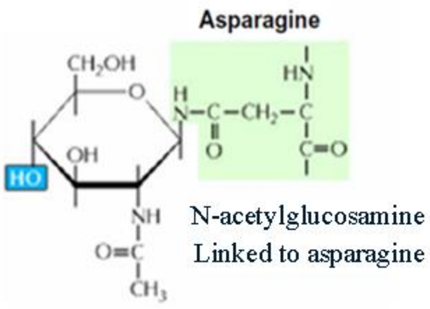


Fig. 33: N-Glycosylation

IV. Techniques for Analyzing and Identifying Amino Acids

The constituent amino acids (standards) of proteins are released by hydrolysis. A protein hydrolysate (**a mixture of amino acids**), obtained after prolonged boiling in hydrochloric acid (HCl) (6N), is a mixture of amino acids that must be identified and quantified. The separation methods used are:

- **Methods based on solubility**
- Paper Chromatography
- Thin-Layer Chromatography
- Gas Chromatography
- **Methods based on charge**
- Electrophoresis
- Ion-exchange Chromatography

IV.1. Electrophoresis

Electrophoresis is a technique for the separation and identification of chemical species. The **principle** is based on the **differential migration** of **charged amino acids** according to their **charge** (depending on the pH of the buffer) under the influence of an **electric field**.

Procedure: The sample to be analyzed is placed in the center of a sheet of paper that has been pre-moistened with the electrophoresis buffer. The sheet is positioned so that its ends are immersed in the buffer solution, where the pH is fixed. An electric field is applied across the ends of the paper for a variable time, depending on the species to be separated (Fig. 34).

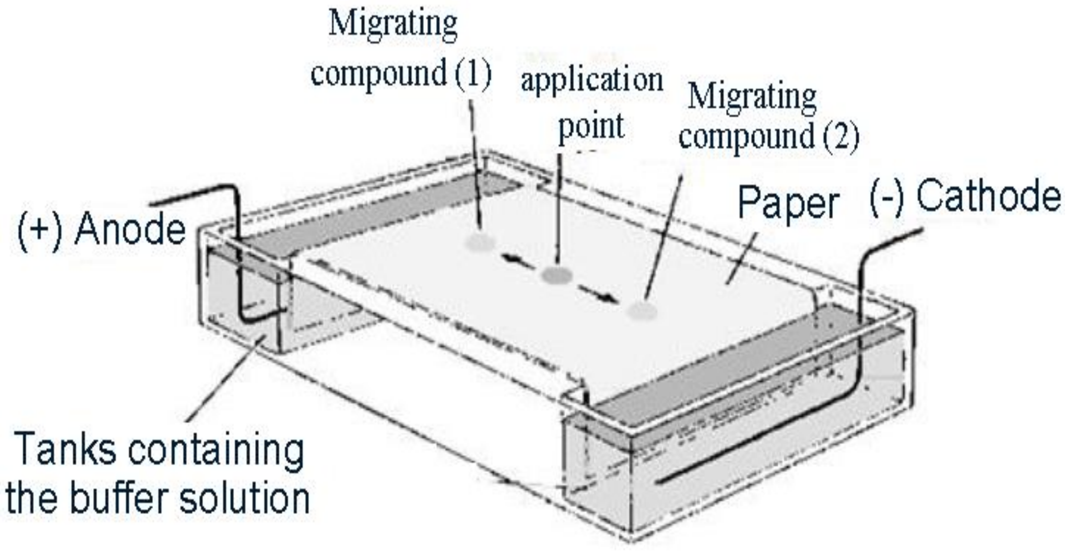


Fig. 34: Electrophoresis Apparatus

Principle of Migration: The amino acids migrate on the support under the influence of the electric field. Depending on their charge (pI and the pH of the buffer solution) (Fig. 35), they will migrate toward the anode (+) or the cathode (-), or will not migrate at all (stationary line).

pH > pHi: AA charged -: migrates towards the anode (positive electrode)
pH = pHi: AA in zwitterion form: does not migrate
pH < pHi: AA charged +: migrates towards the cathode (negative electrode)

Fig.35: Effect of pH on the pHi of Amino Acids

After several hours, the filter paper is dried, and the amino acids are revealed using **ninhydrin.** The NH_3 group of the amino acids reacts with a reduced ninhydrin molecule and an oxidized ninhydrin molecule to form a product that has a **blue-violet** color with primary amines and a **yellow** product with secondary amines (e.g., proline). The result is an electrophoregram (Fig. 36). The migration is faster when the difference between the pH of the solution and the pH of the amino acids is greater.

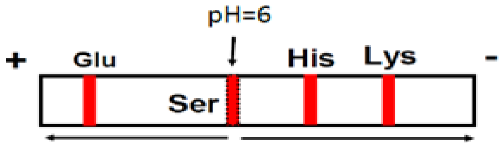


Fig. 36: Electrophoregram

IV.2. Chromatography

IV.2.1. Paper Chromatography

The principle of this method is as follows: a mobile phase (solvent) moves by capillarity in a sealed tank saturated with solvent (stationary phase). The sample to be analyzed (a few µL) is deposited on a starting line located as close as possible to the end of the paper that dips into the mobile solvent (Fig. 37). The amino acids to be separated are chromatographed in the presence of standards. After migration and coloration, the **front ratio (Rf)** of each unknown or standard amino acid (Fig. 38) is calculated using the following formula:

$$RF = \frac{Distance\ traveled\ by\ the\ amino\ acid}{Distance\ trveled\ by\ solvant}$$

The unknown amino acids are identified by comparing their Rf values with those of the standards.

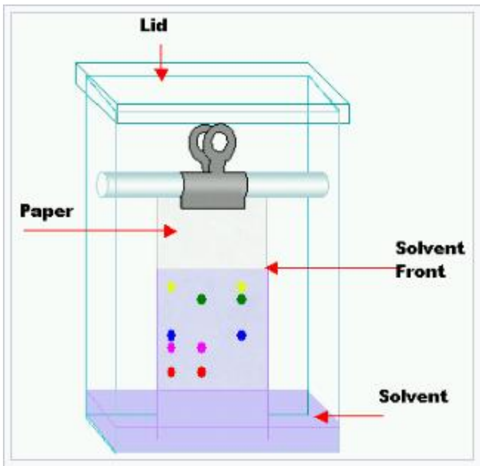


Fig. 37: Diagram representing a paper chromatography setup.

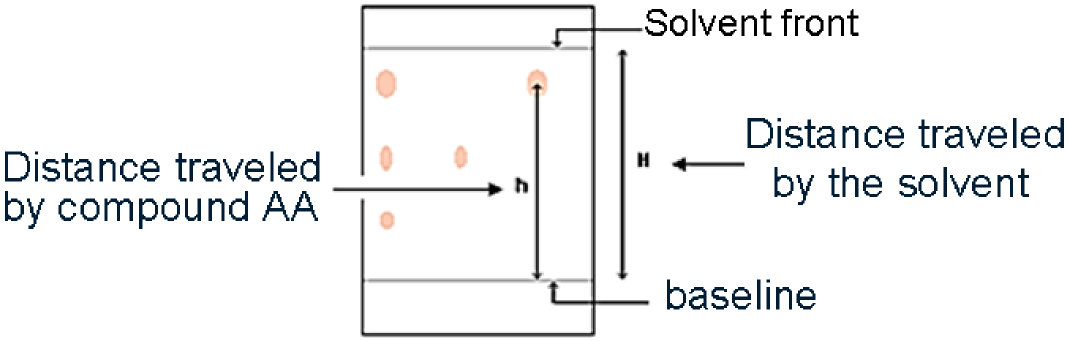


Fig. 38: Diagram representing a chromatogram after revelation.

IV.2.2. Thin-Layer Chromatography (TLC)

It is an adsorption chromatography with a polar stationary phase (cellulose or silica) spread in a thin layer on an inert rigid support (glass or plastic sheet) and a mobile phase that is a less polar organic solvent (Fig. 39).

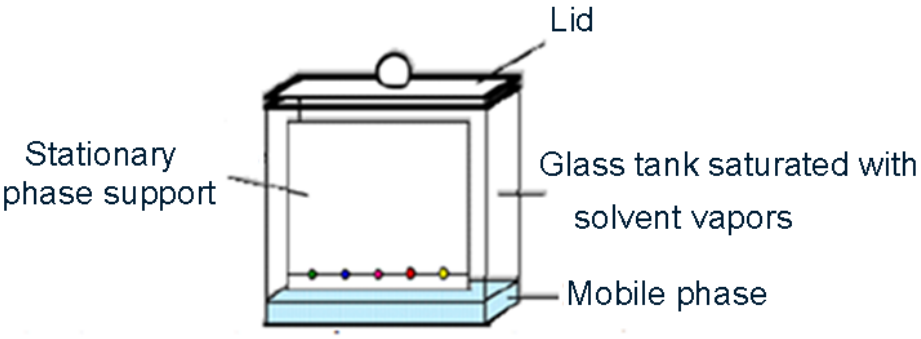


Fig. 39: Diagram representing a setup of Thin-Layer Chromatography (TLC).

The multicolored spots shown above the mobile phase correspond to the deposits of the samples to be analyzed.

The amino acids to be separated are chromatographed in the presence of standards. After migration and coloration, the Rf of each unknown or standard amino acid is calculated. The unknown amino acids are identified by comparing their Rf values with those of the standards.

IV.2.3. Ion-Exchange Chromatography

This is a separation technique that uses two phases: a stationary phase and a mobile phase. The mobile phase is liquid, and the stationary phase is solid. Chemical interactions occur between the stationary phase and the molecules to be separated.

The column is filled with **charged resin** (resin with functional groups that are positively charged [containing NH_3^+] or negatively charged [SO_3^- or COO^-]).

The amino acid mixture is placed on the surface of the resin (Fig. 40). This mixture is dissolved in a buffer with a **specific pH**. At this pH, the amino acids in the mixture may be **positively charged, negatively charged**, or **neutral**, **depending on their pI**. Molecules with a charge opposite to that of the stationary phase **are retained**, while the others are **carried** by the mobile phase.

By gradually changing the pH of the mobile phase either using an **ascending gradient** (e.g., from 2 to 10) or a **descending gradient** (e.g., from 10 to 2) the amino acids that were retained are eluted (Fig. 41).

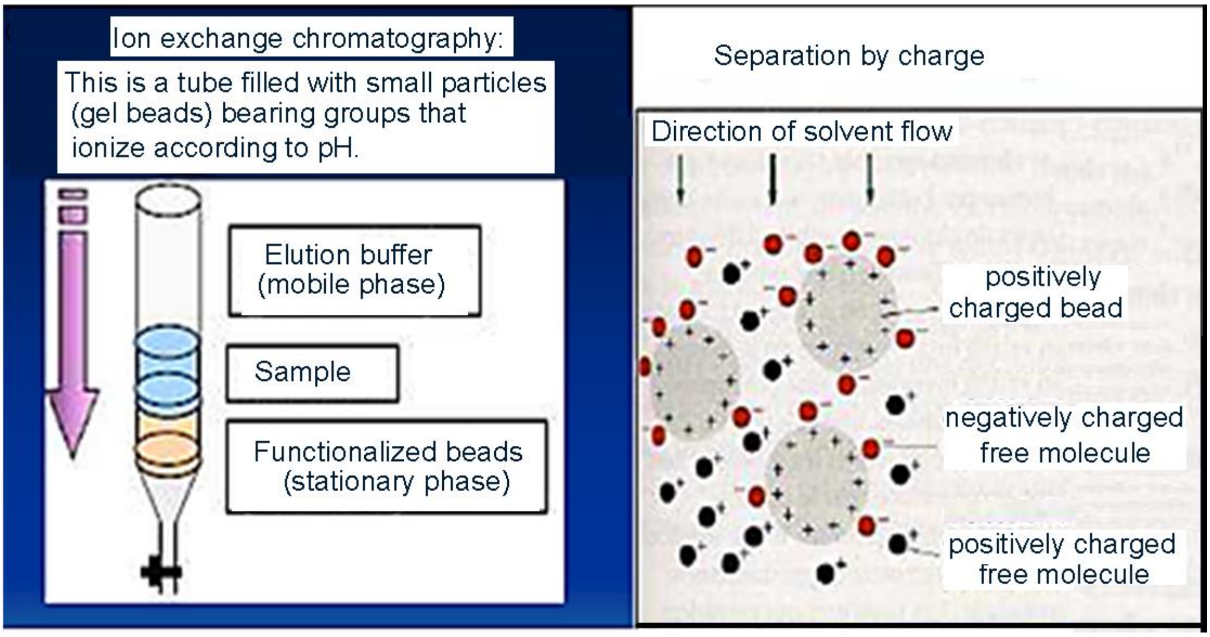


Fig. 40: Ion-Exchange Chromatography

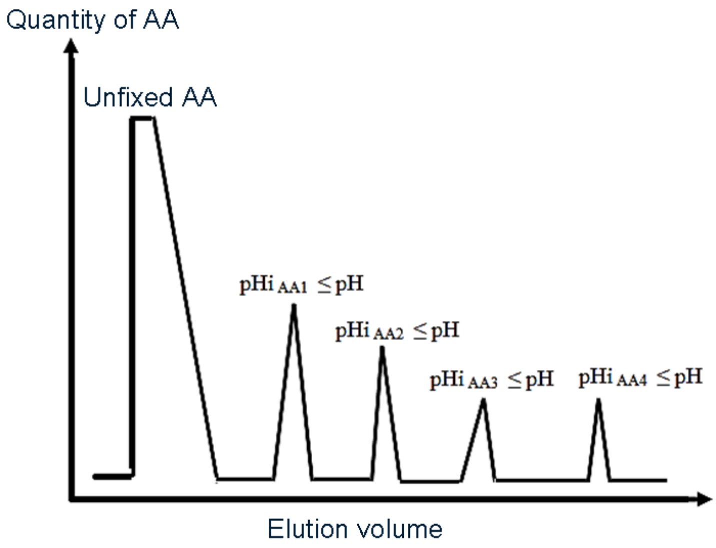


Fig.41: Elution chromatogram of amino acids

Elution of Amino Acids by Increasing or Decreasing pI

The elution of amino acids occurs in increasing or decreasing order of their pI, depending on the type of chromatography used. There are two types of ion-exchange chromatography :

- **Anion-exchange resin**: The resin is **positively** charged, so it retains **anions**. The best **elution** is achieved by using a **descending pH gradient**.
- **Cation-exchange resin**: The resin is **negatively** charged, so it retains **cations**. The best **elution** is achieved by using an **ascending pH gradient**.

Note: The name of the exchanger depends on the charges it captures (the ions it retains).

PART II: PEPTIDES

I. General Information

Peptides are small proteins: they contain fewer than 100 amino acids. The number and sequence of the amino acid residues in a polypeptide constitute its primary structure. A small peptide with fewer than 10 amino acids (n AA < 10) is called an **oligopeptide**, while a peptide with between 10 and 100 amino acids (10 < n AA < 100) is a **polypeptide**.

The reaction between the carboxyl group of one amino acid and the amine group of another amino acid results in the formation of a secondary amide bond. This bond is called a **peptide bond**, which allows the formation of a dipeptide (Fig. 1).

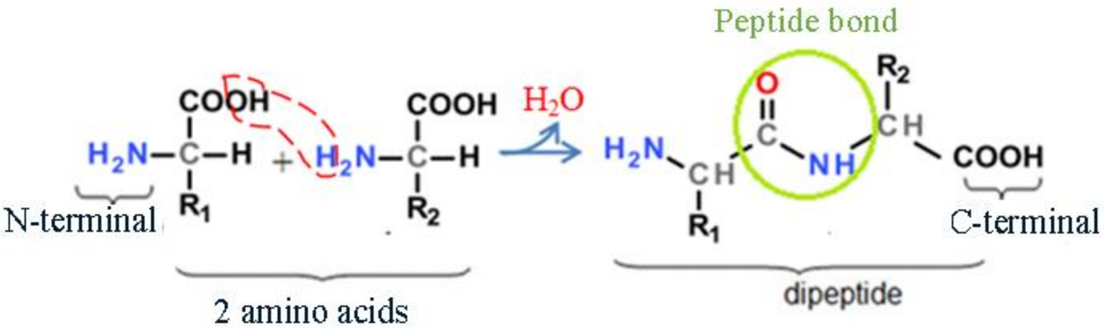


Fig.1: Formation of **dipeptide**

If the peptide bond involves a COOH or NH_2 group located in the side chain (β-COOH, γ-COOH, ε-NH_2), it is called a **peptoid bond** or **pseudo-peptide** bond, e.g., glutathione (Fig. 2).

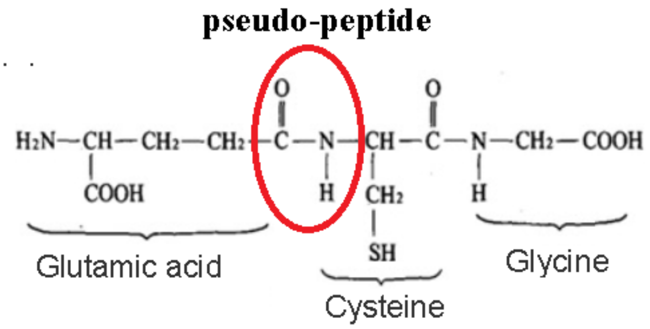


Fig. 2: Peptidoid or pseudopeptide bond (Glutathione).

The peptide has two terminal ends, the N-terminal and C-terminal (Fig. 1). It is important, for nomenclature purposes, to begin with the N-terminal end. The amino acids are numbered by writing the sequence from left to right, starting from the N-terminal end (Fig. 3). The name of the peptide begins with the N-terminal amino acid, which is given the suffix "**yl**" followed by the next amino acid with the same suffix, up to the penultimate amino acid, while the C-terminal end retains **the full name of the amino acid** (Fig. 3).

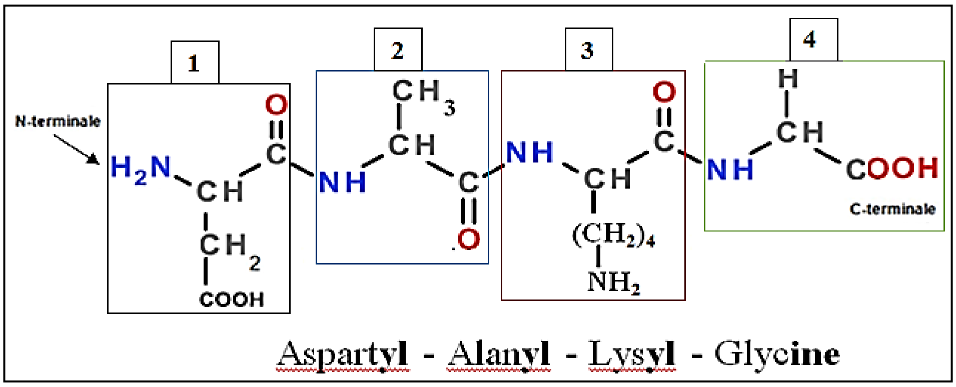


Fig. 3: Nomenclature of peptide

II. Different Types of Peptides

There are four types of peptides:

- **Linear peptide**: Formed by a single chain (monocatenary), with a **free NH_2** group at one end and a **free COOH** group at the other end (Fig. 4).
- **Semi-cyclic peptide**: Formed by a single chain, where one end is free and the other end is occupied by **proline** (Fig. 5).

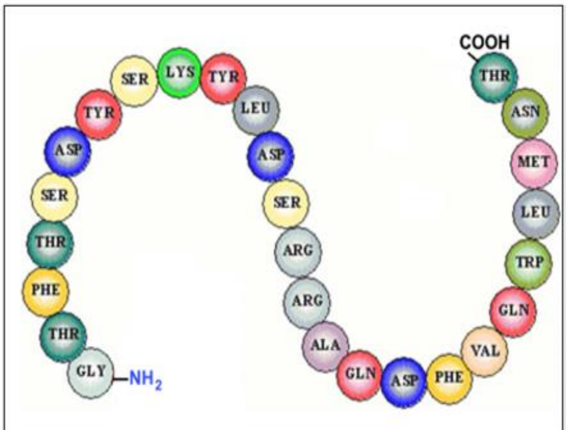


Fig. 4 : Linear peptide structure

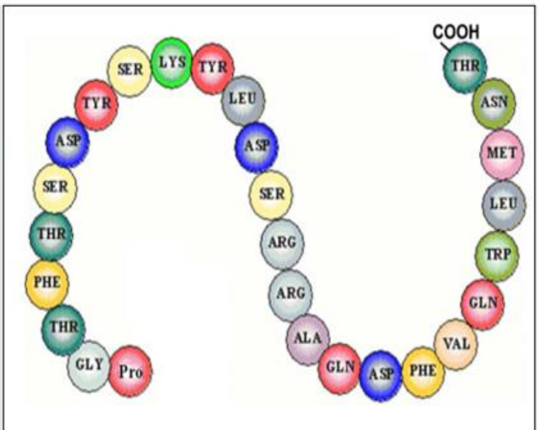


Fig. 5: Semi-cyclic peptide structure

- **Cyclic peptide**: Formed by a single chain, with **no free ends**, neither the N-terminal nor the C-terminal (Fig. 6).

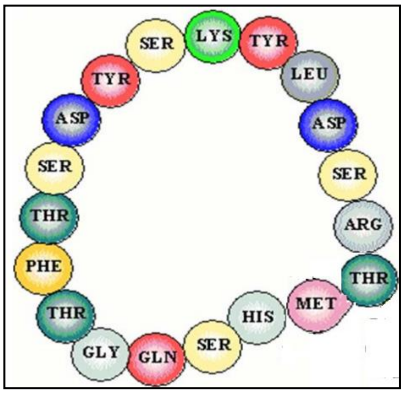


Fig. 6: Form of cyclic peptides.

- **Peptide formed from multiple chains**: A covalent inter-chain bond (S-S bridge) is formed by the oxidation of two thiol groups from two cysteines belonging to different peptide chains. In contrast, the intra-chain bond is formed by the oxidation of two thiol groups from two cysteines within the same peptide chain (Fig. 7).

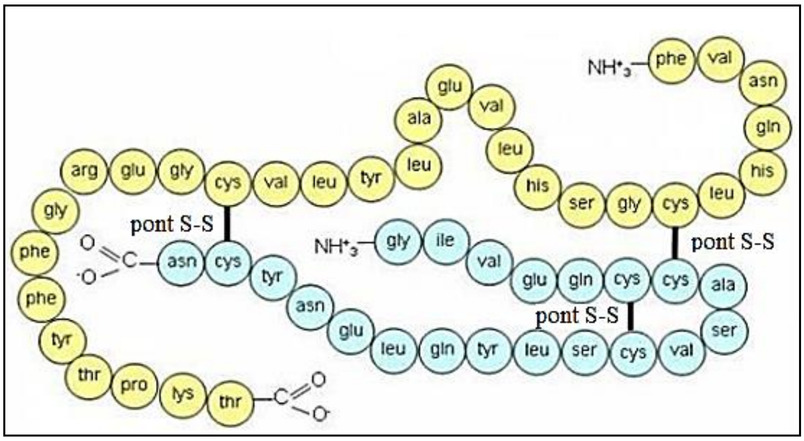


Fig. 7: Peptide formed by two different peptidic chains

III. Properties of Peptides

- Peptides are more soluble in water; they are smaller and contain more hydrophilic amino acids (such as lysine, aspartic acid, etc.).
- They are dialyzable.
- They behave as dipolar ions and can migrate in an electric field.
- They absorb light in the ultraviolet range (if they contain an aromatic amino acid).
- **Ionization of peptides**: Peptides contain an amino-terminal group, a carboxy-terminal group, and ionizable groups on the side chains of the amino acid residues. They can therefore exist in many different ionic forms and possess an isoelectric point (pI) (Fig. 8). The pI, like for amino acids, is the half-sum of the pK values surrounding the zwitterionic form (net charge = 0).

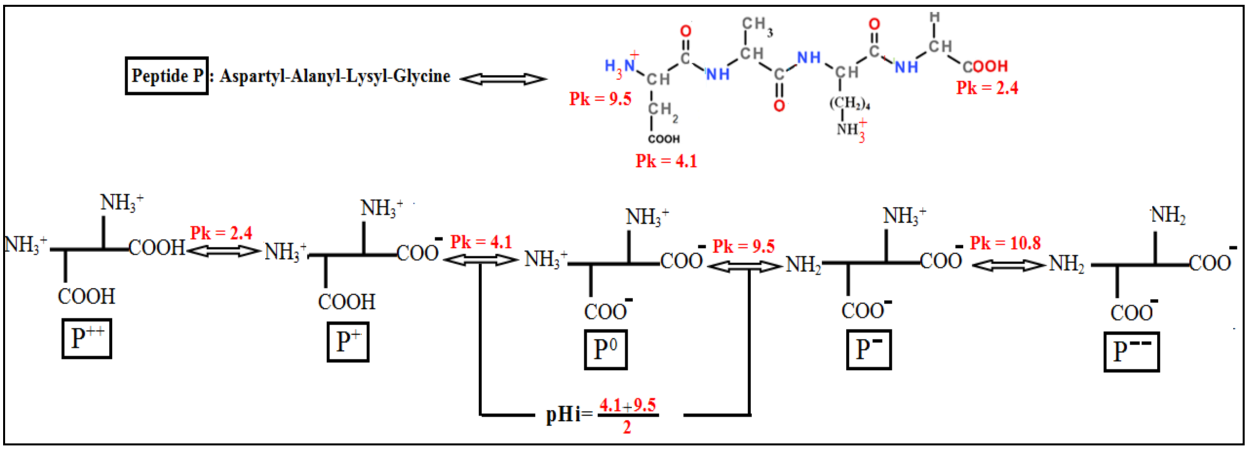


Fig. 8: Ionization of peptide.

Examples of Some Oligopeptides and Polypeptides

- **Glutathione** is a tripeptide γGlu-Cys-Gly; it contains a pseudo-peptide bond (peptoid bond) between the γ-carboxyl group of Glu and the amine group of Cys. It plays a central role in cellular defense against oxygen and its reactive derivatives.
- **Insulin** is a pancreatic hormone polypeptide formed of two chains (one with 21 amino acids and the other with 30 amino acids), which regulates glucose metabolism (hypoglycemia).
- **Glucagon** is a pancreatic hormone, a 29-amino acid peptide, that causes an increase in blood glucose levels (hyperglycemia).
- **Oxytocin**: A nonapeptide, a hormone from the hypothalamus that triggers smooth muscle contractions (uterus for childbirth, mammary gland for milk ejection).
- **Vasopressin**: A nonapeptide, a hormone from the hypothalamus with an antidiuretic effect on the kidneys (hypertensive effect as a medication).

IV. Study of Peptide Sequences

Determining the exact nature, the proportions of each constituent amino acid, and the order of their sequence in a peptide or protein requires, first, the cleavage of covalent bonds.

IV.1. Cleavage of Disulfide Bridges and Separation of Peptide Chains

The covalent structure of peptides involves **disulfide** bridges that can form between chains in the case of polypeptide molecules. These disulfide bonds are formed between two half-residues of **cysteine**. The dissociation of this bond is achieved by cleaving disulfide bridges using:

- **β-Mercaptoethanol**: The protein exchanges its S-S groups with β-Mercaptoethanol, reducing each cystine into two cysteines, while the two molecules of β-Mercaptoethanol are oxidized to form disulfide bonds (Fig. 9). To prevent the re-oxidation of the cysteines formed, **Iodoacetate** is added (Fig.10).

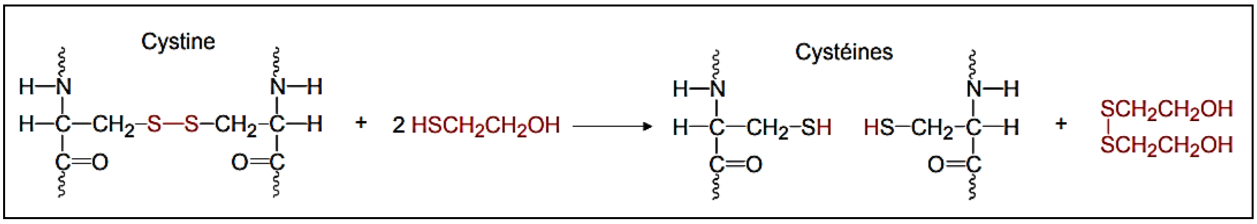


Fig. 9 : Reduction of 2-mercaptoethanol

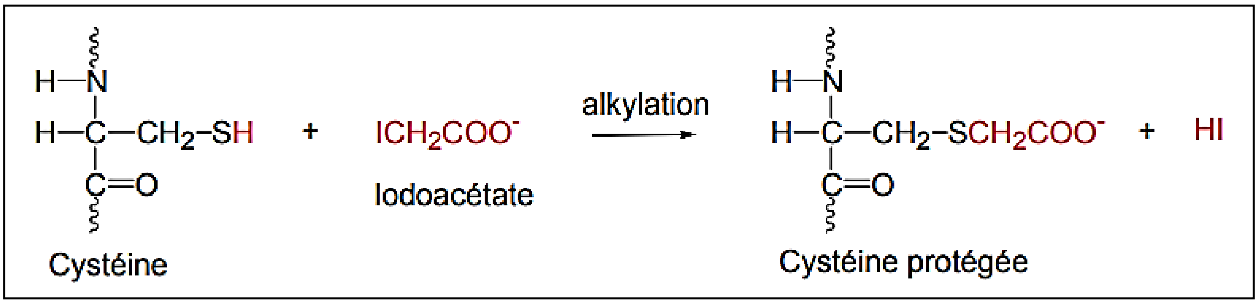


Fig.10 : Prevention of Cysteine Re-oxidation by Iodoacetate

- ➢ **Performic Acid:** All cysteines in a protein are oxidized (those that are linked by disulfide bonds and those in their thiol form). Performic acid cleaves the S-S bonds in proteins and prevents their reformation by converting the cysteine residue into two cysteic acid residues (Fig.11).

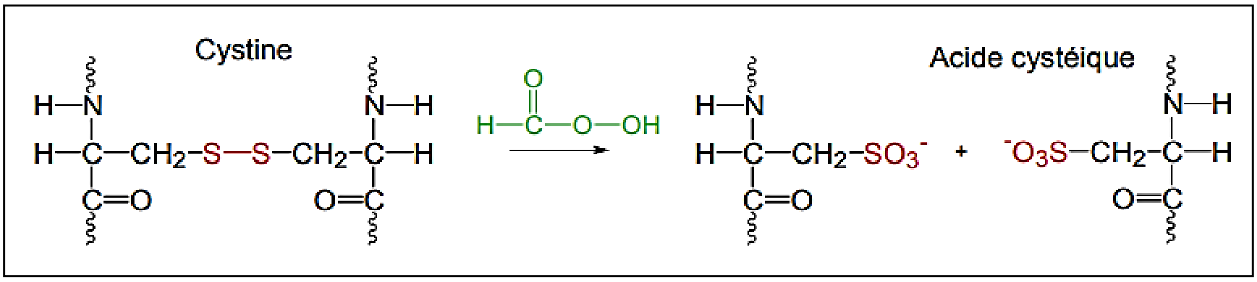


Fig. 11: Oxidation by performic acid.

IV.2. Determination of the Number of Amino Acid Types

The number of amino acids in a peptide chain can be determined via:

IV.2.1. Complete Acid Hydrolysis

Complete hydrolysis of the peptide bond in the presence of strong acid under heat is performed using 6N hydrochloric acid (HCl) at 105°C, boiling for at least 24 hours. This results in a hydrolysate containing amino acids, though with the following **disadvantages**:

- The acidic amino acid **Tryptophan** is completely destroyed due to the sensitivity of the indole ring to acids. Its quantification requires alkaline hydrolysis.
- **Amides** (Asn, Gln) are hydrolyzed into ammonia and their corresponding acids (Asp, Glu).

  This is the most commonly used method, but it requires additional methods to complete the analysis results.

IV.2. 2. Complete Alkaline Hydrolysis

Complete hydrolysis of the peptide bond in the presence of a strong base under heat is carried out using 4N sodium hydroxide (NaOH) at 105°C, boiling for at least 24-72 hours. This results in a hydrolysate containing amino acids, but with the following disadvantages:

- Amino acids **Ser**, **Thr**, **Arg**, and **Cys** are partially destroyed.
- This method is limited to determining the content of **Tryptophan**.

IV.2. 3. Determination of Peptide Chain Structure

To determine the amino acid sequence, it is necessary to first identify the N-terminal and C-terminal amino acids, then the order of the remaining amino acids.

1. Identification of N-terminal Amino Acid

a) **Chemical Methods**

The principle of the chemical method involves fixing the free NH_2 group at the N-terminal of a peptide onto a reagent. Then, hydrolysis of the product is performed, which results in a mixture of amino acids and a derivative of the reagent bound to the N-terminal amino acid (Table I).

Table. I: Identification of AA N^{ter} by chemical methods.

Method	Reagent	Reaction
Sanger	1 Fluoro-2,4-Dinitrobenzene (FDNB)	$\text{FDNB}+\text{NH}_2\text{-AA}_1\text{-AA}_2\text{------AA}_n \longrightarrow \text{DNP-AA}_1+\text{AA Sépares}$
Edman	Phenylisothiocyanate (PITC)	$\text{PITC}+\text{NH}_2\text{-AA}_1\text{-AA}_2\text{....AA}_n \longrightarrow \text{PTC-AA}_1+\text{AA en chaînette}$
Dansyl chloride	1-Dimethyl-Amino-Naphthalene-5-Sulfonyl (DANS)	$\text{DANS}+\text{NH}_2\text{-AA}_1\text{-AA}_2\text{....AA}_n \longrightarrow \text{DNS-AA}_1+\text{AA Sépares}$

b)- Enzymatic Method

The hydrolysis of peptide bonds can be carried out by **proteolytic enzymes** (also called **proteases** or **peptidases**), which are hydrolases. The main specificity of this group of enzymes is the hydrolysis of peptide bonds.

The enzyme **Aminopeptidase** (an exopeptidase) hydrolyzes only the first peptide bond (Fig. 12), releasing the terminal amino acid (except when proline is involved, in which case **Proline Amino-peptidase** will intervene). The released amino acid is identified by chromatography. Since the enzyme can continue hydrolyzing the peptide with **n-1 residues**, several cycles of hydrolysis can be performed.

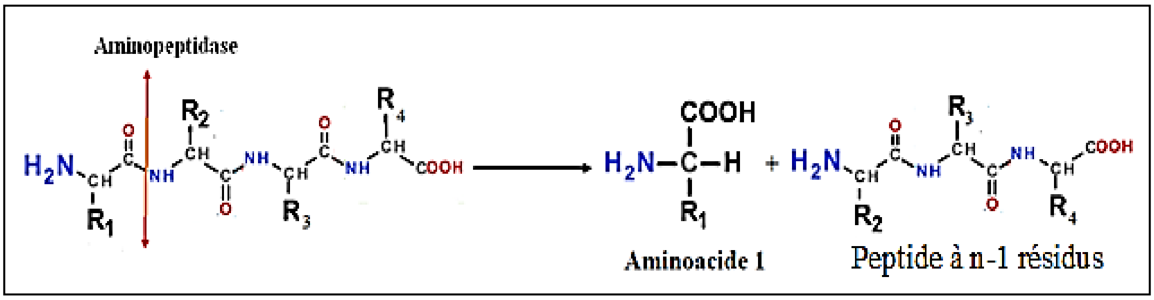


Fig. 12: Effect of Aminopeptidase.

2. Identification of C-terminal Amino Acid

a)- Chemical Methods

Hydrazinolysis: Treatment with **hydrazine** at 100°C hydrolyzes all peptide bonds and releases **hydrazides of all amino acids** except the C-terminal, which remains as a free amino acid (Fig. 13). It is then easy to isolate and identify.

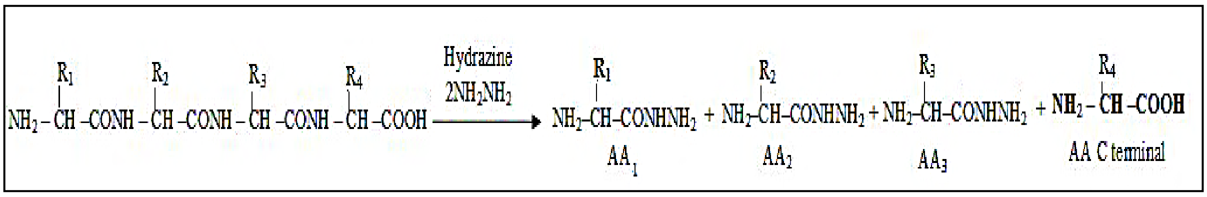


Fig.13: Hydrazinolysis allows the determination of the C-terminal amino acid.

b)- Enzymatic Method

Carboxypeptidase (exopeptidases) hydrolyze peptide bonds starting from the C-terminal residue (Fig. 14); there are several types of carboxypeptidases:

- **Carboxypeptidase A** is a metalloenzyme from pancreatic juice that hydrolyzes all peptide bonds **except** when the C-terminal amino acid is **glycine, proline, or a basic amino acid (Arg, Lys)**. It also does not act if **proline is the penultimate amino acid** in the peptide chain.
- **Carboxypeptidase B**, extracted from the pancreas, removes **basic amino acids** (Lys, Arg) **except** when **proline** is the penultimate amino acid.
- **Carboxypeptidase C** removes all C-terminal amino acids.
- **Proline carboxypeptidase** specifically removes the C-terminal proline.

Note: Since the enzyme can continue hydrolyzing the peptide chain with **n-1 residues**, several cycles can be performed.

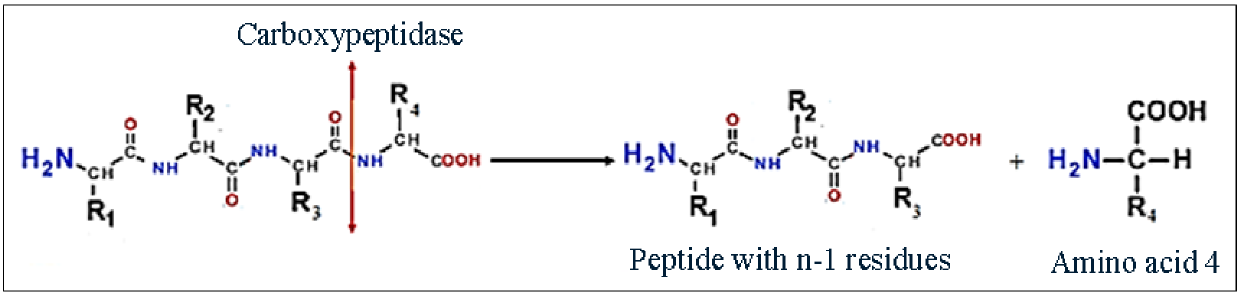


Fig. 14: Effect of Carboxypeptidase

3. Determination of the Amino Acid Sequence

a) Chemical Methods (Specific Chemical Hydrolysis)

- **Cyanogen Bromide (BrCN)**

  BrCN hydrolyzes the peptide bond on the **carboxyl side of methionine** (after **Met↕AA)** (Fig. 15).

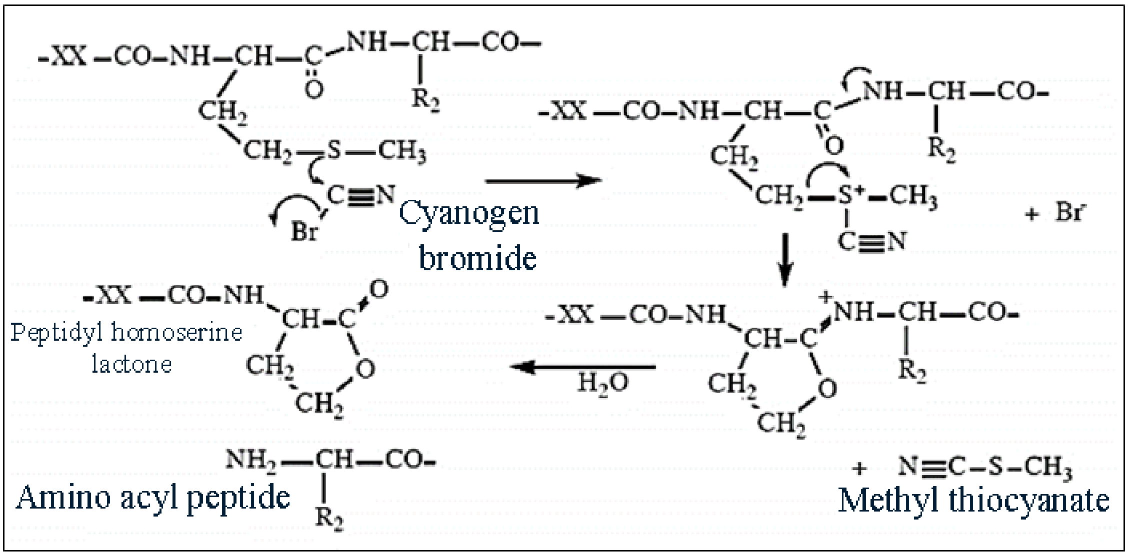


Fig.15: Reaction of Cyanogen Bromide.

- **2-Nitro-5-Thiocyanobenzoate (NTCB)**

NTCB hydrolyzes the peptide bond on the **amine side** of cysteine (before) (**AA ↕ Cys – AA**).

b) Enzymatic Hydrolysis

Endopeptidases are enzymes that **hydrolyze internal** peptide bonds between two **amino acids** [i and (i+1)]. They may be specific to the residue in position i or (i+1). Hydrolysis of a peptide by an endopeptidase will result in multiple peptide fragments (Fig. 16). If there are **m** cleavages (m peptide bonds hydrolyzed), the peptide will be broken down into (**m+1**) peptide fragments. Table II presents some endopeptidases with their specificities.

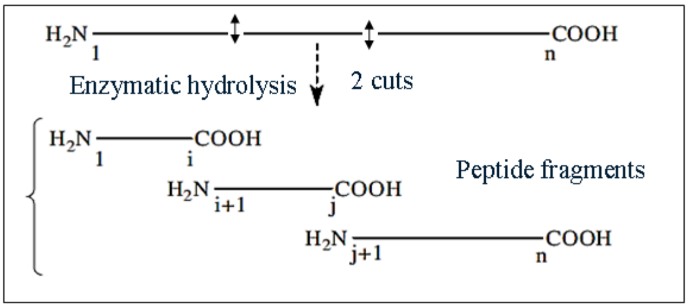


Fig.16: Fragmentation peptidic chain.

Table. II: Some **endopeptidases** and their **specificities**

Enzyme	**Origin**	**Specificity**	**Particularity**
Trypsin	Beef pancreas	C-terminal side of positively charged residues: Arg-↕- and Lys-↕--	Except (i+1) = Pro
Chymotrypsin	Beef pancreas	C-terminal side of aromatic residues: Phe-↕- / Try--↕and Tyr-↕-	Except (i+1) = Pro
Pepsin	Beef gastric mucosa	N-terminal side of aromatic residues: -↕Phe / -↕-Try and -↕-Tyr	Except (i-1) = Pro
Thermolysin	*Bacillus thermoproteolyticus*	N-terminal side of neutral residues: -↕-Leu / -↕-Ile / -↕-Val / -↕Met	Except (i-1) = Pro
Elastase	Beef pancreas	C-terminal side of neutral residues: Gly↕- / Ser-↕- / Val-↕-	Except (i+1) = Pro
Staphylococcus aureus protease	*Staphylococcus aureus*	C-terminal side of negatively charged residues: Asp-↕- and Glu-↕-	Except (i+1) = Pro
Fm Prolinase	*Flavobacterium meningosepticum*	N-terminal or C-terminal side of neutral residues: -↕Pro-↕--	

V. Study of Some Biologically Important Peptides

Peptides contribute to membrane structure as building elements and play important roles in various biological activities. Here are a few examples:

V.1. Peptides with Physico-Chemical Roles

Glutathione is a tripeptide γGlu-Cys-Gly; it contains a pseudo-peptide (peptidoid) bond between the γ-carboxyl group of Glu and the amino group of Cys (Fig. 17). It plays a central role in cellular defense against oxygen and its reactive derivatives. It is a highly effective redox couple against peroxides, since glutathione exists in both a reduced form (thiol) and an oxidized form (in which two molecules are linked by a disulfide bridge, as in cystine).

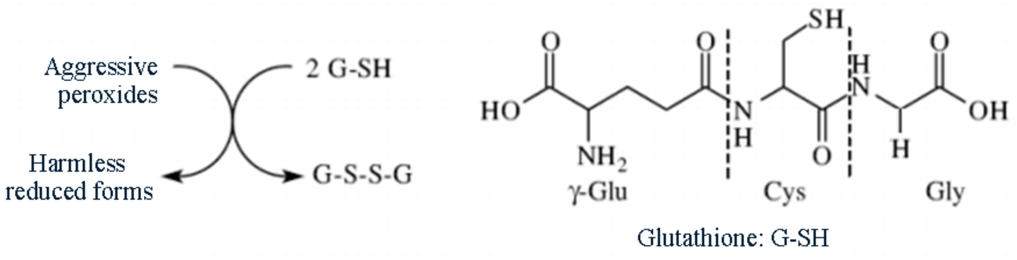


Fig. 17: Structure of reduced Glutathione.

V.2. Peptides with Mediator Activity

This family of peptides is involved in major communication functions within the endocrine system, nerve transmission and modulation, vascular motility, and inflammatory reactions. A few examples are listed below:

V.2.1. Hormonal Peptides

a) Hypothalamus

The hypothalamus is a structure of the central nervous system that contains neurons. One of its most important functions is endocrine activity, through which it secretes two hormonal nanopeptides:

- **Oxytocin**, which triggers contractions of smooth muscles (uterine contractions during childbirth, mammary gland contractions for milk ejection).
- **Vasopressin**, which has an antidiuretic effect on the kidneys (and acts as a hypertensive drug).

b) Anterior lobe of the pituitary gland

It synthesizes and secretes numerous peptide hormones, such as:

- **ACTH (Adrenocorticotropic Hormone)**, a polypeptide of 39 amino acids. It is a hormone of the anterior pituitary that stimulates the synthesis and secretion of steroid hormones by the adrenal cortex.

c)- Endocrine pancreas secretes

- **Insulin**, a polypeptide consisting of two chains (one of 21 and one of 30 amino acids), which regulates glucose metabolism (causing hypoglycemia).
- **Glucagon**, a peptide of 29 amino acids, which increases blood glucose levels (causing hyperglycemia).

V.2.2. Neuropeptides

These are two families of molecules that act as ligands for brain receptors: the **enkephalin family** and the **endorphin family**. These molecules are involved in pain control and share the same N-terminal tetrapeptide sequence (Tyr-Gly-Gly-Phe).

V.2.3. Vasomotor Peptides

Examples of vasoconstrictor peptides include:

- **Angiotensin II**, an octapeptide.
- **Endothelin**, a peptide of 21 amino acids, is a neuropeptide secreted by the vascular endothelium, producing a powerful vasoconstrictive effect on smooth muscle cells.
- **The atria of the heart** secrete a family of **atrial natriuretic peptides (ANP)**, which act on the kidneys by increasing Na^+ excretion and diuresis (water elimination). In humans, **α-ANP** is a polypeptide of 28 amino acids.

V. 2.4. Inflammatory Molecules

Tissue injury triggers a local defense reaction: the **inflammatory response**, characterized by pain, heat, redness, and swelling caused by vasodilator molecules:

- **Amines** such as histamine and serotonin, produced by decarboxylation of histidine (for histamine) and hydroxytryptophan (for serotonin).
- A family of peptides called **kinins**, which relax smooth muscles, causing arterial vasodilation. They act on endothelial cells of blood capillaries to increase their permeability, thus facilitating leukocyte migration. The result of their action is hypotension and pain. The two main kinins are **bradykinin** (a nonapeptide) and **kallidin**.

V.3. Antibiotic Peptides

Certain microorganisms synthesize peptides that inhibit protein synthesis, serving as “weapons” for defense and colonization. These are **cyclic peptides** that incorporate **D- or non-standard amino acids**, features that protect them from proteolytic degradation.

PART III: PROTRIN

I. Generalities

Proteins are hydrolyzable macromolecules composed of sequences of amino acids (more than 100) joined by peptide bonds, which allow for the formation of complex three-dimensional structures: **primary, secondary, tertiary, and quaternary structures**.

II. Classification of proteins

Different types of classification have been proposed:

II.1. Classification based on the shape of the molecules

Proteins can be divided into two main classes based on their shape and certain physical characteristics:

II.1.1. Globular proteins

Also known as **sphero-proteins**, they are spherical or oval in shape. The polypeptide chains forming globular proteins are tightly coiled into a compact structure. They are typically **soluble in aqueous systems** and diffuse quickly. Most have **dynamic functions** (enzymes, antibodies, etc.).

II.1.2. Fibrous proteins

Fibrous proteins, also called **scleroproteins,** are made up of fibers or fibrils and are practically **insoluble in water**. They are elongated, with polypeptide chains extended along an axis rather than coiled into a globular shape. They serve structural or protective roles (keratin, collagen, fibroin, actin, myosin, elastin, etc.).

II.2. Classification based on composition

Two major groups are distinguished:

- **Holoprotein**: A protein whose hydrolysis only yields amino acids.
- **Heteroprotein**: A protein whose hydrolysis yields both amino acids and prosthetic groups. The following are types of heteroproteins :
    - **Phosphoproteins**: Proteins containing phosphoric acid esterifying the alcohol functions of serine and threonine. The most well-known are the caseins found in milk.

- **Glycoproteins**: Result from the covalent association of a protein with a carbohydrate group of varying molecular mass.
- **Chromoproteins**: Colored proteins whose prosthetic group often contains a metal element (Fe, Cu, Mg).

III. Different levels of protein structure

Based on the various interactions between the amino acids that make up proteins, four levels of structure are distinguished:

III.1. Primary structure

The primary structure refers to the linear sequence of amino acids linked together by peptide bonds (Fig. 1).

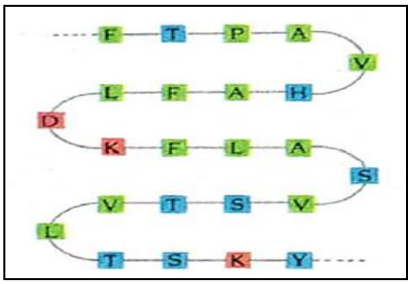


Fig. 1: Primary structure of proteins

III.2. Secondary structure

A chain of amino acids contains many **-CO-** and **-NH-** groups in the peptide bonds, which can form **hydrogen bonds** with each other in space and create a secondary structure. The most common secondary structures are the **α-helix** and the **β-pleated sheet**.

III.2.1. α-Helix

This is a rod-like structure. The main polypeptide chain is tightly coiled, forming the inner part of the rod, while the side chains are positioned outward in a helical arrangement. The helix is stabilized by hydrogen bonds between the NH and CO groups of the main chain. The CO group of each amino acid forms a hydrogen bond with the NH group of the amino acid located 4 residues further along the polypeptide chain (Fig. 2). Each residue is disposed relative to the next with a translation of 1.5 Å along the axis of the helix and a rotation of 100°, which gives 3.6 amino acid residues per turn of the helix.

The helices found in proteins are usually right-handed. Two helices can twist around each other to form a cable. These super-helical coils are found in the **keratin** of hair, **myosin** and **tropomy-**

osin in muscles, the epidermis of the skin, and fibrin in blood clots. These cables serve a mechanical role by forming rigid fiber bundles.

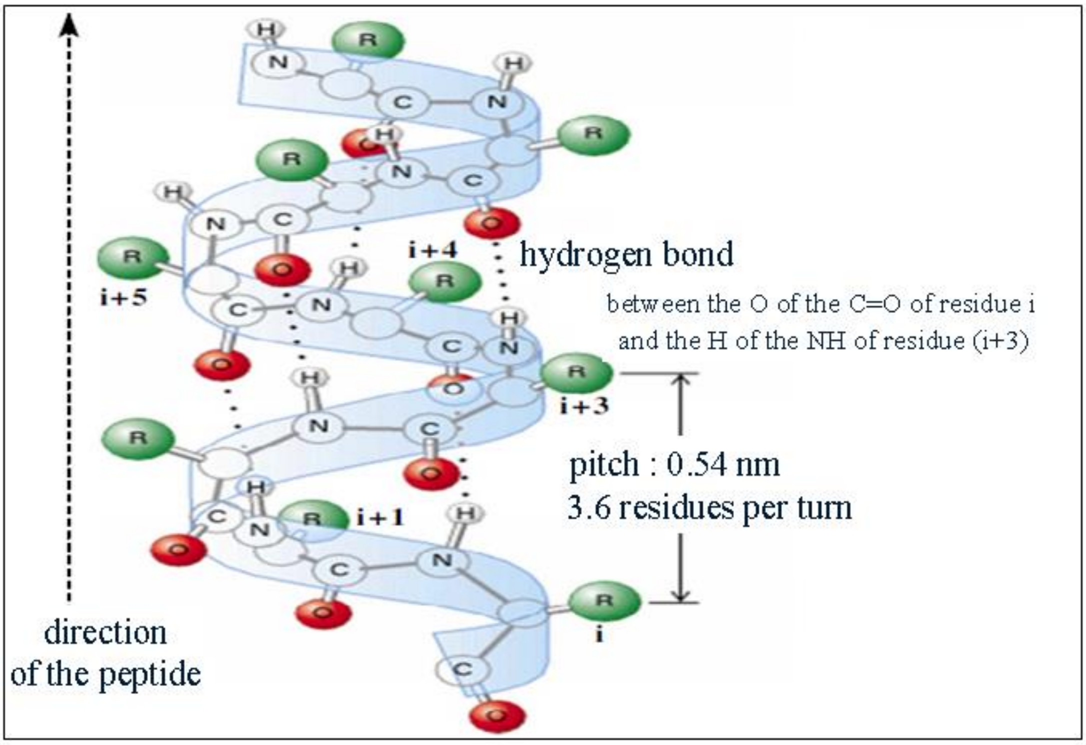


Fig. 2: Structure α-helix

III.2.2. β-sheet

The polypeptide chain in the β-sheet is almost fully extended. The sheet is stabilized by **hydrogen bonds** between the **CO** and **NH** groups of **different polypeptide chains**. The adjacent strands in a β-pleated sheet can run in the same direction, called parallel sheets (Fig. 3), or in opposite directions, called antiparallel sheets (Fig. 4).

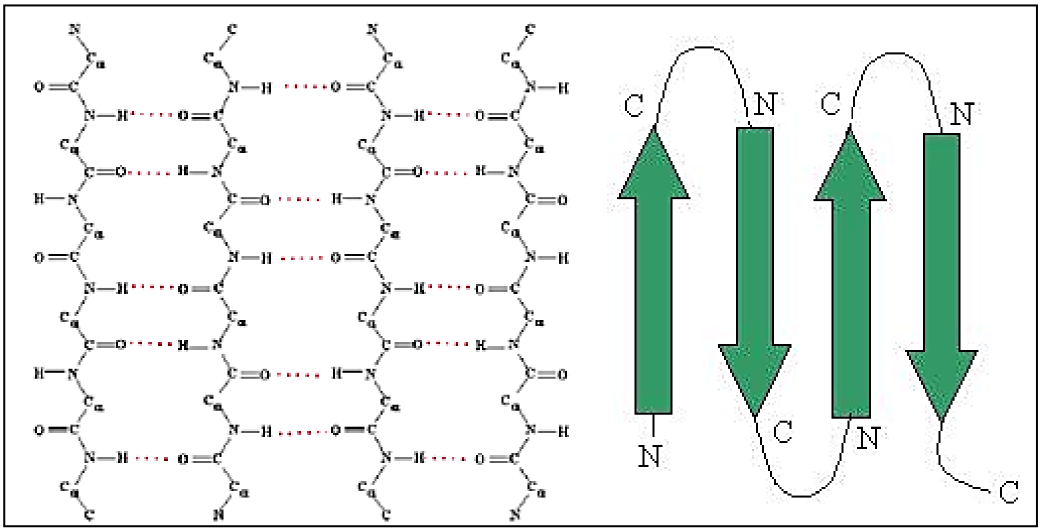


Fig. 3: β-sheet antiparallels

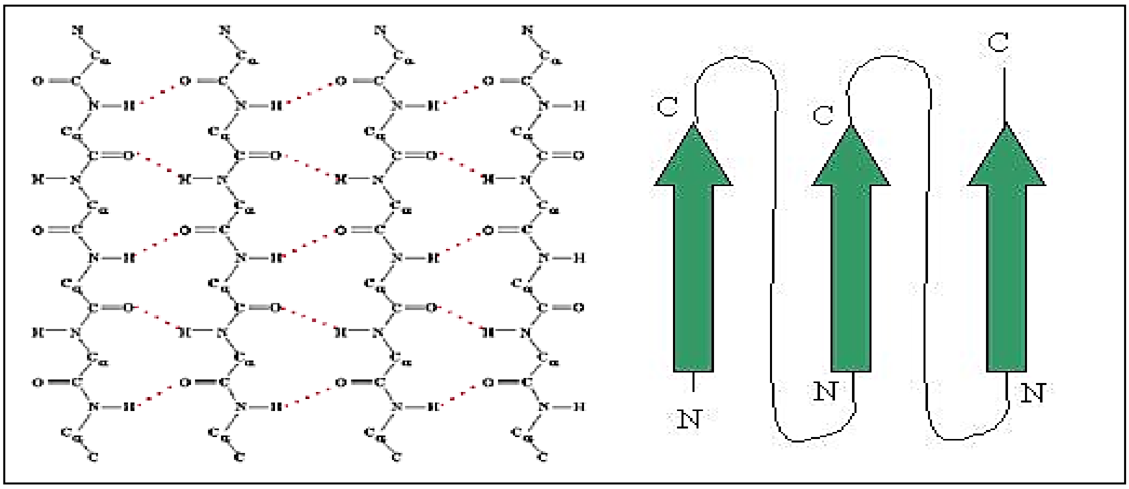


Fig. 4: β-sheet antiparallels

III.3. Tertiary Structure

The term **tertiary structure** refers to the complete three-dimensional conformation of a polypeptide. It describes how elements of secondary structure such as helices, sheets, turns, and loops come together to form domains, and how these domains are connected to each other in three-dimensional space (Fig. 5).

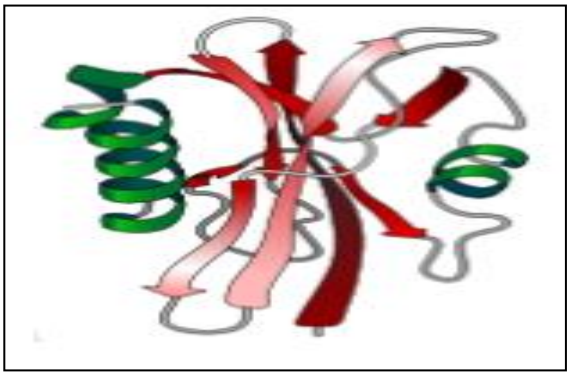


Fig. 5: Tertiary structure

The tertiary structure of a protein is achieved by the folding of the polypeptide chain upon itself, forming various types of bonds (Fig. 6): **hydrogen bonds**, **electrostatic interactions** (between positively charged and negatively charged side chains), **hydrophobic interactions** (van der Waals forces), and **disulfide bonds** (formed between two cysteine thiol groups, denoted as Cys-S-S-Cys).

The tertiary structure is crucial for the biological activity of proteins. Even amino acids that are far apart in the sequence can be brought close together through folding, forming regions essential for the protein's function, such as the active site of enzymes. Polar side chains are typically located on the surface, while hydrophobic residues are hidden in the protein's interior, interacting through hydrophobic bonds.

III.4. Quaternary Structure

The quaternary structure refers to the association of multiple polypeptide chains into a functional complex. Biological activity often exists only at the oligomer level (e.g., dimer, tetramer). The interactions that facilitate the association of these polypeptide subunits are similar to those that occur in the tertiary and secondary structures.

In some cases, the quaternary structure results from the assembly of identical polypeptide subunits, while in other cases, it involves different subunits. For example, hemoglobin consists of four subunits usually two α chains and two β chains (Fig. 7).

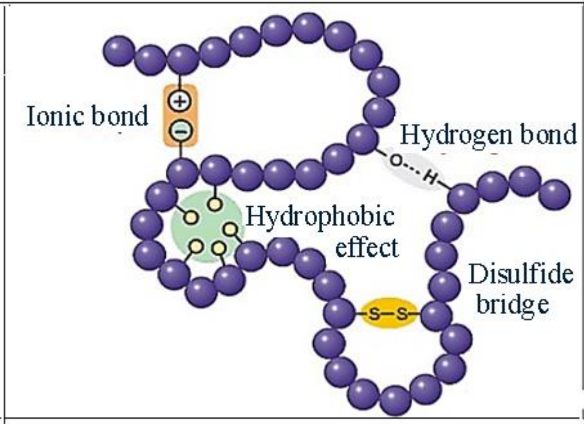
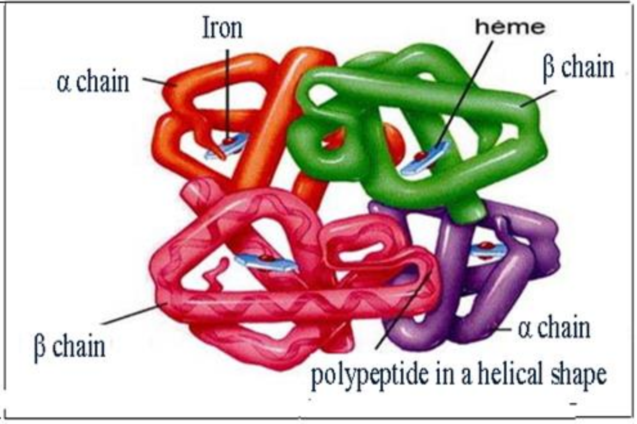
	

Fig. 6: Bonds involved in tertiary structure	**Fig. 7:** Quaternary structure of l'hemoglobulin.

IV. Main properties of proteins

IV .1. Molar masses

The molecular mass ranges from 10 kDa to several million. The molecular mass of a protein is often used as a characteristic element to define or name it.

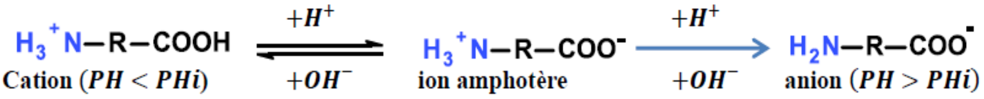
Example: P47 is a 47 kDa protein.

Several methods allow the determination of a protein's molecular mass:

- Dextran gel filtration
- Polyacrylamide gel electrophoresis
- Mass spectrometry

IV.2. Amphoteric nature of proteins

The amphoteric nature of proteins can be described as we did for amino acids, with a higher degree of complexity due to the greater number of charges involved.



$$\underset{\text{Cation }(PH < PHi)}{H_3^{+}N-R-COOH} \underset{+OH^-}{\overset{+H^+}{\rightleftharpoons}} \underset{\text{ion amphotère}}{H_3^{+}N-R-COO^-} \xrightarrow[+OH^-]{+H^+} \underset{\text{anion }(PH > PHi)}{H_2N-R-COO^-}$$

IV.3. Solubility

Solubility is the ability of a substance, called a solute, to dissolve in another substance, called a solvent, to form a homogeneous mixture called a solution. Some proteins are soluble in water, while others are poorly soluble or completely insoluble because protein solubility depends on certain parameters:

- **Influence of electrolytes**: Neutral salts affect solubility depending on their concentration and the charge of the ions, i.e., the ionic strength. At low ionic strength, a dissolving effect is observed, while at high ionic strength, proteins are released, i.e., protein precipitation occurs.
- **Influence of pH**: The solubility of a protein is minimal near its isoelectric point.
- **Influence of organic solvents**: Methyl alcohols, ethanol, and acetone precipitate proteins, provided the temperature is kept around 5°C to prevent protein denaturation by these solvents.

IV.4. Absorption of light by protein solutions

Protein solutions have a characteristic absorption pattern in the UV range.

IV.5. Antigenic properties

Proteins have antigenic properties, meaning that when they are injected into a foreign organism, they induce the production of antibodies in that organism.

As proteins are large molecules, they possess several epitopes or antigenic sites, each capable of inducing the production of a specific type of antibody. It should be noted that antibodies themselves are also protein in nature.

V. Denaturation

Denaturation often leads to protein precipitation and can be reversible or irreversible. The main denaturing agents are:

- **Heat**: High temperatures destroy hydrogen and hydrophobic bonds.
- **Acids and bases**: These act on electrostatic bonds by introducing new charges.
- **Organic solvents**: These destroy hydrophobic bonds.
- **Anionic detergents**: These form electrostatic bonds with $-NH_3^+$ groups and hydrophobic bonds with non-polar side chains.
- **Urea**: This forms many hydrogen bonds with peptide bonds.
- **Reducing or oxidizing agents**: These cause the breaking of disulfide bridges.
- **Heavy metal salts**.
- **UV radiation**.

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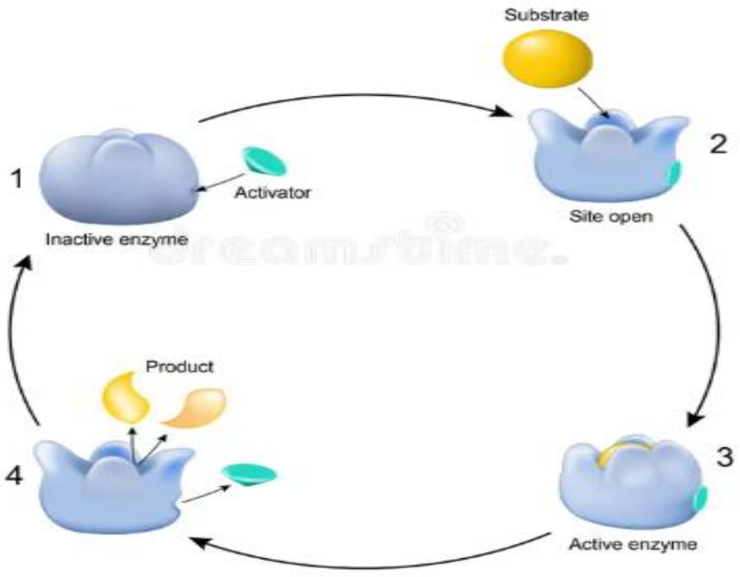
CHAPTRE V : ENZYME



I. Generalities and Biological Importance

Enzymes are biological polymers that catalyze chemical reactions necessary for life. The presence and maintenance of a complete and balanced set of enzymes are crucial for the catabolism of nutrients that provide energy and basic chemical constituents. Enzymes allow the assembly of these basic constituents into proteins, DNA, membranes, cells, and tissues; they help control energy for cellular mobility, neural functioning, and muscle contraction.

The absolute stereospecificity of enzymes is particularly interesting for their use as catalysts in solution or immobilized on a support, to perform specific reactions in the synthesis of a drug or antibiotic. Enzymes play an important role in the production and enhancement of the nutritional value of food products. Chymosin is a protease used in cheese production.

II. Basic Concepts in Enzymology

II.1. Enzyme

Enzymes are the biological catalysts of biochemical reactions:

- **Catalysts:** They increase the rate of chemical reactions without altering the results, by acting at very low concentrations. They remain intact (unchanged) at the end of the reaction.
- **Biological:** They are produced by the cell. Enzymes are proteins (with the exception of ribozymes, which are RNAs with catalytic activity), and their synthesis is genetically determined.

There are two main categories of enzymes:

- **Homogeneous enzymes:** These are purely protein enzymes, consisting only of amino acids.
- **Heteroenzymes:** These enzymes consist of two "parts":
  - **A protein part:** "the apoenzyme"
  - **A non-protein part:** "the cofactor"

The combination of the protein part (apoenzyme) and the non-protein part (cofactor) forms the "heteroenzyme". The cofactor can be linked to the apoenzyme by a weak bond, constituting the co-substrate (coenzyme), or linked by a covalent bond, forming the prosthetic group.

II.2. Substrate

All molecules that enter an enzymatic reaction and are permanently modified are called substrates. The energetic interaction between the enzyme and the substrate is weak (hydrogen bonds, van der Waals forces, electrostatic interactions, hydrophobic interactions).

II.3. Product

A molecule that appears during a reaction catalyzed by an enzyme, that is, a new molecule resulting from this transformation is called a product.

II.4. Cofactor

A chemical substance that is essential in an enzymatic reaction:

- to transport or complete a substrate;
- to accept a product;
- as a participant in the structure of the enzyme.

Cofactors can be ions such as Zn, Mn, Mg, Cu, and Co.

II.5. Coenzymes

A biological molecule acting as an essential cofactor in the enzymatic catalysis of a reaction. Coenzymes are biological molecules, meaning their natural synthesis can only be done by living cells (such as vitamins). Coenzymes can be:

- **Free**
  - ✓ Dissociates from the enzyme at the end of each reaction.
  - ✓ Linked to the enzyme by weak bonds (electrostatic type).
  - ✓ The concentration of the coenzyme is of the same order of magnitude as that of the substrate.
- **Bound**
  - ✓ Does not dissociate from the enzyme.
  - ✓ Linked to the enzyme by strong bonds (covalent type).
  - ✓ Its concentration is the same as that of the enzyme (low).
  - ✓ It is called a prosthetic group.

III. Enzyme Nomenclature and Classification

The name of most enzymes is formed by adding the suffix “-ase” to the term describing the reaction or the nature of the substrate, for example, lactate dehydrogenase. Others are designated by their common name, such as pepsin.

A coherent and suitable naming system has been established to assign names to already existing enzymes and those that may be discovered. For a given enzyme, the following are provided:

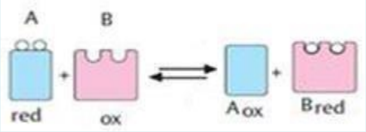
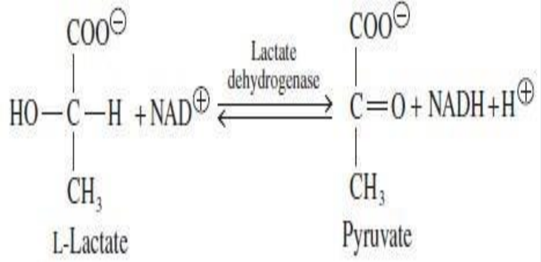
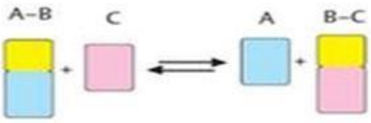
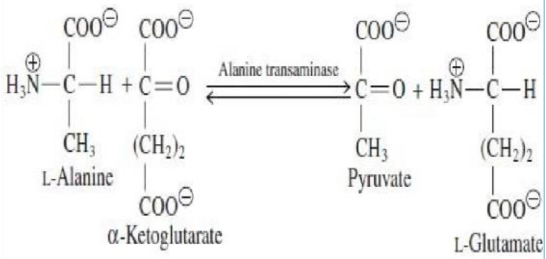
- Code number
- Systematic name
- Recommended common name

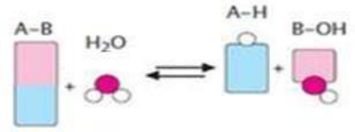
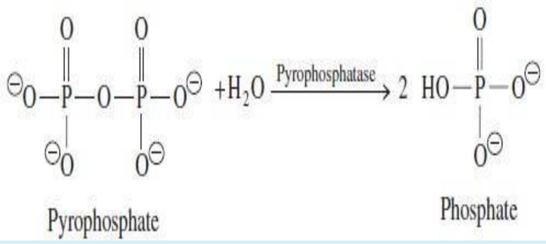
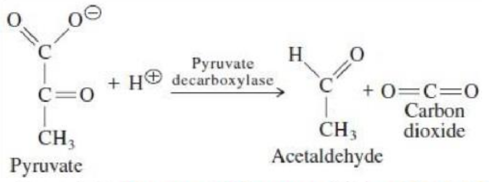
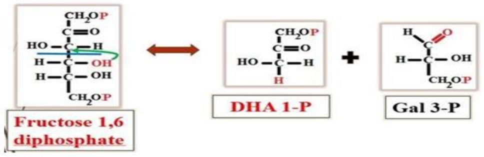
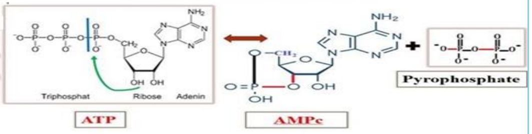
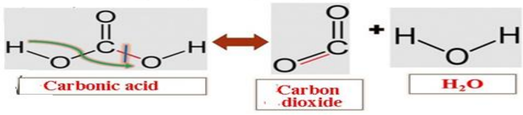
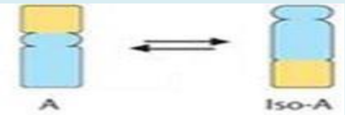
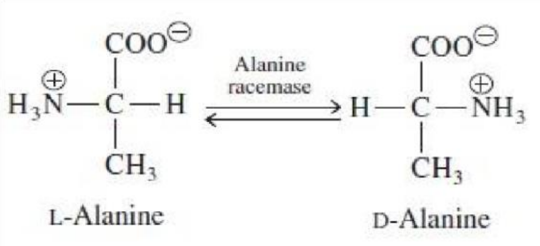
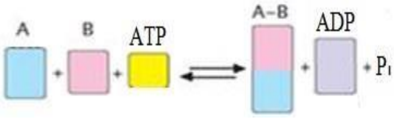
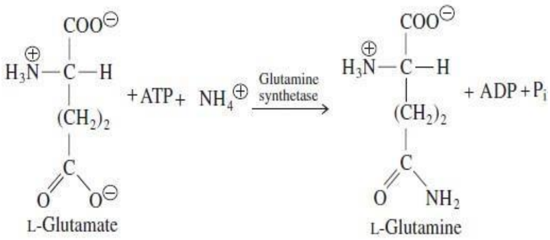
III.1. Official Nomenclature

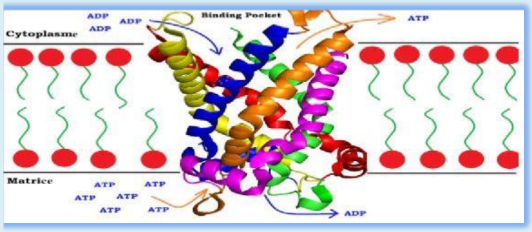
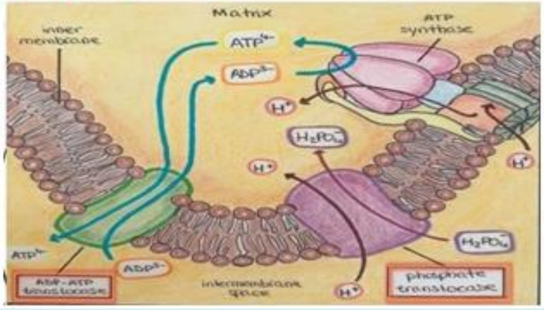
Each enzyme is designated by a number given by the Enzyme Commission of the International Union of Biochemistry and Molecular Biology. This number is preceded by the letters EC and consists of four digits separated by periods: EC (W.X.Y.Z):

- **The first digit (W):** indicates the class of the enzyme, and there are seven classes (Table I).
- **The second digit (X):** represents the subclass, the nature of the chemical group donor, and the type of function of the metabolized substrate.
- **The third digit (Y):** indicates the sub-subclass, describing the chemical nature of the acceptor.
- **The fourth digit (Z):** is the serial number of the enzyme within the sub-subclass, related to the enzyme's substrate.

Table I : Classes of Enzymes.

N°	Classe	Mode of Action	Examples
1	Oxidoreductases	They catalyze oxidation reduction reactions, which involve the transfer of protons and electrons. 	
2	Transferases	They catalyze the transfer reactions of an atom or group of atoms (such as a carbon, phosphorus, or nitrogen group) between two substrates. 	

3	Hydrolases	Catalyze the cleavage of covalent bonds that require the consumption of a water molecule.  	
4	Lyases	Catalyze lytic reactions that are neither hydrolytic nor oxidative, creating double bonds. In the reverse reaction, lyases catalyze the addition of a functional group to the double bond of a substrate.	 Example 2: Fructose diphosphate Aldolase EC 4.1.2.13  Example 3: Adénylate cyclase EC 4.6.1.1  Example 4: Carbonic acid anhydrase EC 4.2.1.1 $H_2CO_3 = CO_2 + H_2O$ 
5	Isomerases (mutases)	Catalyze isomerization reactions. They facilitate the transfer of atoms or groups within the same molecule without changing its overall molecular formula.  	
6	Ligases (synthetases)	Catalyze condensation reactions, which involve the formation of covalent bonds that require the consumption of an energy molecule (ATP).  	

7	**Translocase**	Appearing in 2018. Catalyze the exchange of ions or groups from one side of a membrane to the other. They ensure the movement of ions and molecules across a membrane. For example: ❖ ATP/ADP Translocase ❖ Pyruvate Translocase H^+ Translocase	 **ATP/ADP Translocase** ❖ Example : **ATP/ADP Translocase; H^+ Translocase phosphate translocase** 

III.2. Systematic name

It indicates the nature of the donor, the acceptor and the type of the reaction catalyzed.

III.3. Recommended common name

This is the simple name dedicated to the use, e.g. glucokinase.

IV. Characteristics of enzymes

IV.1. Specificity

A given enzyme is specific to a reaction; it catalyzes the same transformation occurring on the same chemical bodies. This is double specificity:

- **Action specificity:** The enzyme catalyzes only one type of reaction for a given substrate.
- **Substrate specificity:** The enzyme acts only on one substrate or type of substrates. This specificity can be:
  - **Narrow:** The enzyme binds to only one substrate, e.g., salivary amylase binds only to starch.
  - **Broad:** The enzyme acts on a type of substrates, e.g., phosphoesterase hydrolyzes different phosphate esters.

a) - Stereospecificity

Enzymes differentiate between stereoisomers. For example, trypsin only hydrolyzes L-series amino acids. The specificity of an enzyme is due to its active site.

b)- Active Site

The active site is the region of the enzyme that allows for the recognition and binding of the substrate. It is also the site of catalysis. The active site is a spatial structure: a hydrophobic internal pocket that appears when the protein folds into its tertiary structure. Two models have been proposed to elucidate this specificity:

- **Fisher Model (1890):** The lock and key model (Fig.1). The shape of the substrate (key) is complementar y to that of the enzyme's active site (the lock).

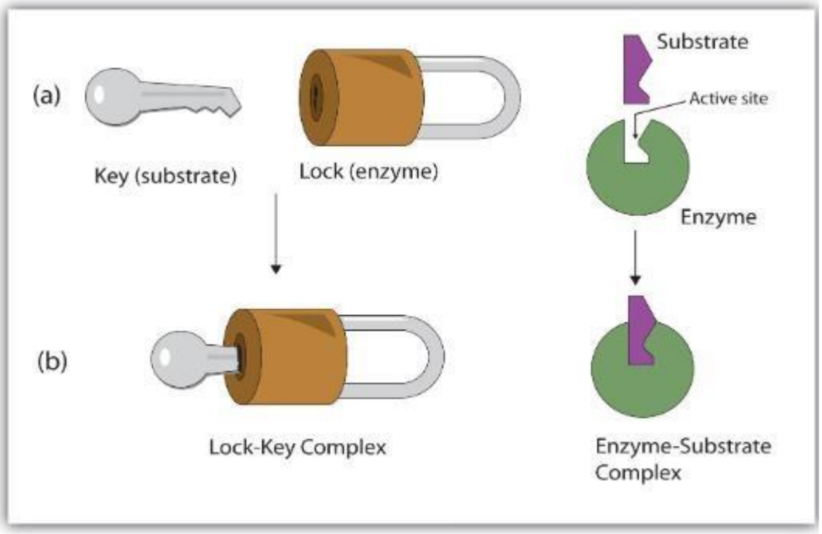


Fig. 1: Fisher's lock-and-key model of the active site

- **Koshland Model (1985):** The induced fit model. The enzyme is not rigid but flexible. The enzyme and the substrate mutually adapt their respective shapes, becoming complementary only within the enzyme-substrate complex (Fig. 2).

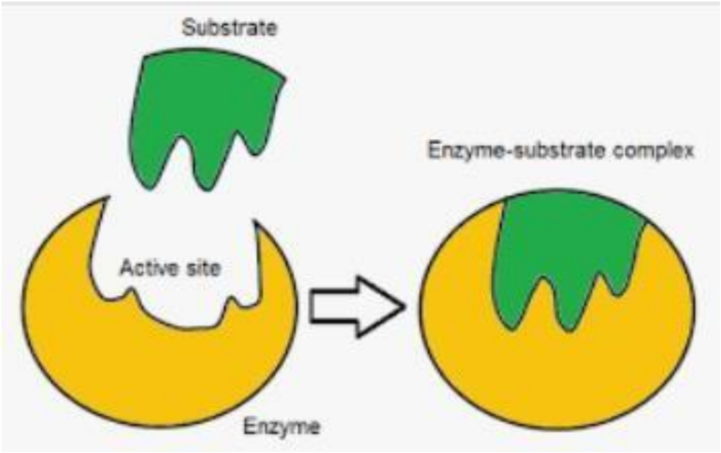


Fig. 2: Active site, Koshland model

IV.2. Efficiency

Enzymes are more efficient than chemical catalysts. At the active site, catalysis is due to:

- The local increase of reactant molecules;
- The proper orientation of the molecules;
- The decrease in the free energy of activation of the reaction (Fig.3).

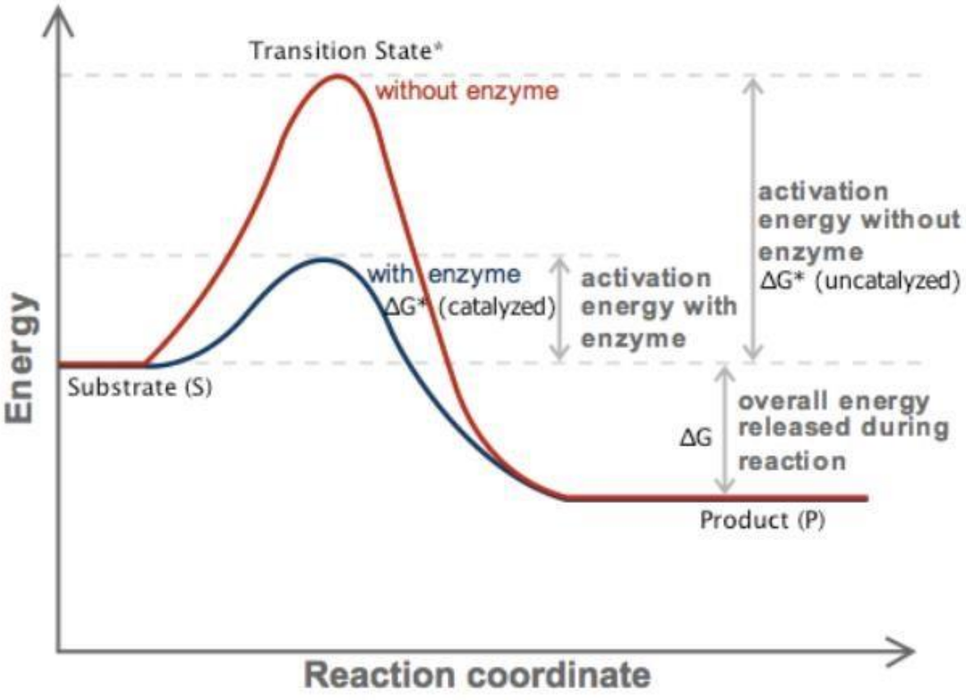


Fig. 3: Free energy of activation in the presence of enzyme

IV.3. Thermolability

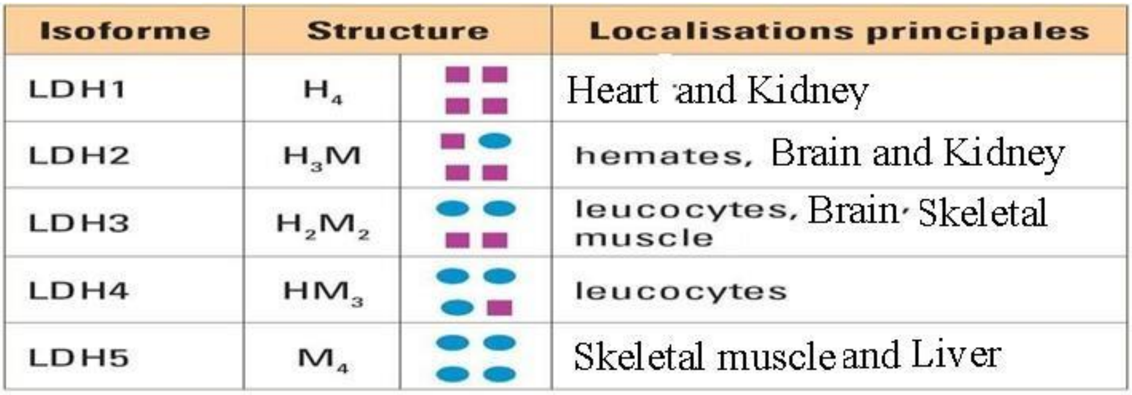
Enzymes are proteins that denature under the effect of heat.

IV.4. Regulated

Some enzymes modify their catalytic activity according to the cellular need.

IV.5. Molecular Variety: Isoenzymes

Isoenzymes are multiple forms of enzymes, with different protein structures and different affinities. They catalyze the same reaction with the same substrate but have different distributions in the organism (Fig. 4). For example, lactate dehydrogenase (LDH).



Isoforme	Structure		Localisations principales
LDH1	H_4	■■ ■■	Heart and Kidney
LDH2	H_3M	■● ■■	hemates, Brain and Kidney
LDH3	H_2M_2	●● ■■	leucocytes, Brain, Skeletal muscle
LDH4	HM_3	●● ●■	leucocytes
LDH5	M_4	●● ●●	Skeletal muscle and Liver

Fig. 4 : Lactate dehydrogenase isoenzymes.

Roles of Isoenzymes

- The difference in affinity allows the regulation of enzyme activity according to the tissue.
- The activity of the isoenzyme provides insight into the state of the tissue to which it belongs.

V. Enzyme Kinetics

V.1. Definition

It is the study of the rates of enzymatic reactions and their changes in response to variations in experimental conditions.

V.2. Reaction Rate or Catalysis Rate

It is defined by the amount of substrate transformed (dS) per unit of time (dt) or the amount of product formed (dP) per unit of time (dt), as seen in Figure 5. This rate is measured by

$V = -dS/dt = dP/dt = K[S]$, where K is a rate constant.

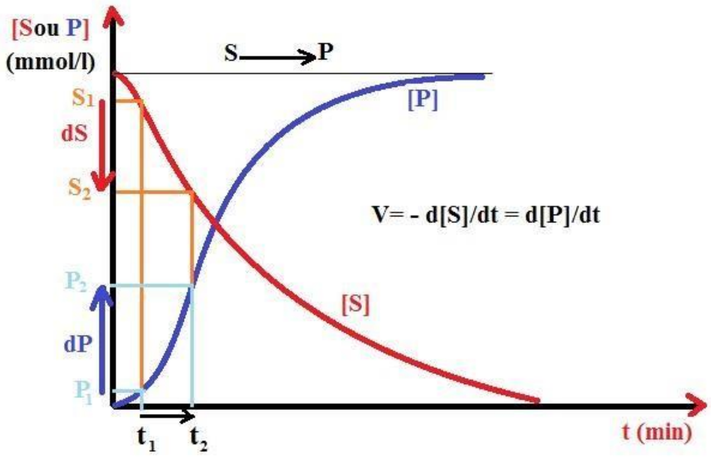


Fig. 5: Enzyme reaction rate

V.3. Influence of Substrate and Enzyme Concentration on Reaction Rate

The rate of the enzymatic reaction increases with the substrate concentration until it reaches the maximum rate (Vmax) when the enzyme is saturated with the substrate. The rate also increases with the increase in enzyme concentration (Fig. 6).

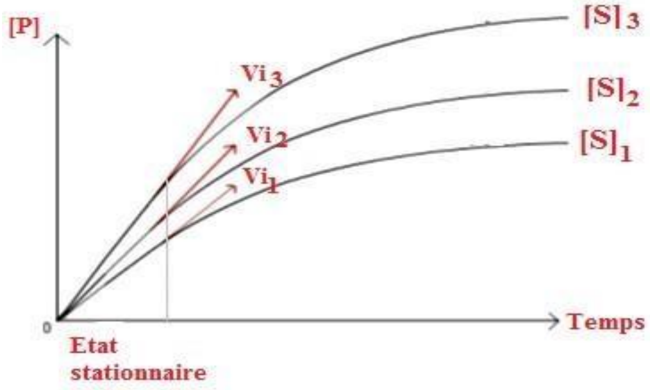


Fig. 6: Effect of substrate concentration on initial reaction rate

V.4. Michaelis-Menten Kinetics

To establish the Michaelis-Menten equation, the following conditions must be met:

- The product concentration must be negligible compared to that of the substrate to avoid the reverse reaction.
- The concentration of the enzyme-substrate complex (ES) must remain constant over time.

V.4.1. Mathematical demonstration: Let the enzymatic reaction be (1):

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} P + E \quad \text{------}(1)$$

Reaction (1) is broken down into two steps: formation of the ES complex (2) and its dissociation (3)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \quad \text{-----}(2)$$

$$ES \xrightarrow{k_2} E + P \quad \text{-----}(3)$$

The rate of disappearance of the substrate is equal to the rate of appearance of the product (rate of the enzymatic reaction):

$$v = -\frac{d[S]}{dt} = \frac{d[P]}{dt} \quad \text{-----}(4)$$

The rate of disappearance of the substrate is equal to the difference between the rates V1 and V2 of the equation (2):

$$-\frac{d[S]}{dt} = V_1 - V_2$$

According to the law of mass action: V1 = k1 [E] [S] and V2 = k -1[ES]

According to reaction (3), the speed of appearance of the product is: V3 = k2 [ES]

$$\frac{d[P]}{dt} = V_3 = k_2\,[ES] \text{ - - - - -} (5)$$

If we replace in: V_1-V_2= V_3

- ⇨ k_1 [E] [S]- k $_{-1}$[ES]= k_2 [ES]
- ⇨ k_1 [E] [S]= [ES] (k_2+ k $_{-1}$) => [E] [S]/ [ES]= (k_2+ k $_{-1}$)/ k_1

K_M = (k_{-1}+ k_2) /k_1 = Michaélis (-Menten) constant, dissociation constant of the enzymesubstrate complex.

V.4.2. Michaelis-Menten equation

Let: [**E_t**] be the total concentration of the enzyme. The free concentration of the enzyme will be:

[E]= [E_t]- [ES]

The expression for the Michaélis constant becomes:

Km = [E][S]/[ES]= ([Et]- [ES]) [S]/[ES]= ([Et][S]/[ES]) - ([ES][S]/ [ES]) = ([Et][S]/[ES]) - [S]

=> Km+ [S] = ([Et] [S]/ [ES]

=> [ES]= [Et] [S]/ (Km+[S]) --------(7)

The rate of the enzymatic reaction is equal to V3: V = V3 = k2 [ES] ------------- (8) By substituting [ES] (7) into equation 8:"

V= k_2 [Et] [S]/ (Km+[S]) ----------------(9)

According to equation (8), the rate will be maximal (Vmax) when [ES] is maximal: when the total enzyme is combined with the substrate: [ES] = [Et]

- ⇨ V_{max}= k_2 [E_t] ----------(10), equation (9) becomes :

$$V_0 = V_{max} \cdot \frac{[S]}{[S] + K_M} \text{ - - - - - Equation de Michaélis Menten}$$

V.4.3. Meaning of maximum speed Vmax and Michaélis constant Km

Vmax :

- Corresponds to the initial speed when the enzyme is saturated by its substrate.
- Provides information on the catalytic efficiency of the enzyme, Vmax= k2[Et] =>Vmax = kcat [Et]
- **kcat** : Turn Over Number « **TNO** », number of substrate molecules transformed into product per unit of time, this is the catalytic efficiency of the enzyme: the frequency of the catalytic act (s^{-1}).
- **Km:** Michaelis Constant. Km is the substrate concentration at which the enzymatic reaction rate is at half of Vmax. It is a measure of the enzyme's affinity for the substrate:
  - **A low Km** indicates a high affinity of the enzyme for the substrate, as a low substrate concentration is sufficient to reach half of the maximum velocity.
  - **A high Km** means a low affinity, requiring a higher substrate concentration to reach half of Vmax.

"its substrate and the reverse.

- Initial substrate concentration at which Vi = 1/2 Vmax, so half of the active sites of the enzyme are occupied by the substrate.
- When [S] >>> Km"

$$V_i = V_{max} = k_{cat}\,[Et]$$

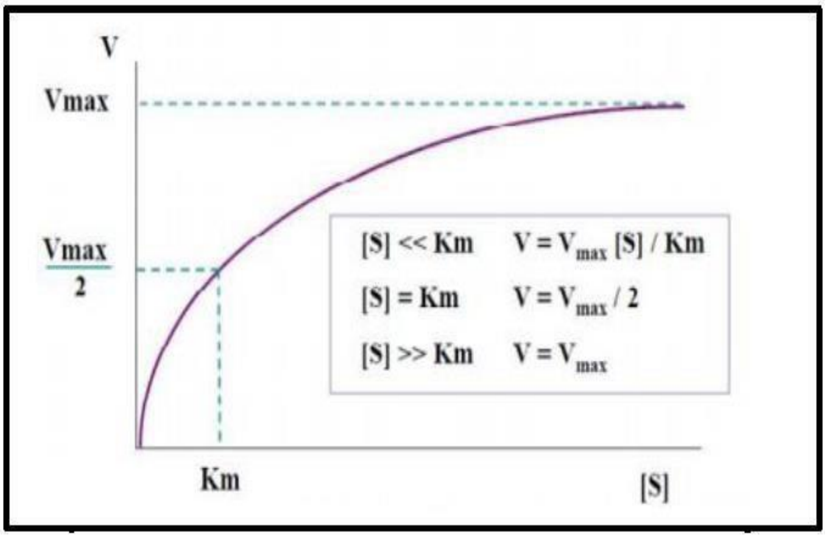


Fig. 7: Direct representation of the Michaelis hyperbola

V.5. Lineweaver-Burk Plot Method

Experimentally, it is not possible to determine Vmax; only an approximate value is known. The two important values, Vmax and Km, can be obtained through various mathematical treatments, among which the double reciprocal plot (Lineweaver-Burk Plot) is one method. Lineweaver and Burk established a rate equation derived from the Michaelis-Menten equation, which allows for the determination of Km and Vmax values. Double reciprocal plot:

$$\mathbf{1/V = f\,(1/[S]) \rightarrow}$$

$$\frac{1}{v} = \frac{K_M}{V_M}\left(\frac{1}{[S]}\right) + \frac{1}{V_M}$$

This is the equation of a line in the form: y = ax + b. The kinetic parameters Km and Vmax are determined graphically by direct reading from the x-axis (abscissa) and the y-axis (ordinate) (Fig. 8).

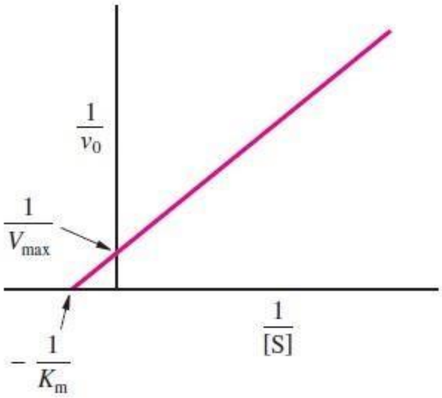


Fig. 8: Graphical representation by Lineweaver and Burk.

V.6. Enzyme Unit

It is the quantity of enzyme that catalyzes the transformation of a certain amount of substrate per unit of time. Currently, there are two units:

- **International Unit (IU):** The amount of enzyme that catalyzes the transformation of one micromole of substrate per minute.
- **Katal (kat):** The amount of enzyme that catalyzes the transformation of one mole of substrate per second.

Specific Enzymatic Activity

The number of substrate molecules transformed per minute per milligram of enzyme. It is used to verify the purity of an enzyme preparation.

$$\text{AES} = \frac{\text{UI}}{\text{mg de protéine}} = \frac{\mu\text{mole}}{\text{min}\cdot\text{mg de protéine}}$$

Molecular Enzymatic Activity

The number of substrate molecules transformed per minute per molecule of enzyme.

$$\text{AEM} = \frac{\text{UI}}{\mu\text{mole de protéine}} = \frac{\mu\text{mole}}{\text{min}\cdot\mu\text{mole de protéine}}$$

V.7. Factors Influencing Enzymatic Reaction

Many factors modulate the activity of enzymes, including:

- ✓ **Physico-chemical parameters:** Such as temperature, pH, and ionic strength.
- ✓ **Chemical parameters:** Including metals, denaturing agents, covalent modifications, and proteolysis.

V.7.1. Physico-chemical parameters

a)- Influence of pH pH affects the ionization state of proteins and impacts enzyme activity:

- **At extreme pH levels,** enzymes are denatured.
- **At neutral pH,** the groups essential for activity are ionized.

b)- Influence of Temperature

- **Above 40 or 45°C:** Heat denatures the secondary and tertiary structures of the enzyme, and its activity rapidly drops to zero.
- **At lower temperatures:** The ambient heat provides additional energy that facilitates the enzymatic reaction.

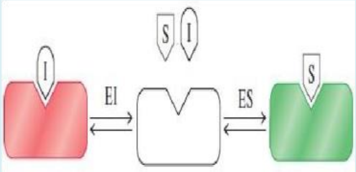
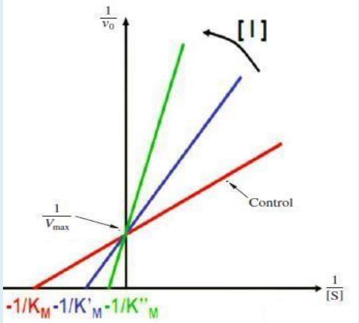
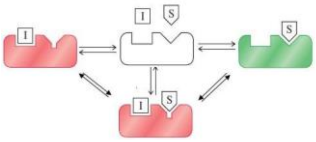
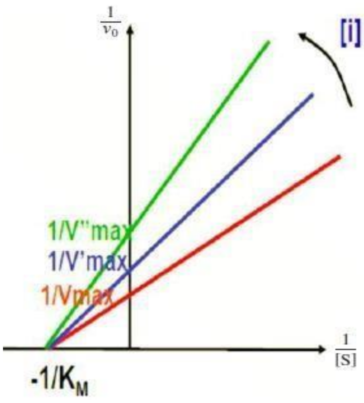
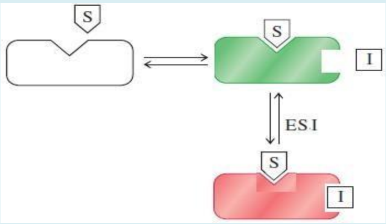
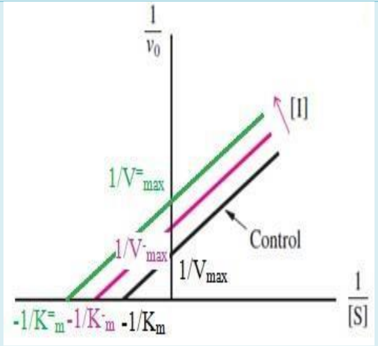
V.7.2. Enzyme Activity Effectors

Any molecule that modifies the rate of an enzymatic reaction is called an effector. Effectors that increase enzymatic activity are activators, whereas those that decrease it are inhibitors; some molecules can act as either an activator or an inhibitor depending on the conditions.

V.7.2.1. Enzyme Inhibitors

An inhibitor is a ligand (a chemical that specifically binds to a protein at a particular binding site) that binds to the enzyme but does not undergo transformation, altering the enzyme's behavior. Many types of inhibition have been described. In this study, we will consider three examples: competitive inhibition, non-competitive inhibition, and uncompetitive inhibition, which will be summarized in Table II.

Table II : Enzymatic inhibitions

Type of inhibition	Mode of action	Consequence	Graphical representation
Competitive	The inhibitor has a structural resemblance to the substrate, and both compete to bind to the same enzymatic site.  	**$V_{max} = V^{-}_{max}$** Affinity decreases => K_m increases => K^{-}_m = **Fi *** K_m $K_{-m} > K_m$ => $1/K^{-}_m < 1/K_m$ => **$-1/K^{-}_m > -1/K_m$**	
Non-Competitive	The inhibitor can bind with equal affinity to both the free enzyme and the enzyme-substrate complex. The substrate binds to the active site, while the inhibitor binds to a different binding site.  	Same affinity => $K_{-m} = K_m$ V_{max} decreases => $V_{-max} = V_{max}$/**Fi** **$V_{max} > V^{-}_{max}$**=> $1/V_{max} < 1/V_{-max}$	
Uncompetitive	The inhibitor never binds to the free enzyme but only to the enzyme-substrate complex (ES), preventing the formation of products.  	Affinity increases => K_m descreases => K^{-}_m = K_m /**Fi** $K_m > K^{-}_m$ => $1/K_m < 1/K^{-}_m$ => **$-1/K_m > -1/K^{-}_m$** **V_{max}** descreases => $V_{-max} = V_{max}$/**Fi** **$V_{max} > V^{-}_{max}$**=> $1/V_{max} < 1/V_{-max}$	

Let us know that: $\mathbf{Fi = 1 + \frac{[I]}{KI}}$

V.7.2.2. Enzyme Activators

Several types exist:

- **Metal ions:** They promote proper enzyme binding and participate in catalysis, e.g., DNA polymerase requires Mg^{2+}.
- **Limited proteolysis (Pro-enzyme):** Through specific cleavage of peptide bonds, allowing the active site to appear, e.g., chymotrypsin.
- **Covalent modifications:** The enzyme can exist in two interconvertible forms, one active and the other inactive, e.g., phosphorylation and dephosphorylation.

VI. Allosteric Enzymes

Some enzymes do not follow Michaelis–Menten kinetics, where the curve Vi = f(S) is a branch of a rectangular hyperbola, but instead display a sigmoidal curve (Fig. 10), these are allosteric enzymes.

Allosteric enzymes have a quaternary protein structure, composed of several subunits, each capable of binding a substrate molecule. The arrangement of these subunits is such that the molecule has an axis of symmetry.

Binding of the substrate to one subunit increases the enzyme's affinity for additional substrate molecules, which gives rise to the sigmoidal shape of the curve; this is known as the cooperative effect.

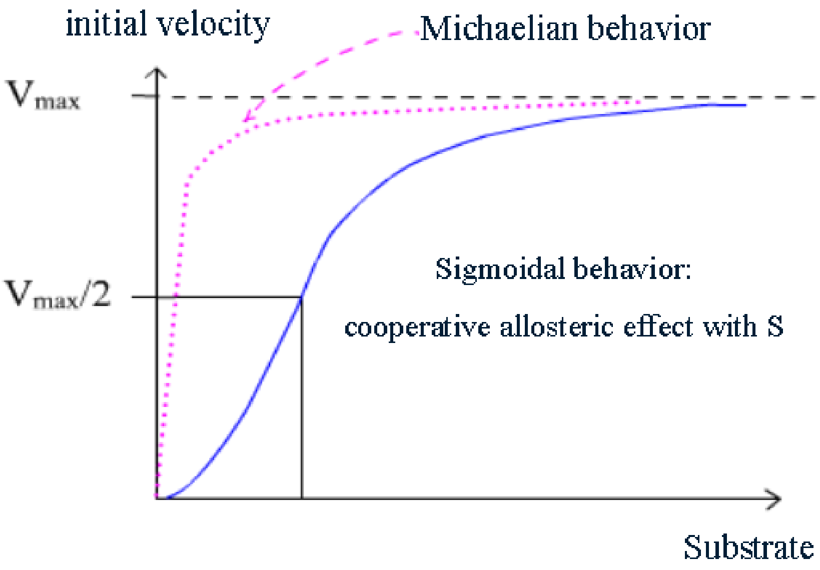


Fig. 10: Kinetics of allosteric enzymes

An allosteric enzyme, in addition to its **catalytic site**, has an **effector site** that allows the binding of molecules other than the substrate (Fig. 11), this is known as the **heterotropic effect**:

- **Positive effector:** allosteric activator
- **Negative effector:** allosteric inhibitor

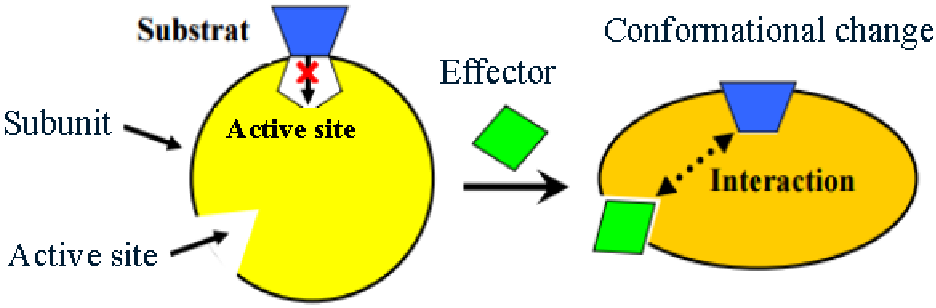


Fig. 11: Structure of an allosteric enzyme.

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