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Ministry of Higher Education and Scientific Research

Ferhat Abbas University Setif1

Faculty of natural and life science

Department of Basic studies

Biochemistry

A Course Designed to **2nd Year Basic studies** Students (LMD)

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Table of content

CHAPITRE III: STRUCRURE AND PHYSICO-CHEMICAL PROPRIETES OF AMINO ACIDS, PEPTIDES AND PROTEINES

CHAPTER IV: STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF LIPIDS

List of figures

List of tables

General Introduction

Biochemistry, study of the chemical substances and processes that occur in plants, animals, and microorganisms and of the changes they undergo during development and life. It deals with the chemistry of life, and as such it draws on the techniques of analytical, organic, and physical chemistry, as well as those of physiologists concerned with the molecular basis of vital processes. All chemical changes within the organism—either the degradation of substances, generally to gain necessary energy, or the buildup of complex molecules necessary for life processes—are collectively called metabolism. These chemical changes depend on the action of organic catalysts known as enzymes, and enzymes, in turn, depend for their existence on the genetic apparatus of the cell. It is not surprising, therefore, that biochemistry enters into the investigation of chemical changes in disease, drug action, and other aspects of medicine, as well as in nutrition, genetics, and agriculture.

The term biochemistry is synonymous with two somewhat older terms: physiological chemistry and biological chemistry. Those aspects of biochemistry that deal with the chemistry and function of very large molecules (e.g., proteins and nucleic acids) are often grouped under the term molecular biology. Biochemistry has been known under that term since about 1900. Its origins, however, can be traced much further back; its early history is part of the early history of both physiology and chemistry.

Course Objectives

The objectives of this course are to familiarize students with knowledge of:

- Fundamentals of chemistry and biology and the key principles of biochemistry and molecular biology.
- Basic biochemistry needed for higher level courses
- Chemical concepts with particular reference to chemical process found within living cells (chemical bonds, functional groups, equilibrium…)
- Building blocks of cellular components
- Structure and properties of amino acids, peptide bond
- Structure and properties of proteins
- Structure and properties of carbohydrates
- Structure and properties of fatty acids and lipids

CHAPTER I: Chemicals Bonds

I.1. Definition

Chemical bonding refers to the force that holds the chemical molecules or atoms together. The combination of atoms leads to the formation of a molecule that has distinct properties different from that of the constituent atoms. These bonds make significant contributions to the structure, stability, and functional competence of macromolecules in living cells.

Strength of the bond varies considerably, depends on the molecules or atoms involved in the process of bond formation. When two atoms of same or different elements approach each other, the energy of the combination of the atoms becomes less than the sum of the energies of the two separate atoms at a large distance.

I.2. Classification

Based on dissociation energy, chemical bonds can be classified as strong or weak.

I.2.1. Strong forces

These forces involve the sharing or transfer of electrons between atoms, resulting in the formation of strong chemical bonds. These bonds require significant energy to break. Examples include: Covalent Bonds**,** Ionic Bonds, Metallic Bonds.

I.2.1.1. Covalent bonds

Covalent chemical bonds are the strongest bonds in biochemistry, it involve the sharing of a pair of valence electrons by two atoms, in contrast to the transfer of electrons in ionic bonds (Fig.1). Such bonds lead to stable molecules if they share electrons in such a way as to create a noble gas configuration for each atom.

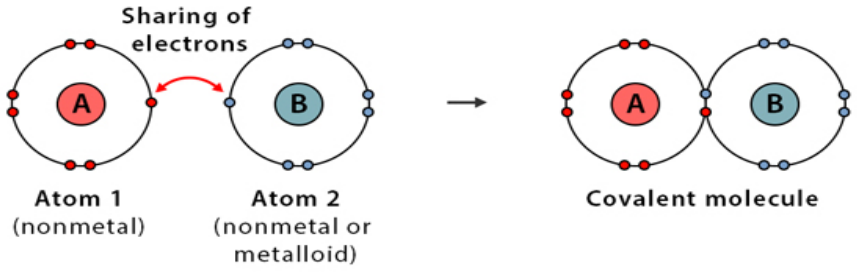


Fig.1: Covalent Bond Formation through Electron Sharing

Hydrogen gas forms the simplest covalent bond in the diatomic hydrogen molecule. The halogens such as chlorine also exist as diatomic gases by forming covalent bonds. The nitrogen and oxygen which makes up the bulk of the atmosphere also exhibits covalent bonding in forming diatomic molecules.

Additionally to simple covalent bonds, covalent bonds can also exist as double or triple bonds, as represented in Fig. 2.

Double Bond: is a covalent bond in which four electrons (two pairs) are shared by the bonding atoms.

Triple Bond: is a covalent bond in which two atoms share three pairs of electrons. Nitrogen gas is an example of a triple bond.

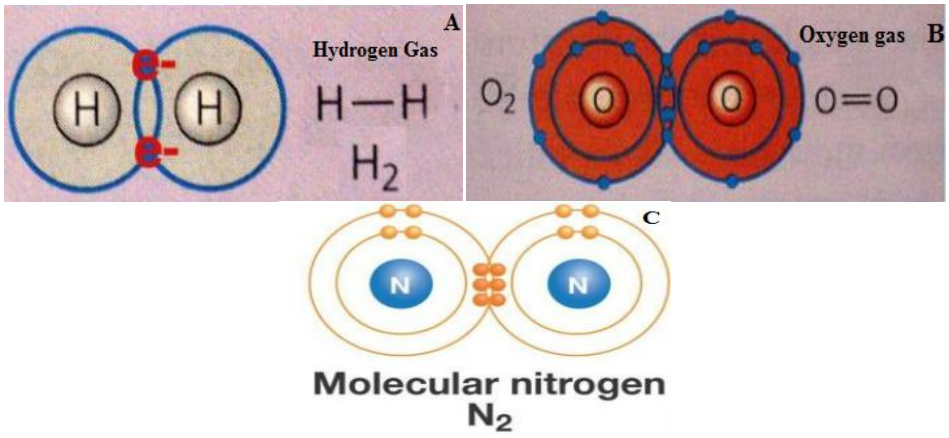


Fig. 2: Covalent bond types; A: single bond, B: double bond, C: triple bond

Covalent bonds can be:

- **Nonpolar:** where electrons are shared equally between atoms (usually when the bonded atoms have similar electronegativities). Example: Covalent bonds that are formed between identical atoms, as in oxygen gas (O_2) and hydrogen gas (H_2), Cl_2
- **Polar:** where electrons are shared unequally due to differences in electronegativity, resulting in partial charges on the atoms. Example: bond between the two atoms H and Cl.

Covalent bonds exhibit high bond dissociation energies because they involve the sharing of electrons between atoms. Moreover, many covalent bonds are stable in aqueous environments because they are not prone to hydrolysis. For instance, the carbon–hydrogen (C–H) and carbon–carbon (C–C) bonds found in amino acids are excellent examples. These bonds contribute to the structural framework of proteins and nucleic acids, and other key biomolecules, ensuring that they maintain their integrity and function under physiological conditions.

I.2.1.2. Ionic bond

Ions are atoms or molecules which are electrically charged. Cations are positively charged and anions carry a negative charge. Ions form when atoms gain or lose electrons. Since electrons are negatively charged, an atom that loses one or more electrons will become positively charged; an atom that gains one or more electrons becomes negatively charged.

Ionic bonding is the attraction between positively- and negatively-charged ions. These oppositely charged ions attract each other to form ionic networks (or lattices). Electrostatics explains why this happens: opposite charges attract and like charges repel. Fig. 3 gives an example of this bond.

When many ions attract each other, they form large, ordered, crystal lattices in which each ion is surrounded by ions of the opposite charge

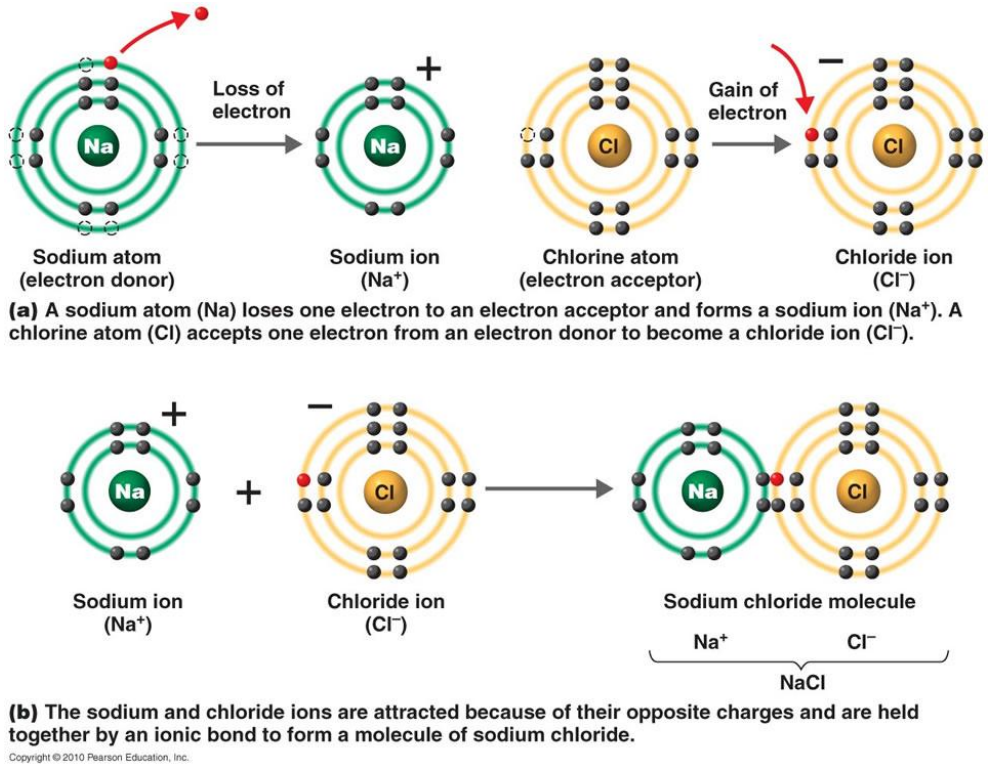


Fig. 3: Ionic bond between sodium ions and chloride ions

I.2.1.3. Metallic bond

Metallic bond is the force that holds atoms together in a metallic substance. Such a solid consists of closely packed atoms. In most cases, the outermost electron shell of each of the metal atoms overlaps with a large number of neighboring atoms. As a consequence, the valence electrons continually move from one atom to another and are not associated with any specific pair of atoms. In short, the valence electrons in metals, unlike those in covalently

bonded substances, are nonlocalized, capable of wandering relatively freely throughout the entire crystal. The atoms that the electrons leave behind become positive ions, and the interaction between such ions and valence electrons gives rise to the cohesive or binding force that holds the metallic crystal together (Fig. 4).

Many of the characteristic properties of metals are attributable to the non-localized or free-electron character of the valence electrons. This condition, for example, is responsible for the high electrical conductivity of metals. The valence electrons are always free to move when an electrical field is applied. The presence of the mobile valence electrons, as well as the nondirectionality of the binding force between metal ions, account for the malleability and ductility of most metals. When a metal is shaped or drawn, it does not fracture, because the ions in its crystal structure are quite easily displaced with respect to one another. Moreover, the nonlocalized valence electrons act as a buffer between the ions of like charge and thereby prevent them from coming together and generating strong repulsive forces that can cause the crystal to fracture.

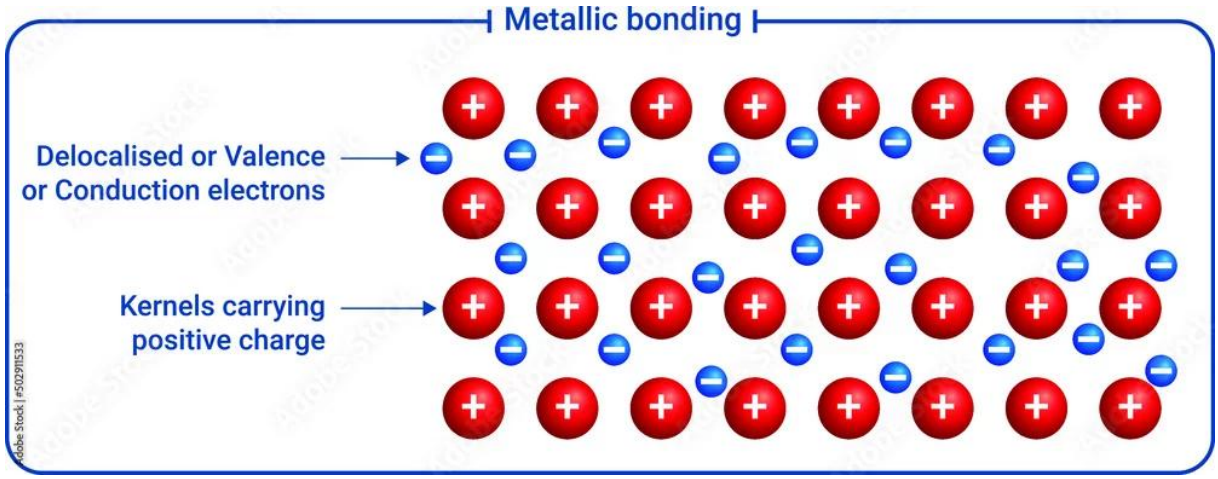


Fig.4: diagram of metallic bond.

I.2.2. Weak forces

These forces are significantly weaker than strong forces and involve interactions between molecules or parts of molecules, rather than the formation of direct chemical bonds between atoms. They require much less energy to disrupt. Examples: Hydrogen Bonds, Hydrophobic interaction, Van Der Waals attraction.

This difference in bond strength and energy has a profound impact on the physical and chemical properties of substances, such as melting and boiling points, solubility, and reactivity.

I.2.2.1. Van der Waals Forces

Van Der Waals forces arise from attractions between transient dipoles generated by the rapid movement of electrons of all neutral atoms, leading to the formation of instantaneous dipoles that interact with one another.

Significantly weaker than hydrogen bonds but potentially extremely numerous, Van der Waals forces decrease as the sixth power of the distance separating atoms. Thus, they act over very short distances, typically 2 to 4 Å. These forces are responsible for many physical properties of substances, such as boiling points, melting points, and solubility (Fig.5).

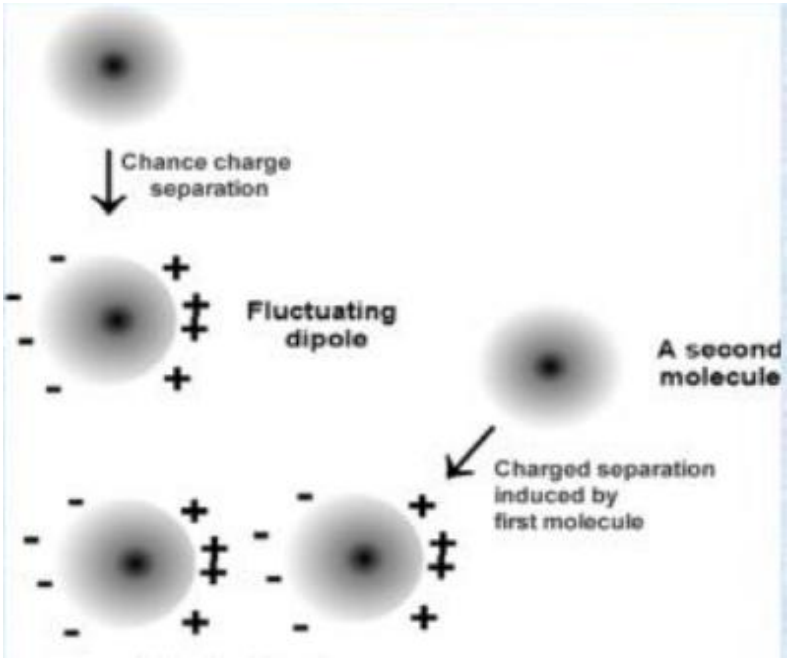


Fig.5: Diagram of Van der Waals forces

There are three main types of Van der Waals forces:

- *London Dispersion Forces:* Also known as instantaneous dipole-induced dipole forces, these are the weakest form of Van der Waals forces. They occur between all atoms and molecules, including nonpolar ones, due to temporary fluctuations in electron distribution around the particles.
- *Dipole-Dipole Interactions:* These forces arise between polar molecules that possess permanent dipoles. The positively charged end of one molecule is attracted to the negatively charged end of another molecule, resulting in an attractive force.

- *Dipole-Induced Dipole Interactions:* These forces occur between a polar molecule with a permanent dipole and a nonpolar molecule. The permanent dipole induces a temporary dipole in the nonpolar molecule, leading to an attractive force between the two.

I.2.2.2 Hydrogen bonds

Hydrogen bonding is a weak to moderate attractive force that exists between a hydrogen atom covalently bonded to a very electronegative atom, X, and a lone pair of electrons on another small, electronegative atom, Y. Hydrogen bonds are strongest when the bonded molecules allow for linear bonding patterns. Ideally, the three atoms involved are in a line as shown in Fig.6.

Hydrogen Bonds have a important role in the unique properties of water, structure and function of proteins, DNA, polysaccharides, binding of substrates to enzymes, binding of hormones to receptors, matching of mRNA and tRNA.

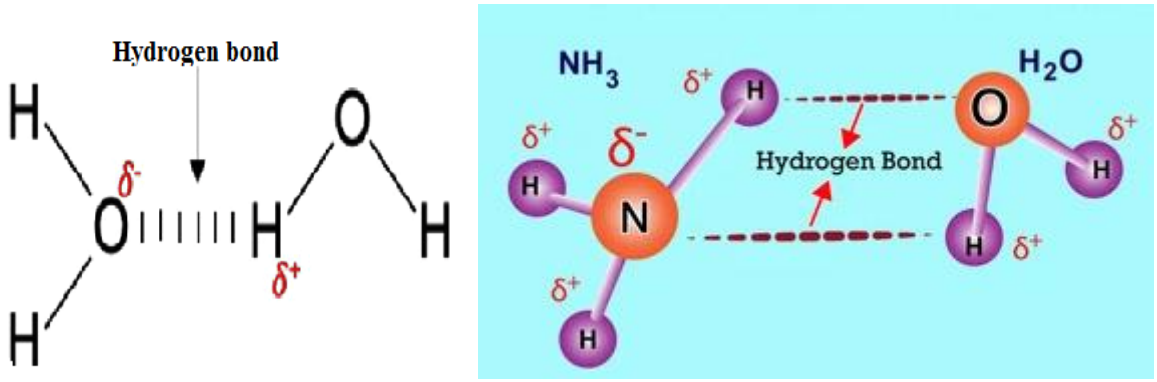


Fig. 6: Hydrogen bonding between different molecules.

I.2.2.3. Hydrophobic interaction

Hydrophobes are nonpolar molecules and usually have a long chain of carbons that do not interact with water molecules. The mixing of fat and water is a good example of this particular interaction.

The Hydrophobic interaction is an association or interaction of nonpolar components of molecules in the aqueous solution as depicted in Fig.7. It is one of the main factors behind: protein folding, protein-protein association, formation of lipid micelles, binding of steroid hormones to their receptors. Binding sites in enzymes and receptors are often Hydrophobic.

Such sites can bind hydrophobic substrates and ligands, such as steroid hormones. Many drugs are designed to take advantage of the hydrophobic effect.

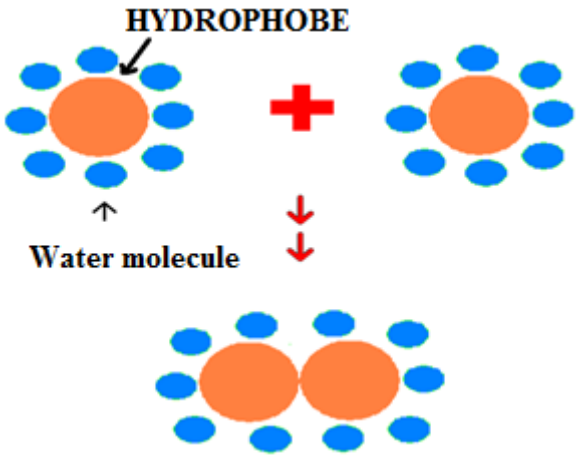


Fig. 7: Diagram of Hydrophobic interaction; the image indicates that when the hydrophobes come together, they will have less contact with water.

Chapter II: STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF CARBOHYDRATE

II.1. Background

Until the 1960s, carbohydrates were thought to have only passive roles as energy sources (e.g., glucose and starch) and as structural materials (e.g., cellulose). Carbohydrates, as we will see, do not catalyze complex chemical reactions as do proteins, or replicate themselves as do nucleic acids. And because polysaccharides are not built according to a genetic "blueprint," as are nucleic acids and proteins, they tend to be much more heterogeneous both in size and in composition than other biological molecules.

However, it has become clear that the innate structural variation in carbohydrates is fundamental to their biological activity. An understanding of carbohydrate structure, from the simplest monosaccharides to the most complex branched polysaccharides, is essential for appreciating the varied functions of carbohydrates in biological systems.

II.2. Biological significance of carbohydrates

Carbohydrates play a crucial role in the biology of all living organisms. They serve as essential components in structural, biological, and genetic functions. Their versatility allows them to participate in various cellular processes, from energy storage to communication between cells.

Energy-related Functions:

- Carbohydrates are an important and rapid source of energy (4.2 Cal/g).
- They serve as energy storage in plants in the form of starch (found in fruits, vegetables, and whole grains) and in mammals as glycogen (found in animal products like meat).

Structural Functions:

- Carbohydrates act as structural components in many organisms, such as cellulose in plants, which provides support.

Cellular Communication and Protection Functions:

- They can be components of cell membranes, playing a role in intercellular communication.
- They serve as cell-surface antigens, contributing to recognition and adhesion between cells.

- They form part of the body's extracellular matrix, where carbohydrate polymers help lubricate skeletal joints.
- Carbohydrates in the form of glycoproteins are found in mucosal secretions (e.g., in saliva and the digestive system), aiding in lubrication and protection.

Associations with Other Macromolecules:

- Carbohydrates are associated with proteins and lipids, forming glycoproteins and glycolipids, respectively.
- Carbohydrates are part of nucleic acids, with ribose and deoxyribose found in RNA and DNA.
- Carbohydrates are involved in the formation of several coenzymes, including NAD+, NADP+, FAD, and CoA.

II.3. Definition

Carbohydrates or **saccharides** (Greek: *sakcharon*, sugar) are the most abundant biological molecules. They are chemically simpler than nucleotides or amino acids, containing just three elements: carbon, hydrogen, and oxygen—combined according to the formula $(CH_2O)n$, where $n \geq 3$.

Carbohydrates can be divided into subcategories based on their complexity. Fig.8 illustrates the main classes of carbohydrates. The simplest carbohydrates are the monosaccharides which are the simple sugars required for the biosynthesis of all the other carbohydrate types. Disaccharides consist of two monosaccharides that have been joined together by a covalent bond called the glycosidic bond. Oligosaccharides are polymers that consist of a few monosaccharides covalently linked together, and Polysaccharides are large polymers that contain hundreds to thousands of monosaccharide units all joined together by glycosidic bonds.

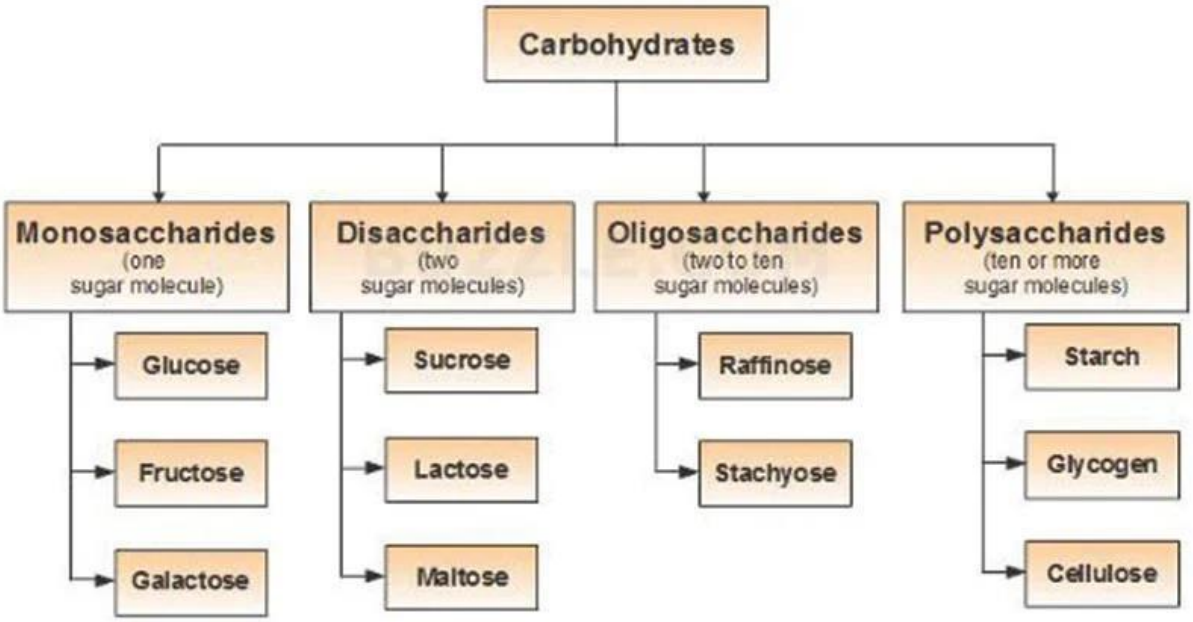


Fig. 8: Carbohydrate classification

II.4. Monosaccharides

Monosaccharides are aldehyde or ketone derivatives of straight-chain polyhydroxyalcohols containing at least three carbon atoms. They are classified according to the chemical nature of their carbonyl group and the number of their C atoms. If the carbonyl group is an aldehyde, the sugar is an **aldose**. If the carbonyl group is a ketone, the sugar is a **ketose (Fig.9)** .

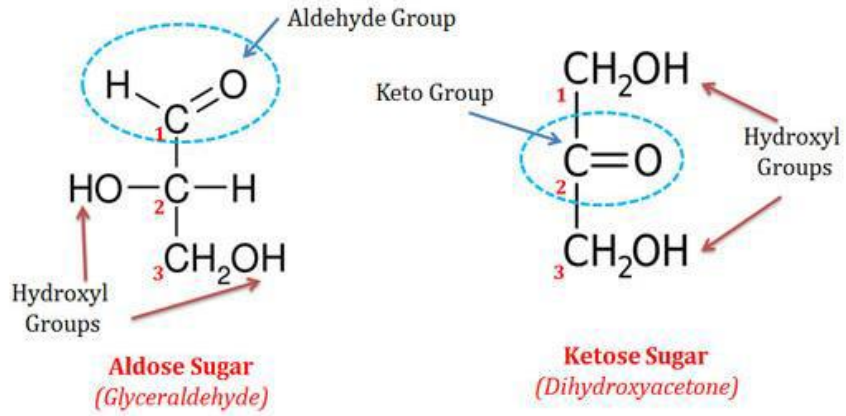


Fig. 9: Difference between aldose and ketose sugars

II.4.1. Nomenclature

There are numerous different types of monosaccharides, which, differ in their number of carbon atoms and in the arrangement of the H and O atoms attached to the carbons. Monosaccharides are named by:

Prefix of the Latin number of the carbon atoms forming the sugar + the suffix –ose Furthermore, monosaccharides can be strung together in almost limitless ways to form polysaccharides. The smallest monosaccharides, those with three carbon atoms, are **trioses**.

Those with four, five, six, seven, etc. C atoms are, respectively, **tetroses, pentoses, hexoses, heptoses**, etc **(Tab. 1).**

Tab.1: Monosaccharides nomenclature.

Arabic number	Latin	sugar	
3	Tri-	AldoTriose	KetoTriose
4	Tetr-	AldoTetrose	KetoTetrose
5	Pent-	AldoPentose	KetoPentose
6	Hex-	AldoHexose	KetoHexose
7	Hept-	AldoHeptose	KetoHeptose
8	Oct-	AldoOctose	KetoOctose

II.4.2. Linear chains of Monosaccharides

II.4.2.1. Fischer projections

Fischer projections are a useful way to represent the 3-dimensional structure of sugars in only two dimensions. The Fischer Projection show sugars in their open chain form and consists of both horizontal and vertical lines.

The carbon atoms of a sugar molecule are connected vertically by solid lines, while carbon-oxygen and carbon-hydrogen bonds are shown horizontally, with carbon atoms sometimes not shown and represented by the center of crossing lines.

In an aldose, C1 is the carbon of the aldehyde group; in a ketose, C1 is the carbon closest to the ketone group, which is typically found at C2. The arrangement of the atoms distinguishes one stereoisomer from the other.

Monosaccharides are further classified stereochemically as D (dextro) and L (levo) on the configuration of the **asymmetric carbon** farthest away from the carbonyl group in straight chained compounds. If the farthest hydroxyl (–OH) group on the carbon atom next to the last CH_2OH is on the right as represented in the Fisher projection, it is classified as D and if on the left, classified as L. Most of the sugars found in nature are in the D-conformation; the much rarer L-sugars are produced primarily by certain fungal and microbial organisms for specialized uses.

Asymmetric carbon A carbon has the ability to make four single bonds and if it bonds to four different chemical groups, their atoms can be arranged around the carbon in two different

ways, giving rise to enantiomers. Each carbon with such a property is referred to as a stereogenic center or chiral carbon. Fig. 10 illustrates the deference between D and L structure of glucose.

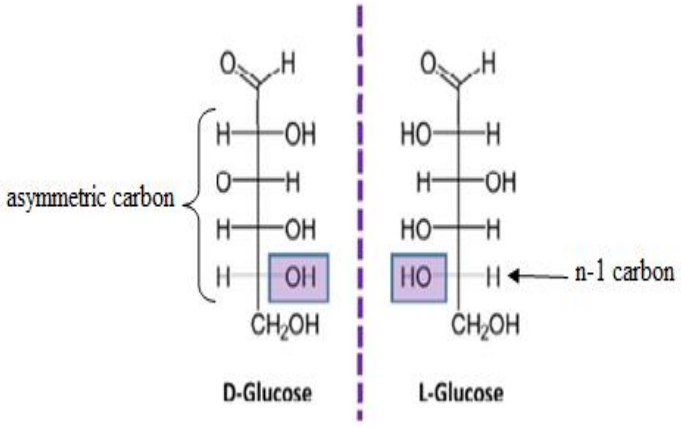


Fig.10: Structure of D. Glucose and L. Glucose

Fig. 11 and 12 represent respectively the Fischer structures of aldoses and ketoses with three to six carbon atoms.

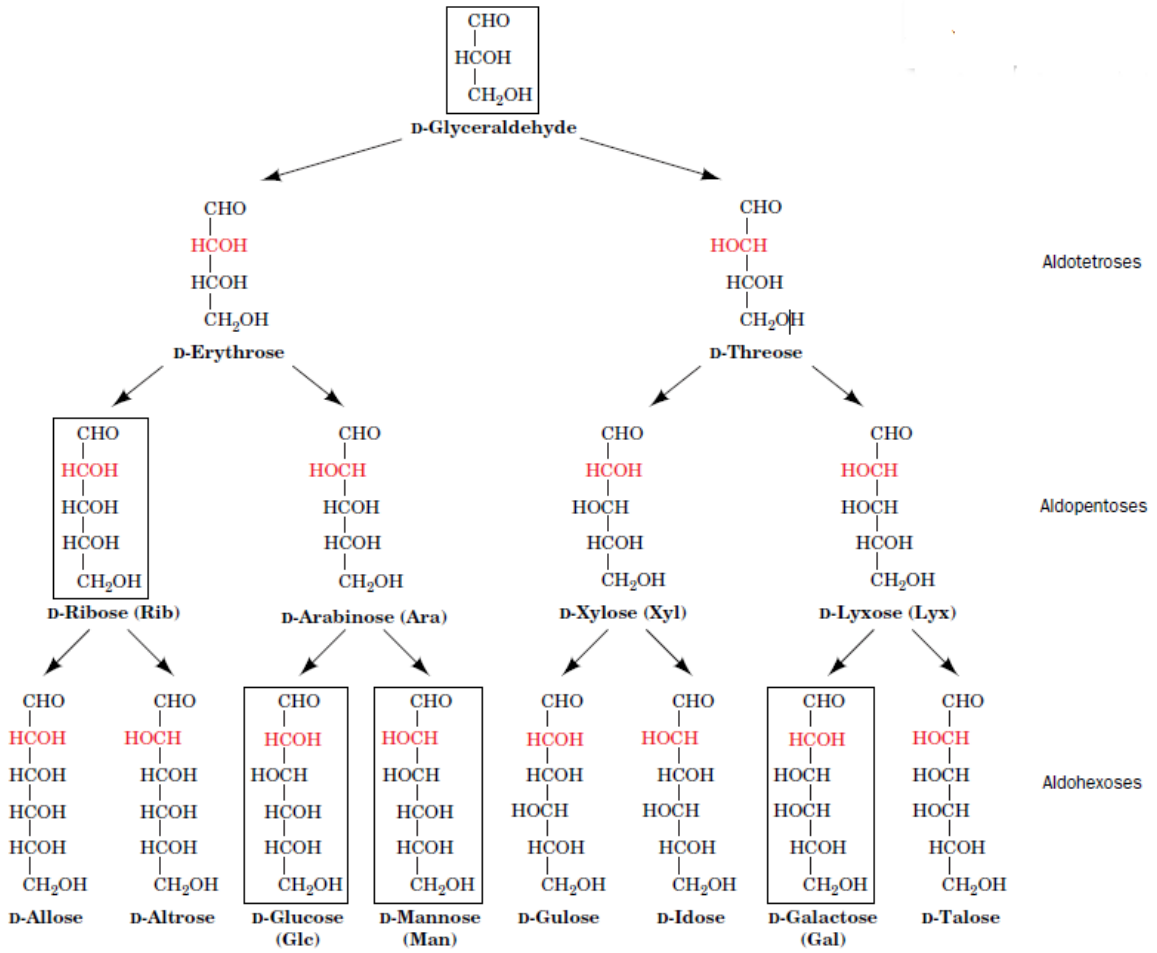


Fig. 11: The D-aldoses with three to six carbon atoms.. The biologically most common aldoses are boxed. Each time a new stereogenic center is added just below the carbonyl group generating two additional stereoisomers;

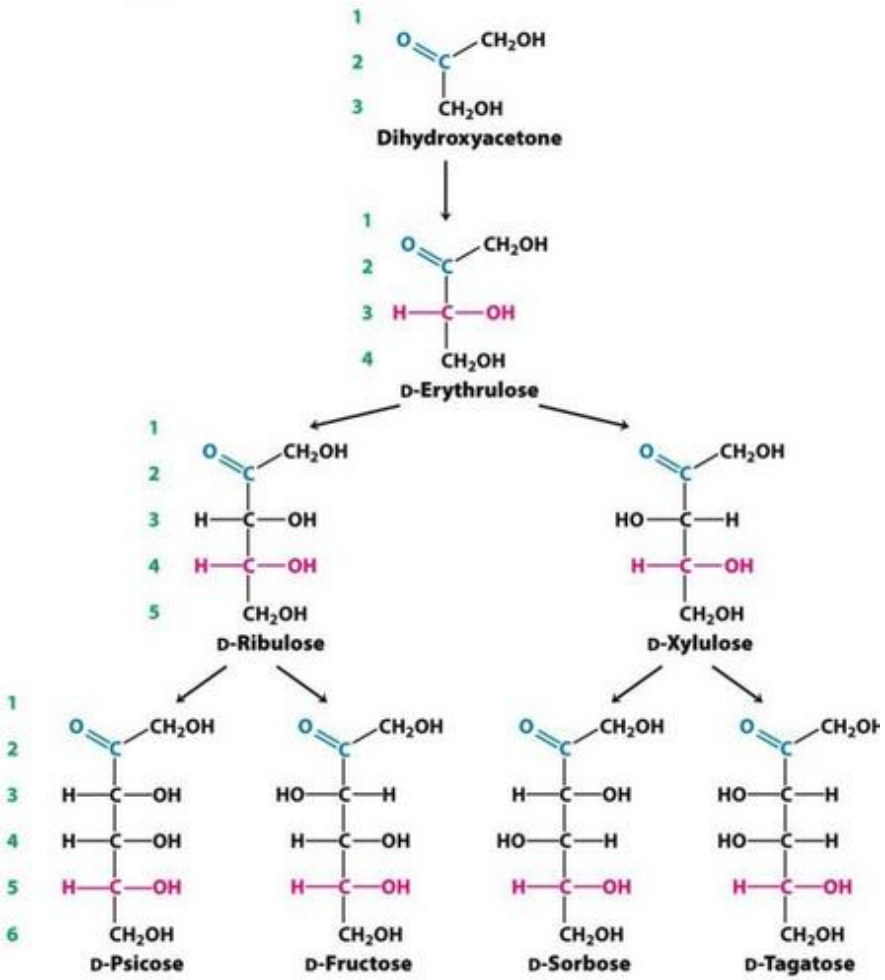
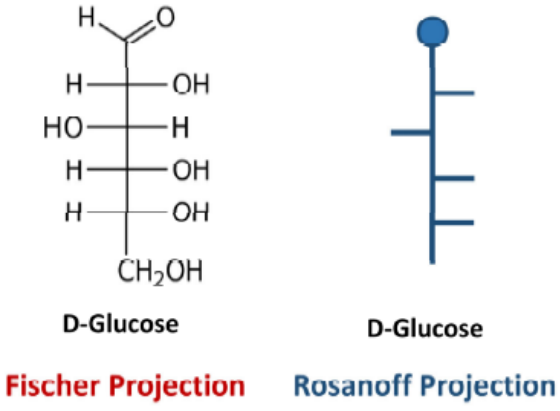


Fig. 12: The D-ketoses with three to six carbon atoms.

II.4.3. Rosanoff projections

Rosanoff further streamlined Fischer's projection in 1906 and curtailed the versions of fisher's projection, which appear to be more simplified. In Rosanoff projections only the direction of the OH group is given by the horizontal bonds. The –H bonds are implied. So for glucose, you can see that the –OH is on the right, the left, and the right and the right.



II.4.4. Degradation and Synthesis of Monosaccharides

II.4.4.1. Kiliani-Fischer Synthesis of Monosaccharides

In order to increase the chain length, Heinrich Kiliani (Freiburg, Germany) suggested a method which involves transformation of an aldose to the epimeric aldonic acids having one additional carbon through the addition of hydrogen cyanide and subsequent hydrolysis of the epimeric cyanohydrins.

Fischer later extended this method by showing that aldonolactones obtained from the aldonic acids can be reduced to aldoses. Therefore this method of transforming aldose to higher aldoses is known as Kiliani-Fisher synthesis.

Addition of hydrogen cyanide to glyceraldehyde produces two epimeric cyanohydrins because the reaction creates a new chirality center.

The cyanohydrins can be separated easily (since they are diastereomers), and each can be converted to an aldose through hydrolysis, acidification, lactonization, and reduction with Na–Hg at pH 3–5. One cyanohydrin ultimately yields D-(-)-erythrose and the other yields D-(-)-threose. A schematic representation is given in Fig.13, summarizing the steps of monosaccharide synthesis.

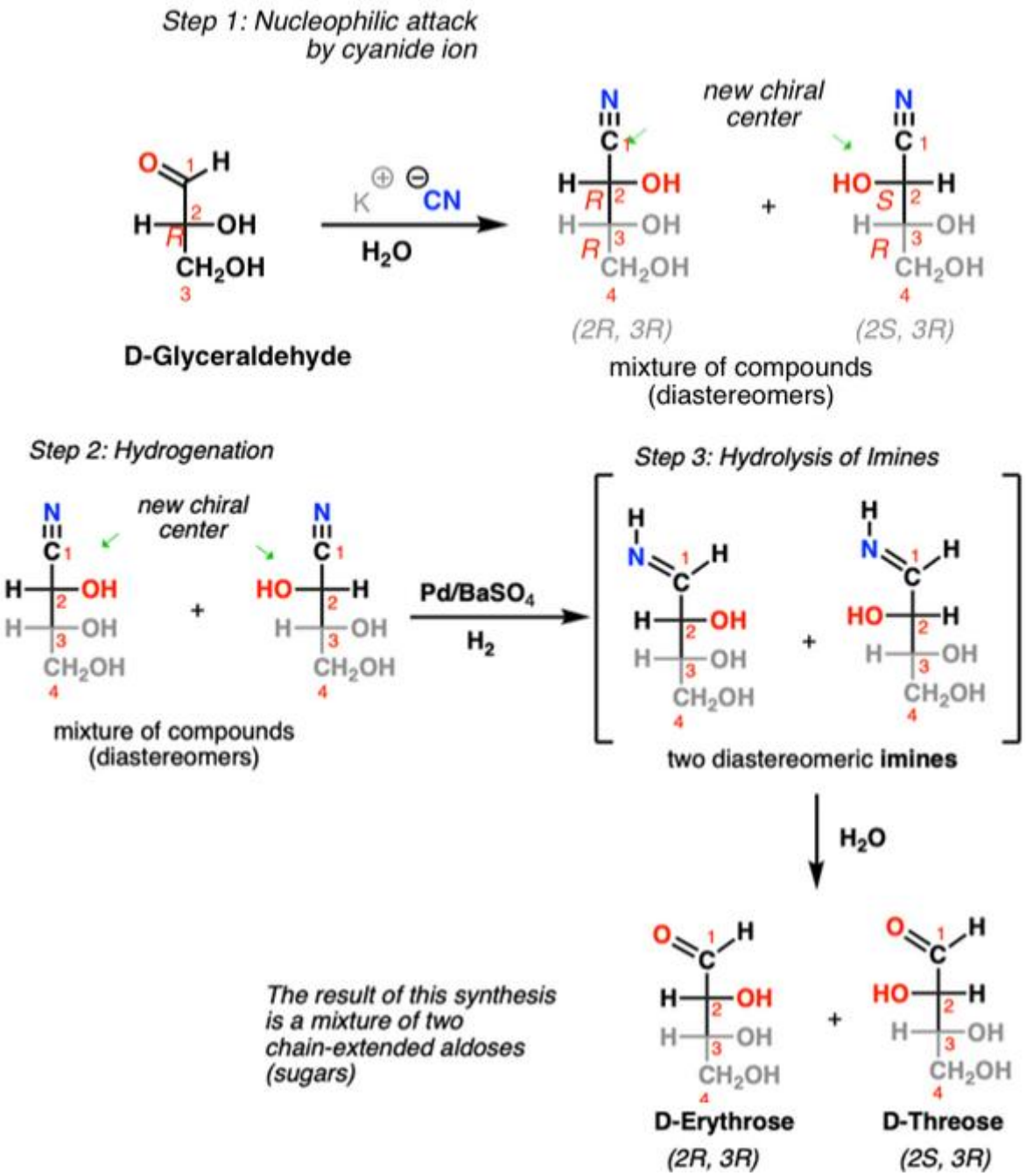


Fig. 13: Different steps involved in Kiliani-Fisher synthesis

II.4.4.2. Ruff Degradation of monosacchride

It's also possible to go in the reverse direction, where an aldose is **reduced** in length by one carbon. The procedure for going in this direction is called the Ruff Degradation, a procedure which dates back to the late 1890's.

In the first step, the aldehyde is selectively oxidized to a carboxylic acid by bromine (Br_2) and water. Note that the secondary and primary alcohols are not oxidized here.

The next step involves adding an iron (III) salt [$Fe_2(SO_4)_3$] with hydrogen peroxide, which involves the loss of carbon dioxide and oxidation of the adjacent C_2-OH to an aldehyde (Fig.14).

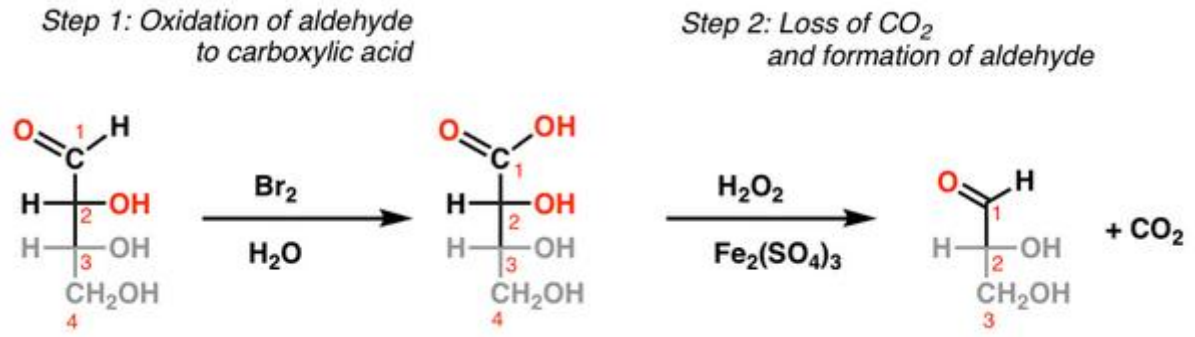


Fig.14 : Principale steps involved in Ruff degradation.

II.4.5. Isomerism

Stereoisomers are compounds with with different conformation around chiral or asymmetric carbon atoms, but they have the same molecular formula, functional groups, and position of functional groups.

In general, a molecule with *n* chiral centers can have 2^n stereoisomers.

e.g. Glyceraldehyde has $2^1 = 2$; Aldohexoses, with four chiral centers, have 24 = 16 stereoisomers. The position of their carbonyl group gives ketoses one less asymmetric center than their isomeric aldoses, so a ketohexose has only 23 = 8 possible stereoisomers (4 D sugars and 4 L sugars) (**Tab. 2)**.

Tab. 2: Number of asymmetric carbon and optical isomers of monosaccharide.

Number of carbon atoms	Number of		Number of optical isomers (2^n)
	Aldose/Ketose	asymmetric carbon (n)	
3	Aldose	1	2
4	Aldose	2	4
5	Aldose	3	8
6	Aldose	4	16
3	Ketose	0	--
4	Ketose	1	2
5	Ketose	2	4
6	Ketose	3	8

II.4.5.1. Enantiomer

Enantiomers are stereoisomers that constitute mirror images from each other. The mirror images can't be superimposed on each other.

Enantiomers have the same chemical and physical properties, but they differ in their biological properties. Enzymes have very specific 3-D structures, so they can differentiate between stereoisomers or enantiomers. Ex. D. glyceraldehydes/ L.glyceraldehyde.

II.4.5.2. Epimers

Sugars that differ only by the configuration around one asymmetric C atom are known as **epimers** of one another. Ex. D-glucose and D-mannose are epimers with respect to C_2 . D-glucose and D- galactose are also epimers at C-4.

II.4.5.3. Diastereomers

Diastereomers are stereoisomers that are not mirror images of each other. They arise when molecules have multiple chiral centers (stereocenters) and differ in the configuration at two or more of these centers, but not all. Ex., D-mannose and D- galactose.

II.4.5.4. Constitutional isomers

Aldoses and ketose can be constitutional (structural) isomers **if** they have the same molecular formula but a different bonded order of the atoms. ex; D Glucose and D Fructose

II.4.5.5.Anomers

Anomers are isomeric forms of **the same** monosaccharide that differ only in their configuration about the hemiacetal or hemiketal carbon atom in the cyclic representation**.**

- The hemiacetal carbon atom (or carbonyl) is called the **anomeric** carbon.

- For example, D-glucose exists in solution as an intramolecular hemiacetal in which the free hydroxyl group at C-5 has reacted with the aldehydic C-1, rendering the latter carbon asymmetric and producing two anomers designated as α and β (**Fig. 15).**

-The designation α means that the -OH on the anomeric carbon is trans to the terminal -CH_2OH

-The designation β means that that the -OH on the anomeric carbon is cis to the terminal -CH_2OH.the figure below ulustrate the position cis and trans.

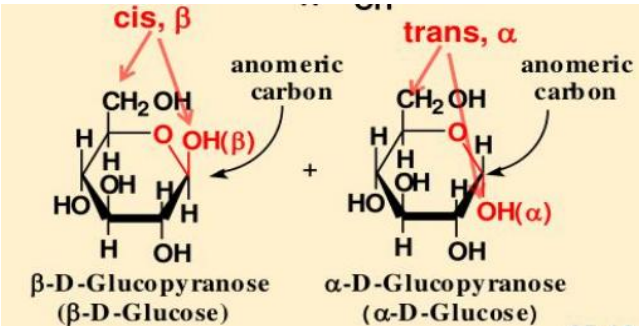


Fig. 15: Anomeric Forms of D-Glucose: Alpha and Beta Anomers

The figure below (Fig. 16) summarizes the different classes of isomers.

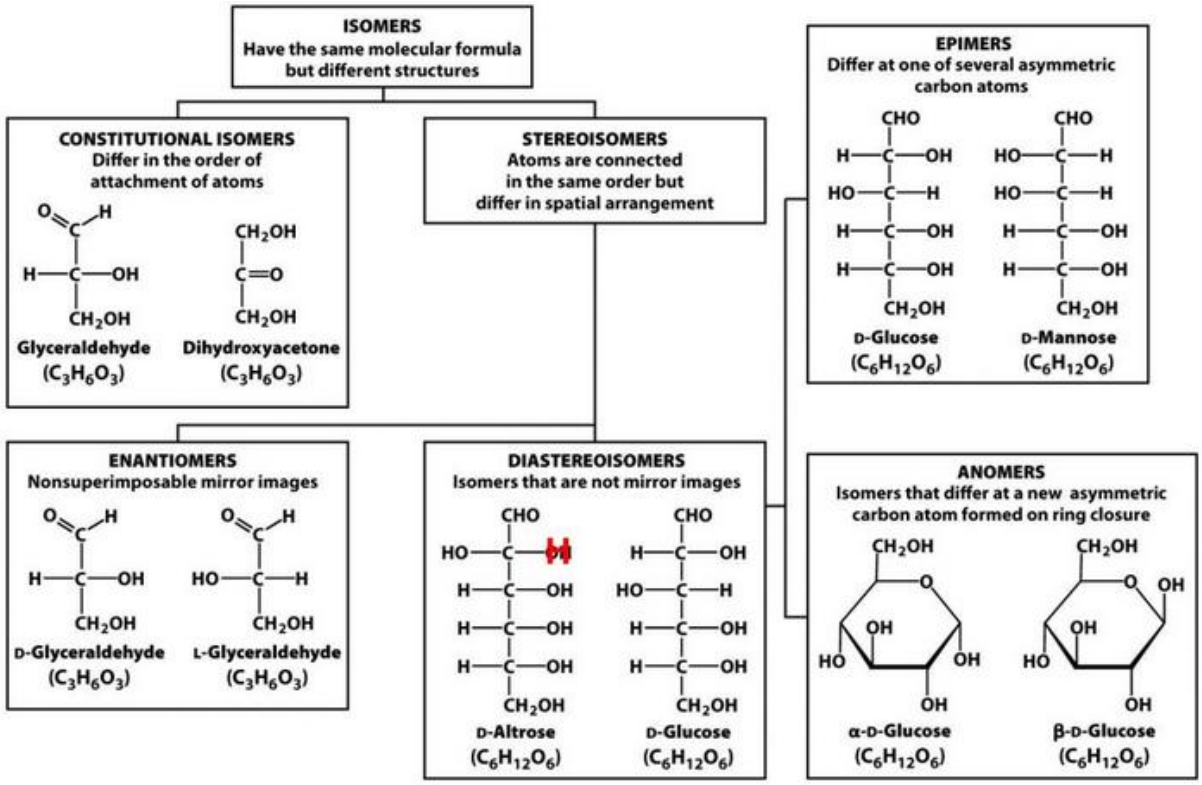


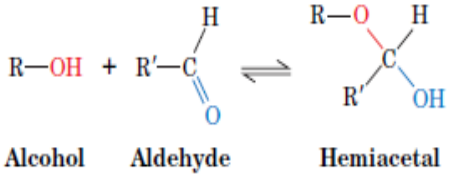
Fig. 16: Isomeric forms of carbohydrate

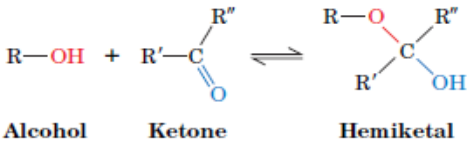
II.4.6. Carbohydrates objection of linear form

Monosaccharides, in their linear form, contain an aldehyde or ketone functional group, making them chemically reactive. However, in aqueous solutions, these linear structures are not the predominant forms. Instead, they undergo an intramolecular reaction between the carbonyl group and a hydroxyl group, leading to the formation of cyclic structures.

II.4.6.1. Formation of hemiacetals and hemiketals

Aldehydes and ketones, in their hydrated form, react with two molecules of alcohol to yield acetals, whereas sugars combine with only one molecule of alcohol to yield a hemiacetal.





II.4.6.2. Mutarotation

Monosaccharides exhibit a phenomenon called **mutarotation** (Latin *mutare*, meaning "to change"), which is the change in optical rotation that occurs when a sugar is dissolved in water. This happens because monosaccharides exist in equilibrium between their cyclic forms (alpha and beta anomers) and their linear form **(Fig. 17).**

- *Mutarotation of Glucose*

It is possible to obtain a sample of crystalline glucose in which all the molecules have the α structure or all have the β structure. The α form melts at 146°C and has a specific rotation of +112°, while the β form melts at 150°C and has a specific rotation of +18.7°. When the sample is dissolved in water. However, a mixture is soon produced containing both anomers as well as the straight-chain form, in dynamic equilibrium. You can start with a pure crystalline sample of glucose consisting entirely of either anomer, but as soon as the molecules dissolve in water, they open to form the carbonyl group and then reclose to form either the α or the β anomer. The opening and closing repeats continuously in an ongoing interconversion between anomeric forms and is referred to as **mutarotation**. At equilibrium, the mixture consists of about 36% α-D-glucose, 64% β-D-glucose, and less than 0.02% of the open-chain aldehyde form. The observed rotation of this solution is +52.7°.

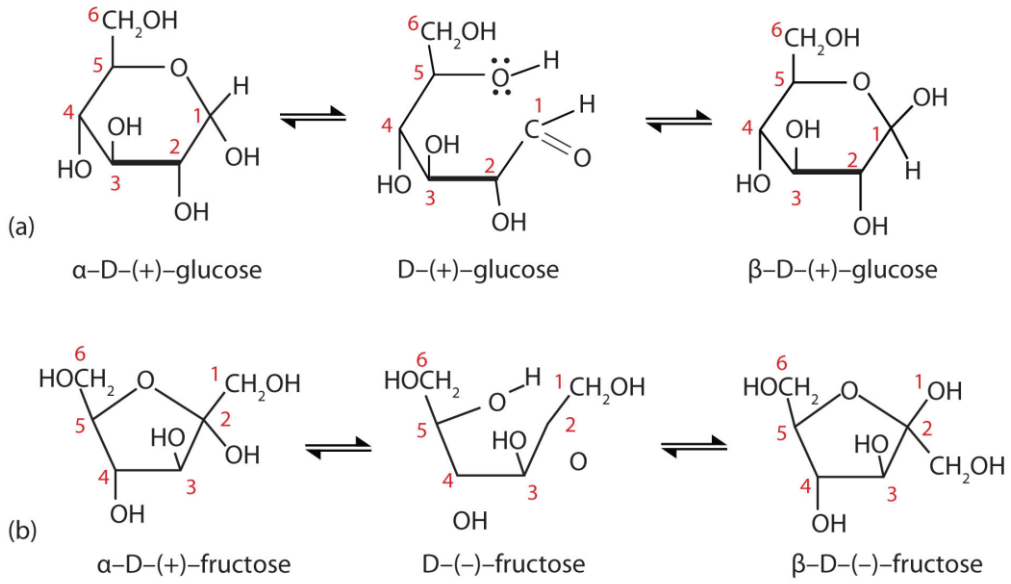


Fig. 17: Mutarotation phenomenon for D-glucose (a) and D-fructose (b). In an aqueous solution, monosaccharides exist as an equilibrium mixture of three forms. The interconversion between the forms is known as *mutarotation*, which is shown for D-glucose (a) and D-fructose (b).

➢ *Biological Importance of Mutarotation*

Mutarotation plays an important biological role in carbohydrate metabolism.

- Isomerases catalyze mutarotation for rapid equilibration. This provides the proper anomer for subsequent enzymatic reactions.
- Mutarotation enables the cyclic conformations needed for transport across membranes.
- It facilitates alternating cyclic-open transitions during glycolys.
- The equilibrium mixture presents both anomers for recognition by carbohydrate-binding proteins.
- Mutarotation forms reducing sugars
- It enables equilibrium between furanose and pyranose forms of pentose sugars like ribose and deoxyribose.

- Because the β anomers are more thermodynamically stable, they are more likely to form β-glycosidic bonds in biological systems.

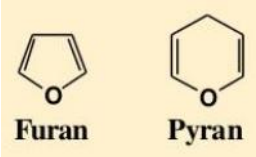
Overall, mutarotation makes it possible for anomeric forms to change balance over time, which is important for the complex chemistry of carbohydrates in living things.

II.4.6.3. Haworth perspective formulas

Reaction between the aldehyde group at C-1 and the hydroxyl group at C-5 forms a hemiacetal linkage, producing either of two stereoisomers, the α and β anomers, which differ only in the stereochemistry around the hemiacetal carbon.

•Six-membered hemiacetal rings are shown by the infix -pyran-.

• Five-membered hemiacetal rings are shown by the infix -furan-.



The six-membered ring of oses are called **pyranoses**

The systematic names for the two ring forms of D-glucose are α-D-glucopyranose and β-D glucopyranose. The Fig**.** 18 illustrates the different steps of the conversion of open chain structure into ring structure and reconstruction of Haworth projection from corresponding Fisher projection.

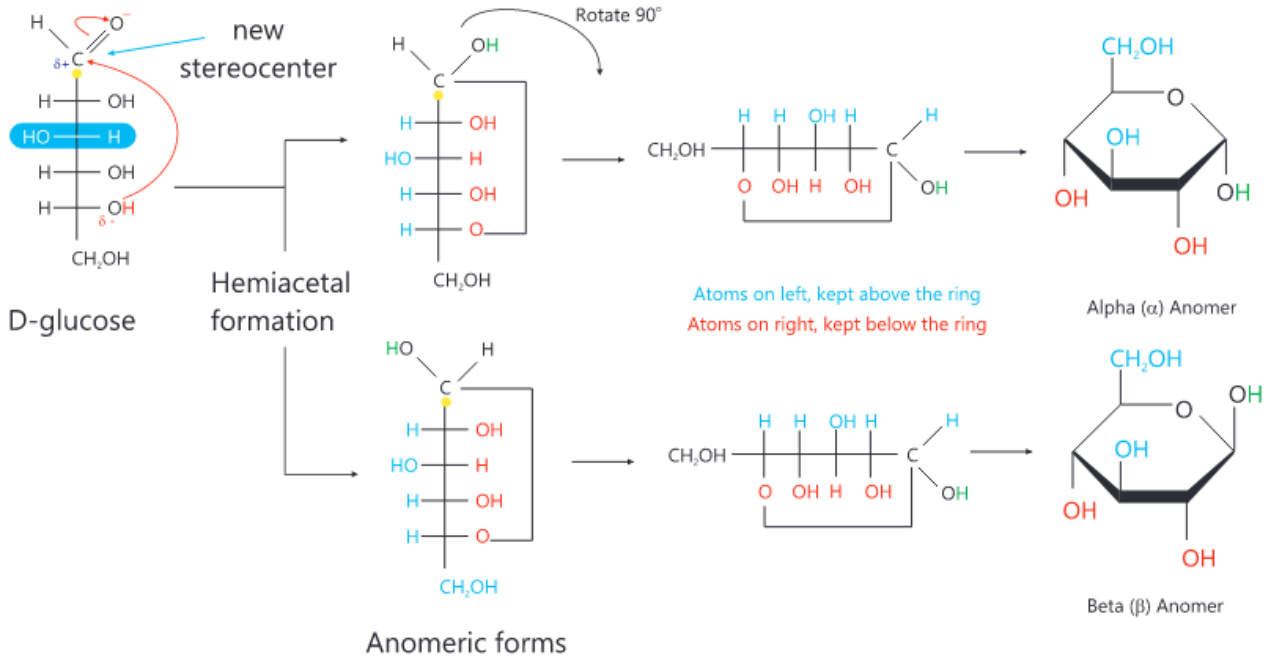


Fig. 18: Converting fisher projection to Haworth projection during cyclization of Aldose

Like glucose (aldohexose), fructose (ketohexose) forms ring structure. Reaction between the ketone group at C-2 and the hydroxyl group at C-5 forms a hemiketal linkage, producing the α and β anomers of fructose, which differ only in the stereochemistry around the hemiacetal carbon (C2). In Haworth perspective formulas of glucose and fructose, the edges of the ring nearest the reader are represented by bold lines.

Surprisingly, fructose forms both pyranose and furanose rings (**Fig. 19).**

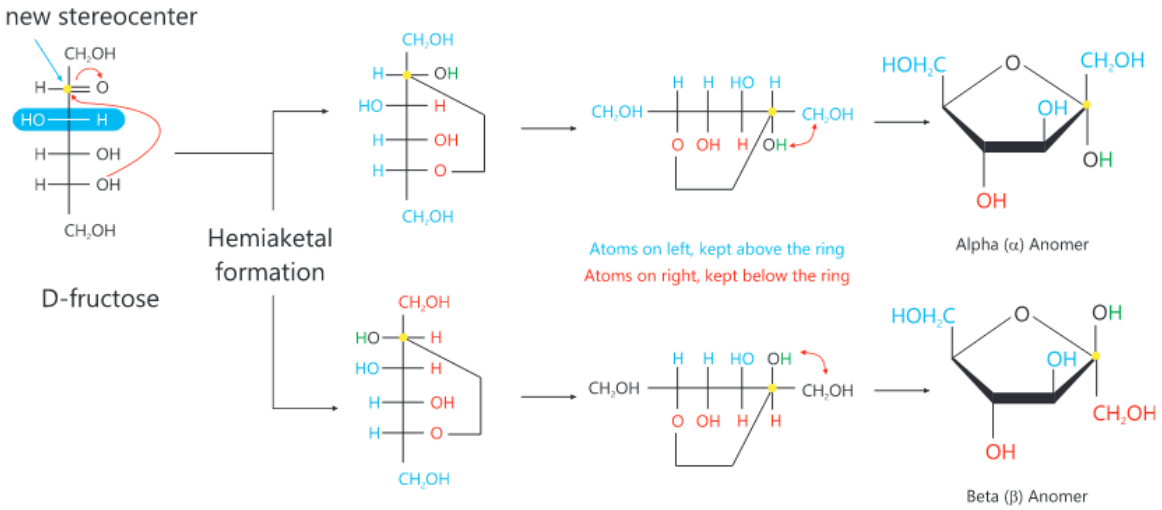


Fig.19: converting fisher projection to Haworth projection during cyclization of cetose

Although the Haworth perspective form is commonly used in biochemistry, the six-membered pyranose ring is not planar, as Haworth perspectives suggest, but tends to assume either of two **"chair"** conformation (**Fig.20**).

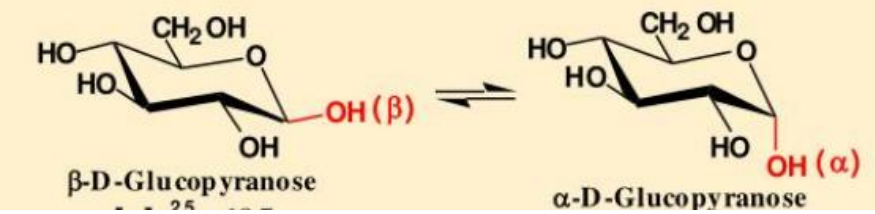


Fig.20: Chair Conformation of D-Glucopyranose

II.4.7. Physico-Chemical Properties of Monosaccharides

II.4.7.1. Physical properties

II.4.7.1.1. Optical properties of monosaccharides

All monosaccharide accept dihydroxy acetone have one or more asymmetric carbon atoms and show optic activity. When polarized light passes through a solution containing an optically active monosaccharide, the plane of polarization is rotated. This property is called **optical rotation**, the direction and angle of rotation can be measured using an instrument known as a **polarimeter** (Fig. 21). Monosaccharides can rotate light either:

- **Dextrorotatory** (D, +): Rotates light clockwise.
- **Levorotatory** (L, -): Rotates light counterclockwise.

For example:

- **D-Glucose** is dextrorotatory and is denoted as **D-(+)-glucose** because it rotates light to the right.

However, the **D and L** prefixes refer to the stereochemistry (configuration) relative to glyceraldehyde, not the direction of optical rotation. So a D. sugar can be levorotatory, and an L. sugar can be dextrorotatory.

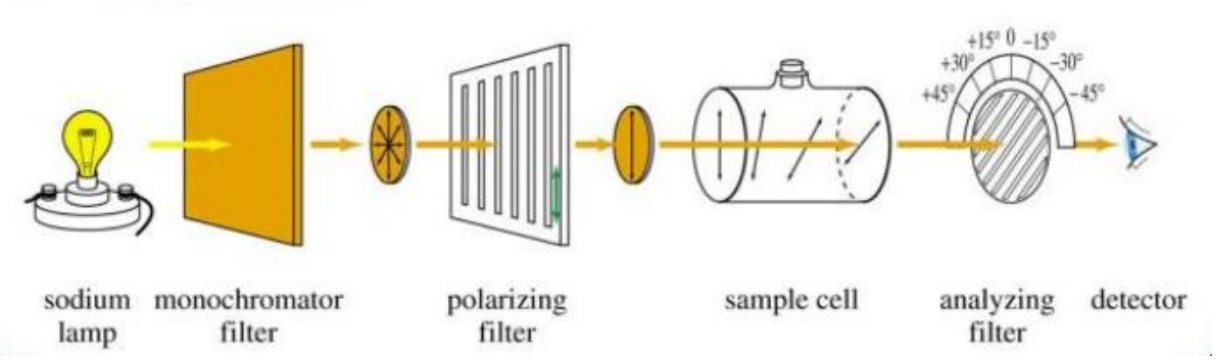


Fig. 21: Rotation of polarized light by chiral molecules. When plane polarized light passes through a solution containing a chiral compound, the chiral compound causes the plane of vibration to rotate.

The optical activity of organic compounds is measured according to the law of Biot:

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

where:

- $[\alpha]_D^{20}$: Specific rotation of the optically active substance. It is a characteristic constant of the substance and depends on the nature of the solute, the solvent, the temperature, and the wavelength at which the measurement is performed. D refers to the wavelength of the sodium D line at 589.3 nm, and 20 indicates the temperature of 20°C.
- α: Observed rotation angle measured with the polarimeter.
- L: Length of the tube containing the solution (dm, decimeters).
- C: Concentration of the substance (g/ml).

Enantiomers rotate the plane of polarized light by exactly the same amount but in opposite direction. A solution containing equal amounts of two enantiomers is called a **racemic mixture** which is optically inactive.

Biot's Law exhibits an additive property; the observed rotation of a mixture of chiral substances is the sum of the individual rotations of each component. For example, if a solution contains a mixture of glucose and fructose, the observed rotation will be the sum of the rotations caused by each sugar. By knowing the specific rotations of glucose and fructose, and measuring the observed rotation, it is possible to determine the relative concentrations of each sugar in the mixture.

II.4.7.1.2. Solubility of carbohydrates

In common monosaccharides, most carbon atoms have a hydroxyl group attached. These accounts for their high solubility in water: the hydroxyl groups are very polar and prone to hydrogen bonding. All monosaccharides are soluble in water; smaller sugars are more soluble in water than larger ones. Oligosaccharides are less soluble than monosaccharides. However, Polysaccharides like starch and glycogen are slightly soluble or nearly insoluble although they have too many –OH groups because of their high molecular weight.

II.4.7.1.3. Spectral proprieties of carbohydrates

Carbohydrates generally do not absorb UV or visible light significantly due to the lack of conjugated double bonds or aromatic rings in their structure. Therefore, they typically appear as colorless or white in the solid state. while they generally show characteristic absorption bands in the infrared spectrum due to the vibrations of functional groups such as hydroxyl (-OH), carbonyl (C=O), and C-H bonds.

II.4.7.2. Chemicals proprieties of carbohydrates

II.4.7.2.1. Oxidation of monosaccharides

Monosaccharides, due to their reactive functional groups, can undergo oxidation reactions that modify their structure. These oxidations can lead to the formation of different types of sugar derivatives. Several types of oxidation reactions are commonly observed in monosaccharides, including:

A. Oxidation with Bromine

Bromine, Br_2, is a mild oxidizing agent and oxidizes the aldehyde group in the aldose to the carboxylic acid group. The resulting compound is an **aldonic acid**. The aqueous solution of bromine, Br_2(aq), is used for this oxidization with a pH in the range of 5~6. No isomerization and fragment reaction for monosaccharides occurs in such mild acidic conditions (Fig.22).

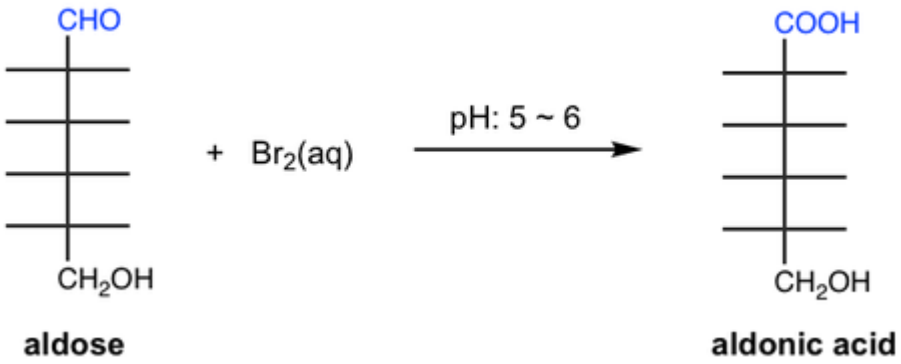


Fig. 22: Oxidation of Aldose with bromine (the OH groups on the middle carbons are skipped).

The oxidation product aldonic acid may undergo a subsequent intramolecular esterification reaction, between COOH and the OH group on C-4, to form a γ-lactone, as shown in the following example (Fig.23).

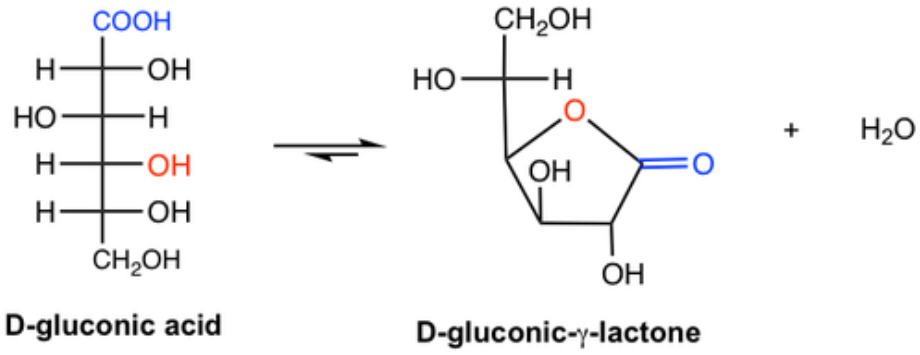


Fig.23: Cyclization reaction of D-gluconic acid to D-gluconic-γ-lactone.

B. Oxidation with Nitric Acid HNO_3

Dilute nitric acid is a stronger oxidizing agent and oxidizes both the aldehyde group and the terminal CH_2OH group of an aldose to carboxylic acid groups, giving dicarboxylic acids that are known as aldaric acids (Fig.24).

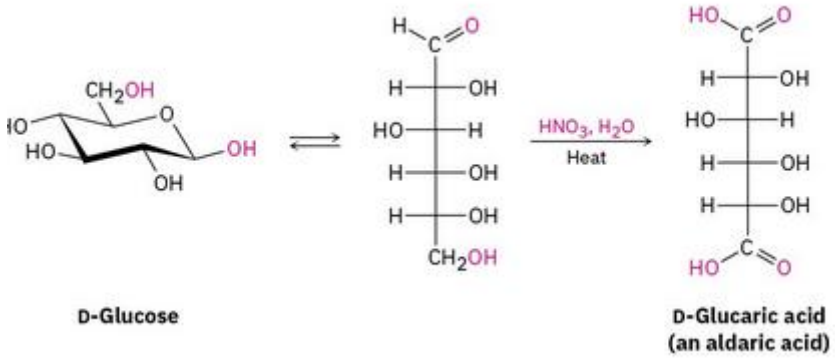


Fig. 24: Oxidation of aldoses with nitric acid (the OH groups on the middle carbons are skipped)

C. Oxidation with Tollens' Reagent

Both aldoses and ketoses are oxidized to aldonic acids by Tollens' reagent. Tollens reagent is prepared by mixing $AgNO_3$ with aqueous ammonia solution and contains $Ag(NH_3)^+$ cation. Although the Tollens' reagent does not react with the ketone group directly, ketose undergoes isomerization in an alkaline solution to aldose, which is then oxidized by the Tollens' reagent **(Fig. 25)**.

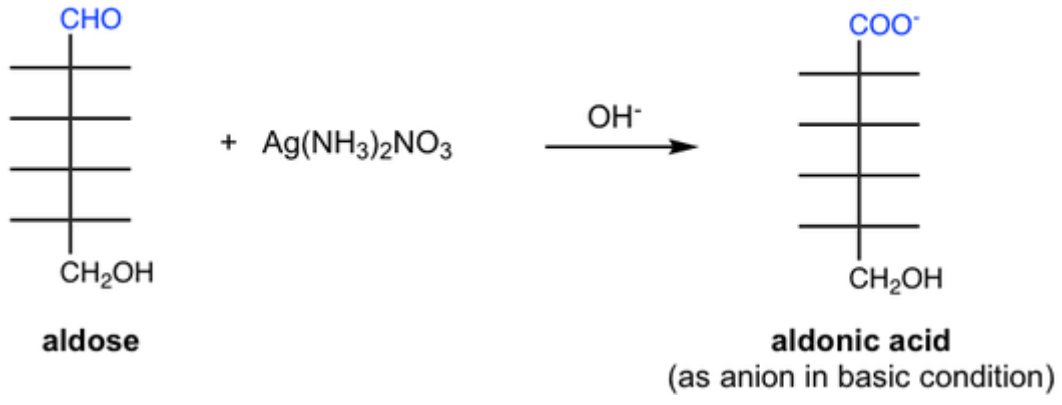


Fig. 25. Oxidation of aldoses with Tollens' reagent (the OH groups on the middle carbons are skipped).

D.Enzymatic oxidation

When the **–CH2OH** (the primary alcohol group) at the terminal end of an aldose is oxidized, but the **–CHO** (the aldehyde group) is not, the product is a **monocarboxylic acid** known as **uronic acid**. The reaction can only be done enzymatically; no chemical reagent is known that can accomplish this selective oxidation in the laboratory. Uronic acids are important in biology, especially in the structure of glycosaminoglycans, which are components of connective tissues (Fig.26).

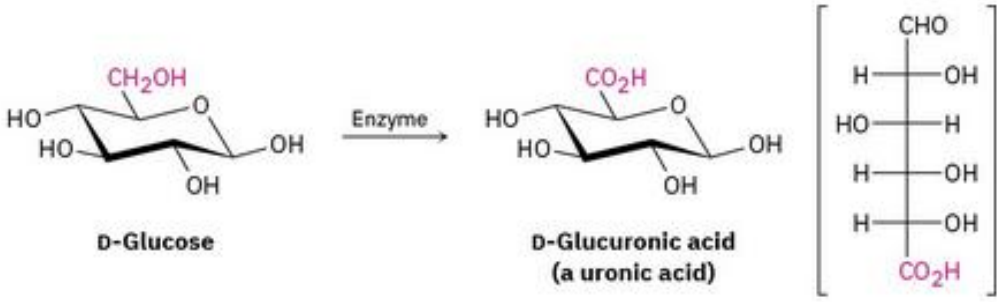


Fig.26: Enzymatic Oxidation of D-Glucose to D-Glucuronic Acid; The enzyme responsible for this reaction is called **glucuronate dehydrogenase.**

E. Oxidation of aldoses using Fehling reagent

The presence of the hydrogen atom in the aldehyde group gives reducing properties to the aldehyde molecule. Aldehydes are easily oxidized by mild oxidizing agents such as Fehling's reagent to carboxylic acids. Thus, aldoses are reducing agents, any sugar that has a free aldehyde group is referred to as a reducing sugar. The product name is made by changing the –ose ending to –onic acid (-onate), exemple: Glucose ⟶ gluconic acid or gluconat (Fig.27).

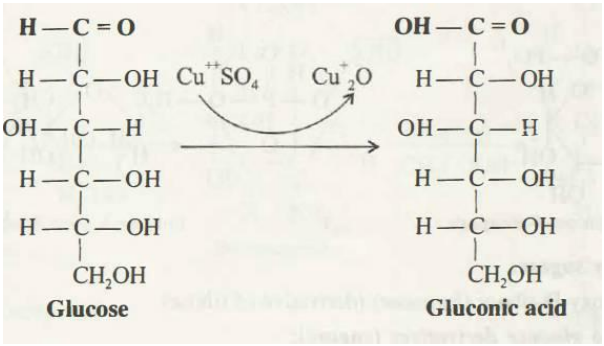


Fig.27: Oxidation of Glucose to Gluconic Acid Using Fehling's Reagent

Fehling's test is used to determine if a reducing sugar is present in a sample using a reagent called the Fehling's reagent. The reagent is an alkaline solution, containing a copper tartrate complex with Cu^{2+} ions. When the reagent reacts with the aldehyde group of a reducing sugar, the Cu^{2+} ions are reduced to Cu^{+} ions, forming a red precipitate of cuprous oxide. The chemical reaction of the key functional groups can be viewed in Fig.28.

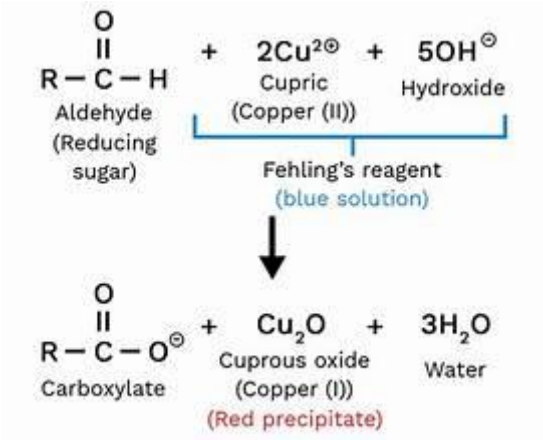


Fig.28: Simplified reaction of a reducing sugar with Fehling's reagent.

F. Oxidation of ketoses (using Fehling reagent)

Ketones do not have such reducing properties of the aldehyde group due to the lack of H attached to C=O and are not oxidized under similar conditions.

BUT, fructose reduces such reagents even though it contains no aldehyde group. Reduction occurs because the reagents are basic solutions and fructose is readily isomerized to a mixture of aldoses (glucose and mannose) under basic conditions. The figure below (fig.29) illustrates the interconversion of fructose (a ketose) to glucose and mannose (aldoses) under basic conditions. The process involves an enediol intermediate, allowing for the shift of the carbonyl group and subsequent formation of different aldose isomers. This explains why fructose, despite being a ketose, can act as a reducing sugar in basic solutions.

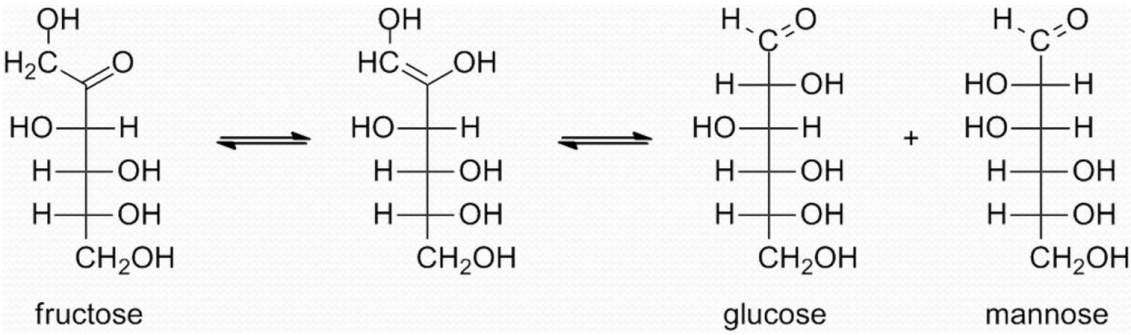


Fig.29 : Fructose Isomerization in Basic Solution

G. Periodic acid cleavage

One important method of elucidating the structure of carbohydrates is using periodic acid degradation. Periodic acid, HIO_4, oxidizes compounds containing OH groups on adjacent carbons in a way called oxidative cleavage, that is, to break the C-C bonds and produce carbonyl compound products.

When multiple neighboring hydroxyl (-OH) groups are present:

- The "primary alcohol" function oxidizes to produce formaldehyde (HCHO).
- The "secondary alcohol" functions oxidize to form formic acid (HCOOH) (Fig.30).

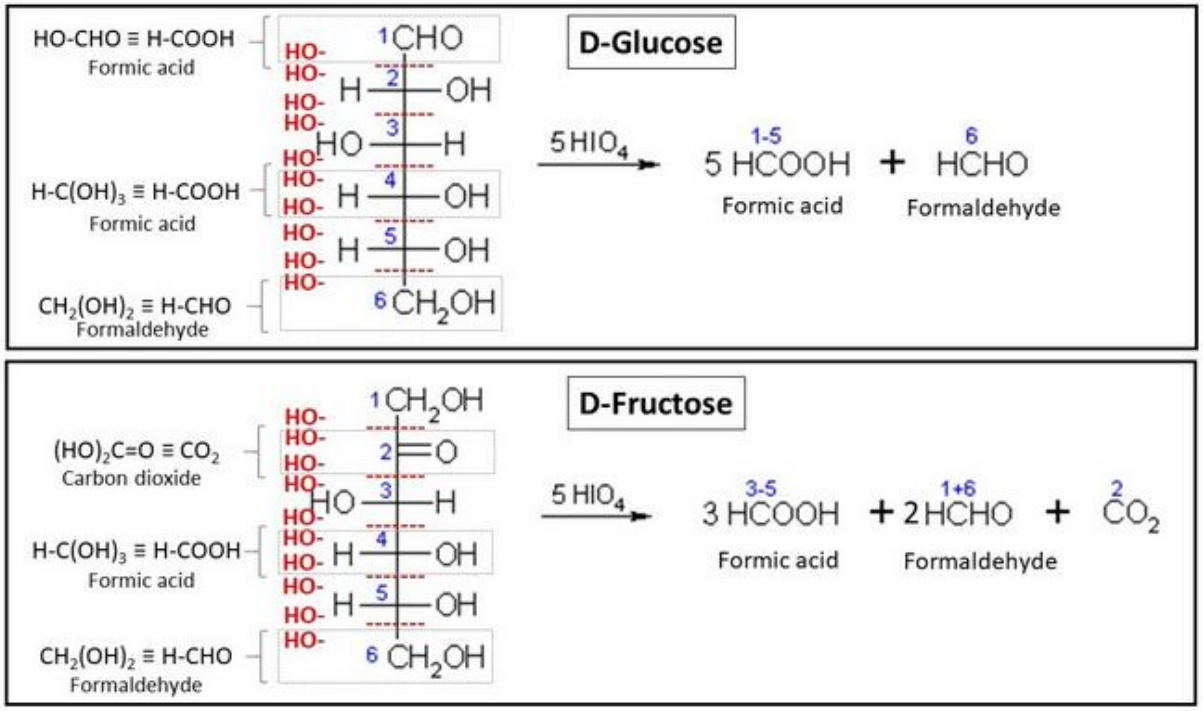


Fig. 30: Oxidation of glucose and fructose with periodate. each C−C oxidative cleavage of C−C bond gives OH group to each carbon.

As shown in Fig.31, The mechanism of this reaction involves a cyclic periodate ester that reacts with two neighboring alcohol functional groups which are oxidized to carbonyl functional groups.

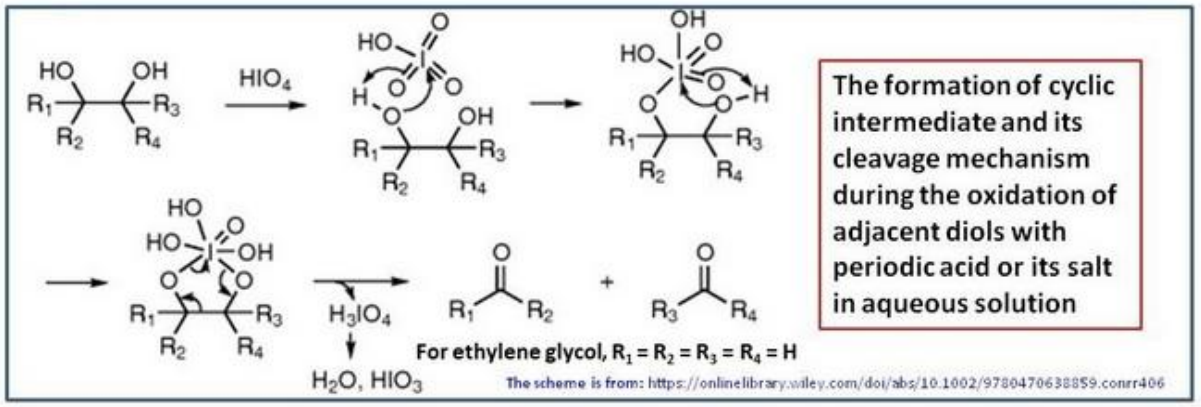


Fig. 31: Mechanism of periodic acid oxidation of vicinal diols

II.4.7.2.2.Reduction of Monosaccharides

Treatment of an aldose or ketose with $NaBH_4$ reduces it to a polyalcohol called an alditol. The reduction occurs by reaction of the open-chain form present in the aldehyde/ketone ⇄ hemiacetal equilibrium. Although only a small amount of the open-chain form is present at any given time, that small amount is reduced, more is produced by opening of the pyranose form, that additional amount is reduced, and so on, until the entire sample has undergone reaction.

D-Glucitol, the alditol produced by reduction of D-glucose, is itself a naturally occurring substance found in many fruits and berries. It is used under the name D-sorbitol as a sweetener and sugar substitute in many foods. Reduction of glucose forms one alditol product as shown in

Fig.32

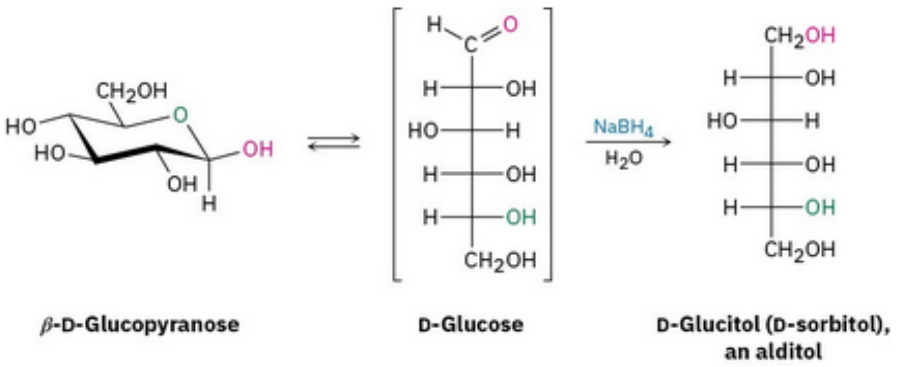


Fig. 32. Reduction of glucose with sodium borohydride (the OH groups on the middle carbons are skipped)

For the reduction of ketoses, however, two alditols are formed because the reduction creates a new chirality center and both stereoisomers are possible. The reduction of D-fructose is given as an example in Fig. 33, in which both D-mannitol and D-Glucitol are produced.

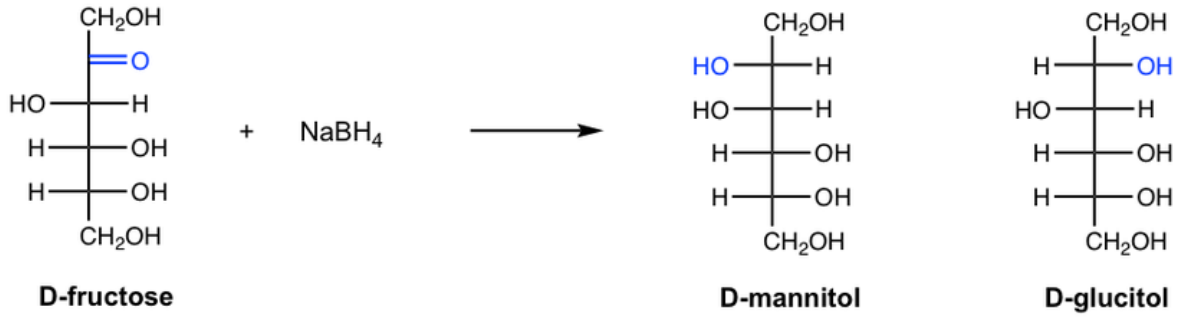


Fig. 33: Reduction of D-fructose with sodium borohydride

II.4.7.2.3. Reaction of monosaccharides with phenylhydrazine

Since aldoses or ketoses have the carbonyl group, they also react with phenylhydrazine, but in a different way than that of common aldehyde or ketone. Aldoses and ketoses react with three equivalents of phenylhydrazine to produce a type of compound called **osazones**. The osazone derivatives of monosaccharides are crystalline solids that are insoluble in water, and could be easily isolated and purified; they were once used to identify monosaccharides (Fig. 34).

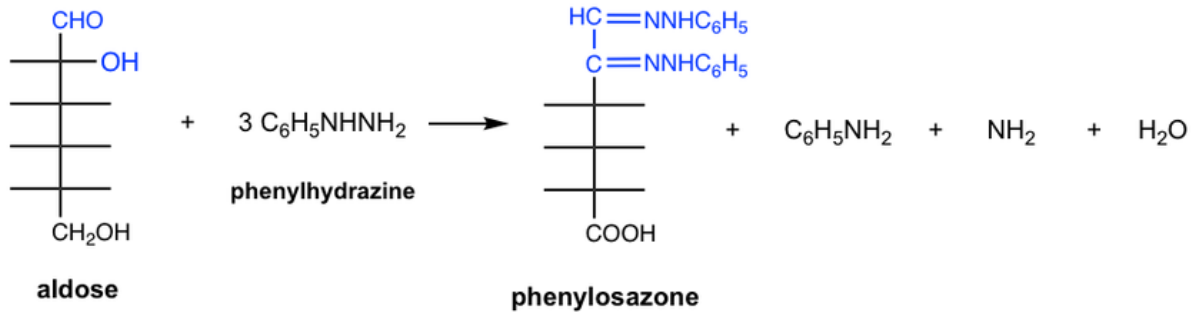


Fig. 34: Formation of osazone between aldose and hydrazine (the OH groups on the middle carbons are skipped)

The simplified way to understand why three equivalents of phenylhydrazine react with aldose or ketose is that one equivalent oxidizes the OH group (the OH at C-2 for aldose, the OH at C-1 for ketose) to a carbonyl group, and two equivalents react with two carbonyl groups to form two imines. The actual mechanism is more complicated though.

Since C-2 in osazone is not chiral carbon, two aldoses that are epimers at C-2 form the identical osazones. For the example below (Fig.35), D-glucose and D-mannose, which are C-2 epimers, both produce the same osazone.

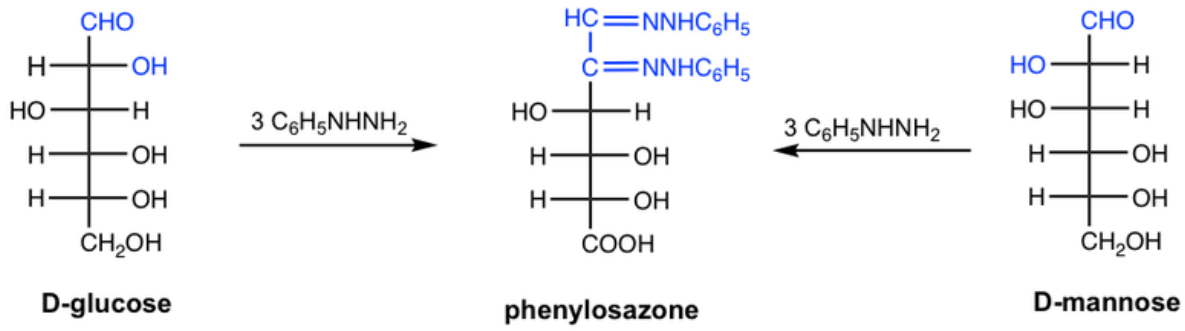


Fig. 35: Phenylosazone Formation from D-Glucose and D-Mannose; both D-glucose and D-mannose produce the same osazone when reacting with hydrazine

II.4.7.2.4. Ester and Ether Formation

Monosaccharides behave as simple alcohols in much of their chemistry. For example, carbohydrate –OH groups can be converted into esters and ethers. Because of their many hydroxyl groups, monosaccharides are usually soluble in water but insoluble in organic solvents such as ether. They are also difficult to purify and have a tendency to form syrups rather than crystals when water is removed. Ester and ether derivatives, however, are soluble in organic solvents and are easily purified and crystallized.

A. Esterification

Esterification is normally carried out by treating the carbohydrate with an acid chloride or acid anhydride in presence of base. All –OH groups react including the anomeric –OH group. For example, β-D-glucopyranose is converted into its pentaacetate by treatment with acetic anhydride in pyridine solution (Fig. 36).

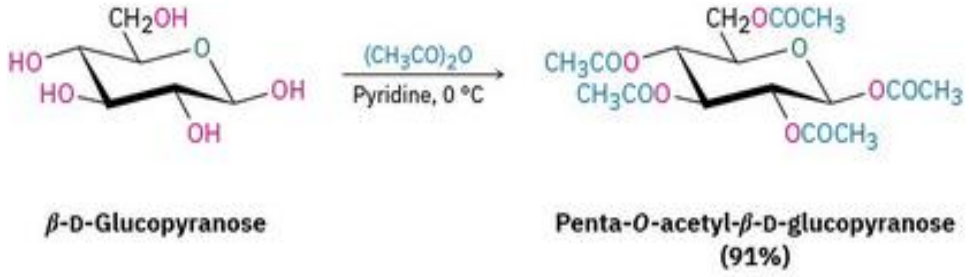
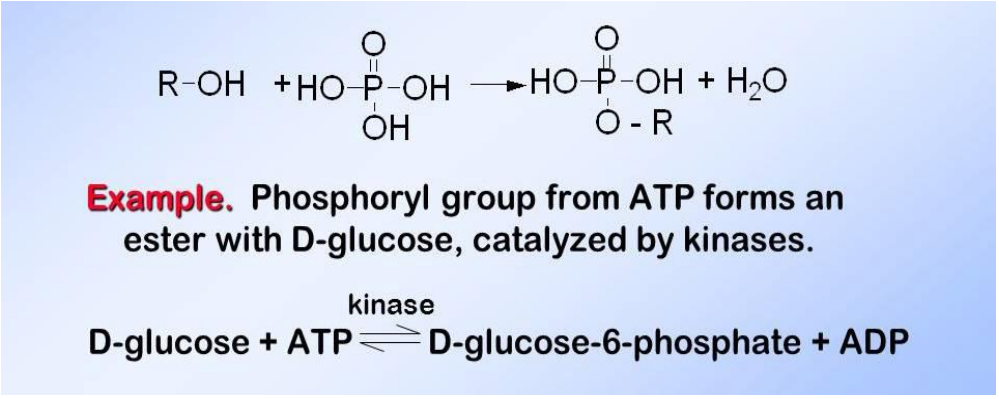


Fig.36: Esterification of β-D-Glucopyranose to Penta-O-acetyl-β-D-glucopyranose

The most important biological esters of carbohydrates are phosphate esters.

In the cell, phosphate esters are produced NOT by using the very acidic phosphoric acid, but most often by transfer of phosphate group from ATP to carbohydrate hydroxyl group, a reaction catalyzed by enzymes called kinases.



B.Formation of ethers

Carbohydrates are converted into ethers by treatment with an alkyl halide in the presence of base. Silver oxide (Ag2O) gives high yields of ethers without degrading the sensitive carbohydrate molecules;T he reaction replaces all hydroxyl (-OH) groups with methoxy (-OCH3) groups, resulting in the formation of α-D-glucopyranose pentamethyl ether with an 85% yield. This is a common reaction used in carbohydrate chemistry to protect hydroxyl groups or to determine the structure of sugars. The figure bellow (Fig.37) illustrates the complete methylation of α-D-glucopyranose using iodomethane (CH3I) and silver oxide (Ag2O).

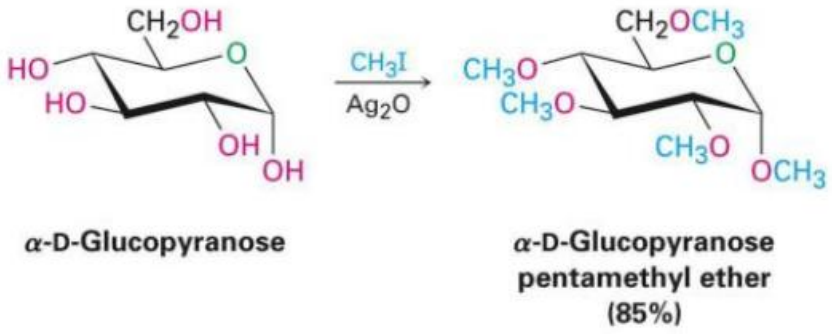


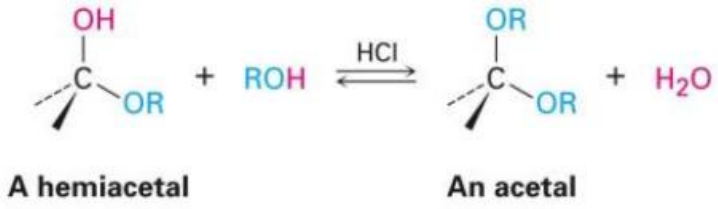
Fig.37: Methylation of α-D-Glucopyranose with Iodomethane and Silver Oxide

II.4.7.2.5. Deoxygenation of Hydroxyl Groups

The replacement of monosaccharide hydroxyl groups with hydrogen atoms forms deoxysugars. Nature has evolved reductases to perform this reaction in one step, whereas chemically multistep procedures are required. Deoxygenation of ribose within a ribonucleotide to form the 2-deoxyribonucleotide is a critical reaction in DNA biosynthesis. Fucose (Fuc), one of the common vertebrate monosaccharides, is deoxygenated at C-6 during its biosynthesis from mannose.

II.4.7.2.6. Glycoside Formation

Treatment of monosaccharide hemiacetals with an alcohol and acid catalyst yields an acetal, called a glycoside. Glycosides are named by first citing the alkyl group and then replacing the ose ending of the sugar with oside.



They are stable in neutral water and do not mutarotate. Wile, they hydrolyze back to free monosaccharide plus alcohol upon treatment with aqueous acid. The reaction forms a glycosidic bond, which is a key linkage in carbohydrates.

II. 5. Disaccharides

II. 5. 1. Definition

Disaccharides consist of two sugar units, the hydroxyl group of one monosaccharide combines with the anomeric carbon of another monosaccharide through a covalent bond, releasing a molecule of water. This covalent bond formed between the two sugar molecules is known as a **glycosidic bond**.

II. 5. 2. Glycosidic Bond

In biochemistry, glycosidic linkages are critical covalent bonds that connect monosaccharides to form larger carbohydrate structures. A glycosidic linkage is a bond formed between the **anomeric carbon of a monosaccharide** and an oxygen or nitrogen atom of another molecule, typically another monosaccharide or an alcohol. The most common type of glycosidic bond in biochemistry is the **O-glycosidic bond**, where the oxygen atom connects two sugar units. Glycosidic bonds can also form between sugars and non-sugar molecules, but for the purpose of carbohydrate digestion and metabolism, the focus is on the sugar-sugar glycosidic bond (Fig.38).

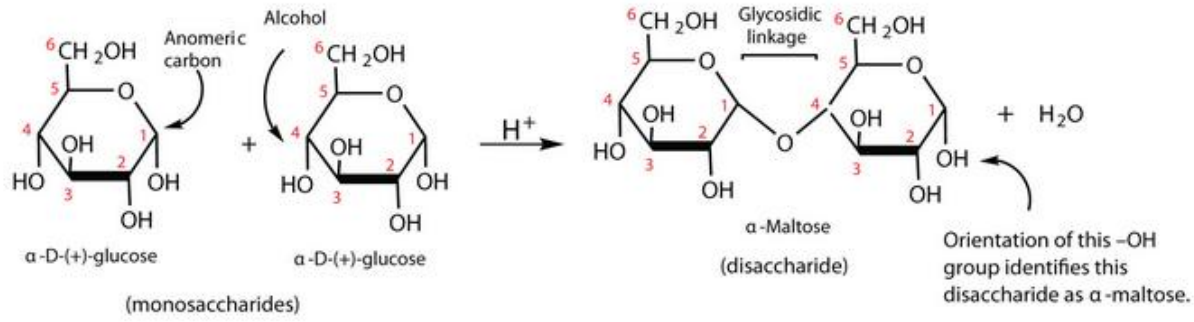


Fig. 38: Mechanism of Glycosidic Bond Formation Between Two Monosaccharides

➢ Types of Glycosidic Bonds

The type of bond depends on the orientation of the anomeric carbon:

- **α-glycosidic bonds**: Formed when the hydroxyl group on the anomeric carbon is in the alpha (α) position (below the plane of the sugar ring). Example: The bond in maltose.
- **β-glycosidic bonds**: Formed when the hydroxyl group on the anomeric carbon is in the beta (β) position (above the plane of the ring). Example: The bond in cellulose ;

The difference between α and β configurations is crucial in biology because they require specific enzymes for their breakdown. Humans, for instance, can hydrolyze α(1→4) bonds in starch but lack the enzymes to break down β(1→4) bonds in cellulose.

II. 5. 3. Rules of Nomenclature of Disaccharides:

To name disaccharides several rules are followed. By convention, the name describes the compound with its non-reducing end to the left, and we can "build up" the name in the following order.

(1) Give the configuration (α or β) at the anomeric carbon joining the first monosaccharide unit (on the left) to the second.

(2) Name the non-reducing residue; to distinguish five- and six-membered ring structures, insert "furano" or "pyrano" into the name.

(3) Indicate in parentheses the two carbon atoms joined by the glycosidic bond, with an arrow connecting the two numbers; for example, (1-4) shows that C-1 of the first-named sugarresidue is joined to C-4 of the second.

(4) Name the second residue. Exemple: β-D-lactose (β-D-Galactopyranosyl-(1-4)D-glucopyranose)

II.5.3. Classes of disaccharides

Disaccharides can be classified into two groups (reducing sugar and non-reducing sugar) based on their ability to undergo oxidation-reduction reactions.

II.5.2.1. Reducing sugar: A disaccharide in which the reducing sugar has a free hemiacetal unit serving as a reducing aldehyde group. Examples include lactose, maltose and cellobiose.

A. Lactose

Lactose is known as *milk sugar* because it occurs in the milk of humans, cows, and other mammals. In fact, the natural synthesis of lactose occurs only in mammary tissue, whereas most other carbohydrates are plant products. Human milk contains about 7.5% lactose, and cow's milk contains about 4.5%. This sugar is one of the lowest ranking in terms of sweetness, being about one-sixth as sweet as sucrose. Lactose is produced commercially from whey, a by-product in the manufacture of cheese. It is important as an infant food and in the production of penicillin. Lactose is a reducing sugar composed of one molecule of D-galactose and one molecule of D-glucose joined by a β-1,4- glycosidic bond.

The systematic name for lactose is β-D-galactopyranosyl-(1→4)-D-glucopyranose. The two monosaccharides are obtained from lactose by acid hydrolysis or the catalytic action of the enzyme *lactase*.

Many adults and some children suffer from **lactose intolerant,** which occurs when the small intestine produces insufficient lactase, This insufficient occurs when the intestinal lining is damaged by conditions such as acute gastroenteritis, celiac disease, or inflammatory bowel diseases (like Crohn's disease). Damage to the mucosal cells reduces lactase production, leading to lactose malabsorption. As a result, undigested lactose moves into the colon, where bacteria ferment it. This fermentation process produces gases such as hydrogen, leading to symptoms like bloating, abdominal cramps, and diarrhea due to increased water retention in the colon (Fig. 39).

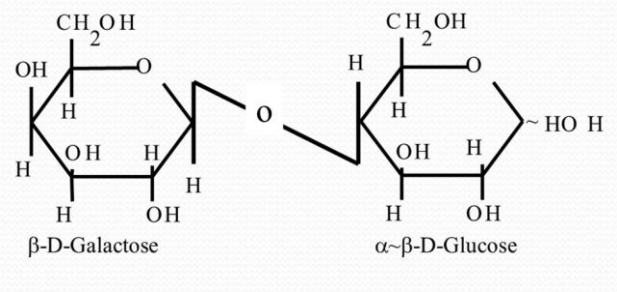


Fig. 39: Lactose Structure

B. Maltose

Maltose is also called maltobiose or malt sugar. Occurs to a limited extent in sprouting grain. It is formed most often by the partial hydrolysis of starch and glycogen.

Maltose is about 30% as sweet as sucrose. The human body is unable to metabolize maltose or any other disaccharide directly from the diet because the molecules are too large to pass through the cell membranes of the intestinal wall. Therefore, an ingested disaccharide must first be broken down by hydrolysis into its two constituent monosaccharide units. In the body, such hydrolysis reactions are catalyzed by enzymes such as *maltase*.

The disaccharide ***maltose*** contains two D-glucose residues joined by a O-glycosidic linkage between C-1 (the anomeric carbon) of one glucose residue and C-4 of the other (Fig. 40)..

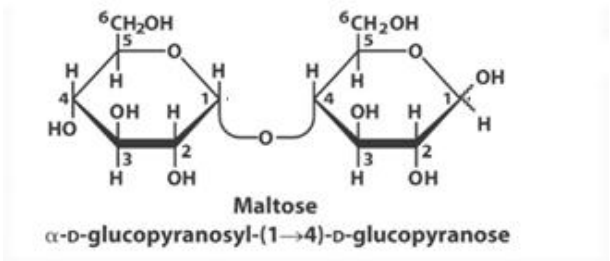


Fig. 40: Maltose structure.

II.5.2.2. Non-reducing Sugar: Disaccharides that do not have a free hemiacetal because they bond through an acetal linkage between their anomeric centers. Examples: sucrose and trehalose.

A. Sucrose

Sucose - the most abundant disaccharide of all- is known as *beet sugar*, *cane sugar*, *table sugar*. Most of the sucrose sold commercially is obtained from sugar cane and sugar beets by evaporation of the water and recrystallization.

The sucrose molecule is unique among the common disaccharides in having an α-1,β-2-glycosidic (head-to-head) linkage. The systematic name for sucrose is α-D-glucopyranosyl-(1→2)-D-fructofuranoside.

Because this glycosidic linkage is formed by the OH group on the anomeric carbon of α-D-glucose and the OH group on the anomeric carbon of β-D-fructose, it ties up the anomeric carbons of both glucose and fructose.

This linkage gives sucrose certain properties that are quite different from those of maltose and lactose. As long as the sucrose molecule remains intact, neither monosaccharide "uncyclizes" to form an open-chain structure. Thus, sucrose is incapable of mutarotation and exists in only one form both in the solid state and in solution. In addition, sucrose does not undergo reactions that are typical of aldehydes and ketones. Therefore, sucrose is a nonreducing sugar.

The hydrolysis of sucrose in dilute acid or through the action of the enzyme *sucrase* (also known as invertase) gives an equimolar mixture of glucose and fructose. This 1:1 mixture is referred to as *invert sugar* because it rotates plane-polarized light in the opposite direction than sucrose. Sucrose typically rotates plane-polarized light to a certain degree (around +34°). However, glucose and fructose have different optical activities—glucose is also dextrorotatory, but fructose is strongly levorotatory. When combined, the overall optical rotation of the mixture becomes negative (usually around -20°). This change in the direction of rotation is what is observed by polarimetry and is the reason the product is called "inverted sugar." (Fig. 41).

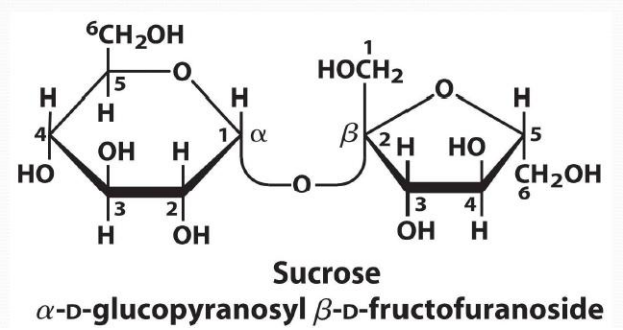


Fig. 41: Sucrose structure

B.Trehalose

Trehalose is a homodisaccharide in which two glucose units are linked by α -1, 1-glycosidic linkage. Its systematic name is α D-glucopyranosyl- (1-1)- α -D-glucopyranoside (Fig. 40). As anomeric carbons of both glucose molecules are involved in glycosidic bond formation, therefore it is a non- reducing sugar. Trehalose is important component of hemolymph (insect blood). It protects the organisms against temperature variations. Hence, it is found in organisms that are naturally subjected to variations in temperature and other environmental stress such as bacterial spores, yeasts and insects.

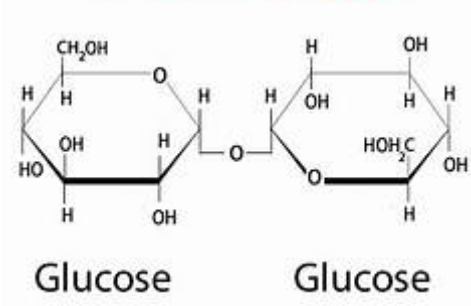


Fig. 42: Structure of Trehalose: Note the inverted glucose

Some other examples of disaccharides include lactulose, chitobiose, kojibiose, nigerose, isomaltose, sophorose, laminaribiose, gentiobiose, turanose, maltulose, trehalose, palatinose, gentiobiulose, mannobiose, melibiose, melibiulose, rutinose, rutinulose, and xylobiose **(Tab. 3).**

Tab. 3: List of disaccharides with their monomer units

Disaccharide	Monomer Units
Sucrose	Glucose and Fructose
Lactose	Galactose and Glucose
Maltose	Glucose and Glucose (alpha-1,4 linkage)
Trehalose	Glucose and Glucose (alpha-1, alpha-1 linkage)
Cellobiose	Glucose and Glucose (beta-1,4 linkage)
Gentiobiose	Glucose and Glucose (beta-1,6 linkage)

II.5.3. Functions of Disaccharides

- Sucrose is a product of photosynthesis, which functions as a major source of carbon and energy in plants.
- Lactose is a major source of energy in animals. Maltose is an important intermediate in starch and glycogen digestion.
- Trehalose is an essential energy source for insects.
- Cellobiose is essential in carbohydrate metabolism.
- Gentiobiose is a constituent of plant glycosides and some polysaccharides.

II.5.4. Hydrolysis of Glycosidic Linkages

II.5.4.1.Definition

Hydrolysis is the process of breaking a bond through the **addition of water**. In the case of glycosidic linkages, the bond between two monosaccharides is cleaved by adding a water molecule, separating the sugars into their individual components. The hydrolysis of glycosidic bonds is an essential step in carbohydrate metabolism, particularly in the digestion of dietary sugars and polysaccharides.

The hydrolysis of glycosidic linkages is catalyzed by enzymes known as glycoside hydrolases (or glycosidases). These enzymes are highly specific, targeting either α- or β-glycosidic linkages depending on the type of carbohydrate. Here are a few examples:

- **Amylase**: This enzyme breaks down **α(1→4) glycosidic bonds** in starch, a polysaccharide made of glucose units. Salivary amylase initiates this process in the mouth, and pancreatic amylase continues it in the small intestine.

- **Lactase**: Lactase hydrolyzes the **β(1→4) glycosidic bond** between glucose and galactose in lactose (the sugar in milk). A deficiency in lactase leads to lactose intolerance.
- **Cellulase**: While humans lack cellulase, certain bacteria and fungi produce this enzyme to break down the **β(1→4) glycosidic bonds** in cellulose, enabling them to digest plant cell walls.

Many glycosidases use acid-base catalysis, where an acidic residue in the enzyme's active site donates a proton (H^+) to the glycosidic oxygen, destabilizing the bond. A base in the active site activates a water molecule, which acts as a nucleophile to attack the anomeric carbon, breaking the bond and forming two free sugar molecules. For example, lysozyme, an enzyme that hydrolyzes glycosidic bonds in bacterial cell walls, uses a glutamic acid residue to donate a proton and a nearby aspartic acid to activate water.

II.5.4.2.Biological importance of glycosidic hydrolysis

1. **Energy Metabolism**: The hydrolysis of glycosidic linkages is central to releasing glucose and other sugars from complex carbohydrates like starch and glycogen. These monosaccharides are then used in cellular respiration to produce ATP, the energy currency of the cell.
2. **Digestion**: In humans, the digestion of dietary carbohydrates begins with the hydrolysis of glycosidic bonds. Polysaccharides like starch are broken down into smaller oligosaccharides, disaccharides (e.g., maltose), and ultimately monosaccharides (e.g., glucose) that can be absorbed into the bloodstream.
3. **Cell Structure**: In plants and fungi, polysaccharides like cellulose and chitin provide structural integrity. The hydrolysis of their β-glycosidic bonds by specialized enzymes enables the decomposition of these biological materials in nature, recycling nutrients in ecosystems.

II.5.4.3.Factors affecting glycosidic hydrolysis

1. **pH**: The activity of glycosidase enzymes is highly pH-dependent, as their catalytic residues (acidic and basic groups) need to be properly protonated or deprotonated. For example, amylase functions optimally at the slightly alkaline pH of the small intestine, while lysosomal enzymes function best in acidic conditions.

2. **Temperature**: As with most enzymatic reactions, hydrolysis rates increase with temperature up to a point. Beyond the enzyme's optimal temperature, denaturation can occur, reducing activity.
3. **Substrate Specificity**: Glycosidase enzymes are highly specific for their substrates, meaning that enzymes that hydrolyze α-glycosidic bonds (e.g., amylase) will not typically hydrolyze β-glycosidic bonds (e.g., those in cellulose). This specificity is crucial in biological systems, preventing unintended breakdown of important polysaccharides.

II.6. Oligosaccharides

Oligosaccharides are compounds that yield 3 to 10 molecules of the same or different monosaccharides on hydrolysis. All the monosaccharides are joined through glycosidic linkage. Based on the number of monosaccharides attached, the oligosaccharides are classified as trisaccharides, tetrasaccharides, pentasaccharides, and so on. The oligosaccharides are normally present as glycans. oligosaccharides are not as abundant in nature as disaccharides. They are normally found in plants and part of some antibiotics

The most common example of oligosaccharide is raffinosen, Stachyose, Verbascose.

A. Raffinose,

also called melitose, is a non-reducing trisaccharide that is widely found in legumes and cruciferous vegetables, including beans, peas, cabbage, brussels sprouts, and broccoli. It consists of galactose connected to sucrose (glucose and fructose) via α (1-6) glycosidic linkage (Fig. 43). Humans lack the enzyme α -galactosidase (α -GAL) which hydrolyses this linkage. As a result, raffinose passes undigested through small intestine and subjected to fermentation by the bacteria present in the large intestine releasing CO2, hydrogen and methane. That’s why beans and these vegetable are sometimes called flatulence food.

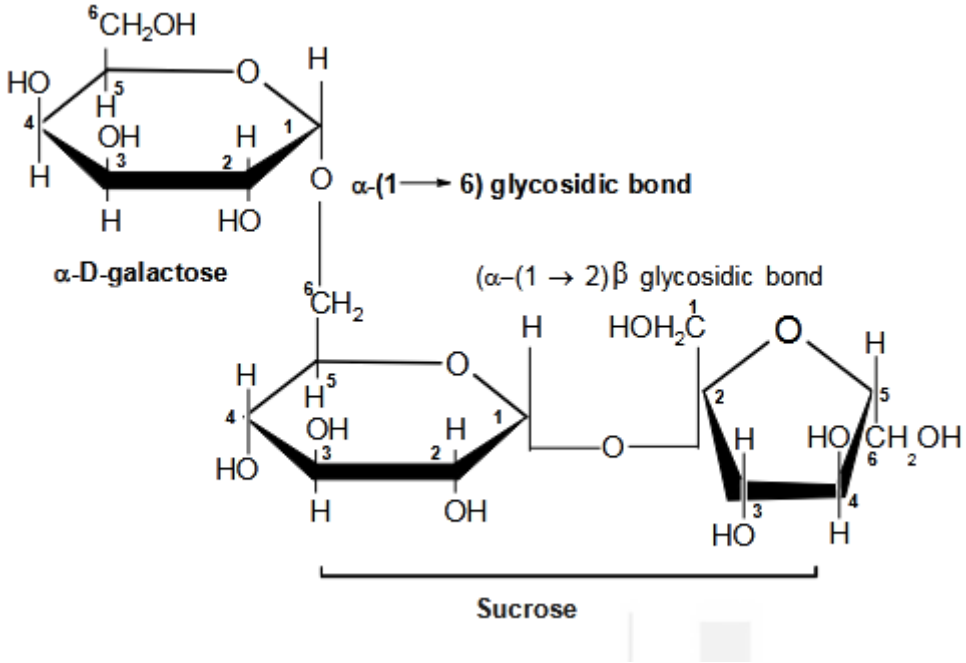


Fig.43: Raffinose structure

B. Stachyose

Stachyose is a non-reducing tetrasaccharide which is found together with raffinose in beans and other plants. It consists of two α -D-galactose units, one α -D-glucose unit, and one β -D-fructose unit sequentially linked as galactose (1-6) galactose (1- 6) glucose (1-2) fructose. Just like raffinose (Fig. 44), it cannot be digested in human intestine and hence causes flatulence.

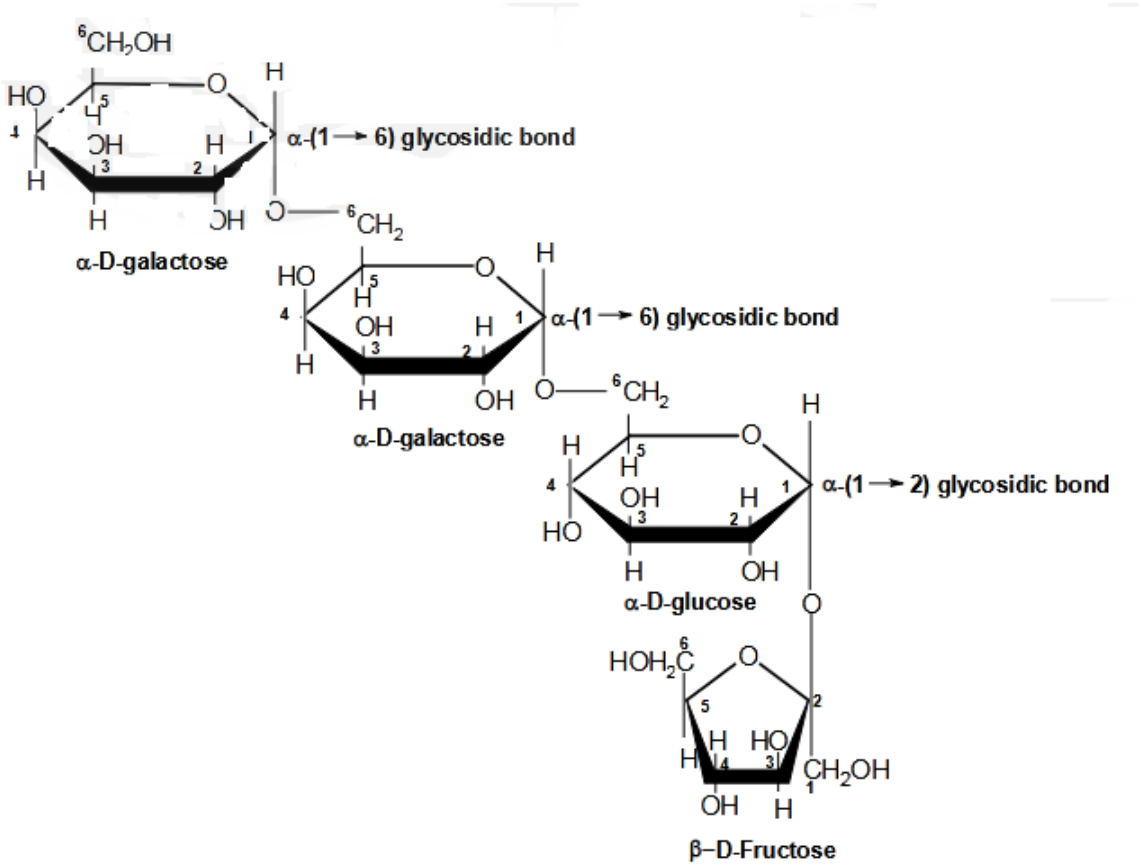


Fig.44: Structure of Stachyose.

C. Verbascose

It is a pentasaccharide consisting of three units of galactose, one of glucose and one of fructose. Three galactose units are linked by α (1 - 6) linkage to each other and to glucose which is then linked to fructose by α (1 - 5) linkage (Fig. 45). Thus it has one reducing end and another non reducing end.

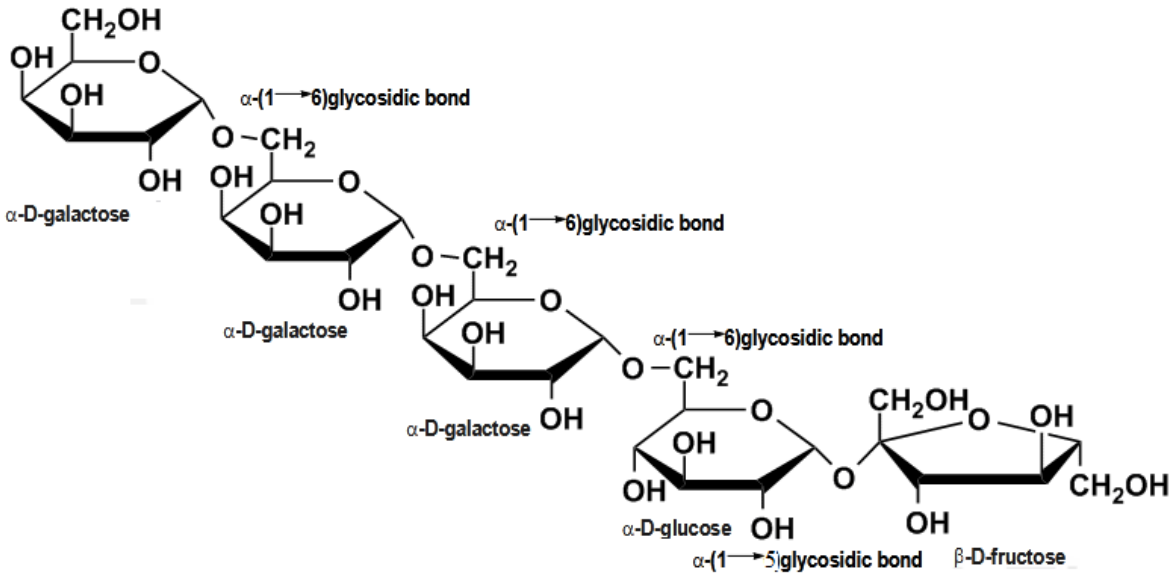


Fig.45: Verbascose Structure

All these poorly digested oligosaccharides act as soluble fibers and may help in excretion. However, when consumed in excess can trigger abdominal bloating, excessive gas and diarrhoea.

II.7. Polysaccharides

Polysaccharides are a chain of more than 10 carbohydrates joined together through glycosidic bond formation. They are ubiquitous and mainly involved in the structural or storage functions of organisms. They are also known as glycans. These compounds' physical and biological properties depend on the components and the architecture of their binding or reacting molecules and their interaction with the enzymatic machinery.

• Polysaccharides are classified based on their functions, the type of monosaccharide units they contain, or their origin. Based on the type of monomers involved in the formation of polysaccharide structures, they are classified into two groups: homopolysaccharides and heteropolysaccharides. And based on their functional roles, these compounds are classified into structural polysaccharides and storage polysaccharides (fig.46).

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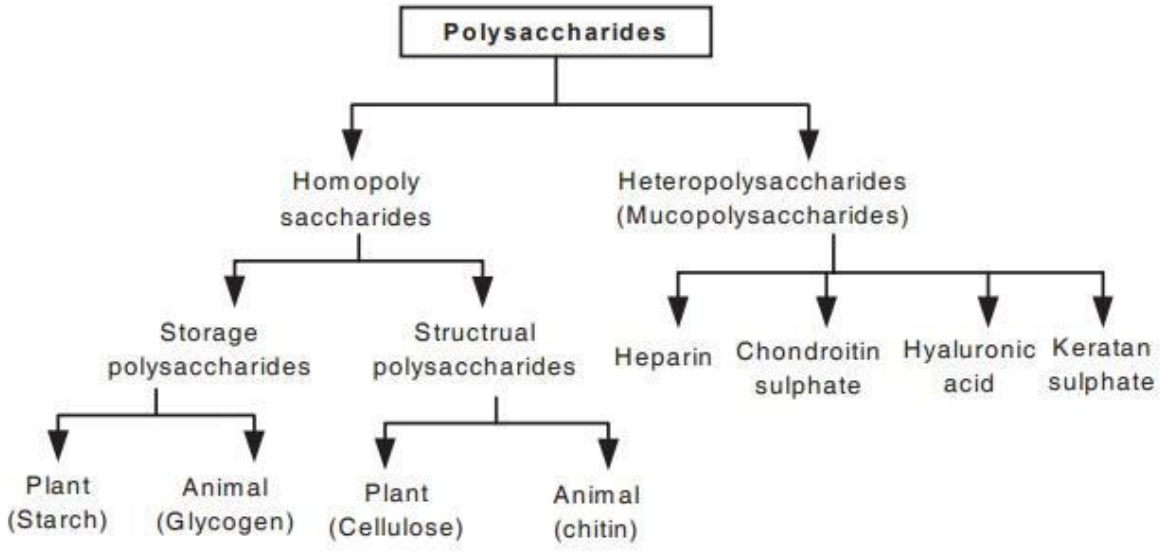


Fig. 46: Classification summary of polysaccharides into different sub-groups**.**

II.7. 1. Homopolysaccharides

They are composed of repeating units of only one type of monomer (monosaccharides) or sugar derivatives. A few examples of homopolysaccharides include: storage polysaccharides (starches (amylose and amylopectin), and glycogen…) and structural polysaccharides (cellulose, chitin),

II.7.1.1 Storage polysaccharides

A. Starch

Starch is made of repeating units of D-glucose that are joined together by alpha-linkages. It's one of the most abundant polysaccharides found in plants and it is an important source of carbohydrates in the human diet. Starch is a mixture of two polymers: amylose and amylopectin. Natural starches consist of about 10%–30% amylose and 70%–90% amylopectin. Amylose is a linear polysaccharide composed entirely of D-glucose units joined by the α-1,4- glycosidic linkages. Because of hydrogen bonding, amylose acquires a spiral structure that contains six glucose units per turn.

Amylopectin is a branched-chain polysaccharide composed of glucose units linked primarily by α-1,4-glycosidic bonds but with occasional α-1,6-glycosidic bonds, which are responsible for the branching. A molecule of amylopectin may contain many thousands of glucose units with branch points occurring about every 25–30 units. In the human body, several enzymes known collectively as amylases degrade starch sequentially into usable glucose units (Fig. 47).

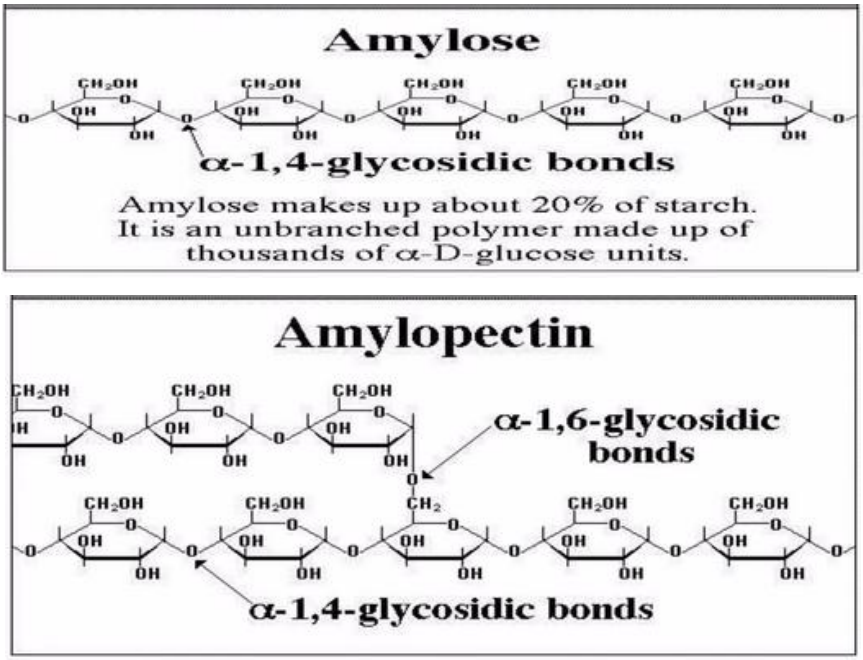


Fig. 47: Starch structure.

B. Glycogen

Glycogen is the energy reserve carbohydrate of animals. Practically all mammalian cells contain some stored carbohydrates in the form of glycogen, but it is especially abundant in the liver (4%–8% by weight of tissue) and in skeletal muscle cells (0.5%–1.0%). Like starch in plants, glycogen is found as granules in liver and muscle cells. When fasting, animals draw on

these glycogen reserves during the first day without food to obtain the glucose needed to maintain metabolic balance.

Glycogen is structurally quite similar to amylopectin, although glycogen is more highly branched (8–12 glucose units between branches) and the branches are shorter. When treated with iodine, glycogen gives a reddish brown color. Glycogen can be broken down into its D-glucose subunits by acid hydrolysis or by the same enzymes that catalyze the breakdown of starch. In animals, the enzyme phosphorylase catalyzes the breakdown of glycogen to phosphate esters of glucose (Fig. 48).

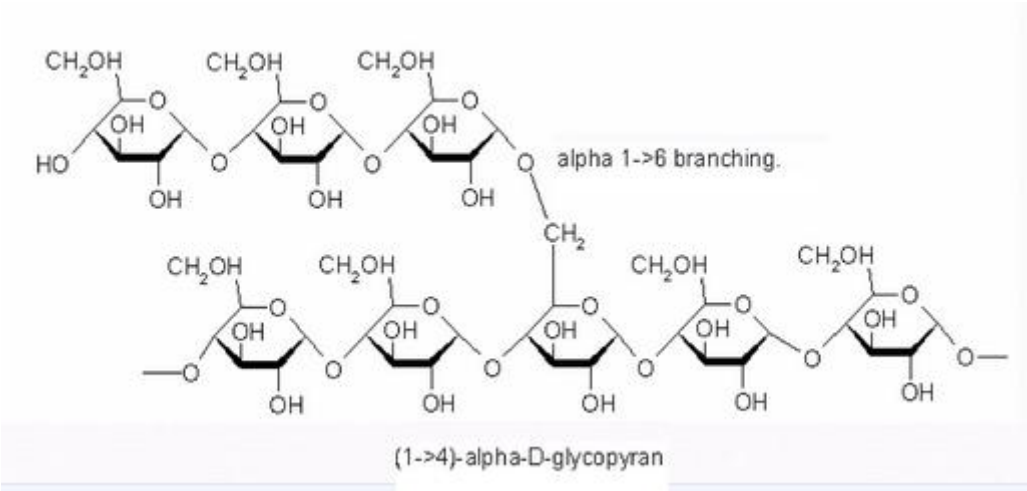


Fig. 48: Glycogen structure.

II.7.1.2. Structural polysaccharides

A. Cellulose

Cellulose, a fibrous carbohydrate found in all plants, is the structural component of plant cell walls. Because the earth is covered with vegetation, cellulose is the most abundant of all carbohydrates, accounting for over 50% of all the carbon found in the vegetable kingdom. Cotton fibrils and filter paper are almost entirely cellulose (about 95%), wood is about 50% cellulose, and the dry weight of leaves is about 10%–20% cellulose.

Like amylose, cellulose is a linear polymer of glucose. It differs, however, in that the glucose units are joined by β-1,4- glycosidic linkages, producing a more extended structure than amylose. This extreme linearity allows a great deal of hydrogen bonding between OH groups on adjacent chains, causing them to pack closely into fibers. As a result, cellulose exhibits little interaction with water or any other solvent. Cotton and wood, for example, are completely insoluble in water and have considerable mechanical strength. Because cellulose

does not have a helical structure, it does not bind to iodine to form a colored product (Fig. 39).

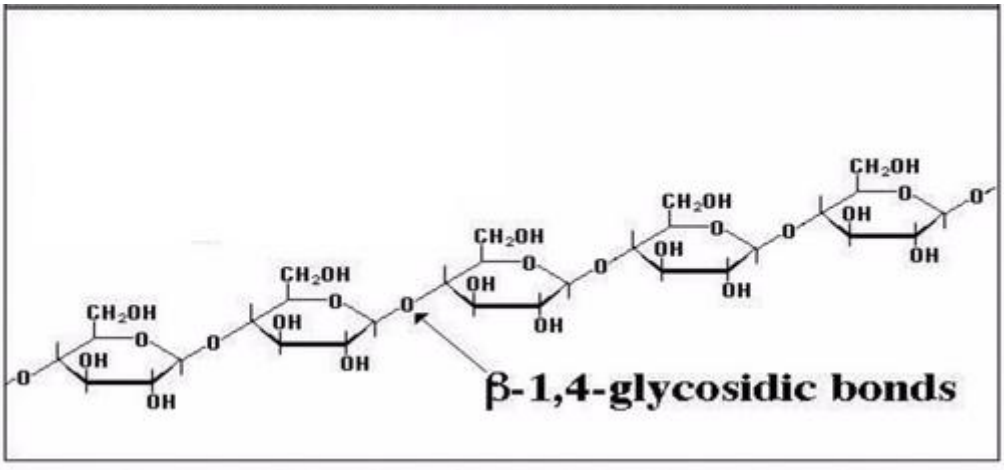


Fig. 49: Cellulose structure

Cellulose yields D-glucose after complete acid hydrolysis, yet humans are unable to metabolize cellulose as a source of glucose. Our digestive juices lack enzymes that can hydrolyze the β-glycosidic linkages found in cellulose, so although we can eat potatoes, we cannot eat grass. However, certain microorganisms can digest cellulose because they make the enzyme cellulase, which catalyzes the hydrolysis of cellulose. The presence of these microorganisms in the digestive tracts of herbivorous animals (such as cows, horses, and sheep) allows these animals to degrade the cellulose from plant material into glucose for energy.

B. Chitin

Chitin, earth's second most abundant carbohydrate polymer (after cellulose) is a homopolysaccharide that is similar to cellulose, both in its biological function and its primary, secondary, and tertiary structure, is chitin. Chitin is present in the cell walls of fungi and is the fundamental material in the exoskeletons of crustaceans, insects, and spiders. The structure of chitin, an extended ribbon, is identical to cellulose, except that the -OH group on each C-2 is replaced by N-acetylglucosamine (-NHCOCH3), so that the repeating units are N-acetyl-D-glucosamines in β (1-4) linkage. Like cellulose, the chains of chitin form extended ribbons and pack side by side in a crystalline, strongly hydrogen-bonded form (Fig.50).

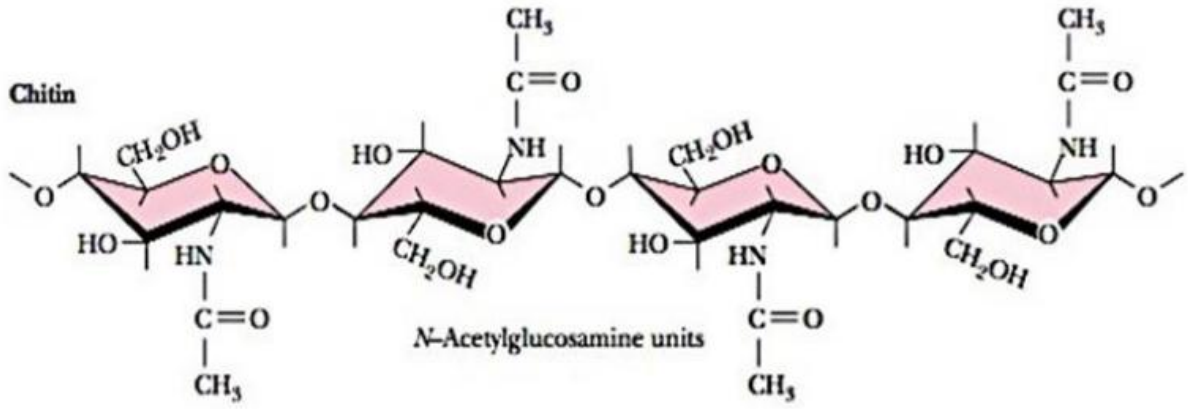


Fig.50: a short fragment of chitin

One significant difference between cellulose and chitin is whether the chains are arranged in parallel (all the reducing ends together at one end of a packed bundle and all the non-reducing ends together at the other end) or antiparallel (each sheet of chains having the chains arranged oppositely from the sheets above and below). Natural cellulose seems to occur only in parallel arrangements. Chitin, however, can occur in three forms, sometimes all in the same organism. α-Chitin is an all-parallel arrangement of the chains, whereas β-chitin is an antiparallel arrangement. In γ-chitin, the structure is thought to involve pairs of parallel sheets separated by single antiparallel sheets.

Chitin's availability and abundance offer opportunities for industrial and commercial applications. Chitin-based coatings can extend the shelf life of fruits, and a chitin derivative that binds to iron atoms in meat has been found to slow the reactions that cause rancidity and flavor loss.

II.7. 2. Heteropolysaccharides

They are composed of two or more repeating units of different types of monomers. Examples include Proteoglycans, Glycoproteins, and Glycolipids. In natural systems, they are linked to proteins, lipids, and peptides. The process in which a protein or lipid is linked to a saccharide to form a glycoprotein or glycolipid is termed glycosylation.

II.7. 2.1. Glycosylation

In biology, glycosylation refers to the process of covalently attaching a carbohydrate to an organic molecule to produce structures like glycoproteins and glycolipids.

A. N-Linked Oligosaccharides

Asparagine is attached to an oligosaccharide during N-linked glycosylation by a beta bond to the side chain's amine nitrogen. N-linked glycosylation takes place concurrently with or cotranslationally with the translation of the proteins. Because sugars are hydrophilic, it is thought that N-linked glycosylation, which is added cotranslationally, influences how

polypeptides fold. Every N-linked oligosaccharide is a pent saccharide, which is composed of five monosaccharides. In eukaryotes, this process occurs at the membrane of the endoplasmic reticulum. Whereas in prokaryotes, it occurs at the plasma membrane.

B. O-Linked Oligosaccharides

The hydroxyl group of the side chain is where oligosaccharides that take part in O-linked glycosylation are connected to threonine or serine. In the Golgi apparatus, where monosaccharide units are added to a whole polypeptide chain, O-linked glycosylation takes place. O-glycosylation occurs on extracellular and cell surface proteins. The secondary and tertiary structures of the polypeptide define where glycosyltransferases will add sugars to O-linked oligosaccharide glycosylation sites (Fig.51).

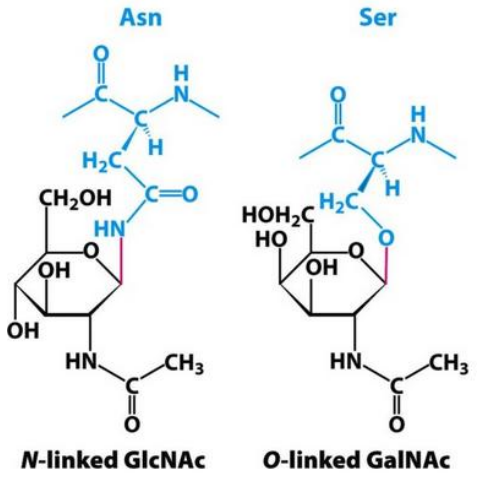


Fig.51: Differences between **N-linked glycosylation** and **O-linked glycosylation** in proteins.

C. Glycosylation of lipids

Glycosylation of lipids the process of attaching sugar molecules to lipids, is a fundamental biological process with significant roles in cell signaling, membrane structure, and cell recognition. Glycolipid biosynthesis primarily occurs in the Golgi apparatus. Glycosyltransferases are the enzymes responsible for adding sugar residues to lipids which catalyze the formation of **glycosidic bonds** between the lipid and sugar.

II.7.2.2. Glycosaminoglycans (GAG) are negatively charged unbranched heteropolysaccharides. They are composed of repeating units of disaccharides with the general structural formula n. Amino acids like N-acetylglucosamine or N-acetylgalactosamine and uronic acid (like glucuronic acid) are commonly found in the GAG structure.

The classification of Glycosaminoglycans is based on:

-Monomeric (acidic & amino sugar) composition

-Degree & location of sulfation

-Type of glycosidic linkages

-Chain length of the disaccharides

-Nature of the core protein

-Their tissue distribution

-Their biologic functions

- A list containing major GAGs is mentioned in the table below:

GAGs	Acidic sugar	Amino sugar
Hyaluronic acid	D-Glucuronic acid	N-acetylglucosamine
Chondroitin sulfate	D-Glucuronic acid	N-acetylgalactosamine
Heparan sulfate	D-Glucuronic acid or L-iduronic acid	N-acetylglucosamine
Heparin	D-Glucuronic acid or L-iduronic acid	N-acetylglucosamine
Dermatan sulfate	D-Glucuronic acid or L-iduronic acid	N-acetylgalactosamine

A. Hyaluronic acid

Hyaluronic acid is unique among the GAGs in that it does not contain any sulfate and is not found covalently attached to proteins as a proteoglycan. It is, however, a component of non-covalently formed complexes with proteoglycans in the Extra cell matrix (ECM). It is the only GAG present both in animals and bacteria. Found in synovial fluid, vitreous humor, ECM of loose connective tissue, Umbilical cord, Cartilage

Hyaluronic acid is found with high concentration in embryonic tissues and is thought to play an important role in permitting cell migration during morphogenesis and wound repair. It Act also as lubricators and shock absorbers (Fig.52)

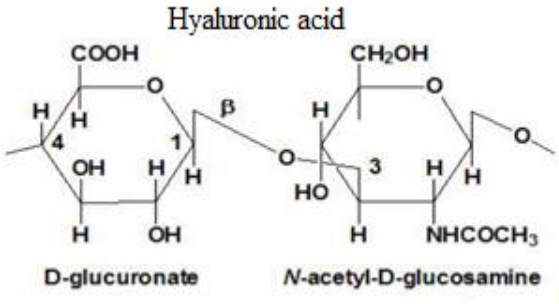


Fig.52: Hyaluronic acid.

B. Heparin (Natural anticoagulant)

The heparin is an intracellular GAG composed of alternating unit of N-sulfo, D-glucosamine 6-sulphate and glucronate 2- sulphate. It is a component of intracellular granules of mast cells lining the arteries of the lungs, liver and skin and has a highest negative charge density of any known biological macromolecule.

Heparin and warfarin are widely used in the treatment of thrombotic and thromboembolic conditions, such as deep vein thrombosis and pulmonary embolus. Heparin is administered first, because of its prompt onset of action, whereas warfarin takes several days to reach full effect. The figure below demonstrates the structure of heparin (Fig.53).

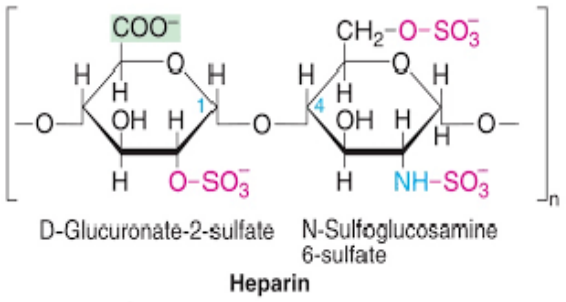


Fig.53: heparin structure.

C. Chondroitin sulfate

Chondroitin sulfate is composed of glucuronic acid and N-acetylgalactosamine sulfate and is the most abundant glycosaminoglycan in the body. It plays a crucial role in cartilage by binding to collagen and firmly holding the collagen fibers together, thereby contributing to the tissue's strength and resilience. Additionally, chondroitin sulfate is found in tendons, ligaments, and heart valves, where it helps maintain structural integrity and elasticity (fig.54).

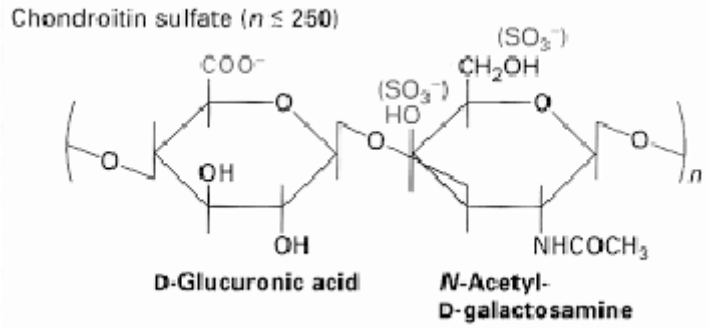


Fig.54: Chondroitin sulfate structure.

D. Dermatan sulfate

Dermatan sulfate is a glycosaminoglycan composed of L-iduronic acid and N-acetylgalactosamine sulfate. It plays several important biological roles, including contributing to the structure and function of various tissues. In the sclera, dermatan sulfate helps provide shape and support to the eye. It also binds low-density lipoprotein (LDL), which is involved in the development of atherosclerosis. Additionally, dermatan sulfate contributes to the elasticity of skin, blood vessels, and heart valves, playing a crucial role in maintaining the flexibility and integrity of these tissues (Fig.55).

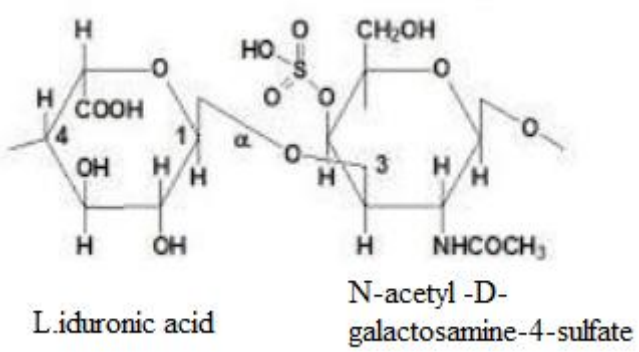


Fig.55: Dermatan structure

II.7. 2.2. Peptidoglycan

The term peptidoglycan was derived from the peptides and the sugars (glycan) that make a molecule; it is also called ‘murein’ or ‘mucopeptide.’ Peptidoglycan consists of a carbohydrate backbone (glycan chain) composed of alternating units of **N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM)** molecules attached through β-1,4-glycosidic bonds (Fig.56) . Peptidoglycan is found only in bacterial cell walls but not in human cells. Peptidoglycan is also absent from the cell walls of *Archaea*, so it is regarded as a key biomarker of bacteria. Peptidoglycan is a good target for antibacterial drugs such as penicillins, cephalosporins, and vancomycin, which inhibit the synthesis of peptidoglycan by inhibiting transpeptidase reactions.

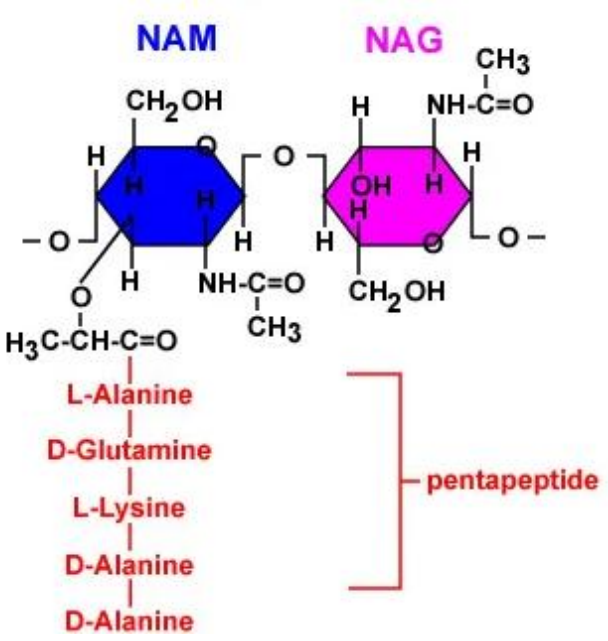


Fig. 56. Peptidoglycan monomer**;** NAG: N-acetylglucosamine and NAM: N-acetylmuramic acid

II.7. 2.3. Proteoglycans

These are macromolecules of the cell surface or extracellular matrix in which one or more glycosaminoglycan chains are joined covalently to a membrane protein or a secreted protein. The glycosaminoglycan moiety commonly forms the greater fraction (by mass) of the proteoglycan molecule, dominates the structure, and is often the main site of biological activity. In many cases the biological activity is the provision of multiple binding sites, rich in opportunities for hydrogen bonding and electrostatic interactions with other proteins of the cell surface or the extracellular matrix. Proteoglycans are major components of connective tissue such as cartilage, in which many noncovalent interactions with other proteoglycans, proteins, and glycosaminoglycans provide strength and toughness. These molecules act as tissue organizers, influence the development of specialized tissues, mediate the activities of various growth factors, and regulate the extra- cellular assembly of collagen fibrils.

II.7. 2.4. Glycoproteins

These have one or several oligosaccharides of variable complexity joined covalently to a protein. They are found on the outer face of the plasma membrane, in the extracellular matrix, and in the blood. Inside cells they are found in specific organelles such as golgi complexes, secretory granules, and lysosomes. The oligosaccharide portions of glycoproteins are less monotonous than the glycosaminoglycan chains of proteoglycans; they are rich in

information, forming highly specific sites for recognition and high- affinity binding by other proteins.

II.7. 2.5. Glycolipids

Glycolipids are carbohydrates attached to lipids, they are essential for changing how membrane proteins those act as receptors and for identifying cells. They may also serve as cellular signaling and recognition receptors. The oligosaccharide's head serves as a binding partner in receptor activity. The binding mechanisms of receptors to the oligosaccharides that are exposed or presented above the surface of the membrane are influenced by the makeup of those oligosaccharides. Because glycolipids' binding mechanisms are so varied, they are essential to numerous biological activities.

Pathogens use as a target as a point of contact and entry. For instance, research on the chaperone action of glycolipids in relation to HIV infection.

Major Functions of glycoproteins and glycolipids

Cell identification: Both glycoproteins and glycolipids, which both aid in classifying different cell types, are found on the surface of all cells. Specific oligosaccharides can be recognized by lectins, or proteins that bind carbohydrates, and this knowledge is helpful for identifying cells based on oligosaccharide binding.

The involvement of glycolipids in classifying blood types is a crucial illustration of oligosaccharide cell recognition. The glycan modification seen on the blood cell surface helps to identify the different blood types. Mass spectrometry can be used to see these. The non-reducing ends of the oligosaccharide are where the oligosaccharides on the A, B, and H antigens are located.

Cell adherence: Many different types of cells make lectins, which are unique carbohydrates-binding proteins that work with oligosaccharides to mediate cell adhesion. A class of lectins called selectins mediates some types of cell-cell adhesion; including the adherence of leukocytes to endothelial cells.

Endothelial cells can temporarily express specific selectins during an immune response in reaction to cell damage or injury.

CHAPITRE III: STRUCRURE AND PHYSICO-CHEMICAL PROPRIETES OF AMINO ACIDS, PEPTIDES AND PROTEINES

III.1. Amino acids

III.1.1. Biomedical importance

L-α-Amino acids provide the monomer units of the long polypeptide chains of proteins. In addition, these amino acids and their derivatives participate in cellular functions as diverse as nerve transmission, and the biosynthesis of porphyrins, purines, pyrimidines, and urea. The neuroendocrine system employs short polymers of amino acids called *peptides* as hormones, hormone-releasing factors, neuromodulators, and neurotransmitters. Humans and other higher animals cannot synthesize 10 of the L-α-amino acids present in proteins in amounts adequate to support infant growth or to maintain adult health. Consequently, the human diet must contain adequate quantities of these *nutritionally essential* amino acids. Each day the kidneys filter over 50 g of free amino acids from the arterial renal blood. However, only traces of free amino acids normally appear in the urine because amino acids are almost totally reabsorbed in the proximal tubule, conserving them for protein synthesis and other vital functions.

Certain microorganisms secrete free D-amino acids, or peptides that may contain both D- and L-α-amino acids. Several of these bacterial peptides are of therapeutic value, including the antibiotics bacitracin and gramicidin A, and the antitumor agent bleomycin. Certain other microbial peptides are, however, toxic. The cyanobacterial peptides microcystin and nodularin are lethal in large doses, while small quantities promote the formation of hepatic tumors. The ingestion of certain amino acids present in the seeds of legumes of the genus *Lathyrus* can result in lathyrism, a tragic irreversible disease in which individuals lose control of their limbs. Certain other plant seed amino acids have also been implicated in neurodegenerative disease in natives of Guam.

III.1.2. Structure of amino acids

Although more than 300 different amino acids have been described in nature, only 20 are commonly found as constituents of mammalian proteins. [Note: These are the only amino acids that are coded by DNA, the genetic material in the cell]

Each amino acid (except for proline, which has a secondary amino group),) consist of α-carbon atom that attached to

- α carboxyl group
- α primary amino group

- α hydrogen atom
- α distinctive side chain("R group")

The general structure of an α-amino acid can be represented as given in Fig. 57 (a). The structure of glycine as an example is given in Fig. 57 (b).

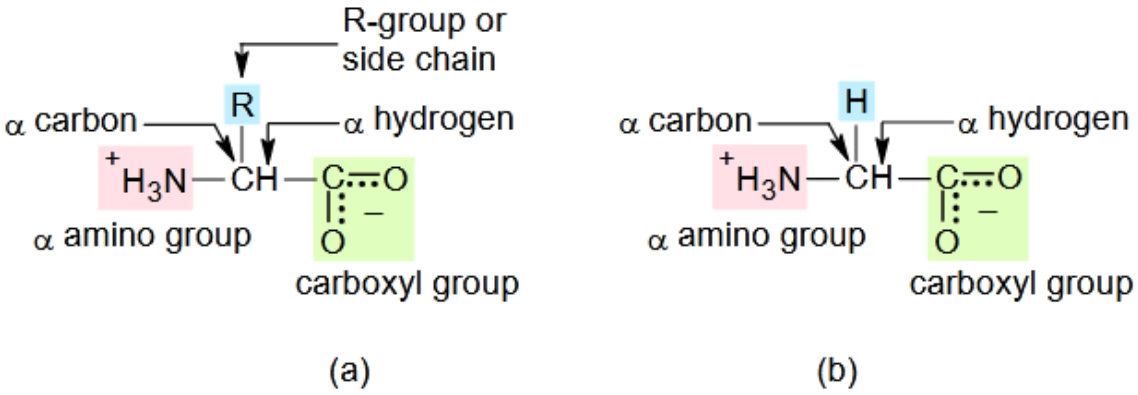


Fig. 57: a) General structure of an amino acid; b) Structure of glycine.

L- and D-amino acids;

Every amino acid comes in two forms, a 'left-handed' (L) and a 'right-handed' (D) form. These two forms are identical in every respect except for the conformation of the subunits of which they are composed. That is to say, although chemically they contain the same elements, in precisely the same quantities and in the same sequence, they are the mirror image of each other. In a Fischer projection of an amino acid, if the amino group ($-NH_2$) is on the **right**, the amino acid is designated as **D**. If the amino group is on the **left**, the amino acid is designated as **L**. Protein chains cannot be formed from a combination of L and D amino acids (Fig.58).

The body is constructed almost without exception from the L forms of amino acid. However, the D forms, which occur in nature, are often found to have therapeutic value. Although D-amino acids do occur in nature, they are never present in proteins.

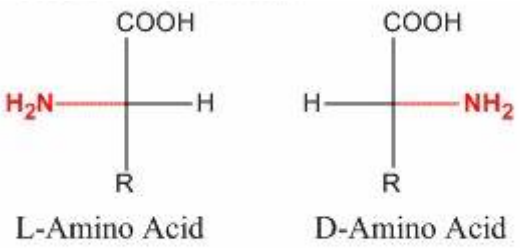


Fig.58: D-and L-forms of amino acid

III.1.4.5. D-Amino Acids

D-Amino acids that occur naturally include free D-serine and D-aspartate in human brain tissue, D-alanine and D-glutamate in the cell walls of gram-positive bacteria, and D-amino acids in certain peptides and antibiotics produced by bacteria, fungi, reptiles, and amphibians. *Bacillus subtilis* excretes D-methionine, D-tyrosine, D-leucine, and D-tryptophan to trigger biofilm disassembly, and *Vibrio cholerae* incorporates D-leucine and D-methionine into the peptide component of its peptidoglycan layer.

III.1.3. Abbreviations and symbols of amino acids

Each amino acid name has an associated three-letter abbreviation and a one-letter symbol**.** The one-letter codes are determined by the following rules.

- *Unique first letter:* If only one amino acid begins with a given letter, then that letter is used as its symbol. For example, V = valine.
- *Most commonly occurring amino acids have priority:* If more than one amino acid begins with a particular letter, the most common of these amino acids receives this letter as its symbol. For example, glycine is more common than glutamate, so G = glycine.
- *Similar sounding names:* Some one-letter symbols sound like the amino acid they represent. For example, F = phenylalanine, or W = tryptophan.
- *Letter close to initial letter:* For the remaining amino acids, a one-letter symbol is assigned that is as close in the alphabet as possible to the initial letter of the amino acid, for example, K = lysine **(Tab.4).**

Tab.4: amino acid name, three-letter abbreviation and one-letter symbol

Amino Acid	Three Letter Code	One Letter Code
Alanine	Ala	A
Arginine	Arg	R
Aspartic Acid	Asp	D
Asparagine	Asn	N
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y

III.1.4. Classification of amino acids

There are different ways of classifying the amino acids based on the structure and polarity, nutritional requirement, metabolic fate etc.

III.1.4.1. Classification of amino acids based on polarity

Amino acids are classified into four groups based on their polarity.

A. Amino acids with nonpolar side chains

Each of these amino acids has a nonpolar side chain that does not gain or lose protons or participate in hydrogen or ionic bonds (Fig. 59). The side chains of these amino acids can be thought of as “oily” or lipid-like, a property that promotes hydrophobic interactions.

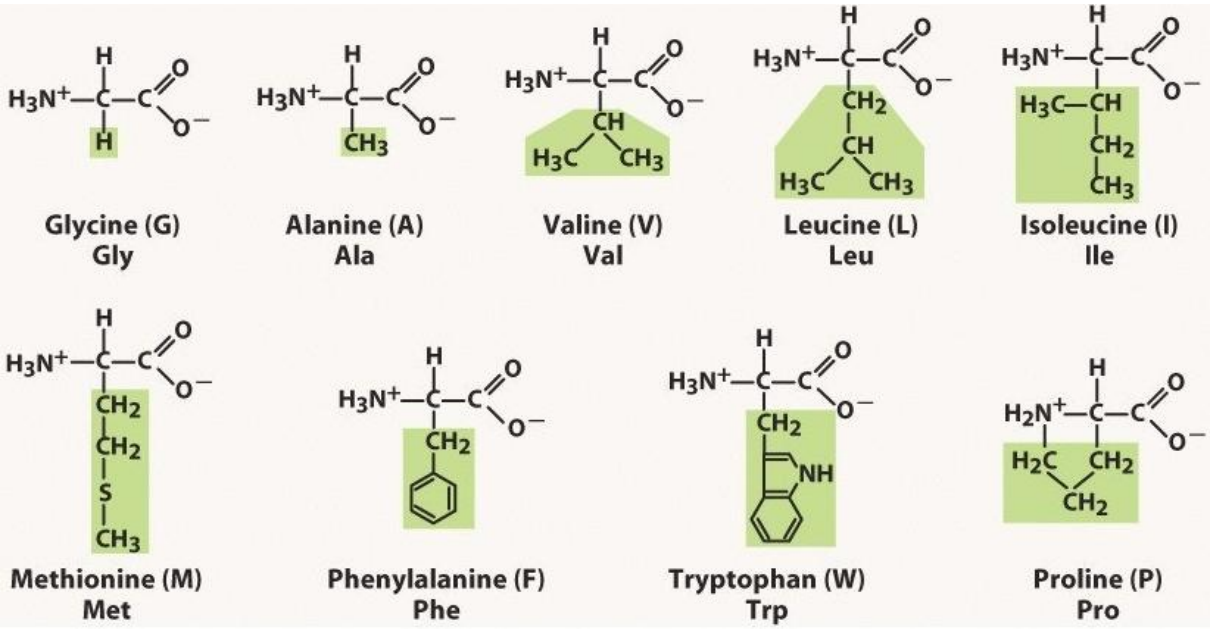


Fig.59: Structure of Amino acids with nonpolar side chains

B. Amino acids with uncharged polar side chains

These amino acids have zero net charge at physiologic pH, although the side chains of cysteine and tyrosine can lose a proton at an alkaline pH (Fig. 60). Serine, threonine, and tyrosine each contain a polar hydroxyl group that can participate in hydrogen bond formation. The side chains of asparagine and glutamine each contain a carbonyl group and an amide group, both of which can also participate in hydrogen bonds

C. Polar amino acids with negative R groups

The amino acids aspartic and glutamic acid are proton donors. At physiologic pH, the side chains of these amino acids are fully ionized, containing a negatively charged carboxylate group (–COO–). They are, therefore, called aspartate or glutamate to emphasize that these amino acids are negatively charged at physiologic pH (Fig. 60).

D. Polar amino acids with positive R groups

The side chains of the basic amino acids accept protons (Fig. 60). At physiologic pH, the R groups of lysine and arginine are fully ionized and positively charged. In contrast, histidine is weakly basic.

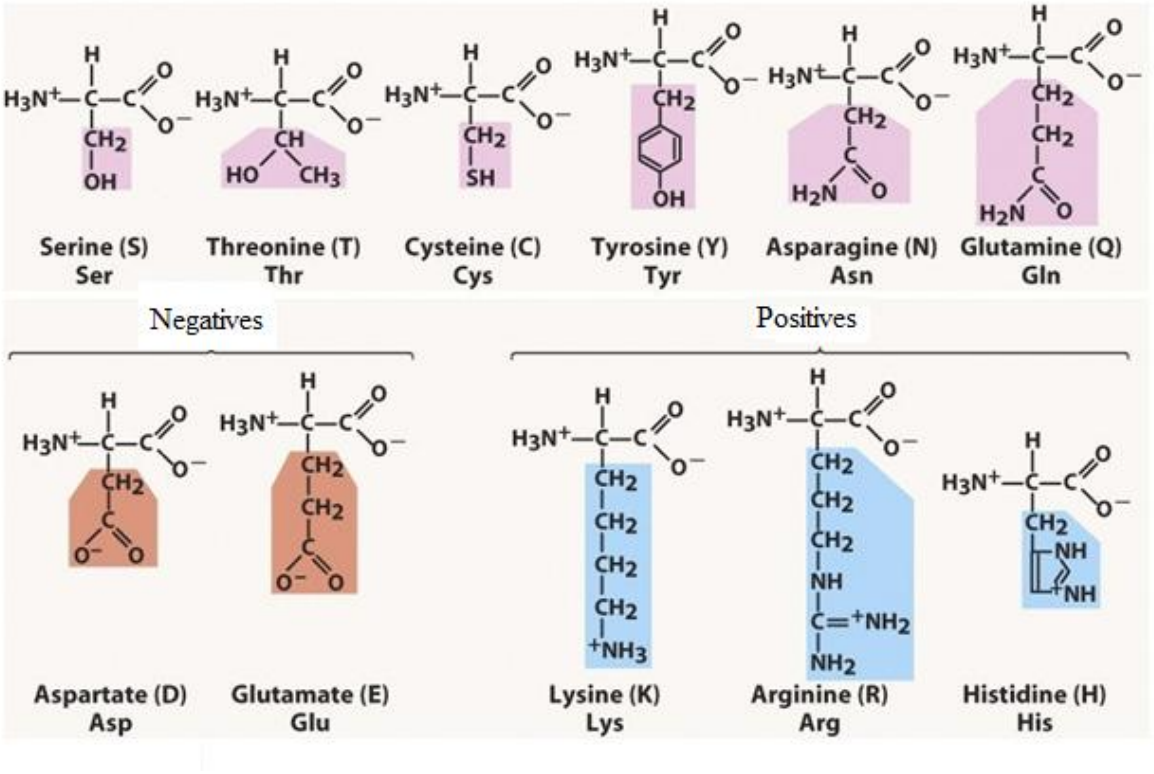


Fig. 60: Structure of Amino acids with uncharged polar side chains, positives and negatives side chains

III.1.4.2.. Classification of amino acids based on structure

A. Amino acids with aliphatic side chain

Aliphatic amino acids have nonpolar, hydrophobic side chains consisting of hydrocarbon chains (essentially, chains of carbon and hydrogen atoms). These are the most simple amino acids having one amino and one carboxylic acid. For example, Glycine, alanine, valine, leucine, and isoleucine.

B.Hydroxy amino acids

There are some amino acids in which the hydroxyl group is present in their side chain. Serine, threonine, and Trysosine containing hydroxyl group. But, Tyrosine is aromatic in nature. Therefore, it is considered an aromatic amino acid.

C. Sulphur containing Amino acids

Cysteine and Methionine, are sulphur-containing amino acids. Cysteine contains a sulfhydryl group and methionine contains a thioether group.

D.Acidic amino acids

Aspartic acid and glutamic acids are acidic amino acids in which two carboxylic acids and one amino acids are present. Asparagine and glutamine are their respective amide derivatives.

E. Basic amino acids

Three amino acids lysine, arginine, and histidines are dibasic monocarboxylic acids. Arginine contains a guanidine group and histidine contains an imidazole ring.

F.Aromatic amino acids

Phenylalanine, Tyrosine, and Tryptophan are aromatic amino acids. Tryptophan contains an indole ring.

G.Imino acids

There is a unique amino acid Proline that contains an imine group(=NH) instead of an amino group ($-NH_2$). Therefore, these are also called alpha-imino acid.

III.1.4.3. Nutritional classification of amino acids

Amino acids can be classified into:

A. Essential amino acids

These amino acids can't be formed in the body and so, it is essential to be taken in diet. Their deficiency affects growth, health and protein synthesis. Essential amino acids include: valine, leucin, isoleucine, phenylalanine, tryptophan, methionine, lysine and threonine.

B. Semi essential amino acids

These are formed in the body but not in sufficient amount for body requirements especially in children. The two amino acids namely arginine and histidine can be synthesized by adults but in growing children the rate of their biosynthesis cannot cope with rate of protein synthesis, so must be supplied in diet, hence these are considered as semi–essential amino acids

C. Non essential amino acids

These are the rest of amino acids that are formed in the body in amount enough. These include: alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine.

Tyrosine is produced from phenylalanine, so if the diet is deficient in phenylalanine, tyrosine will be required as well.

III.1.4.4. Selenocysteine, the 21st Protein L-α-Amino Acid

Selenocysteine (Fig. 61) is an L-α-amino acid present in proteins from every domain of life. Humans contain approximately two dozen selenoproteins that include certain peroxidases and reductases, selenoprotein P, which circulates in the plasma, and the iodothyronine deiodinases

responsible for converting the prohormone thyroxine (T4) to the thyroid hormone 3,3′5-triiodothyronine (T3). Peptidyl selenocysteine is not the product of a posttranslational modification, but is inserted directly into a growing polypeptide during *translation.*

Selenocysteine thus is commonly termed the "21st amino acid.

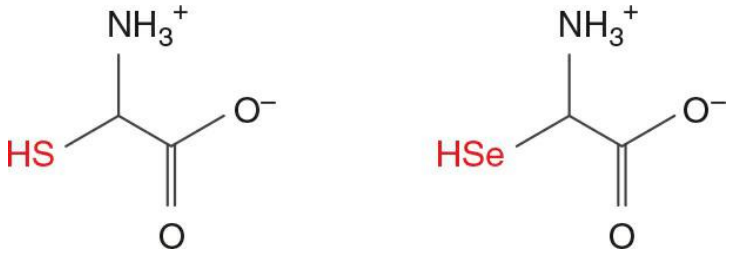


Fig 61: Cysteine (left) & selenocysteine (right). p*K*3, for the selenyl proton of selenocysteine is 5.2. Since this is 3 pH units lower than that of cysteine, selenocysteine represents a better nucleophile at or below pH 7.4.

III.1.4.5. Pyrrolysine ; the 22st Protein L-α-Amino Acid

Pyrrolysine (symbol: Pyl, abbreviation: O) is a unique amino acid that is not found in proteins in most organisms but is crucial in certain archaea and bacteria. Its structure includes a pyrroline ring fused to a lysine side chain (Fig. 62). The incorporation of pyrrolysine into proteins is mediated by a unique mechanism involving a specific tRNA (tRNA^Pyl) and mRNA sequence. The precise functions of pyrrolysine-containing proteins vary. They are often enzymes involved in specialized metabolic pathways, particularly those related to methanogenesis or methylamine metabolism. The presence of pyrrolysine expands the genetic code, allowing organisms that use it to encode additional functional diversity beyond the standard 20 amino acids. This evolutionary adaptation reflects the unique metabolic niches occupied by these organisms.

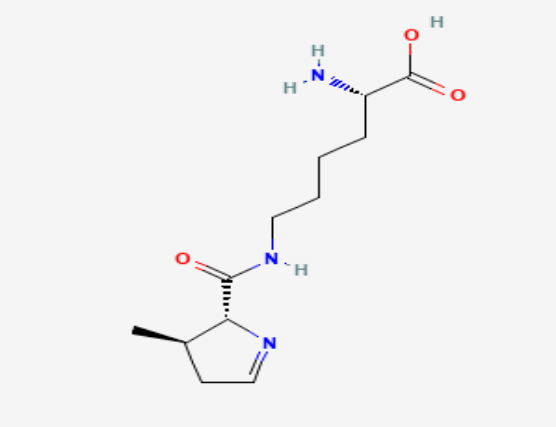


Fig. 62: Pyrrolysine structure

III.1.5. Physicochemical properties of amino acids

III.1.5.1. Physical properties of amino acids

A. Absorption of visible light

Amino acids do not absorb visible light and thus are colorless. However, tyrosine, phenylalanine, and tryptophan absorb high-wavelength (250-290 nm) ultraviolet light. Because it absorbs ultraviolet light about 10 times more efficiently than either phenylalanine or tyrosine, tryptophan makes the major contribution to the ability of most proteins to absorb light in the region of 280 nm (Fig.63).

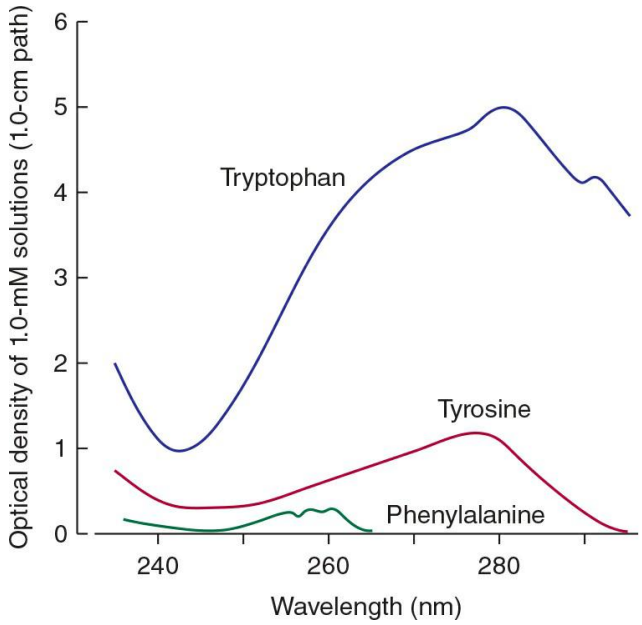


Fig.63: Ultraviolet absorption spectra of tryptophan, tyrosine, and phenylalanine**.**

B. Optical properties of amino acids

With the exception of glycine, all the amino acids recovered from polypeptides are **optically active**; that is, they rotate the plane of polarized light and exist as dextrorotatory or laevorotatory isomers. Optical activity depends on the Ph and side chain.. The Cα atoms of the amino acids (except glycine) are asymmetric centers. Glycine, which has two H atoms attached to its Cα atom, is superimposable on its mirror image and is therefore not optically active. Few amino acids like isoleucine and threonine have an additional asymmetric carbon in their structures.

The optical activity of amino acid is measured using a polarimeter and according to the law of Biot (see previous chapter).

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

Compounds that rotate the plane polarized light in clockwise direction are termed as **dextrorotatory** and designated by plus sign (+), while the compounds that rotate the plane polarized light in anti or counterclockwise are termed as **levorotatory** and designated by minus sign (-).

C. Solubility of Amino Acids

The solubility of amino acids reflects their ionic character. The charges conferred by the dissociable functional groups of amino acids ensure that they are readily solvated and thus soluble in-polar solvents such as water and ethanol, but insoluble in nonpolar solvents such as benzene, hexane, or ether.

D. Melting Point

Amino acids are generally melted at a higher temperature of ten above 200^0C.

E. Taste

Amino acids may be sweet (Gly, Ala & Val), tasteless (Leu) or Bitter (Arg & Ile).

III.1.5.2. Chemical properties of amino acids

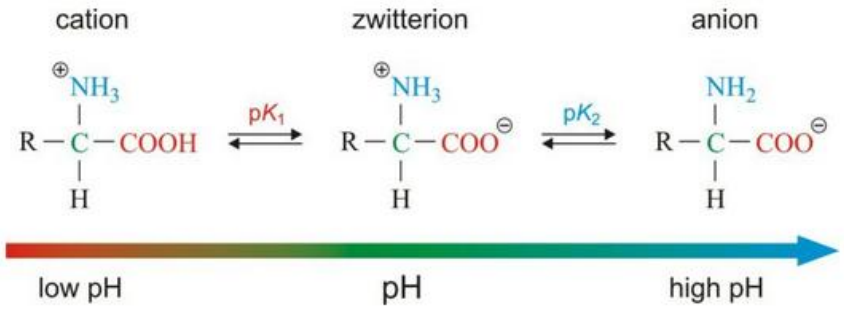
The study of chemical reactions of amino acids is important:

- For identification and analysis of amino acids in protein.
- For identification of amino acid sequences in protein.
- For identification of specific amino acid residue of native protein that are required for biological functioning e.g. haemoglobin (Histidine has role in it).
- For chemical modification of amino acids residue in protein molecules to produce change in biological activity.
- For chemical synthesis of the polypeptides (for medical purpose).

All alpha carboxyl and alpha amino groups of amino acids share common chemical reactivity. Amino acid side chains, on the other hand, exhibit specific chemical reactions depending on the type of functional groups they carry.

III.1.5.2.1. Acidic and basic properties of amino acids

Amino acids may have positive, negative, or zero net charge (zweeterion).



In aqueous solution, the charged and uncharged forms of the ionizable weak acid groups α COOH and α NH_3^+ exist in dynamic protonic equilibrium:

$$R—COOH \leftrightarrows R—COO^- + H^+$$
$$R—NH_3^+ \leftrightarrows R—NH_2 + H^+$$

While both R—COOH and R—NH_3^+ are weak acids, R—COOH is a far stronger acid than R—NH_3+. Thus, at physiologic pH (pH 7.4), carboxyl groups exist almost entirely as R—COO- and amino groups predominantly as R—$NH3^+$. The imidazole group of histidine and the guanidino group of arginine exists as resonance hybrids with positive charge distributed between two nitrogens (histidine) or three nitrogens (arginine).

- *pKa Values Express the Strengths of Weak Acids*

pK_a is defined as the negative $\log_{10}$ of the dissociation constant of an acid, its K_a. Therefore, the pK_a is a quantitative measure of how easily or how readily the acid gives up its proton [H^+] in solution and thus a measure of the "strength" of the acid. Strong acids have a small pKa, weak acids have a larger pKa.

The relationship between pKa and pH is mathematically represented by Henderson-Hasselbach equation shown below, where [A-] represents the deprotonated form of the acid and [HA] represents the protonated form of the acid.

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

- *At Its Isoelectric pH (pI), an Amino Acid Bears No Net Charge*

Zwitterions are one example of an isoelectric species. The form of a molecule that has an equal number of positive and negative charges and thus is electrically neutral. The isoelectric pH, also called the pI, is the pH midway between p*K*a values for the ionizations on either side of the isoelectric species.

- *Titration of Amino Acids*

Amino acids contain ionizable groups. The predominant ionic form of these molecules in solution therefore depends on the pH. Titration of an amino acid illustrates the effect of pH on

amino acid structure (Fig. 64). Titration is also a useful tool in determining the reactivity of amino acid side chains. Consider alanine, a simple amino acid, which has two titratable groups. During titration with a strong base such as NaOH, alanine loses two protons in stepwise fashion. In a strongly acidic solution (e.g., at pH 0), alanine is present mainly in the form in which the carboxyl group is uncharged. Under this circumstance the molecule's net charge is -1 because the ammonium group is protonated. If the H- concentration is lowered, the carboxyl group loses its proton to become a negatively charged carboxylate group. Once the carboxyl group has lost its proton, alanine has no net charge and is electrically neutral. The pH at which this occurs is called the **isoelectric point** (pI). As the titration continues, the ammonium group loses its proton, leaving an uncharged amino group. The molecule then has a net negative charge because of the carboxylate group.

The isoelectric point for alanine may be calculated as follows:

$$\mathrm{pI} = \frac{\mathrm{p}K_1 + \mathrm{p}K_2}{2} = \frac{2.35 + 9.69}{2} = 6.02$$

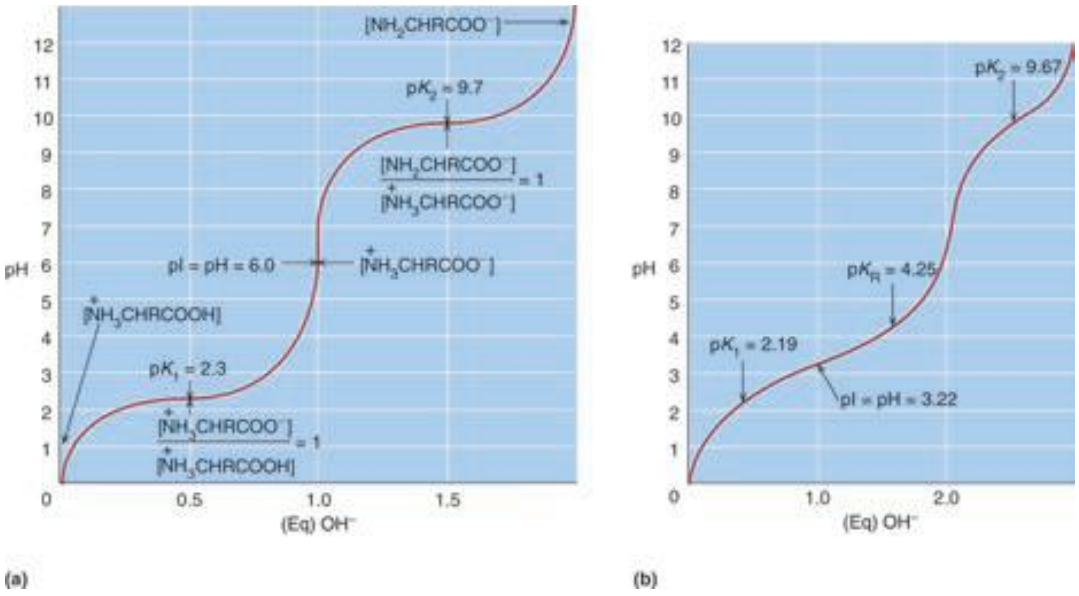


Fig. 64: Titration of Two Amino Acids (a) Alanine and (b) Glutamic Acids.

Amino acids with ionizable side chains have more complex titration curves. Glutamic acid, for example, has a carboxyl side chain group. At low pH, glutamic acid has net charge +1. As base is added, the α carboxyl group loses a proton to become a carboxylate group. Glutamate now has no net charge (Fig. 65).

As more bases are added, the second carboxyl group loses a proton, and the molecule has a -1 charge. Adding additional base results in the ammonium ion losing its proton. At this point, glutamate has a net charge of –2. The pI value for glutamate is the pH halfway between the p*K*a values for the two carboxyl

groups (i.e., the p*K*a values that bracket the zwitterion): pI = pk1+ pkr/2

pI =2.19 + 4.25/2=3.22

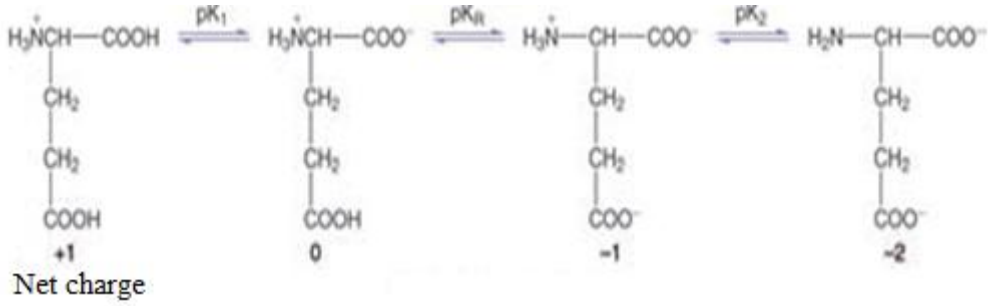


Fig. 65: Titration of glutamic acid

When amino acids are incorporated in polypeptides, the α amino and α carboxyl groups lose their charges. Consequently, except for the α amino and α carboxyl groups of the amino acid residues at the beginning and end, respectively, of a polypeptide chain all the ionizable groups of proteins are the side chain groups of seven amino acids: histidine, lysine, arginine, aspartate, glutamate, cysteine, and tyrosine. In the clinical laboratory, knowledge of the pI guides selection of conditions for electrophoretic separations (**Tab. 5**).

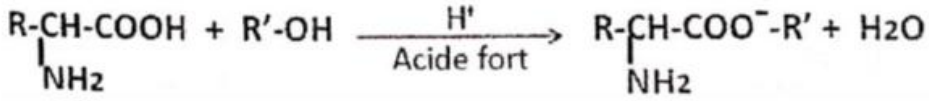
Tab.5: p*K* Values for ionizable groups in proteins

Amino Acid	pK_a of α-COOH	pK_a of α-NH_3^+	pK_a of Side Chain	Isoelectric Point (pI)
Alanine	2.35	9.87	-	6.11
Arginine	2.01	9.04	12.48	10.76
Asparagine	2.02	8.80	-	5.41
Aspartic acid	2.10	9.82	3.86	2.98
Cysteine	2.05	10.25	8.00	5.02
Glutamic acid	2.10	9.47	4.07	3.08
Glutamine	2.17	9.13	-	5.65
Glycine	2.35	9.78	-	6.06
Histidine	1.77	9.18	6.10	7.64
Isoleucine	2.32	9.76	-	6.04
Leucine	2.33	9.74	-	6.04
Lysine	2.18	8.95	10.53	9.74
Methionine	2.28	9.21	-	5.74
Phenylalanine	2.58	9.24	-	5.91
Proline	2.00	10.60	-	6.30
Serine	2.21	9.15	-	5.68
Threonine	2.09	9.10	-	5.60
Tryptophan	2.38	9.39	-	5.88
Tyrosine	2.20	9.11	10.07	5.63
Valine	2.29	9.72	-	6.00

III.1.5.2.2. Reaction due to -COOH group

A. Esterification

Esterification of amino acids involves the reaction of the carboxyl group (-COOH) with an alcohol (typically an alcohol like methanol or ethanol) to form an ester.

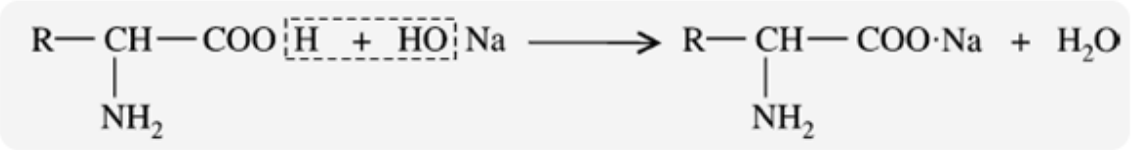


In this reaction:

- The carboxyl group (-COOH) of the amino acid reacts with the hydroxyl group (-OH) of the alcohol.
- This condensation reaction results in the formation of an ester linkage (-COO-R) and water (H_2O).

B. Formation of salts

The carboxyl group of amino acids can release an H^+ ion with the formation of carboxylate (COO^-) ions. These may be neutralized by cations like Na^+ and Ca^{+2} to form salts.



The salts are typically more water-soluble than the free amino acids due to the ionic nature of the salt forms.

Salts of amino acids are important in biochemical processes and are commonly used in analytical techniques, protein purification, and as additives in pharmaceuticals and food industries.

C. Decarboxylation

Decarboxylation is a biochemical reaction where an alpha carboxyl group (-COOH) is removed from an amino acid, releasing carbon dioxide (CO_2) and forming an amine as follow: R-CH(NH_2)-COOH → R-CH_2(NH_2) + CO_2.

Decarboxylation reactions are catalyzed by enzymes called decarboxylases; these enzymes often require cofactors.

- **Histidine** ⟶ Histamine + CO_2
- **Tyrosine** ⟶ Tyramine + CO_2
- **Tryptophan** ⟶ Tryptamine + CO_2
- **Lysine** ⟶ Cadaverine + CO_2
- **Glutamic acid** ⟶ Gamma Amino Butyric Acid (GABA) + CO_2

This reaction is crucial for various biological processes, including neurotransmitter synthesis and the production of biogenic amines.

D) Amidation

Amidation is the process of forming an amide bond. An amide bond is a covalent bond formed between a carboxylic acid and an amine. In biological systems, amidation is crucial for: Peptide bond formation (linking amino acids in proteins) and the synthesis of various biomolecules. Exemple: as shown in the reaction below; the reaction between an amino acid's carboxyl group and another amino acid's amino group to form a peptide bond is an amidation reaction.

III.1.5.2.3. Properties due to the NH2 group

A. Salt Formation with Acids:

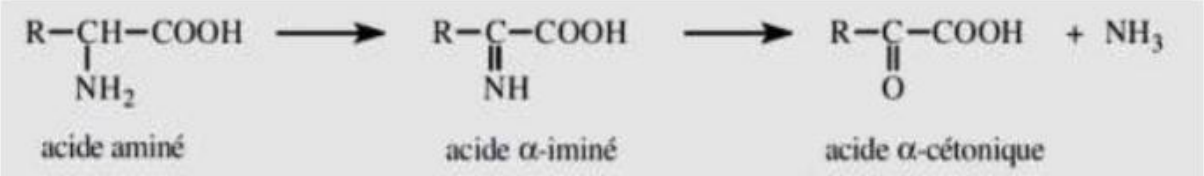
Amino acids can react with acids (e.g., hydrochloric acid, sulfuric acid) to form salts. In these reactions, the amino group ($-NH_2$) of the amino acid reacts with the acid, resulting in the formation of an ammonium ion ($+NH_3$) and a conjugate base of the amino acid.

Example: Amino Acid+HCl→Amino Acid Hydrochloride Salt

B. Deamination

To maintain the intracellular reserve of amino acids used for protein synthesis, the metabolism will go through deaminations with oxidation which will produce α ketone acids.

Deamination is a chemical reaction that involves the removal of an amino group ($-NH_2$) from a molecule. The general reaction is as follow



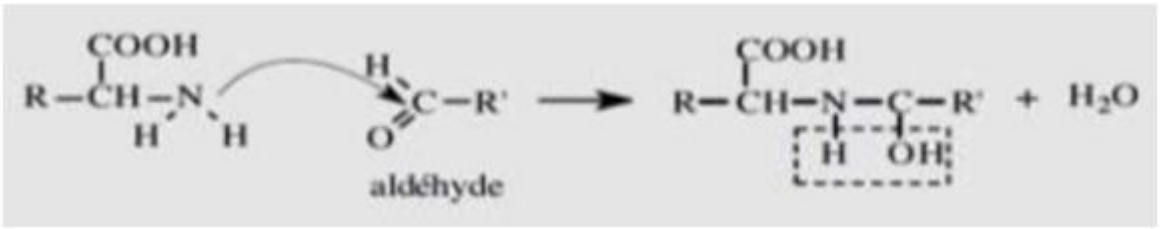
Various enzymes catalyze deamination reactions, including:

Glutamate dehydrogenase: Catalyzes the oxidative deamination of glutamate.

Amino acid oxidases: Catalyze the oxidative deamination of various amino acids.

C. Addition of carbonyl

The α amino functions of amino acids react reversibly with aldehydes to give a very fluorescent product. It is one of the very sensitive means of detecting amino acids. The general reaction is the following :



D. Reaction with Formaldehyde

When the amino acid reacts with two molecules of formaldehyde, it forms an "N-dimethylol derivative" (hydroxy-methyl derivative). This reaction is done in two steps. These derivatives are insoluble in water and resistant to attack by microorganisms (fig. 66).

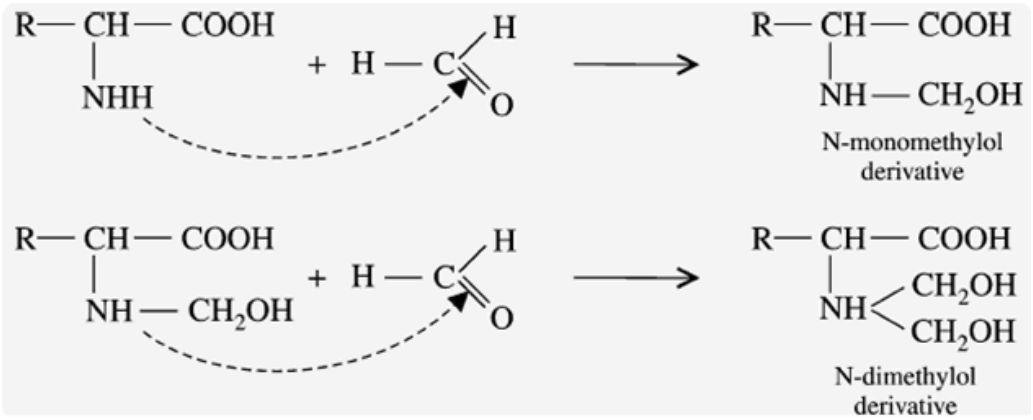


Fig. 66: Formaldehyde Reaction with Amino Acids; The reaction involves a nucleophilic addition of the amino group to the carbonyl carbon of formaldehyde. This forms an unstable intermediate, which then loses a water molecule to produce the N-hydroxymethyl derivative.

This reaction is utilized generaly for the determination of amino acid concentration through formol titration,

E. Reaction with Benzaldehyde

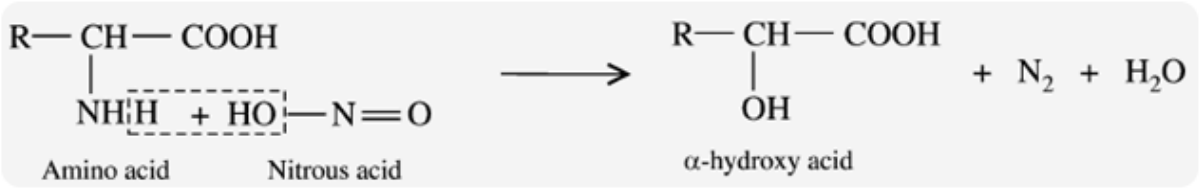
The reaction of amino acids with benzaldehyde is a classic organic chemistry reaction used in qualitative analysis and some synthetic applications. It's often used to detect the presence of amino acids.When the amino acid reacts with Benzaldehyde, it gives "Schiff's base".

General Reaction is as follow:

The amino group ($-NH_2$) of the amino acid acts as a nucleophile, attacking the electrophilic carbonyl carbon (C=O) of benzaldehyde. This leads to the formation of an imine (a C=N bond), also known as a Schiff base. Water is eliminated during this process.

F) Reaction with Nitrous acid (Van Slyke reaction)

The reaction of amino acids with nitrous acid (HNO_2) is known as the **Van Slyke reaction**.When the amino acids react with nitrous acid (HNO_2) to liberate N_2 gas and produce the corresponding "-hydroxyl acid". It's a classic method used to quantify amino acids by measuring the volume of nitrogen gas (N_2) released. The imino acids proline and hydroxyproline do not respond to this reaction. General Reaction is as follow:



Nitrous acid reacts with the amino group of the amino acid to form an unstable diazonium ion ($R\text{-}CH(N_2^+)\text{-}COOH$). The diazonium ion rapidly decomposes, releasing nitrogen gas (N_2) and forming an α-hydroxy acid.

G) Reaction with acylating agents (Acylation)

Acylation of amino acids involves the introduction of an acyl group (R-C=O) into the amino acid molecule. This reaction is often used for protection of amino groups or for modifying the properties of amino acids. Two exemples of acylation reaction are shown below (fig.67):

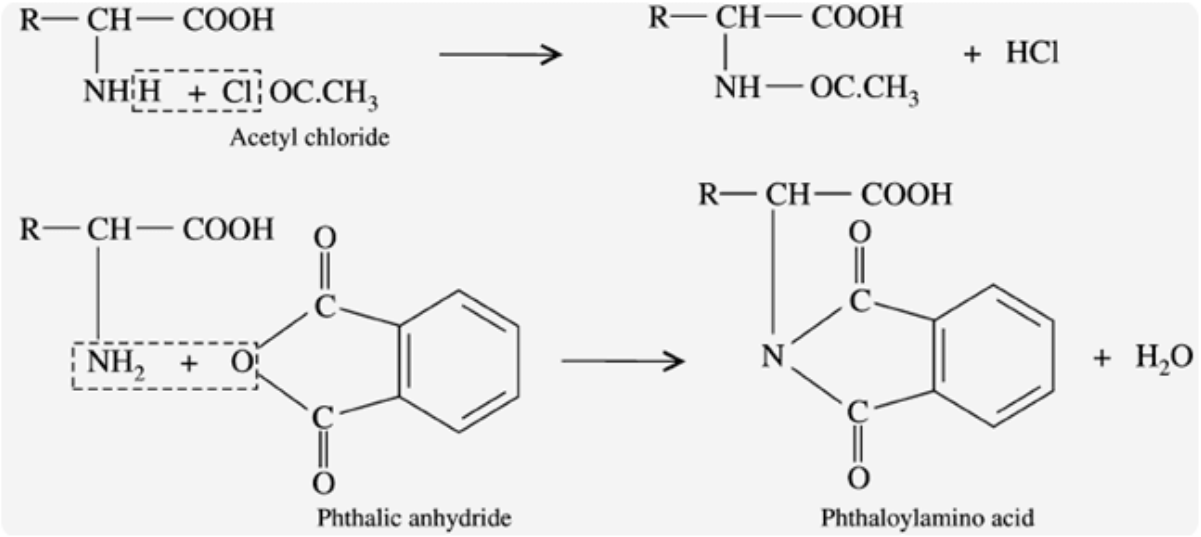


Fig.67: Amino Acid Acylation Reactions

The previous reactions depict two common acylation reactions of amino acids. In the first, an amino acid reacts with acetyl chloride, resulting in the amino group being acetylated, forming an N-acetyl amino acid and releasing HCl. In the second, an amino acid reacts with phthalic anhydride, forming a phthaloylamino acid and water. This also protects the amino group through the formation of a cyclic imide structure. Both reactions are important for modifying amino acids in chemical synthesis and peptide chemistry.

H.Transamination

Transamination is a crucial biochemical reaction involving the transfer of an amino group (-NH_2) from one amino acid to an α-keto acid (Fig.68). This process is essential for amino acid metabolism, allowing for the interconversion of amino acids and the synthesis of non-essential amino acids. Transamination does not result in net deamination, since one amino acid is replaced by another amino acid. It is accomplished by enzymes known as transaminases or aminotransferases, having pyridoxal phosphate as a coenzyme (a derivative of vitamin B6). An aminotransferase may be specific to an amino acid or particular to any member of a group of similar amino acids. Alanine aminotransferase and aspartate aminotransferase are two common types of aminotransferases. Transamination occurs in the

mitochondria and cytoplasm of eukaryotic cells and requires the synthesis of non-essential amino acids from essential amino acids obtained from dietary intake. Aminotransferases are widely distributed in human tissues, including heart muscle, liver, skeletal muscle, and kidney.

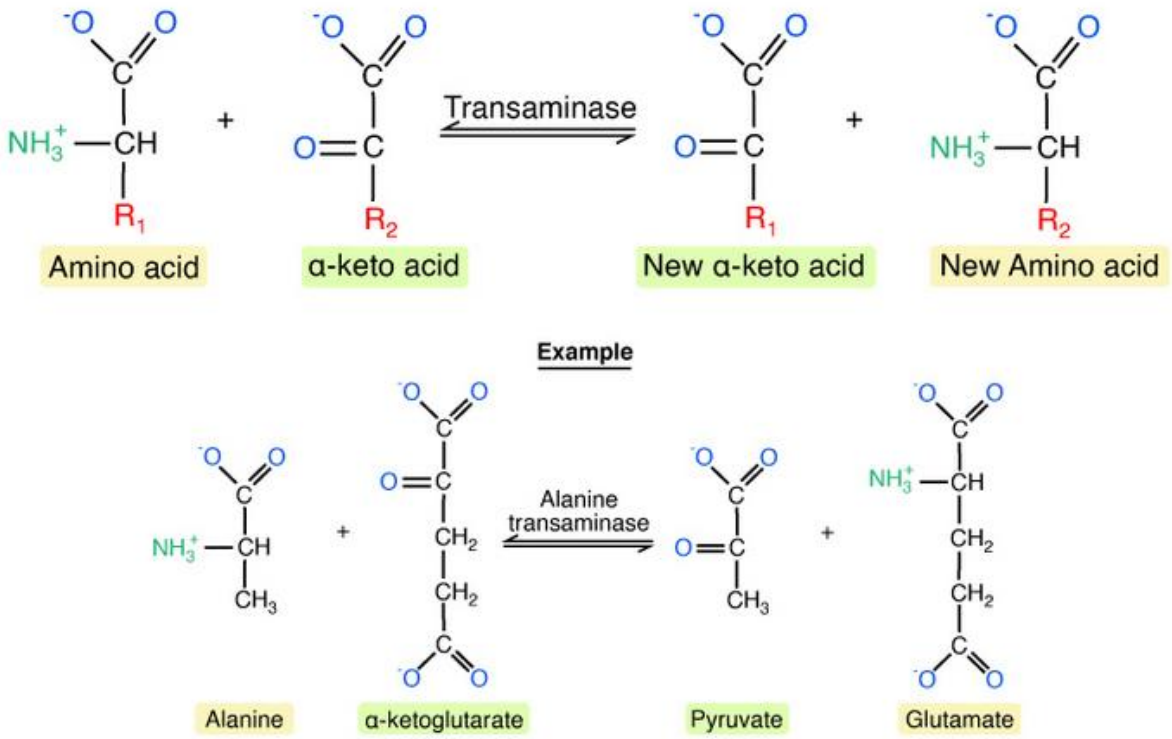


Fig. 68: Transamination Reaction

Some common transamination reactions are tabulated below (tab. 06):

Tab. 06: Exemple of transamination reactions

Examples	Enzymes	Product
1. Alanine to Glutamate Transamination	Alanine transaminase	Pyruvate and glutamate
2. Aspartate-α-Ketoglutarate Transamination	Aspartate transaminase	Oxaloacetate and glutamate
3. Glutamate-α-Ketoglutarate Transamination	Glutamate aminotransferase	α-ketoglutarate and glutamate
4. Serine-Pyruvate Transamination	Alanine aminotransferase	Alanine and hydroxypyruvate
5. Phenylalanine-Pyruvate Transamination	Phenylalanine transaminase	Phenylpyruvate and alanine
6. Tyrosine to α-Ketoglutarate	Tyrosine aminotransferase	p-Hydroxyphenylpyruvate + glutamate
7. Asparagine-Oxaloacetate Transamination	Asparagine aminotransferase	Aspartate and α-ketoglutarate
8. Histidine to α-Ketoglutarate Transamination	Histidine transaminase	Imidazolepyruvate and glutamate

III.1.5.2.4. Due to amino & carboxyl group

Ninhydrin reaction

This assay is based on the fact that two molecules of ninhydrin (2, 2- dihydroxyindane-1, 3-dione) react with a free alpha-amino acid to produce a deep purple or blue color known as Ruhemann's purple (Fig.69).

In this reaction, ninhydrin acts as an oxidizing agent and causes the deamination and decarboxylation of the amino acids at an elevated temperature. This reaction is then followed by condensation between the reduced ninhydrin molecules, released ammonia, and the second molecule of ninhydrin. By the end of the reaction, a diketohydrin complex is formed which has a deep purple color. In amino acids like proline and hydroxyproline, this test yields an iminium salt, which is yellow-orange in color.

The intensity of the formed complex is proportional to the concentration of amino acids in the solution. The color intensity, in turn, depends on the type of amino acid present. The importance of this reaction is in its application in the spectrophotometric quantification of amino acids at 570 nm. [α-imino acids,e.g Proline and Hydroxyproline, yield yellow complex instead of purple, with absorption maxima at 440nm].

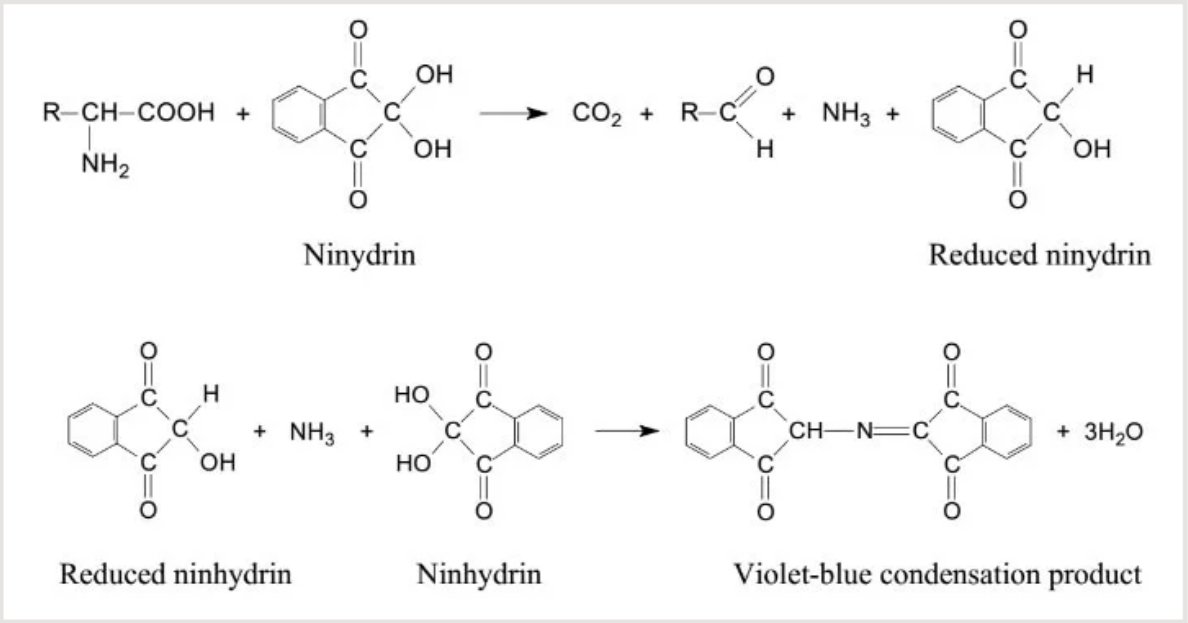


Fig.69: Ninhydrin Reaction with Amino Acids.

This reaction is a very sensitive and it is used for amino acid and imino acid identification.

III.1.5.2.5. Reactions of Specific Functional Groups of the Side Chain

Specific reaction of amino acids is important for degradation, sequencing and chemical synthesis of peptides and proteins. These reactions are useful in identifying specific functional groups and hence specific amino acids possessing such functional groups.

A. Reactions of sulfyhydryl group

The thiol groups of cysteine can react with one another to form disulfide species.

Cysteine is also known to react with iodoacetic acid to yield S–carboxymethyl cysteine derivatives.

B. Xanthoproteic Reactions of Aromatic Ring

Amino acids with aromatic nuclei form yellow nitro derivatives on heating with concentrated nitric acid. This reaction is based on the nitration of benzene ring with concentrated HNO3 yielding yellow derivatives of nitrobenzenes. The reaction is given by tyrosine, tryptophan,

and polypeptide which contain these amino acids. Phenylalanine is more difficult to nitrate and so requires H_2SO_4 as a catalyst.

C. Millon's Reaction

The amino acid called tyrosine, a hydroxybenzene-radical containing compound, reacts with Millon's reagent (a solution of mercuric and mercurous ions in nitric and nitrous acids) to form red complexes.

D. Reaction of tryptophan with formaldehyde

Tryptophan reacts with formaldehyde reagent in the presence of concentrated H_2SO_4, to form a bluish- purple compound appearing as ring at the interface of the liquids. This reaction is characteristic of the indole- ring of tryptophan.

E. Sakaguchi Reaction

Arginine, an amino acid containing guanidinium group, reacts with α- naphthol and an oxidizing agent, such as bromine water, to give a red colour compound.

III.1.6. Separation of amino acids

Amino acids, peptids and proteins can be separated using various methods depending on theirs specific characteristics and the desired separation criteria. Some commonly used methods for separating amino acids are: Electrophoresis methods, chromatographymethods and centrifugation.

III.1.6.1. Electrophoresis

In this method, the amino acids can be separated on the basis of their pI values. A few drops of a solution of amino acid mixture are applied to the middle of the piece of filter paper or to a gel. When this paper or gel is placed in a buffered solution between two electrodes and an electric field is applied, an amino acid having a pI less than the pH of the medium will have an overall positive charge and will move toward the cathode. While an amino acid with a pI greater than the pH of the buffer will have an overall negative charge and will move toward anode. In case of the molecules have the same charge, the larger one will migrate more slowly compared to that of the smaller one during the electrophoresis (Fig. 70). After the separation, the filter paper is sprayed with ninhydrin and dried in a warm oven to give purple colored spot. The amino acids are identified by their location on the paper comparing with a standard.

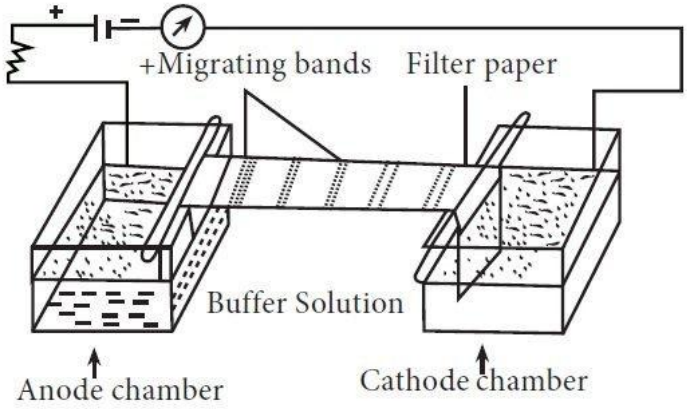


Fig. 70: Separation of Amino Acids with Electrophoresis; The image depicts the core principle of electrophoresis, which is the separation of charged molecules, including amino acids, based on their differential migration in an electric field.

III.1.6.2. Chromatographic methods for separation:

A. Size Exclusion Chromatography

Size exclusion chromatography is one of chromatographic separation modes. The column used is filled with material containing many pores. When dissolved molecules of various sizes flow into the column, smaller dissolved molecules flow more slowly through the column because they penetrate deep into the pores, whereas large dissolved molecules flow quickly through the column because they do not enter the pores. Consequently, larger molecules elute from the column sooner and smaller molecules later, which effectively sorts the molecules by size. This is the separation principle of size exclusion chromatography.

b. Ion-Exchange Chromatography

In this technique, a column is packed with an insoluble ion-exchange resin. Then, a solution of a mixture of amino acids is loaded on the top of the column, and eluted with aqueous solutions of increasing pH. Since the amino acids bind with the resin at different extent, during the elution, the weakly bound amino acid can flow faster compared to that bound strongly, which can be collected as different fractions. Fig.71 presents the structure of section of a commonly used resin and diagram of the ion-exchange chromatography separation.

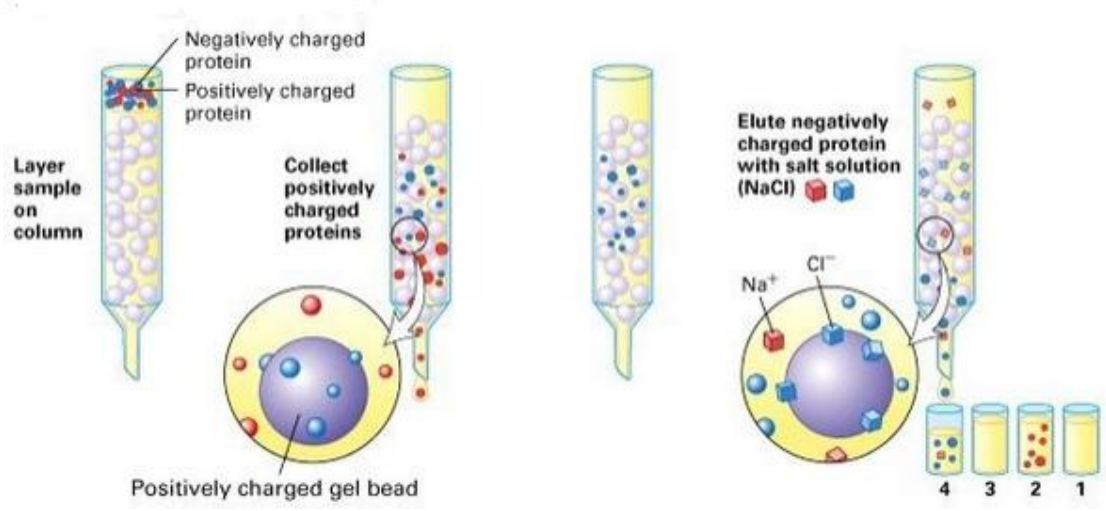


Fig. 71: Cation-exchange chromatography. A sample containing both positively and negatively charged proteins is loaded onto a column packed with positively charged beads. Positively charged proteins bind to the beads, while negatively charged proteins pass through and are collected. To elute the bound positively charged proteins, a salt solution (NaCl) is added. The salt ions compete with the proteins for binding sites on the beads, releasing the positively charged proteins which are then collected in separate fractions.

C. Thin Layer Chromatography (TLC)

TLC separates amino acids based on their polarity. Amino acids are spotted onto a thin layer of adsorbent material (like silica gel or cellulose) on a plate. The plate is then placed in a solvent system that moves up the plate by capillary action. Different amino acids will move up the plate to different extents based on their polarity, allowing for separation (Fig.72).

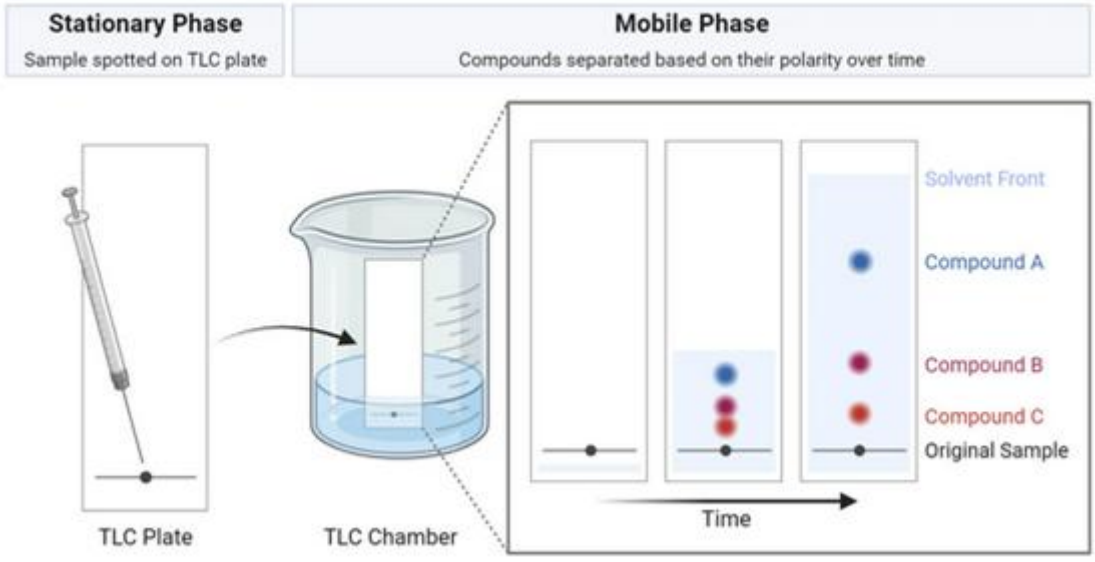


Fig.72: Thin Layer Chromatography (TLC) of Amino Acids. A sample containing a mixture of amino acids is spotted onto a TLC plate (stationary phase), which is then placed in a TLC chamber containing a solvent (mobile phase). As the solvent moves up the plate, the amino acids separate based on their polarity and interaction with the stationary phase. The image depicts the separation over time, showing the original amino acid mixture and

the resulting separated amino acids (represented as spots A, B, and C) at different heights on the plate, culminating in the solvent front. This technique allows for the identification and separation of amino acids in a sample.

D. Affinity Chromatography

Proteins are known to have high affinity for various substances such as substrates, prosthetic groups, receptors, inhibitor and antibodies raised against them. When any of these affinity compounds (called ligands) is covalently attached (immobilised) to insoluble resin (matrix), it can be used to purify its conjugate protein by allowing a mixture containing the protein of interest to pass through a column of the immobilized ligand.

During their passage, only the protein with complimentary site to that of the immobilized ligand is retarded and hence separated from others. The desired protein can then be recovered from the column by changing the elution conditions (Fig.73).

A ligand ideal for affinity chromatography should exhibit the following characteristics:

-Specific and reversible binding;

-Presence of chemically modifiable groups that can be used for attachment to the matrix while it still retain its binding properties;

-Its affinity for the binding site of the biomolecule should be within 10-4 and 10-8 M in free solution;

-It should interact with the biomolecule noncovalently

Affinity chromatography can be used to purify proteins and peptide such as enzymes, antibodies, lectins, hormones and even whole cells.

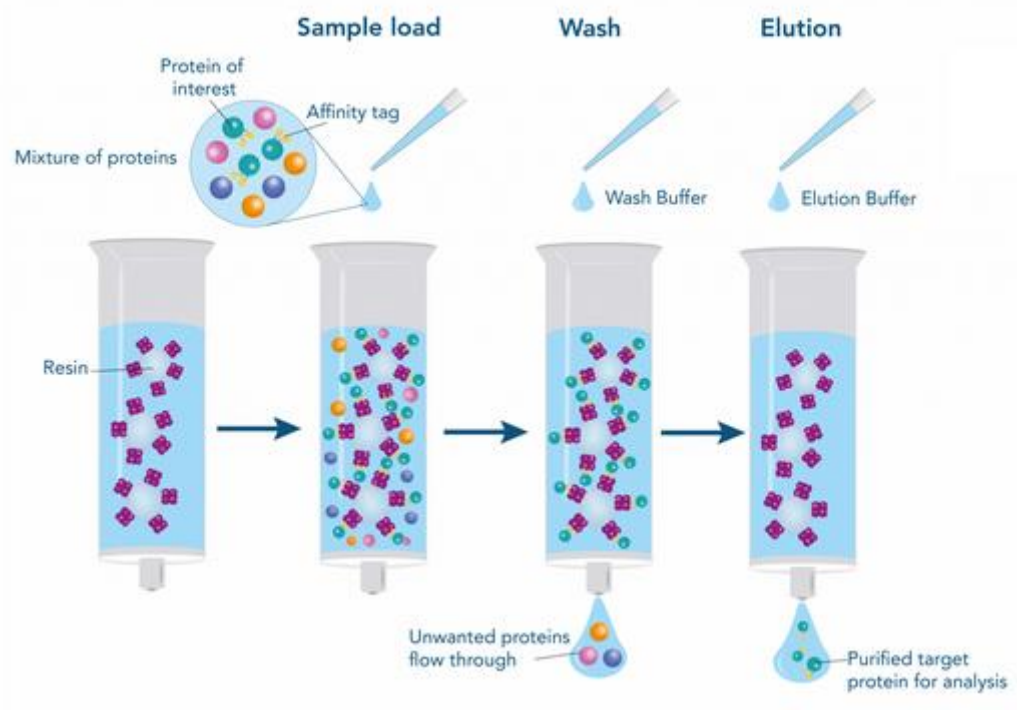


Fig.73: Principle of affinity-based protein purification

E. High Performance Liquid Chromatography (HPLC)

HPLC is a more advanced form of liquid chromatography that can separate amino acids based on various factors such as size, polarity, and affinity for the stationary phase. It is highly sensitive and can separate amino acids quickly and efficiently.

In column chromatography the smaller and more tightly packed a resin is the greater the separation capability of the column. In gravity flow columns the limitation column packing is the time it takes to pass the solution of proteins through the column. HPLC utilizes tightly packed fine diameter resins to impart increased resolution and overcomes the flow limitations by pumping the solution of proteins through the column under high pressure. Like standard column chromatography, HPLC columns can be used for size exclusion or charge separation. An additional separation technique commonly used with HPLC is to utilize hydrophobic resins to retard the movement of nonpolar proteins. The proteins are then eluted from the column with a gradient of increasing concentration of an organic solvent. This latter form of HPLC is termed reversed-phase HPLC.

III.1.6.3. Centrifugation

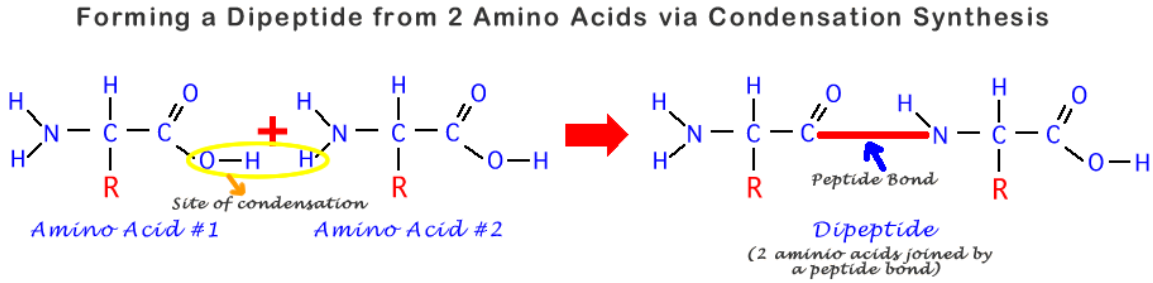
Amino acid, peptid or proteins will sediment through a solution in a centrifugal field dependent upon their mass. The most common solution utilized is a linear gradient of sucrose

(generally from 5-20%). Proteins are layered atop the gradient in an ultracentrifuge tube then subjected to centrifugal fields in excess of 100,000 x g. The sizes of unknown proteins can then be determined by comparing their migration distance in the gradient with those of known standard proteins.

III.2. PEPTIDE

III.2.1. Definition

Peptides are biologically occurring short chains (< 100) of amino acid monomers linked by peptide (amide) bonds. The covalent chemical bonds are formed when the carboxyl group of one amino acid reacts with the amine group of another.

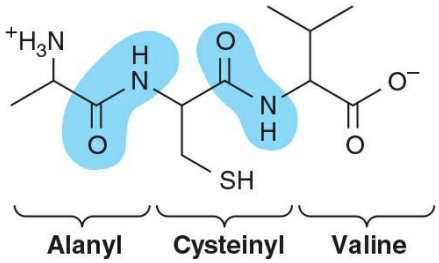


Peptides can be classified into **oligopeptides** and **polypeptides** based on the number of amino acids they contain:

Oligopeptides: Contain a small number of amino acids, typically **2 to 20**. They can be further classified depending on the number of amino acids, The shortest peptides are dipeptides, consisting of 2 amino acids joined by a single peptide bond, followed by tripeptides, tetrapeptides, etc. Examples: Glutathione (a tripeptide) and enkephalins (a pentapeptide).

Polypeptides: Contain between 20 and 100 amino acids. Examples: Insulin (a peptide hormone with 51 amino acids), *Glucagon (*29-amino acid)

Each peptide chain starts on the left side by free amino group of the first amino acid enter in chain formation. It is termed (N- terminus) and ends on the right side by free COOH group of the last amino acid and termed (C-terminus).



III.2.2. Primary Structure of peptides

The *number* and *order* of the amino acid residues in a polypeptide constitute its *primary* structure. Amino acids present in peptides, called *aminoacyl residues*, are referred to by replacing the *ate* or *ine* suffixes of free amino acids with *yl* (eg, alany*l*, aspart*yl*, tyros*yl*). Peptides are then named as derivatives of the *carboxy* terminal aminoacyl residue. For example, Lys-Leu-Tyr-Gln is called lys*yl*-leuc*yl*-tyros*yl*-glutam*ine*. The *ine* ending on the carboxy-terminal residue (eg, glutam*ine*) indicates that its α-carboxyl group is *not* involved in a peptide bond. Three-letter abbreviations linked by straight lines represent an unambiguous primary structure. Lines are omitted when using single-letter abbreviations. Prefixes like *tri-* or *octa-* denote peptides with three or eight *residues*, respectively. By convention, peptides are written with the residue that bears the free α-amino group at the left. This convention was adopted long before it was discovered that peptides are synthesized in vivo starting from the amino-terminal residue.

Glu—Ala—Lys—Gly—Tyr—Ala
E A K G Y A

III.2.3. Unusual Amino Acids in Peptides

In mammals, peptide hormones typically contain only the 20 codon specified α-amino acids linked by standard peptide bonds. Other peptides may, however, contain nonprotein amino acids, derivatives of the protein amino acids, or amino acids linked by an atypical peptide bond. For example, the amino terminal glutamate of glutathione, a tripeptide that participates in the metabolism of xenobiotics and the reduction of disulfide bonds, is linked to cysteine by a non-α peptide bond (Fig.74).

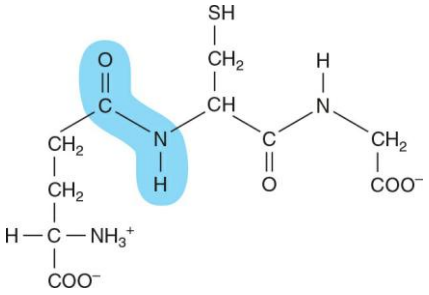


Fig.74: Glutathione structure (γ-glutamyl-cysteinyl-glycine).

The amino terminal glutamate of thyrotropin-releasing hormone (TRH) is cyclized to pyroglutamic acid, and the carboxyl group of the carboxyl terminal prolyl residue is amidated. The nonprotein amino acids D-phenylalanine and ornithine are present in the cyclic peptide antibiotics tyrocidin and gramicidin S, while the heptapeptide opioids dermorphin and deltophorin in the skin of South American tree frogs contain D-tyrosine and D-alanine.

III.2.4. Peptide bond character

- *The peptide bond has partial double-bond character*

Although peptide structures are written as if a single bond linked the α-carboxyl and α-nitrogen atoms, this bond in fact exhibits partial double bond character:

The bond that connects a carbonyl carbon to the α-nitrogen therefore cannot rotate, as this would require breaking the partial double bond. Consequently, the O, C, N, and H atoms of a peptide bond are *coplanar*. The imposed semirigidity of the peptide bond has important consequences for the manner in which peptides and proteins fold to generate higher orders of structure. Encircling brown arrows indicate free rotation about the remaining bonds of the polypeptide backbone (Fig. 75).

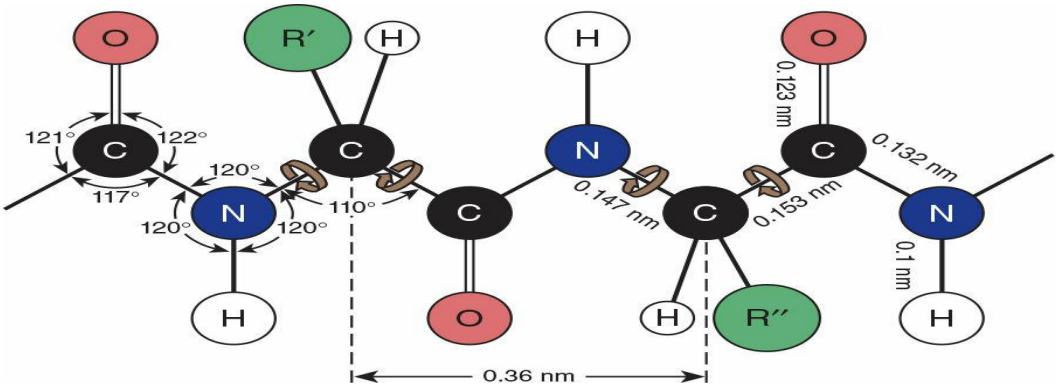


Fig.75: Dimensions of a fully extended polypeptide chain. The four atoms of the peptide bond are coplanar. Free rotation can occur about the bonds that connect the α-carbon with the α-nitrogen and with the α- carbonyl carbon (**brown arrows**). The extended polypeptide chain is thus a semirigid structure with two-thirds of the atoms of the backbone held in a fixed planar relationship to one another. The distance between adjacent α-carbon atoms is 0.36 nm (3.6 Å). The interatomic distances and bond angles, which are not equivalent, are also shown.

- *Peptides Are Polyelectrolytes*

The peptide bond is uncharged at any pH of physiologic interest. Formation of peptides from amino acids is therefore accompanied by a net loss of one positive and one negative charge per peptide bond formed.

Peptides nevertheless are charged at physiologic pH, owing to their terminal carboxyl and amino groups and, where present, their acidic or basic R groups. As for amino acids, the net

charge on a peptide depends on the pH of its environment and on the p*K*a values of its dissociating groups.

III.2.5. Noncovalent forces constrain peptide conformations

Folding of a peptide probably occurs coincident with its biosynthesis. The mature, physiologically active conformation reflects the collective contributions of the amino acid sequence, noncovalent interactions (eg, hydrogen bonding, hydrophobic interactions), and the minimization of steric hindrance between residues. Common repeating conformations include α-helices and β-pleated sheets.

III.2.6. Examples and Functions of Biologically Active Peptides

- *Oxytocin:* A nine-amino acid peptide hormone involved in social bonding, childbirth, and lactation.
- *Vasopressin (Antidiuretic Hormone, ADH):* An eight-amino acid peptide hormone that regulates water retention in the kidneys and blood pressure.
- *Glutathione:* The tripeptide glutathione (glutamyl-L-cysteinyl-glycine) contains an unusual amide bond. Found in almost all organisms, glutathione (GSH) is involved in protein and DNA synthesis, drug and environmental toxin metabolism, amino acid transport, and other important biological processes.
- *Angiotensin:* A peptide hormone involved in regulating blood pressure and electrolyte balance. It exists in several forms, including angiotensin I (10 amino acids) and angiotensin II (8 amino acids).
- *Insulin:* Although typically thought of as a protein, insulin in its active form is an oligopeptide composed of two polypeptide chains (A chain and B chain) linked by disulfide bonds, totaling 51 amino acids.
- *Glucagon:* A 29-amino acid peptide hormone that plays a role in glucose metabolism and regulation of blood sugar levels.
- *Bombesin:* A tetradecapeptide (14 amino acids) found in the gastrointestinal tract and central nervous system, involved in the regulation of food intake and gastrointestinal motility.

III.2.7. Amino acid sequence

The order of the amino acids in a polypeptide or protein is called the amino acid sequence: The methods available if protein sequencers are not available include the use of chemical and enzymatic techniques. Sequencing plays a very vital role in Proteomics as the information

obtained can be used to deduce function, structure, and location which in turn aids in identifying new or novel proteins as well as understanding of cellular processes. Better understanding of these processes allows for creation of drugs that target specific metabolic pathways.

It is often desirable to know the unordered amino acid composition of a protein prior to attempting to find the ordered sequence, as this knowledge can be used to facilitate the discovery of errors in the sequencing process or to distinguish between ambiguous results. Knowledge of the frequency of certain amino acids may also be used to choose which protease to use for digestion of the protein. A generalized method often referred to as amino acid analysis for determining amino acid frequency is as follows:

1. Hydrolyse a known quantity of protein into its constituent amino acids.

2. Separate and quantify the amino acids in some way.

Hydrolysis: Hydrolysis is done by heating a sample of the protein in 6 M hydrochloric acid to 100–110°C for 24 hours or longer.

Separation: The amino acids can be separated by ion-exchange chromatography or hydrophobic interaction chromatography.

Quantitative analysis: Once the amino acids have been separated, their respective quantities are determined by adding a reagent that will form a coloured derivative. If the amounts of amino acids are in excess of 10 nmol, ninhydrin can be used for this; it gives a yellow colour when reacted with proline, and a vivid purple with other amino acids. The concentration of amino acid is proportional to the absorbance of the resulting solution.

III.2.7.1. Chemical techniques.

III.2.7.1.1. separation of peptide strands

2-mercaptoethanol or dithiothreitol used in order to permit separation of peptide strands and prevent protein conformations that are dependent upon disulfide bonds. Both of these chemicals reduce disulfide bonds. To prevent reformation of the disulfide bonds the peptides are treated with iodoacetic acid in order to alkylate the free sulfhydryls.

III.2.7.1.2. *N*-terminal Residue Identification

N-terminal residue identification encompasses a technique which chemically determines which amino acid forms the *N*-terminus of a peptide chain. This information can be used to aid in ordering of individual peptide sequences that were generated using other sequencing techniques that fragment the peptide chain. There are three major chemical techniques for sequencing

peptides and proteins from the N-terminus. These are the Sanger, Dansyl chloride and Edman techniques.

A. Sanger's Reagent

This sequencing technique utilizes the compound, 2,4-dinitrofluorobenzene (DNF) which reacts with the N-terminal residue under alkaline conditions. The derivatized amino acid can be hydrolyzed and will be labeled with a dinitrobenzene group that imparts a yellow color to the amino acid. Separation of the modified amino acids (DNP-derivative) by electrophoresis and comparison with the migration of DNP-derivative standards allows for the identification of the N-terminal amino acid (Fig. 76).

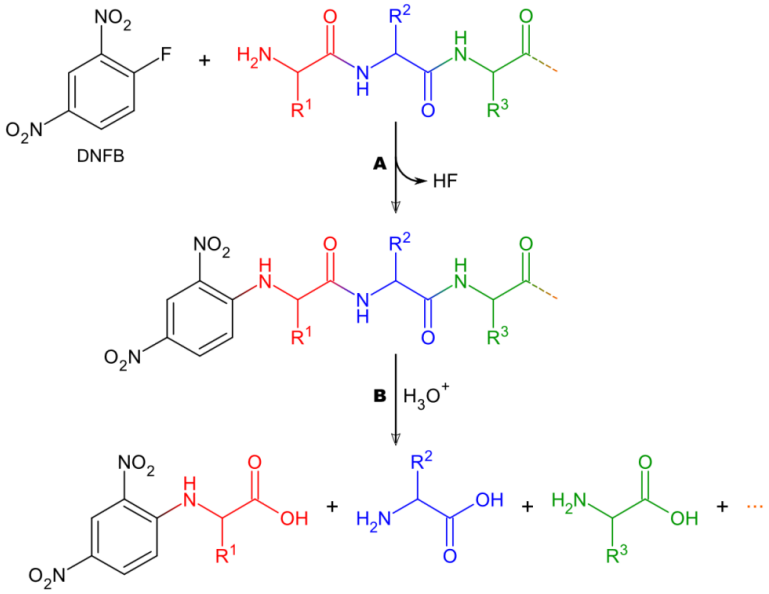


Fig.76: Sanger's method of peptide end-group analysis: A) complex of *N*-terminal end with Sanger's reagent (DNFB), B) total acid hydrolysis of the dinitrophenyl peptide.

B**. Dansyl chloride**: Like DNF, dansyl chloride reacts with the N-terminal residue under alkaline conditions. Analysis of the modified amino acids is carried out similarly to the Sanger method except that the dansylated amino acids are detected by fluorescence. This imparts a higher sensitivity into this technique over that of the Sanger method (Fig. 77).

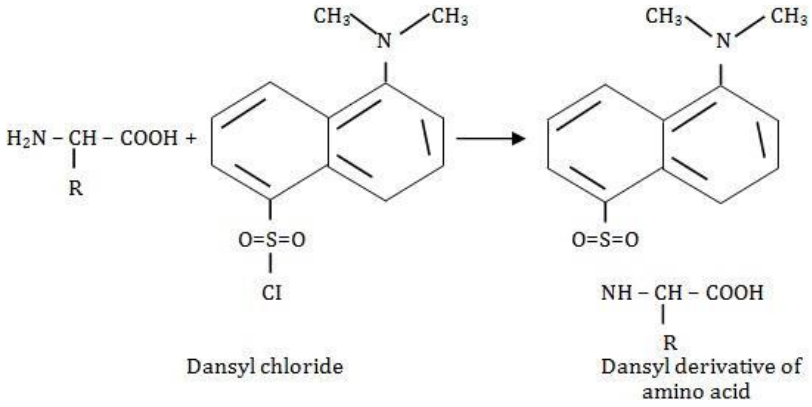


Fig.77: Dansyl chloride complex with amino acid.

C. Edman degradation

This method utilizes phenylisothiocyanate to react with the N-terminal residue under alkaline conditions. The resultant phenylthiocarbamyl derivatized amino acid is hydrolyzed in anhydrous acid. The added advantage of the Edman process is that the remainder of the peptide is intact. The entire sequence of reactions can be repeated over and over to obtain the sequences of the peptide. This process has subsequently been automated to allow rapid and efficient sequencing of even extremely small quantities of peptide (Fig. 78).

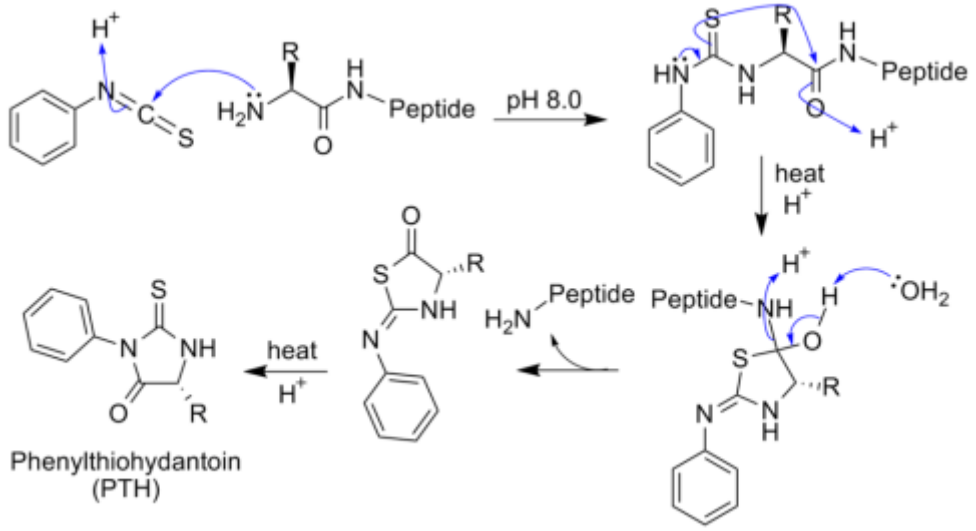


Fig.78: Formation of PTH from the reaction of Phenyl isothiocyanate with peptide.

III.2.7.1.3. *C*-terminal Residue Identification

The most reliable chemical technique for C-terminal residue identification is hydrazinolysis. A peptide is treated with hydrazine, NH_2–NH_2, at high temperature (90°C) for an extended length of time (20-100hr). This treatment cleaves all of the peptide bonds yielding amino-acyl hydrazides of all the amino acids excluding the C-terminal residue which can be identified chromatographically compared to amino acid standards.

III.2.7.1.4. Chemical Digestion

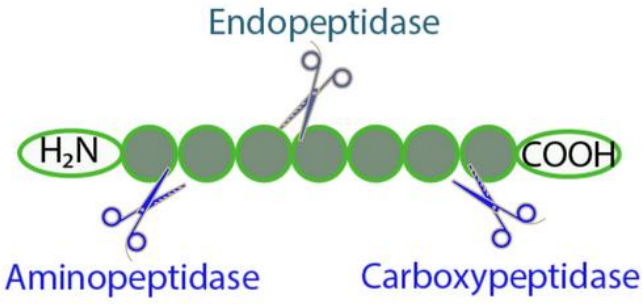
The most commonly utilized chemical reagent that cleaves peptide bonds by recognition of specific amino acid residues is cyanogen bromide (CNBr). This reagent causes specific cleavage at the C-terminal side of Met residues. The number of peptide fragments that result from CNBr cleavage is equivalent to one more than the number of M residues in a protein. Some of chemical methods are listed in Tab. 7.

Tab.7: Some of chemical method used for determining protein sequence without the use of sequencers.

Chemical Analyses		
Method/ Reagent	**Specificity**	**Additional use**
DNFB (2, 4-dinitrofluorobenzene) or the Sanger reagent	Determination of the N-terminal amino acid producing a dinitrophenol (DNP)-amino acid	Detection of N-terminal residues only; results in complete hydrolysis of the protein
Phenylisothiocyanate (Edman reagent)	Determination of N-terminal amino acid producing a phenylthiohydantoin (PTH)-amino acid and cleaving this from the parent peptide	This reagent is currently used in protein sequencers as it cleaves only the N-terminal amino acid while keeping the rest of the peptide intact
Hydrazine	Determination of the C-terminal amino acid	
Cyanogen bromide (CNBr)	Cleaves peptide bonds in which the carboxyl groups are contributed by the amino acid, methionine	At least one hydrolytic product will have methionine for its C-terminal amno acid
Hydroxylamine HCl	Cleaves peptide bonds joining asN and gly resides only.	If the cleavage products are two shorter peptides, one of these will have asN as its C terminal while the other will have gly as its C-terminal.
Iodosobenzoate	Cleaves at the carboxyl side of tryptophan residues	If there are 2 hydrolytic products, one will have trp as its C-terminal residue

III.2.7.2.Enzymatic methods

The enzymes that achieve amino acids sequencing and break down proteins can be divided into exopeptidases and endopeptidases.



III.2.7.2.1. Exopeptidases

break the peptide links of the amino acids at the ends of the chains. Endopeptidase break peptide links (the bonds which hold amino acids together) in the middle of polypeptide chains.

A. *N*-terminal Residue Identification

Aminopeptidases catalyze the cleavage of amino acids from the amino terminus of protein or peptide substrates. They are widely distributed throughout the animal and plant kingdoms and are found in many subcellular organelles, in cytoplasm, and as membrane components. Many, but not all, of these peptidases are zinc metalloenzymes. Some are monomeric, and others are assemblies of relatively high mass (50 kDa) subunits.

B. Carboxy-Terminal Sequence Determination

Carboxypeptidases are a class of enzymes that cleave peptides at the C-terminal residue. The resulting free amino acids can then be analyzed chromatographically and compared to known amino acid standards for identification.

No reliable chemical techniques exist for sequencing the C-terminal amino acid of peptides. However, there are enzymes, exopeptidases, that have been identified that cleave peptides at the C-terminal residue which can then be analyzed chromatographically and compared to standard amino acids. This class of exopeptidases are called, carboxypeptidases. There are several classes of carboxypeptidases. The table below summarizes these classes (Tab. 08).

Tab. 08: Specificities of Several Exopeptidases.

Enzyme	**Source**	**Specificity**
Carboxypeptidase A	Bovine pancreas	will not cleave when C-terminal residue = R, K or P or if P resides next to terminal residue
Carboxypeptidase B	Bovine pancreas	cleaves when C-terminal residue = R or K; not when P resides next to terminal reside
Carboxypeptidase C	Citrus leaves	all free C-terminal residues, pH optimum = 3.5
Carboxypeptidase Y	yeast	all free C-terminal residues, slowly at G residues

III.2.7.2.2. Endopeptidases

Enzymes, endopeptidases, cleave at specific sites within the primary sequence of proteins. The resultant smaller peptides can be chromatographically separated and subjected to Edman degradation sequencing reactions. Peptides longer than around 50 residues cannot be

sequenced completely by Edman degradation technique. Generally, endopeptidases do not readily cleave peptide bonds where proline (Pro, P) is involved.

Example: Trypsin can cleavage peptide bond C-terminal to R, K, but not if next to P.

Pepsin: cleavage peptide bond N-terminal to L, F, W, Y.

The table below summarizes some endopeptidases and their cleavage sites

Tab.09: Endopeptidases used for determining protein sequence without the use of sequencers.

Enzymatic hydrolysis		
Trypsin	Cleaves peptide bonds in which the carbonyl group is contributed by basic residues, lys and arg	All hydrolytic products except the product towards the C-terminal will have lys or arg as its C-terminal.
Chymotrypsin	Cleaves peptide bonds at the carboxyl side of aromatic amino acids, tyr, phe and trp	All hydrolytic products except the product towards the C-terminal will have tyr or phe or trp as its C-terminal.
Thermolysin	Cleaves amino side of residues where R is contributed by leu and val	All hydrolytic products except N-terminal will have leu or val as N-terminal
Streptococcal protease	Cleaves peptide bonds at the carboxyl side of acidic amino acids	All hydrolytic products except the product towards the C-terminal will have glu or asp as its C-terminal residue.
Thrombin	Cleaves peptide bonds at the carboxyl side of arginine	If there are 2 hydrolytic products, one will have arg as its C-terminal amino acid
Elastase	Cleaves peptide bonds at the carboxyl side of ala	If there are 2 hydrolytic products, one will have ala as its C-terminal amino acid

III.3. PROTEIN

The term “protein” is derived from the Greek word “Proteios”, meaning “of first importance”. It is the most abundant cellular component making up approximately 70% of the organic matter in a cell.

III.3.1. Orders of protein structure

The modular nature of protein synthesis and folding are embodied in the concept of orders of protein structure:

III.3.1.1. Primary structure

Every polypeptide has a specific amino acid sequence. The interactions between amino acid residues determine the protein's three dimensional structure and its functional role and relationship to other proteins.

Polypeptides that have similar amino acid sequences and have arisen from the same ancestral gene are said to be **homologous**. Sequence comparisons among homologous polypeptides have been used to trace the genetic relationships of different species.

III.3.1.2. Secondary structure

After synthesis, polypeptide chains are folded or pleated into different shapes, called their **Secondary Structure**. Two common examples of secondary structures are **Alpha Helices** and **Beta Pleated Sheets**. Secondary structure is held together by many Hydrogen bonds, overall giving the shape great stability.

Free rotation is possible about only two of the three types of covalent bonds comprising the polypeptide backbone: the bond linking the α-carbon (Cα) to the carbonyl carbon (Co) and the bond linking Cα to nitrogen. The partial double-bond character of the peptide bond that links Co to the α-nitrogen requires that the carbonyl carbon, carbonyl oxygen, and α-nitrogen remain coplanar, thus preventing rotation. The angle about the Cα—N bond is termed the phi (**ϕ**) angle, and that about the Co—Cα bond the psi (**ψ**) angle.

- *Alpha Helix*

The polypeptide backbone of an α helix is twisted by an equal amount about each α-carbon with a phi angle of approximately –57° and a psi angle of approximately –47°. A complete turn of the helix contains an average of 3.6 aminoacyl residues, and the distance it rises per turn (its *pitch*) is 0.54 nm. The R groups of each aminoacyl residue in an α helix face outward. Proteins contain only L-amino acids, for which a right-handed α helix is by far the more stable, and only righthanded α helices are present in proteins. Schematic diagrams of proteins represent α helices as coils or cylinders.

The stability of an α helix arises primarily from hydrogen bonds formed between the oxygen of the peptide bond carbonyl and the hydrogen atom of the peptide bond nitrogen of the fourth residue down the polypeptide chain.

Since the peptide bond nitrogen of proline lacks a hydrogen atom, it is incapable of forming a hydrogen bond with acarbonyl oxygen. Consequently, proline can only be stably

accommodated within the first turn of an α helix. When present elsewhere, proline disrupts the conformation of the helix, producing a bend (Fig**.** 79).

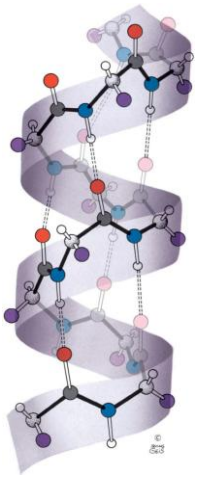


Fig. 79: The **α** helix. This right-handed helical conformation has 3.6 residues per turn. Dashed lines indicate hydrogen bonds between C-O groups and N—H groups that are four residues farther along the polypeptide chain.

- *Beta Sheet*

The second recognizable regular secondary structure in proteins is the β sheet. The amino acid residues of a β sheet, when viewed edge-on, form a zigzag or pleated pattern in which the R groups of adjacent residues project in opposite directions. Unlike the compact backbone of the α helix, the peptide backbone of the β sheet is highly extended. However, like the α helix, β sheets derive much of their stability from hydrogen bonds between the carbonyl oxygen and amide hydrogen of peptide bonds. However, in contrast to the α helix, these bonds are formed with adjacent segments of the β sheet (Fig. 80).

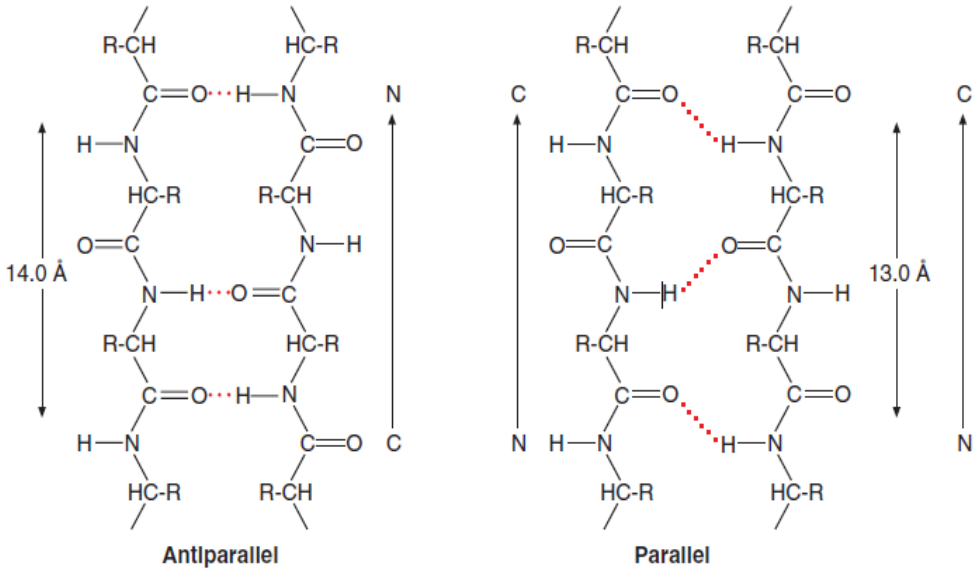


Fig.80: Spacing and bond angles of the hydrogen bonds of antiparallel and parallel pleated β sheets.

III.3.1.3. Tertiary structure

The overall three-dimensional structure of a polypeptide is called its **tertiary structure**. The tertiary structure is primarily due to interactions between the R groups of the amino acids that make up the protein.

Tertiary structure is held together by four different bonds and interactions:

- *Disulfide Bonds:* Where two **Cysteine** amino acids are found together, a strong double bond (S=S) is formed between the Sulphur atoms within the Cysteine monomers.
- *Electrostatic interaction*: If two oppositely charged 'R' groups are found close to each other, and electrostatic interaction forms between them.
- *Hydrogen Bonds:* the typical Hydrogen bonds.
- *Hydrophobic and Hydrophilic Interactions*: Some amino acids may be hydrophobic while others are hydrophilic. In a water based environment, a globular protein will orientate itself such that it's hydrophobic parts are towards its centre and its hydrophilic parts are towards its edges.

III.3.1.4. Quaternary structure

Many proteins, especially those with high molecular weights, are composed of several polypeptide chains. As mentioned,

Each polypeptide component is called a subunit. Some proteins are made up of multiple polypeptide chains, sometimes with an inorganic component (*for example, a haem group in hemoglobin*) called a **Prosthetic Group**. These proteins will only be able to function if all subunits are present.

III.3.2. Types of protein

The functions and properties of proteins are related to their diversified structures. It is quite difficult to classify these macromolecules on the basis of one single property or characteristic. The classification of proteins is dependent on the following.

- Solubility
- Shape and structure
- Chemical composition
- Biological function

III.3.2.1. Based on Solubility

The types of proteins can be classified based on their solubility in different solvents, such as water, salt solution and alcohol as follows. Some proteins are:

- soluble in water and salt solution e.g., albumin
- soluble in salt solution but sparingly soluble in water e.g., globulin
- soluble in 70-80% ethanol, are usually rich in proline amino acid e.g., protamines.
- nucleoproteins soluble in salt solution e.g., histones

III.3.2.1.2 Based on Shape

On the basis of their shape proteins can be divided into two classes:

A. Fibrous proteins

These proteins form long fibres and mostly consist of repeated sequences of amino acids which are insoluble in water and physically tough. Fibrous proteins have structural and protective functions such as the keratins found in skin, hair, and nails, and Collagen in bone and cartilage, Keratin in fingernails and hair (Fig. 81).

B. Globular proteins

are compact spherical molecules that are usually water- soluble. Typically, globular proteins have dynamic functions. For example, nearly all enzymes have globular structures. Other examples include the immunoglobulins and the transport proteins hemoglobin and albumin (a carrier of fatty acids in blood).

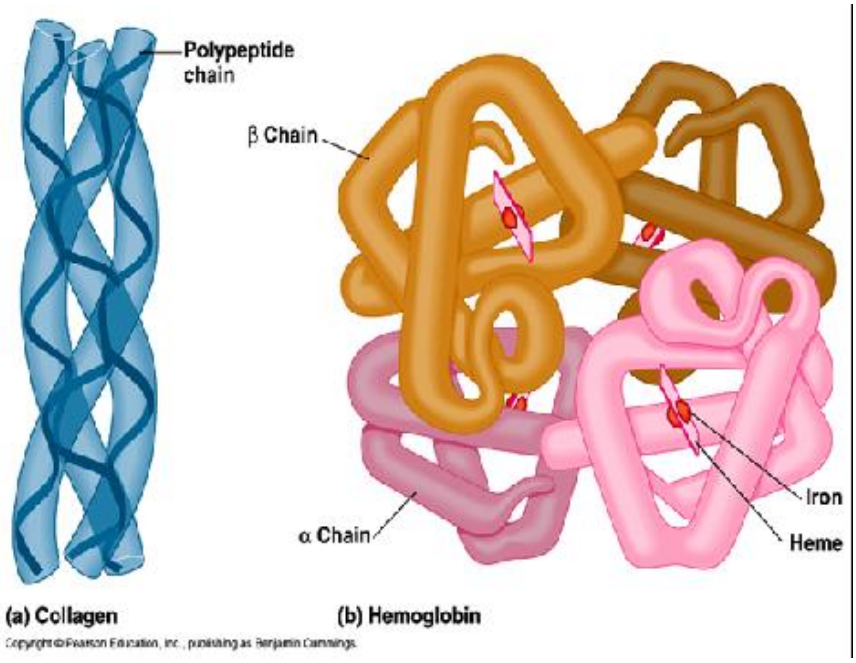


Fig.81: Protein form: A. collagen (fibrous protein), B. Hemoglobin (globular protein)

III.3.2.1.3. Based on Chemical Composition

On the basis of their chemical composition or the product of hydrolysis, proteins may be divided into following classes.

- *Simple proteins or homoproteins:* are made up of only amino acids and

do not contain any non-protein part. Examples are plasma albumins, collagens, protamines, prolamines, keratins, etc.

- *Conjugated proteins or heteroproteins* contain in their structure a non-protein part or group. The protein part of conjugated proteins is called apoprotein and the nonprotein part is referred to as the prosthetic group. The entire molecule is called a haloprotein. The proteins and their prosthetic groups are listed below in Tab. 10.

Tab.10: Some proteins and their prosthetic groups

Class	*Prosthetic group*	*Example*
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

- *Derived proteins:* are the proteins derived by the action of different physical or chemical agents like, heat, acid, alkali or enzyme on the native protein.

III.3.2.1.4. Based on Biological Functions

The multitude of functions that proteins perform is the consequence of both the folding of the polypeptide chain, and the presence of many different functional groups in the amino acid side chains. From the functional point of view, they may be divided into several groups

Catalysis. Catalytic proteins called the enzymes accelerate thousands of biochemical reactions in such processes as digestion, energy capture, and biosynthesis.

Structure. Structural proteins often have very specialized properties. For example, collagen (the major components of connective tissues) and fibroin (silkworm protein) have significant mechanical strength. Elastin, the rubberlike protein found in elastic fibers, is found in blood vessels and skin that must be elastic to function properly.

Movement. Proteins are involved in all cell movements. Actin, tubulin, and other proteins comprise the cytoskeleton. Cytoskeletal proteins are active in cell division, endocytosis, exocytosis, and the ameboid movement of white blood cells.

Defense. A wide variety of proteins are protective. In vertebrates, keratin, a protein found in skin cells, aids in protecting the organism against mechanical and chemical injury. The blood-clotting proteins fibrinogen and thrombin prevent blood loss when blood vessels are damaged.

Regulation. Binding a hormone molecule or a growth factor to cognate receptors on its target cell changes cellular function. For example, insulin and glucagon are peptide hormones that regulate blood glucose levels. Growth hormone stimulates cell growth and division. Growth factors are polypeptides that control animal cell division and differentiation. Examples include platelet-derived growth factor (PDGF) and epidermal growth factor (EGF).

Transport. Many proteins function as carriers of molecules or ions across membranes or between cells. Examples of membrane transport proteins include the enzyme Na -K ATPase and the glucose transporter. Other transport proteins include hemoglobin, which carries O_2 to the tissues from the lungs, and the lipoproteins LDL and HDL, which transport water-insoluble lipids in the blood from the liver. Transferrin and ceruloplasmin are serum proteins that transport iron and copper, respectively.

Storage. Certain proteins serve as a reservoir of essential nutrients. For example, ovalbumin in bird eggs and casein in mammalian milk are rich sources of organic nitrogen during development. Plant proteins perform a similar role in germinating seeds.

Stress response. The capacity of living organisms to survive a variety of abiotic stresses is mediated by certain proteins. Examples include cytochrome P450, a diverse group of enzymes found in animals and plants that usually convert a variety of toxic organic contaminants into less toxic derivatives, and metallothionein, a cysteine-rich intracellular protein found in virtually all mammalian cells that binds to and sequesters toxic metals such as cadmium, mercury, and silver.

III.3.3. Perturbation of protein conformation

The perturbation of protein conformation may have pathologic consequences

III.3.3.1. Prions

The transmissible spongiform encephalopathies, or **prion diseases**, are fatal neurodegenerative diseases characterized by spongiform changes, astrocytic gliomas, and neuronal loss resulting from the deposition of insoluble protein aggregates in neural cells.

III.3.3.2. Alzheimer Disease

Refolding or misfolding of another protein endogenous to human brain tissue, β-amyloid, is a prominent feature of the Alzheimer disease. While the main cause of the Alzheimer disease remains elusive, the characteristic senile plaques and neurofibrillary bundles contain aggregates of the protein β-amyloid, a 4.3-kDa polypeptide produced by proteolytic cleavage of a larger protein known as amyloid precursor protein. In Alzheimer disease patients, levels of β-amyloid become elevated, and this protein undergoes a conformational transformation from a soluble α helix–rich state to a state rich in β sheet and prone to self-aggregation.

Apolipoprotein E has been implicated as a potential mediator of this conformational transformation.

III.3.3.3. Beta-Thalassemias

Thalassemias are caused by genetic defects that impair the synthesis of one of the polypeptide subunits of hemoglobin. During the burst of hemoglobin synthesis that occurs during erythrocyte development, a specific chaperone called α-hemoglobin–stabilizing protein (AHSP) binds to free hemoglobin α-subunits awaiting incorporation into the hemoglobin multimer. In the absence of this chaperone, free α-hemoglobin subunits aggregate, and the resulting precipitate has cytotoxic effects on the developing erythrocyte. Investigations using genetically modified mice suggest a role for AHSP in modulating the severity of β -thalassemia in human subjects

Some protein misfolding diseases are summarized in the tab. 11.

Tab. 11: Some protein misfolding diseases

Disease	Defective Protein
Alzheimer's disease	Amyloid-β protein
Amyotrophic lateral sclerosis	Superoxide dismutase
Fibrinogen amyloidosis	Fibrinogen α chain
Huntington's disease	Huntingtin with polyglutamate expansion
Light chain amyloidosis	Immunoglobulin light chain
Lysozyme amyloidosis	Lysozyme
Parkinson's disease	α-Synuclein
Transmissible spongiform encephalopathies (TSEs)	Prion protein

CHAPTER IV: STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF LIPIDS

IV.1. INTRODUCTION

Lipids are a diverse group of molecules that all share the characteristic that at least a portion of them is hydrophobic. Lipids play many roles in cells, including serving as energy storage (fats/oils), constituents of membranes (glycerophospholipids, sphingolipids, cholesterol), hormones (steroids), vitamins (fat soluble), oxygen/ electron carriers (heme), among others. For lipids that are very hydrophobic, such as fats/ oils, movement and storage in the aqueous environment of the body requires special structures. Other, amphipathic lipids, such as glycerophospholipids and sphingolipids spontaneously organize themselves into lipid bilayers when placed in water (Fig. 82)**.**

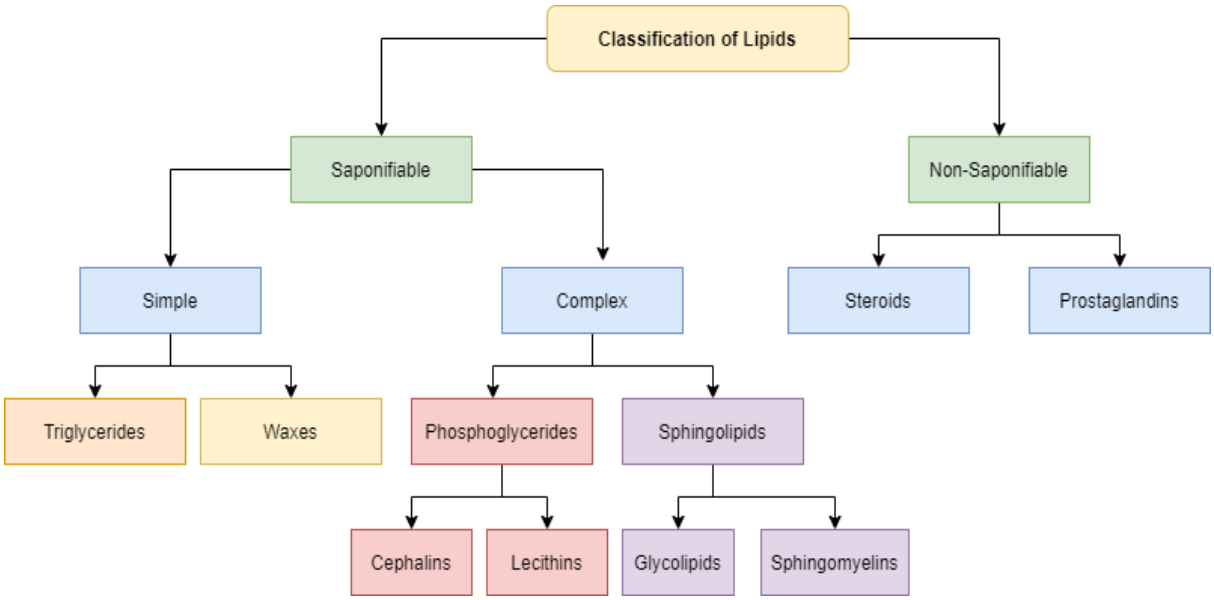


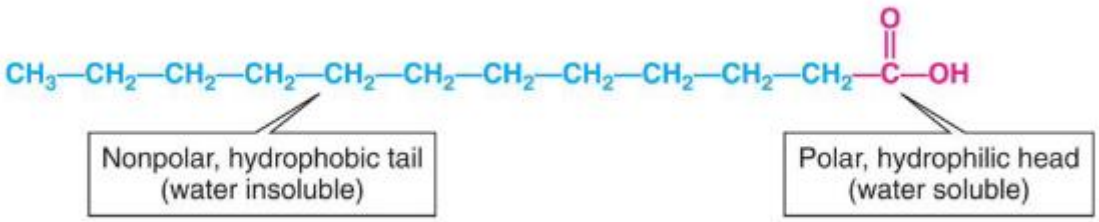
Fig. 82: Lipid organization based on structural relationships.

IV.2. Fatty acids

The most ubiquitous lipids in cells are the fatty acids. They serving as membrane anchors for proteins and other biomolecules, fatty acids are important for energy storage, membrane structure, and as precursors of most classes of lipids. The hydrocarbon chain of fatty acids ends with a carboxylic acid group. They are the fundamental building blocks of lipid molecules. Fatty acids are responsible for

Fatty acids are long-chain carboxylic acids characterized by a polar head group (COOH) and a long hydrocarbon tail. Since fatty acid are synthesized from 2C units. They have varying length that ranges **from 4 to 30 carbon atoms,**. Fatty acids with hydrocarbon tails that lack

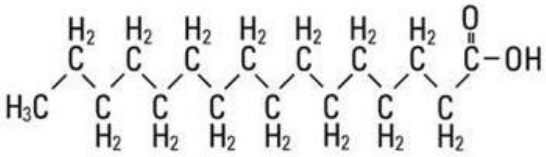
any double bonds are described as **saturated**, while those with one or more double bonds in their tails are known as **unsaturated** fatty acids.



IV.2.1. Saturated fatty acids

Saturated fatty acids are generally solids at room temperature due to large number of carbon atoms. They have hydrophobic tail of zig-zag configuration and because of single bond they are free to rotate and are thus more flexible and elongated. They form a fully extended and stable configuration in which the steric hindrances of neighboring atoms are minimized. These molecules can thus pack together tightly to form a nearly crystalline structure. Their general formula is **$CH_3(CH_2)nCOOH$**, where n represents the number of carbon atoms in the chain (typically between 12 and 20). This formula indicates a terminal methyl group (CH_3), a chain of methylene groups (CH_2), and a carboxylic acid group (COOH). Exemple:

Palmitic acid
$C_{16}H_{32}O_2$



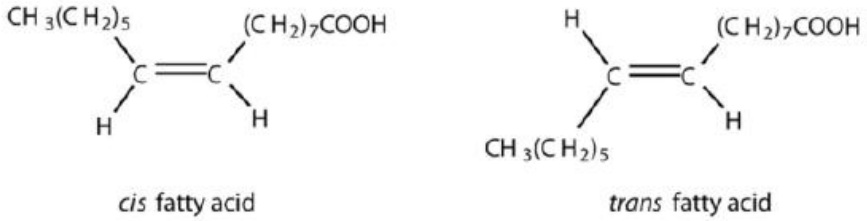
The table 12 summurize the major saturated faty acid.

Tab. 12: Most Saturated fatty acids

Common name	Chemical structure	
Caprylic acid	$CH_3(CH_2)_6COOH$	8
Capric acid	$CH_3(CH_2)_8COOH$	10
Lauric acid	$CH_3(CH_2)_{10}COOH$	12
Myristic acid	$CH_3(CH_2)_{12}COOH$	14
Palmitic acid	$CH_3(CH_2)_{14}COOH$	16
Stearic acid	$CH_3(CH_2)_{16}COOH$	18
Arachidic acid	$CH_3(CH_2)_{18}COOH$	20
Behenic acid	$CH_3(CH_2)_{20}COOH$	22
Lignoceric acid	$CH_3(CH_2)_{22}COOH$	24
Cerotic acid	$CH_3(CH_2)_{24}COOH$	26

IV.2.2. Unsaturated fatty acids

They have one or more double bonds in their tails and characterized by lowers melting point and therefore lipids with unsaturated acids are mostly oils at room temperature. The number of double bond shows the degree of unsaturation. Double bonds produce rigid bends in the HC tail resulting in geometric isomerism. Most common are the less stable 'cis' form rather than 'trans' forms which are more stable.



cis fatty acid *trans* fatty acid

Fatty acids with double bonds have kinks in their structure and therefore cannot pack together like saturated FA and their interaction with each other is poor. These double bonds thus give a large bulky structure to fatty acids and thus require less thermal energy to disorder them resulting in lower melting points and therefore lipids with unsaturated acids are mostly oils at room temperature. The main difference between fats and oils is for oils being liquid at room temperature, whereas, fats are solids. This is mainly due to presence of larger percentage of unsaturated fatty acids in oils than fats that has mostly saturated fatty acids. Its general formula is $C_nH_{2n-2x}O_2$. **n**: number of carbon atoms, **x**: nember of double bond (Fig.83).

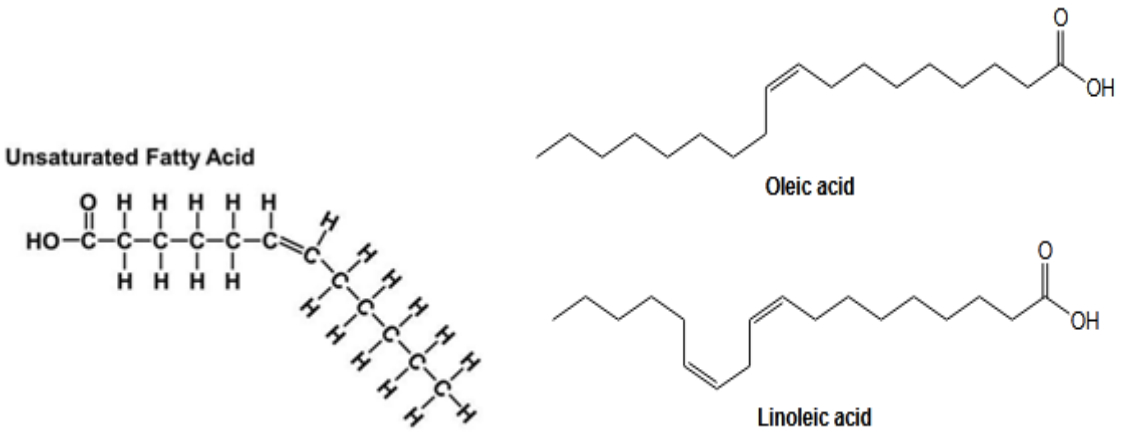


Fig .83: General structure and exemple of unsaturated fatty acid

According to the number of double bonds unsaturated acid may be:

1. **Monoenoic or monoethenoid acid** exemple: oleic acid (C18), erucic acid (20 C)

2. Dienoic or diethenoid acid exemple: Linoleic acid (18C) in cotton seed, linseed and soybean.

3. Trienoic or triethenoid acid exemple: Linolenic acid (18 C) in palm and linseed oil.

4. **Tetraenoic or Tetraethenoid acid**: exemple: Arachidonic acid (20) in peanut oil.

IV.2.3. Chemical name

The systematic name for a fatty acid is derived from the name of its parent hydrocarbon.

- In ***saturated fatty* acids** the Latin number of carbon atoms + the suffix **a**noic *Examples*

-Palmetic acid contains 16 carbons and is saturated, its name is Hexadecanoic

-Stearic acid contains 18 carbons and saturated. Its name is Octadecanoic.

- In the ***unsaturated fatty acids*** more details are required to indicate the position and the number of double bonds.

Site of the double bond + Latin number of the carbon atoms + number of double bonds + the suffix enoic

Example

-Linoleic acid contains 18 carbon atoms, 2 double bonds between $C_{9,10}$ and $C_{12,13}$

Its name is 9,12 –octadecadi enoic

-Linolenic acid contains 18 carbon atoms, 3 double bonds between C6,7; C9,10 and $C_{12,13}$. Its name is 6,9,12 –octadecatri enoic.

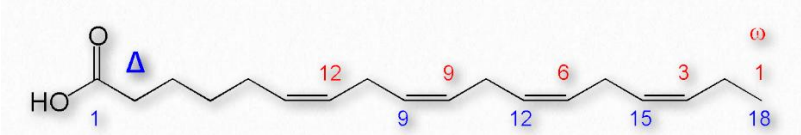
- A simplified nomenclature specifies the chain length and the number of double bonds and the site of the double bonds

Example: Palmetic acid is 16-carbon saturated fatty acid. It is abbreviated C16:0, Stearic acid C18:0.

- The positions of any double bonds are specified by superscript numbers following Δ or ω.

Δ : indicates a double bond on the carbon counting from the COOH carbon as the first carbon.

ω : indicates a double bond on the carbon counting from the CH_3-carbon as the first carbon.



Examples:

Linoleic acid C18:2$\Delta^{9,12}$

Linolenic acid C18:3 $\Delta^{6, 9,12}$

In animals, additional double bonds are introduced only between the existing double bond (eg, ω9,ω6, or ω3) and the carboxyl carbon, leading to three series of fatty acids known as the ω9,ω6, and ω3 families, respectively. The table 13 summurizes the most insaturated fatty acids.

Tab. **13:** most insaturated fatty acids

Name	Double Bond Info	
Myristoleic acid	*cis*-Δ^9	14:1
Palmitoleic acid	*cis*-Δ^9	16:1
Sapienic acid	*cis*-Δ^6	16:1
Oleic acid	*cis*-Δ^9	18:1
Elaidic acid	*trans*-Δ^9	18:1
Vaccenic acid	*trans*-Δ^{11}	18:1
Linoleic acid	*cis,cis*-Δ^9,Δ^{12}	18:2
Linoelaidic acid	*trans,trans*-Δ^9,Δ^{12}	18:2
α-Linolenic acid	*cis,cis,cis*-$\Delta^9,\Delta^{12},\Delta^{15}$	18:3
Arachidonic acid	*cis,cis,cis,cis*-$\Delta^5\Delta^8,\Delta^{11},\Delta^{14}$	20:4
Eicosapentaenoic acid	*cis,cis,cis,cis,cis*-$\Delta^5,\Delta^8,\Delta^{11},\Delta^{14},\Delta^{17}$	20:5
Erucic acid	*cis*-Δ^{13}	22:1
Docosahexaenoic acid	*cis,cis,cis,cis,cis,cis*-$\Delta^4,\Delta^7,\Delta^{10},\Delta^{13},\Delta^{16},\Delta^{19}$	22:6

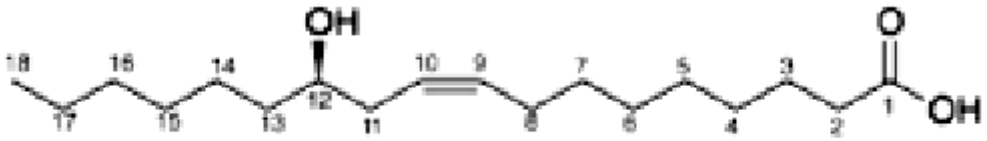
IV.2.3. Essential Fatty acids

Fatty acids are described as essential fatty acids if they must be in the diet (can't be synthesized by the organism) and nonessential fatty acids if the organism can synthesize them. Humans and other animals lack the desaturase enzymes necessary to make double bonds at positions greater than Δ-9, so fatty acids with double bonds beyond this position must be obtained in the diet. Linoleic acid and linolenic acid, both fall in this category. Related unsaturated fatty acids can be made from these fatty acids, so the presence of linoleic and linolenic acids in the diet eliminates the need to have all unsaturated fatty acids in the diet. Both linoleic and linolenic acid contain 18 carbons, but linoleic acid is an ω-6 fatty acid, whereas linolenic acid is an ω-3 fatty acid. Notably, ω-6 fatty acids tend to be proinflammatory, whereas ω-3 fatty acids are lesser so.

IV.2.4. Unusual fatty acid

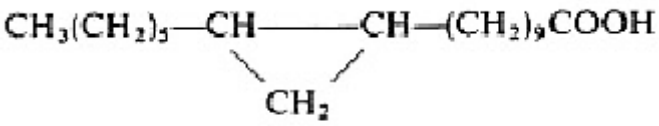
Unusual fatty acids are fatty acids that **differ structurally or functionally** from common saturated and unsaturated fatty acids found in most animals and plants. These differences may involve chain length, branching, rings, functional groups**, or** position and number of double bonds**.** The most important are:

Hydroxy or oxygenated fatty acid eg. ricinoleic acid (18C) castor oil with OH group on C12.



Ricinoleic acid (C18)

Cyclic fatty acid – example – lactobacillic acid with a cyclopropyl group and methylene group across the double bond of vaccenic acid.



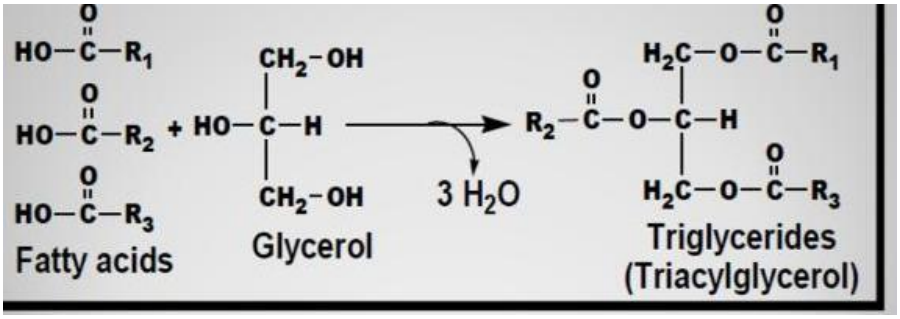
Lactobacillic acid

IV.3. Simple lipids

Lipids containing only fatty acids and glycerol or long chain alcohols (Monohydric) are called as simple lipids which include glycerid and waxes.

IV.3.1. Glycerides

Glycerides (or *acylcylgerols*) are esters composed of fatty acid units joined to *glycerol*. Accordingl to the number of fatty acid esterified to glycerol they may be called mono-,di- or triacylglyceral. Most common are triacylglycerol but mono and diacylglycerol are also common. Because the polar OH group and the polar carboxyl of fatty acid are bound in ester linkage triacylglycerol are non-polar hydrophobic molecules. They have lower specific gravity than water hence they float on it.



When the glycerol is esterified with fatty acid then C-2 becomes asymmetric centre and the 2nd hydroxyl group of glycerol is always show to left of C-2 while C atom above C-2 is called C-1 and below C-2 is C-3. This is stereo specific numbering (SN) (Fig.84).

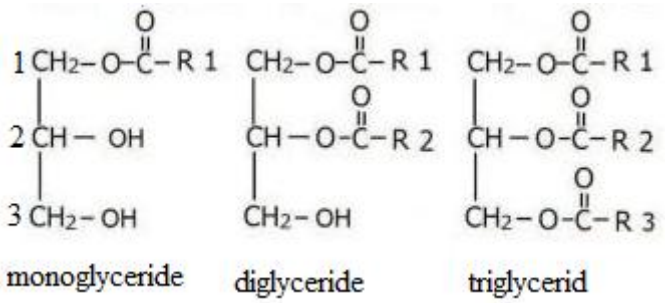


Fig. 84: Differences between mono,di and triglyceride

IV.3.1.1.Types of triglycerides

Based on the nature of the fatty acids that constitute them, triglycerides can be classified into simple and mixed triglycerides**.**

a) Simple triglycerides

If the three fatty acids connected to glycerol are of the same type, the triglyceride is called simple triglyceride.

b) Mixed Triglycerides

If the three fatty acids connected to glycerol are of different type, the triglyceride is called mixed triglyceride.

Natural fats are mixture of mixed triglycerides with a small amount of simple triglycerides.

The commonest fatty acids in animal fats are palmitic, stearic and oleic acids (Fig.85).

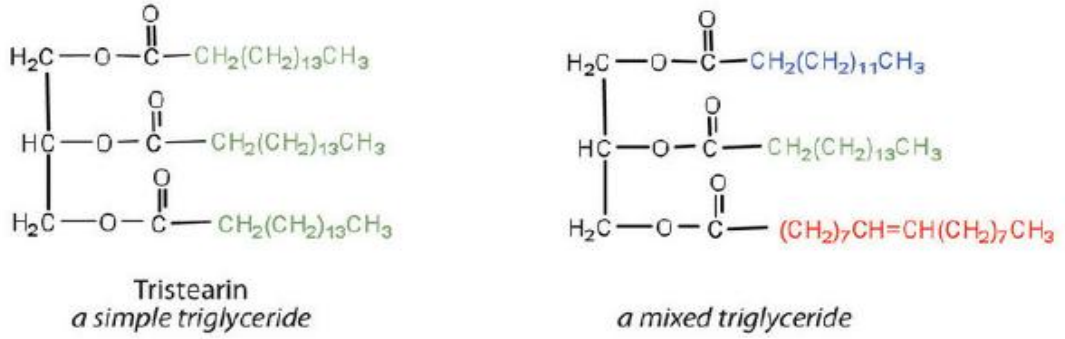


Fig.85: Structure of Simple and Mixed Triglycerides

We can also classify triglycerides based on their rigidity into fats and oils.

a) Oils

oils are typically liquid at room temperature and are mostly derived from plant sources, such as olive oil, sunflower oil, and soybean oil. They can also come from fish, such as cod liver oil. Oils generally have a higher proportion of unsaturated fatty acids, including both monounsaturated and polyunsaturated types. These fatty acids contain one or more double

bonds in their hydrocarbon chains, which create kinks that prevent the molecules from packing closely together, keeping the oil in a liquid state at room temperature.

b) Fats

On the other hand, are usually solid at room temperature. They are primarily derived from animal sources, such as butter, lard, and tallow, though some plant sources like coconut oil and palm oil also contribute to the fat category. Fats tend to have a higher proportion of saturated fatty acids, which have no double bonds in their hydrocarbon chains. This allows the fatty acids to pack closely together, forming a solid structure at room temperature.

IV.3.2. Waxes

Waxes occur as protective coating on fruits and leaves or secreted by insects (beewax). These are mixtures of long chain alkanes with odd number of carbon atom C25-C35 and oxygenated derivative such as secondary alcohols and ketones. They are insoluble in water and are chemically enert resistant to atmospheric oxygen and therefore protect plants from water loss and abrasive damage. Mostly they are fatty acyl ester of long chain alcohols of long length. The myricyclic alcohol of beewax has 30C ($C_{30}H_{61}OH$-- triacontanol) and is myricyl palmitate RCOOR' R is C17-C29 C atoms and R' C18 – C30. Sperm whale wax is composed of palmitic acid esterified with hexacoscenol ($C_{26}H_{53}OH$). This was primary wax source for many years but now Simmondsia chinensis a desert plant has large oxygen waxesters as storage lipid in its seed. These wax esters act as superior machine lubricants.

Sebum, secreted by the sebaceous glands of the skin, contains waxes that help to keep skin soft and prevent dehydration. Some exemples are monsioned in the figure bellow (fig.86)

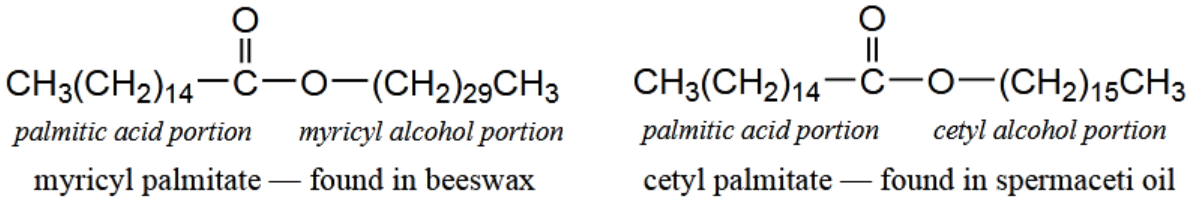


Fig.86: Structure and natural sources of some waxes

IV.3.3. Physical properties of fats and oils

IV.3.3.1. Melting points

A triglyceride is called a fat if it is a solid at 25°C; it is called oil if it is a liquid at that temperature. These differences in melting points reflect differences in the degree of unsaturation and number of carbon atoms in the constituent fatty acids (Fig.87).

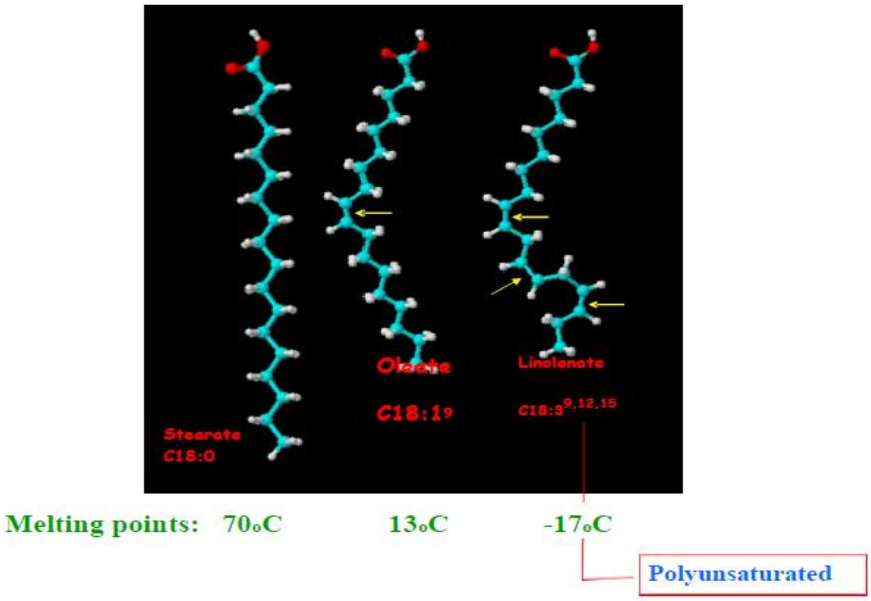


Fig.87: Melting Points of Saturated, Monounsaturated, and Polyunsaturated Fatty Acids.

IV.3.3.2. Others properties

Pure fats and oils are colorless, odorless, and tasteless. The characteristic colors, odors, and flavors that we associate with some of them are imparted by foreign substances that are lipid soluble and have been absorbed by these lipids. For example, the yellow color of butter is due to the presence of the pigment carotene; the taste of butter comes from two compounds diacetyl and 3-hydroxy-2-butanone produced by bacteria in the ripening cream from which the butter is made.

Fats and oils are lighter than water, having densities of about 0.8 g/cm. They are poor conductors of heat and electricity and therefore serve as excellent insulators for the body, slowing the loss of heat through the skin.

IV.3.4. Chemical reactions of fats and oils

Fats and oils can participate in a variety of chemical reactions

IV.3.4.1. Saponification

Triglycerides, being esters, can undergo hydrolysis in the presence of an acid, a base, or specific enzymes known as lipases. The hydrolysis of fats and oils in the presence of a base is used to make soap and is called **saponification**. Today most soaps are prepared through the hydrolysis of triglycerides (often from tallow, coconut oil, or both) using water under high pressure and temperature [700 lb/in (~50 atm or 5,000 kPa) and 200°C]. Sodium carbonate or sodium hydroxide is then used to convert the fatty acids to their sodium salts (soap molecules): The reaction is mentioned in the fig. 88.

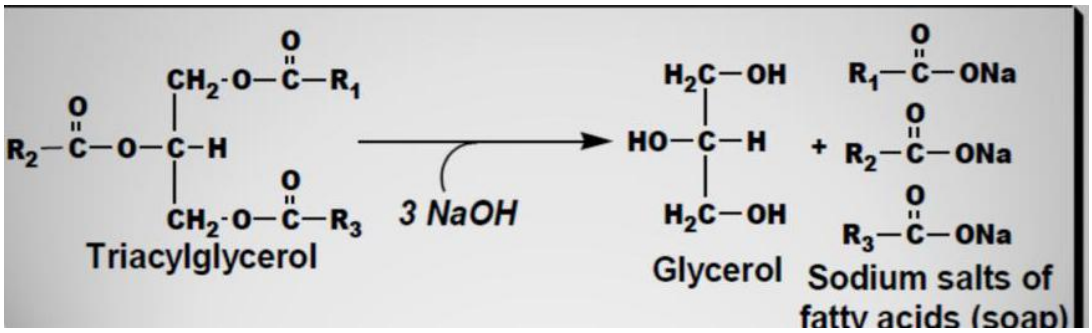


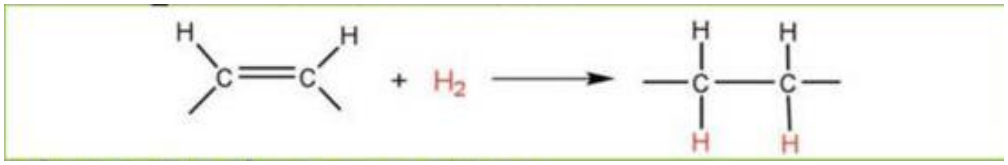
Fig.88: Saponification: The Production of Soap from Triacylglycerols

Saponification value or saponification number represents the number of milligrams of potassium hydroxide or sodium hydroxide required to saponify one gram of fat under the conditions specified. It is a measure of the average molecular weight (or chain length) of all the fatty acids present in the sample.

IV.3.4.2. Hydrogenation

Oils containing unsaturated fatty acids can be hydrogenated in the presence of high temperature, pressure and finally divided nickel. Unsaturated fatty acids either free or combined in lipids react with gaseous hydrogen to yield the saturated fatty acids. The addition of hydrogen takes place at the C-C double bonds.

In **complete hydrogenation**, all double bonds are fully converted into single bonds, resulting in a fully saturated fat. In **incomplete or partial hydrogenation**, some cis double bonds remain, while others are converted into trans bonds, resulting in the formation of **trans fats**. This reaction is of great commercial importance since it permits transformation of inexpensive and unsaturated liquid vegetable fats into solid fats. The latter are used in the manufacture of candles, vegetable shortening and margarine



IV.3.4.4. Dehydration

Fatty acids can lose water molecules when heated, particularly in the presence of acids or bases. Fatty Acid → Dehydrated Product + H_2O. Dehydration reactions are involved in the formation of conjugated acids.

IV.3.4.5. Halogenation

Halogenation of fatty acids involves the addition of halogens (such as chlorine, bromine, or iodine) to the carbon-carbon double bonds of unsaturated fatty acids.

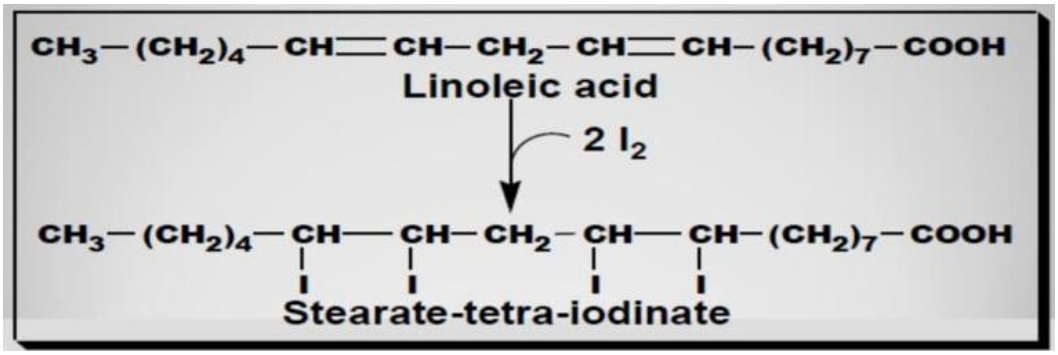


Fig. 88: Halogenation reaction of linoleic acid

Halogenation is used to test for unsaturation in fatty acids and oils. The extent of halogenation indicates the degree of unsaturation. Halogenated fatty acids are used in various industrial processes, including the production of surfactants, lubricants, and other chemical intermediates. The iodine value (also known as iodine number or iodine index) is a measure of the degree of unsaturation in fats and oils. the iodine value (IV; also iodine absorption value, iodine number or iodine index) is the mass of iodine in grams that is consumed by 100 grams of a chemical substance.The higher iodine value indicate the higher degree of unsaturation.

IV.3.4.6. Rancidity

The development of an unpleasant taste and odor in fats or oils upon storage is known as rancidity. Oils and fats, when stored for extended periods in contact with heat, light, air, and moisture, develop an unpleasant smell. These oils and fats are referred to as rancid. Rancidity occurs due to certain chemical changes that take place in the fat, including:

A. Hydrolytic rancidity:

This type of rancidity occurs due to the hydrolysis of triglycerides (fats). The process is catalyzed by lipase enzymes**.** Bacterial contamination leading to the liberation of free fatty acids and glycerol at high temp and moisture.Volatile short-chain fatty acids have unpleasant odor. C-O-CO-R + H2O → C-O-H + HO-CO-R

The water present in the food and the high temperature will increase the rate of hydrolysis to fatty acids

B. Ketonic Rancidity

Occurs due to contamination with certain fungi, such as *Aspergillus niger*, on fats like coconut oil. During this process, various compounds are formed, including **ketones**, **fatty aldehydes**, **short-chain fatty acids**, and **fatty alcohols**. These compounds contribute to the

unpleasant characteristics associated with rancidity. Additionally, moisture accelerates the development of ketonic rancidity in oils and fats.

C. Oxidative rancidity

Oils containing highly unsaturated fatty acids are spontaneously oxidized by atmospheric oxygen at ordinary temperature. The oxidation takes place slowly and results in the formation of short chain fatty acids (C4- C10) and aldehydes which give a rancid taste (Fig.89). This type of rancidity is called as oxidative rancidity and is due to a reaction called auto oxidation. Oxidative rancidity is observed more frequently in animal fats than in vegetable fats. This is due to the presence of natural antioxidants in vegetable fats such as tocopherol, phenols, and napthols which check autoxidation. Vitamin E is therefore sometimes added to foods to prevent rancidity.

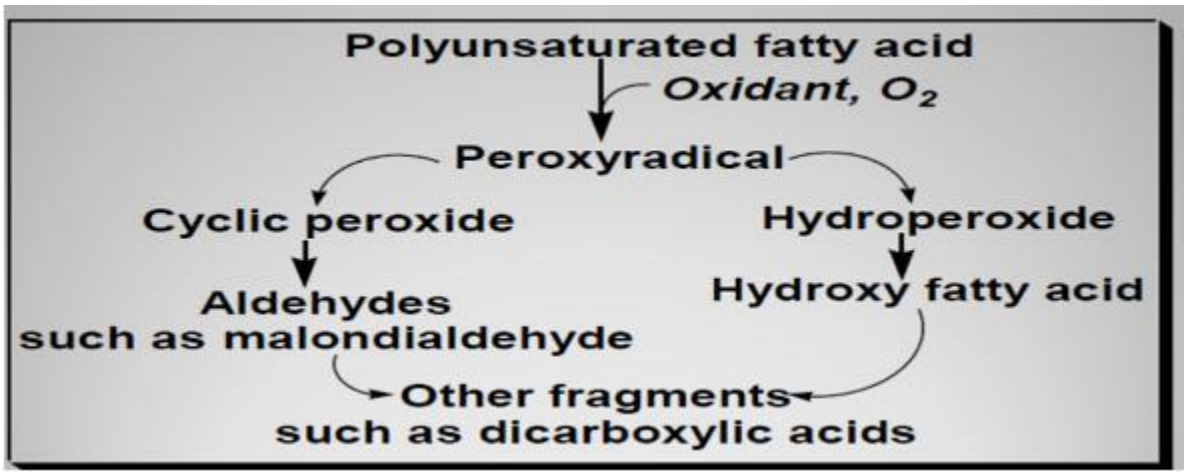


Fig. 89: Oxidative Rancidity of Polyunsaturated Fatty Acids; when a polyunsaturated fatty acid reacts with an oxidant (like oxygen), it forms a peroxyradical**.** This peroxyradical can then lead to the formation of cyclic peroxides or hydroperoxides. These peroxides further break down into various volatile compounds responsible for the rancid odor and flavor, including aldehydes (such as malondialdehyde**),** hydroxy fatty acids and other fragments like dicarboxylic acids.

IV.4. Complex lipids

Complex lipids are lipids that contain additional components besides fatty acids and glycerol (or a similar alcohol). These extra components are typically polar or charged groups, making complex lipids amphipathic.These group of lipids include glycerophospholipids, sphingo phospholipids, glycolipids, sulpholipids and lipoproteins. The presence of additional components such Phosphate groups, carbohydrate, proteins…allows complex lipids to perform a wider range of functions in biological systems compared to simple lipids (like

triglycerides). Their amphipathic nature is crucial for the formation of biological membranes and for the transport of other lipids.

IV.4.1. Phospholipids

Phospholipids in animal membranes are derived from either **glycerol** or **sphingosine**.

IV.4.1.1. Glycerophospholipids

Glycerophospholipids (also known as phosphoglycerides) consists of a glycerol backbone (3-OH, 3-C alcohol), two fatty acid chains and a phosphorylated alcohol. The fatty acid chain usually contain even number of C atom, between 14-24. The 16 and 18 are most common.

-Fatty acids may be saturated or unsaturated.

-The configuration of double bonds in unsaturated fatty acids is nearly always *cis*.

-The length and the degree of unsaturation of fatty acids chains affect the membrane fluidity (Fig.90).

Glycerophospholipids are important components of the lipid bilayer of cellular membranes. Phosphoglycerides are structurally related to fats, as both are derived from phosphatidic acid.

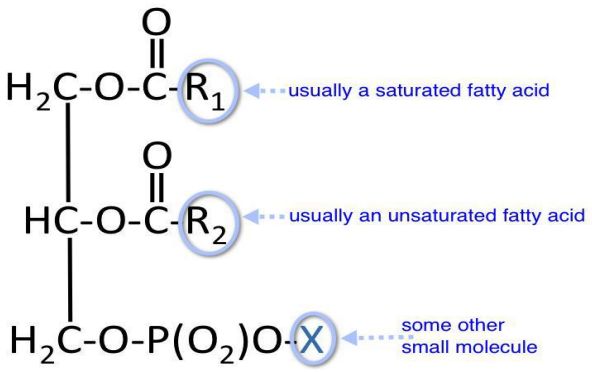


Fig. 90: Basic Structure of a Glycerophospholipid

Phosphatidic acid is a simple glycerophospholipid that is usually converted into phosphatidyl compounds. These are made by esterifying various groups, such as ethanolamine, serine, choline, inositol, and others (Fig. 91) to the phosphate of phosphatidic acid. All of these compounds form lipid bilayers in aqueous solution , due to their amphiphilic nature.

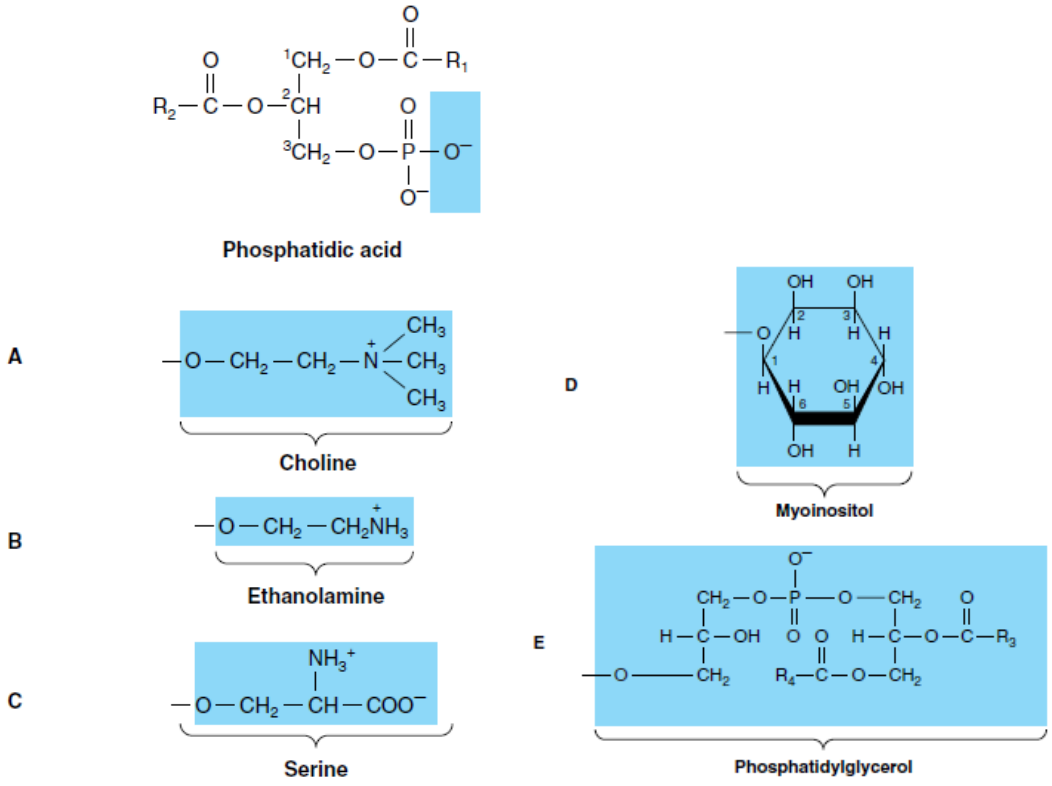


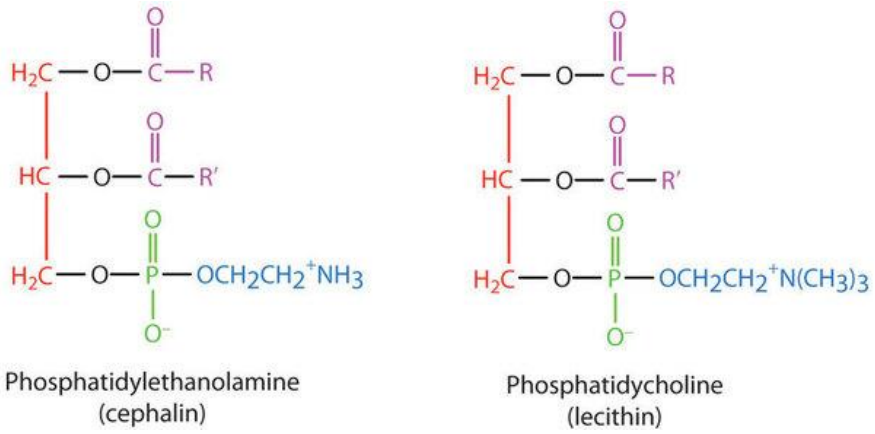
Fig. 91: basic structure of phosphatidic acid and common head groups found in phosphoglycerides.

A. Phosphatidylethanolamines

Phosphoglycerides containing ethanolamine as the amino alcohol are called *Phosphatidyl-ethanolamines* or *cephalins*. Cephalins are found in brain tissue and nerves and also have a role in blood clotting.

B. Phosphatidylcholines

Phosphoglycerides containing choline as the amino alcohol unit are called *phosphatidylcholines* or *lecithins*. Lecithins occur in all living organisms.



C. Phosphatidyl inositol

Phosphatidyl inositol 4,5-bisphosphate is an important constituent of cell membrane phospholipids. Upon stimulation by a suitable hormone agonist, it is cleaved into

diacylglycerol and inositol trisphosphate, both of which act as internal signals or second messengers.

D. Cardiolipin

Phosphatidic acid is a precursor of phosphatidyl glycerol which, in turn, gives rise to cardiolipin.This phospholipid is found only in mitochondria and is essential for mitochondrial function.

E. Plasmalogens

These compounds constitute as much as 10% of the phospholipids of brain and muscle.

Structurally, the plasmalogens are glycerophospholipids resemble phosphatidyl-ethanol amine but in which the C1 substituent of the glycerol moiety is linked via an α,β-unsaturated ether linkage in the cis configuration rather than through an ester linkage. Typically, the alkyl radical is an unsaturated alcohol (Fig. 92).

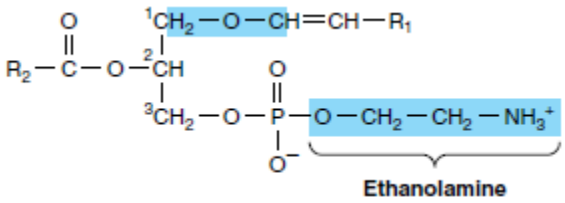


Fig.92: plasmalogens structure

IV.3.1.6. Enzymatic hydrolyse of glycerophospholipids.

Enzymatic hydrolysis of glycerophospholipids is a critical process in biological systems, involving the breakdown of these lipids by enzymes called phospholipases. These enzymes play a crucial role in membrane remodeling and signal transduction. An important intermediate product formed during this process is the lysophospholipid. Lysophospholipids are formed when one of the fatty acyl chains of a glycerophospholipid is removed, leaving a single fatty acyl chain attached to the glycerol backbone. Based on their site of action on the phospholipid molecule, phospholipases are classified into four main types:

- **Phospholipase A1 (PLA1):** This enzyme hydrolyzes the sn-1 acyl bond, releasing a fatty acid and generating a 2-lysophospholipid.
- **Phospholipase A2 (PLA2):** This enzyme hydrolyzes the sn-2 acyl bond, releasing a fatty acid (often arachidonic acid) and generating a 1-lysophospholipid.
- **Phospholipase C (PLC):** This enzyme cleaves the phosphodiester bond between the glycerol backbone and the phosphate group, producing diacylglycerol (DAG) and a phosphate-containing head group.

- **Phospholipase D (PLD):** This enzyme cleaves the phosphodiester bond between the phosphate group and the head group, yielding phosphatidic acid (PA) and the head group.

Lysophospholipids, resulting from the action of PLA1 or PLA2, are important intermediates with diverse biological functions. They play important roles in cell membrane dynamics, signaling, and various biological processes. The following figure summarizes the sites of action for phospholipases. The figure below (Fig.93) illustrates the points of cleavage for phospholipases.

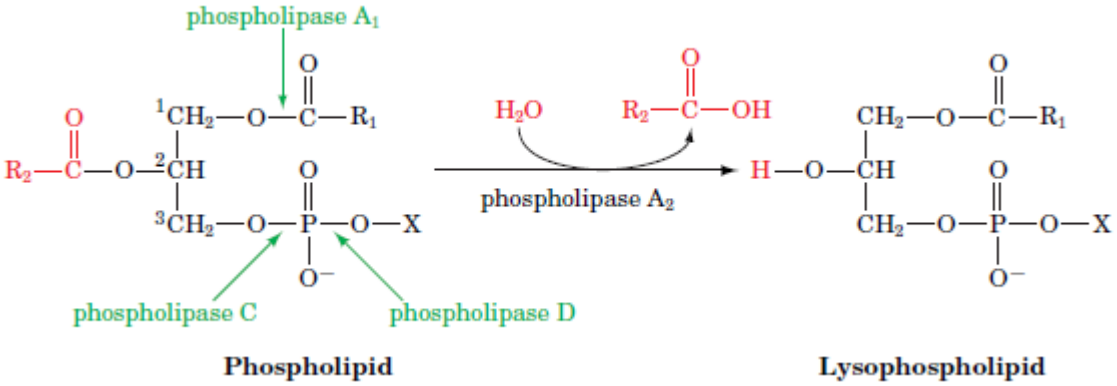


Fig.93: Action of phospholipases**.** Phospholipase A2 hydrolytically excises the C2 fatty acid residue from a phospholipid to yield the corresponding lysophospholipid. The bonds hydrolyzed by other types of phospholipases, which are named according to their specifi cities, are also indicated.

IV.4.1.2. Sphingophospholipids

Sphingophospholipids consists of sphingosine (complex alcohol) and a fatty acid. Sphingosine is *amino* alcohol that contains a long, *unsaturated* hydrocarbon chain ($C_{18}H_{37}NO_2$. Sphingosine can bind to ONE fatty acid through AMIDE bond. It may also bind to the lateral CH_2OH group to give many derivatives.

Sphingosine+ 1 fatty acid ⟶ Ceramide

Ceramide+ Phosphocholine ⟶ A Sphingomyelin (Phospholipid)

Ceramide+ Phosphoethanolamine ⟶ A Sphingomyelin (Phospholipid)

Ceramide+ Sugar ⟶ A Cerebroside (Glycolipid)

Ceramide+ many Sugars ⟶ A Ganglioside (Glycolipid)

A. Sphingomyelins

The simplest sphingolipids, each contain a fatty acid, a phosphoric acid, sphingosine, and choline (Fig.94). Because they contain phosphoric acid, they are also classified as

phospholipids. Sphingomyelins are important constituents of the myelin sheath surrounding the axon of a nerve cell. Multiple sclerosis is one of several diseases resulting from damage to the myelin sheath.

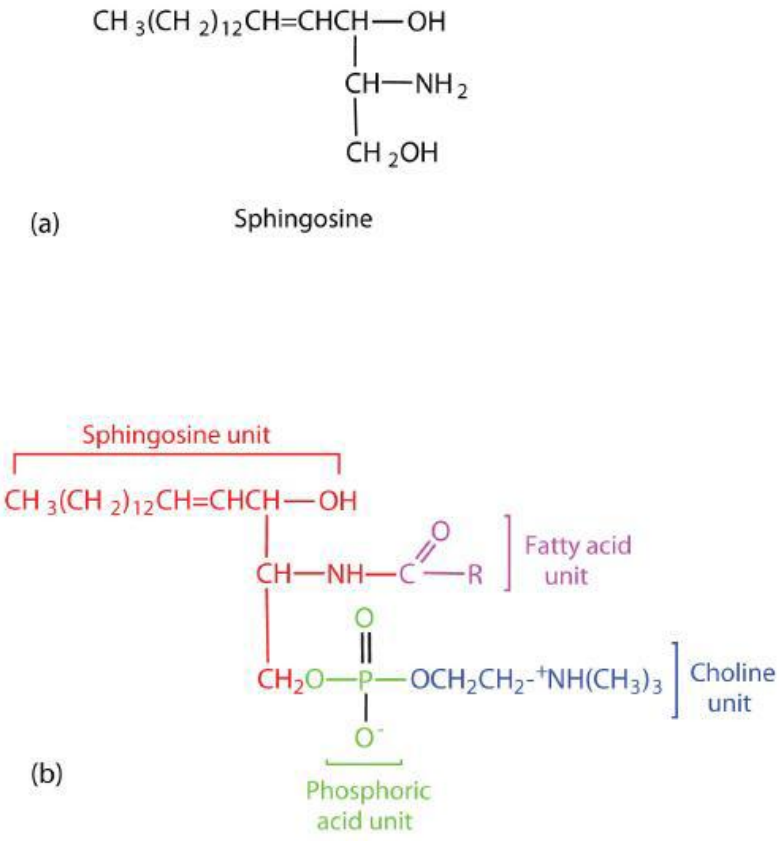


Fig.94: Sphingolipids structure. (a) Sphingosine, an amino alcohol, is found in all sphingolipids. (b) A sphingomyelin is also known as a phospholipid, as evidenced by the phosphoric acid unit in its structure.

B. Cerebrosides

Most animal cells contain sphingolipids called cerebrosides (Fig.95). Cerebrosides are composed of sphingosine, a fatty acid, and galactose or glucose. They therefore resemble sphingomyelins but have a sugar unit in place of the choline phosphate group.

Cerebrosides are important constituents of the membranes of nerve and brain cells.

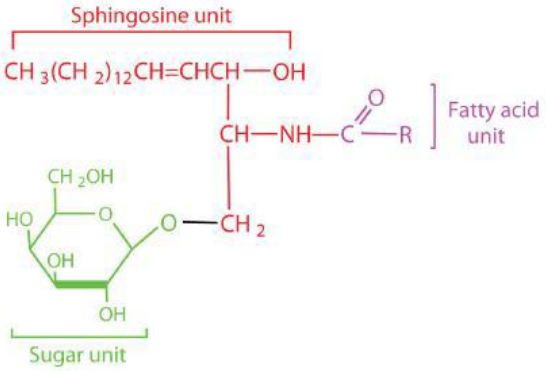


Fig. 95: Cerebrosides structure.

C. Gangliosides

The sphingolipids called gangliosides are more complex, usually containing a branched chain of three to eight monosaccharides and/or substituted sugars (Fig.96). Because of considerable variation in their sugar components, about 130 varieties of gangliosides have been identified. Most cell-to-cell recognition and communication processes (e.g., blood group antigens) depend on differences in the sequences of sugars in these compounds. Gangliosides are most prevalent in the outer membranes of nerve cells, although they also occur in smaller quantities in the outer membranes of most other cells. Because cerebrosides and gangliosides contain sugar groups, they are also classified as glycolipids.

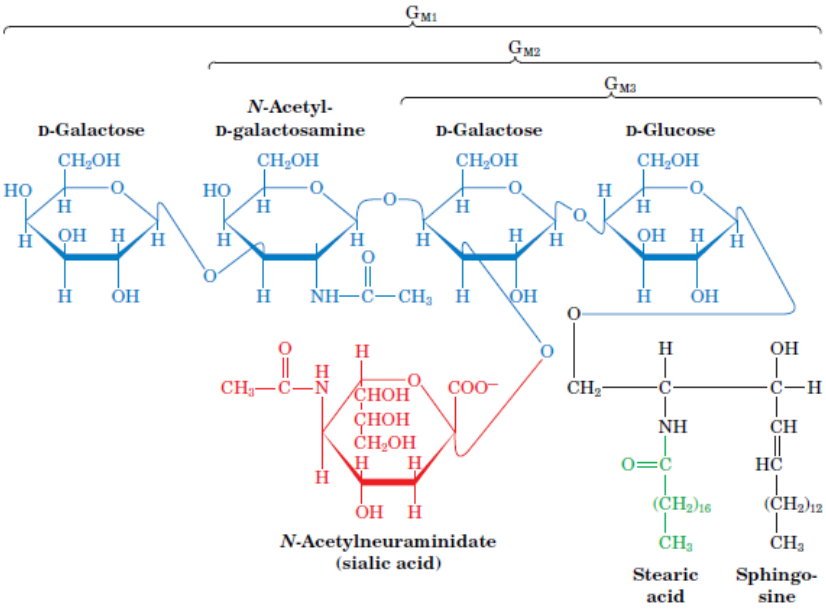


Fig. 96: Structural formula of gangliosides. Gangliosides GM2 and GM3 differ from GM1 only by the sequential absences of the terminal D-galactose and *N*-acetyl-D-galactosamine residues.

IV.4. Unsaponifiable lipids

Unsaponifiable lipids are lipids that cannot be hydrolyzed by alkali (like sodium hydroxide) in a process called saponification. This is because they lack the ester linkages that are broken down during saponification. They are a diverse group of lipids (Steroids, Terpenes, Eicosanoids…).

IV.4.1. Steroids

Steroids are classified as lipids because they are soluble in nonpolar solvents, but they are non-saponifiable because the components are not held together by ester linkages.

The basic steroid structure contains four fused rings:

They have a basic structure derived from fully saturated ring system sterane or perhydrocyclopentanephenanthrene which has 3 complex ring fused in a linear or phenanthrene manner and a terminal cyclopentane ring.

Cholesterol is the most abundant steroid in the body. It is an essential component of cell membranes, and is a precursor for other steroids, such as the bile salts, sex hormones, vitamin D, and the adrenocorticoid hormones. There is apparently a correlation between high levels of cholesterol in the blood and atherosclerosis.

Cholesterol can also be esterified to long chain fatty acids to form **cholesteryl esters**, such as **cholesteryl stearate** (Fig.97).

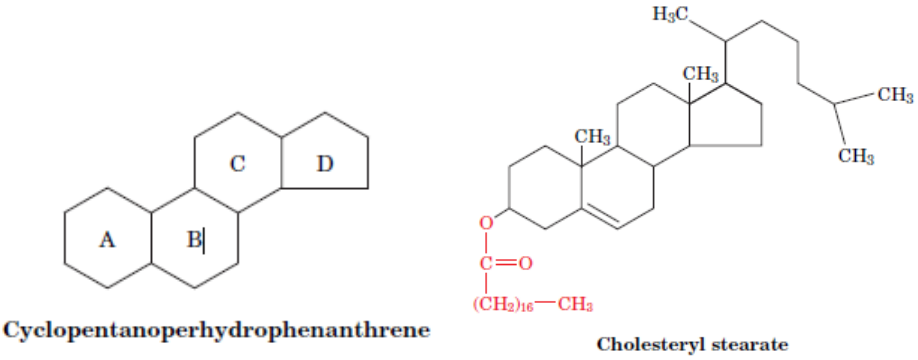


Fig.97: The Basic Steroid Ring System and the structure of cholesteryl stearate.

IV.4.2. Steroid hormones

Hormones are chemical messengers that are released in one tissue and transported through the circulatory system to one or more other tissues. One group of hormones is known as steroid hormones because these hormones are synthesized from cholesterol, which is also a steroid. There are two main groups of steroid hormones: adrenocortical hormones and sex hormones.

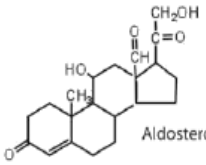
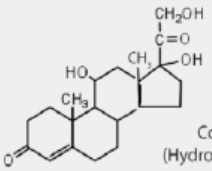
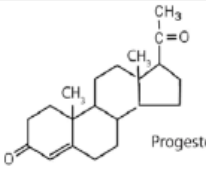
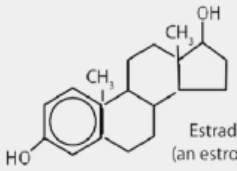
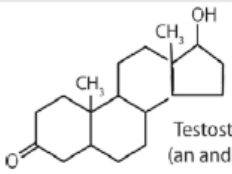
The adrenocortical hormones, such as aldosterone and cortisol, are produced by the adrenal gland, which is located adjacent to each kidney.

- *Aldosterone*: acts on most cells in the body, but it is particularly effective at enhancing the rate of reabsorption of sodium ions in the kidney tubules and increasing the secretion of potassium ions and/or hydrogen ions by the tubules. Because the concentration of sodium ions is the major factor influencing water retention in tissues, aldosterone promotes water retention and reduces urine output.
- *Cortisol:* regulates several key metabolic reactions (for example, increasing glucose production and mobilizing fatty acids and amino acids). It also inhibits the inflammatory response of tissue to injury or stress.

Cortisol and its analogs are therefore used pharmacologically as immunosuppressants after transplant operations and in the treatment of severe skin allergies and autoimmune diseases, such as rheumatoid arthritis.

- The sex hormones are a class of steroid hormones secreted by the gonads (ovaries or testes), the placenta, and the adrenal glands.
- **Testosterone** and **androstenedione** are the primary male sex hormones, or *androgens*, controlling the primary sexual characteristics of males, or the development of the male genital organs and the continuous production of sperm. Androgens are also responsible for the development of secondary male characteristics, such as facial hair, deep voice, and muscle strength.
- Two kinds of sex hormones are of particular importance in females: **progesterone,** which prepares the uterus for pregnancy and prevents the further release of eggs from the ovaries during pregnancy, and **the estrogens**, which are mainly responsible for the development of female secondary sexual characteristics, such as breast development and increased deposition of fat tissue in the breasts, the buttocks, and the thighs. Both males and females produce androgens and estrogens, differing in the amounts of secreted hormones rather than in the presence or absence of one or the other (**Tab. 14**).

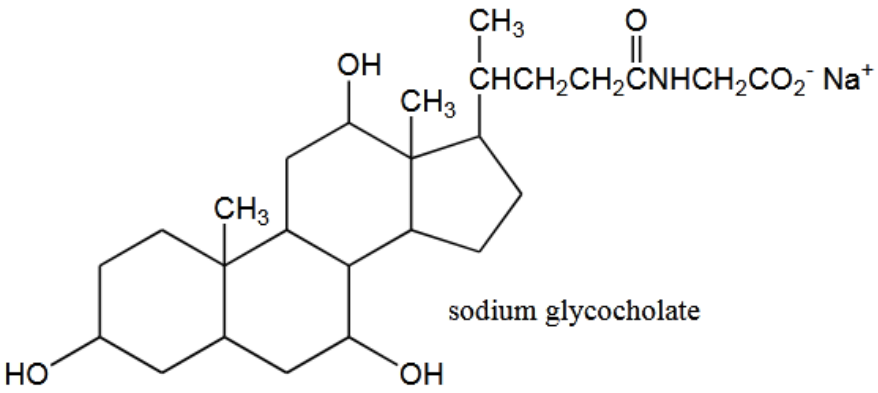
Tab. 14: Representative Steroid Hormones and Their Physiological Effects

Hormone	Effect
 Aldosterone	regulates salt metabolism; stimulates kidneys to retain sodium and excrete potassium
 Cortisol (Hydrocortisone)	stimulates the conversion of proteins to carbohydrates
 Progesterone	regulates the menstrual cycle; maintains pregnancy
 Estradiol (an estrogen)	stimulates female sex characteristics; regulates changes during the menstrual cycle
 Testosterone (an androgen)	stimulates and maintains male sex characteristics

IV.4.3. Bile Salts

Biles; yellowish brown or green fluid are steroid-derived molecules synthesized in the liver and secreted into the small intestine via bile.

Bile salts act like soaps and other emulsifiers: they contain both polar and nonpolar regions, helping to break fats in foods into smaller pieces, allowing them to be hydrolyzed more easily (Fig.98).

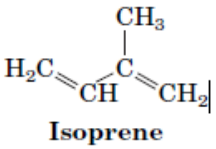


sodium glycocholate

Fig.98: Chemical structure of sodium glycocholate; a bile salt molecule.

IV.4.4. Terpenoids

Terpenes are diverse class of organic compounds, produced by a variety of plants, and some insects. They are derived biosynthetically from units of *isoprene*,



Isoprene

The plant kingdom is rich in isoprenoid compounds, which serve as pigments, molecular signals (hormones and pheromones), and defensive agents. Indeed, over 50,000 isoprenoids (also known as terpenoids), which are mostly of plant, fungal, and bacterial origin, have been characterized.

IV.4.5. Fat soluble vitamins

Vitamins are organic substances that an animal requires in small amounts but cannot synthesize and hence must acquire in its diet. Fat soluble vitamins (A, D, E & K) are derived lipids and they have important functions. The table bellows (tab.15) summarizes key information about the fat soluble vitamins. For each vitamin, it lists its primary function within the body and the characteristic deficiency symptoms that can arise when intake or absorption of that vitamin is inadequate.

Tab. 15: Functions and deficiencies of fat-soluble vitamins.

Vitamin	Function	Deficiency
A	Roles in vision, growth, reproduction	Night blindness, cornea damage, damage to respiratory and gastrointestinal tract
D	Regulation of calcium and phosphate metabolism	Rickets (children): skeletal deformaties, impaired growth Osteomalacia (adults): soft, bending bones
E	Antioxidant	Inhibition of sperm production; lesions in muscles and nerves (rare)
K	Blood coagulation	Subdermal hemorrhaging

IV.4.5.1. Vitamin A, or retinol

Vitamin A is derived mainly from plant products such as **β**-carotene [a red pigment that is present in green vegetables as well as in carrots. Retinol is oxidized to its corresponding aldehyde, **retinal**, which functions as the eye's photoreceptor at low light intensities. Light causes the retinal to isomerize, triggering, via a complex signaling pathway, an impulse through the optic nerve. A severe deficiency of vitamin A can lead to blindness. Retinoic acid

also has hormone like properties in that it stimulates tissue repair. It is used to treat severe acne and skin ulcers and is also used cosmetically to
eliminate wrinkles (Fig.99).

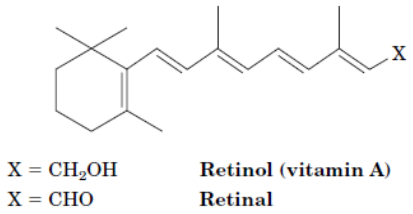


Fig.99: vitamin A structure

IV.4.5.2. Vitamin K

Vitamin K is a lipid synthesized by plants (as phylloquinone) and bacteria (as menaquinone). About half of the daily requirement for humans is supplied by intestinal bacteria. Vitamin K participates in the carboxylation of Glu residues in some of the proteins involved in blood clotting.

Vitamin K deficiency prevents this carboxylation, and the resulting inactive clotting proteins lead to excessive bleeding. Compounds that interfere with vitamin K function are widely used as anti-clotting drugs (e.g., to prevent clot formation after surgery), as well as being the active ingredients in some rodent poisons the figure below shows the structure of vitamin K (Fig.100).

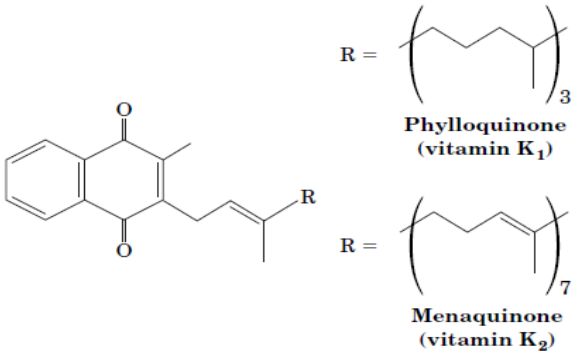


Fig.100: vitamin K structure

IV.4.5.3. Vitamin E

Vitamin E is actually a group of compounds whose most abundant member is **tocopherol:**

This highly hydrophobic molecule is incorporated into cell membranes, where it functions as an antioxidant that prevents oxidative damage to membrane proteins and lipids. A deficiency

of vitamin E elicits a variety of nonspecific symptoms, which makes the deficiency difficult to detect. The popularity of vitamin E supplements rests on the hypothesis that vitamin E protects against oxidative damage to cells and hence reduces the effects of aging. However, clinical trials have shown that this is not the case and that excessive intake of vitamin E may even increase the risk of certain cancers **(Fig.101).**

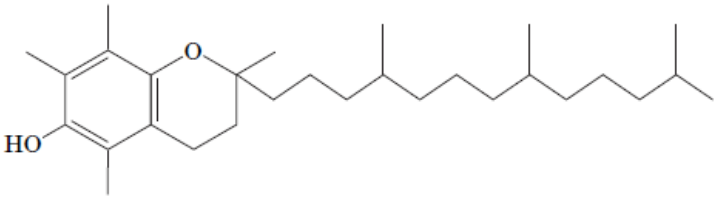


Fig.101: vitamin E structure

IV.4.6. Eicosanoids

Other less common lipids are derived from relatively abundant membrane lipids. prostacyclins, thromboxanes, leukotrienes, and lipoxins are known collectively as eicosanoids because they are all C20 compounds (Greek: *eikosi*, twenty). The eicosanoids act at very low concentrations and are involved in the production of pain and fever, and in the regulation of blood pressure, blood coagulation, and reproduction. The figure below shows the different classes of eicosanoids (Fig. 102).

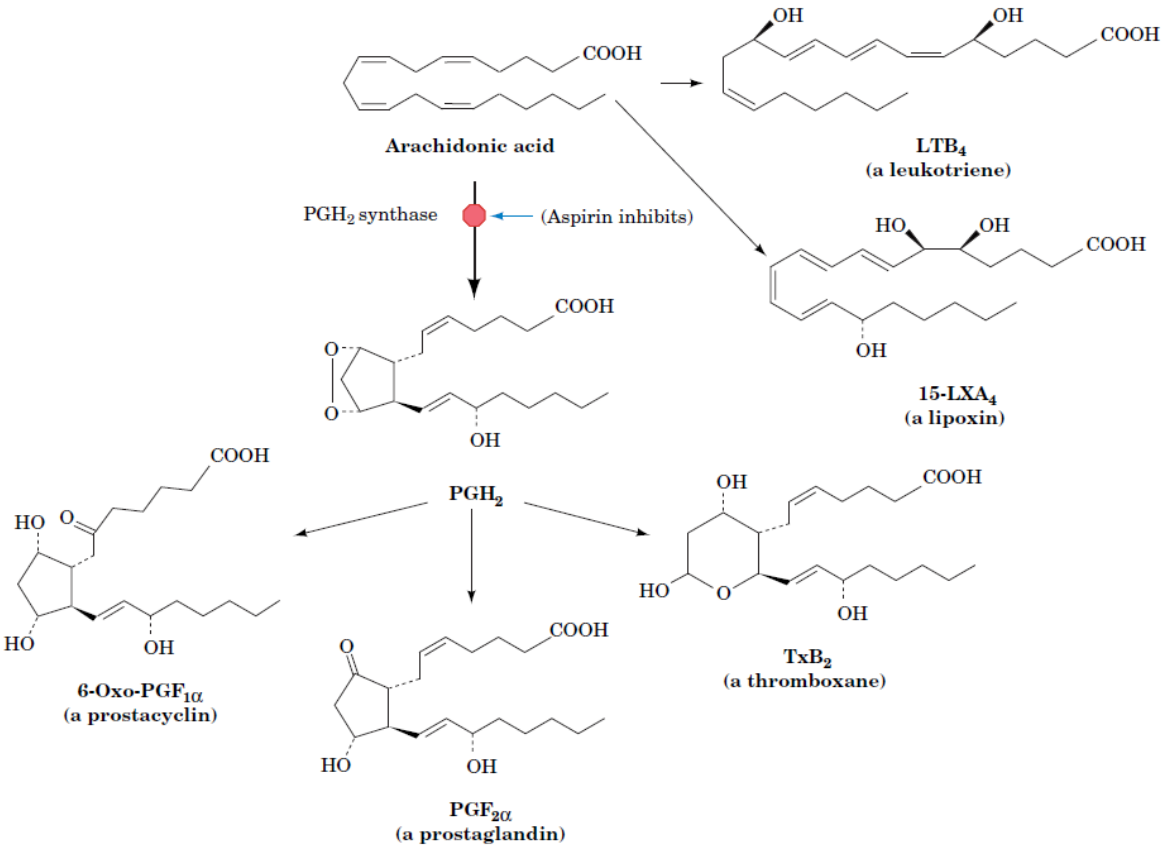


Fig. 102: Eicosanoids. Arachidonate is the precursor of prostaglandins (PG), prostacyclins, thromboxanes (Tx), lipoxins (LX) and leukotrienes.

IV.4.7. Lipoproteins

Plasma lipids consist of triacylglycerols (16%), phospholipids (30%), cholesterol (14%), cholesterylesters (36%) and unesterified long-chain fatty acids (free fatty acids) (4%).

- lipoproteins have a single layer phospholipid and cholesterol outer shell, with the hydrophilic portions oriented outward toward the water and lipophilic portions oriented inwards toward the lipids molecules within the particles. The protein moiety of a lipoprotein is known as an ***apolipoprotein*** or **apoprotein,** constituting nearly 70% of some HDL and as little as 1% of chylomicrons.

Some apolipoproteins are integral and cannot be removed, where as others are free to transfer to other lipoproteins.

Four major groups of lipoproteins that can be separated according to their electrophoretic properties and have been identified as physiologically important compounds and used in clinical diagnosis.

These are:

(1) chylomicrons, derived from intestinal absorption of triacylglycerol and other lipids;

(2) very low density lipoproteins (VLDL, or pre-β-lipoproteins), derived from the liver for the export of triacylglycerol;

(3) low-density lipoproteins (LDL, or β-lipoproteins), represent the final stage in the catabolism of VLDL;

(4) high-density lipoproteins (HDL, or α-lipoproteins), involved in VLDL and chylomicron metabolism and also in cholesterol transport.

Triacylglycerol is the predominant lipid in chylomicrons and VLDL, whereas cholesterol and phospholipid are the predominant lipids in LDL and HDL, respectively.

The table below (Tab.16) provides a classification of the major lipoprotein particles found in the blood, categorized by their source, diameter, density, and composition. It details the protein and lipid percentages within each lipoprotein, along with their main lipid components (like triacylglycerols, phospholipids, and cholesterol) and the key apolipoproteins associated with them.

Tab. 16: Composition of the lipoproteins in plasma of humans.

Lipoprotein	Source	Diameter (nm)	Density (g/mL)	Composition		Main Lipid Components	Apolipoproteins
				Protein (%)	Lipid (%)		
Chylomicrons	Intestine	90–1000	< 0.95	1–2	98–99	Triacylglycerol	A-I, A-II, A-IV,[1] B-48, C-I, C-II, C-III, E
Chylomicron remnants	Chylomicrons	45–150	< 1.006	6–8	92–94	Triacylglycerol, phospholipids, cholesterol	B-48, E
VLDL	Liver (intestine)	30–90	0.95–1.006	7–10	90–93	Triacylglycerol	B-100, C-I, C-II, C-III
IDL	VLDL	25–35	1.006–1.019	11	89	Triacylglycerol, cholesterol	B-100, E
LDL	VLDL	20–25	1.019–1.063	21	79	Cholesterol	B-100
HDL	Liver, intestine, VLDL, chylo-microns					Phospholipids, cholesterol	A-I, A-II, A-IV, C-I, C-II, C-III, D,[2] E
HDL_1		20–25	1.019–1.063	32	68		
HDL_2		10–20	1.063–1.125	33	67		
HDL_3		5–10	1.125–1.210	57	43		
Preβ-HDL[3]		< 5	> 1.210				A-I
Albumin/free fatty acids	Adipose tissue		> 1.281	99	1	Free fatty acids	

Abbreviations: HDL, high-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low density lipoproteins.
[1]Secreted with chylomicrons but transfers to HDL.
[2]Associated with HDL_2 and HDL_3 subfractions.
[3]Part of a minor fraction known as very high density lipoproteins (VHDL).

Chapter V: ENZYMES

V.1. Definition and biomedical importance

Enzymes are catalysts that increase the rate or velocity of physiologic reactions by decreasing the energy needed for substrate activation and are not consumed during the reaction. They are found in all tissues and fluids of the body and have a high degree of specificity for types of reaction catalized and for their substrate. Enzymes are also stereospecific catalysts for specific stereoisomers (L & D).

The presence and maintenance of a complete and balanced set of enzymes is essential for the breakdown of nutrients to supply energy and chemical building blocks; the assembly of those building blocks into proteins, DNA, membranes, cells, and tissues; and the harnessing of energy to power cell motility and muscle contraction. With the exception of a few catalytic RNA molecules, or ribozymes, the vast majority of enzymes are proteins. Deficiencies in the quantity or catalytic activity of key enzymes can result from genetic defects, nutritional deficits, or toxins. Defective enzymes can result from genetic mutations or infection by viral or bacterial pathogens (eg, *Vibrio cholerae*). Medical scientists address imbalances in enzyme activity by using pharmacologic agents to inhibit specific enzymes and are investigating gene therapy as a means to remedy deficits in enzyme level or function.

Estimation of enzymes activities in the serum has many applications in the diagnosis, differential diagnosis (e.g. in myocardial infarction both AST and LDH are increased in the serum but in case of pulmonary embolism AST is normal but LDH is increased), assessing prognosis of diseases, and early detection of disease (e.g. increase level of ALT in serum in viral hepatitis before the occurrence of jaundice).

V.2. Essential notion in enzymology

V.2.1. Substrate

The substrate is the molecule upon which an enzyme acts. It is the reactant in an enzymatic reaction that binds to the enzyme's active site, where the enzyme catalyzes the conversion of the substrate into products.

V.2.2. Product

The product is the molecule(s) formed as a result of the enzymatic reaction. It is the outcome of the substrate being altered by the enzyme. After the reaction, the product(s) are released

from the enzyme's active site, allowing the enzyme to catalyze further reactions with other substrate molecules.

V.2.3. Catalysts

Catalysis is when the substrate is changed. It could be broken down or combined with another molecule to make something new. It will break or build chemical bonds.

IV.2.4. Ribozymes

Nearly all enzymes are proteins. However, some RNA molecules are also capable of speeding reactions. The most famous of these molecules was discovered by Tom Cech in the early 1980s. Cech was puzzled at his inability to find any proteins catalyzing a process his group was studying in a microorganism named Tetrahymena. Ultimately, the catalysis was recognized as coming from RNA itself. Since this discovery, many other examples of catalytic RNAs have been found. Ribozymes catalyze the cleavage and synthesis of phosphodiester bond in RNA at specific sites (Fig. 103).

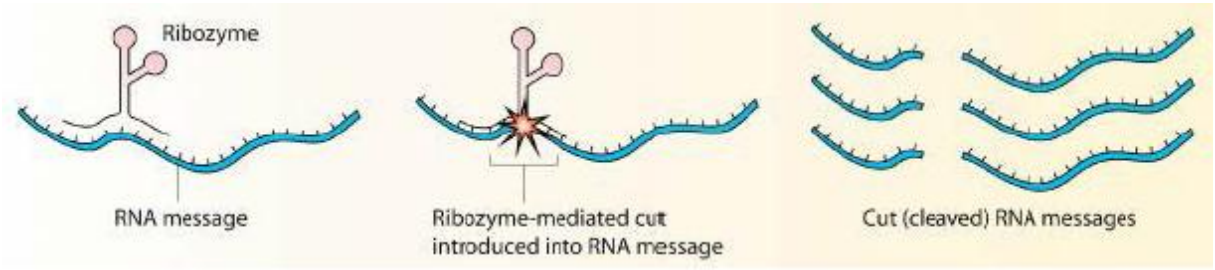


Fig. 103: Cleavage of an RNA by a ribozyme.

V.2.5. Active site of an enzyme

The active sits of an enzyme is that region of the enzyme where catalysis takes place. It is also the region that binds the substrate and contributes the residues that directly participates in the making and breaking of bonds.

Some features of active site are:

- it is a relatively small portion of the total enzyme volume
- it is a three dimensional entity
- substrate binds with relatively weak forces
- it is a cleft or crevice
- the specificity of binding depends on the precisely defined arrangement of atom in an active site.

Generally certain amino acid side chains have important role in enzyme catalysis e.g. SH of cysteine, OH of hydroxy-amino acids, carboxylic group of acidic amino acids and amino group of basic amino acids. They help in binding of the enzyme with its substrate and with the groups undergoing transfer (added or removed).

V.2.6. Specificity

Enzymes are highly specific with varying degrees of specifity.

- Absolute specifity: they act on one substrate and only on that substrate.
- Stereospecifity : such enzymes that can detect the difference between optical isomers (mirror images) and select only one of such isomers.

-Reaction specifity: enzymes that catalyze certain types of reactions.

-Group specifity : enzymes that catalyzes a group of substances that contain specific compounds.

V.2.7. Cofactors

The catalytic activity of many enzymes depends on the presence of small molecules termed *cofactors,* although the precise role varies with the cofactor and the enzyme. Generally, these cofactors are able to execute chemical reactions that cannot be performed by the standard set of twenty amino acids. An enzyme without its cofactor is referred to as an ***apoenzyme;*** the complete, catalytically active enzyme is called a ***holoenzyme*****.**

Cofactors can be subdivided into two groups: (1) metals and (2) small organic molecules called ***coenzymes*****.** Often derived from vitamins, coenzymes can be either tightly or loosely bound to the enzyme. Tightly bound coenzymes are called ***prosthetic groups***. Loosely associated coenzymes are more like cosubstrates because, like substrates and products, they bind to the enzyme and are released from it. The use of the same coenzyme by a variety of enzymes sets coenzymes apart from normal substrates, however, as does their source in vitamins. Enzymes that use the same coenzyme usually perform catalysis by similar mechanisms; Some enzyme cofactors are summarized in Tab.17**.**

Tab. 17: Some enzyme cofactors

Cofactor	Enzyme
Coenzyme	
Thiamine pyrophosphate	Pyruvate dehydrogenase
Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Pyridoxal phosphate	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
5′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase
Mn	Superoxide dismutase
K^{+}	Propionyl CoA carboxylase

V.2.8. Isoenzymes

Isoenzymes (also known as isozymes) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction,They vary with respect to their kinetic parameters, electrophoretic mobility, localization; and all have independent action. Believed to be originating from closely linked genes or from multiple gene loci

Eg.Lactate dehydrogenase have 5 isoenzymes (LDH1, LDH2, LDH3, LDH4 & LDH5). They can be used to identify the specific affected tissues and can be clinically quantified in the lab;Tab. 18 gives an example of the LDH isoenzyme.

Tab. 18: LDH isoforms

Isoenzyme name	Composition	Electrophoretic migration	Present in	Elevated in
LDH 1 Heat resistant	(H_4)	Fastest moving	Myocardium, RBC, kidney	myocardial infarction
LDH2 Heat resistant	(H_3M_1)		Myocardium, RBC, kidney	Kidney disease, megaloblastic anemia
LDH3	(H_2M_2)		brain	Leukemia, malignancy
LDH4 Heat labile	(H_1M_3)		Lung, spleen	Pulmonary infarction
LDH5 Heat labile Inhibited by urea	(M_4)	Slowest moving	Skeletal muscle, Liver	Skeletal muscle and liver diseases

V.3. Steps of Enzyme Action

The enzyme reaction generally involves four key steps, which are as follows:

1. The enzyme and the substrate are in the same area. Some situations have more than one substrate molecule that the enzyme will change.
2. The enzyme grabs on to the substrate at a special area called the active site that can only pick up one or two parts. The combination is called the enzyme/substrate complex. Enzymes are very, very specific and don't just grab on to any molecule.
3. A process called catalysis happens. Catalysis is when the substrate is changed. It could be broken down or combined with another molecule to make something new. It will break or build chemical bonds. When done, you will have the enzyme/products complex.
4. The enzyme releases the product. When the enzyme lets go, it returns to its original shape. It is then ready to work on another molecule of substrate (Fig. 104).

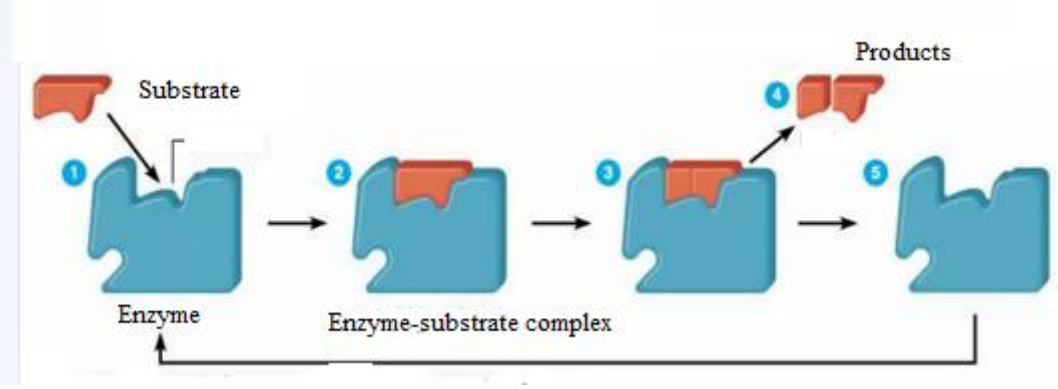


Fig. 104: Enzymatic reaction steps

V.3.1. The free energy of an enzymatic reaction

All chemical reactions have an energy barrier separating the reactants and the products. This barrier, called the free energy of activation, is the energy difference between that of the reactants and a high-energy intermediate that occurs during the formation of product.

For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state. Enzyme accelerates the rate of reaction by lowering the free energy of activation (Fig. 105).

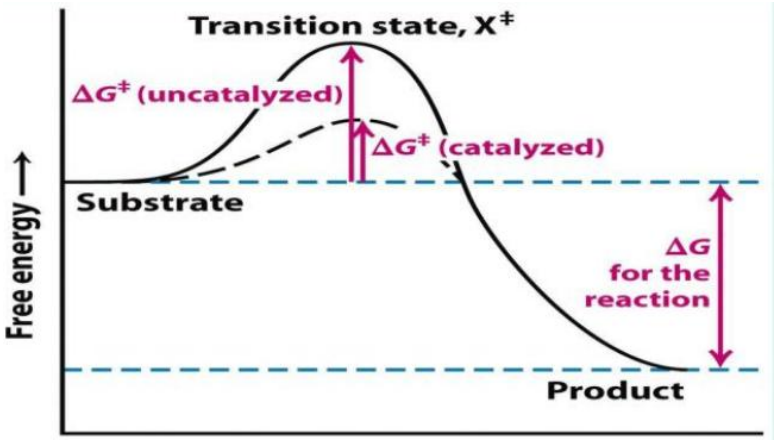


Fig. 105 : The free energy profile of an enzymatic reaction

V.3.2. The theories of enzyme action

The theories of enzyme action can be explained by the following two models:

V.3.2.1. The key and lock (Fisher model) theory

The active site of the enzyme is complementary in conformation to the substrate, so that enzyme and substrate recognize each other. This theory postulates that active site has fixed shape.

The substrate binds to a specific site on the enzyme to from enzyme substrate complex, this is followed by activation of the substrate, then formation of the reaction products and

the enzyme sets free to catalyze a new reaction. The only disadvantage of this model is the rigidity of active site (Fig. 106).

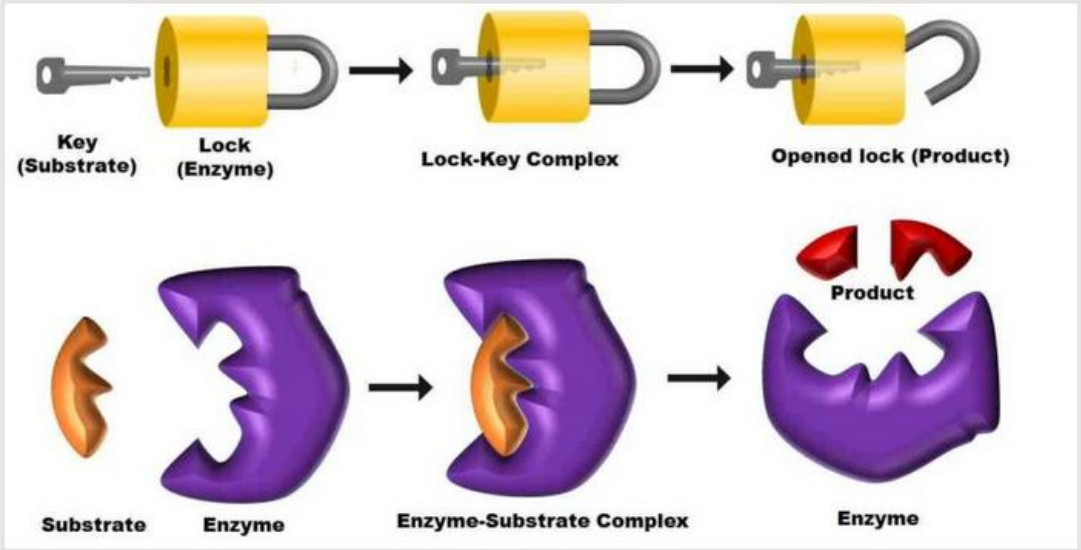


Fig. 106: key and lock (Fisher model) theory diagram

V.3.2.2. The induced fit theory (Khoshland model)

The enzyme changes its shape upon binding the substrate, so that catalytic site is suggested to be pre-shaped to fit with substrate.

In this model, the substrate induces a conformational changes in the enzyme, which make the catalytic site (or groups) more fit or more suitable for both binding of substrate and catalysis (Fig. 107).

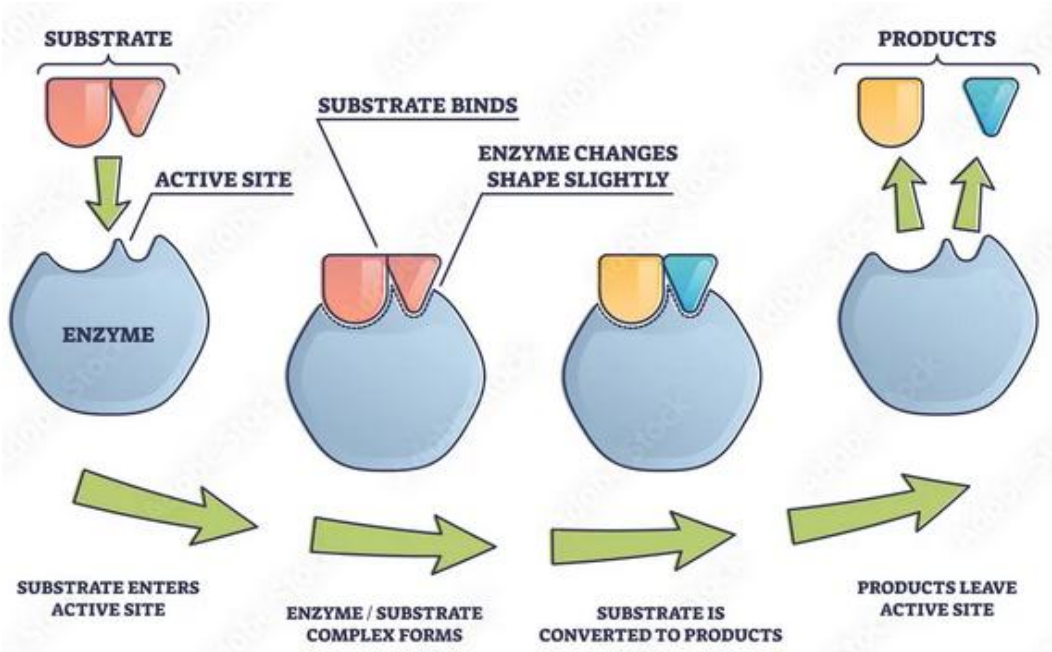


Fig. 107: fit theory diagram

V.4. Enzymes nomenclature and classification

According to the International union Of Biochemistry an enzyme **Systematic Name** has two parts:

-First part is the name of the substrates for the enzyme.

-Second part is the type of reaction catalyzed by the enzyme. This part ends with the suffix “ase”. Example: Lactate dehydrogenase

-One or more modifiers usually identify the **source of the enzyme** (pancreatic ribonuclease), specify its **mode of regulation** (hormone-sensitive lipase), or name a distinguishing characteristic of its mechanism (a cysteine protease).

Some names describe both the substrate and the function For example, alcohol dehydrogenase

-When it was discovered that multiple forms of some enzymes existed, alphanumeric designators were added to distinguish between them (eg, RNA polymerase III; protein kinase C).

- Sometimes **common names** are used, particularly for the digestion enzymes such as pepsin and trypsin.

-To address the ambiguity and confusion arising from these inconsistencies in nomenclature and the continuing discovery of new enzymes, the International Union of Biochemists (I.U.B.)., in its report in 1961, devised a system for classification of enzymes that also serves as a basis for assigning code numbers to them. These code numbers, prefixed by EC (Enzyme Commission number), which are now widely in use, contain four elements separated by points, with the following meaning: EC:X.Y.Z.W

- X: the first number shows to which of the six main divisions (classes) the enzyme belongs,
- Y: the second number indicates the subclass, generally contains information

about the type of compound or group involved.

- Z: the third number gives the sub-subclass, further specifies the type of reaction involved. For instance, EC 1.x.1.- indicates that NAD+ or NADP+ is the acceptor, while 1.x.2.- has a cytochrome as the acceptor, etc.
- W: the fourth number is the serial number of the enzyme in its sub-subclass.

Enzymes are classified into six functional Classes, on the basis of the types of reactions that they catalyze

EC 1. Oxidoreductases

EC 2. Transferases

EC 3. Hydrolases

EC 4. Lyases

EC 5. Isomerases

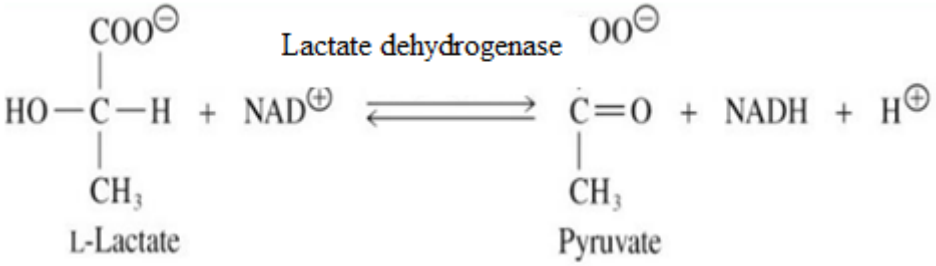
EC 6. Ligases.

V.4.1. Oxidoreductases

Oxidoreductases catalyze the transfer of hydrogen or oxygen atoms or electrons from one substrate to another, also called oxidases, dehydrogenases, or reductases.

Note that since these are 'redox' reactions, an electron donor/acceptor is also required to complete the reaction. Examples: Lactate dehydrogenase, Glucose, Oxidase,

Peroxidase, Catalase, Phenylalanine hydroxylase.

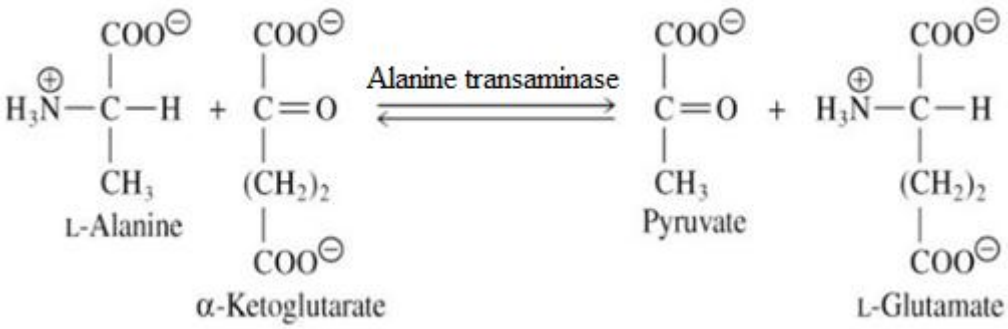


V.4.2. Transferases

Transferase catalyze transfer of groups such as methyl or glycosyl groups from a donor molecule to an acceptor molecule excluding oxidoreductases (which transfer hydrogen or oxygen and are EC 1).

These are of the general form: A-X + B ↔ BX + A

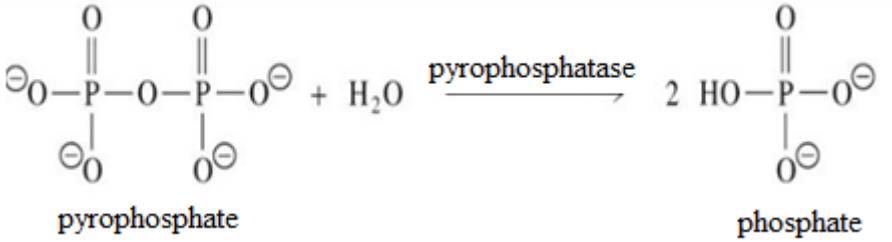
Examples: Transaminases, Phosphotransferases, Transmethylases, Transacylase.



V.4.3. Hydrolases

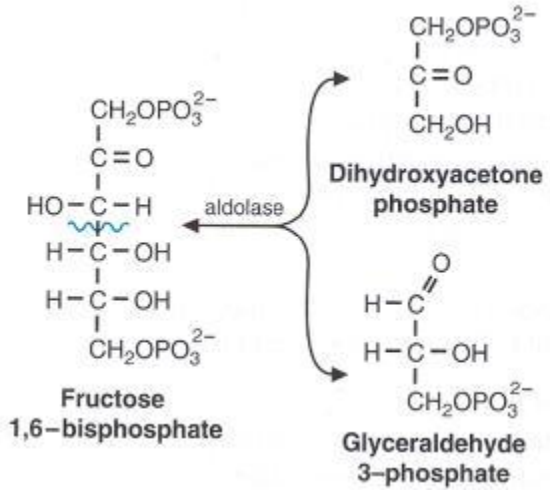
These enzymes catalyze the hydrolytic cleavage of C-C, C-O, C-N, P-O, and certain other bonds, including acid anhydride bonds, by adding water across the bond. These are of the general form: A-X + H2O ↔ X-OH + HA

Examples: Protein hydrolysing enzymes (Peptidases), Carbohydrases (Amylase, Maltase, Lactase),Lipid hydrolysing enzymes (Lipase),Deaminases, Phosphatases…..



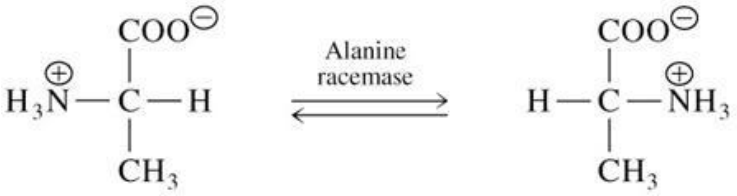
V.4.4. Lyases

Lyase cleave various bonds by means other than hydrolysis and oxidation. Add Water, Ammonia or Carbon dioxide across double bonds, or remove these elements to produce double bonds. Examples: Fumarase, carbonic anhydrase**,** decarboxylases and aldolases;



V.4.5. Isomerases

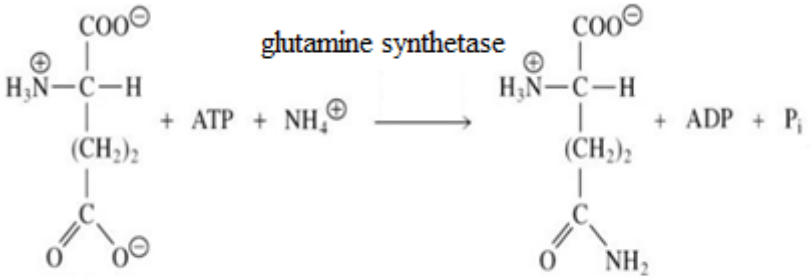
Isomerase catalyzes geometric or structural changes within a single molecule.Carry out many kinds of isomerization: L to D isomerizations and Mutase reactions. Examples: Isomerase, Mutase.



L-Alanine D-Alanine

V.4.6. Ligases

Ligase catalyze the joining together of two molecules, coupled to the hydrolysis of a pyrophosphoryl group in ATP or a similar nucleoside triphosphate. Examples: Acetyl~CoA Carboxylase. Glutamine synthetase;



L. glutamate L. glutamine

7. Translocase: translocase catalyze the movement of ions or molecules across membranes or their separation within membranes; the reaction is designated as a transfer from "side 1" to "side 2".

$$AX + B\,||_{(side\ 1)} = A + X + ||\,B_{(side\ 2)}$$

The Tab.19 gives a schematic explanation and more examples of enzyme classes.

Tab.19. Enzyme classes

Class	Reaction type	Important subclasses
1 Oxidoreductases	○ = Reduction equivalent $A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}$	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	$A\text{–}B + C \rightleftharpoons A + B\text{–}C$	C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	$A\text{–}B + H_2O \rightleftharpoons A\text{–}H + B\text{–}OH$	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	$A + B \rightleftharpoons A\text{–}B$	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	$A \rightleftharpoons Iso\text{-}A$	Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")	$A + B + XTP \rightleftharpoons A\text{–}B + XDP + P$	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

V.5. Enzyme Kinetics

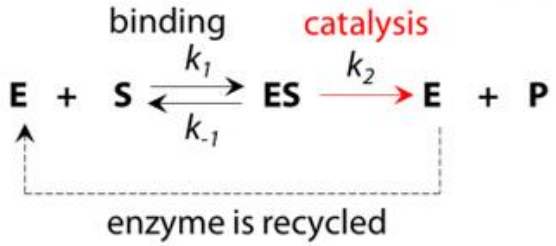
Enzyme Kinetics is the study of the rate or velocity of reactions catalyzed by enzymes. Initial reaction velocity (V0) is the rate at which reaction proceeds and it is measured as decrease in concentration of substrates or increase in concentration of products with time**.**

V.5.1. The Michaelis-Menten Equation

We can gain some understanding of enzyme behaviour through the study of rates of enzyme-catalyzed reactions and by the mathematical analysis of their rate.

The mathematical relationship between initial velocity and [S] was first worked out by Henri (1905, France), and subsequently verified experimentally by Michaelis and Menten (USA/Canada, 1913). Henri, Michaelis and Menten all assumed that the binding step was at equilibrium, which is only an approximation.

Although the overall reaction appears to be S→P, the underlying process is more complex and can be interpreted as a **two-step reaction**.



1. The enzyme is a **catalyst** and is recycled in an unchanged state by the end of the reaction process, so the total quantity of enzyme **[E]total** does not change as reaction proceeds.
2. If we deal only with **initial reaction rate v_o,** when product P is not yet present, there will be no reverse reaction ES → E + P at the catalytic step to complicate matters. Omitting this reverse reaction will make the algebra more manageable.
3. Each stage in the reaction has an associated **rate constant, k_1, k_2** and k_{-1} for the reverse of step 1. k_2 is sometimes described as k_{cat}, the rate constant for catalysis.

The rate of appearance of product P describes the overall rate of the complete enzyme reaction. It can be determined by taking the rate of step 2, which is a **fundamental chemical process**, for which we can write the **first order rate equation:**

$$v_o = k_2 [ES]$$

where **[ES]** is the concentration of enzyme actually occupied by substrate.

Although we know $[E]_{total}$, because that's how much enzyme we put into the reaction, we don't explicitly know what fraction of enzyme is empty E and what fraction is occupied ES, so [E] and [ES] are unknown values, but related by the equation:

$[E]_{total} = [E] + [ES]$. (Conservation of Mass).

We can determine [ES] algebraically

We can write the fundamental rate equations for formation and breakdown of ES.

Rate of formation of ES = $k_1[E][S]$

Rate of breakdown of ES = $k_2[ES] + k_{-1}[ES]$ (forward component + reverse component)

The **steady state condition** states that **rate of breakdown = rate of formation**

If the rate of breakdown happens to be **greater than** the rate of formation, **[ES] will decrease**, causing the rate of breakdown to slow down. If the rate of breakdown happens to be **less than** the rate of formation, **[ES] will increase**, causing the rate of breakdown to speed up. **Hence the two rates rapidly tend to match each other**.Thus, we can write:

$$(k_2 + k_{-1})[ES] = k_1[E][S]$$

Divide by k_1 to put all the constants on the left, and replace

K_M[ES] = [E][S] but both **[ES]** and **[E]** are unknown.

Now use the relationship **[E] = $[E]_{total}$ – [ES]** to eliminate the second unknown value **[E]**

K_M[ES] = ([E]total – [ES]) [S]

then take the term [ES][S] over to the left side

(K_M + [S]) [ES] = $[E]_{total}$[S]

and divide by (K_M + [S])

where **$[E]_{total}$** and **[S]** are known values, and **K_M** is a constant

This is used to replace the **[ES]** in the rate equation **$v_o = k_2$[ES]**

The maximum observable rate **V_{max}** occurs when **100% of the enzyme is occupied by substrate**, so **$[ES]_{max} = [E]_{total}$** where **$[E]_{total}$** is a measurable quantity.

Thus we can write **$V_{max} = k_2[E]_{total}$** and replace **$k_2[E]_{total}$** in the equation above with **V_{max}** to get the **Michaelis-Menten equation**

$$v_o = \frac{Vmax\,[S]}{K_M + [S]}$$

The Michaelis-Menten equation shows how v_o varies as a function of substrate concentration [S], Every enzyme has characteristic values of K_M and V_{max} that describe its catalytic behavior (Fig. 108).

Km is numerically equal to the substrate concentration at which the reaction velocity is equal to ½ Vmax.

- Substrates are usually present in physiological fluids in amounts nearly equal to Km values.
- Km reflects the affinity of the enzyme to substrate.
- Small (low) Km = high affinity of the enzyme for substrate i.e. low concentration of substrate is needed to half saturate enzyme.
- Large (high) Km = low affinity of the enzyme for the substrate (high concentration of substrate is needed to half saturate enzyme).
- Km does not vary with the concentration of enzyme.

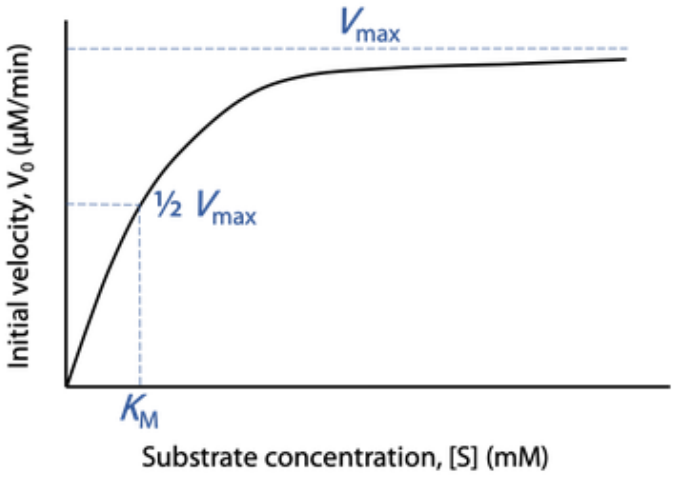


Fig. 108: Michaelis-Menten curve

V.5.2. Units of enzyme activity

Enzyme activity never expressed in terms of their concentration (mg or µg), but are expressed only as activities.

- Katal: amount of enzyme that catalyze conversion of 1 mol of substrate to product per second (mol/s).
- International Unit (IU): amount of enzymes that catalyze conversion of 1 µmol of substrate to product per minute; IU = 60 µkatal.

V.5.3. Lineweaver Burk Plot

In order to rectify the shortcomings of hyperbolic curve, Hans Lineweaver and Dean Burk in 1934, developed a simple method by inverting the existing Michaelis-Menten equation, which fits well into the equation of a straight line.

Original Michaelis-Menten equation:

$$v_0 = \frac{V_{max}[S_0]}{K_m + [S_0]}$$

On inverting both the sides we get,

$$\frac{1}{v_o} = \frac{K_m + [S_0]}{V_{max}[S_0]} \quad \text{or} \quad \frac{1}{v_o} = \frac{K_m}{V_{max}[S_0]} + \frac{[S_0]}{V_{max}[S_0]} \quad \text{or} \quad \frac{1}{v_o} = \frac{K_m}{V_{max}[S_0]} + \frac{1}{V_{max}}$$

This equation, can now be compared with equation of line y = mx +c, Where,

$$y = \frac{1}{v_o}, x = \frac{1}{[S_0]}, m = \frac{K_m}{V_{max}} \quad c = \frac{1}{V_{max}}$$

Now, if we plot inverse of rate of reaction (v0) on y axis and inverse of substrate concentration [S0] on x axis, a straight line is obtained. It must be noted that point at which

the line intersectsy axis (c) represents 1/Vmax and the point at which the same line intersects x axis represents -1/Km. the slope of this line represents km/Vmax (**Fig. 109)**.

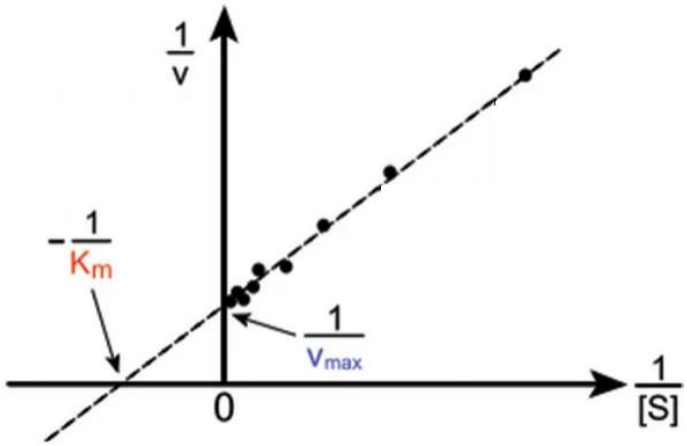


Fig. 109: Lineweaver Burk Plot

V.6. Factors affecting the rate of enzyme catalysis

V.6.1. Effect of Temperature

With increase in the temperature, kinetic energy of the reacting molecules increases. Chances of collision of molecules increases so that the rate of reaction increases.

▪ Velocity of enzyme reaction increases with increase in temperature up to a maximum called optimum temperature and then declines.

The optimum temperature for most of the enzyme is between 40° to 45°c. Its nearly 35.5°c for the enzyme of human body. However, an optimum temperature for reaction may be various from enzyme to enzyme depends on their origin.

Example: Taq polymerase is a heat stable DNA polymerase synthesized by thermophilic bacterium *Thermus aquaticus*. In research labs, the name of the enzyme termed TaqPol or simply Taq. This enzyme can replicate DNA even at above 90°C. The half-life of Taq is more than 2 hours at 92.5°C.

At low-temperature state, enzymes are temporarily inactive. When the temperature rises to a normal state then the enzymes will gain their lost activity. This low-temperature state is generally used in laboratory conditions to preserve the enzymes for a longer period.

Higher temperature is an enemy for most of the enzymes. At this state, enzymes are denatured and lost their activity by destroying the weak hydrogen bonds of their tertiary structure. These structural changes make the enzymes lost their catalytic activity. Once the

enzyme (protein) is denatured, then it remains inactive even if the temperature comes back to a normal state (Fig. 110).

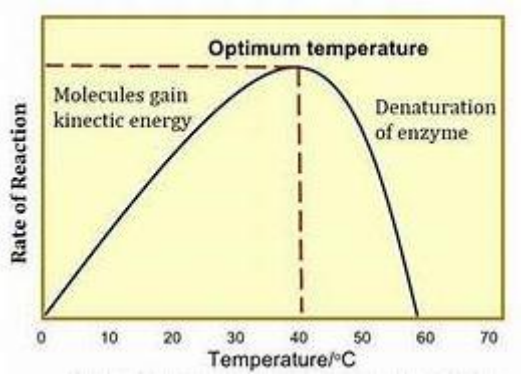


Fig.110: Effect of temperature on enzymatic activity

V.6.2. Effect of pH

Enzyme catalysis requires appropriate interaction between the enzyme and the substrate. As the pH changes, the charge of the substrate and the amino acid residues in the active site change, thus decreasing the interaction between the enzyme and substrate with a resulting decrease in catalysis. Thus increase in the hydrogen ion concentration pH considerably influences the enzyme activity. A bell shaped curve is normally obtained.

Each and every enzyme has an optimum pH for catalysis at which the velocity is maximum. Most of the enzymes of higher organisms show optimum activity around neutral pH (6-8) (Fig.111).

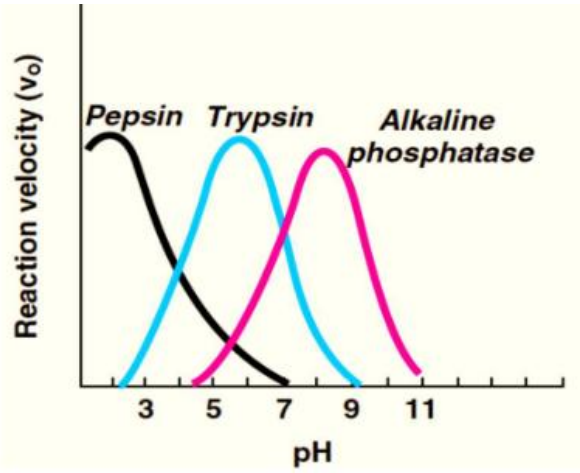


Fig.111: effect of pH on enzymatic activity

V.6.3. Effect of concentration of enzyme

As the concentration of the enzyme is increased, the velocity or rate of the enzyme catalyzed reaction proportionately increases. If the temperature and pH are kept constant and the

substrate concentration is high, the rate of reaction increases with increasing enzyme concentration. In fact, this property of enzyme is made use in determining the activities of serum enzymes for diagnosis of disease (Fig.112**)**.

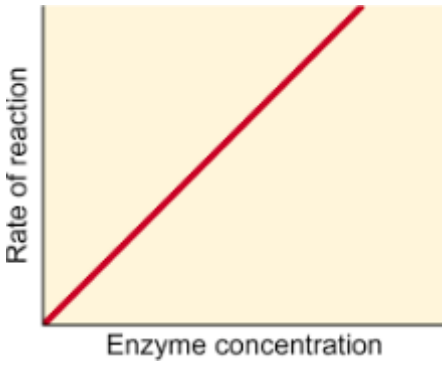


Fig.112: effect of concentration of enzyme on enzymatic activity

V.6.4. Effect of concentration of substrate

▪ Increase in the substrate concentration gradually increases the velocity of enzyme reaction within the limited range of substrate levels i.e. at a given enzyme concentration the rate of reaction increases with increase in substrate concentration up to a point called saturation point. A rectangular hyperbola is obtained when velocity is plotted against the substrate concentration. At saturation point further increase in substrate concentration produce no significant change in reaction rate because the binding sites of the enzyme at a given instant are all saturated with substrate (Fig.113).

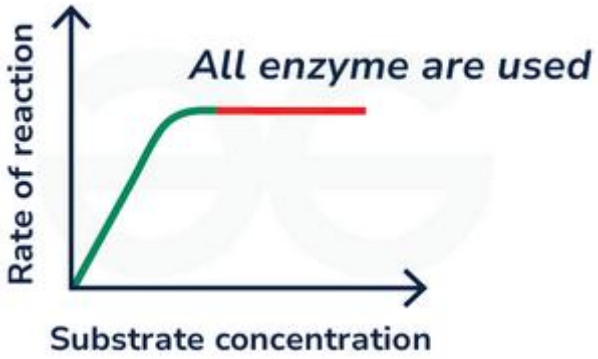


Fig.113: effect of concentration of substrat on enzymatic activity

V.6.5. Effect of product concentration

The accumulation of end-products can inhibit enzyme activity through feedback inhibition which is a regulatory mechanism that helps maintain homeostasis. When end-products

accumulate to high levels, they can bind to the enzyme's allosteric site, altering its conformation and reducing its catalytic activity. This inhibition prevents excessive production of end-products and conserves resources within the cell. As end-product levels decrease, inhibition is removed. This allows enzyme activity to resume.

V.6.6. Effect of inhibitor on enzyme activity

The activity of many enzymes can be inhibited by the binding of specific small molecules and ions. This means of inhibiting enzyme activity serves as a major control mechanism in biological systems, typified by the regulation of allosteric enzymes. In addition, many drugs and toxic agents act by inhibiting enzymes. Inhibition can be a source of insight into the mechanism of enzyme action: specific inhibitors can often be used to identify residues critical for catalysis.Enzyme inhibition can be either irreversible or reversible.

V.6.6.1. Irreversible inhibitor

An *irreversible inhibitor* dissociates very slowly from its target enzyme because it has become tightly bound to the enzyme, either covalently or noncovalently. Some irreversible inhibitors are important drugs. Penicillin acts by covalently modifying the enzyme transpeptidase, thereby preventing the synthesis of bacterial cell walls and thus killing the bacteria. Aspirin acts by covalently modifying the enzyme cyclooxygenase, reducing the synthesis of signaling molecules involved in inflamation.

Reversible inhibition, in contrast with irreversible inhibition, is characterized by a rapid dissociation of the enzyme–inhibitor complex (**Tab. 15,16**).

V.6.6.2. Competitive inhibition

In this type, an enzyme can bind substrate (forming an ES complex) or inhibitor (EI) but not both (ESI). The competitive inhibitor often resembles the substrate and binds to the active site of the enzyme. The substrate is thereby prevented from binding to the same active site.
A competitive inhibitor diminishes the rate of catalysis by reducing the proportion of enzyme molecules bound to a substrate. At any given inhibitor concentration, competitive inhibition can be relieved by increasing the substrate concentration. Under these conditions, the substrate successfully competes with the inhibitor for the active site. Methotrexate is an especially potent competitive inhibitor of the enzyme dihydrofolate reductase, which plays a role in the biosynthesis of purines and pyrimidines. This compound is a structural analog of

dihydrofolate, a substrate for dihydrofolate reductase. What makes it such a potent competitive inhibitor is that it binds to the enzyme 1000-fold more tightly than the natural substrate and inhibits nucleotide base synthesis. It is used to treat cancer.

V.6.6.3. Uncompetitive inhibition

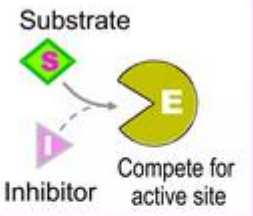
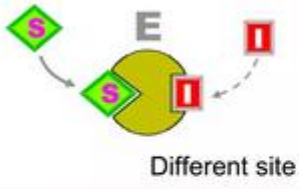
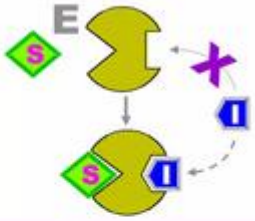
Uncompetitive inhibition is distinguished by the fact that the inhibitor binds only to the enzyme–substrate complex. The uncompetitive inhibitor's binding site is created only on interaction of the enzyme and substrate. Uncompetitive inhibition cannot be overcome by the addition of more substrate.

V.6.6.4. Noncompetitive inhibition

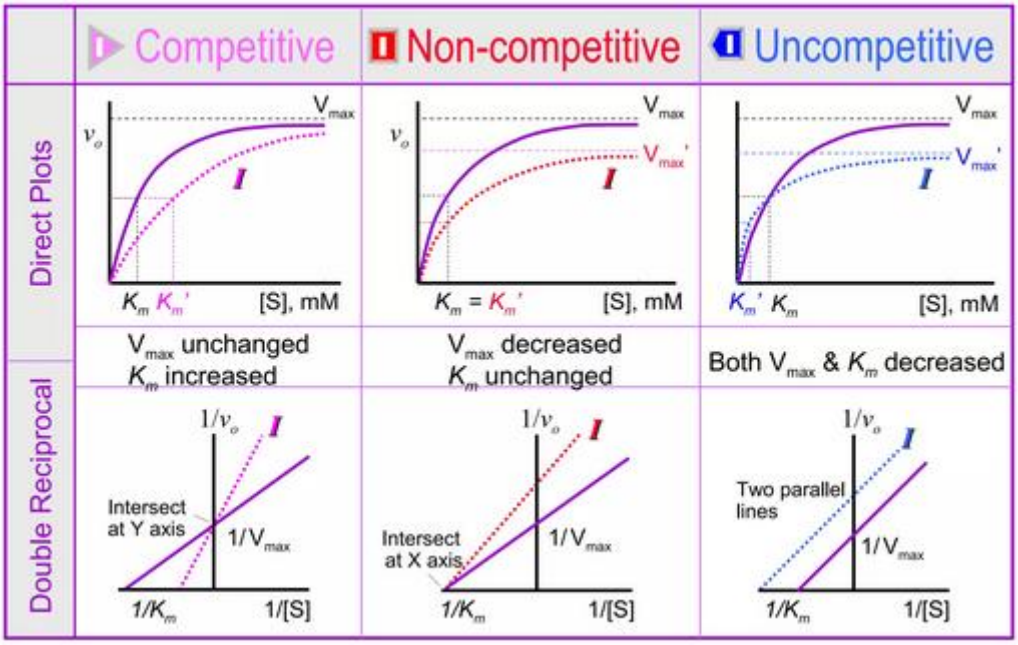
In *noncompetitive inhibition,* the inhibitor and substrate can bind simultaneously to an enzyme molecule at different binding sites. A noncompetitive inhibitor acts by decreasing the turnover number rather than by diminishing the proportion of enzyme molecules that are bound to substrate. Noncompetitive inhibition, like uncompetitive inhibition, cannot be overcome by increasing the substrate concentration.

The table 20 and 21 summurize the mechanism and the plots of enzyme inhibition.

Tab. 20: Mechanism of enzyme inhibition

	Competitive	Non-competitive	Uncompetitive
Cartoon Guide			
Equation and Description	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\downarrow\uparrow$ EI	$E + S \rightleftarrows ES \rightarrow E + P$ $+ \quad +$ $I \quad I$ $\downarrow\uparrow \quad \downarrow\uparrow$ $EI + S \rightarrow EIS$	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\downarrow\uparrow$ EIS
	[*I*] binds to free [E] only, and competes with [S]; increasing [S] overcomes Inhibition by [*I*].	[*I*] binds to free [E] or [ES] complex; Increasing [S] can not overcome [*I*] inhibition.	[*I*] binds to [ES] complex only, increasing [S] favors the inhibition by [*I*].

Tab.21: Plots of enzyme inhibition



V.7. Allosteric enzymes

Allosteric enzymes constitute an important class of enzymes whose catalytic activity can be regulated. These enzymes, which do not conform to Michaelis–Menten kinetics, consist of multiple subunits and multiple active sites which display cooperativity. Allosteric enzymes often display sigmoidal plots of the reaction velocity *V*0 versus substrate concentration [S], rather than the hyperbolic plots predicted by the Michaelis–Menten equation. In allosteric enzymes, the binding of substrate to one active site can alter the properties of other active sites in the same enzyme molecule. A possible outcome of this interaction between subunits is that the binding of substrate becomes *cooperative;* that is, the binding of substrate to one active site facilitates the binding of substrate to the other active sites. In addition, the activity of an allosteric enzyme may be altered by regulatory molecules that are reversibly bound to specific sites other than the catalytic sites. The catalytic properties of allosteric enzymes can thus be adjusted to meet the immediate needs of a cell. For this reason, allosteric enzymes are key regulators of metabolic pathways (Fig.114).

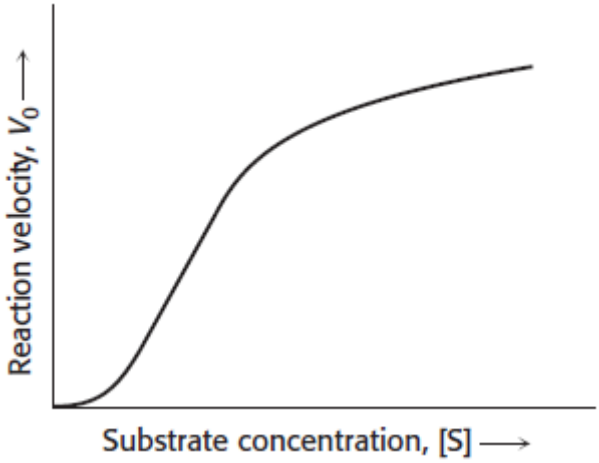


Fig.114: Kinetics for an allosteric enzyme.

V.8. Mechanisms of Enzyme Activations

The mechanisms of Enzyme Activations include the following:

1- Activation of zymogens. 2- Activation by metal ions.

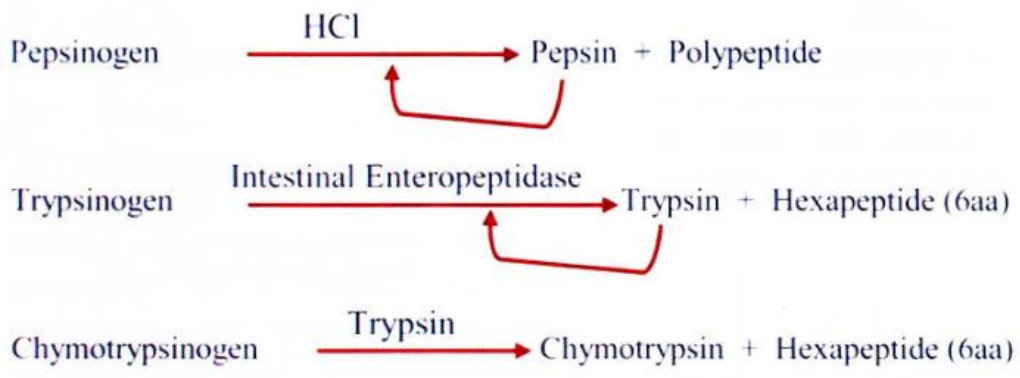
3- Allosteric activation. 4- Covalent modification.

V.8.1. Activation of Zymogens (Pro-enzymes)

Many enzymes are formed in the form of proenzymes or zymogens, in this form they are inactive, and there activation requires proteolysis (removal of a part of the polypeptide chain which masks the active site or substrate site).

A good example is the formation of digestive proteolytic enzymes as zymogens inside the cells to prevent digestion of cellular proteins. When these zymogens are released to the gut, they are activated to digest food proteins.

Many of these enzymes after activation can activate its zymogen in a process termed autocatalytic activation (autocatalysis).



V.8.2. Metal Ion Activation

There are three main possible mechanisms by which the metals interact with the enzyme and substrate as follows:

A. The metal helps to maintain an active conformation of the enzyme e.g. Mg^{2+} in glutamine synthase.

B. The metal binds with the substrate, then it helps binding of the substrate to the enzyme as in phosphotransferase reactions.

C. The metal is associated with the active center of the enzyme and helps in binding of the substrate to the enzyme e.g. cytochromes.

V.8.3. Allosteric Activation

Certain enzymes contain specific site (away from the catalytic site). The binding of an allosteric activator with the allosteric site produces conformational changes in the protein structure of the enzyme which result in increased velocity of the reaction.

V.8.4. Covalent Modification for Activation by Phosphorylation and Dephosphorylation)

Many enzymes are activated by phosphorylation and inactivated by dephosphorylation and vice versa. This means that the enzyme is present in two interconvertible forms (phosphorylated and dephosphorylated). The phosphate groups are usually attached to the hydroxyl group of amino acid residues (mainly serine or tyrosine) present in the polypeptide chain of the enzyme (Fig.115).

Good examples are the activation of glycogen phosphorylase and hormone sensitive lipase by phosphorylation and activation of glycogen synthase by dephosphorylation

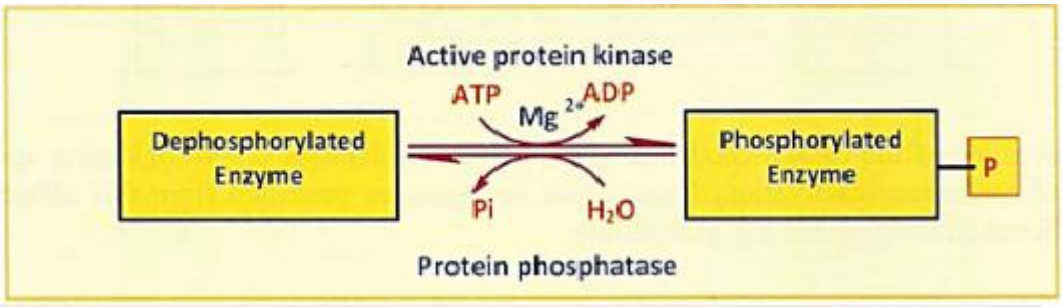


Fig. 115: Regulation of Enzyme Activity by Phosphorylation and Dephosphorylation; A **dephosphorylated enzyme** can be converted to a **phosphorylated enzyme** by an **active protein kinase**. This reaction utilizes **ATP** as a phosphate donor, converting it to **ADP**, and often requires **magnesium ions (Mg^{2+})** as a cofactor. The addition of a phosphate group (represented by "P") can either activate or inhibit the enzyme's function. The reverse reaction, the removal of the phosphate group to return the enzyme to its dephosphorylated state, is catalyzed by a **protein phosphatase**. This process involves the hydrolysis of the phosphate group, releasing **inorganic phosphate (Pi)** and water. This cycle of phosphorylation and dephosphorylation provides a rapid and dynamic way for cells to control metabolic pathways and respond to various signals.

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