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Ministère de l'Enseignement Supérieur
et de la Recherche Scientifique
Ecole Normale Supérieure
El cheikh Mohammed El Bachir El Ibrahimi, Kouba – Alger
Département des Sciences Naturelles

وزارة التعليم العالي والبحث العلمي
المدرسة العليا للأساتذة الشيخ محمد البشير الإبراهيمي، القبة، الجزائر
قسم العلوم الطبيعية

This course material is designed to support the academic needs of students pursuing advanced studies

Structural and Functional Biochemistry

Program: Master 1 Students

Prepared by: Dr. Lakache

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Content

General Introduction

Structural and functional biochemistry is a crucial branch of molecular biology that focuses on understanding how the structure of biomolecules determines their function in biological systems. Biomolecules such as proteins, carbohydrates, lipids, and nucleic acids are fundamental components of life, each playing specific and essential roles in cellular processes. By studying their molecular architecture and interactions, scientists can decipher the biochemical mechanisms that sustain life, regulate metabolism, and drive cellular communication.

Proteins are among the most versatile biomolecules, serving as enzymes, transporters, receptors, and structural components. Their function is directly linked to their three-dimensional structure, which is dictated by the sequence of amino acids and stabilized by various interactions, such as hydrogen bonds and disulfide bridges. Enzyme kinetics and mechanisms of catalysis are key topics in functional biochemistry, helping to understand how proteins facilitate biochemical reactions with remarkable specificity and efficiency.

Carbohydrates, composed of simple sugars (monosaccharides) and complex polysaccharides, are essential for energy storage, cellular communication, and structural integrity. Glycogen and starch act as energy reserves in animals and plants, respectively, while cellulose and chitin provide structural support. Beyond their metabolic functions, carbohydrates play critical roles in cell signaling and recognition, particularly in glycoproteins and glycolipids that mediate immune responses and intercellular communication.

Lipids, which include phospholipids, triglycerides, and steroids, are crucial for membrane structure, energy storage, and signaling. The amphipathic nature of membrane lipids, with hydrophilic heads and hydrophobic tails, enables the formation of the biological membranes that define cell boundaries and organelles. The fluid mosaic model describes the dynamic organization of membranes, where proteins and lipids interact to regulate transport, signaling, and cellular homeostasis. The role of sugars in membrane function further enhances communication and stability, influencing processes such as immune recognition and cell adhesion.

To study biomolecules, biochemical techniques such as chromatography are employed to analyze, separate, and identify molecular components. Chromatographic methods, including gas chromatography (GC), high-performance liquid chromatography (HPLC), and affinity chromatography, allow researchers to purify and characterize biomolecules with high precision, providing valuable insights into their structure and function. These analytical techniques are fundamental in research, medicine, and pharmaceutical applications.

Beyond primary biomolecules, secondary metabolites play a significant role in biological activity and ecological interactions. These compounds, including alkaloids, flavonoids, and terpenoids, are not directly involved in basic cellular metabolism but contribute to antimicrobial defense, signaling, and adaptation to environmental stress. Many secondary metabolites have pharmaceutical applications, serving as the basis for antibiotics, anticancer drugs, and other therapeutic agents.

Understanding the structure and function of biomolecules is essential for advances in medicine, biotechnology, and drug development. The integration of structural and functional biochemistry provides a comprehensive framework for unraveling the molecular mechanisms of life, enabling researchers to design targeted therapies, develop biomaterials, and improve industrial bioprocesses. This field continues to drive scientific innovation, offering solutions to global challenges in health, agriculture, and environmental sustainability.

Chapter 1: Structure and Functions of Biomolecules

1.Proteins

1.1.Introduction

Proteins are vital, highly abundant biological macromolecules found in all living cells. Composed of amino acids, often referred to as the "building blocks" of proteins, they are essential for numerous structural and functional roles within the body. The term "protein" originates from the Greek word proteios, meaning "of primary importance," and was introduced by Jöns Jakob Berzelius in 1838.

Proteins play a fundamental role in body-building functions, encompassing key structural and metabolic activities. They are primarily composed of carbon, hydrogen, oxygen, and nitrogen, with phosphorus and sulfur as minor components. As polymers of amino acids, proteins exhibit remarkable diversity; approximately 3,000 molecular species have been identified, collectively accounting for about 17% of the wet mass of a cell.

1.2.Amino Acids

12.1.Chemistry of Amino Acids

Amino acids are the basic structural units of proteins. They link together via peptide bonds to form larger protein molecules. The general structure of an amino acid is depicted in Figure 1. These molecules exhibit versatile chemical properties that contribute to the diverse functions of proteins (Figure 1).

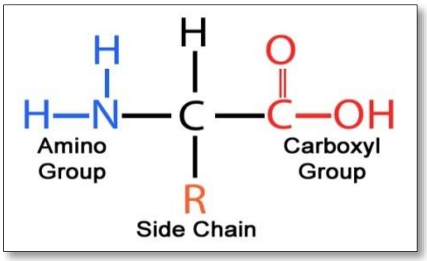


Figure 1.Structure of amino acids

Each α-amino acid is characterized by a central carbon atom, known as the α-carbon**,** which is bonded to four distinct groups: a side chain (R group), a hydrogen atom, an amino group (-NH_2), and a carboxylic acid group (-COOH). This unique arrangement gives rise to the term "amino acid."

1.2.2.Ionization and Amphoteric Nature

Amino acids are amphoteric, meaning they can act as both acids and bases. Their ionization state varies with pH, resulting in different ionic forms:

- **Acidic form:** Predominates at low pH when the amino group is protonated and the carboxylic acid group remains un-ionized.
- **Zwitterion form:** At physiological pH, the amino group is positively charged (-NH_3^+) while the carboxylic group is negatively charged (-COO^-).
- **Basic form:** At high pH, the carboxylic group is deprotonated, and the amino group loses its proton.

Each ionic form differs in structure, size, electrical charge, and solubility in water (Figures 2).

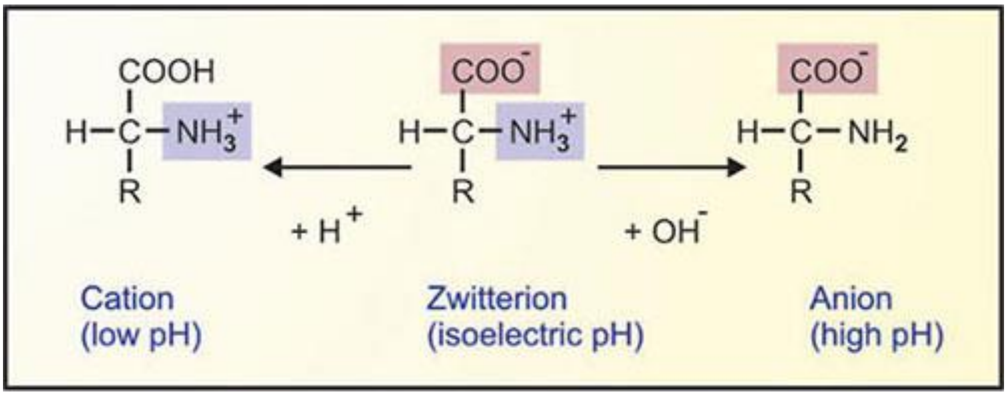


Figure 2. Ionization and amphoteric nature

1.2.3.Optical Isomerism of Amino Acids

Optical isomerism is a significant feature of amino acids, stemming from the asymmetry of the α-carbon atom when the side chain (R group) is not hydrogen. This makes the α-carbon a chiral center, allowing the molecule to exist in two mirror-image configurations:

- **Dextrorotatory (D):** Rotates plane-polarized light to the right.
- **Levorotatory (L):** Rotates plane-polarized light to the left.

Proteins exclusively incorporate L-amino acids, which are predominantly derived from mammalian sources. In contrast, D-amino acids are rare in nature but are found in certain microbial peptide antibiotics (Figure 3).

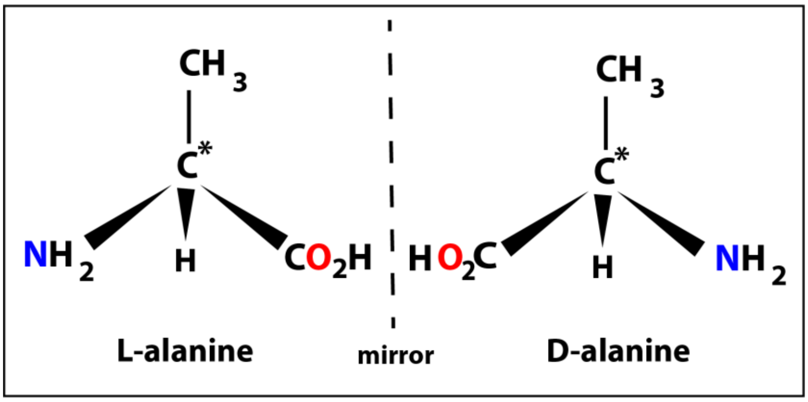


Figure 3. **Optical Isomerism of amino acids**

1.2.4.Classification of amino acids

A total of 20 amino acids are commonly found in proteins. They can be classified based on the polarity of their side chains (R groups) or their nutritional importance (Table 1).

1.2.4.1. Classification by Polarity of Side Chains

- **Nonpolar (hydrophobic):**

  Amino acids with nonpolar side chains, which tend to avoid water. Examples: Glycine, Alanine, Valine, Leucine, Isoleucine, Methionine, Proline, Phenylalanine, Tryptophan.

- **Polar, uncharged:**

  Amino acids with side chains that can form hydrogen bonds but lack a charge. Examples: Serine, Threonine, Cysteine, Tyrosine, Asparagine, Glutamine.

- **Acidic (negatively charged):**

  Amino acids with carboxylate groups in their side chains. Examples: Aspartic acid (Asp), Glutamic acid (Glu).

- **Basic (positively charged):**

  Amino acids with amino groups in their side chains. Examples: Arginine (Arg), Histidine (His), Lysine (Lys).

1.2.4.2. Classification by Nutritional Aspects

- **Essential amino acids:**

  These cannot be synthesized by the body and must be obtained from the diet.

  Examples:

  - Histidine (His), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Threonine (Thr), Tryptophan (Trp), Valine (Val).

- **Non-essential amino acids:**

  These can be synthesized by the body. Examples:

  - Alanine (Ala), Arginine (Arg), Asparagine (Asn), Aspartic acid (Asp), Cysteine (Cys), Glutamic acid (Glu), Glutamine (Gln), Glycine (Gly), Proline (Pro), Tyrosine (Tyr), Serine (Ser).

- **Conditionally essential amino acids:**

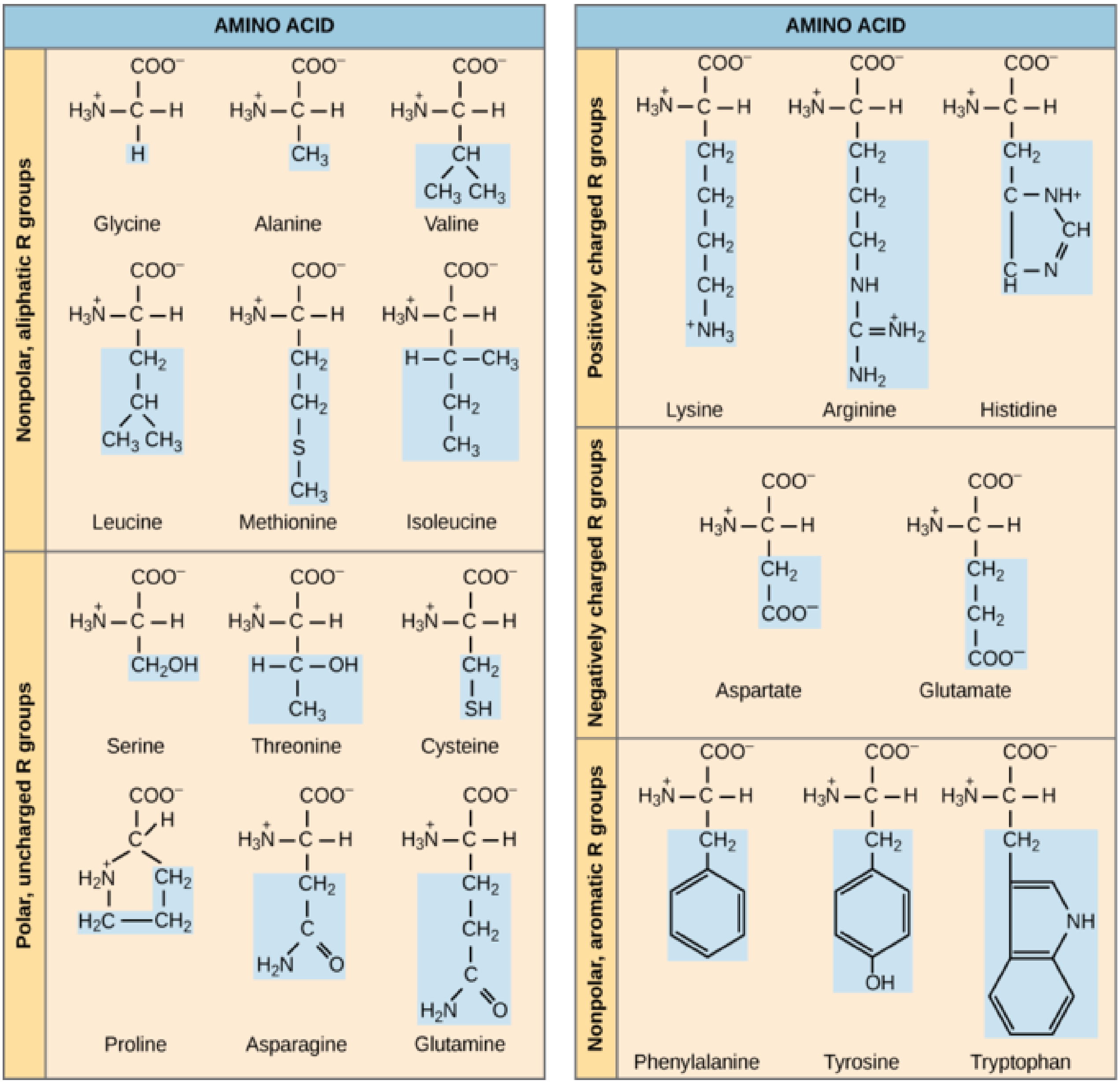
  These are usually non-essential but become essential under certain conditions, such as illness or stress. Examples:

  - Arginine (Arg), Cysteine (Cys), Glutamine (Gln), Glycine (Gly), Proline (Pro), Tyrosine (Tyr).

1.2.4.3. Classification by Functional Groups

Amino acids can be classified based on the functional groups present in their side chains, which influence their chemical properties and biological roles. **Monoaminodicarboxylic acids**, also known as acidic amino acids, contain an additional carboxyl group in their side chain, making them negatively charged at physiological pH. Examples include aspartic acid (Asp) and glutamic acid (Glu)**,** both of which play key roles in protein structure, metabolism, and neurotransmission. In contrast, **diaminomonocarboxylic acids**, or basic amino acids, possess an extra amino group in their side chain, giving them a positive charge at physiological pH. This group includes arginine (Arg), histidine (His), and lysine (Lys), which are essential for protein stability, enzymatic activity, and cellular signaling. This classification highlights the chemical versatility and functional significance of amino acids, which are fundamental to protein structure and biological processes (Table 1).

Table 1. Classification by functional groups



1.3.Peptides

Peptides are formed when two amino acids are covalently linked by an amide bond, also called a peptide bond or peptide link. This bond forms between the carbonyl group (C=O) of one amino acid and the amino group (NH_2) of the next, with the elimination of one water molecule (H_2O), as shown in Figure 4. The amino acids that are incorporated into peptides are known as amino acid residues.

Depending on the number of amino acids involved, peptides can be classified as:

- **Dipeptide:** 2 amino acid residues.
- **Tripeptide:** 3 amino acid residues.
- **Tetrapeptide:** 4 amino acid residues.
- **Oligopeptide:** 6-10 amino acid residues.
- **Polypeptide:** A chain of more than 10 amino acid residues.

When the number of amino acid residues is large enough, it forms proteins.

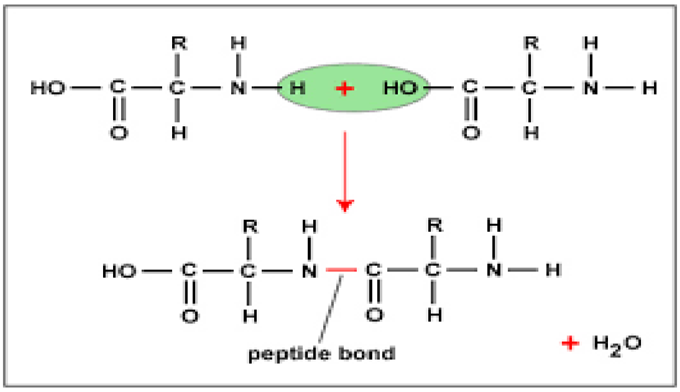


Figure 4. Peptide structure

a. N-Terminal and C-Terminal Residues

In a peptide chain, the N-terminal residue is the amino acid located at one end, characterized by a free amino group (-NH_2). Conversely, the C-terminal residue is the amino acid at the opposite end, possessing a free carboxyl group (-COOH). These terminal residues define the orientation of the peptide and play a crucial role in protein synthesis, stability, and function. Peptide sequences are conventionally named based on their amino acid residues, with numbering typically starting from the N-terminal end, following the standard convention in biochemical nomenclature.

b. Side Chains (R1 and R2)

The R1 and R2 groups represent the side chains of the two different amino acids that are linked by the peptide bond. These side chains contribute to the unique chemical properties and function of the peptide or protein.

2.Protein Structure

Proteins are organized into four levels of structural complexity, each level building upon the previous one. These structural levels provide insight into the functional properties of proteins.

2.1. Primary Structure

The primary structure of a protein refers to the unique sequence of amino acids in its peptide chain. This sequence determines the identity, composition, and concentration of each amino acid in the protein. The primary structure is primarily formed by covalent peptide bonds that link the α-carboxyl carbon of one amino acid to the α-nitrogen of the next amino acid, as shown in Figure 5. The primary structure is fundamental because any change in the sequence can result in a different protein or function.

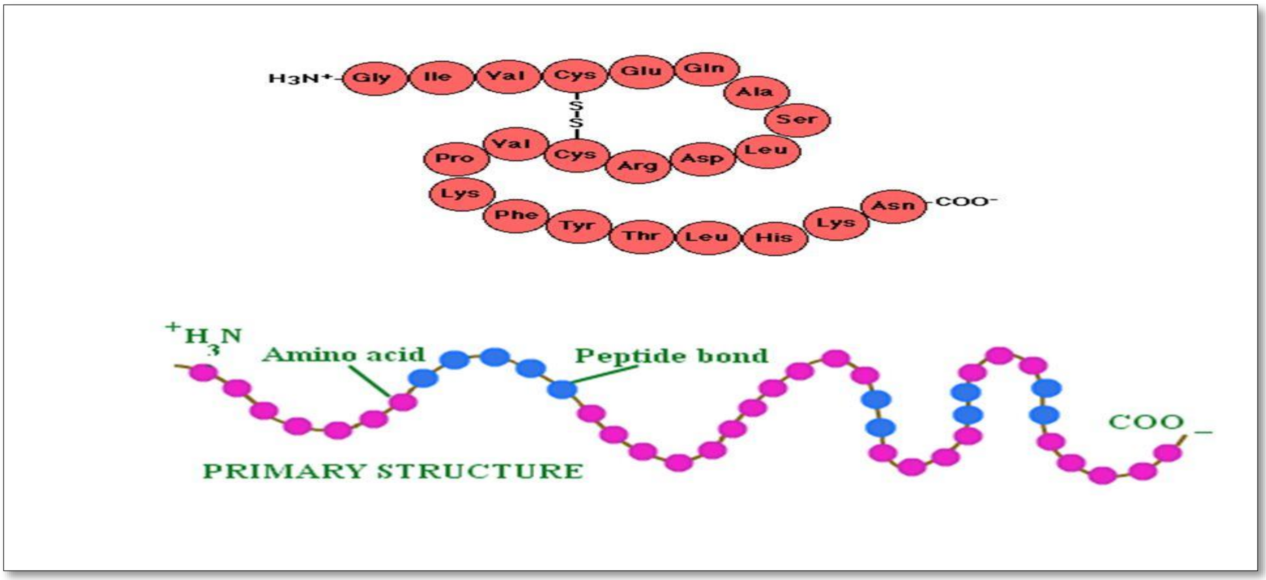


Figure 5. Primary structure of protein

2.2. Secondary Structure

The secondary structure of a protein refers to the three-dimensional conformation that forms through local interactions between amino acid residues, particularly through hydrogen bonding (Figure 6). These interactions stabilize the protein's folded shape. There are three main types of secondary structures:

- **α-helix:** A right-handed, coiled structure (Figure 5a) that is compact and rigid. Each turn of the helix has a pitch of 540 pm and contains approximately 3.6 amino acid residues. The α-helix is stabilized by hydrogen bonds between the hydrogen atom attached to the nitrogen of one peptide bond and the carbonyl oxygen of the fourth amino acid in the chain. The amount of α-helix content varies between different proteins.
- **β-pleated sheet:** A fully extended, slightly zig-zag sheet-like structure (Figure 5b). It is stabilized by regular hydrogen bonds between the amide nitrogen (N) of one peptide strand and the carbonyl oxygen (O) of another peptide strand on an adjacent chain.
- **Triple helix:** This structure involves three coiled polypeptide chains that wind around each other, forming a stiff, cable-like structure (Figure 5c). Also known as a superhelical structure, the triple helix is particularly strong and rigid. Examples include collagen-like proteins and triplex DNA or RNA.

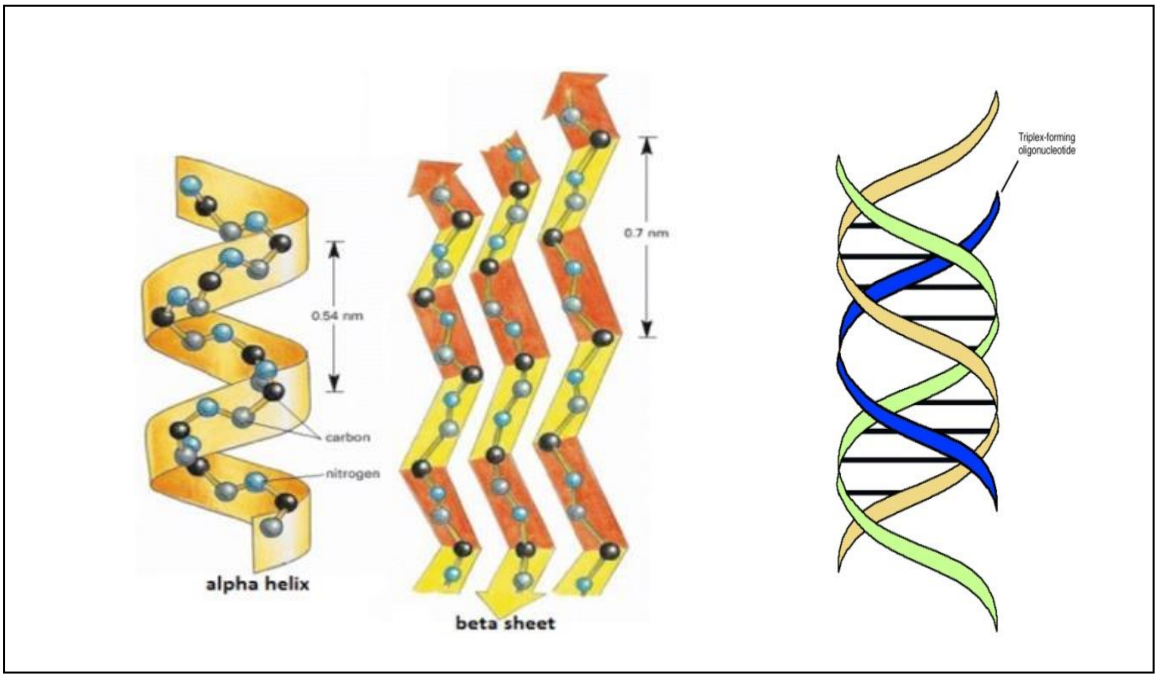


Figure 6. Secondary Structure of protein

2.3.Tertiary Structure of Proteins

The tertiary structure of a protein refers to its final three-dimensional shape, which is critical for the protein's functionality. This structure is formed by the folding of the polypeptide chain into a compact, three-dimensional form (Figure 6). The tertiary structure dictates the protein's biological activity, such as enzyme catalysis, binding, or transport, and allows it to interact with other molecules (Figure 7).

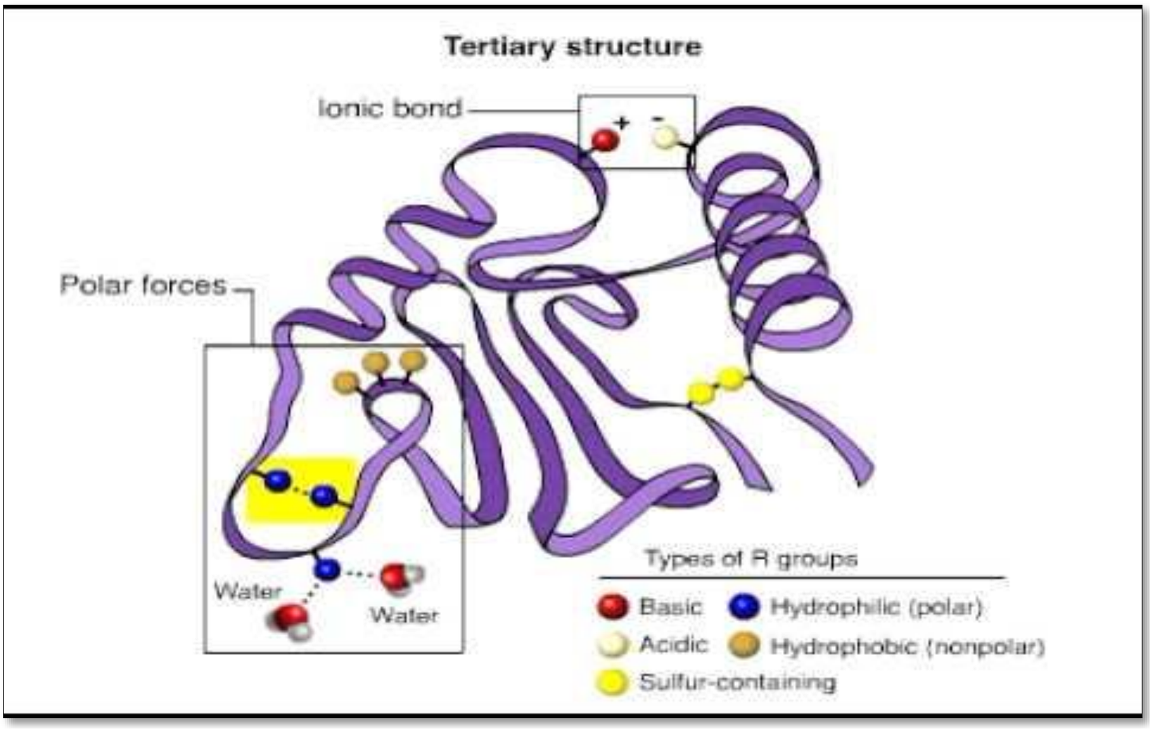


Figure 7. **Tertiary structure of proteins**

2.3.1. Forces Stabilizing the Tertiary Structure

The stability of the tertiary structure is maintained by several types of intermolecular forces between amino acid residues in different parts of the polypeptide chain:

a. Disulfide Bonds:

Covalent disulfide bonds form between the sulfur atoms of two cysteine residues. These bonds are particularly important for stabilizing the protein's structure in the extracellular environment, where the protein is exposed to harsh conditions.

b. Electrostatic Interactions (Ionic Bonds):

Electrostatic interactions occur between ionic groups of opposite charge, such as the amino group ($-NH_3^+$) of a basic amino acid and the carboxyl group ($-COO^-$) of an acidic amino acid. These interactions help to stabilize the folded protein.

c. Hydrogen Bonds:

Hydrogen bonds form between the hydrogen atom of one amino acid's side chain and an electronegative atom (such as oxygen or nitrogen) in another amino acid's side chain. These bonds contribute significantly to the overall stability of the protein structure.

d.Hydrophobic Interactions:

Nonpolar side chains tend to cluster in the interior of the protein, away from the aqueous environment. This hydrophobic effect helps drive the folding of the protein, contributing to its stability.

e. Van der Waals Interactions:

These weak interactions occur between atoms that are in close proximity to each other, and they help to further stabilize the folded protein.

2.4.Quaternary Structure of Proteins

The quaternary structure refers to the spatial arrangement of multiple polypeptide chains, or subunits, that come together to form a functional protein. These subunits are usually held together by non-covalent interactions and occasionally by covalent bonds, giving the protein its final, functional shape (Figure 8).

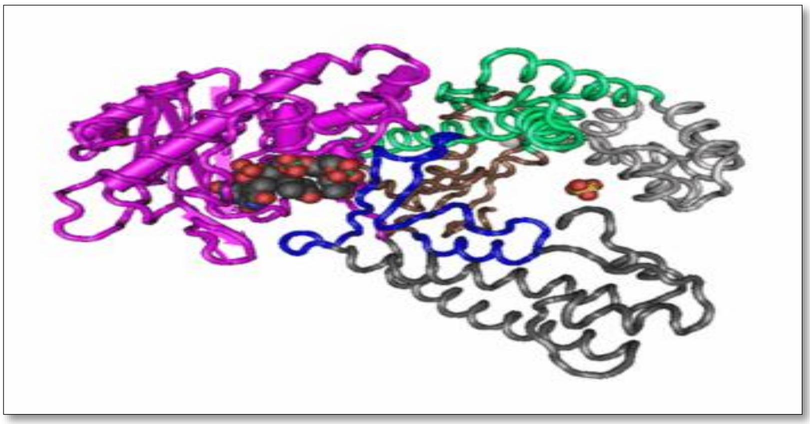


Figure 8. Quaternary structure of a protein

2.4.1. Subunits and Oligomers:

Many proteins are composed of multiple polypeptide chains, each of which is referred to as a subunit. These subunits can function independently or interact to form a larger protein complex. When a protein consists of more than one subunit, it is classified as an oligomer. Oligomeric proteins can contain two or more subunits, which may be identical or different, and their assembly often enhances structural stability and functional efficiency. These protein complexes play crucial roles in various biological processes, including enzymatic activity, signal transduction, and structural support.

2.4.2. Forces Stabilizing Quaternary Structure:

The subunits in quaternary structure are primarily held together by:

a. Hydrogen Bonds:

Hydrogen bonds form between polar amino acid side chains on the surface of the subunits, helping to stabilize the overall structure.

b. Ionic Bonds:

Electrostatic interactions between oppositely charged side chains of polar amino acids also contribute to the stability of the quaternary structure.

c. Hydrophobic Interactions:

Nonpolar amino acid side chains interact with each other in the interior of the protein, avoiding the aqueous environment, further stabilizing the structure.

2.4.3. Functional Importance of Quaternary Structure:

The quaternary structure plays a critical role in catalytic activity and receptor activation:

- **Enzyme Catalysis:** Enzymes, for instance, often require multiple subunits to bring about the catalytic activity necessary for biochemical reactions. The arrangement of subunits in the quaternary structure is vital for their function.
- **Hormone Receptor Regulation:** In hormones and their receptors, the quaternary structure facilitates the interaction between subunits, which is crucial for receptor activation and signal transduction.

2.5. Properties of Proteins

Proteins are macromolecules with high molecular weight and exhibit various unique properties that are essential for their biological functions. These properties are largely determined by their structure, composition, and interactions with their environment. Below are some key properties of proteins:

a. Amphoteric Nature

Proteins are amphoteric, meaning they can act as either an acid or a base depending on the pH of their environment. This behavior is due to the presence of both amino (—NH_2) and carboxyl (—COOH) groups in the amino acids that form proteins. At different pH levels, these groups ionize, allowing proteins to function in a range of biological processes.

- The isoelectric point (pI) of a protein is the pH at which the protein has no net charge. It depends on the relative numbers and ionization of the amino acid residues' ionic side chains and their pK values (the pH at which half of the ionizable groups are ionized).

b. Solubility and Relationship with Water

The solubility of proteins is determined by the distribution of polar and non-polar groups along the protein chain and how they interact with the surrounding environment. Proteins have a complex relationship with water, influenced by their structure and the nature of the solvent.

In polar solvents, such as water, glycerol, or formic acid, proteins are generally soluble because these solvents stabilize the hydrophilic (water-attracting) side chains, allowing the protein to maintain its structural integrity. This interaction is essential for proteins functioning in aqueous environments, such as enzymes and transport proteins in biological fluids.

In contrast, proteins are insoluble in non-polar solvents like ethanol, as these solvents lack the ability to hydrate the hydrophilic regions of the protein. Instead, the proteins tend to aggregate or precipitate, as the exposure of hydrophobic (water-repelling) residues leads to instability.

Several factors influence protein solubility, including salt concentration, which can enhance or reduce solubility through salting-in or salting-out effects; pH, which affects protein charge and interactions; molecular size, where larger proteins may be less soluble due to increased aggregation tendencies; and protein hydration, as water binding plays a key role in maintaining solubility. These factors determine whether a protein remains in solution or precipitates, impacting its biological function and industrial applications.

c. Denaturation

Proteins can undergo denaturation, a process in which they lose their native, functional three-dimensional structure without breaking the peptide bonds between amino acids. This structural disruption leads to the loss of biological activity and can be irreversible or reversible depending on the conditions. Denaturation can be triggered by various factors, including heat, which disrupts hydrogen bonds and hydrophobic interactions; X-rays and UV rays, which can cause molecular damage and alter protein conformation; and chemical agents such as urea, which interfere with hydrogen bonding and destabilize the protein's structure. Understanding protein denaturation is crucial in fields such as biochemistry, food science, and medicine, as it influences enzymatic activity, protein stability, and therapeutic applications.These agents lead to the unfolding of the polypeptide chain, causing the protein to lose its biological function. However, denaturation does not involve the hydrolysis of peptide bonds, meaning the primary structure (the sequence of amino acids) remains intact, but the protein loses its specific shape necessary for its activity.

2.6.Classification of Proteins

Proteins can be classified in a variety of ways based on their structure, function, and composition. Below are the main classification categories:

2.6.1. Classification Based on Shape

Proteins can be classified based on their shape into globular and fibrous proteins, each with distinct structural and functional characteristics.

Globular proteins are compact and spherical in form, typically soluble in water due to their flexible and dynamic structure. They play diverse functional roles in biological systems, including enzymatic activity, molecular transport, immune defense, and hormonal regulation. Examples of globular proteins include enzymes such as amylase and DNA polymerase, hormones like insulin, antibodies such as immunoglobulins, and transport proteins like hemoglobin and albumin, which facilitate oxygen and nutrient distribution in the body.

In contrast, fibrous proteins have an elongated, thread-like structure and are primarily insoluble or only slightly soluble in water. They serve as structural components, providing mechanical strength and support to tissues. Fibrous proteins are typically rigid and contribute to the integrity of biological structures. Examples include collagen, which reinforces the structure of skin, bones, and connective tissue; elastin, which grants elasticity to tissues like blood vessels and lungs; keratin, a key component of hair, nails, and skin; and fibrin, which plays a crucial role in blood clotting.

2.6.2. Classification Based on Biological Function

Proteins play diverse roles in biological systems and can be classified based on their function into several categories.

Enzymes are proteins that act as biocatalysts, accelerating biochemical reactions with high specificity for their substrates. They are essential for metabolic processes, including energy production and cellular signaling. Common examples include dehydrogenases, which are involved in oxidation-reduction reactions, and kinases, which transfer phosphate groups in phosphorylation reactions.

Storage proteins function in the storage of essential molecules such as ions, minerals, and oxygen. For example, ferritin stores iron, preventing toxicity while ensuring its availability, and myoglobin facilitates oxygen storage in muscle tissues for efficient energy production.

Structural proteins provide mechanical support and integrity to cells and tissues. They are critical in maintaining the strength and stability of biological structures. Examples include collagen, which is a key component of connective tissues, and proteoglycans, which contribute to the extracellular matrix and joint lubrication.

Regulatory proteins are involved in gene expression, cell signaling, and homeostasis. They help control biological processes by modulating transcription, translation, and hormone signaling. Examples include DNA-binding proteins, which regulate gene expression, and peptide hormones like insulin, which controls glucose metabolism.

Protective proteins play a crucial role in the immune response and defense mechanisms. These proteins help combat infections, facilitate blood clotting, and protect against harmful agents. Examples include immunoglobulins (antibodies), which recognize and neutralize pathogens, and blood clotting factors, which prevent excessive bleeding after injury.

Transport proteins facilitate the movement of molecules such as gases, ions, and lipids across biological membranes or through the bloodstream. Hemoglobin, for example, transports oxygen in the blood, while plasma lipoproteins help carry lipids such as cholesterol and triglycerides throughout the body.

Contractile or motile proteins are involved in movement and cellular dynamics, playing essential roles in muscle contraction and intracellular transport. Actin and tubulin are key examples; actin is a major component of muscle fibers, while tubulin forms microtubules that assist in intracellular transport and cell division.

2.6.3. Classification Based on Composition

Proteins can be classified based on their composition into three major categories: simple proteins, conjugated proteins, and derived proteins, each with distinct structural and functional characteristics.

Simple proteins are composed exclusively of amino acids and do not contain any additional non-protein components. These proteins play essential roles in biological processes such as transport, enzymatic activity, and immune defense. Examples include albumins, which are water-soluble proteins involved in maintaining osmotic pressure in blood, and globulins, which are important for immune function and molecular transport.

Conjugated proteins consist of a simple protein covalently or non-covalently bound to a non-protein component, which can be either organic or inorganic. These additional groups contribute to the protein's function and stability. Depending on the type of non-protein component, conjugated proteins can be classified into various subtypes: glycoproteins, which contain carbohydrates and are involved in cell recognition and signaling; lipoproteins, which are associated with lipids and play a key role in lipid transport; and metalloproteins, which contain metal ions such as zinc or copper and are often involved in enzymatic reactions and electron transport.

Derived proteins are modified or degraded forms of natural proteins, resulting from physical, chemical, or enzymatic processes. These proteins may retain partial biological activity or have completely altered properties. Examples include peptides, which are short chains of amino acids with various regulatory functions, and denatured proteins, which have lost their native structure due to environmental changes such as heat, pH shifts, or chemical exposure.

2.7. Functions of Proteins

Proteins are vital to nearly all biological processes and contribute significantly to the structure and function of cells. They account for about 17% of the wet cell mass, and their roles span across a wide variety of essential functions in the body. Below are the key functions of proteins:

2.7.1. Energy Source

Although proteins are not the primary energy source like carbohydrates and fats, they can provide energy when needed. Proteins contribute to the body's energy balance, particularly in situations of starvation or extreme physical stress.

2.7.2. Structural Components

Proteins are key structural elements of cells and tissues. They form the bulk of muscle mass and other body tissues, providing both mechanical support and shape. The most well-known structural proteins include:

- **Collagen:** Provides tensile strength and is a major component of connective tissues such as tendons, ligaments, and skin.
- **Elastin:** Provides elasticity to tissues such as the lungs, arteries, and the uterus.
- **Keratin:** Found in hair, nails, and skin, providing structural integrity and protection.

2.7.3. Catalytic Activity (Enzymes)

One of the most important and diverse roles of proteins is as enzymes. Enzymes are biological catalysts that speed up chemical reactions without being consumed in the process. Almost all biochemical reactions in living organisms, from digestion to DNA replication, are catalyzed by enzymes. Each enzyme is highly specific to the reaction it catalyzes, ensuring efficient metabolic control.

2.7.4. Transport Proteins

Proteins also function to transport various substances across cell membranes or through the bloodstream:

- **Transport proteins** are responsible for moving metal ions, organic molecules, and gases such as oxygen across membranes.
    - **Hemoglobin** is an example that transports oxygen in the blood.
    - **Membrane transport proteins** move nutrients (like glucose and amino acids) across cell membranes. These proteins ensure the uptake of essential molecules into cells and their release when needed.

2.7.5. Motor Proteins

Proteins such as actin and myosin are involved in muscle contraction and cellular movement, enabling locomotion and mobility at the cellular and organismal levels. These motor proteins are essential for processes such as muscle contraction, intracellular transport, and the movement of cells.

2.7.6. Immune Function

Proteins like immunoglobulins (antibodies) play a crucial role in the immune system. Produced by lymphocytes, antibodies recognize and neutralize foreign invaders like bacteria, viruses, and other pathogens. They are a key part of the body's defense mechanisms.

2.7.7. Storage Proteins

Certain proteins act as storage proteins, binding and storing essential molecules for later use. For example:

- **Ferritin** stores iron in the liver and spleen, releasing it when the body needs it for processes such as red blood cell production.

2.7.8. Hormonal Regulation

Many proteins act as hormones, regulating various physiological functions within the body. Hormones such as insulin (which regulates blood sugar levels) are essential for maintaining metabolic processes. Protein and peptide hormones help regulate growth, metabolism, and reproductive processes.

2.7.9. Fluid and Ionic Balance

Proteins also help maintain the balance of fluids and ions across cell membranes. This is essential for processes such as nerve signaling and muscle contraction, as well as maintaining osmotic pressure in cells.

2. Carbohydrates

2.1.Introduction

Carbohydrates are vital biomolecules necessary for the survival and function of all living organisms, including humans, plants, and microbes. They are an essential component of our diet, found in a wide variety of foods such as fruits, grains, vegetables (e.g., potatoes), milk, honey, and table sugar. These biomolecules are central to the energy metabolism and structural framework of life.

Among the four major macromolecules—proteins, fats, carbohydrates, and nucleic acids—carbohydrates were the last to capture the scientific community's attention for detailed study and exploration.

2.2. Definition

Carbohydrates are defined as biomolecules containing naturally occurring carbonyl groups (either aldehydes or ketones) and multiple hydroxyl groups. Their basic chemical composition includes carbon (C), hydrogen (H), and oxygen (O), typically in a hydrogen-to-oxygen ratio of 2:1, resembling the ratio found in water.The general empirical formula is

$(CH_2O)_n(CH\_{_2}O)\_{_n}(CH_2O)_n$.

2.3.Classification of Carbohydrates

Carbohydrates are classified based on their complexity, the number of sugar units, and functional groups (Figure 9). They fall into three main categories:

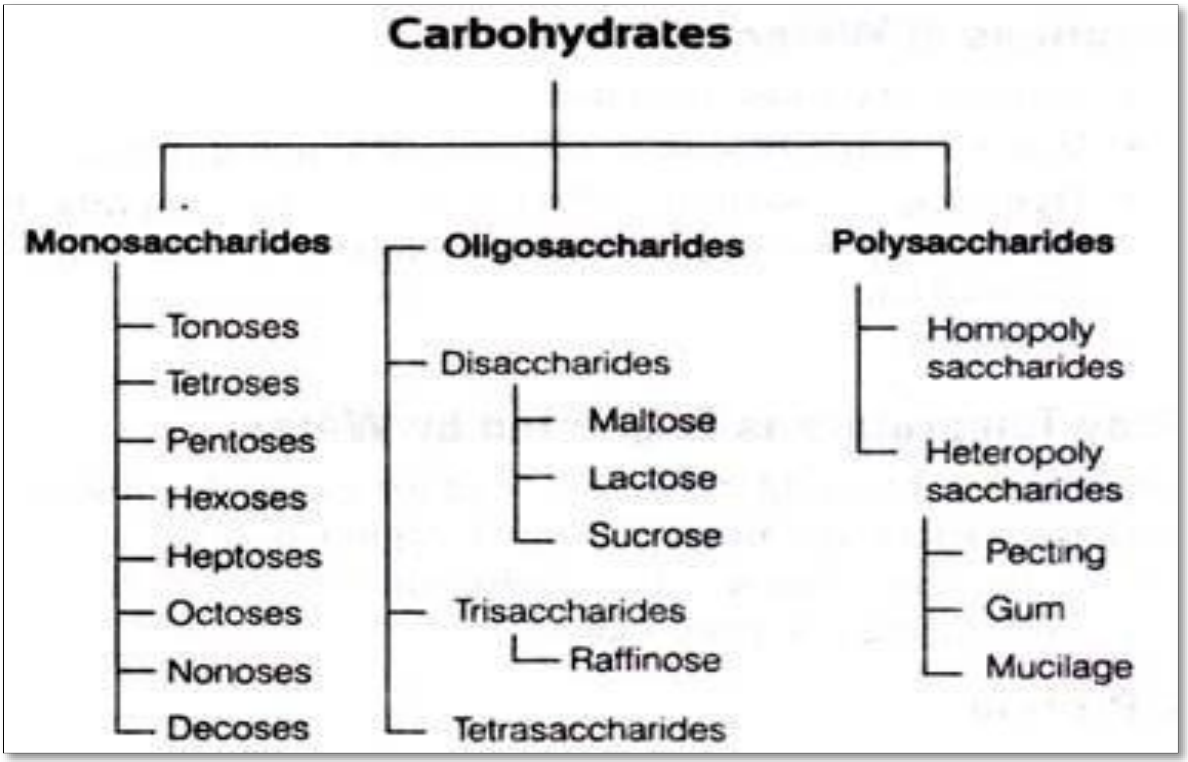


Figure 9. Classification of carbohydrates

2.3.1. Monosaccharides

Monosaccharides are the most basic form of carbohydrates. These simple sugars cannot be broken down into smaller carbohydrate molecules via hydrolysis. They serve as the building blocks for more complex carbohydrates, such as disaccharides and polysaccharides.

a. General Characteristics

Carbohydrates are essential biomolecules that serve as a primary source of energy and structural components in living organisms. The simplest carbohydrates, known as monosaccharides, contain only one sugar unit and cannot be hydrolyzed into simpler forms.

Their general chemical formula is typically represented as $C_n(H_2O)_nC\_n(H\_2O)\_nC_n(H_2O)_n$ or $C_nH_{2n}O_nC\_nH\_\{2n\}O\_nC_nH_{2n}O_n$, indicating that they are composed of carbon, hydrogen, and oxygen in a 1:2:1 ratio.

In terms of physical properties, carbohydrates are generally colorless and crystalline solids, which contribute to their ability to form stable structures. They are highly soluble in water due to the presence of hydroxyl (-OH) groups that facilitate hydrogen bonding. However, they are insoluble in nonpolar solvents, as their polar nature prevents interactions with hydrophobic molecules.

Common examples of simple carbohydrates include glucose, a primary energy source for cells; fructose, a naturally occurring sugar found in fruits; ribose, a crucial component of nucleotides like RNA; and erythrose, an important intermediate in metabolic pathways. These fundamental properties make carbohydrates indispensable in various biological processes.

b. Functional Groups

Monosaccharides are classified based on the type of functional group they contain, which determines their chemical behavior and reactivity.

- Aldoses are monosaccharides that contain an aldehyde group (-CHO) at the end of their carbon chain. This functional group makes them reactive in oxidation-reduction reactions and contributes to their role in energy metabolism. A common example of an aldose is glucose, which is a primary energy source for cells.
- Ketoses contain a ketone group (-CO) within the carbon chain, typically at the second carbon atom. This structure influences their reactivity and metabolism. Fructose, found in fruits and honey, is a well-known example of a ketose and is widely used as a natural sweetener.

The presence of either an aldehyde or ketone group significantly affects the chemical properties and biological functions of monosaccharides, playing a crucial role in metabolic pathways and cellular energy production.

Monosaccharides are classified based on the **number of carbon atoms** in their structure, which determines their size and biological function. The naming convention follows a numerical prefix combined with the suffix "-ose."

1. **Trioses (3 carbons)** : The simplest monosaccharides, essential intermediates in metabolic pathways.

   - Examples: Glyceraldehyde (an aldose) and Dihydroxyacetone (a ketose).
2. **Tetroses (4 carbons)**: Play roles in biosynthetic processes and metabolic cycles.
   - Example: Erythrose, involved in the pentose phosphate pathway.
3. **Pentoses (5 carbons)**: Crucial components of nucleotides and coenzymes.
   - Examples: Ribose (a key sugar in RNA and ATP) and Xylose (found in plant cell walls).
4. **Hexoses (6 carbons)**: The most common monosaccharides, serving as primary energy sources.
   - Examples: Glucose (a fundamental energy molecule), Galactose (a component of lactose), and Fructose (a natural sugar in fruits).
5. **Heptoses (7 carbons)**: Less common but important in carbohydrate metabolism.
   - Example: Sedoheptulose, involved in the pentose phosphate pathway.

d. Structure and Isomerism

Monosaccharides exhibit structural diversity due to their ability to exist in **different forms and isomeric variations**, which influence their chemical behavior and biological functions.

- **Open-Chain and Cyclic Forms:** Monosaccharides, such as glucose, can exist in both linear (open-chain) and cyclic structures. In aqueous solutions, most monosaccharides prefer the cyclic form, where the carbonyl group reacts with a hydroxyl group to form a stable ring structure. This conversion is common in hexoses and pentoses, leading to the formation of furanose (5-membered) or pyranose (6-membered) rings.
- **Optical Isomerism:** Due to the presence of asymmetric (chiral) carbon atoms, monosaccharides can exist as D- and L- isomers. These forms are mirror images of each other and are classified based on the orientation of the hydroxyl (-OH) group on the chiral carbon farthest from the carbonyl group. In biological systems, D-isomers are the most commonly utilized form.
- **Structural Variations:** Some monosaccharides share the same molecular formula (e.g., $C_6H_{12}O_6$ for glucose, galactose, and fructose) but differ in their structural arrangement. These differences arise due to variations in the position of functional groups around asymmetric carbons, leading to distinct biological properties. For example, glucose and galactose differ only in the configuration of the hydroxyl group on carbon 4, whereas

glucose and fructose differ in their functional groups, with glucose being an aldose and fructose a ketose.

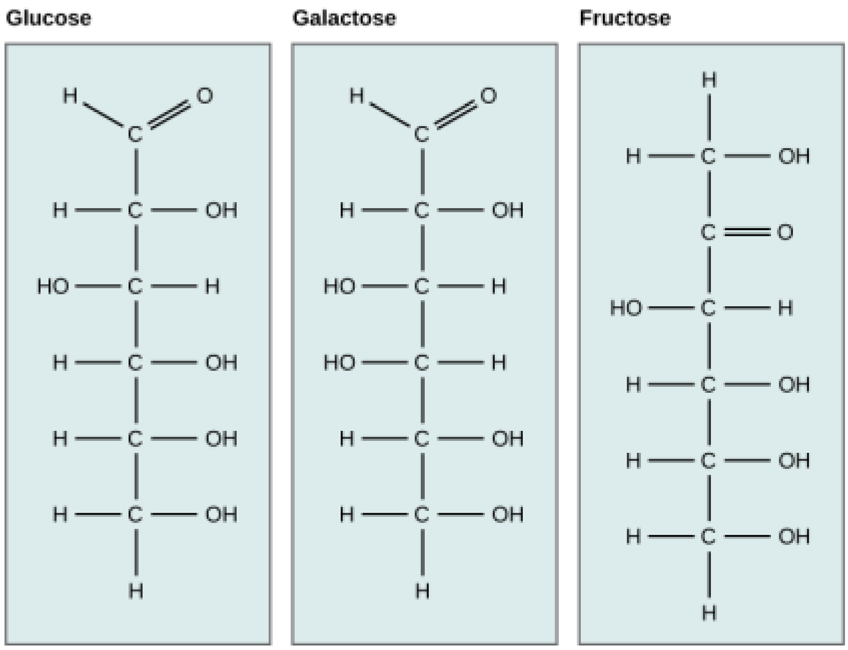


Figure 10. A structural representation of glucose, fructose, and galactose

2.3.1.1. Structure of monosaccharides

Monosaccharides can exist in two structural forms: linear chains or ring-shaped molecules. When monosaccharides like glucose form a ring structure, the hydroxyl group (-OH) attached to carbon-1 (the anomeric carbon) can adopt two different orientations, leading to the formation of two isomers: alpha (α) and beta (β). In the alpha configuration, the hydroxyl group is positioned below the plane of the ring, while in the beta configuration, it is positioned above the plane. This difference in the position of the hydroxyl group around the anomeric carbon is crucial for the properties and function of the sugar. The interconversion between these two forms occurs in solution and is known as mutarotation.

Cyclic structure of glucose

- In the aqueous environment, glucose forms a hemiacetal by intramolecular reaction between its aldehyde group (−CHO-CHO−CHO) and a hydroxyl group (−OH-OH−OH) on carbon 5.
- This creates a six-membered ring structure known as a pyranose ring.

Anomeric carbon and isomerism

- The carbon atom (C-1) involved in ring formation becomes asymmetric and is referred to as the anomeric carbon.
- The orientation of the hydroxyl group (−OH-OH−OH) attached to this carbon determines the type of anomer:
    - **Alpha (α) Anomer**: The −OH-OH−OH group is below the plane of the ring.
    - **Beta (β) Anomer**: The −OH-OH−OH group is above the plane of the ring.

2.3.1.2. Functions of monosaccharides

Monosaccharides play essential roles in various biological functions. Glucose ($C_6H_{12}O_6$) is a primary source of energy for both humans and plants. Plants produce glucose through photosynthesis, using carbon dioxide and water, and store excess glucose as starch, which is later consumed by humans and herbivores. Galactose, found in milk sugar (lactose), and fructose, present in fruits and honey, contribute to the sweetness of these foods. Ribose is a crucial component of nucleic acids (RNA and DNA) and certain coenzymes, vital for cellular processes. Additionally, mannose is important in the formation of mucoproteins and glycoproteins, which are necessary for the proper functioning of the body, particularly in cellular communication and immune responses.

2.3.2. Disaccharides

Disaccharides are carbohydrates composed of two sugar units linked by a covalent bond called a glycosidic bond. This bond forms during a dehydration reaction, also known as a condensation reaction or dehydration synthesis, in which the hydroxyl group of one monosaccharide reacts with the hydrogen atom of another, releasing a molecule of water. Glycosidic bonds can be classified as either alpha or beta, depending on the position of the hydroxyl group on the carbon-1 of the first monosaccharide. An alpha bond occurs when the hydroxyl group is below the plane of the sugar ring, while a beta bond forms when the hydroxyl group is above the plane.

Common examples of disaccharides include sucrose, lactose, and maltose. Sucrose, the most abundant disaccharide, consists of one D-glucose molecule and one D-fructose molecule linked by an alpha-1→2 glycosidic bond. Its systematic name is O-α-D-glucopyranosyl-(1→2)-D-fructofuranoside. Lactose, found in mammalian milk, is made up of one D-galactose and one D-glucose molecule connected by a beta-1→4 glycosidic bond, with the systematic name O-β-D-galactopyranosyl-(1→4)-D-glucopyranose. Maltose, another disaccharide, consists of two glucose molecules linked by an alpha-1→4 bond.

Disaccharides are further classified based on their ability to undergo oxidation-reduction reactions. Reducing disaccharides, such as maltose and cellobiose, have a free hemiacetal group capable of acting as a reducing agent. Non-reducing disaccharides, like sucrose and trehalose, lack a free hemiacetal group because their anomeric carbons are involved in an acetal linkage. Additional examples of disaccharides include lactulose, chitobiose, kojibiose, nigerose, isomaltose, sophorose, laminaribiose, gentiobiose, turanose, maltulose, trehalose, palatinose, melibiose, and rutinose, showcasing the diversity within this group of carbohydrates.

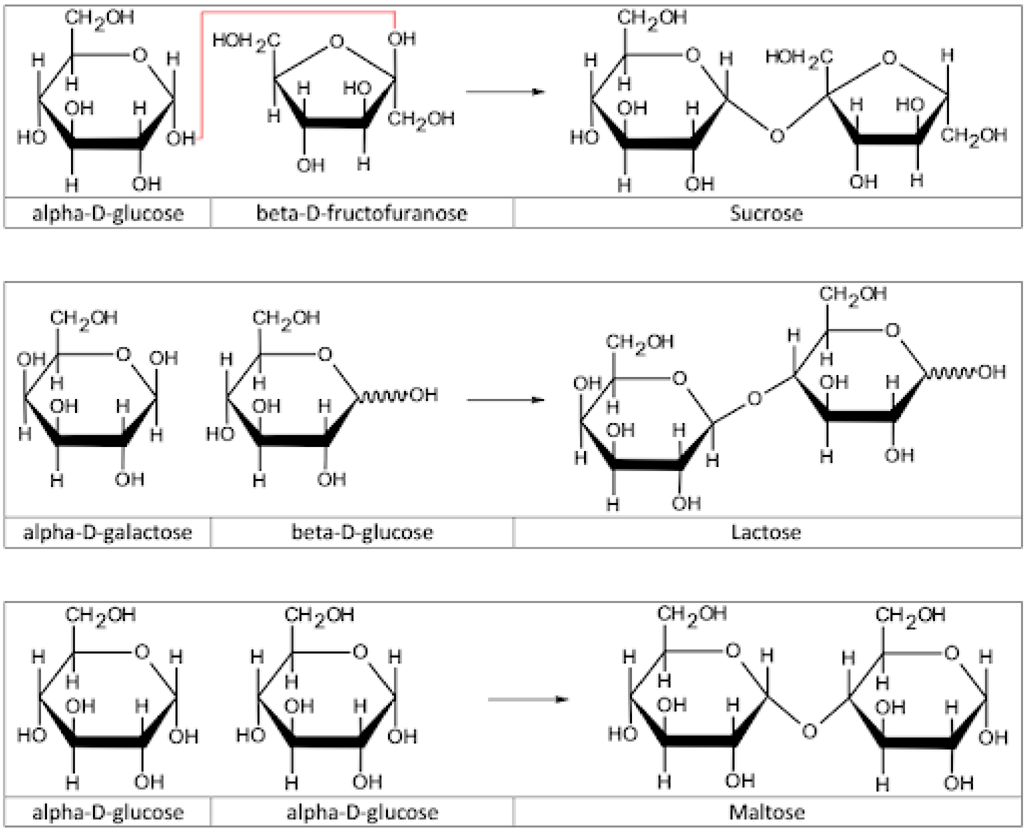


Figure 11. A structural representation of lactose and maltose.

2.3.2.1. Functions of Disaccharides

Disaccharides also play vital roles in various biological functions:

- **Sucrose** is produced during photosynthesis and serves as a major source of carbon and energy in plants. It is commonly found in table sugar.
- **Lactose**, the sugar found in milk, provides a major source of energy for newborn mammals, including humans, as it is easily digested and absorbed.
- **Maltose** acts as an important intermediate in the digestion of starch and glycogen, breaking them down into simpler sugars for energy.
- **Trehalose** is a key energy source for many insects, providing them with the necessary fuel for their metabolic needs.
- **Cellobiose** plays an essential role in carbohydrate metabolism, especially in the breakdown of cellulose.

- **Gentiobiose** is found in plant glycosides and some polysaccharides, contributing to plant metabolism and the synthesis of other important compounds.

2.3.3.Oligosaccharides

Oligosaccharides are carbohydrates that yield 3 to 10 monosaccharide molecules upon hydrolysis. These monosaccharides are connected through glycosidic linkages. Depending on the number of monosaccharides, oligosaccharides are classified as trisaccharides (three monosaccharides), tetrasaccharides (four monosaccharides), pentasaccharides (five monosaccharides), and so on. The general formula for trisaccharides is $C_n(H_2O)_{n-2}$, and for tetrasaccharides, it is $C_n(H_2O)_{n-3}$.

Oligosaccharides are typically found as glycans, which are carbohydrate chains linked to either lipids or proteins. When attached to lipids or proteins, they form glycolipids or glycoproteins. This attachment occurs through N-glycosidic or O-glycosidic bonds in a process called glycosylation, where a carbohydrate is covalently bonded to an organic molecule.

There are two types of glycosylation:

- **N-Linked Oligosaccharides**: These involve the attachment of an oligosaccharide to an asparagine residue via a beta linkage to the amine nitrogen of the side chain. In eukaryotes, this process happens in the membrane of the endoplasmic reticulum, while in prokaryotes, it occurs at the plasma membrane.
- **O-Linked Oligosaccharides**: These involve the attachment of oligosaccharides to serine or threonine residues at the hydroxyl group on the side chain. This process takes place in the Golgi apparatus, where monosaccharide units are added to a polypeptide chain.

Both types of glycosylation play important roles in cellular recognition, signaling, and the stability of proteins and lipids.

2.3.3.1.Functions of Oligosaccharides

Oligosaccharides play vital roles in various biological processes due to their involvement in glycoproteins, glycolipids, and other cellular interactions:

1. **Glycoproteins**: These are proteins that have carbohydrates attached to them and are involved in several critical functions, including antigenicity (immune system recognition), solubility, and resistance to proteases (proteins that break down other proteins). Glycoproteins serve as cell-surface receptors, cell-adhesion molecules, immunoglobulins (antibodies), and tumor antigens, helping in cellular communication, immune response, and the protection of cells.
2. **Glycolipids**: These are carbohydrates attached to lipids and play a crucial role in cell recognition. They also help in modulating membrane proteins that act as receptors, contributing to cell signaling and interactions.
3. **Lectins**: Cells produce lectins, which are carbohydrate-binding proteins. Lectins mediate cell adhesion by recognizing specific oligosaccharides, playing a role in cellular interactions and immune functions.
4. **Dietary Fiber**: Oligosaccharides are components of dietary fiber found in plant tissues. Fiber is important for digestive health and plays a role in regulating the gut microbiota.

Overall, oligosaccharides are involved in a variety of processes, including immune response, cell communication, and maintaining cellular structures, as well as contributing to the fiber content in the diet.

2.3.4.Polysaccharides

Polysaccharides are large carbohydrate molecules consisting of chains of more than ten monosaccharides linked by glycosidic bonds. Known as glycans, they are widely distributed in nature and serve primarily structural and storage functions in living organisms. The physical and biological properties of polysaccharides are influenced by their monosaccharide components, the structure of their bonds, and their interactions with enzymatic systems.

Polysaccharides can be classified based on their functions, the types of monosaccharide units they contain, or their origin. One key classification is based on the monosaccharides involved in their structure, dividing polysaccharides into two main categories:

Homopolysaccharides are polysaccharides composed of repeating units of a single type of monosaccharide. These biomolecules play crucial structural and storage roles in living organisms. Examples include cellulose, chitin, starches (amylose and amylopectin), glycogen, and xylans. Based on their functional roles, homopolysaccharides are further classified into structural polysaccharides and storage polysaccharides.

- **Cellulose**: A linear, unbranched polymer of glucose units linked by β-1,4 glycosidic bonds. It is one of the most abundant organic compounds on Earth, forming the structural framework of plant cell walls. The β-linkages make cellulose resistant to hydrolysis, providing rigidity and strength (Figure 12).

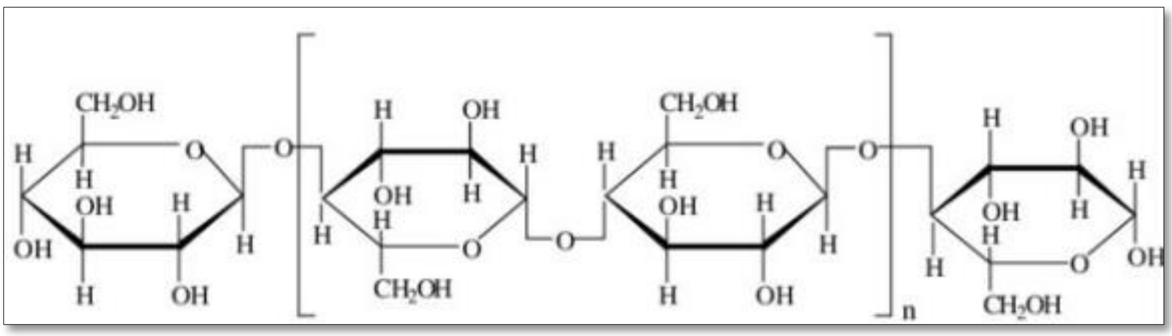


Figure 12. Cellulose structure

- **Chitin**: Composed of long chains of N-acetyl-D-glucosamine (a glucose derivative) linked by β-1,4 glycosidic bonds. It is the second most abundant natural biopolymer after cellulose, serving as a structural component in the exoskeletons of arthropods (like insects and crustaceans) and fungal cell walls (Figure 13).

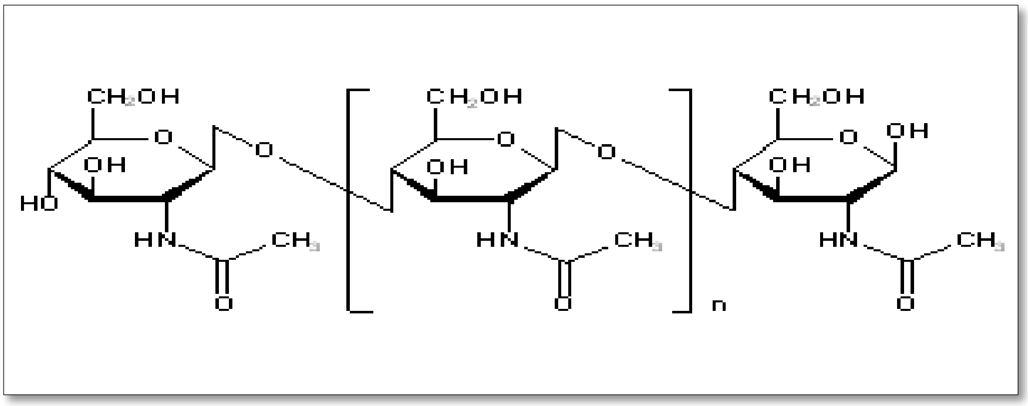


Figure 13. Chitin structure

- **Starch**: A storage polysaccharide in plants, made up of D-glucose units joined by α-linkages (Figure 14). It exists as a mixture of two polymers:
    - **Amylose**: A linear chain of glucose molecules (15-20% of starch), primarily linked by α-1,4 bonds.
    - **Amylopectin**: A highly branched polymer (80-85% of starch), containing both α-1,4 and α-1,6 linkages.

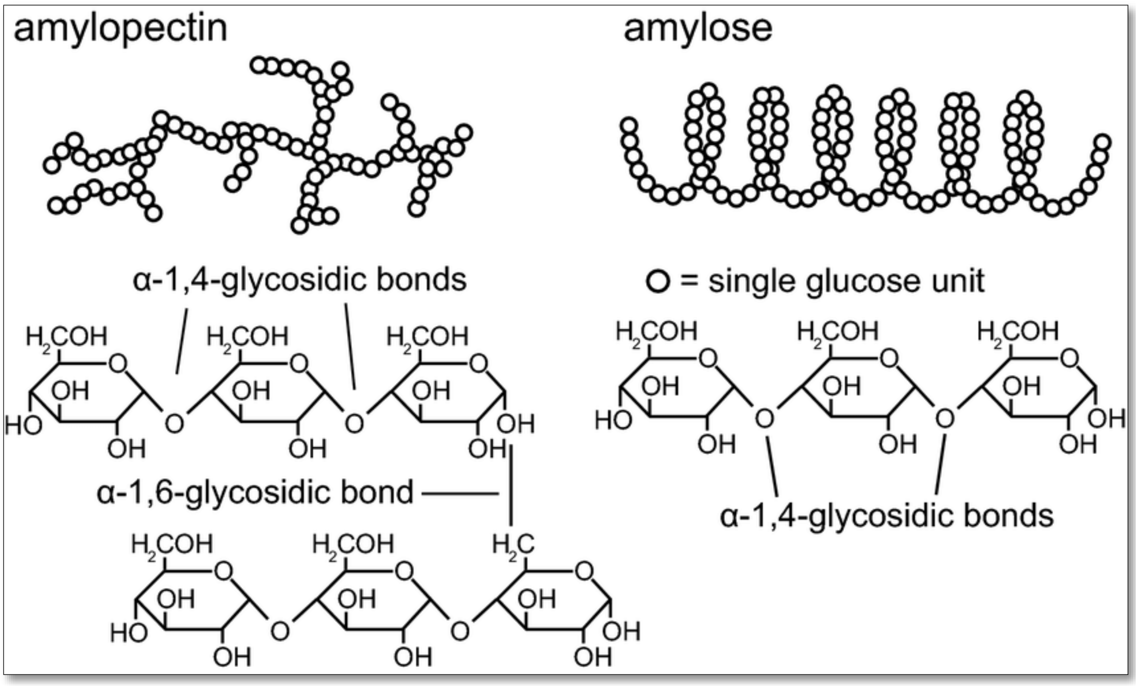


Figure 14. Starch structure

Heteropolysaccharides are polysaccharides composed of two or more types of monosaccharide units. They are widely distributed in natural systems, often forming complex structures by linking with proteins, lipids, or peptides. Examples of heteropolysaccharides include glycosaminoglycans (GAGs), agarose, and peptidoglycans.

- **Glycosaminoglycans (GAGs)**: These are unbranched, negatively charged heteropolysaccharides consisting of repeating disaccharide units. The disaccharide typically includes amino sugars such as N-acetylglucosamine (GlcNAc) or N-acetylgalactosamine (GalNAc), paired with a uronic acid like glucuronic acid. GAGs are essential components of connective tissue, contributing to structural integrity and biological functions such as hydration and shock absorption.
- **Peptidoglycans**: These are heteropolymers consisting of alternating units of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), linked by β-1,4-glycosidic bonds. They form a crucial part of bacterial cell walls, providing rigidity and protection while maintaining cellular shape.

- **Agarose**: A polysaccharide derived from red algae, agarose is composed of repeating disaccharide units called agarobiose. Each unit contains D-galactose and 3,6-anhydro-L-galactopyranose. Agarose is widely used in molecular biology, particularly in gel electrophoresis for the separation of DNA and RNA.

Heteropolysaccharides play essential roles in various biological systems, from providing structural support and maintaining cell integrity to facilitating interactions in extracellular matrices and microbial surfaces (Figure 15).

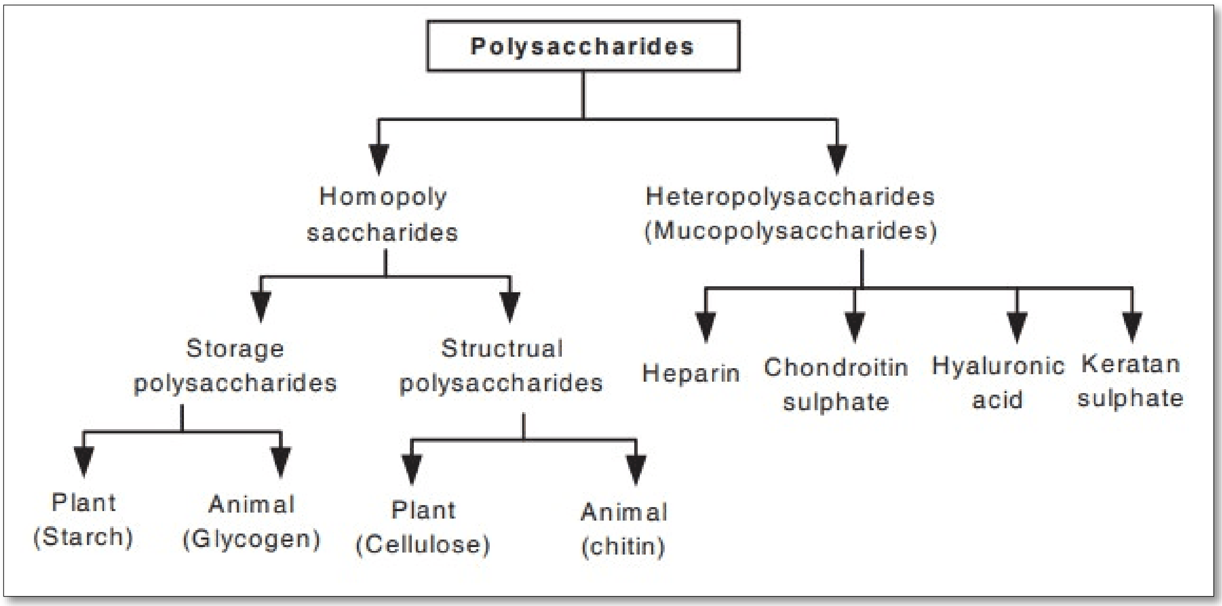


Figure 15. A classification summary of polysaccharides into different sub-groups.

2.3.4.1.Functions of Polysaccharides

Polysaccharides perform a wide array of vital roles in biological systems, categorized primarily into structural, storage, and specialized functions:

- **Structural Polysaccharides**: These provide mechanical stability to cells and organisms. For example, cellulose forms the primary structural component of plant cell walls and serves as a crucial dietary fiber for ruminants, while chitin contributes to the fungal cell wall structure and exoskeletons in arthropods.

- **Storage Polysaccharides**: These serve as carbohydrate reserves, releasing sugar monomers when energy is required. Examples include:
    - **Starch**: Acts as the primary energy reserve in plants and is broken down by amylase enzymes in animals for energy.
    - **Glycogen**: Functions as the main storage polysaccharide in animals, bacteria, and fungi.
    - **Inulin**: Found in plants, it acts as an energy reserve.
- **Agarose**: Provides structural support in the cell walls of marine algae.
- **Peptidoglycan**: A key component of bacterial cell walls, it provides structural integrity, participates in binary fission, and protects against osmotic pressure.
- **Hyaluronic Acid**: Found in the vitreous humor of the eye and synovial fluid in joints, it acts as a lubricant and participates in processes like tumor metastasis, angiogenesis, and blood coagulation.
- **Heparin**: Serves as a natural anticoagulant, preventing blood clot formation.
- **Keratan Sulfate**: Present in the cornea, cartilage, and bones, it cushions joints and absorbs mechanical shocks.
- **Chondroitin**: A major component of cartilage, providing resistance to compression and contributing to joint health.
- **Dermatan Sulfate**: Plays roles in wound repair, infection response, regulation of blood coagulation, and the management of cardiovascular diseases.

3. Lipids

Lipids are a class of biological molecules defined by their low solubility in water and high solubility in non-polar solvents like chloroform. Structurally, they are organic molecules primarily composed of hydrocarbons, and their oxidation during metabolism releases large amounts of energy. Lipids include a diverse range of naturally occurring molecules, such as fats, waxes, sterols, triglycerides, and fat-soluble vitamins (A, D, E, and K).

Lipids play essential roles in the human body, from providing energy to producing hormones. They also have industrial applications in cosmetics, food production, and nanotechnology. Their primary biological functions are energy storage, signaling, and serving as structural components of cell membranes.

3.1.Functions of Lipids

3.1.1. Energy Production and Storage:

Lipids are a major energy source for the body. Triglycerides, stored in adipose tissue, serve as the primary form of energy storage. Lipids yield high caloric energy upon oxidation, providing about 9 kcal per gram compared to 4 kcal per gram for carbohydrates and proteins. During rest and everyday activities, nearly half of the body's energy needs are met by lipids.

3.1.2. Structural Function:

Essential lipids like linolenic acid and linoleic acid are crucial for health. They are used in building cell membranes, producing hormones, and supporting immune and visual systems. Lipids contribute to the structural integrity of cell walls throughout the body.

3.1.3. Insulation and Protection:

Lipids help regulate body temperature by forming a layer of fat beneath the skin, which insulates against external temperature changes. Fat also surrounds vital organs like the kidneys, acting as a protective cushion against physical damage.

3.1.4. Hormone production

Cholesterol, a type of lipid, is vital for synthesizing important steroid hormones such as estrogen, testosterone, progesterone, and the active form of vitamin D. These hormones are essential for various bodily functions, including maintaining pregnancy, regulating calcium levels, and developing sex characteristics.

3.1.5. Chemical messaging:

Lipids like steroid hormones act as primary messengers, transmitting signals between cells, tissues, and organs. Other lipid molecules facilitate intracellular communication between biochemical systems.

3.2. Classification of lipids

Lipids can be classified into two main categories based on their ability to undergo a saponification reaction: saponifiable lipids and non-saponifiable lipids (Figure 16).

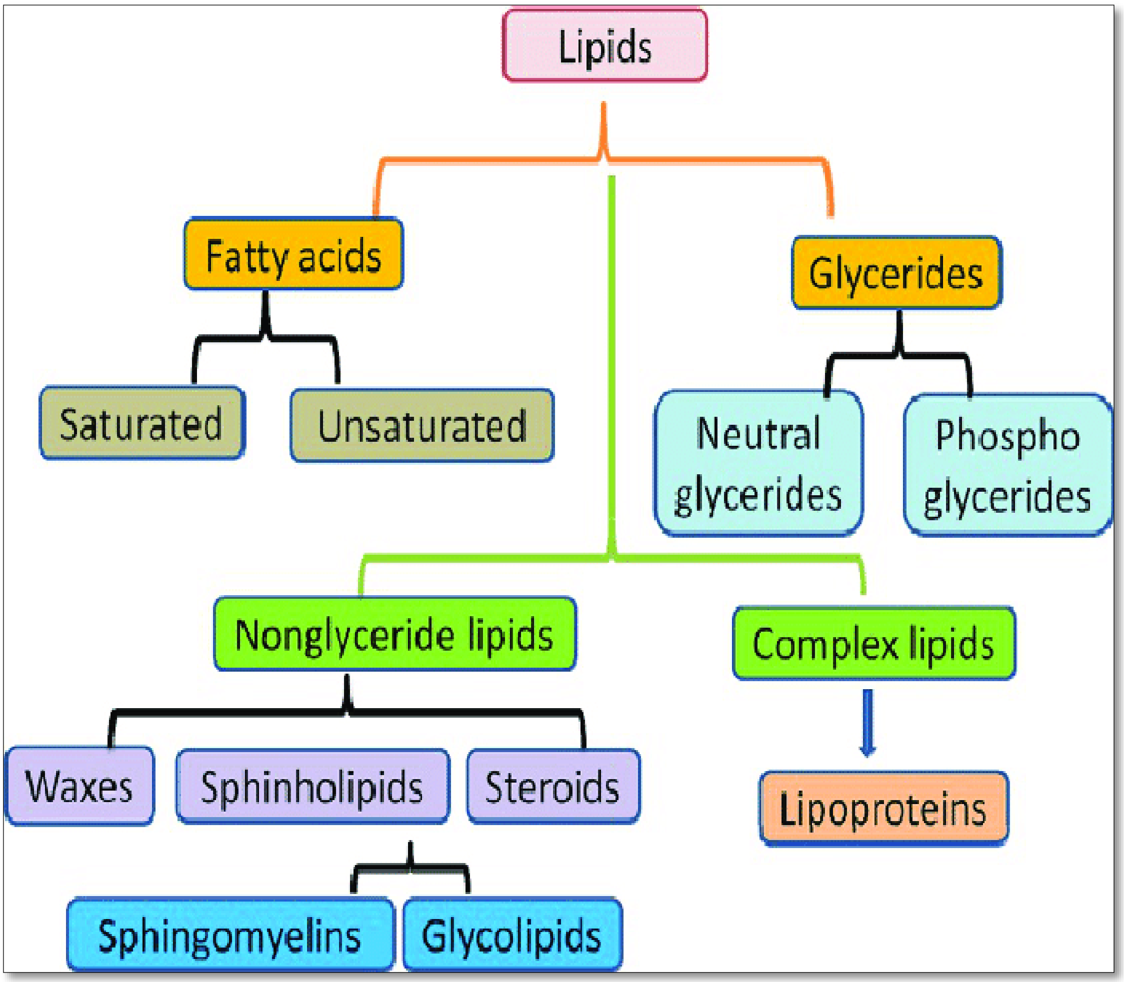


Figure 16. Classification of lipids

3.2.1. Saponifiable Lipids

These lipids can undergo a saponification reaction, where an alkali (like sodium hydroxide, NaOH, or potassium hydroxide, KOH) cleaves the ester bonds to yield a carboxylic acid salt (soap) and alcohol. Saponifiable lipids are further classified based on their structural complexity:

a. Simple Lipids

Simple lipids are a class of lipids that consist of fatty acid esters combined with various alcohols. They serve essential biological functions such as energy storage, insulation, and protection. The two main types of simple lipids are fats and waxes**.** Fats are esters of fatty acids and glycerol and can exist in either a solid or liquid state. When solid at room temperature, they are commonly referred to as fats (e.g., butter, animal fat), while in a liquid state, they are known as **oils** (e.g., olive oil, sunflower oil), which generally contain a higher proportion of unsaturated fatty acids. Waxes, on the other hand, are esters of fatty acids and long-chain monohydric alcohols. They are highly hydrophobic and primarily function as **protective coatings** in plants and animals. Examples include beeswax, lanolin (wool wax), and cutin (found in plant cuticles)**,** which help prevent water loss and provide structural integrity. Simple lipids are crucial in biological membranes, waterproofing, and energy metabolism, making them indispensable in both plant and animal systems

.b. Compound Lipids

Compound lipids are esters of fatty acids that, in addition to alcohol and fatty acids, contain other functional groups, making them structurally and functionally diverse. These lipids play critical roles in cell membrane structure, signaling, and metabolic processes.

- **Phospholipids** are essential components of biological membranes and contain fatty acids, an alcohol (either glycerol or sphingosine), and a phosphoric acid residue. They often include nitrogenous bases and other substituents, contributing to their amphipathic nature. Based on the type of alcohol present, phospholipids are classified into glycerophospholipids (which contain glycerol, e.g., phosphatidylcholine) and sphingophospholipids (which contain sphingosine, e.g., sphingomyelin).

- **Glycolipids (Glycosphingolipids)** consist of a fatty acid, sphingosine, and a carbohydrate instead of a phosphate group. These lipids are abundant in the nervous system, where they contribute to cell recognition, signaling, and stability in the plasma membrane.
- **Other Complex Lipids** include sulfolipids, aminolipids, and lipoproteins, which play various specialized roles in metabolism, immune response, and transport of hydrophobic molecules in the bloodstream.

3.2.2. Non-saponifiable lipids

These lipids do not contain ester bonds and thus cannot undergo saponification. Examples include:

- **Steroids**: Lipids with a characteristic fused ring structure (e.g., cholesterol).
- **Prostaglandins**: Lipid-derived signaling molecules involved in various physiological functions.

3.2.3.Saponification reaction

This reaction is a chemical process in which an alkali cleaves an ester bond, producing a carboxylic acid salt (soap) and alcohol. It is most commonly used in the production of soaps, with NaOH and KOH being the preferred alkalis.

3.3.Fatty acids

A fatty acid is a carboxylic acid consisting of a long aliphatic chain, which can be either saturated (no carbon-carbon double bonds) or unsaturated (one or more carbon-carbon double bonds). Most naturally occurring fatty acids have an unbranched chain with an even number of carbon atoms, typically ranging from 4 to 28. Fatty acids are commonly derived from triglycerides or phospholipids and serve as essential dietary fuel sources for animals. Upon metabolism, fatty acids yield substantial amounts of ATP, making them a critical energy source. Many cell types can utilize either glucose or fatty acids for energy production.

3.3.1.Nomenclature of fatty acids

The carbon atoms in a fatty acid chain are numbered starting from the carboxyl (-COOH) group, designated as C-1. The positions of double bonds are indicated using the symbol (Δ). For instance, in an 18-carbon fatty acid, a double bond between C-12 and C-13 is denoted as Δ12. The fatty acid can also be represented as 18:1(Δ12), where "18" indicates the number of carbons, "1" the number of double bonds, and "Δ12" the double bond's position.

Fatty acids with an odd number of carbon atoms are termed odd-chain fatty acids, while those with an even number are known as even-chain fatty acids, which are more common in nature. Two naming systems are used for fatty acids: systematic names (IUPAC) following standard organic chemistry rules and trivial names, which are more commonly used in literature and often reflect historical naming conventions.

3.3.2.Micelles

Micelles are spherical aggregates of lipid molecules that form in aqueous solutions, driven by the amphipathic nature of lipids like fatty acids. Amphipathic molecules have both hydrophilic (water-attracting) regions and hydrophobic (water-repelling) regions. In the case of fatty acids, the hydrophilic part consists of polar head groups, such as acidic carboxyl groups, while the hydrophobic part consists of long hydrocarbon chains.

In a micelle, the hydrophilic head groups face outward, interacting with water, while the hydrophobic tails are tucked inward, away from the water. This arrangement minimizes the energy of the system by reducing the exposure of the hydrophobic regions to the aqueous environment.

Fatty acids that form micelles typically have a single hydrocarbon chain, which allows them to adopt a spherical shape with minimal steric hindrance. In contrast, lipids like glycolipids and phospholipids, which have two hydrophobic tails, are too bulky to form micelles and instead arrange themselves into bilayers. This difference arises because bilayers provide a more stable structure for molecules with larger hydrophobic regions.

Micelles form spontaneously in water, driven by the amphipathic nature of the lipid molecules, which self-organize into this spherical configuration to minimize unfavorable interactions between their hydrophobic tails and water.

3.3.3.Physical properties of fatty acids

The physical properties of fatty acids, as well as the lipids that contain them, are primarily influenced by the length and degree of unsaturation of their hydrocarbon chains. These properties are significant in determining the physical state of fats and oils at room temperature, as well as their melting points.

3.3.3.1.Saturated vs. unsaturated fatty acids:

Fatty acids can be classified into saturated and unsaturated based on the presence or absence of double bonds in their hydrocarbon chains. Saturated fatty acids have no double bonds, resulting in a straight-chain structure that allows them to pack closely together. This tight packing leads to higher melting points, making these fats solid at room temperature. They are commonly found in animal fats, such as butter, lard, and dairy products.

In contrast, unsaturated fatty acids contain one or more double bonds, introducing kinks in their structure, which prevents tight packing. As a result, they have lower melting points and are typically liquid at room temperature. Unsaturated fatty acids are prevalent in plant oils, such as olive oil, sunflower oil, and flaxseed oil. These fatty acids can be further classified into monounsaturated fatty acids (MUFA), which have one double bond, and polyunsaturated fatty acids (PUFA), which have multiple double bonds.

The physical properties of saturated and unsaturated fatty acids significantly impact their biological roles, dietary effects, and health implications, with unsaturated fats often being considered healthier alternatives due to their potential benefits for heart health and cholesterol regulation.

3.3.3.2.Melting point of fatty acids

The melting point of fats depends on both the chain length and the degree of unsaturation of the constituent fatty acids:

- **Saturated fats**: Fatty acids with short chains (C4 to C8) are usually liquid at room temperature. However, fats with longer saturated chains (C10 and above) are typically solid. The melting point of these fats increases with chain length.
- **Unsaturated fats**: The presence of double bonds (unsaturation) significantly lowers the melting point of fats. Each double bond introduces a kink in the hydrocarbon chain, which prevents the molecules from packing closely together, resulting in weaker interactions and lower melting points.

3.3.3.3.Effect of chain length and unsaturation on fluidity:

The fluidity of fatty acids and their derivatives is influenced by chain length and the degree of unsaturation. Shorter chain lengths and a higher number of double bonds (unsaturation) increase molecular mobility, reducing intermolecular interactions and making the fat more fluid. As a result, these fats are less likely to be solid at room temperature. This characteristic is particularly important in biological membranes, where increased fluidity allows for better membrane flexibility and permeability.

Conversely, longer fatty acid chains and higher saturation (absence of double bonds) promote stronger intermolecular interactions due to increased van der Waals forces. This leads to tighter molecular packing, making the fat more rigid and solid at room temperature. For example, saturated fats (e.g., palmitic acid, stearic acid) tend to be solid, while unsaturated fats (e.g., oleic acid, linoleic acid) remain liquid under similar conditions.

In biological systems, the balance of fatty acid composition is crucial for membrane dynamics, ensuring proper functionality of membrane-bound proteins, cell signaling, and adaptability to temperature changes.

3.3.3.4.Geometric isomerism

The presence of double bonds introduces cis-trans isomerism. In cis-unsaturated fatty acids, the double bond creates a bend or kink in the hydrocarbon chain, making it less flexible and preventing tight packing of molecules. This contrasts with saturated fatty acids, which have straight chains and can pack tightly, maximizing interactions and increasing the melting point.

3.3.3.5.Packing and crystallization:

- **Saturated fatty acids** can pack tightly into nearly crystalline arrays due to their straight chains, allowing for stronger van der Waals interactions between molecules. This results in higher stability and a higher melting point.
- **Unsaturated fatty acids**, due to the kinks in their chains, cannot pack as tightly, leading to weaker intermolecular interactions and lower melting points.

3.4.Glycerides (acylglycerols)

Glycerides, also known as acylglycerols, are esters formed by the reaction of glycerol (a three-carbon alcohol) with fatty acids. The esterification of glycerol can occur at one, two, or all three hydroxyl groups, forming **monoglycerides**, **diglycerides**, and **triglycerides**. The majority of lipids in both vegetable oils and animal fats are **triglycerides**, although they are broken down by natural enzymes called **lipases** into mono and diglycerides, along with free fatty acids and glycerol.

3.4.1.Triglycerides:

Triglycerides are the simplest lipids formed from fatty acids, and they consist of three fatty acid molecules attached to a single glycerol backbone. When all three fatty acids in a triglyceride molecule are the same, it is called a **simple triglyceride**. For example:

- **Tristearin** (C18:0),
- **Tripalmitin** (C16:0),
- **Triolein** (C18:1).

In contrast, mixed triglycerides contain two or more different fatty acids, and to describe them precisely, the type and position of each fatty acid must be specified. Because of the ester linkage between the polar hydroxyl groups of glycerol and the polar carboxylate groups of the fatty acids, triglycerides are nonpolar and hydrophobic, meaning they are insoluble in water.

At room temperature, fats (which are solid) and oils (which are liquid) are both mixtures of triglycerides, but oils generally contain higher levels of unsaturated fatty acids than fats. This contributes to the lower melting point of oils.

3.4.2. Hydrolysis of glycerides:

Glycerides can be hydrolyzed (broken down by water) into glycerol and fatty acids under either acidic or basic conditions. In biological systems, this reaction is catalyzed by lipase enzymes. When glycerides are hydrolyzed in the presence of a base, the resulting fatty acids are neutralized, forming carboxylate ions, which can be used to produce soap. This process is known as saponification.

- **Saponification** is the hydrolysis of triglycerides using a base, such as sodium hydroxide (NaOH) or potassium hydroxide (KOH), and it produces soap as a result. The saponification number refers to the number of milligrams of KOH required to neutralize the fatty acids from 1 gram of fat or oil (Figure 17).

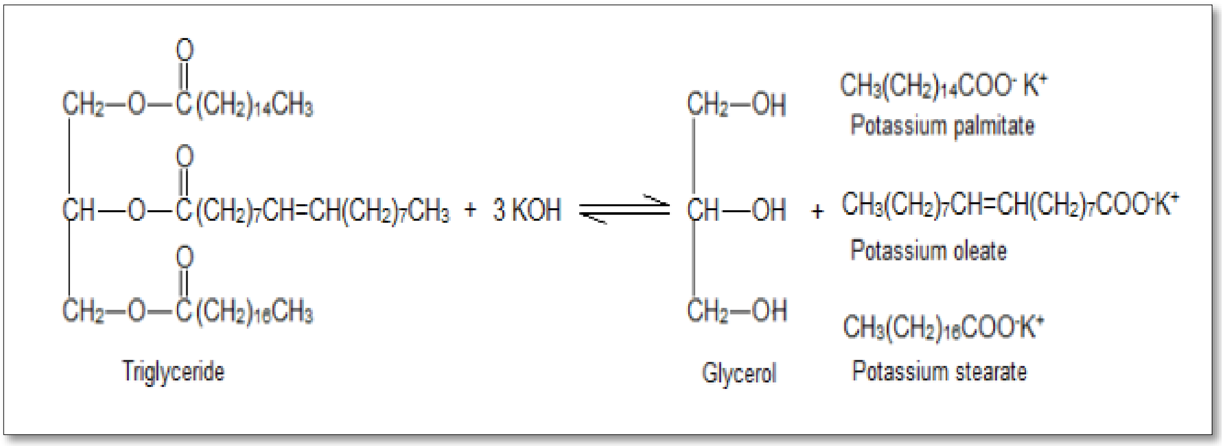


Figure 17. Saponification of lipid

2. Reaction with iodine:

The degree of unsaturation in the fatty acids of glycerides can be determined by reacting the fats with iodine. This is called the iodine number, which indicates the number of grams of iodine that react with 100 grams of fat. A higher iodine number corresponds to a higher level of unsaturation in the fat. The iodine number is an important indicator of the quality and characteristics of the fat.

3. Hydrogenation:

The presence of double bonds in the fatty acid chains of glycerides makes them prone to oxidation by atmospheric oxygen, which leads to rancidity. To prevent this, hydrogenation is used, a process where hydrogen atoms are added to the fatty acid chains, converting double bonds into single bonds. This process not only increases the stability of oils and fats but also improves their taste and texture.

- The hydrogenation process reduces the number of double bonds per fatty acid molecule, resulting in more saturated fats. This is particularly useful for producing margarines, shortenings, and other products that require a solid fat content that is suitable for culinary uses. The level of solid fat content can be adjusted by controlling the hydrogenation conditions and catalysts used. Typically, nickel is used as the catalyst for hydrogenation due to its high performance-to-price ratio.

3.5.Waxes:

Waxes are a group of neutral lipids that are of significant physiological importance. They consist of a long-chain fatty acid esterified to a long-chain alcohol. Waxes are completely water-insoluble and are generally solid at room temperature due to their hydrophobic nature. This strong hydrophobic characteristic allows waxes to serve various functions, such as acting as water repellents on plant leaves, bird feathers, and insect cuticles. Waxes are also important energy-storage molecules in organisms such as plankton, whales, and many fish species.

A well-known example of an animal wax is beeswax, which is used by honeybees to construct their honeycombs. The main component of beeswax is the ester myricyl palmitate, which is formed by the esterification of triacontanol (a long-chain alcohol) and palmitic acid (a fatty acid).

In addition to their biological roles, waxes are widely used in industry. They are important components in complex formulations and are commonly used in coatings. Waxes are used to make candles, which are used for lighting and decoration, as well as wood finishes and coatings. In the pharmaceutical, cosmetic, and other industries, biological waxes such as lanolin (from lamb's wool), beeswax, and carnauba wax are used in products like lotions, ointments, and polishes.

3.6.Phosphoglycerides or phospholipids:

Phosphoglycerides are a type of glycerol-based phospholipid that play a critical role as main components of lipid bilayers in cell membranes. They share similarities with triglycerides, with some key differences. While triglycerides contain three fatty acids linked to a glycerol backbone, phosphoglycerides are made up of two fatty acids, along with phosphoric acid and glycerol. The fatty acids are attached to the glycerol at the 1 and 2 positions through ester bonds.

The third hydroxyl group on glycerol is esterified to phosphoric acid through a phosphate ester bond, and this phosphate group is often further linked to a complex amino alcohol, such as choline, ethanolamine, or serine. The presence of these additional polar head groups makes phospholipids amphipathic, meaning they have both a hydrophilic (polar) head and hydrophobic (non-polar) tail.

The phosphatidic acid (diacylglycerol 3-phosphate) is the simplest form of phosphoglyceride, formed when glycerol's hydroxyl groups are esterified with two fatty acids and a phosphate group. More complex phosphoglycerides are named based on the amino alcohol present in the head group. For example:

- Phosphatidylserine has serine as the polar head,
- Phosphatidylcholine has choline,
- Phosphatidylethanolamine has ethanolamine.

The properties of phospholipids are largely influenced by the fatty acid chains (hydrophobic) and the polar head (hydrophilic) that contains the phosphate group and amino alcohol. This amphipathic nature allows phospholipids to form structures like lipid bilayers, which are crucial for the structure and function of cell membranes. The phosphate group provides an ionic character, while the fatty acid chains remain non-polar, giving the molecule unique polar and non-polar regions (Figure 18).

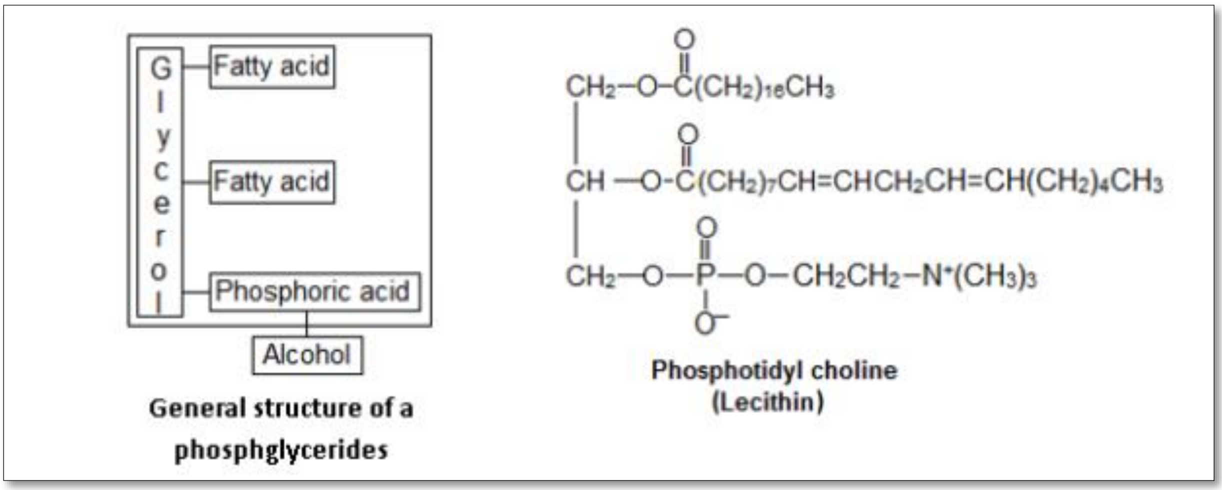


Figure 18. General structure of a phosphoglycerides

3.7.Lecithin:

Lecithin is one of the most common phospholipids found in various natural sources such as egg yolks, wheat germ, and soybeans. It is widely extracted from soybeans and used as an emulsifying agent in the food industry. Lecithin's ability to act as an emulsifier arises from its amphipathic nature-containing both polar (hydrophilic) and non-polar (hydrophobic) properties. This enables it to facilitate the mixing of fats and oils with water, making it essential in food preparation, especially in the creation of smooth and stable mixtures.

In terms of structure, lecithin contains the ammonium salt of choline, which is attached to a phosphate group via an ester linkage. The choline molecule contains a positively charged nitrogen atom, similar to an ammonium ion, with four methyl groups attached to the nitrogen. Lecithin is also an important component in the lipid bilayers of cell membranes, contributing to the structural integrity and function of cells.

3.7.Cephalins:

Cephalins are another type of phosphoglyceride, similar to lecithin, but differ in the amino alcohol or amino acid attached to the phosphate group. Instead of choline, cephalins contain ethanolamine or serine, which are attached to the phosphate group via phosphate ester bonds. Like lecithin, cephalins contain a variety of fatty acids that make up the rest of the molecule (Figure 19).

Cephalins are found in most cell membranes, with a significant concentration in brain tissues. They also play a critical role in blood clotting due to their presence in blood platelets. These phospholipids are essential for maintaining cellular functions, particularly in the nervous and circulatory systems.

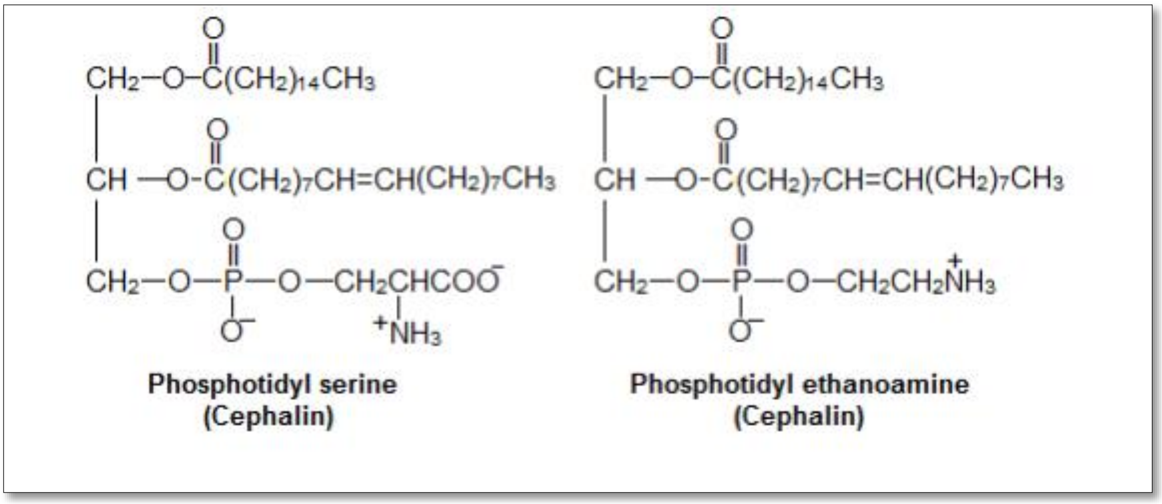


Figure 19. Cephalins structure

3.8.Sphingolipids:

Sphingolipids are a class of lipids that contain a sphingoid base as their backbone, which is a family of aliphatic amino alcohols, including sphingosine. These lipids are composed of a long-chain amino alcohol (sphingosine or its derivatives), a long-chain fatty acid, and a polar head group. The head group can be attached to the sphingoid base either through a glycosidic linkage or a phosphodiester bond (Figure 20).

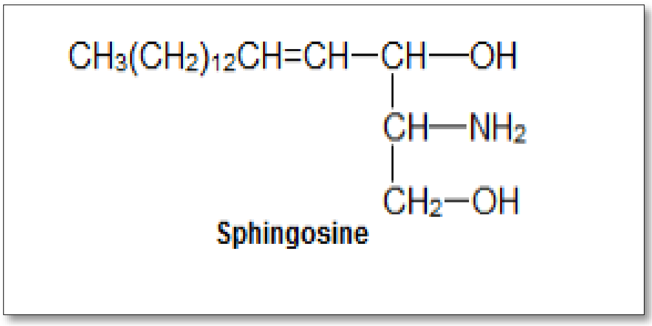


Figure 20. Sphingosine structure

Sphingolipids are important in cell signaling, cell recognition, and the structure of cell membranes. Defects in sphingolipid metabolism can lead to sphingolipidoses, disorders that often have significant impacts on neural tissue.

The structure of sphingolipids is similar to glycerophospholipids in that C-1, C-2, and C-3 of the sphingosine molecule resemble the three carbons of glycerol. When a fatty acid attaches via an amide linkage to C-2 of sphingosine, a ceramide is formed. Ceramide serves as the structural precursor to all sphingolipids and is similar to diacylglycerols in structure.

There are three major subclasses of sphingolipids, all derivatives of ceramide, but differing in their head groups:

1. **Sphingomyelins**:

   These sphingolipids have phosphocholine or phosphoethanolamine as their polar head group. They are structurally similar to phosphatidylcholines and do not carry a net charge on their head groups. Sphingomyelins are particularly prominent in myelin, the membranous sheath that surrounds and insulates the axons of some neurons, and are also found in plasma membranes of animal cells.

2. **Glycosphingolipids**:

   Glycosphingolipids have sugar molecules as their polar head groups, which are connected to the ceramide moiety via the OH group at C-1. These lipids are phosphate-free and occur mainly in the outer face of plasma membranes. There are two major types:

   - **Cerebrosides**: These contain one sugar (galactose or glucose) attached to ceramide. Cerebrosides with galactose are found in neural tissues, while those with glucose are found in non-neural tissues.
   - **Globosides**: These are neutral glycosphingolipids that contain two or more sugars (often glucose, galactose, or N-acetylgalactosamine). They are neutral at physiological pH.

3. **Gangliosides**:

   Gangliosides are the most complex sphingolipids. They have oligosaccharides as their polar head groups, often containing N-acetylneuraminic acid (also known as sialic acid) at the termini. The presence of sialic acid imparts a negative charge to gangliosides at physiological pH, distinguishing them from globosides. Gangliosides are classified based on the number of sialic acid residues they contain:

   - **GM** (one sialic acid residue),
   - **GD** (two sialic acid residues),
   - **GT** (three sialic acid residues),
   - **GQ** (four sialic acid residues).

3.9.Steroids:

Steroids are a large group of lipids derived from terpenoid precursors, which are constructed from five-carbon isoprene units. Terpenoids are a major class of naturally occurring organic compounds and are found in many organisms. About 60% of known natural products are terpenoids. The steroids in animals, such as sterols, are biologically produced from these terpenoid precursors.

Steroids share a common structural feature: the steroid nucleus, which consists of four fused rings (three six-membered rings and one five-membered ring). This structure is rigid and almost **planar**, which makes the steroid nucleus quite different from other types of lipids. The fused rings prevent rotation about the C-C bonds.

3.10.Cholesterol:

Cholesterol is the primary sterol in animal tissues. It has an amphipathic nature, meaning it has both polar and nonpolar parts. The polar head group is the hydroxyl group at C-3, while the nonpolar hydrocarbon body includes the steroid nucleus and a hydrocarbon side chain at C-17. Cholesterol plays several key roles in the body:

- It is an essential membrane constituent, stabilizing the fluidity of cell membranes.
- It serves as a precursor for the synthesis of other important molecules, including steroid hormones (which regulate gene expression) and bile acids (which emulsify fats in the digestive tract).

Steroid Hormones and Bile Acids:

- Steroid hormones are biological signals that regulate various physiological processes, including the immune system, metabolism, and reproductive functions.

- Bile acids**,** derived from cholesterol, are involved in digestive processes. They act as detergents in the intestine, breaking down dietary fats and making them more accessible to digestive enzymes.

3.11.**Prostaglandins:**

Prostaglandins are physiologically active lipids that have hormone-like effects in animals. They are derived from arachidonic acid, a fatty acid, and consist of a 20-carbon skeleton with a 5-carbon ring. These molecules are involved in various biological functions and have diverse physiological effects:

1. **Inflammatory response**: Prostaglandins contribute to pain, fever, and inflammation when tissues are damaged. They stimulate white blood cells to the site of damage to minimize tissue destruction.
2. **Blood clotting**: Prostaglandins help regulate blood clot formation. For example, thromboxane promotes platelet aggregation and blood vessel constriction, while PGI2 has the opposite effect.
3. **Gastrointestinal and renal functions**: Prostaglandins inhibit acid production in the stomach and increase the secretion of protective mucus. They also increase blood flow to the kidneys and play a role in regulating bronchi constriction during asthma through leukotrienes.

3.12.**Lipoproteins:**

Lipoproteins are complex molecules consisting of both **lipids** and proteins. They serve the vital function of transporting hydrophobic lipids (such as cholesterol and triglycerides) through aqueous environments like blood or extracellular fluid. The structure of a lipoprotein includes:

- A single-layer phospholipid shell, with the hydrophilic (water-loving) parts facing outward and the lipophilic (fat-loving) parts facing inward toward the lipid molecules inside.

Lipoproteins are categorized based on their protein-to-lipid ratio and the types of apoproteins and lipids they contain. Some major types of lipoproteins include:

1. **Chylomicrons**: The largest lipoproteins, with the lowest density due to their high lipid content (about 1% protein by weight).
2. **VLDL (Very Low-Density Lipoproteins)**: These have a protein content of about 10% by weight.
3. **LDL (Low-Density Lipoproteins)**: Often referred to as "bad cholesterol," they have about 25% protein by weight.
4. **HDL (High-Density Lipoproteins)**: Known as "good cholesterol," they have the highest protein content at approximately 45% by weight.

Chapter 2: Techniques in Biochemistry

Separation techniques in biochemistry are critical methodologies used to isolate, purify, and study the various components of biological systems. These techniques enable researchers to understand the structure, function, and interactions of biomolecules such as proteins, nucleic acids, lipids, and small metabolites. Here are some widely used separation methods in biochemistry:

1.Chromatography:

Chromatography is a powerful technique for separating components of a mixture based on their interaction with a stationary phase and a mobile phase. The principle is based on the differential migration of components in a mixture, where they interact differently with the two phases, resulting in their separation over time

2.Types of chromatography

2.1.Column chromatography

Proteins and other biomolecules can be separated and purified based on their unique characteristics, such as size, shape, net charge, and binding affinity. Column chromatography is one of the most widely used techniques for this purpose.

In this method, the stationary phase (a solid or gel-like material) is packed into a column, while the mobile phase (a liquid buffer) carries the sample through the column. The sample containing the mixture to be separated is first applied to the column, followed by the mobile phase, which ensures the flow of the components through the stationary phase (Figure 1).

As the sample passes through the column, the individual components interact differently with the stationary phase due to their distinct properties. Components with stronger interactions move more slowly, while those with weaker interactions flow faster. The separated fractions are collected at the bottom of the column in a time- and volume-dependent manner.

Column chromatography is a versatile and efficient method for purifying biomolecules and is widely employed in research and industrial applications.

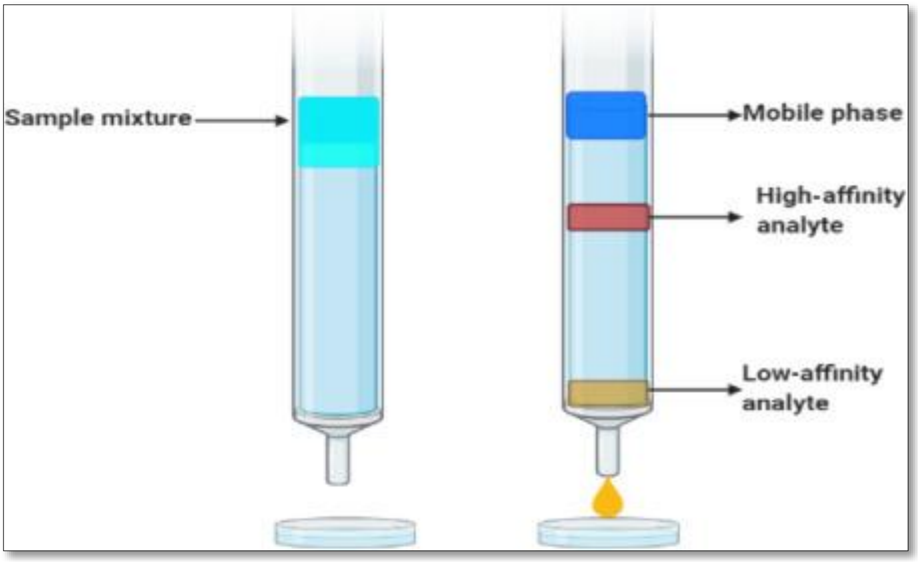


Figure 1. Column chromatography

2.2.Ion-Exchange Chromatography

Ion-exchange chromatography relies on electrostatic interactions between charged protein groups and a solid support material, known as the matrix. The matrix is designed to carry an opposite charge to that of the target protein, allowing the formation of ionic bonds that facilitate binding.

Proteins are bound to the matrix through these ionic interactions and can be eluted (released) from the column by altering conditions such as pH, salt concentration, or the ionic strength of the buffer solution (Figure 2).

- **Anion-Exchange Chromatography**: Utilizes a positively charged matrix that binds negatively charged proteins.
- **Cation-Exchange Chromatography**: Utilizes a negatively charged matrix that binds positively charged proteins.

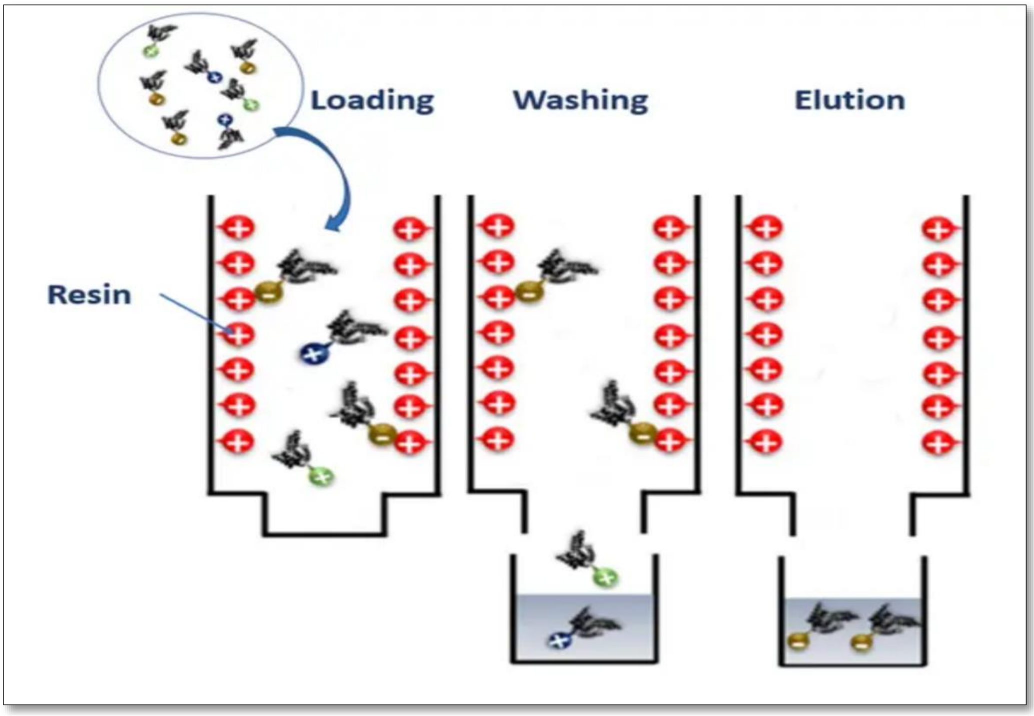


Figure 2. Ion-Exchange Chromatography

2.3.Gel- permeation (molecular sieve) chromatography

Gel-permeation chromatography, also known as molecular sieve chromatography, separates macromolecules based on differences in their molecular sizes using materials like dextran. This technique is primarily employed to determine the molecular weight of proteins and to reduce salt concentrations in protein solutions. The stationary phase in the column consists of inert molecules with small pores. When a solution containing molecules of varying sizes flows through the column at a constant rate, larger molecules, which cannot penetrate the pores, are restricted to the spaces between the particles and pass through the column more quickly. In contrast, smaller molecules diffuse into the pores, with their retention time increasing as their size decreases. The most commonly used column material is Sephadex G-type gels, although other materials like dextran, agarose, and polyacrylamide are also widely utilized. This technique is essential for size-based separation and purification of biomolecules (Figure 3).

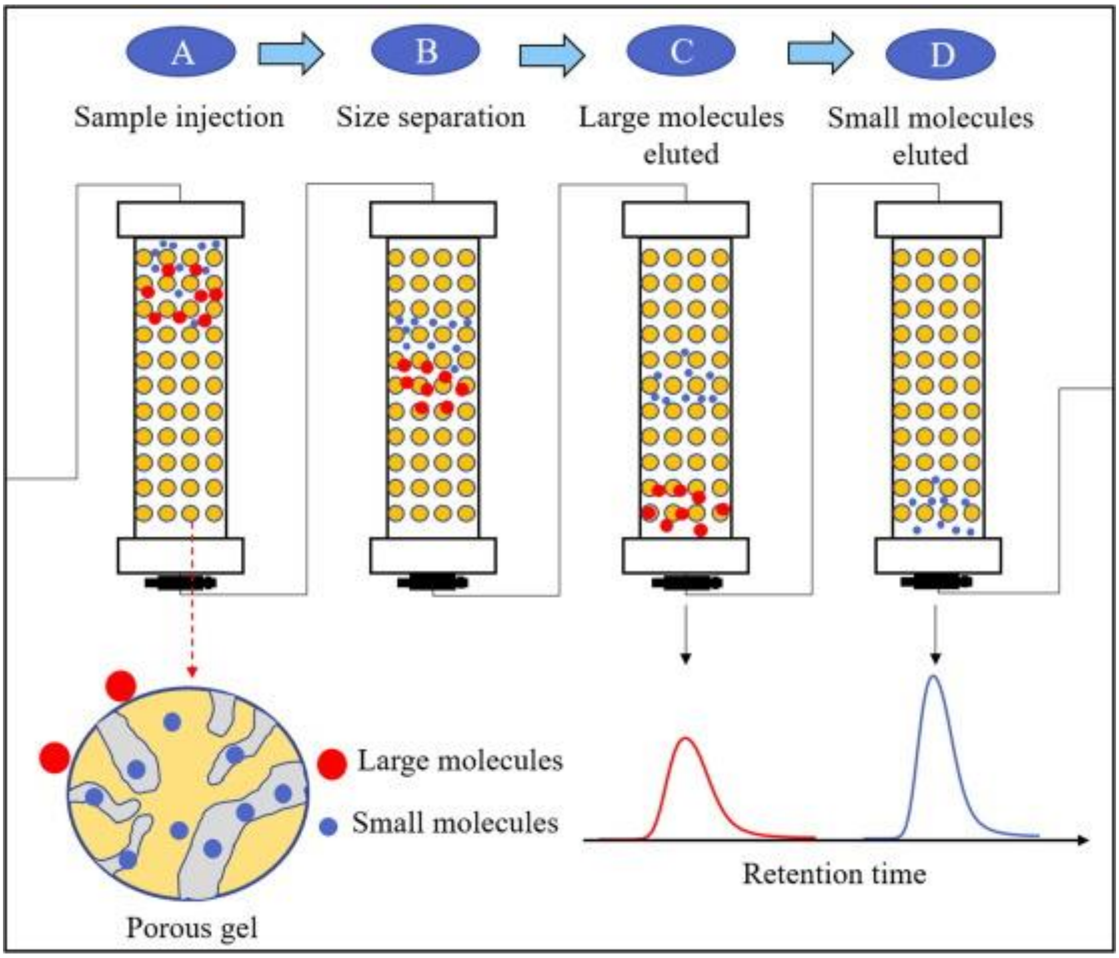


Figure 3. Gel- permeation (molecular sieve) chromatography

2.4.Affinity chromatography

Affinity chromatography is a powerful technique used to purify enzymes, hormones, antibodies, nucleic acids, and specific proteins. It relies on the specific interaction between a target molecule and a ligand, which is attached to the stationary phase (such as dextran, polyacrylamide, or cellulose). The ligand binds selectively to the target protein, forming a complex that retains the protein in the column while unbound molecules pass through. To elute the bound protein, the interaction is disrupted by altering the ionic strength, such as by changing the pH or adding a salt solution (Figure 4). This method provides high specificity and is widely used for the purification of biomolecules.

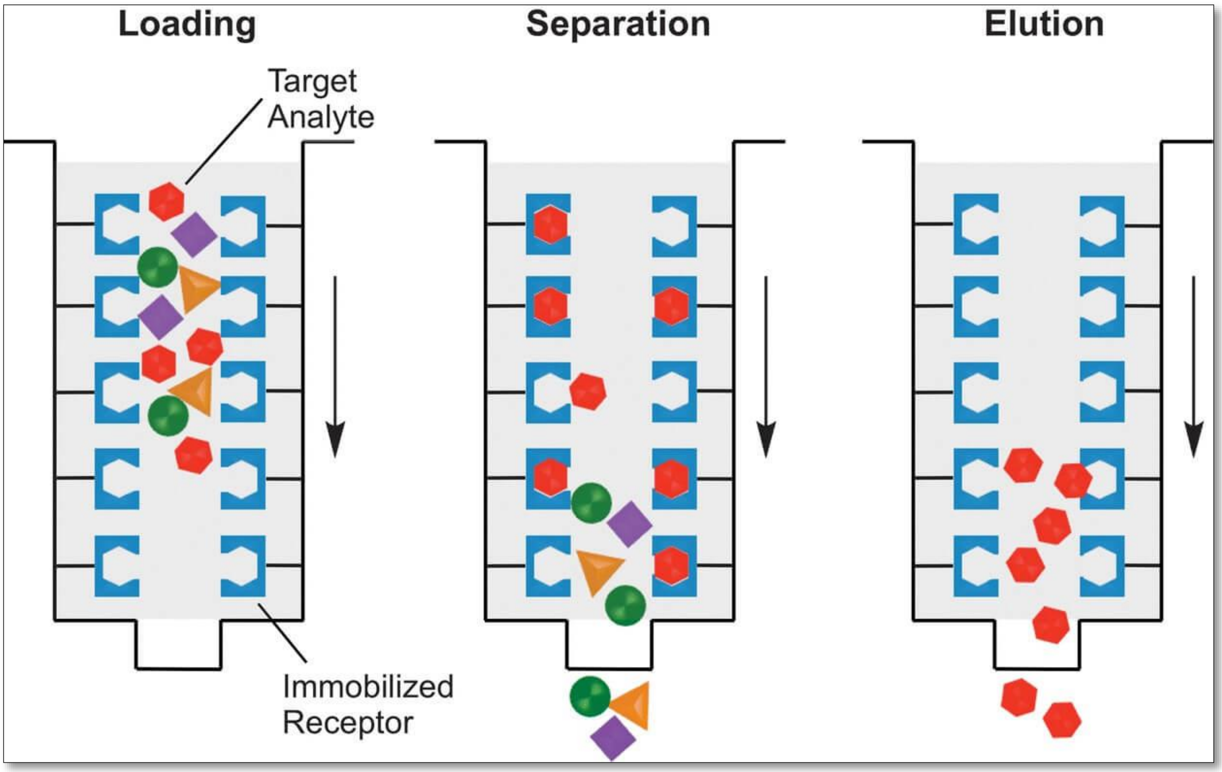


Figure 4. Affinity chromatography

2.5.Paper chromatography

Paper chromatography is a type of liquid-liquid chromatography where the support material is a layer of cellulose, typically in the form of thick filter paper saturated with water (Figure 5). The water within the paper's pores acts as the stationary liquid phase. The mobile phase is a suitable solvent or fluid, which is introduced into a developing tank. When a sample is applied to the paper and the mobile phase flows through it, the components of the sample separate based on their differential solubility and interaction with the two liquid phases. This simple and effective method is widely used for analyzing small molecules.

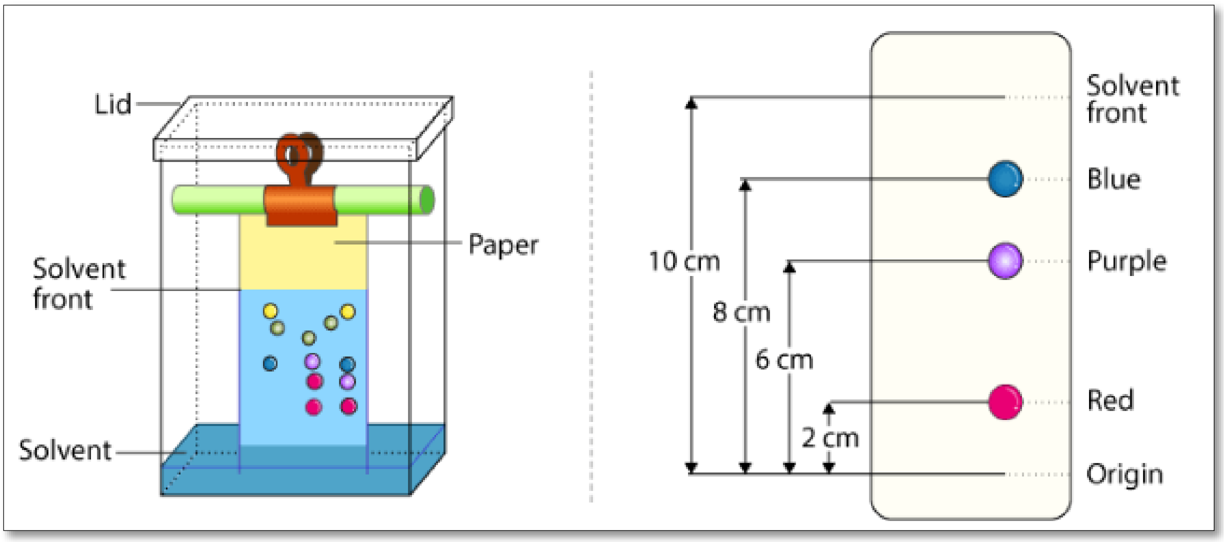


Figure 5. Paper chromatography

2.6.Thin-layer chromatography

Thin-layer chromatography (TLC) is a type of solid-liquid adsorption chromatography, where the stationary phase consists of a solid adsorbent material, such as alumina, silica gel, or cellulose, coated onto glass plates (Figure 6). The mobile phase, a solvent, moves upward through the stationary phase by capillary action. A sample mixture applied near the base of the plate is carried upward at different rates depending on the polarity of the analytes, the stationary phase, and the solvent. This differential movement allows the components to separate.

When the sample contains colorless molecules, techniques such as fluorescence, radioactivity, or chemical reactions can be used to produce visible colored products, enabling their detection. The separation results in a chromatogram, where the position of each molecule can be quantified using the relative mobility (Rf) value. The Rf value, calculated as the ratio of the distance traveled by the molecule to the distance traveled by the solvent front, provides a qualitative means of identifying the analytes. TLC is widely used for rapid and effective analysis of mixtures.

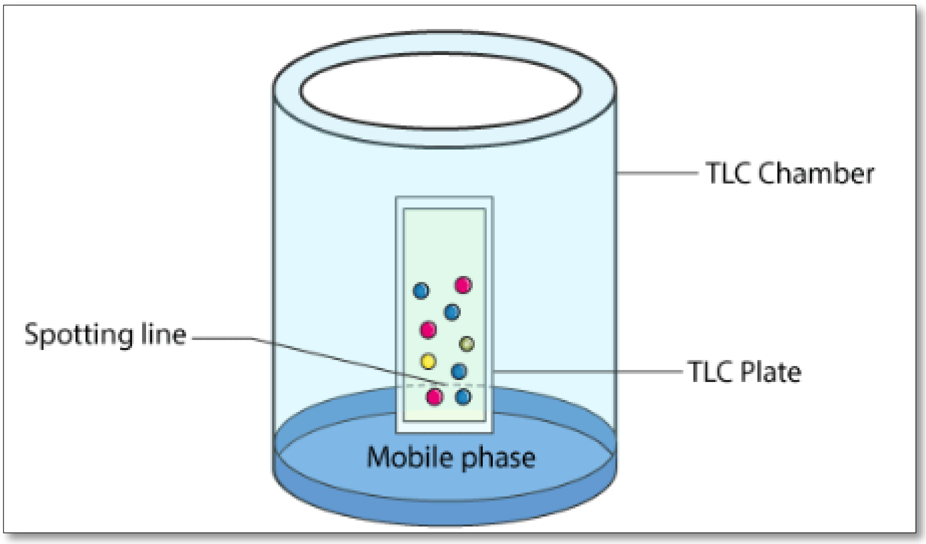


Figure 6. Diagram of Thin Layer Chromatography

2.8.Gas chromatography

Gas chromatography (GC) is a gas-liquid chromatography technique in which the stationary phase is a liquid adsorbed onto the surface of an inert solid within a column. The mobile phase is an inert carrier gas, such as helium (He) or nitrogen (N_2), which flows through the column under high pressure. The sample to be analyzed is vaporized and introduced into the mobile phase, where its components interact with the stationary phase. The separation occurs as different components distribute between the stationary and mobile phases based on their chemical properties (Figure 7).

GC is known for its simplicity, versatility, high sensitivity, and rapid performance. It is particularly effective for separating and analyzing very small amounts of analytes, making it a powerful tool for detecting trace compounds in complex mixtures. This technique is widely used in fields such as environmental analysis, pharmaceuticals, and food safety.

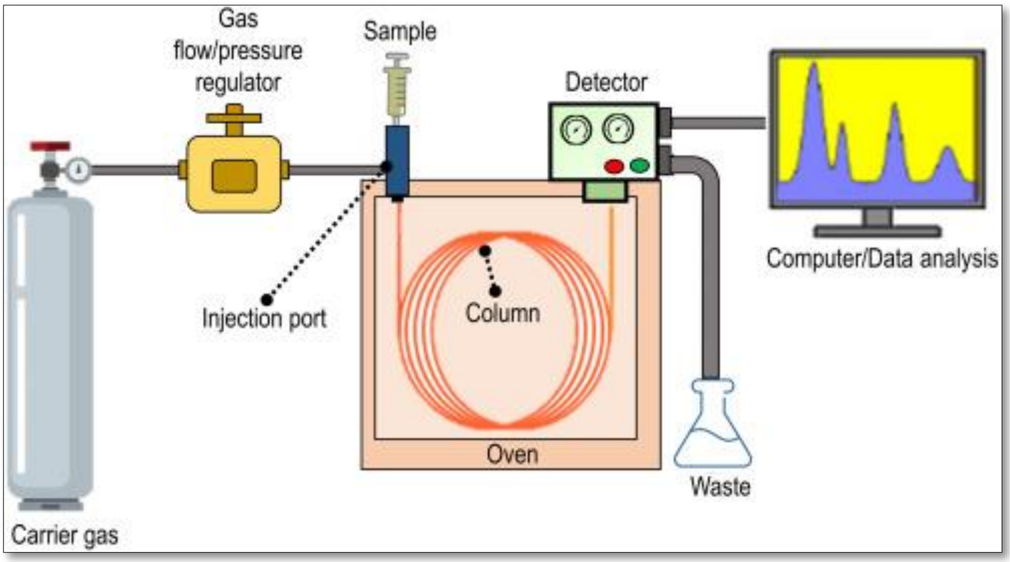


Figure 7. Gas chromatography

2.9.Dye-ligand chromatography

Dye-ligand chromatography is a technique developed based on the ability of certain enzymes to bind purine nucleotides, such as NAD, to the Cibacron Blue F3GA dye (Figure 8). The dye's planar ring structure, which contains negatively charged groups, is structurally similar to NAD, particularly to its adenine and ribose binding sites. This analogy has been confirmed by studies showing that Cibacron Blue F3GA binds to these specific sites on NAD, behaving as an analogue of ADP-ribose. The binding capacity of Cibacron Blue F3GA is 10 to 20 times stronger than that of other affinity adsorbents.

In this technique, proteins that interact with the dye are adsorbed onto the stationary phase. Under optimal pH conditions, the adsorbed proteins can be eluted by applying high-ionic strength solutions, utilizing the ion-exchange properties of the adsorbent. This results in the separation of the bound proteins from the column, offering a highly efficient method for protein purification based on specific molecular interactions.

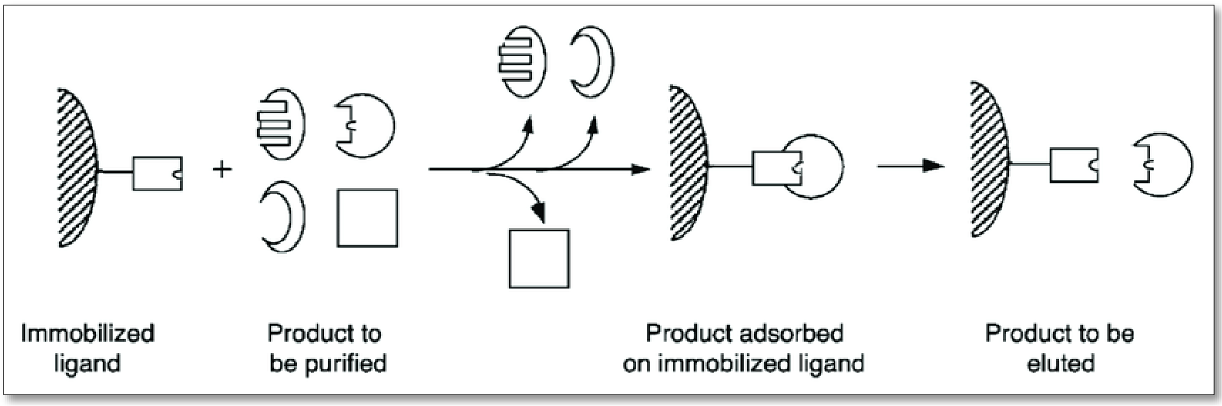


Figure 8. Dye-ligand chromatography

2.10.Hydrophobic interaction chromatography

Hydrophobic interaction chromatography (HIC) is a technique that utilizes adsorbents similar to those used in affinity chromatography, where the matrix is designed to bind ligands. HIC is based on the principle of hydrophobic interactions, where the separation occurs due to the varying degrees of hydrophobicity of the molecules in the sample. In this method, proteins or other biomolecules with hydrophobic side chains interact with the hydrophobic groups on the chromatography matrix. The strength of these interactions is influenced by the salt concentration in the mobile phase, with higher salt concentrations promoting stronger hydrophobic interactions, leading to the retention of more hydrophobic molecules. This technique is commonly used to purify proteins and other biomolecules based on their surface hydrophobicity (Figure 9).

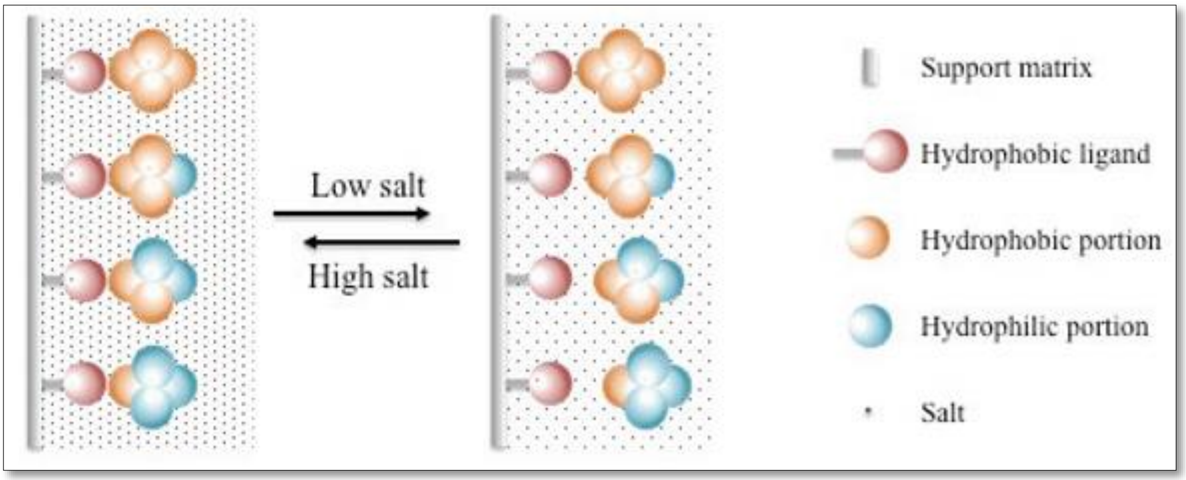


Figure 9. Hydrophobic interaction chromatography

2.11.High-pressure liquid chromatography

High-pressure liquid chromatography (HPLC) is a highly efficient technique used for the structural and functional analysis, as well as the purification, of various biomolecules within a short period. It is particularly effective for separating and identifying amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules. In HPLC, the mobile phase is pumped through the column at high pressures ranging from 10 to 400 atmospheres and at high flow rates (0.1–5 cm/sec). The use of small particle sizes in the stationary phase, combined with high pressure, enhances the separation power, allowing for rapid and precise analysis (Figure 10).

The essential components of an HPLC system include a solvent reservoir, a high-pressure pump, a pre-packed column, a detector, and a recorder. The entire process is typically controlled and monitored by a computerized system, which ensures accurate separation and collection of the analytes. HPLC is widely recognized for its speed, sensitivity, and versatility, making it a cornerstone technique in biochemical and analytical laboratories.

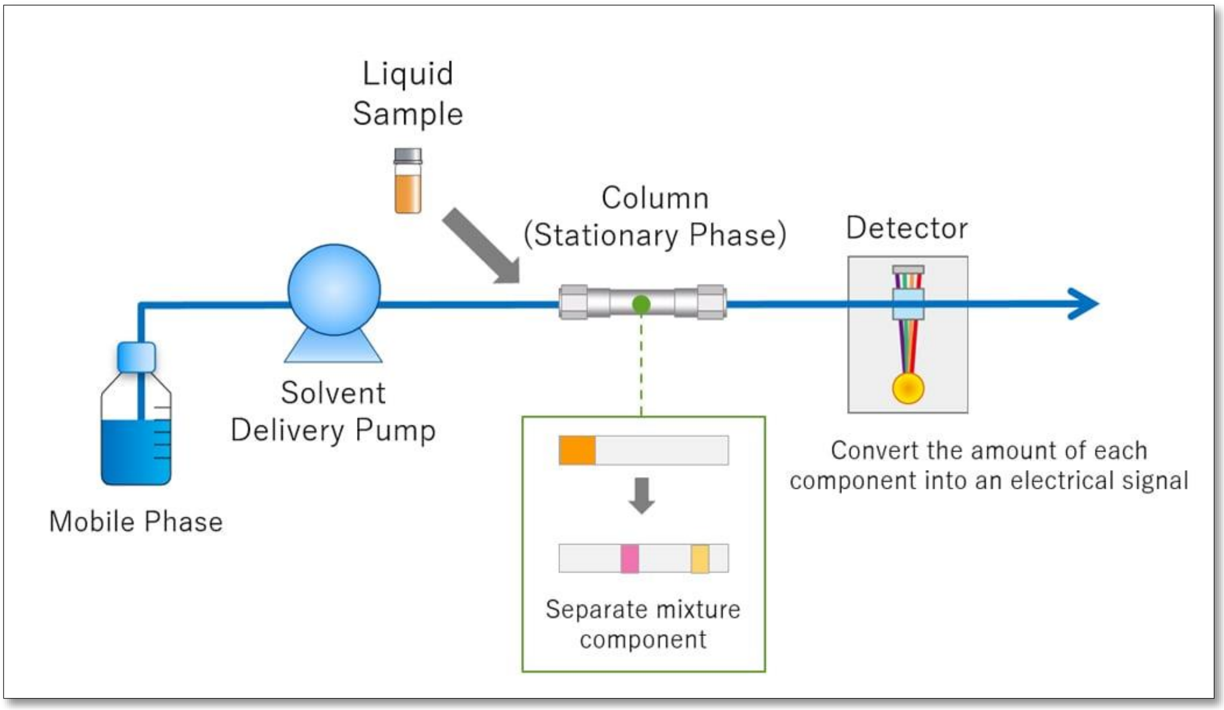


Figure 10. High-pressure liquid chromatography

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Chapter 3: Structure and Organization of Membranes

1.Introduction

Biological membranes are complex structures composed primarily of lipids, proteins, and sugars, with the phospholipid bilayer forming the foundation. This bilayer is made of lipid molecules arranged in two layers, with hydrophilic heads facing outward and hydrophobic tails inward. In addition to lipids, membrane proteins and sugars are essential components. Proteins are crucial for maintaining structural integrity, organization, and regulating material flow across membranes. Sugars, covalently attached to specific lipids or proteins, are localized to one side of the bilayer.

Membrane lipids are categorized into three types: phospholipids, glycolipids, and sterols. Phospholipids, the primary lipids in membranes, consist of two fatty acid chains attached to glycerol and a phosphate group. A common subtype, glycerophospholipids, includes molecules like phosphatidylcholine (PC), where choline is bound to the phosphate group. Variants include phosphatidylserine (PS) and phosphatidylethanolamine (PE), where serine or ethanolamine replace choline. Another class, sphingophospholipids, includes sphingomyelin, which is based on sphingosine.

Glycolipids, which can be based on either glycerol or sphingosine, have a sugar molecule like glucose instead of a phosphate group. Sterols, such as cholesterol in animal membranes and stigmasterol in plant membranes, differ structurally from phospholipids and glycolipids. Cholesterol, for instance, comprises a hydrophilic hydroxyl group, a four-ring steroid structure, and a short hydrocarbon tail. Sterols are largely absent from bacterial membranes but play vital roles in animal and plant membranes.

Together, these components form dynamic and functional structures, enabling membranes to act as barriers, mediators of transport, and platforms for biochemical processes (Figure 1).

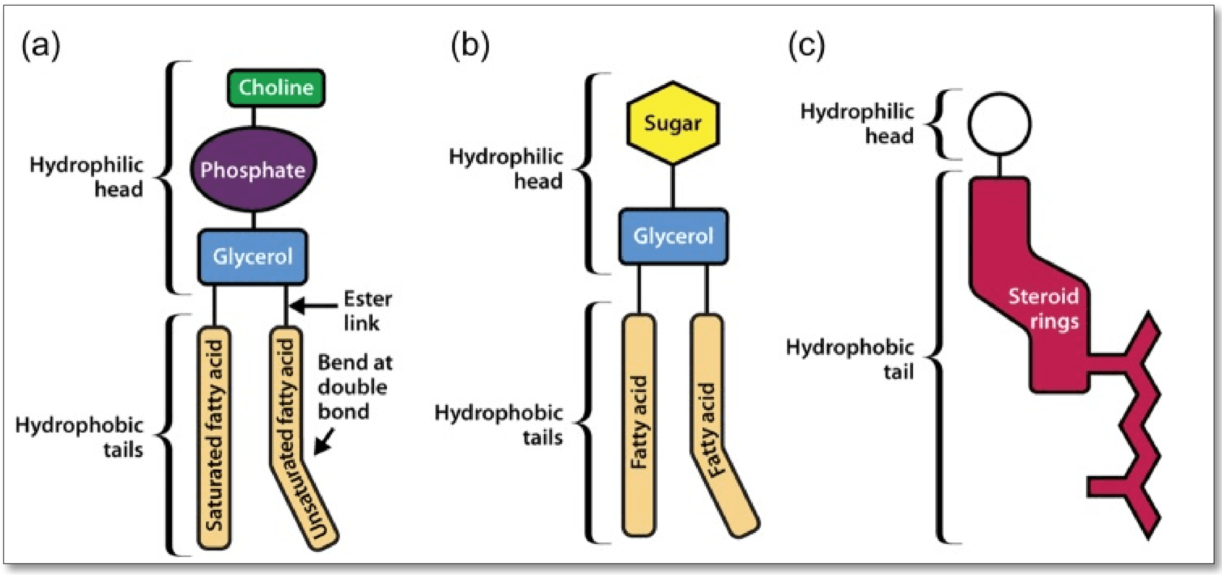

Figure 1. Schematic representation of three types of membrane lipid. (a)phosphatidylcholine, (b) Glycolipid, (c) A sterol

2.Role of Sugars and Amphipathic Nature of Membrane Lipids

The sugars attached to lipids and proteins in biological membranes serve as important markers due to their structural diversity. These sugar chains can function in cell recognition processes, such as determining blood group antigens on the surface of red blood cells. These antigens, composed of specific sugar chains, interact with antibodies and can trigger immune responses. This is why matching blood groups is critical in transfusions to avoid adverse reactions. Additionally, specific carbohydrate markers found on diseased cells, such as cancer cells, play a role in diagnosis and treatment, offering valuable targets for medical interventions (Figure 2).

Membrane lipids are amphipathic, meaning they possess both hydrophilic (water-attracting) and hydrophobic (water-repelling) regions. This dual nature drives their ability to form bilayers, a hallmark of biological membranes. The hydrophilic heads of these lipids orient towards the aqueous external and internal environments, while the hydrophobic tails align inward, away from water, forming a lipid-friendly core. This configuration creates a stable, functional barrier.

In an aqueous environment, amphipathic lipids spontaneously organize into structures such as liposomes-spherical bilayer formations with water on both sides. These liposomes mimic the structural properties of cellular membranes, highlighting the inherent self-organizing capabilities of membrane lipids. This natural bilayer arrangement ensures optimal interactions between the hydrophilic and hydrophobic regions, maintaining the integrity and functionality of cellular membranes.

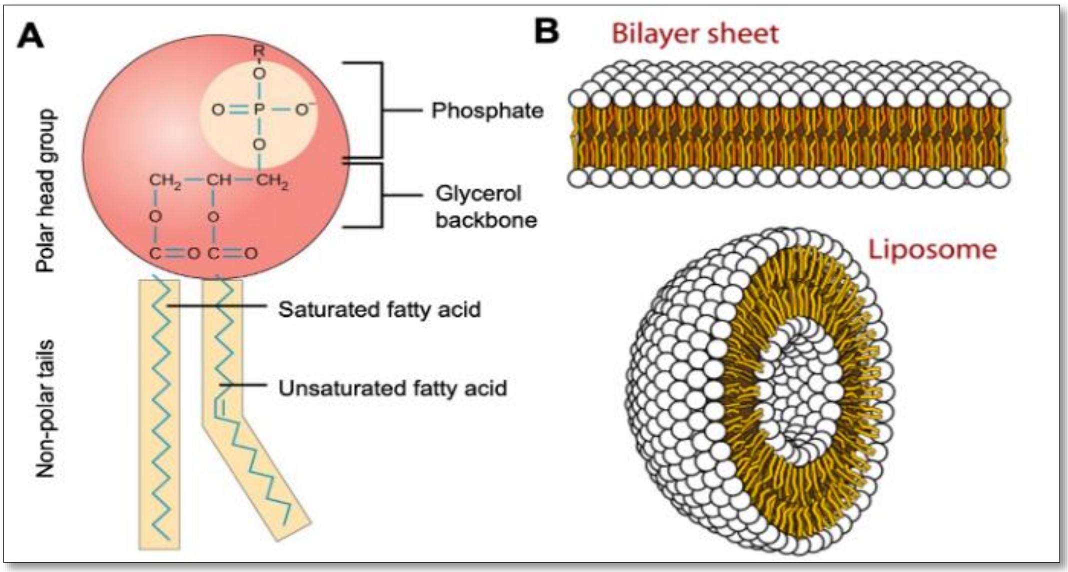

Figure 2. A membrane bilayer and liposome

3.Biological membranes and the fluid mosaic model

The bilayer structure of biological membranes was first demonstrated by E. Gorter and F. Grendel in 1925. By extracting lipids from red blood cells and measuring their surface area, they observed that the lipids occupied twice the surface area of the cell. Since red blood cells lack internal membranes, they deduced that the plasma membrane consists of two layers of lipids.

Building on this foundational understanding, the fluid mosaic model, proposed by Jonathan Singer and Garth Nicolson in 1972, describes membranes as dynamic and fluid structures. In this model, lipids and proteins are not static but can diffuse laterally within the membrane. Phospholipids, for example, exhibit significant mobility: they can move around the perimeter of a red blood cell within approximately 12 seconds or traverse the length of a bacterial cell in just 1 second. Additionally, phospholipids rotate along their axis and display flexibility in their lipid tails, further contributing to the membrane's fluidity (Figure 3).

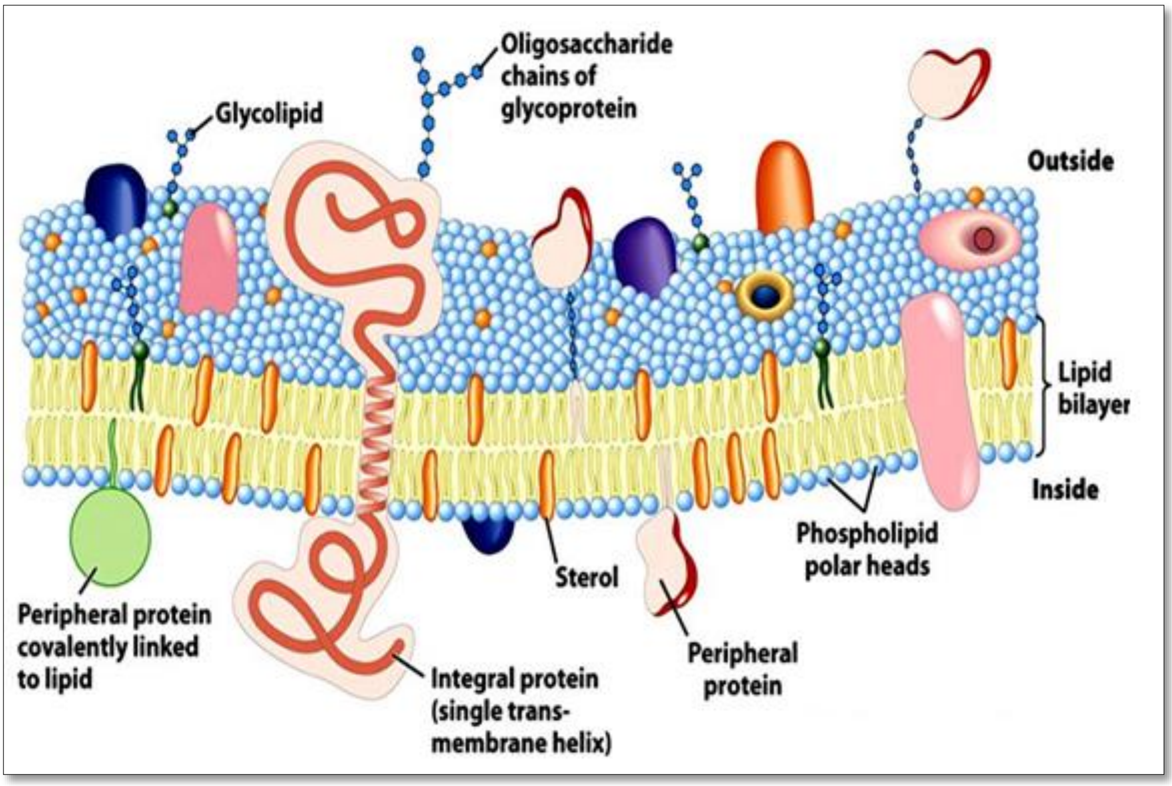

Figure 3. Biological membranes and the fluid mosaic model

Membrane proteins also exhibit lateral movement, though at slower rates than lipids. In some cases, proteins are localized to specific regions of the membrane to support cellular polarity, enabling distinct functional domains within the cell. An example is the use of glycosyl-phosphatidylinositol (GPI) anchors to target proteins to the apical membrane of epithelial cells, ensuring their exclusion from the basolateral membrane.

Experimental methods, such as fluorescence photobleaching, provide visual evidence of the fluid nature of membranes. This technique involves tagging a lipid or protein with a fluorescent marker, such as green fluorescent protein (GFP). A small region of the membrane is then bleached using a focused laser beam, rendering it non-fluorescent. Over time, fluorescence reappears in the bleached area as tagged molecules diffuse into it, demonstrating the lateral mobility of lipids and proteins within the bilayer.

4.Lipid and Membrane Dynamics

Despite the dynamic lateral movement of lipids and proteins in the bilayer, their vertical movement, or "flip-flop," from one leaflet to the other occurs at an extremely low rate. This limited vertical movement results from the significant energetic barrier associated with passing the hydrophilic head (in lipids) or hydrophilic regions (in proteins) through the hydrophobic interior of the membrane. This restriction helps maintain distinct lipid compositions between the inner and outer leaflets of the bilayer and ensures that membrane proteins are inserted with the correct orientation for proper function. However, specialized enzymes called flippases (or phospholipid translocators) can overcome this barrier. Using ATP, flippases facilitate the flip-flop of lipids between leaflets. In eukaryotic cells, flippases are found in organelles such as the endoplasmic reticulum (ER), where they play a key role in translocating newly synthesized lipids.

5.Membrane Synthesis

Membranes are formed by the addition of lipids to pre-existing membranes. In prokaryotes, this process occurs on the inner leaflet of the plasma membrane, which faces the cytoplasm. In eukaryotes, membrane synthesis primarily takes place at the ER on the cytoplasmic leaflet of its membrane (Figure 4).

Enzymes embedded in the ER membrane catalyze the assembly of membrane lipids. Two fatty acids from the cytoplasm are sequentially attached to glycerol phosphate, forming a diacylglycerol phosphate that becomes anchored in the ER membrane via its fatty acid chains. Subsequently, the phosphate group is replaced with a specific head group, such as phosphate and choline, completing the lipid structure. Flippases in the ER membrane can then move some of these newly synthesized lipids to the luminal leaflet. Similarly, in prokaryotic membranes, flippases transfer lipids from the cytoplasmic leaflet to the outer leaflet, ensuring proper lipid distribution.

In eukaryotic cells, once lipids are synthesized, they are distributed to various intracellular membranes. Vesicles bud from the ER and travel through the secretory pathway, starting with the ER-Golgi intermediate compartment (ERGIC) and then the Golgi apparatus, where lipids are sorted. From the Golgi, vesicles deliver lipids to specific destinations, such as the plasma membrane or lysosomes.

Some organelles, such as mitochondria, acquire lipids from the ER through a different mechanism involving phospholipid-exchange proteins. These water-soluble proteins extract phospholipids from the ER and insert them into the mitochondrial membranes, ensuring that these organelles maintain their required lipid composition. This intricate system of vesicle transport and lipid exchange enables cells to create and maintain the distinct lipid profiles necessary for the functions of various membranes.

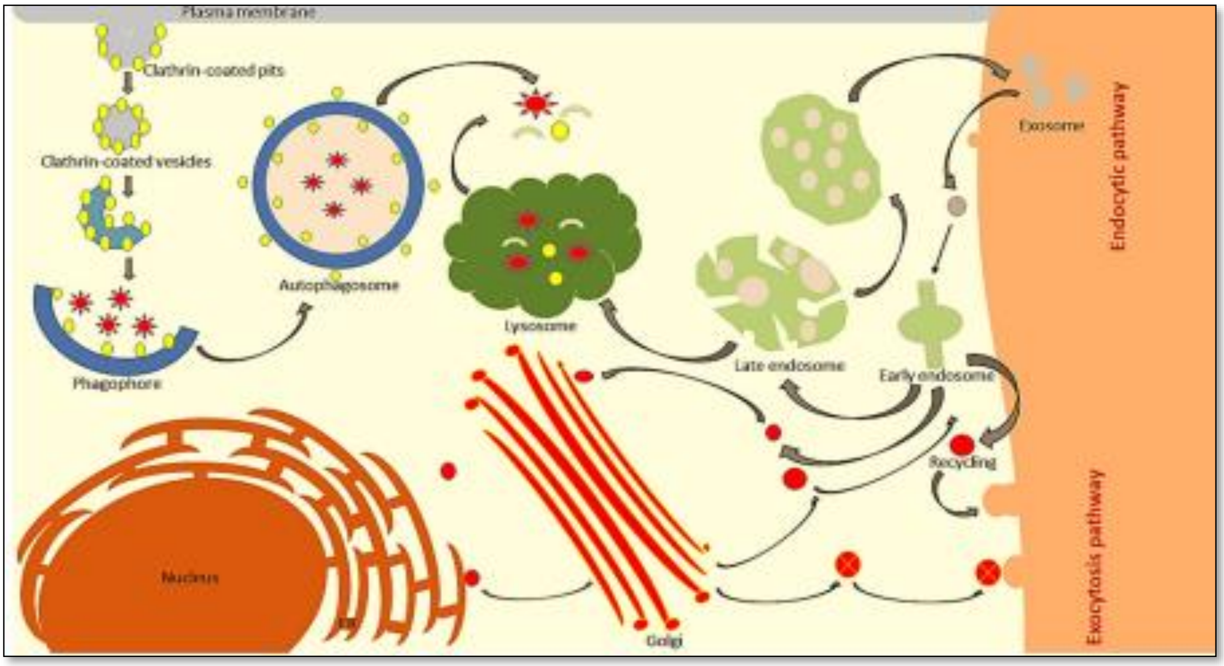

Figure 4. Membrane traffic in eukaryotic cells

6.Lipid Distribution in Cellular Membranes

The lipid composition of bilayers is asymmetrical, with distinct differences between the inner and outer leaflets. In mammalian plasma membranes, phosphatidylcholine (PC) and sphingomyelin are primarily found in the outer leaflet, while phosphatidylserine (PS) and phosphatidylethanolamine (PE) are concentrated in the inner leaflet. This asymmetry is crucial for maintaining normal cell function.

During apoptosis (programmed cell death), this asymmetry breaks down as PS, a negatively charged lipid, is translocated to the outer leaflet by the enzyme scramblase, a specialized type of flippase. The exposure of PS on the cell surface changes the plasma membrane's charge and acts as a signal for phagocytic cells, such as macrophages, to recognize and engulf the dying cell.

Lipid distribution also varies across different organelles within eukaryotic cells. For example, cholesterol, synthesized in the endoplasmic reticulum (ER), is found in low concentrations in the ER membrane itself but is progressively enriched as it is transported through the secretory pathway. The cholesterol content increases in the Golgi apparatus, particularly in the trans-Golgi network, and peaks in the plasma membrane.

This gradient of cholesterol correlates with membrane thickness, as cholesterol contributes to membrane rigidity and thickness. Membranes in the late Golgi and plasma membrane are slightly thicker compared to those in the ER. This thickness difference is thought to aid in protein sorting, as transmembrane proteins in the plasma membrane typically have longer hydrophobic domains than those residing in the ER. This lipid composition gradient, therefore, plays a role in maintaining the functional and structural organization of cellular membranes.

7.Membrane Proteins: Structure and Synthesis

Membrane proteins are essential nanomachines that enable cells to interact with their environment, communicate, and transport molecules. These proteins convert the phospholipid bilayer, which would otherwise act as an impenetrable barrier, into a dynamic interface capable of facilitating nutrient uptake, waste removal, and response to stimuli. Both unicellular and multicellular organisms rely on membrane proteins to survive. The specific proteins embedded in a membrane determine its permeability and the signals it can recognize, thus defining the membrane's functionality.

7.1.Synthesis of Membrane Proteins

In eukaryotic cells, membrane protein synthesis begins on cytosolic ribosomes. For proteins destined for the plasma membrane, ER, or other membranes, the process involves co-translational insertion into the membrane. This mechanism was elucidated in the 1970s by Günter Blobel, David Sabatini, and Bernhard Dobberstein, who discovered the existence of a "binding factor" that directs ribosomes to the ER (Figure 5).

The process begins with the synthesis of a short protein segment, which contains an N-terminal signal sequence. These sequences are hydrophobic stretches of 20–30 amino acids flanked by a basic region at the N-terminus and a polar domain at the C-terminus. This motif is recognized by the signal recognition particle (SRP), a molecule that binds the signal sequence, ribosome, and SRP receptor located in the ER membrane.

Upon binding SRP, the ribosome pauses translation. The SRP-ribosome complex docks at the SRP receptor, which is located adjacent to a protein pore called the translocon in the ER membrane. The translocon allows the nascent protein to thread through and insert into the membrane. The translocon also features a lateral gate, enabling the protein to integrate into the lipid bilayer. Once docking is complete, SRP dissociates, and protein synthesis resumes. This process, known as co-translational targeting, ensures that membrane proteins are correctly inserted and oriented in the ER membrane.

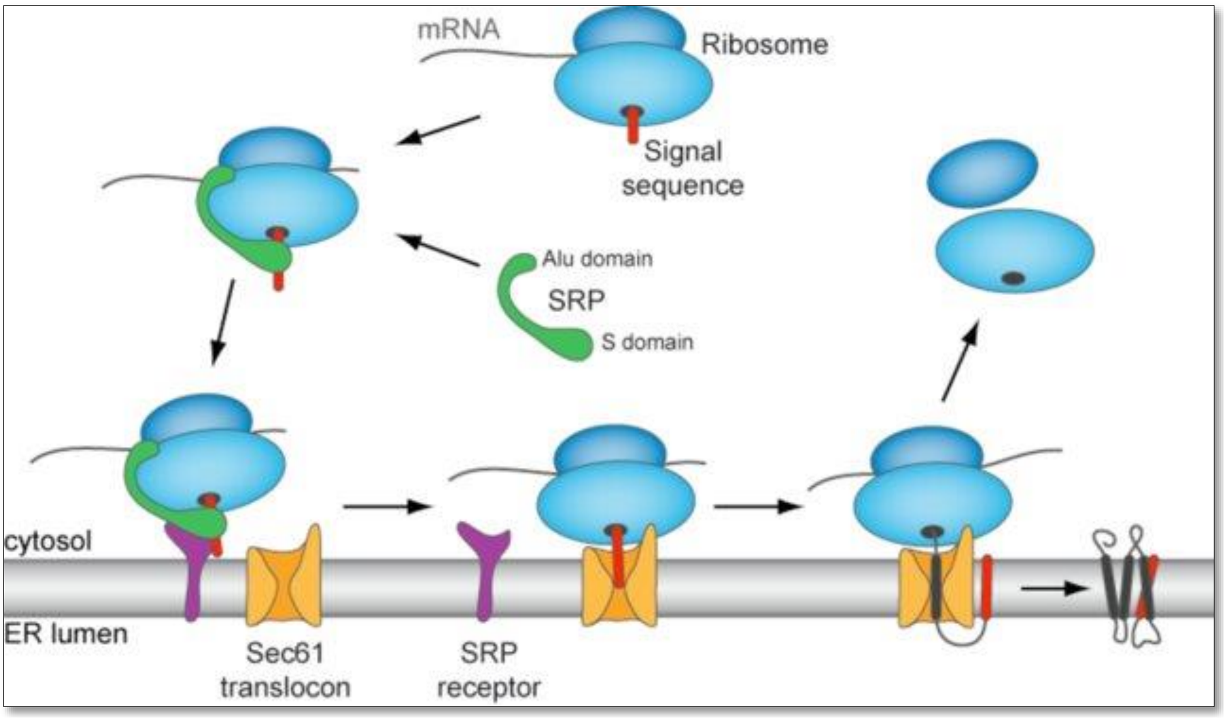

Figure 5. Synthesis of Membrane Proteins

7.2.Co-Translational and Post-Translational Targeting of Membrane Proteins

In higher eukaryotes**,** co-translational targeting is the dominant mechanism for delivering proteins to the ER. This involves the simultaneous synthesis and insertion of proteins into the ER membrane. However, post-translational targeting-where proteins are delivered to the ER after their synthesis is complete—is more prevalent in yeast and prokaryotes**.**

In higher eukaryotes, post-translational targeting may occur when the protein is small and the signal sequence emerges only after synthesis is completed. This process can operate through SRP-dependent or SRP-independent pathways, enabling flexibility in the delivery of membrane proteins to the ER.

7.3.Structure and Function of Membrane Proteins

Membrane proteins play essential roles in controlling molecular traffic and communication across cell membranes. Their structures can be diverse:

- **α-Helices**: Found in many membrane proteins, these structures span the bilayer with hydrophobic stretches of ~20 amino acids, which corresponds to the ~30 Å thickness of the lipid bilayer. For example, the Ca^{2+}-ATPase spans the membrane ten times with ten hydrophobic segments.
- **β-Barrels**: These proteins form pore-like structures, with hydrophobic residues facing the bilayer and hydrophilic residues lining the pore.
- **Non-spanning Proteins**: These proteins associate with membranes through hydrophobic anchors but do not fully traverse the bilayer.

7.4.Transport Functions of Membrane Proteins

Membrane proteins control the movement of molecules across biological membranes, enabling the cell to take in nutrients, expel waste, and maintain homeostasis (Figure 6).

7.4.1.Passive Transport

In passive transport, molecules move across membranes without requiring energy, following their concentration or electrochemical gradients. Examples include:

- **Simple Diffusion**: Small uncharged molecules (e.g., water, gases) pass directly through the membrane.
- **Facilitated Diffusion**: Larger or charged molecules move through channel proteins or carrier proteins without energy input, traveling "downhill" from high to low concentration.

7.4.2.Active Transport

Active transport involves the movement of molecules against their concentration gradients, requiring energy. Sources of energy include:

- **ATP Hydrolysis**: Direct energy from ATP powers transport (e.g., ion pumps).
- **Light**: In phototrophic organisms, light can drive active transport.
- **Secondary Active Transport**: The "downhill" movement of one molecule (e.g., H^+) provides energy for the "uphill" movement of another molecule within the same transporter.

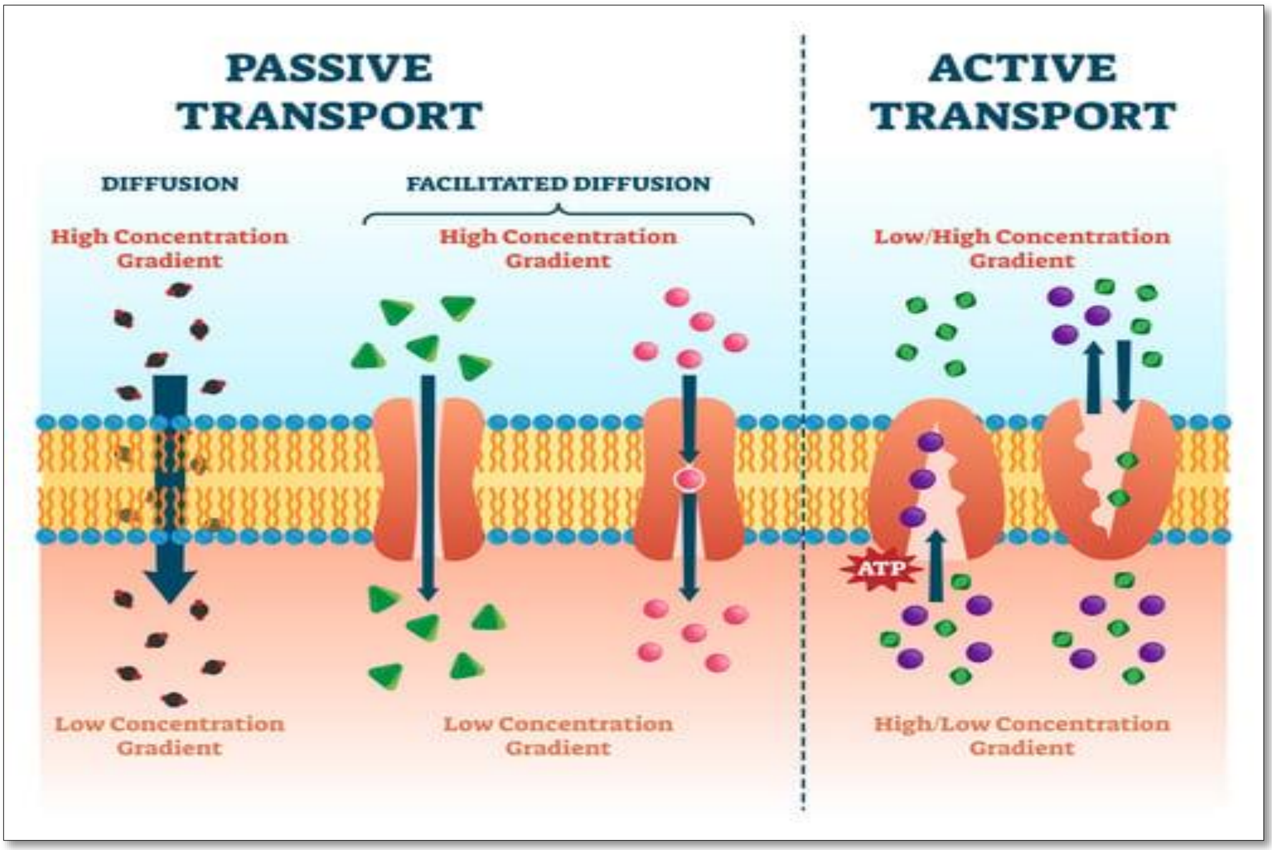

Figure 6. Passive and active Transport

7.4.2.1. Energy Sources for Active Transport

Active transport mechanisms rely on various energy sources to move substances across membranes against their gradients. One primary source is ATP hydrolysis, used directly in primary active transport. For example, SERCA (Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase), a P-type ATPase, uses energy from ATP breakdown to pump two Ca^{2+} ions into the endoplasmic or sarcoplasmic reticulum per ATP molecule hydrolyzed. This process prevents toxic cytoplasmic Ca^{2+} accumulation, with SERCA undergoing phosphorylation during its transport cycle.

Another energy source is the electrochemical gradient, which powers secondary active transport. In this mechanism, the movement of one molecule down its gradient is coupled with the uphill transport of another molecule. Additionally, light energy can drive active transport in specialized systems, such as bacteriorhodopsin in photosynthetic bacteria, where light powers the movement of ions or molecules across the membrane.

Examples of P-Type ATPases

- **SERCA**: Maintains low cytoplasmic Ca^{2+} concentrations and undergoes significant ATP-powered conformational changes during its cycle.
- **Na^+/K^+-ATPase**: Pumps three Na^+ ions out of the cell and two K^+ ions into the cell per ATP molecule hydrolyzed, playing a critical role in maintaining resting membrane potential and cellular homeostasis.

Key Takeaway

Membrane proteins, such as carrier proteins and ion pumps, demonstrate the adaptability of cellular systems in regulating transport. While passive carriers rely on existing gradients, active transport systems like ATPases allow cells to establish and maintain ionic environments essential for functions such as muscle contraction, neurotransmission, and metabolism.

To transport glucose into cells, the Na^+–glucose symporter utilizes the electrochemical gradient of Na^+ across the plasma membrane. Na^+ is present at much higher concentrations outside the cell, and the inside of the cell is negatively charged relative to the outside. This allows Na^+ to flow down its electrochemical gradient, enabling the symporter to move glucose against its concentration gradient into the cell. Since both Na^+ and glucose move in the same direction—into the cell—this is referred to as symport. To maintain this process, the Na^+ gradient must be preserved, which is accomplished by the Na^+/K^+-ATPase pump that actively transports Na^+ out of the cell, ensuring a low intracellular Na^+ concentration.

Similarly, both Na^+ and Ca^{2+} are present at higher concentrations outside the cell than inside. The Na^+–Ca^{2+} exchanger, like the Na^+–glucose symporter, relies on the Na^+ electrochemical gradient to transport Ca^{2+} against its concentration gradient. However, this transporter functions as an antiporter, with Na^+ flowing in to drive the movement of Ca^{2+} out of the cell. The exchanger operates with a ratio of three Na^+ ions entering the cell for every two Ca^{2+} ions it pumps out. It moves Ca^{2+} more efficiently than the plasma membrane versions of SERCA but has a lower affinity for Ca^{2+} than these P-type ATPases. As with the Na^+–glucose symporter, the Na^+/K^+-ATPase pump is essential for maintaining the low intracellular Na^+ concentration needed for proper function of the Na^+–Ca^{2+} exchanger.

Chapter 4: Biosynthesis Overview

1.Introduction

Biosynthesis encompasses the metabolic pathways that produce both primary and secondary metabolites, essential for various biological functions. These processes are energy-intensive, fueled by the energy released during carbohydrate glycolysis and the citric acid cycle.

The oxidation of glucose, fatty acids, and amino acids generates ATP (adenosine triphosphate), a high-energy molecule central to energy transfer in cells. ATP, produced during the catabolism of primary compounds, is recycled to drive anabolic (biosynthetic) reactions. While catabolism involves oxidation, biosynthesis (or anabolism) primarily consists of reduction reactions, requiring reducing agents such as NADP (nicotinamide adenine dinucleotide phosphate). These reactions are catalyzed by coenzymes, with coenzyme A (CoA) being one of the most prevalent. CoA is composed of adenosine diphosphate (ADP) and pantetheine phosphate.

1.1. Terpenoids

Terpenoids represent a vast and diverse group of phytoconstituents with limited functional and structural similarities. This group includes compounds such as steroids, carotenoids, and gibberellic acid, making it one of the most significant classes of bioactive plant compounds, with over 23,000 known structures.

- **Structure and Biosynthesis**

  Terpenoids are polymeric derivatives of isoprene units, synthesized from acetate through the mevalonic acid pathway. Their formation involves the head-to-tail linkage of isoprene units, with the number of these units determining the specific classification of the terpene.

- **Pharmacological and Industrial Importance**

  Terpenoids have extensive pharmacological applications, including their use in treating diseases in both humans and animals. For example, diterpenes, which are particularly abundant in the Lamiaceae family, are known for their antimicrobial and antiviral properties.

Beyond their medicinal properties, terpenoids play a significant role in the industrial sector, where they are widely utilized in the production of flavors, fragrances, and spices. These compounds contribute to the distinctive aromas and tastes found in essential oils, perfumes, and food additives, making them highly valuable in the cosmetic, pharmaceutical, and food industries.

To date, thousands of terpenoid molecules have been isolated and characterized from a vast array of plant species. Despite their structural diversity, these bioactive compounds are synthesized through a limited number of biosynthetic pathways, which regulate their formation from simple molecular precursors. The key steps in these pathways, along with the enzymatic transformations involved, are illustrated in Figure 2. Understanding these biosynthetic mechanisms is essential for biotechnological applications, including the sustainable production of terpenoids through synthetic biology and metabolic engineering.

1.1.1. Functions

Terpenoids play a vital role in plant adaptation to both abiotic and biotic stressors, such as herbivores and pathogens. Their diverse chemical structures enable plants to defend themselves against environmental threats while also contributing to ecological interactions. Additionally, the high volatility and reactivity of certain terpenoids allow them to influence atmospheric composition, further extending their impact beyond the plant itself.

One of the key features of terpenoids is their volatility, which enables plants to communicate with other organisms through airborne infochemicals. This mechanism allows sessile plants to interact with neighboring plants, pollinators, and natural enemies of herbivores, enhancing their survival strategies.

Beyond their role in communication, plant-derived volatile terpenoids are integral to several physiological processes, including:

1. Plant-to-plant interactions, allowing plants to detect and respond to environmental cues.
2. Symbiotic signaling, fostering beneficial relationships with microorganisms and other species.
3. Attracting pollinators, which is essential for plant reproduction and biodiversity.

These biological functions have important agricultural and commercial implications, including the development of sustainable pest control methods that reduce the need for synthetic pesticides. Additionally, the production of flavors and fragrances highlights the economic and ecological significance of terpenoids. Their multifunctional nature makes them key targets for biotechnological advancements in crop protection, ecosystem management, and industrial applications.

1.1.2. Pharmacological Activities

Extensive research on terpenoids has revealed their biological and physiological significance. Numerous studies have highlighted their broad spectrum of pharmacological properties, which have led to the discovery of terpenoids with medicinal potential **and** agricultural applications**.**

- **Medicinal Applications**

  Recent findings indicate that certain nitrogenous terpene derivatives demonstrate anti-hypertensive activity, suggesting their potential use in medicine. Furthermore, terpenoids exhibit notable antimicrobial and insecticidal properties, positioning them as candidates for synthetic terpenoid pathways for therapeutic use.

- **Agricultural Applications**

The antimicrobial and fungicidal activities of terpenoids have led to their application as pesticides and fungicides in agriculture and horticulture.

1.2. Essential Oils

Essential oils are natural aromatic and volatile compounds extracted from various parts of plants, such as leaves, flowers, stems, and roots. These oils are a rich source of secondary metabolites and play significant roles in both plants and human applications.

1.2.1.Biological Role in Plants

In plants, essential oils serve as chemical defenses against herbivores, pathogens, and pests. They also function in plant-to-plant communication, pollinator attraction, and symbiotic signaling. Their volatility allows for the emission of airborne signals that support survival and reproduction.

1.2.2. Industrial and Medicinal Applications

Essential oils are widely used in the pharmaceutical, cosmetic, and food industries for their antimicrobial, antioxidant, and anti-inflammatory properties. They are also prominent in aromatherapy and the production of flavors, fragrances, and natural pesticides, contributing to sustainable agricultural practices.

1.3. Alkaloids

Alkaloids are a group of secondary metabolites characterized by the presence of basic nitrogen atoms. Some related compounds with neutral or weakly acidic properties are also considered alkaloids. In addition to carbon, hydrogen, and nitrogen, alkaloids may contain elements such as oxygen, sulfur, and, more rarely, chlorine, bromine, or phosphorus.

1.3.1. Sources and Production

Alkaloids are produced by a wide range of organisms, including bacteria, fungi, and animals, but are most abundantly found in plants as secondary metabolites. These compounds are often toxic to other organisms and can be extracted using acid-base techniques.

1.3.2. Pharmacological and Historical Significance

Alkaloids are bioactive compounds that exhibit a wide range of pharmacological effects, making them essential in both traditional and modern medicine. For centuries, various cultures have relied on alkaloid-rich plants for their therapeutic properties, using them to treat ailments ranging from pain and infections to cardiovascular and neurological disorders. Many historically significant remedies, such as opium-derived morphine and cinchona bark-derived quinine, have laid the foundation for modern pharmaceuticals.

Beyond their medicinal uses, alkaloids have influenced the development of synthetic drugs and continue to serve as models for novel therapeutic agents. Their physiological effects—including analgesic, stimulant, sedative, and antimicrobial properties—have led to their incorporation into modern clinical treatments. Moreover, their role in plant defense mechanisms against herbivores and pathogens underscores their biological significance and potential applications in agriculture and biotechnology.

1.3.3.Structural Diversity and Classification

Alkaloids exhibit remarkable structural diversity, distinguishing them from other classes of secondary metabolites. Their complex molecular architectures make classification challenging, and over time, different methods have been used to categorize them. Historically, alkaloids were classified based on their natural sources, such as plant families, due to the limited understanding of their chemical structures. However, as analytical techniques advanced, modern classifications shifted towards structural and biosynthetic criteria.

Currently, alkaloids are classified based on their carbon skeletons and precursor molecules. The major categories include true alkaloids, which contain a nitrogen atom within a heterocyclic ring and are derived from amino acids, and protoalkaloids, where the nitrogen is not part of a heterocyclic system. Additionally, alkaloids can be grouped into subclasses such as indole alkaloids (e.g., strychnine), isoquinoline alkaloids (e.g., morphine), pyrrolizidine alkaloids (e.g., senecionine), and tropane alkaloids (e.g., atropine). This structural variation underlies the diverse pharmacological activities of alkaloids, reinforcing their significance in both medicine and natural ecosystems.

1.3.4.Biosynthesis

Alkaloids are primarily synthesized from amino acids, serving as fundamental precursors in their biosynthetic pathways. Among these, tyrosine, tryptophan, ornithine, and lysine are the most common starting materials. The biosynthesis of alkaloids involves multiple enzymatic transformations, including decarboxylation, oxidation, methylation, and cyclization, which contribute to their complex structures and diverse biological activities.

A well-known example is the biosynthesis of morphine, a potent opioid alkaloid derived from the benzylisoquinoline pathway. This process begins with tyrosine, which undergoes hydroxylation and decarboxylation to form dopamine. Dopamine then combines with another tyrosine-derived molecule, 4-hydroxyphenylacetaldehyde, leading to the formation of the benzylisoquinoline skeleton. Subsequent enzymatic modifications, including phenol coupling reactions, methylations, and reductions, ultimately yield morphine.

1.4. Phenolics

Phenolic compounds, derived from plants, form one of the largest groups of secondary metabolites, synthesized by fruits, vegetables, teas, cocoa, and various other plants. These compounds are known for their health benefits, including antioxidant, anti-inflammatory, and anti-carcinogenic properties. They play a protective role against **oxidative stress** and help in preventing certain diseases..

1.4.1. Biological Activity

Simple phenolics exhibit bactericidal**,** antiseptic, and anthelmintic properties, with phenol itself serving as the benchmark for antimicrobial agents. These compounds are widely present in nearly all plants and are the subject of extensive research in chemical, biological, agricultural, and medical sciences**.**

1.4.2. Structural Characteristics

Phenolics are structurally diverse but share a common feature: hydroxylated aromatic rings (e.g., flavan-3-ols). Many phenolic compounds polymerize into larger molecules, such as proanthocyanidins (condensed tannins**)** and lignans. Phenolic acids occur in food plants, often as esters or glycosides conjugated with other natural compounds like flavonoids, alcohols, hydroxyfatty acids, sterols, or glucosides.

1.4.3. Prevalence in Foods

Hydroxybenzoic acids and hydroxycinnamic acids represent the primary phenolic compounds found in plants. Foods like tea, coffee, berries, and fruits are particularly rich in phenolics, with total concentrations reaching up to 103 mg/100 g of fresh weight**.**

1.4. Flavonoids

Flavonoids are a diverse group of water-soluble secondary metabolites found in plants. These compounds are largely responsible for the **coloration** of flowers and other plant parts, such as the red, blue, or yellow pigmentation in many plant tissues. They are mainly present in plant cell vacuoles and are categorized into various types based on their structure, such as anthocyanins, flavones, and flavonols.

1.4.1. Classification and Structure

Flavonoids, a large class of plant secondary metabolites, are classified into three primary groups: flavones, flavonols, and anthocyanins. These compounds play crucial roles in plant pigmentation, defense, and signaling.

Structurally, flavonoids share a common C6-C3-C6 backbone, consisting of two phenolic rings (A and B) connected by a three-carbon bridge (C-ring). This core structure is modified through various hydroxylations, methylations, and glycosylations, leading to extensive structural diversity.

Each subgroup exhibits unique physicochemical properties and biological functions. Flavones and flavonols are typically colorless or yellow compounds, contributing to UV protection and defense mechanisms in plants. In contrast, anthocyanins are highly pigmented molecules responsible for red, blue, and purple hues in flowers and fruits, playing a key role in pollination and seed dispersal. Due to their antioxidant, anti-inflammatory, and antimicrobial properties, flavonoids are also widely studied for their potential health benefits in human nutrition and medicine.

1.4.2. Functions in Plants

Flavonoids are involved in several critical processes in plants, including:

- **Pigmentation**: They contribute to the coloration of flowers, fruits, and leaves, which helps attract pollinators and seed dispersers.
- **UV Protection**: Flavonoids act as natural sunscreens, protecting plants from ultraviolet radiation.

- **Symbiotic Relationships**: Certain flavonoids help regulate nitrogen fixation in symbiotic relationships, particularly with rhizobial bacteria**.**
- **Defense Mechanisms**: They serve as chemical messengers **to** protect plants from herbivores and pathogens. For example, flavonoids can act as inhibitors of microbial growth, which helps plants resist infections.

1.4.3. Agricultural and Medicinal Applications

Flavonoids play a significant role in plant health and can have pharmacological effects in humans. Due to their antioxidant, anti-inflammatory, and antimicrobial properties, flavonoids are of interest in medicine and agriculture. In the agricultural sector, they are explored for pest control and promoting plant growth, while in medicine, they are investigated for their potential health benefits, including their role in reducing the risk of chronic diseases. Flavonoids are also important for their dietary benefits, being found in various fruits, vegetables, and beverages like tea and wine, which are known for their flavonoid content.

1.5 Tannins

Tannins are a group of natural polyphenolic compounds that are derived from the French word "Tanin" (meaning tanning substance). These compounds are known for their ability to precipitate proteins and have a broad range of biological activities. They are formed through the shikimic acid pathway, also called the phenylpropanoid pathway, which also produces other phenolic compounds like isoflavones, coumarins, lignins, and aromatic amino acids.

Tannins are diverse in structure, comprising a variety of oligomers and polymers. They are usually **water-soluble**, although some high-molecular-weight tannins may not dissolve in water. Tannins are commonly classified into two main categories:

1. **Hydrolysable tannins (HT)**: This group includes gallotannins, ellagatannins, and other complex tannins.
2. **Condensed tannins (PA)**: Also referred to as **proanthocyanidins**, these are the most common form of tannins and are formed through the condensation of flavonoid units.

1.5.1. Biological and Medicinal Functions

Tannins have various biological functions in plants, such as protecting against herbivores and pathogens by their antimicrobial and antioxidant properties. In terms of medicinal use, tannin-containing plants have long been utilized for their therapeutic properties. They are commonly used in plant-based medicines for the following purposes:

- **Astringents**: Tannins are used to treat diarrhea due to their ability to constrict tissues and reduce secretions.
- **Diuretic**: They can act as diuretics and have been used to treat conditions such as stomach and duodenal tumors.
- **Anti-inflammatory**: Tannins are known to exhibit anti-inflammatory properties, helping to reduce inflammation and treat various inflammatory conditions.

1.6. Glycosides

Glycosides are a group of compounds that can be composed of phenols, alcohols, or sulfur, and are characterized by a sugar molecule (known as the glycone) attached to a non-sugar portion (the aglycone) via a special type of bond. This structural arrangement makes glycosides distinct in that they store bioactive molecules in an inactive form, often in plants, until they are hydrolyzed by enzymes.

In plants, glycosides are commonly stored as inactive compounds, which can be activated through enzyme hydrolysis, usually in the large intestine. This enzymatic process breaks the bond between the sugar and the aglycone, releasing the active non-sugar part, which can then exert its biological effects.

Because of this feature, glycosides are often considered prodrugs, as they remain biologically inactive until they are hydrolyzed into their active form. This ability to release active components after hydrolysis makes glycosides important for medicinal purposes, as many of them have therapeutic effects once converted into their aglycone form.

Saponins are a class of naturally occurring compounds found in various plants. They are glycosides with a sugar molecule linked to a non-sugar (aglycone) part, which is usually a steroidal or triterpenoid structure. The name "saponin" comes from the Latin word "sapo," meaning soap, because of their ability to form foam when mixed with water, similar to soap.

1.7.Structure of Saponins:

Saponins are made up of two main components:

a. Aglycone (Sapogenin): The non-sugar part, which is typically a steroid or a triterpene.

b. Sugar Moiety: The sugar part, which is usually a monosaccharide or oligosaccharide.

The aglycone is the biologically active part, while the sugar portion helps to make the saponin soluble in water. The combination of these components allows saponins to interact with cell membranes and cause foaming when shaken in water.

1.7.1. Types of Saponins:

a. Triterpenoid Saponins: Derived from triterpenes, these saponins are the most common form. They are often found in plants like ginseng, quinoa, and soybeans.

b. Steroidal Saponins: Derived from steroids, these saponins are found in plants like yucca, asparagus, and certain species of solanaceous plants like potatoes.

1.7.2. Properties and Functions of Saponins:

a. Foaming Agents: The ability to form foam in water is one of the most distinctive properties of saponins. This property is why they are sometimes used in detergents, cosmetics, and as emulsifiers in foods and medicines.

b. Antimicrobial and Antiviral: Saponins exhibit antimicrobial properties, which makes them useful in defending plants against pathogens. Some saponins have been shown to have antibacterial, antifungal, and antiviral effects, making them of interest for pharmaceutical applications.

c. Immune-Boosting: Some studies suggest that saponins may help stimulate the immune system by enhancing the activity of macrophages and increasing the production of antibodies.

d. Anti-inflammatory: Saponins have shown anti-inflammatory effects in various animal models, making them potential candidates for treating inflammatory diseases.

e. Toxicity and Safety: While saponins have health benefits, they are also known for their toxic effects when consumed in large amounts. Saponins can be toxic to red blood cells (hemolytic), causing cell lysis. However, many of the saponins found in common foods, like soybeans and quinoa, are present in non-toxic concentrations, and their toxicity can often be reduced through cooking or processing.

f. Cardiovascular Effects: Some saponins can affect the cardiovascular system. For example, saponins from certain plants may lower blood cholesterol levels, leading to interest in their potential use for heart health. However, their exact role in cardiovascular health needs more research.

1.7.3. Uses of Saponins:

a. Pharmaceutical Applications: Due to their biological activity, saponins are used in the development of various drugs, particularly for their antimicrobial, anticancer, and immune-modulating properties.

b. Agriculture: Saponins are sometimes used in natural pesticides due to their toxic effects on insects and fungi. They can act as natural insecticides, reducing the need for synthetic chemicals.

c. Food Industry: Saponins are used as emulsifiers in the food industry. They help in the preparation of products that require stable mixtures of oil and water. They are also studied for their potential in reducing cholesterol and as dietary supplements.

d. Traditional Medicine: Many plants containing saponins have been used in traditional medicine for various purposes, such as treating skin diseases, improving digestion, and alleviating symptoms of respiratory infections.

Chapter 5: Biological activity

1.1.antioxidant activity

1.1.1.Oxidants

1.1.1.1.Free radicals

Free radicals are atoms, or a group of atoms, with an odd number of electrons on the outer orbit, and they can form when oxygen interacts with certain molecules. Reactive oxygen species (ROS) can be free radicals: superoxide anions (O_2-), hydroxyl radical (OH °), or non-radical but highly unstable molecules (singland O_2, H_2O_2); most free radicals come from the respiratory chain (Table 1).

Table 1.Main free radicals with their chemical formulas

Free radicals	Chemical structures
Hydroxyl radical	**OH°**
hydroperoxid Radical	**HOO°**
Peroxyd Radical	**ROO°**
AlkoxyleRadical	**RO°**
Hydrogenperoxide	**H2O2**
Peroxynitrit	**ONOO°**
Superoxide anion	**O2°-**

In order to stabilize, the radical chemical species will interact very quickly with the surrounding molecules in order to separate from their single electron (reducing radicals) or to acquire an additional electron (oxidizing radicals). This creates reactions in a single electron exchange chain leading to a cascade production of new radical species.

1.1.1.1.2.Sources of free radicals

Free radicals are permanently and in small quantities in the body and according to their origins, there are two categories:

• **Endogenous**: free radicals occur spontaneously and continuously within the organism, within the framework of numerous biological phenomena, either during the symmandrical rupture of a covalent bond, or during a reaction redox with a nonradical compound, during cellular respiration.

• **Exogenous**: free radicals can be of environmental origin following prolonged exposure to the sun, air pollution, ozone, radiation

1.1.2.Oxidative stress

1.1.2.1.Definition

Oxidative stress involves any condition in which oxidative mandabolisms can exert their toxic effects due to high production or alteration of cellular protective mechanisms. It is defined as a deep imbalance in the balance bandween prooxidants and antioxidants in favor of the former and involving the production of reactive oxygen species, which leads to irreversible cell damage (Figure 1).

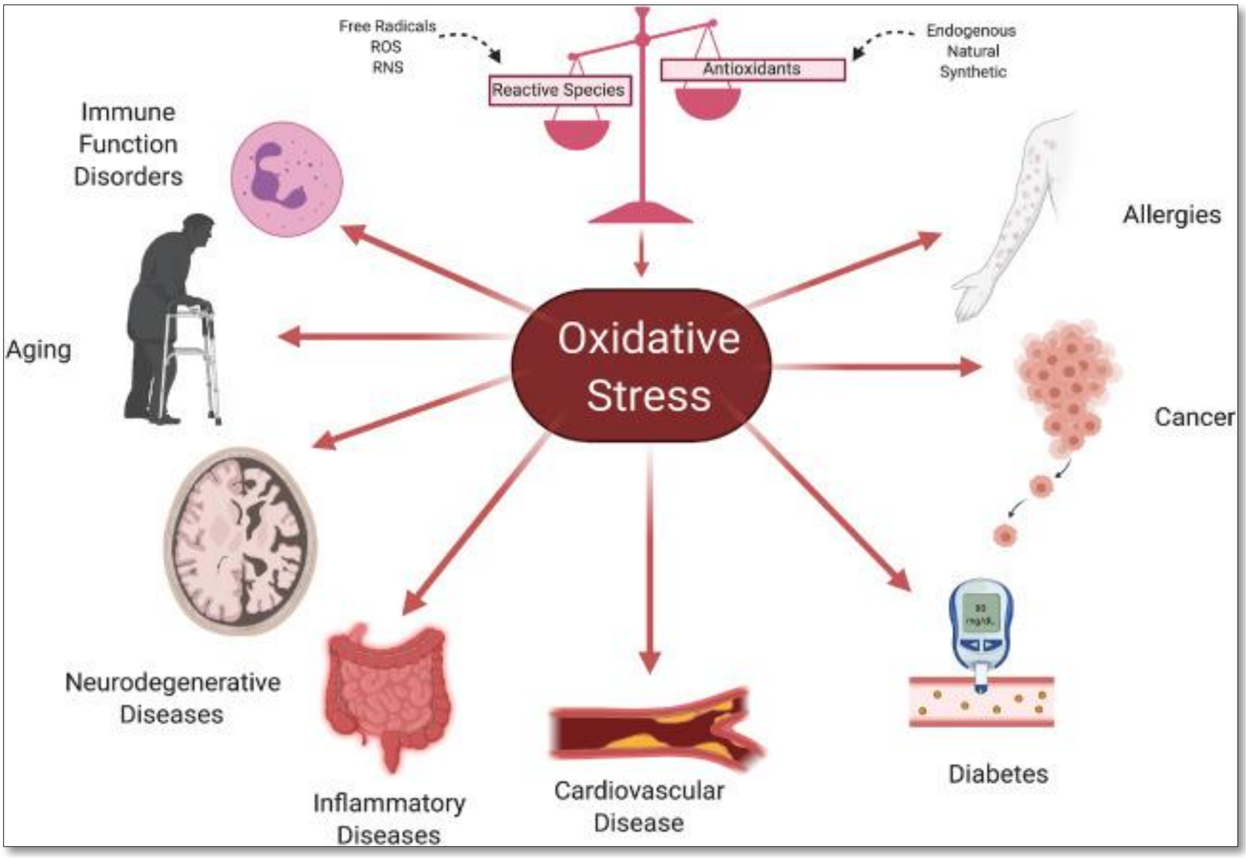

Figure 1. Oxidative stress-induced diseases in humans

1.1.3.Antioxidants

Antioxidants are defined as "any substance, which in low concentration compared to the substrate, capable of being oxidized, thus it prevents or slows down the oxidation of this substrate.

Antioxidants are widely present in our food, either in natural form or in the form of additives used in the food industry. The endogenous system is formed by the enzymes, namely superoxide dismutase, labcatalase and glutathione peroxidase, while the exogenous system is represented by vitamin C, vitamin E, carotenoids and phenolic compounds.

1.2. Evaluation of antioxidant activity

In recent decades, tests for antioxidant activity have been widely developed to evaluate the effectiveness of new compounds. Many mandhodologies are available, making it possible to assess the different physicochemical aspects of the potential antioxidant under different conditions. In this section, the most popular experimental mandhods more widespread will be described as well as the relatively new mandhods called theorandical.

1.2.1. DPPH test

The DPPH (or 2,2-diphenyl-1-picrylhydrazyle) is a temperature stable radical ambient and characteristic blue color. Its stability comes from the high relocation π electrons along the molecule. It is one of the first radicals to have been used to study the structure / antioxidant activity relationship of phenolic compounds. He owns in its structure an unpaired electron on an atom of the nitrogen-nitrogen bridge. His particuliarityn comes from the modification of its UV/Visible absorption properties depending on its state: the shape reduced (e.g., after addition of electron) absorbs at 515-518 nm while its oxidized form does not shows no absorption peak (Figure 2).

The effectiveness of an antioxidant can be measured by its ability to reduce the radical. This was observed historically by the change in color from blue-violand (form oxidized) to yellow (reduced form).

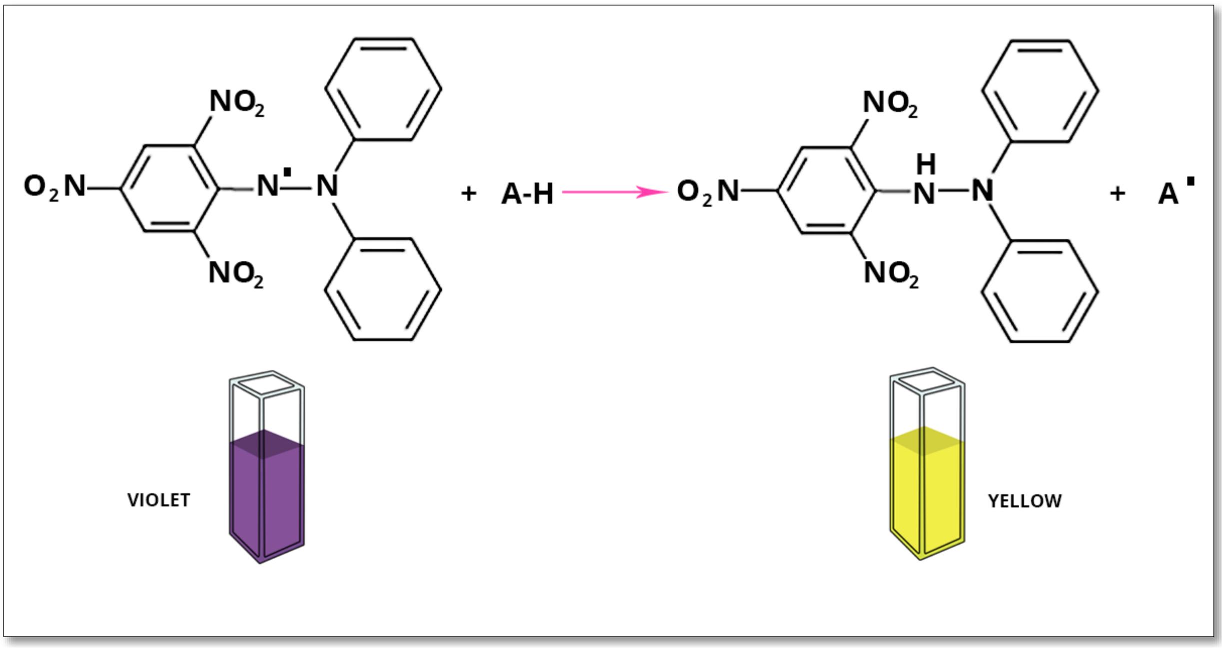

Figure 2. Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant.

1.2.2.TEAC test

The TEAC mandhod (Trolox Equivalent Antioxidant Capacity) is used to measure the ability of a candidate to scavenge the ABTS + cation radical (obtained from ammonium salts 2,2'-Azinobis- (3-andhylbenzothiazolin-6-sulfonic acid). The particularity of this mandhod is the companditive aspect since the measurement will be compared to the capacity of a benchmark antioxidant Trolox. It is important to note that the Trolox is a chemical analogue of vitamin E.

1.2.3.ORAC test

The ORAC (or Oxygen Radical Absorbance Capacity) test is a measurement mandhod of the antioxidant capacity of biological samples in vitro. This mandhod measures oxidative degradation of a fluorescent molecule after adding a generator free radicals, 2,2'-azobis (2-amidinopropane) (AAPH). The thermal degradation of this molecule in the presence of oxygen will cause the generation of free radicals so that can attack the red blood cell membrane (Figure 3).

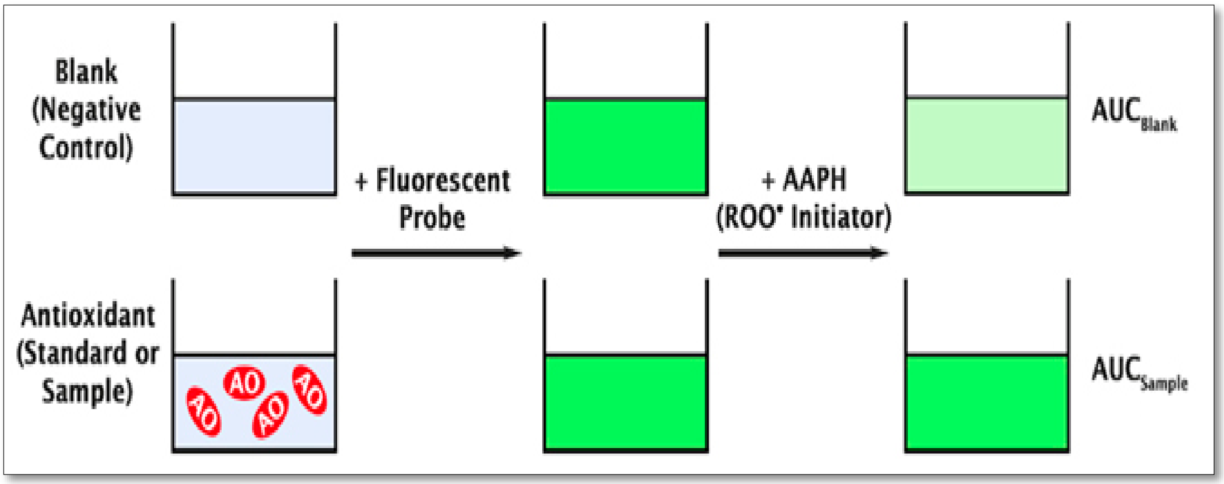

Figure 3. ORAC test

1.2.4.FRAP test

The FRAP (or Ferric Reducing Ability of Plasma) test is a mandhod based on the color change upon reduction of iron, e.g., ferric ion (Fe^{3+}) to the ion ferrous (Fe^{2+}) by electron transfer (Figure 4). This reduction is made a presence of a antioxidant. By the nature of the reduction reaction, the antioxidant must present an electron donor capacity. The hydrogen atom transfer will not be the privileged mechanism. Absorbance is measured at 593 nm.

This test is inexpensive, simple, reproducible and rapid. However he is not able to evaluate the antioxidant activity of thiols (SH), therefore including polypeptides and cysteine group proteins.

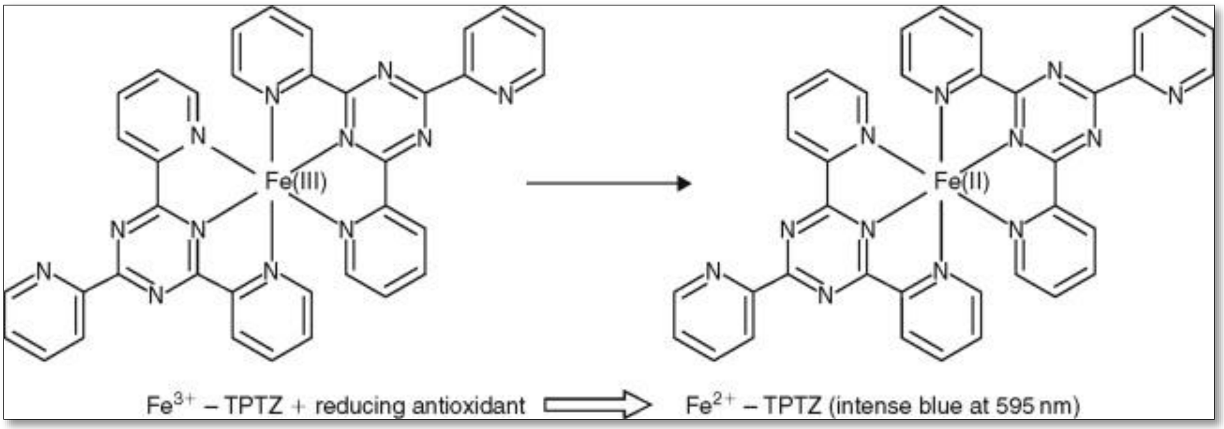

Figure 4. Reaction for FRAP assay.

1.2.5.TRAP test

This TRAP (or Telomeric Repeat Amplification Protocol) test is specific to the action antioxidants on peroxyl radicals ROO •. These radicals will be produced by free radical generators. For this test, the BAP [2,2-azo-bis (2-amidinopropane) hydrochloride] or AAPH [2,2'-azobis (2-amidinopropane)] will be used. This mandhod makes it possible to quantify non-enzymatic antioxidants (glutathione, andc.) as well as to measure the antioxidant capacity of plasma and serum. On the other hand, this mandhod is based on the fact that each antioxidant has a latency time before its action. Thus the correlation with other evaluation mandhods is particularly complicated.

1.2.6.β-carotene–linoleic acid test

This is one of the rapid mandhod to screen antioxidants, which is mainly based on the principle that linoleic acid, which is an unsaturated fatty acid, gands oxidized by ‘‘Reactive Oxygen Species’’ (ROS) produced by oxygenated water.

The antioxidant potential can be assessed by dandermining the ability to inhibit the oxidation of ß-carotene. In this test, the oxidation of linoleic acid produces peroxide radicals which attack the double bonds of ß-carotene (figure 32), which leads to discoloration of the latter measured spectrofiguremandrically at 490 nm .In the absence of an antioxidant rapidly bleaches the typically orange of β-carotene. The antioxidants reduced the extent of β-carotene bleaching by neutralising the linoleate-free radical and other free radicals formed in the system (Figure 4).

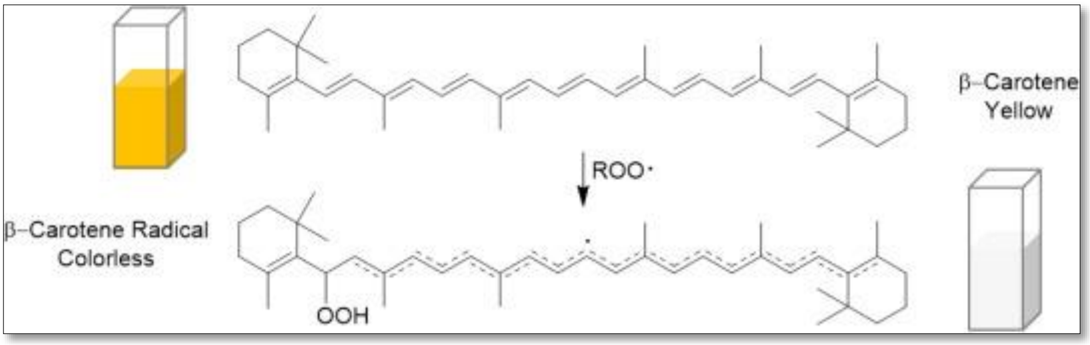

Figure 4. β-carotene reaction mechanism.

2.Antimicrobial activity

From birth, man is in contact with microorganisms which will gradually colonize his skin and mucous lining. Many means are used to resist these microorganisms. We can schematically distinguish 3 groups: anatomical barriers, natural (or innate) resistance mechanisms and acquired immunity**.**

The treatment of bacterial infections is mainly based on the use of antibiotics. The large-scale and somandimes inappropriate prescription of these agents can lead to the selection of multidrug-resistant strains, hence the importance of directing research towards the discovery of new pathways which constitute a source of inspiration for new herbal medicines.

Polyphenols, in particular flavonoids and tannins, are recognized by their toxicity vis-à-vis microorganisms. The mechanism of toxicity can be linked to the inhibition of hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactivate microbial adhesins, transport and cell envelope proteins.

2.1.Mechanisms of Action of the Essential Oils and/or Their Components

The antimicrobial activity of EOs, similar to all natural extracts, is dependent on their chemical composition and the amount of the single components. Many of the antimicrobial compounds are constitutively expressed by the plants, and others can be synthesised as mechanism of self-defence in response to pathogens. Vegandables, spices and fruits with high level of EOs are excellent sources of natural elements with activity against microorganisms of agricultural and health interest. These molecules can be naturally present in their active form in the plant or can be activated by specific enzymes when the vegandal organism is subjected to particular biotic or abiotic stress Different amounts of specific compounds can affect the antimicrobial activity of EOs. For example, high concentrations of cinnamic aldehyde, eugenol or citral confer antimicrobial properties to Eos.

The monoterpenes and phenols present in thyme, sage and rosemary EOs possess noticeable antimicrobial, antifungal and antiviral activity. Some EOs, such as those found in basil, sage, hyssop, rosemary, oregano and marjoram, are active against *E. coli, S. aureus, B. cereus* and *Salmonella spp*. but are less effective *against Pseudomonas spp*. due to the formation of exopolysaccharides that increase resistance to EOs. The mechanism of action of EOs depends on their chemical composition, and their antimicrobial activity is not attributable to a unique mechanism but is instead a cascade of reactions involving the entire bacterial cell; togandher, these properties are referred to as the “essential oils versatility”. In general, EOs act to inhibit the growth of bacterial cells and also inhibit the production of toxic bacterial mandabolites. Most EOs have a more powerful effect on Gram-positive bacteria than Gram-negative species, and this effect is most likely due to differences in the cell membrane compositions (Figure 4)

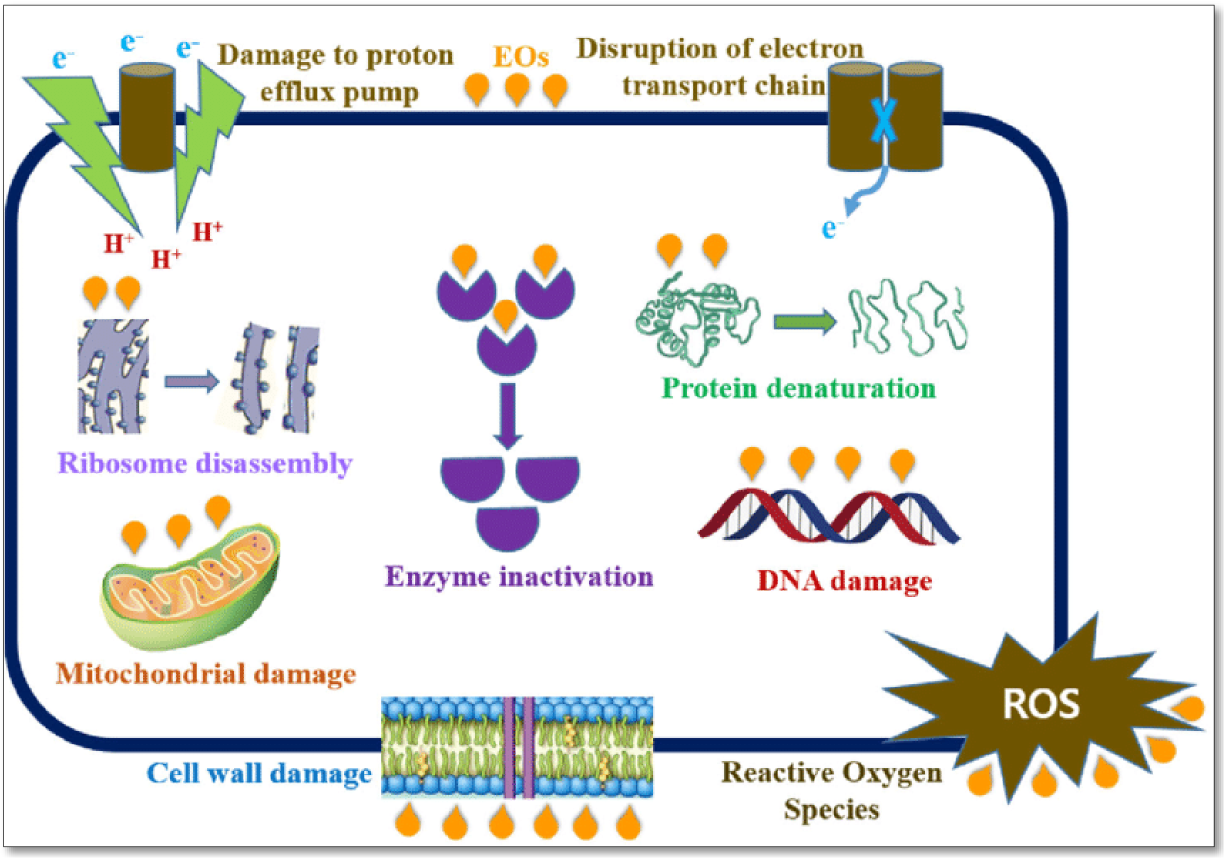

Figure 5. Proposed mechanism of antibacterial action of essential oils.

3. Healing activity

3.1 Definition of the Skin

The skin is the largest organ of the human body, acting as a protective barrier between the internal environment and external factors. It serves multiple essential functions, including protection against pathogens, regulation of body temperature, sensory perception, and immune defense. Structurally, the skin is composed of three main layers: the epidermis, dermis, and hypodermis. The epidermis, the outermost layer, consists mainly of keratinized epithelial cells that provide a waterproof shield and protection against harmful environmental agents. Beneath it, the dermis contains connective tissue, blood vessels, nerve endings, and hair follicles, contributing to skin elasticity, structural support, and sensory reception. The deepest layer, the hypodermis, is composed of fat and connective tissue, playing a key role in insulation, energy storage, and cushioning against mechanical impacts. Additionally, the skin is involved in vital physiological processes such as vitamin D synthesis, wound healing, and thermoregulation through sweat production and blood vessel adjustments. Its complex structure and multifunctionality make it an essential organ for maintaining overall health and homeostasis.

3.2 Functions of the Skin

Skin healing is a phenomenon that allows damaged skin to recover its initial properties and functions. It is a dynamic and interactive process, which involves: soluble mediators; blood cells and the extracellular matrix.

The skin is considered the largest and heaviest organ in the human body.

In adults, the average surface area of the skin is around 2 square meters, with a mass of approximately 5 kilograms, varying depending on the size and weight of individuals.

Its primary function is protective: it serves as a barrier against the environment while also limiting water loss. It is also involved in the perception of sensory stimuli detected through the nerve endings present in all skin compartments.

The skin participates in thermoregulation through various mechanisms. The main way to regulate the body's internal temperature is a balance in the capillaries of the skin between

3.3 Skin Structure

The skin plays a crucial role in thermoregulation through mechanisms like vasoconstriction, which conserves heat, and vasodilation, which helps eliminate excess heat. Adipose tissue and hair provide thermal insulation, while sweating facilitates heat dissipation.

Additionally, the skin serves a vital metabolic function. Exposure to UV-B radiation from sunlight enables the synthesis of vitamin D, a nutrient essential for calcium absorption and bone health.

To perform these diverse functions effectively, the skin is organized into distinct layers:

- The epidermis, which interfaces directly with the external environment.
- The dermis**,** located beneath the epidermis, providing structural support and housing various functional components.
- The hypodermis, the deepest layer, primarily composed of adipose tissue for insulation and energy storage.
- Cutaneous appendages, such as hair follicles, sebaceous glands, and sweat glands, which contribute to the skin's protective and regulatory functions (Figure 6).

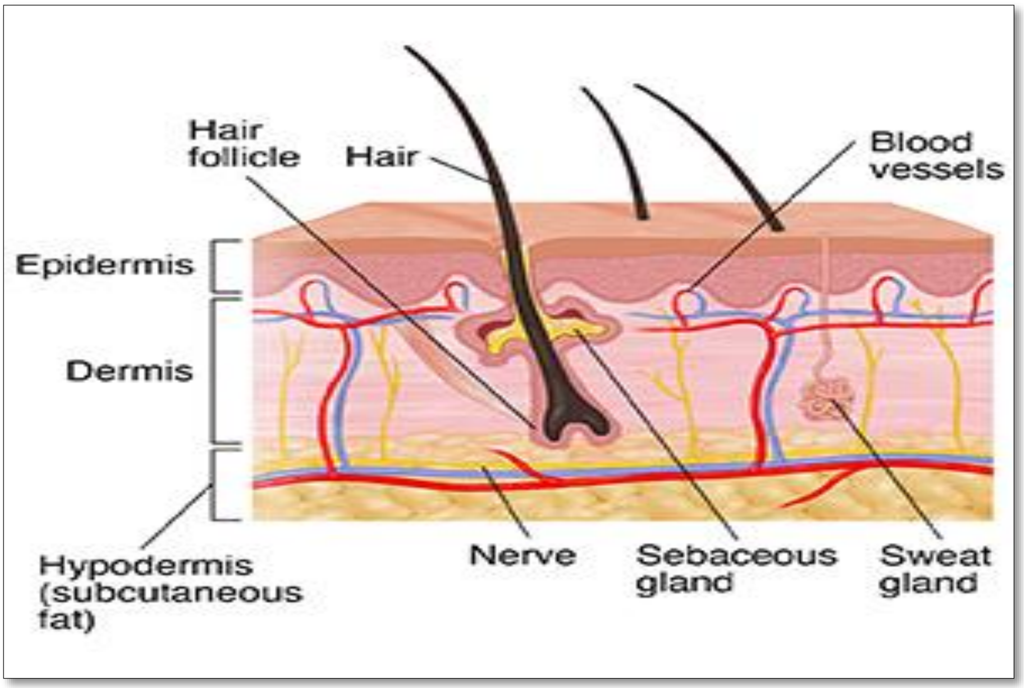

Figure 6. Skin Structure

3.3.1 Epidermis :

3.3.2 Dermis :

It is a multi-stratified epithelial tissue of ectodermal origin, corresponding to the outermost layer of the skin. One of its main functions is to serve as a barrier between the organism and its external environment.

The dermis not only provides nutrients, immunity, and other support to the epidermis but also plays a role in the regulation of temperature, pressure, and pain. The dermis has a

3.3.3 Hypodermis

The hypodermis, also known as the subcutaneous tissue, is the deepest layer of the skin, located beneath the dermis. It is primarily composed of adipose tissue and connective tissue, which provide insulation, cushioning, and energy storage. The hypodermis plays a crucial role in protecting underlying muscles, bones, and organs from mechanical shocks and external pressures. Additionally, it serves as an important site for metabolic functions, including lipid storage and hormone production. This layer also contains blood vessels and lymphatic vessels that help regulate body temperature and facilitate nutrient exchange between the skin and deeper tissues. Due to its composition, the thickness of the hypodermis varies depending on factors such as age, sex, and body region. It contributes to the overall flexibility and mobility of the skin while acting as a structural support system for the upper layers.

3.4.1 Physiology of Wound Healing

3.4.2 Definition of Healing

Healing is the biological process through which the body repairs damaged tissues and restores their structural and functional integrity. It involves a complex series of cellular and molecular events aimed at replacing injured or lost tissue with new, functional tissue. Healing can occur through regeneration, where the original tissue is fully restored, or through repair, where scar tissue forms to close the wound. This process typically consists of four overlapping phases: hemostasis (blood clot formation), inflammation (immune response and removal of debris), proliferation (cell growth and tissue formation), and remodeling (strengthening and maturation of the new tissue). The speed and effectiveness of healing depend on various factors, including the extent of the injury, overall health, nutrition, and underlying medical conditions.

3.4.3 Healing phases

3.4.3.1 Hemostasis phase

The hemostasis phase is the first step in the wound healing process, occurring immediately after tissue injury. Its primary function is to stop bleeding and establish a stable environment for subsequent healing phases. This phase involves vascular constriction, platelet aggregation, and the activation of the coagulation cascade. Upon injury, blood vessels constrict (vasoconstriction) to minimize blood loss. Platelets then adhere to the damaged endothelium, releasing clotting factors and forming a temporary platelet plug. The coagulation cascade is activated, leading to the formation of a fibrin clot, which stabilizes the wound and serves as a scaffold for incoming cells involved in tissue repair. Additionally, platelets release growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β), which signal the recruitment of inflammatory cells to the injury site. This phase typically lasts from a few minutes to several hours, depending on the severity of the wound and the individual's physiological condition.

3.4.1 Wounds

Wound healing is a complex biological process that restores the integrity of damaged tissues. There are two main types of wounds: acute wounds and chronic wounds. Acute wounds result from trauma, such as cuts or surgical incisions, and generally follow a predictable healing process, often resolving spontaneously. In contrast, chronic wounds fail to progress through the normal healing stages and require specialized medical care. These wounds, such as leg ulcers and pressure sores, often persist due to underlying conditions like poor circulation, infection, or diabetes.

One of the advanced treatment options for chronic wounds is alginate dressings, which are derived from seaweed and known for their high absorbency. These dressings help manage wound exudate, promote a moist healing environment, and facilitate autolytic debridement.

Healing is a dynamic physiological process that involves multiple cellular and molecular mechanisms to restore damaged tissue. The healing duration varies significantly depending on the wound type and external factors, such as infection, underlying diseases, and patient health status. The process unfolds in four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Each phase plays a crucial role in ensuring effective wound closure and tissue regeneration.

This initial phase occurs when the tissue is injured and consists of multiple steps. Initially, vasoconstriction of the blood vessels in the wound bed occurs. This vessel constriction lasts for 15 to 60 seconds and aims to slow bleeding to facilitate the establishment of primary hemostasis.

3.4.3.2 Inflammatory phase

The inflammatory phase is the second stage of the wound healing process and plays a crucial role in preventing infection and preparing the wound for tissue repair. This phase typically lasts between 24 to 48 hours, but it can extend up to several days depending on the severity of the wound and external factors such as infection or immune response.

During this phase, vasodilation occurs, allowing increased blood flow to the wound site. This leads to the classic signs of inflammation: redness, swelling, heat, and pain. The process begins with the activation of platelets, which release chemical mediators such as cytokines and growth factors (e.g., platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β)). These molecules attract immune cells to the wound.

3.4.3.3 Proliferation or Granulation phase

During this stage, a platelet plug is formed to address any residual bleeding. The next step is the formation of a fibrin network, which serves both as a matrix for fibroblast development and as protection against microorganisms that could lead to infection. This secondary hemostasis stage lasts between 3 and 5 minutes and allows the coagulation process to take effect. The formation of this network stimulates the growth of fibroblasts and smooth muscle cells within the vascular wall, initiating the repair process and ultimately leading to the dissolution of the clot or fibrinolysis. The total time for this initial hemostasis stage can range from 1 to 3 days.

The second phase of the wound healing process involves the removal of foreign bodies and dead tissue at the wound site. It begins with the secretion of cytokines and growth factors by fibroblasts present in the wound bed. Among these pro-inflammatory molecules are interleukins such as interleukin 1β and tumor necrosis factor or TNFα. These factors initiate an inflammatory response, including the migration of neutrophils. Subsequently, monocytes will be recruited to the wound site and differentiate into macrophages. These inflammatory cells are responsible for phagocytosis, angiogenesis, and cellular recruitment for debridement and the formation of a provisional extracellular matrix at the wound bed. In parallel, they contribute to the recruitment of specific molecules such as lysosomal enzymes like lipases or proteases and oxygen species involved in cleaning the wound. From a macroscopic perspective, this phase is characterized by the appearance of specific signs called the cardinal signs of inflammation. These signs include the development of edema and erythema at the wound site, a sensation of warmth, and a pulsatile pain.

This third phase of the wound healing process occurs 2 to 10 days after the appearance of the injury, with the goal of restoring a stable epithelial barrier for the formation of scar tissue. Following the inflammatory phase, macrophages and neutrophils secrete cytokines and chemokines to initiate cellular recruitment at the lesion site.

During this cellular proliferation, the phenomenon of granulation occurs. Granulation allows for the formation of a new, highly vascularized connective tissue. Fibroblasts are activated by growth factors to secrete collagen and fibrinogen, creating a new extracellular

3.4.3.4 Remodeling or Maturation phase

matrix to close the wound. Angioblasts form a new network of capillaries, and keratinocytes aggregate at the edges of the wound to facilitate tissue epithelialization.

Remodeling or maturation is the longest phase of the wound healing process. It occurs two to three weeks after the injury and can last up to a year. During this phase, the immune response gradually decreases, and a reorganization of the extracellular matrix and granular tissue takes place to achieve scar tissue with a structure as close as possible to the original tissue. This includes a decrease in vascularization and an increase in the proportion of collagen.

Some wounds do not follow this traditional healing process and remain in a prolonged inflammatory phase. These wounds are referred to as chronic wounds. The prevalence of this type of wound has increased alarmingly in recent years, particularly due to the rising number of diabetic individuals, a predominant factor in people with chronic wounds (Figure 6).

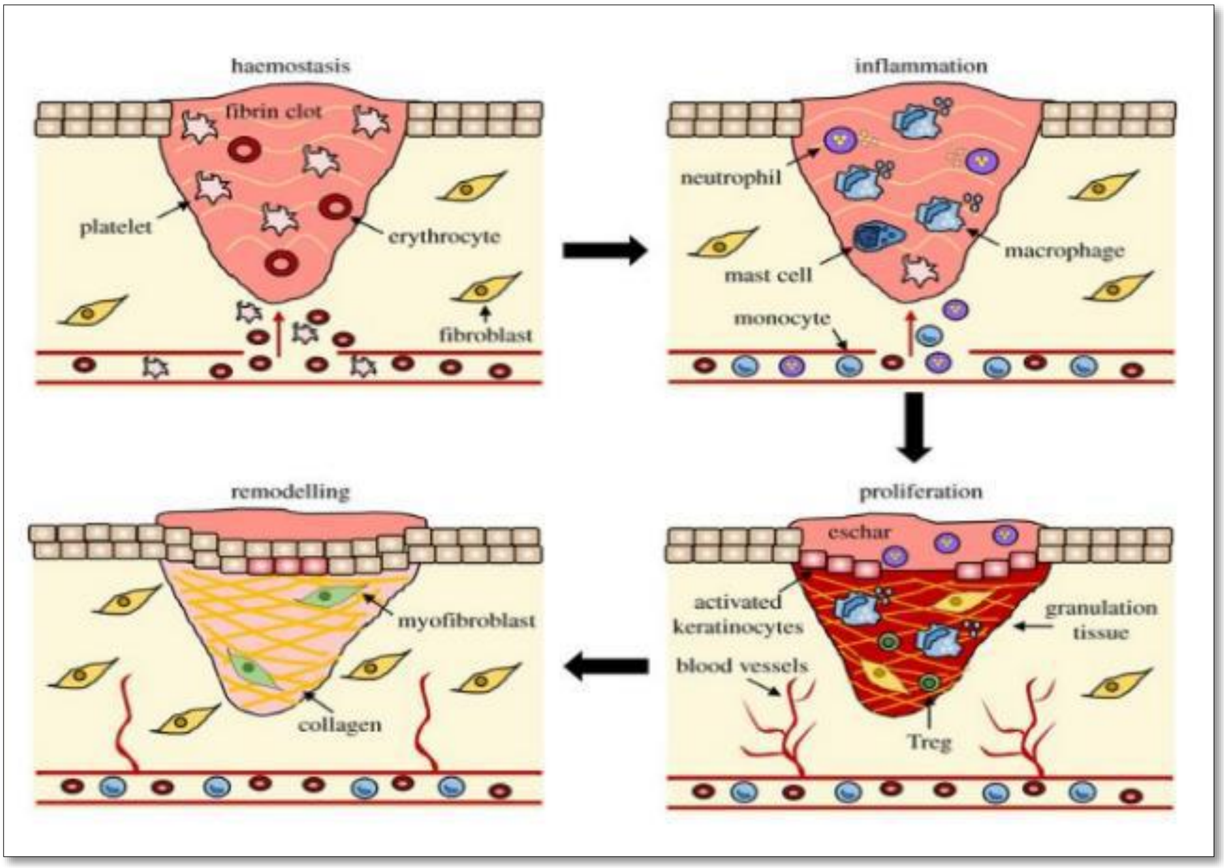

Figure 6. The stages of wound repair and their major cellular components.

Diabetes and anti-diabetic activities

2.3.1. Definition and Diagnostic

Diabetes is a serious metabolic disease that increasingly threatens public health around the world. It affects around 4% of the world's population and is expected to an increase of 5% in

2025.It is characterized by a disorder at the level of regulation lipid, carbohydrate and protein metabolism. and due thus to an excess of sugar (glucose) in the blood. who defines it as blood sugar greater than 1.26g / L (7 mmol / L) fasting characteristic, confirmed by two blood tests consecutive.

Diagnosis of all types of diabetes is simple, it is based essentially on the measurement of fasting blood sugar and induced hyperglycemia. The diagnostic criteria for diabetes have changed over time as studies show a relationship between the onset of complications and blood sugar levels.

2.4. Cytotoxicity

2.4.1. Definition

Is defined as the toxicity caused due to the action of chemotherapeutic agents on living cells, a cytotoxic compound can cause cell damage or death either through necrosis or apoptosis.

2.4.2. Toxicological study

Toxicology seeks to understand the response of living organisms exposed to toxic substances by studying the fate of these toxic substances and their effects in the body. More specifically, it seeks to define the bioavailability of these substances by the various routes of entry into the body, to understand how the absorbed dose is distributed over time among the various organs and tissues, and to establish the link between, on the one hand, the exposure dose and the concentration in the target organs and on the other hand the link between the concentration in the target organs and the appearance of effects on these action organisms involved at the level of organs and cells.

2.4.3. Acute toxicology

All chemicals are toxic when dosages are administered. Sufficient. So that a drug with pharmacological effects can possibly be used as a medicine, it is necessary that the activity occurs at therapeutic doses. The toxicity tests therefore accompany the testing of pharmacological activities during the selection of new substances. The toxicity can be assessed, among other things, by determining the LD_{50}.

Acute toxicity of a chemical is all the effects on the body caused by short-term exposure to a high dose, usually a single one. In fact, in experimental studies in animals, acute toxicity is determined by the LD50 (oral route - intraperitoneal route). The LD_{50} (median lethal dose) is the single statistically deduced dose, believed to cause death of 50% of animals to whom the substance was administered.

2.4.4. Chronic toxicity

Purpose of a chronic toxicity study is to determine the effects of a substance test, in a given mammalian species, following prolonged exposure and repeated. Repeated administration toxicity tests in animals are always carried out when a substance is of possible therapeutic interest.

Conclusion

Structural and functional biochemistry is fundamental to understanding the molecular mechanisms that sustain life. Biomolecules such as proteins, carbohydrates, lipids, and nucleic acids play essential roles in cellular structure, metabolism, and communication. Proteins serve as enzymes, structural components, and signaling molecules, while carbohydrates function as energy sources and structural elements. Lipids contribute to membrane integrity and energy storage, and nucleic acids encode genetic information.

The study of biochemical techniques, including chromatography and spectroscopy, allows for the isolation and characterization of these biomolecules, advancing research in medicine, biotechnology, and pharmaceuticals. Additionally, the structural organization of biological membranes, the role of secondary metabolites such as alkaloids and flavonoids, and the complex processes of wound healing illustrate the intricate biochemical interactions essential for life.

Ultimately, structural and functional biochemistry provides a molecular foundation for understanding physiological processes, disease mechanisms, and biotechnological applications, making it a cornerstone of modern biological and medical sciences.