Bachelor of Science
(B.Sc.-CBZ)

FUNDAMENTALS OF BIOCHEMISTRY
(DBSZCO303T24)

Self-Learning Material
(SEM-III)

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Centre for Distance and Online Education

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EXPERT COMMITTEE

Prof. D.S. Bhatia
Former Director, Life and basic Sciences
JNU, Jaipur

Prof. (Dr.) Subhash Chandra
Head, Department: Zoology
Dean, PG Studies,
MDS University, Ajmer

COURSE COORDINATOR

Dr. Saurabh Singh
Dept. of Life Sciences
Jaipur National University, Jaipur

UNIT PREPARATION

Unit Writers

Dr. Saurabh Singh (Unit1-4)
Dept. of Life Sciences,
JNU,Jaipur

Dr. Kritika Pathak (Unit5-8)
Dept. of Life Sciences, JNU,
Jaipur

Assisting & Proof Reading

Dr. Juhi Sharma
Dept. of Life Sciences, JNU,
Jaipur

Unit Editor

Dr. Kritika Pathak
Dept. of Life Sciences,
JNU, Jaipur

Secretarial Assistance:
Mr. Suresh Sharma

COURSE INTRODUCTION

Overall biochemistry lays the molecular foundation for an organism's phenotype and behavioral outcomes in the plants, animals and microbial worlds. This book therefore covers the essentials of biomacromoles structural and functional characteristics. Content starts with structural and functional diversity of carbohydrates prioritizing significance of their role in biomolecular recognition, calory provision, structural support, storage role, protective function etc. Lipids comes next with their different classes and their role in defining cellular boundaries, other vesicular structures, signaling, storage and calorie provision etc. This is followed by amino acids and their different structural classes, their polymerization in to proteins, hierarchy of its structural components, their configurationally and conformational diversity. Different nucleic acids, components, polymers and their role in inheritance and information conservation, message transcription and translation into response is also emphasized upon. Enzymes, its catalytic action and kinetics is dealt separately to emphasize upon its importance in academic and industrial world. Likewise Immunoglobulins are also explained in a separate chapter to emphasis upon its diverse prophylactic, therapeutic and diagnostic significance.

The course Fundamentals Of Biochemistry is of 3 Credits. This course is divided into 08 units and each Unit is divided into sub topics..

Course Outcomes: After completion of the course, the students will be able to:

1. Describe and differentiate between structural isomers, stereoisomers, and anomers.
2. Classify carbohydrates into their main types: monosaccharides, disaccharides, oligosaccharides, and polysaccharides.
3. Apply techniques for amino acid analysis, including chromatography (e.g., high-performance liquid chromatography - HPLC) and mass spectrometry.
4. Investigate recent advancements in amino acid research, including novel applications and discoveries related to amino acid functions and metabolism.
5. Explain how amino acids contribute to energy production and the synthesis of other biomolecules.
6. Create the structural formulas of common carbohydrates (e.g., glucose, fructose, sucrose, starch, cellulose).

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Unit I

Carbohydrates

Learning Objective

- To understand the structure and behavior of carbohydrates
- Describe the different types of simple and complex carbohydrates

1.1.Definition:

Carbohydrates are the hydrates for carbon. The chemical structure contains atoms of carbon, hydrogen, and oxygen generally in the ration of 2:1 to that of hydrogen. The main food sources of carbohydrates are fruits, vegetables, whole grains, milk, and dairy products

Molecular formula: C_{2n} H_nO_{2n}

1.2.Functional characterization of carbohydrates:

- Aldoes: these carbohydrates contains aldehydes as functional group,
- Ketoses: these carbohydrates contains ketones as functional group

Thus, chemically carbohydrates are either simple derivatives or polymers of polyhydroxy aldehydes or ketones.

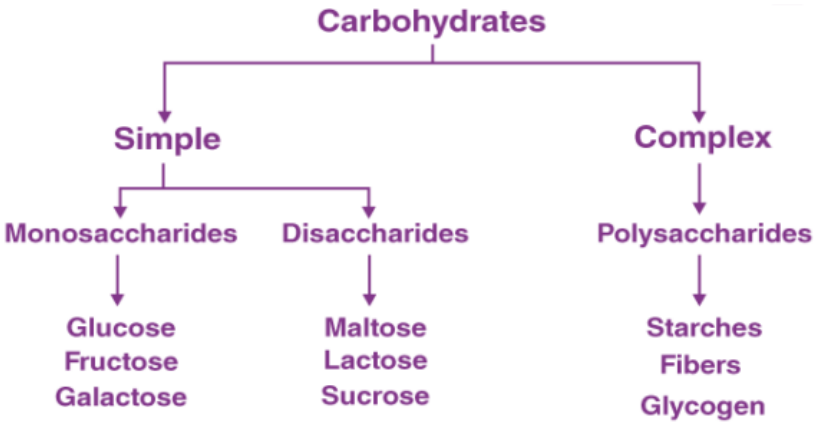


Figure 1.1: Classification of Carbohydrates

Carbohydrates can exist in monomer (simple carbohydrate) or in the multiple (polysaccharides) units linked via ester bonds known as glycoside bonds

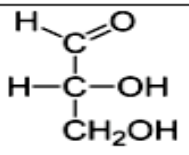
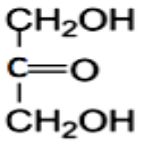
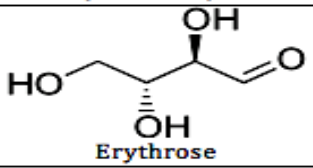
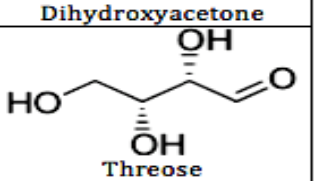
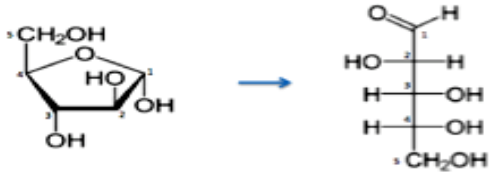
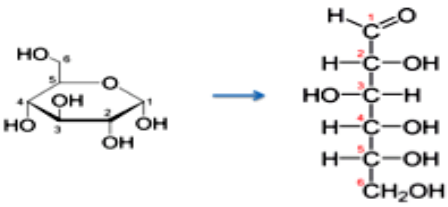
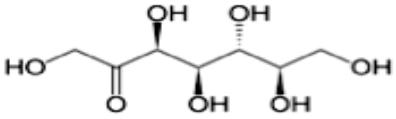
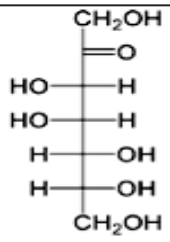
Table 1.1: Common Carbohydrates and their sources

Name	Source
Monosaccharide's	
Glucose	Wine; grape sugar, blood sugar, dextrose.
Galactose	Milk
Fructose	Fruits and honey; sweetest sugar.
Ribose	backbone structure of RNA and DNA
Disaccharides - contain two monosaccharides	
Sucrose	momeric unit of fructose and glucose Found in cane and beet sugar.
Lactose	momeric unit of **galactose and glucose**.
Maltose	Contains 2 **units of glucose**; found in germinating grains
Common Polysaccharides	
Starch	Plants store glucose as the polysaccharide starch. Found in cereals and tuber
Cellulose	Present in cell walls in plants and made up of glucose units
Glycogen	Present in animals and humans

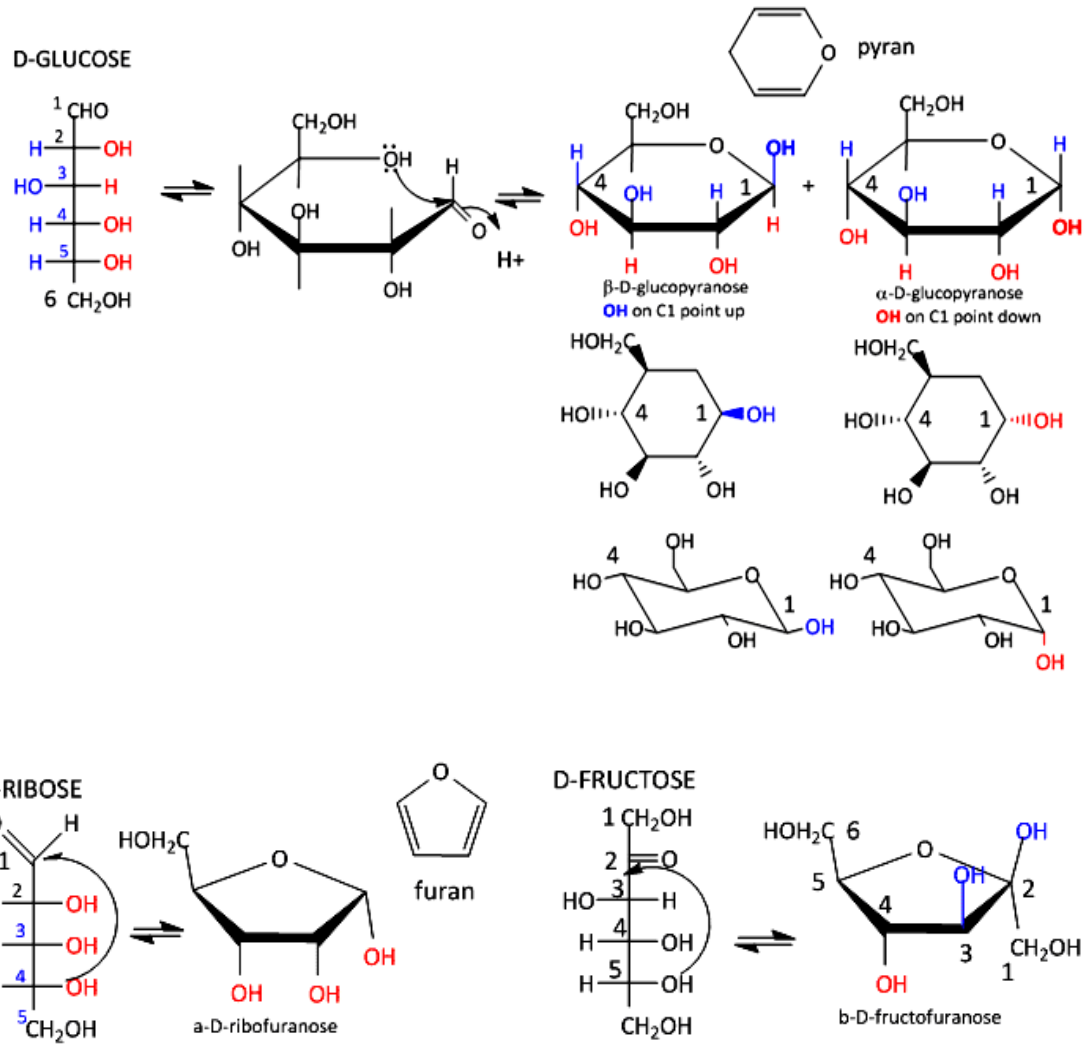
1.3.Simple Carbohydrates:

Simple carbohydrates also are found in natural sugars. Simple sugar may range 3C to 7C.

Table 1.2: CommonSim Carbohydrates and their sources

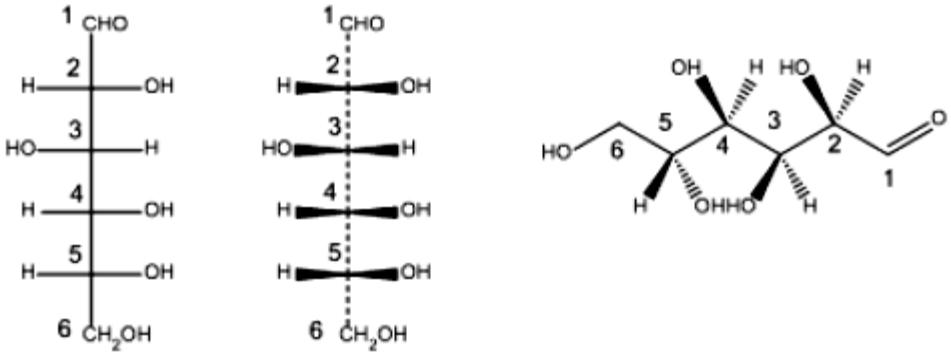
Number of Carbon Atoms (n)	Name	Examples	
3	Triose	 Glyceraldehyde	 Dihydroxyacetone
4	Tetrose	 Erythrose	 Threose
5	Pentose	 Arabinose	
6	Hexose	 Glucose	
7	Heptose	 Seduheptulose	 Mannoheptulose

Monosaccharide exists in linear (Fisher projection) as well cyclic (Haworth projection) structure (see figure below). Glucose is 6C ring (Pyranose), while fructose is a 5C ring (Furanose). These ring structures are formed through intermolecular nucleophillic attack of one of the Hydroxyl group on the carbonyl group of the aldehyde or ketone leading to cyclization.



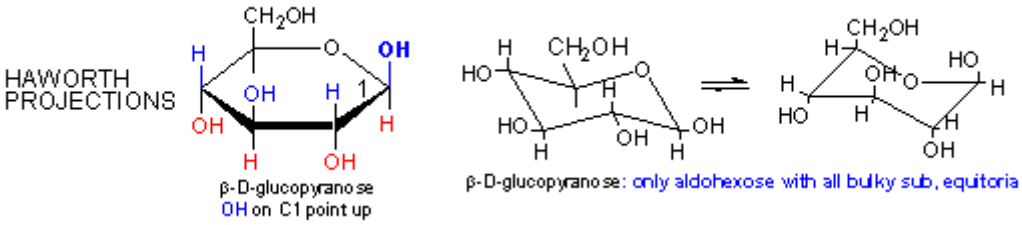
Fisher Projection:

It is a planar projection (2D). The hydroxyl group are represented with the help of wedges and dash lines on a plane next to carbon atom.



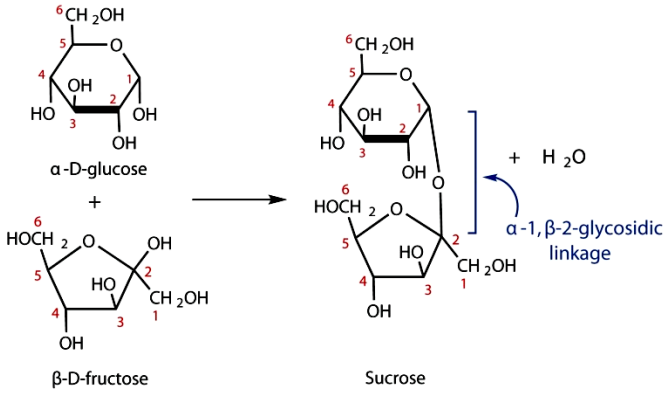
Haworth Projection:

It is a cyclic and planar with substituents (-OH and H) above or below the ring resulting in the chair or boat conformations.



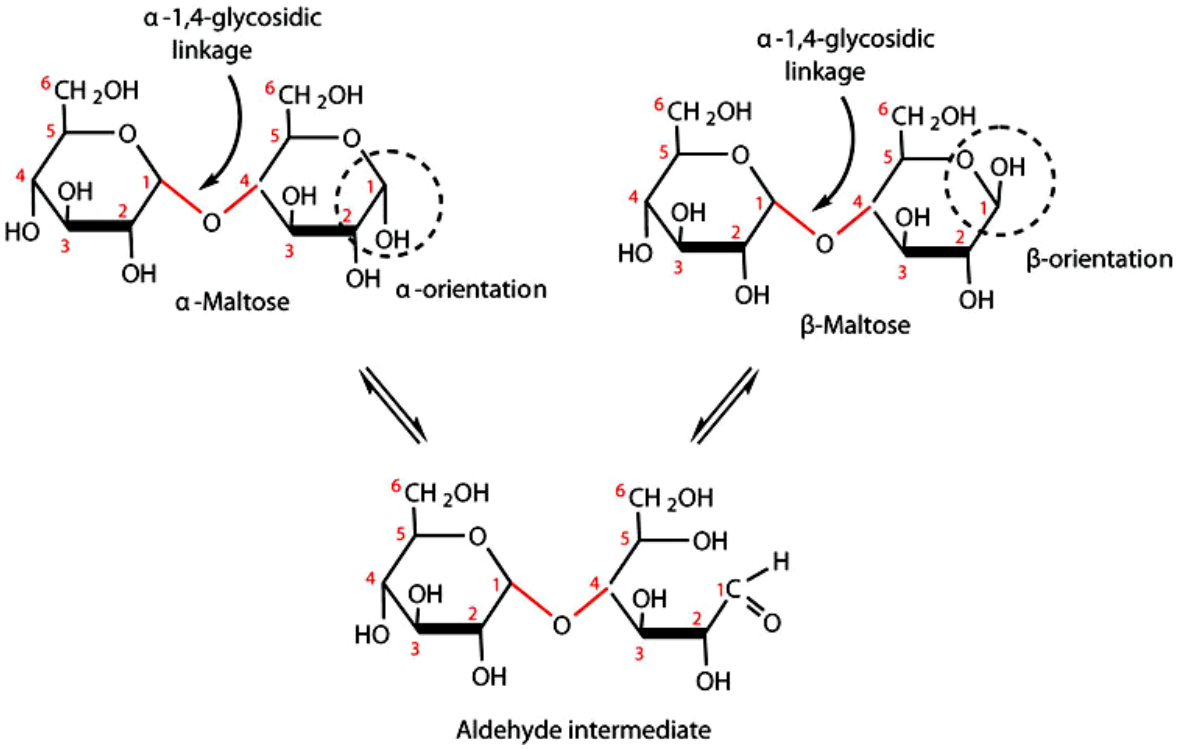
1.4.Disaccharides:

- A disaccharide are formed by bonding of two monosaccharide molecules via glycosidic bond where -OH groups of the two monomeric units combine to produce glycosidic linkages.



Examples:

Maltose: Commonly known as malt sugar. It is a redusing sugar and form α-linkage in head to tail fashion



1.5.Polysaccharides:

- It consists of more the 25 units of monosaccharides. For example Amylose, Amylopectin, Glycogen
- Amylose is the most basic polysaccharide of repetitive units glucose units connected by an alpha-1-4 bond.. While amylopectin is liner as well as branched whereAlpha 1-4 linkage is linear but every 30 to 50 residues, an alpha 1-6 linkage gives rise to a "branch."
- Glycogen is similar to amylopectin, glycogen is a polysaccharide composed primarily of glucose and containing a mixture of alpha 1-4 and alpha 1-6 linkages bus has significantly higher number of alpha 1-6 branches, occurring every ten residues.

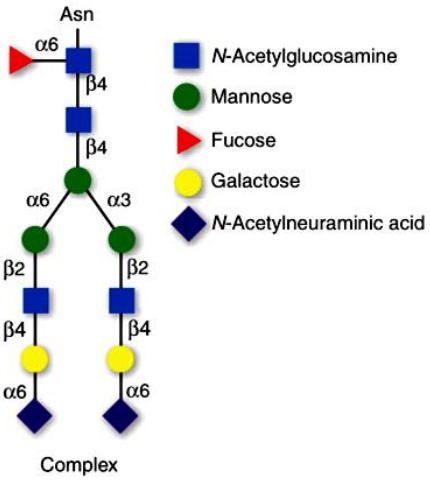
1.6. Glucocongugates:

- Glycans, or carbohydrates that are covalently attached to other biomolecules like proteins, peptides, lipids, are known as called glycoconjugates.
- Glycoconjugates,can be classified as glycoproteins, glycopeptides, peptidoglycans, glycolipids, glycosides, and lipopolysaccharides. They participate in interactions between

cells, including the recognition of other cells, interactions between cells and matrixes, and detoxification procedures.

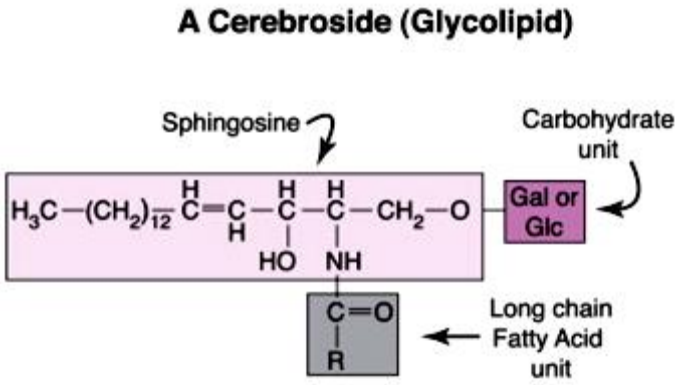
1.6.1. Glycoproteins:

- Proteins with oligosaccharide chains covalently bonded to the side chains of amino acids are known as glycoproteins.
- The protein and the carbohydrate are linked through a cotranslational or posttranslational alteration.



1.6.2. Glycolipids

- Glycolipids are lipids that have a glycosidic link securing a carbohydrate. For e.g. cerebroside
- They function to keep the cell membrane stable and also assist with cellular recognition.



1.6.3. Peptidoglycan:

- These are also known as murein, a polysaccharide made of sugars and amino acids that encircles the bacterial cytoplasmic membrane in the form of a mesh.
- The sugar component is made up of alternating units of N-acetylmuramic acid and β-linked N-acetylglucosamine that alternate.

Self Assessment

1. Define and differentiate among monosaccharides, disaccharides, and polysaccharides?
2. Describe carbohydrates with their structure.

Unit 2:
Lipid

Learning Objective:

- To understand the structure and functions of lipid
- Describe the different types of simple and complex lipids

2.1. Introduction

- Lipids are long chains of hydrocarbons which are soluble in organic solvents but insoluble in polar solvents like water.
- Lipids are composed by log chains of hydrogen and carbon atoms.
- There are two categories: simple and sophisticated. Non-polar soluble organic molecules, such as benzene and chloroform, are known as simple lipids. Fatty acids with alcohol are called complex lipids; examples of these include blood platelets, nerve tissues, and cell membranes.

2.2. Simple Lipid

Simple lipids are the free or ester linked fatty acids with different alcohols.

- **Fats:** Esters linked fatty acids with glycerol.
- **Waxes**: Esters of fatty acids with alcohols of higher molecular weight.

2.3. Complex lipids

- These are fatty acid esters that also contain groups in addition to alcohol. Phospholipids are lipids that contain alcoholic and phosphate group, in addition to fatty acids. They usually contain bases that contain nitrogen as well as other substituents. For example, glycerophospholipids, and sphingosine.
- Glycolipids, also known as glycosphingolipids, are lipids made up of a fatty acid, a carbohydrate, and sphingosine.
- Additional complicated lipids: Lipids like amino lipids and sulfolipids. This group may also include lipoproteins.

2.4. Fatty Acid Classification

Saturated Fatty Acids:

Saturated fatty acids consists of long chains of carbon with single bonds.

Table: Common examples of saturated fatty acid

Common Name	Structural Formula
Stearic acid (18 C)	$CH_3(CH_2)_{16}COOH$
Palmitic acid (16 C)	$CH_3(CH_2)_{14}COOH$
Myristic acid (14C)	$CH_3(CH_2)_{12}COOH$
Lauric acid (12C)	$CH_3(CH_2)_{10}COOH$
Capric acid (10C)	$CH_3(CH_2)_8COOH$
Caprylic acid (8C)	$CH_3(CH_2)_6COOH$
Caproic acid (6C)	$CH_3(CH_2)_4COOH$

Unsaturated fatty acid:

Fatty acid with at least one double bondin the chain are considered unsaturated. They may be monosaturated or polyunsaturated.

These may be Cis i.e. hydrogens are present in the same plane or trans if the hydrogen atoms are on two different planes

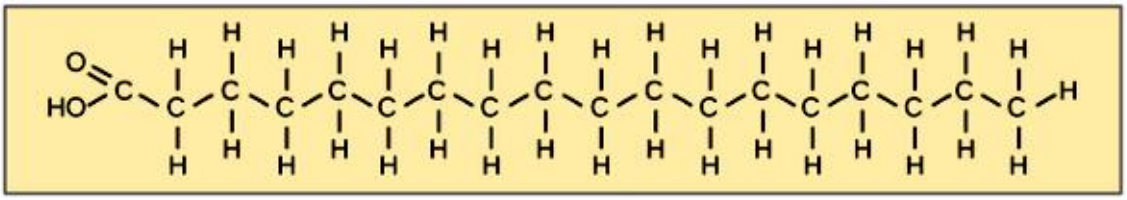
Table: Examples of Unsaturated Fatty Acid

Common Name	Structural Formula and Representation	Configuration
α-Linolenic acid (C18:3)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$ Or $\Delta^{9,12,15}$	cis
Stearidonic acid (C18:4)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_4COOH$ Or $\Delta^{6,9,12,15}$	cis

Eicosapentaenoic acid (C20:5)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2$ $CH=CH(CH_2)_3COOH$ Or $\Delta^{5,8,11,14,17}$	cis
Cervonic acid(C22:6)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2$ $CH=CHCH_2CH=CH(CH_2)_2COOH$ Or $\Delta^{4,7,10,13,16,19}$	cis
Linoleic acid (C18:2)	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$ Or $\Delta^{9,12}$	cis
Linolelaidic acid (C18:2)	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	trans

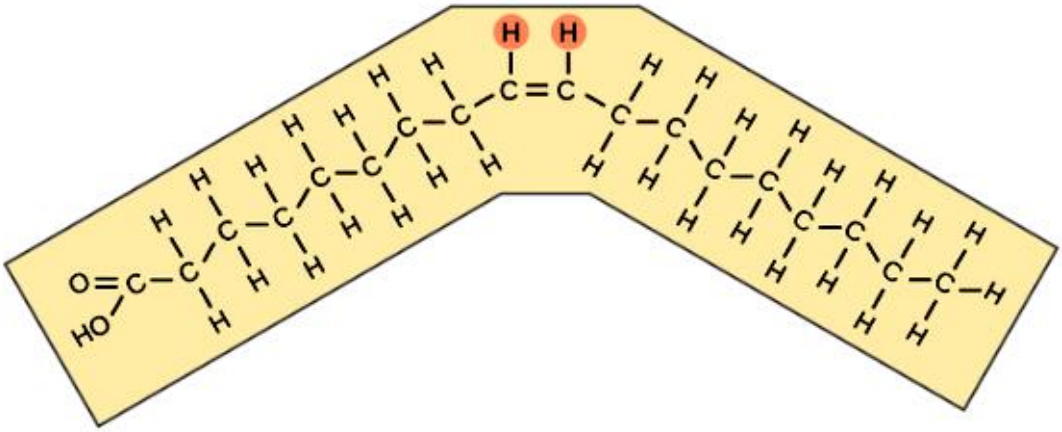
Saturated fatty acid

Stearic acid

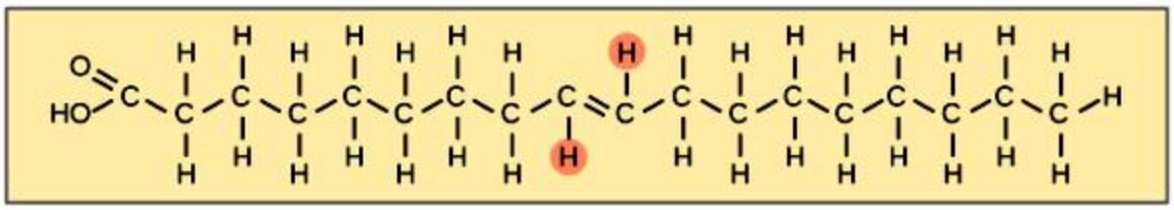


Unsaturated fatty acids

Cis oleic acid



Trans oleic acid



2.5. Tri-acylglycerols

- A **triglyceride** is an ester derived from glycerol and three fatty acids

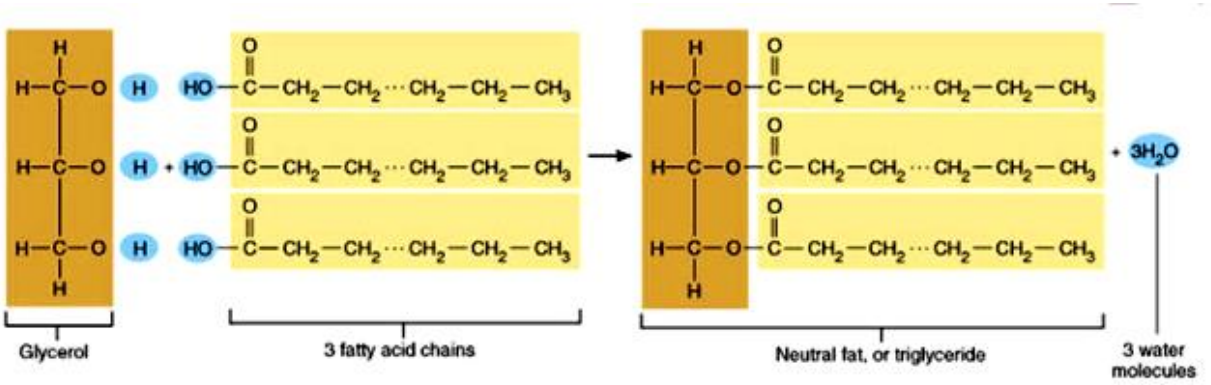


Figure: Formation of Tri-acylglycerols

- Fatty acids linked to glycerols or other substituent can be the same or different.
- The simplest triglycerides have identical fatty acids. These can be identified on the basis of fatty acids derivatives i.e. stearin derived from stearic acid, palmitin derived from palmitic acid, etc.
- Hydrolysis of fatty acid in the presence of acid and heat, or under biological conditions, generates glycerol and three fatty acids.

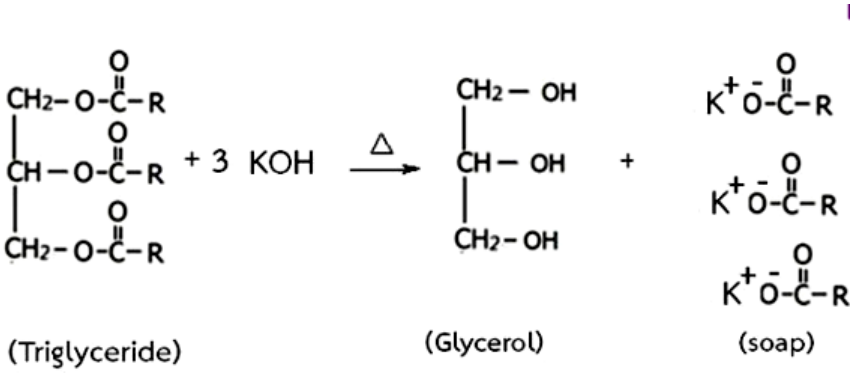


Figure: Hydrolysis of Tri-acylglycerols

Properties:

- Triglycerides are colorless, sometimes yellowish.
- They are a simple, saturated and symmetrical
- They are solid at temperature, but most are oils.
- Their density (ρ)is near 0.9 g/cm^3.

2.6. Phospholipids:

- Phospholipids are complex lipids,which consists of fatty acids, alcohol, nitrogen base and phosphoric acids.
- These phospholipids have a hydrophilic head and a hydrophobic tail, which forms the inside of the bilayer while head remains exposed to the surface
- They commonly occur in cell membrane and aids in dynamic character to the membranes.

2.6.1. Phospholipids types

2.6.1.1. Glycerophospholipids

- They consists of glycerol-based phospholipidsand majorly occur in the biological membrane. It consists of glycerol-based phospholipids. With the non-polar hydrophobic tails pointing inside and the polar hydrophilic heads protruding to the aqueous environment, they are often arranged into a bilayer in membranes. Phosphatidates are the most fundamental structure in the bilayer.
- Glycerol-3-phosphate initiates the de novo synthesis of glycerophospholipids. The phosphate group and the glycerol form an ester bond. In bacteria and eukaryotes, ester bonds are usually used to attach the long-chained hydrocarbons, whereas in archaea, ether linkages are used.
- With the non-polar hydrophobic tails pointing inside and the polar hydrophilic heads protruding to the aqueous environment; they are often arranged into a bilayer in membranes. Phosphatides are the most fundamental structure in the bilayer.

2.6.1.2. Sphingo-phospholipids

- It consists of sphingosine (phopsphocholine) as alcohol and fatty acid.
- It is among the rare membrane phospholipids that aren't made of glycerol and occur in nervous tissue .
- Sphingomyelin is produced at the trans Golgi and endoplasmic reticulum (ER), where it is present in trace amounts. It is more concentrated in the outside leaflet of the plasma membrane than the inner, where it is richer.

Properties of Phospholipids

1. They act as mediators of signals.
2. The molecules are amphipathic.
3. They serve as an anchor for proteins inside cell membranes.
4. They make up the majority of cell membrane components.
5. They are the parts of lipoproteins and bile.

Functions

- It control membrane permeability
- Help better absorption of fat from the intestine.
- Assist in Electron Transport Chain (ETC) in the mitochondria.
- Phospholipids preventsbuild-up of fats in the liver.
- It plays a vital role in the transportation and removal of cholesterol from the cells.
- Involved in the coagulation of blood cells.
- Aids in the synthesis of different lipoproteins, prostacyclins, prostaglandins and thromboxanes.

2.7. Glycolipids

- Lipids bound to a carbohydrate via a glycosidic (covalent) link are known as glycolipids.
- They play a vital function in preserving the integrity of the cell membrane and promote cellular recognition, essential for the immune response and the connections that enable cells to join to form tissues.

Structure

- The existence of a mono- or oligosaccharide attached to a lipid molecule is the fundamental characteristic of a glycolipid.
- Glycerolipids have glycerol backbones bonded to fatty acid are the most prevalent lipids found in cellular membranes.
- The ligand components of the saccharides are those that are connected to the polar head groups outside the cell while fatty acid remain embedded in the cell membrane

Types:

The types of glyceroglycolipidsare of following types:

- **Galactolipids:**

  A galactose sugar bonded to a glycerol lipid molecule is known as a galactolipid. They are connected to photosynthesis processes and are present in chloroplast membranes.
- **Sulfolipids:**

  Sulfolipids are lipid-attached sugar moiety compounds that have a sulfur-containing functional group. Sulfoquinovosyldiacylglycerols, which are connected to the sulfur cycle in plants, are a significant category.
- **Glycosphingolipids**

  A subclass of glycolipids called glycosphingolipids is derived from sphingolipids. The majority of glycosphingolipids are found in nervous tissue, where they are involved in cell signaling.
- **Ganagliosides:**

  The most intricate animal glycolipids are called gangliosides. They comprise one or more sialic acid residues attached to negatively charged oligosacchrides. They are found in nerve cells most abundantly.
- **Globosides:**

  These are the glycosphingolipids with more than one sugar as part of the carbohydrate complex.

2.8. Steroids:

- Steroids are the complex lipids having four fused rings (called A, B, C, and D)

- They play important role assignaling molecules and as crucial parts of cell membranes that change the fluidity of the membrane.
- Cholesterol, stradiol, testosterone are few of many examples of steriods.

Structure:

The basic cyclopentaneperhydrophenanthrene (cyclopentano) structure at the center of steroids is referred to as a "gonane".

It is made up of 17 carbon atoms fused into four rings: three six-membered cyclohexane rings and one five-membered cyclopentane ring (the D ring).

Different steroids are distinguished by differences in the oxidation state of the rings and the functional groups that are connected to this core.

Sterols are cholestane derivatives that belong to the steroid class and have a hydroxy group at position three.

Furthermore, steroids can experience substantial changes such structural or ring cutting.

Steroids can display variations in the order of connected groups, the presence and quantity of methyl groups on the rings, and ring scissions, expansions, and contractions.

Functions:

- Steroids, are anti-inflammatory medicines used to treat a range of condition
- They play an vital role in a varied range of physiological functions, comprisingof growth, homeostasis ,reproductiondevelopment, and energy metabolism.

SELF-ASSESSMENT

1. Classify the lipid on the basis of saturation
2. Write a note on phosopholipids

UNIT 3
AMINO ACIDS

LEARNING OBJECTIVES

By the end of this course, students will:

- **Understand the Structural Composition of Amino Acids**:
- **Classify Amino Acids Based on Their Side Chains**:.
- **Analyze the General characteristic of α-Amino Acids**:
- **Evaluate the Physiological Significance of -Amino Acids**:
- **Apply Knowledge to Real-world Scenarios**:

3.1 INTRODUCTION

Amino acids are a distinct group of neutral compounds set apart from other natural substances both chemically, due to their ampholytic nature, and biochemically, as fundamental components of proteins. An amino acid typically consists of a carboxylic acid with an aliphatic primary amino group positioned α to the carboxyl group, showcasing a characteristic stereochemistry.

In the intricate process of protein biosynthesis, these amino acids, numbering 20 in total, are orchestrated by strict genetic mechanisms, thereby serving as the fundamental building blocks of proteins.

While over 300 amino acids exist in nature, only the 20 standard ones, encoded by genes, are utilized in protein formation. The remaining amino acids, termed non-protein amino acids, either undergo posttranslational modifications within synthesized proteins or exist in organisms independently of protein composition.

Proteins, intricate macromolecules present in all living cells, consist of chains of amino acids, making amino acids the elemental constituents of proteins. Approximately 500 naturally occurring amino acids have been identified.

The structure of amino acids delineates them as organic compounds featuring both amino (-NH2) and carboxylic (-COOH) functional groups. The amino group can be attached to any carbon atom besides the carboxyl group.

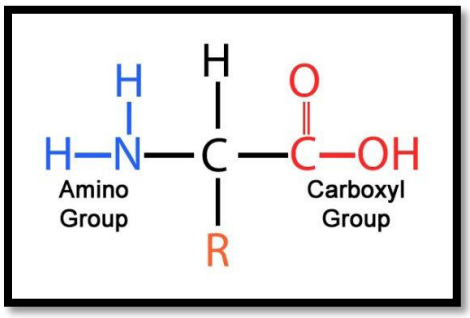


Fig 3.1 Structure of amino acid

Commonly, all 20 amino acids (aa) are classified as α--amino acids, characterized by a –C=O group, $NH_4^{+,}$ and a variable side chain (R group), all linked to the α-carbon. Except glycine The L-configuration characterizes all but one of the 20 amino acids, owing to the presence of an asymmetric carbon at the α position, except for glycine. Glycine lacks an asymmetric carbon, rendering it optically inactive and thus not falling under the categories of D or L.

3.2 PROPERTIES OF AMINO ACIDS

Physical Properties

- Amino acids exhibit colorless, crystalline solidity.
- Their melting points exceed 200°C.

  Solubility varies among amino acids: they dissolve readily in water, have slight solubility in alcohol, and demonstrate limited solubility in methanol, ethanol, and propanol.

  This trait is influenced by both the R-group of the amino acids and the pH of the solvent. When exposed to high temperatures, amino acids decompose. With the exception of glycine, all are optically active.

  They can form peptide bonds by linking their –C=O andNH_4^+groups,This covalent linkage involves the alpha-amino group of one amino acid bonding with the alpha-carboxyl group of another, resulting in a -CO-NH- linkage.

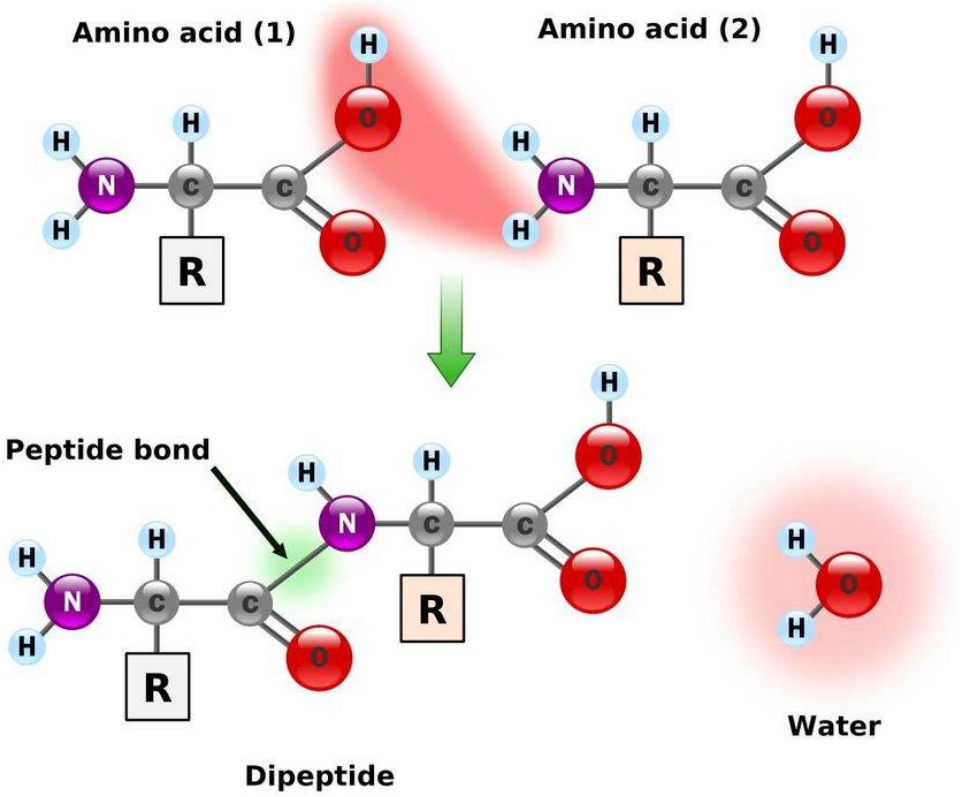


Fig 3.2 structure of amino acid

Chemical Properties

- **Zwitterionic Property**: Amino acids manifest zwitterionic properties, characterized by the presence of functional groups with both positive and negative electrical charges.
- The -NH2 group, being the stronger base, accepts H+ from the -COOH group, resulting in the formation of a neutral zwitterion, the prevalent form of aa in solution.

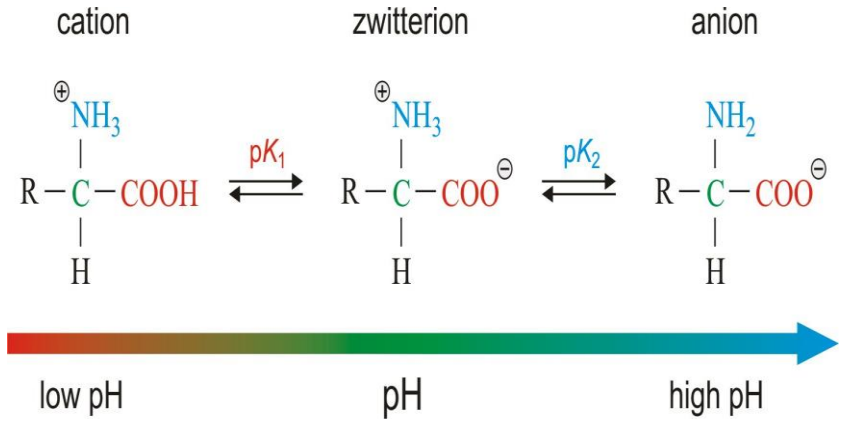


Fig 3.3 Zwitterion or dipolar ion:

- **Amphoteric Property**: Amino acids demonstrate amphoteric behavior, acting as both acids and bases because they contain both amine and carboxylic groups..

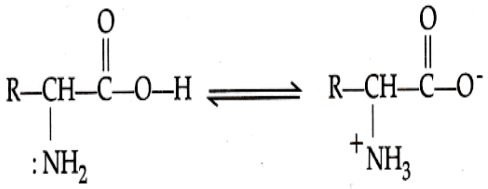


Fig 3.4 amphoteric behaviour of amino acid

- **Ninhydrin Test**: This test, involving the addition of Ninhydrin solution to a protein solution followed by heating, leads to the formation of a violet color, indicating the presence of α-amino acids.

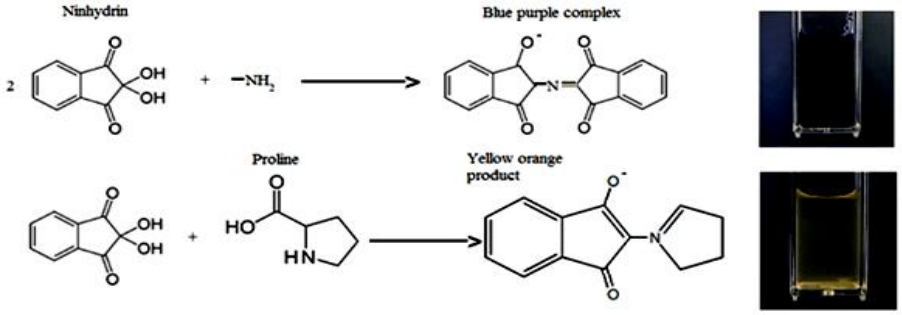


Fig 3.5 Ninhydrin reaction

- **Xanthoproteic Test**: This test, employed to detect protein containing aromatic aalike tyrosine, phenylalanine etc. which involves the nitration of benzoid radicals within the amino acid chain upon their reaction with nitric acid. This reaction leads to a yellow coloration of the solution..

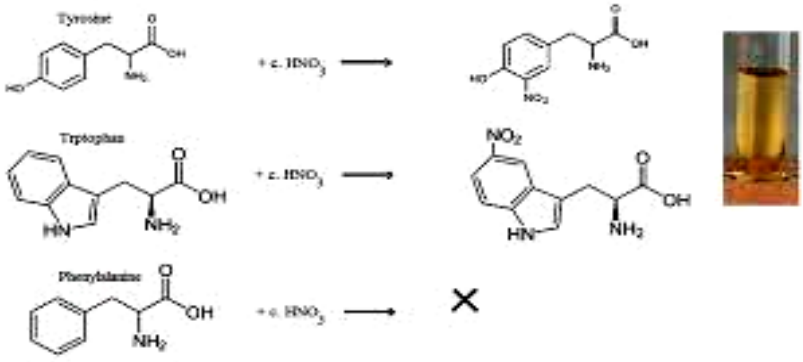


Fig 3.6 detecting aromatic amino acids by Xanthoproteic Test

- **Reaction with Sanger's Reagent**: this reagent (1-fluoro-2, 4-dinitrobenzene) respondsto free amino groups within the peptide chain

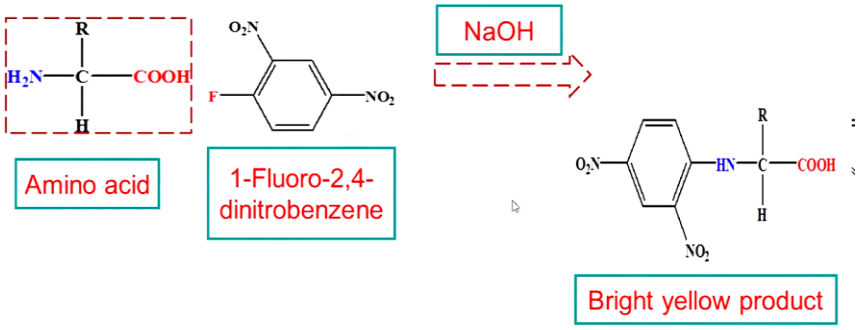


Fig 3.7 sanger reaction

- **Nitrous Acidreaction :**The amino group reacts with nitrous acid, releasing nitrogen and forming the corresponding hydroxyl compound..

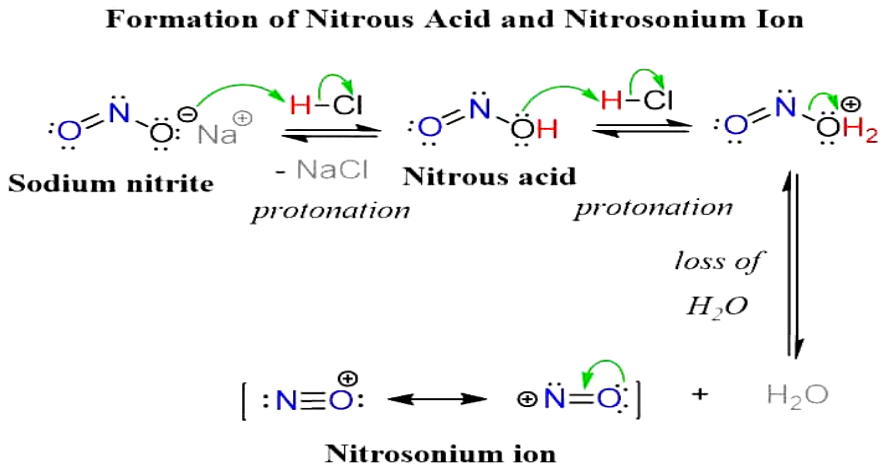


Fig 3.8 Nitrous acid reacts with the amino group

3.3 CLASSIFICATION BASED ON R-GROUP:

1. **Nonpolar, Aliphatic Amino Acids:** These amino acids feature nonpolar, hydrophobic R groups. Valine, Leucine, Methionine, Proline., Alanine, Isoleucine, Glycine.
2. **Aromatic Amino Acids:**Tyrosine, Phenylalanine (phe), and Tryptophan(trp) possess aromatic side chains, rendering them moderately nonpolar (hydrophobic)
3. **Polar, Uncharged Amino Acids:** Amino acids in this category have R groups that are water-soluble or hydrophilic due to functional groups forming hydrogen bonds with water. Serine, Threonine, Cysteine, Asparagine, Glutamine belong to this class.

4. **Acidic Amino Acids:** These feature acidic or negatively charged R groups. for ex Glutamic Acid and Aspartic Acid
5. **Basic Amino Acids:** Amino acids with basic or positively charged R groups include HistidineLysine, Arginine

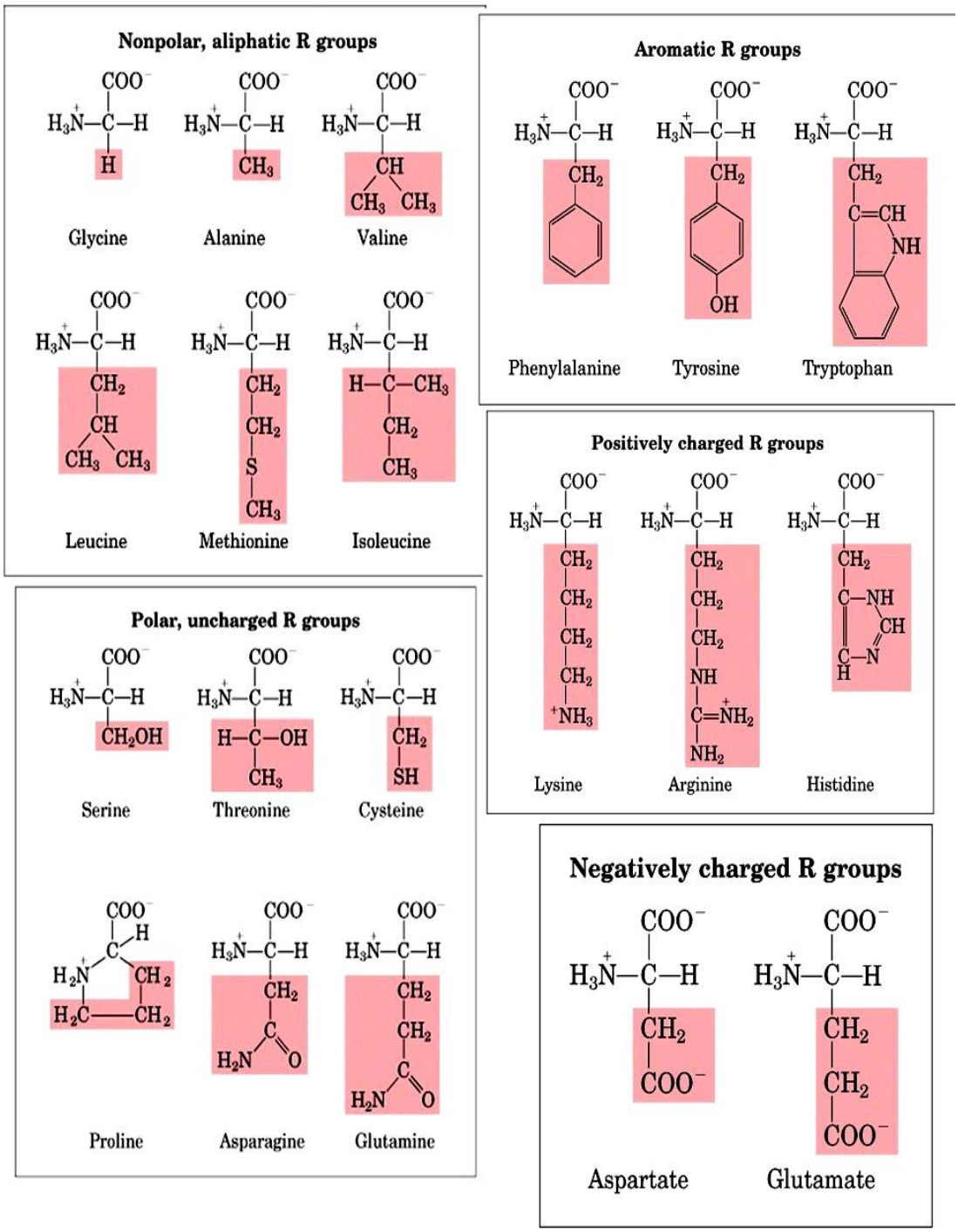


Fig 3.9 types of amino acid based on R group

3.4 CLASSIFICATION OF AMINO ACIDS BASED ON NUTRITION:

- **Essential Amino Acids (9):** These cannot be produced within the body and requisites from the diet for protein synthesis. Phenylalanine, Threonine Histidine, Isoleucine, Leucine, Lysine, Methionine, , Tryptophan, Valine.

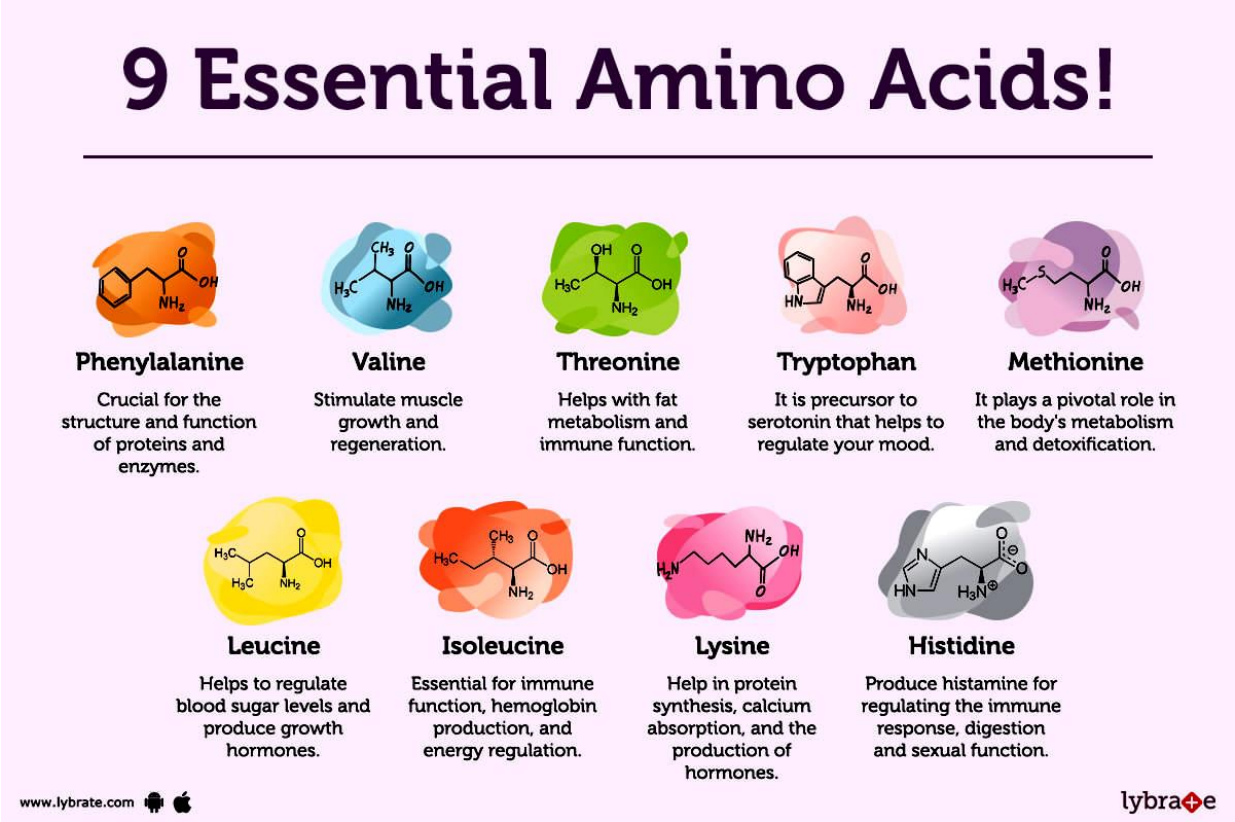


Fig 3.10 importance of essential amino acid

- **Non-essential Amino Acids (11):**body can produce these internally, so they do not essentially need to be consumed through the diet. Serine, Ornithine, Alanine Arginine, AsparagineGlutamine, Tyrosine, Cysteine, Glycine, Proline, Aspartate fall into this category.

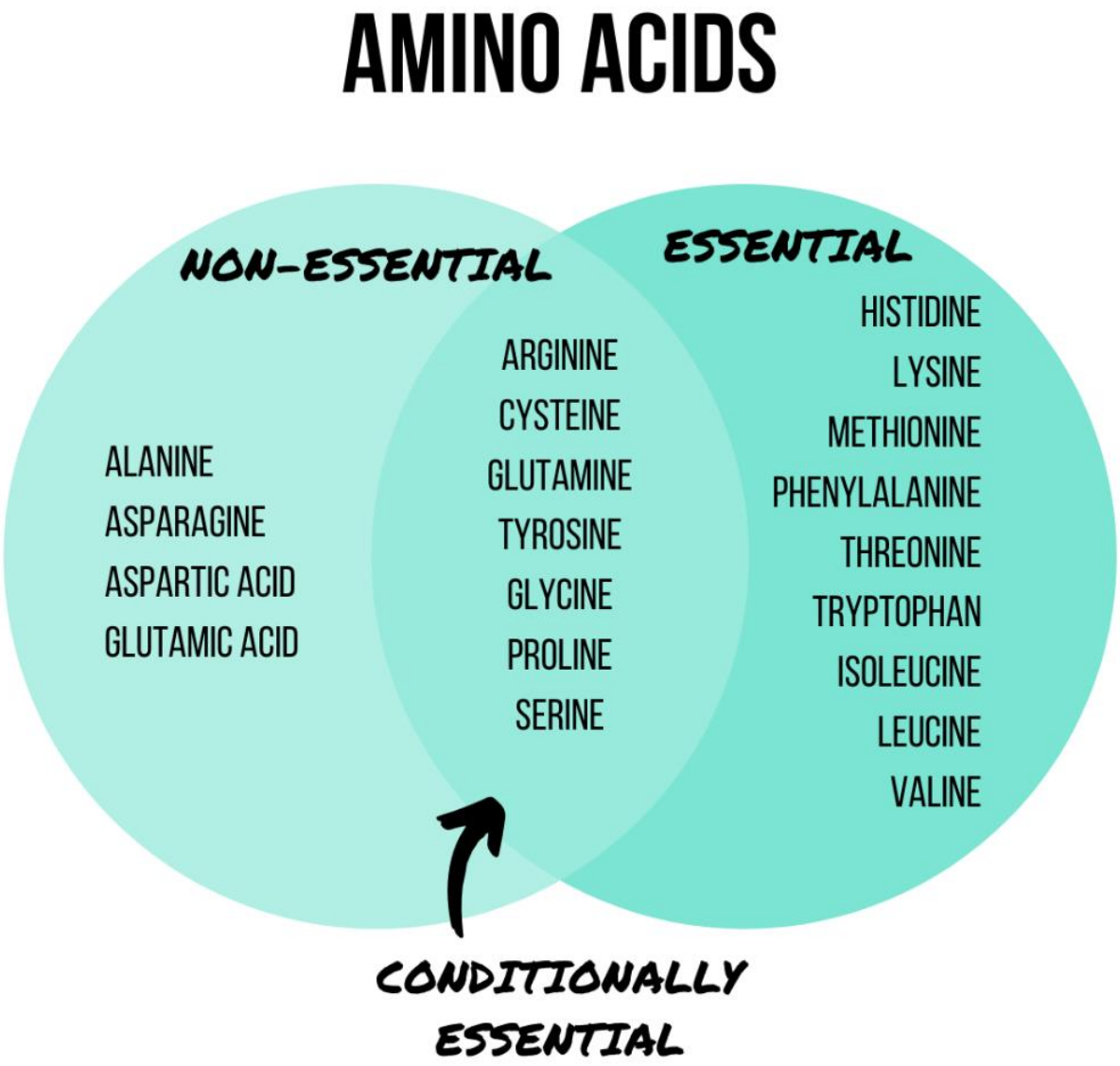


Fig **3.11 classification of amino acid**

3.5 CLASSIFICATION BASED ON METABOLIC FATE:

1. **Glucogenic Amino Acids:**being precursors for gluco-neogenesis, contributing to formation of glucose. Gly, Ala, Ser, His Aspartic Acid, Asparagine, Glutamic Acid, Valine, Methionine, Cysteine, Glutamine, Proline, , Arginine are examples.
2. **Ketogenic Amino Acids:**Leucine and Lysine break down to produce ketone bodies.
3. **Both Glucogenic and Ketogenic Amino Acids:** Isoleucine, Phenylalanine, Tryptophan, and Tyrosine can be metabolized to yield precursors for both ketone bodies and glucose.

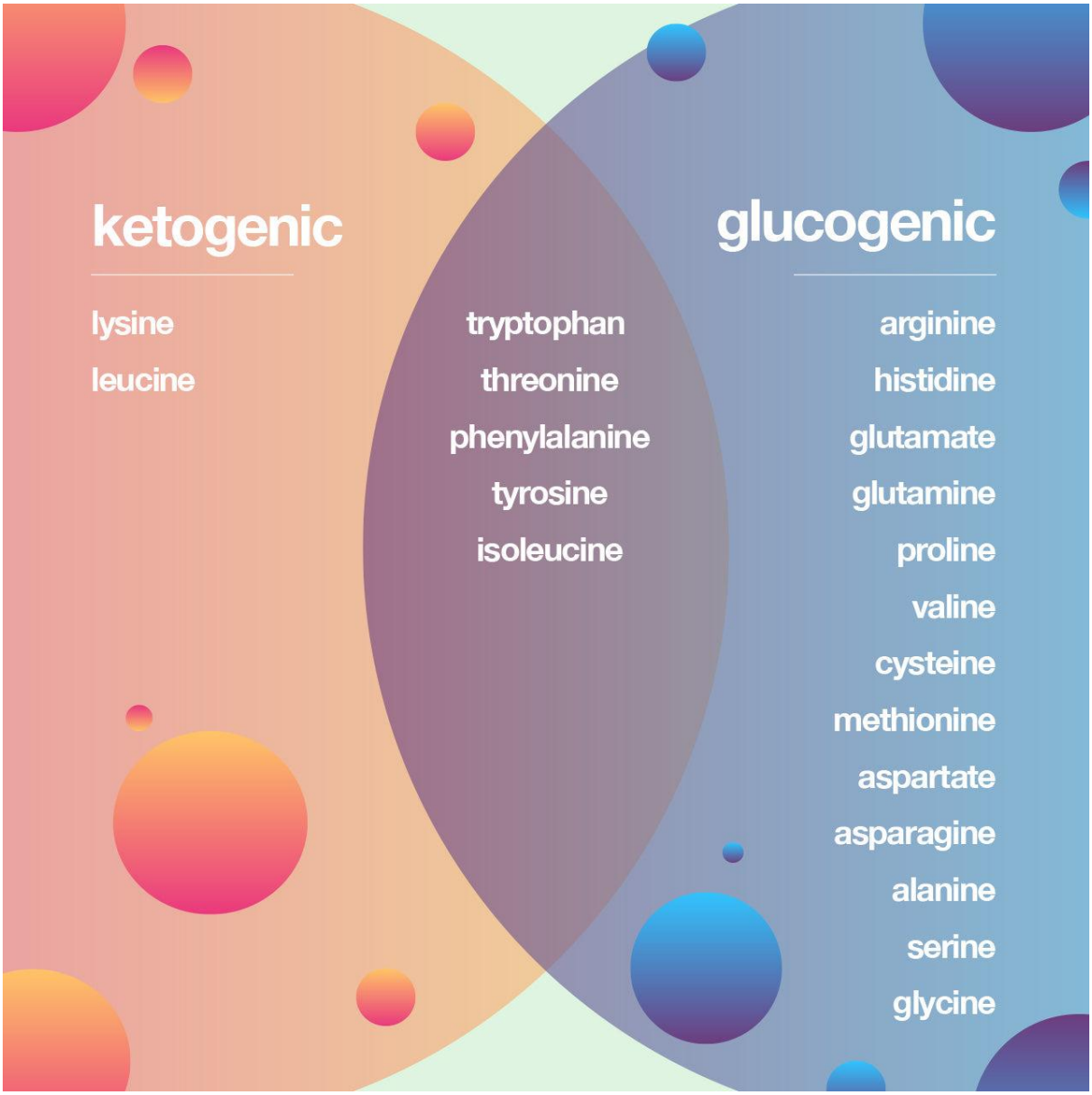


Fig 3.12 classification of amino acids based on metabolic fate

3.6 PROTEIN FORMATION

When aaassociate to form proteins, the NH_2 group of one aa molecule chemically bonds with the carboxylic group of another.

• This bonding process results in the formation of a peptide bond.

• Successive linking of amino acids continues until all required amino acids for protein synthesis are connected.

• One water molecule is lost in each bonding event between two amino acids. This union forms a larger unit called a peptide.

• A polypeptide comprises multiple peptides linked together, and these polypeptides further assemble to constitute a complete protein.

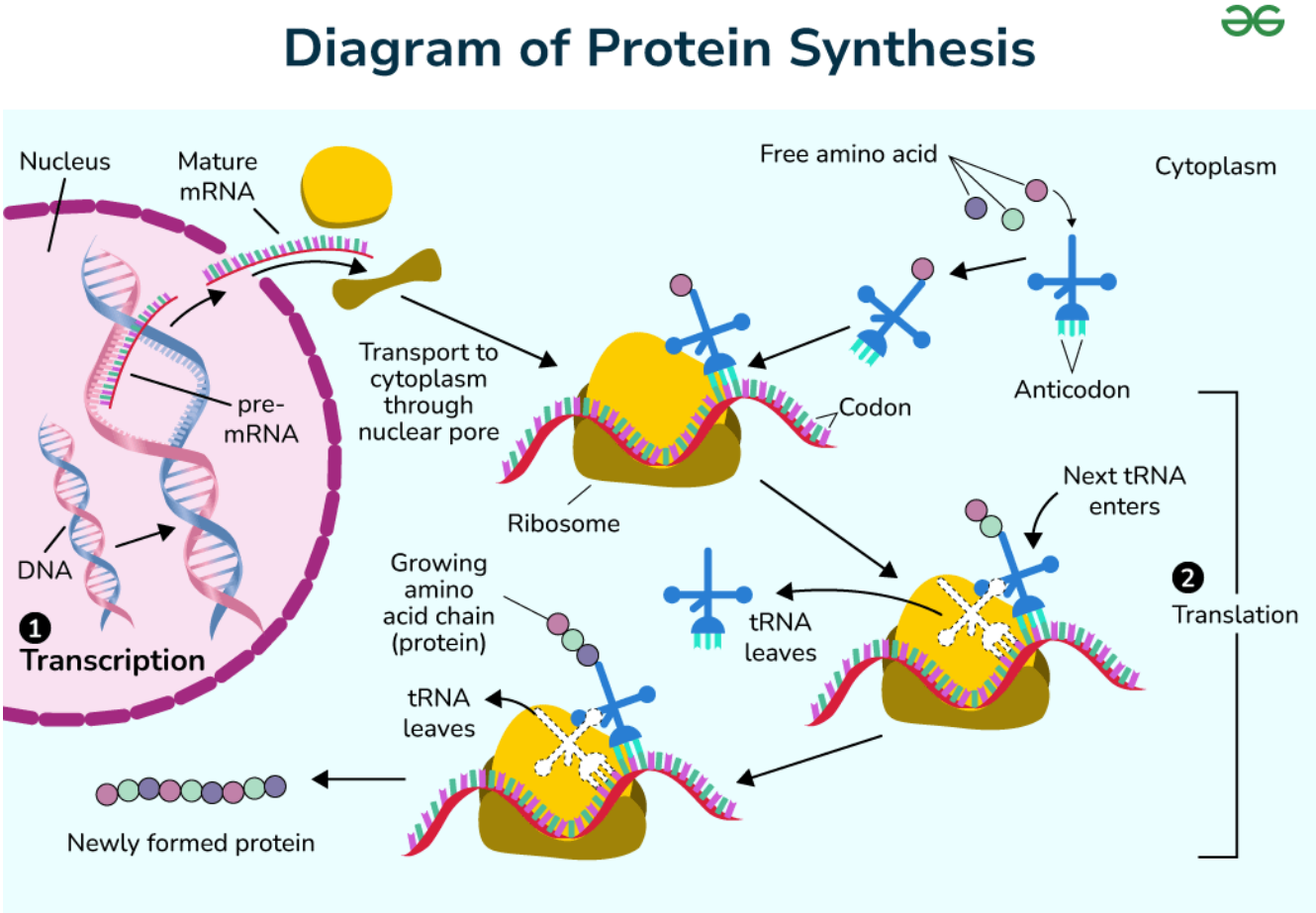


Fig 3.13 Process of protein synthesis

3.7 FUNCTIONS

• Twenty essential amino acids show a pivotal role in life as they aid as the central constituents of peptides and proteins, forming the cornerstone of all living organisms.

• The sequential arrangement of amino acid residues along a polypeptide chain dictates the three-dimensional structure of a protein, which in turn determines its functionality.

• Amino acids are indispensable for maintaining human health, contributing significantly to:

- Hormone production
- Muscular structure

- Optimal function of the nervous system
- Vital organ health
- Cellular integrity
- Various tissues utilize amino acids for protein synthesis and the production of N_2-containing compounds for egheme,
- Both dietary and tissue proteins undergo breakdown, yielding nitrogen-containing substrates and carbon skeletons.
- Carbon skeletons serve as aenergybasis in the kerb cycle, support gluconeogenesis.

3.8 PHYSIOLOGICAL IMPORTANCE OF ESSENTIAL AND NON-ESSENTIAL AMINO ACIDS:

Essential Amino Acids:

1. Phenylalanine: Main constituent of artificial sweeteners, involved in melanin synthesis inhibition and hunger regulation.
2. Leucine: Essential for muscle building and maintenance, blood sugar regulation, wound healing, and growth hormone stimulation.
3. Isoleucine: Stimulates protein synthesis, muscle development, pancreas insulin secretion, and glucose consumption.
4. Valine: Crucial during fasting for energy production, muscle growth, and regeneration.
5. Lysine: Essential for L carnitine production, collagen constituent, calcium fixation in bones, and wound angiogenesis.
6. Methionine: Aids in various syntheses, shows lipolytic activity, promotes tissue growth, and aids in mineral absorption.
7. Histidine: Precursor of histamine, essential for blood cell formation, and myelin maintenance.
8. Threonine: Promotes digestion, lowers cholesterol, vital for collagen and elastin, and prevents fatty liver.
9. Tryptophan: Precursor of serotonin and melatonin, increasing pain tolerance.

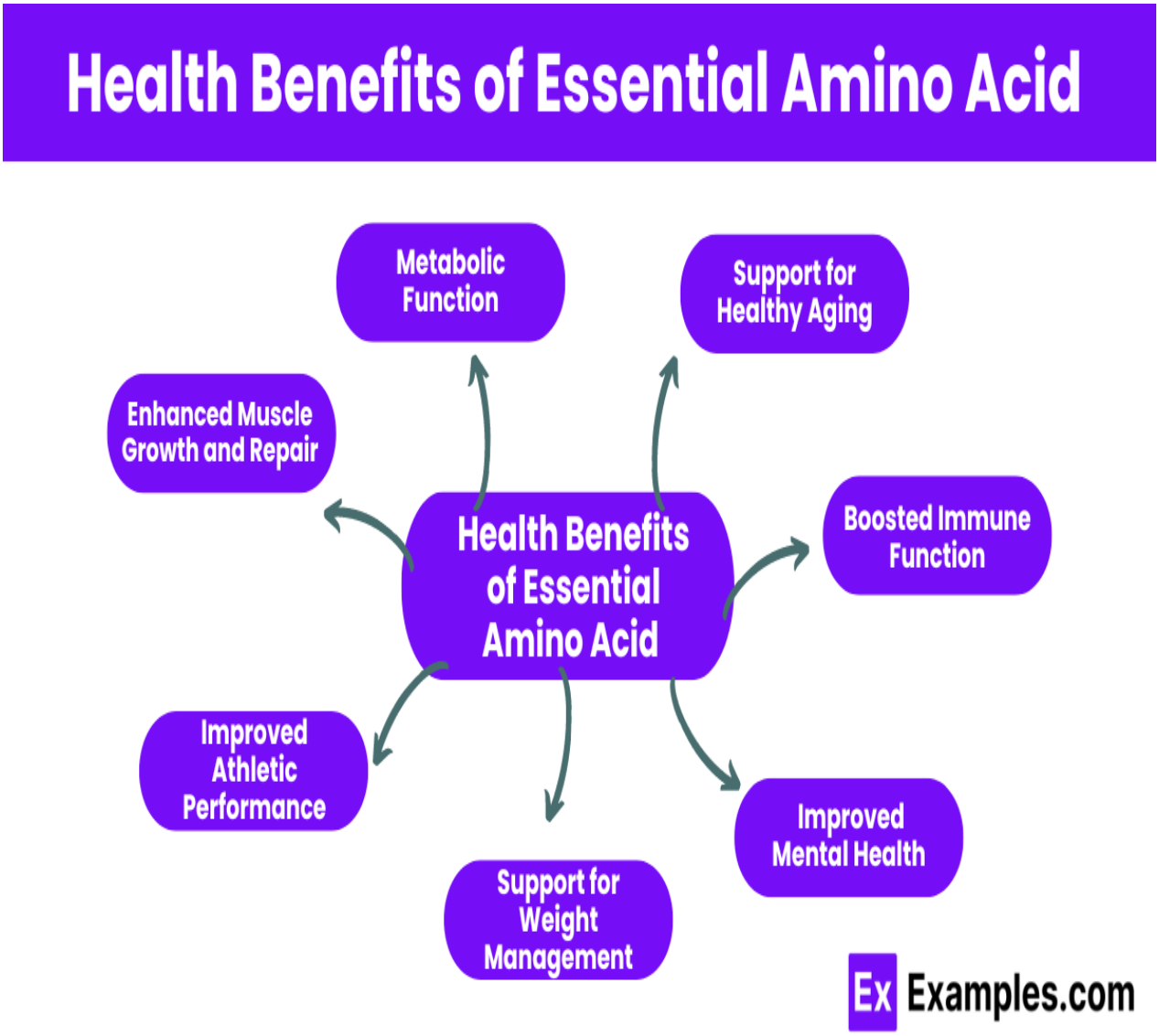


Fig 3.14 Importance of essential amino acid

Non-essential Amino Acids:

1. Glycine: Component of collagen, neurotransmitter, anti-inflammatory, antioxidant, aids in wound healing, and improves sleep.
2. Alanine: Associated with toxin removal during exercise, important for energy production, and can cause fatigue.
3. Arginine: Essential in newborns, participates in the urea cycle, wound healing, immune system maintenance, and nitric oxide production.

4. Proline: Important for cartilage and collagen production, intracellular signaling, and cell signaling.
5. Glutamine: Aids in acid-base balance, DNA/RNA synthesis, and helps in sickle cell anemia.
6. Glutamic Acid: Neurotransmitter, ammonia transport, ulcer healing, and sugar level control.
7. Asparagine: Essential for brain development and function, emotion balance, and ammonia synthesis.
8. Aspartic Acid: Neurotransmitter, urea cycle involvement, and precursor of other amino acids.
9. Serine: Methyl group transfer, epinephrine precursor, nucleic acid synthesis, and muscle growth.
10. Tyrosine: Precursor of thyroid hormone, melanin, and dopamine.
11. Cysteine: Source of sulfide, glutathione precursor, nail/hair component, liver damage cure, and vascular disease risk.

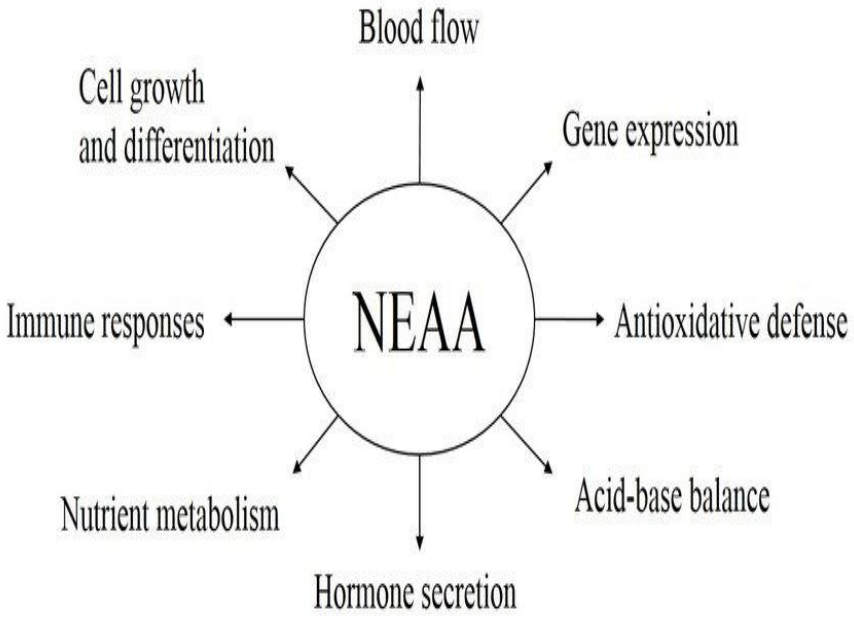


Fig 3.15 Importance of non essential amino acid

3.9 FORMATION OF PROTEINS FROM AMINO ACIDS:

Proteins are synthesized through a series of chemical reactions where the amino group of one amino acid molecule combines chemically with the carboxylic group of another, forming a peptide bond.

This process continues, linking amino acids until all necessary amino acids for protein synthesis are connected. One water molecule is released with each bond formation.

When two amino acids bond, they create a larger unit known as a peptide. Multiple peptides link to form a polypeptide, which then combines to form a complete protein.

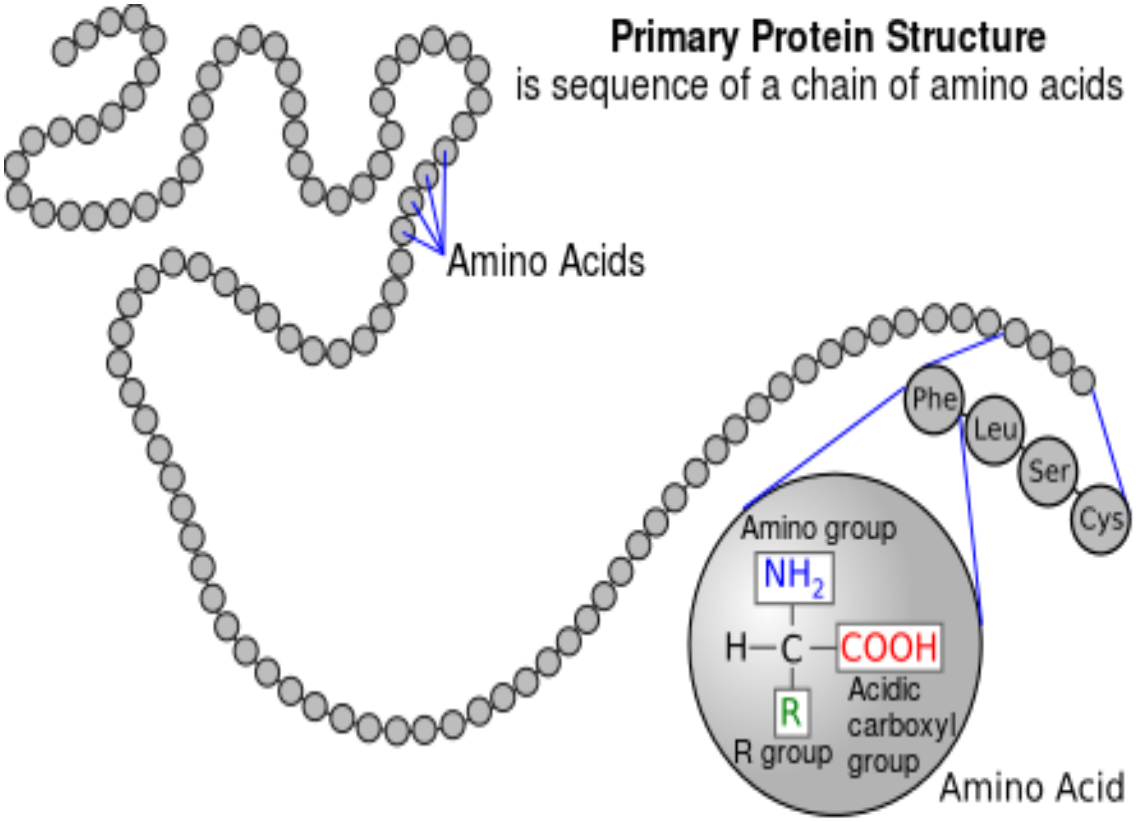


Fig 3.16 Formation Of Proteins From Amino Acids

SELF ASSESSMENT

- Explain structure of amino acid
- Classify amino acid
- Differentiate between essential and non-essential amino acid
- Explain denaturation of protein

UNIT 4
PROTEINS

LEARNING OBJECTIVES

- Understand the concepts which provides a foundation for exploring the diverse roles that proteins play in biological systems
- Explore Bonds stabilizing protein structure
- Explain Levels of organization in proteins
- Differentiate simple and conjugate proteins

4.1 **INTRODUCTION**

Proteins constitute large biomolecules, also known as macromolecules, comprised of 1 or more lengthy chains made up of amino acid filtrates. Within organisms, proteins undertake a diverse range of functions, comprisingofcatalyzing metabolic reactions, facilitating DNA replication, responding towards stimuli, if cellular and organismal structure, and transporting molecules between locations.

A key distinguishing feature among proteins lies in their unique order of amino acids, determined by the nucleotide arrangement of their genes.

This sequence typically consequences in the folding of proteins into precise 3D structures, crucial for their respective activities.

Protein Bonds Proteins are essentially amino acids polymer, with amino acids linked composed by a specialized kind of covalent bond known as a peptide bond, forming linear structures termed polypeptides.

These polypeptides subsequently fold into precise configurations to assume the functional conformation of the protein. The foldaway process is facilitated and stabilized by various types of bonds present within proteins.

These bonds encompass a spectrum from robust to delicate interactions. Significant bond types included in shaping protein conformation and structure include disulfide bonds peptide bonds, hydrogen bonds ionic bonds and hydrophobic interactions.

4.2 TYPES OF BONDS

There exist five pivotal chemical bonds crucial in shaping and stabilizing the three-dimensional structure of proteins. They are:

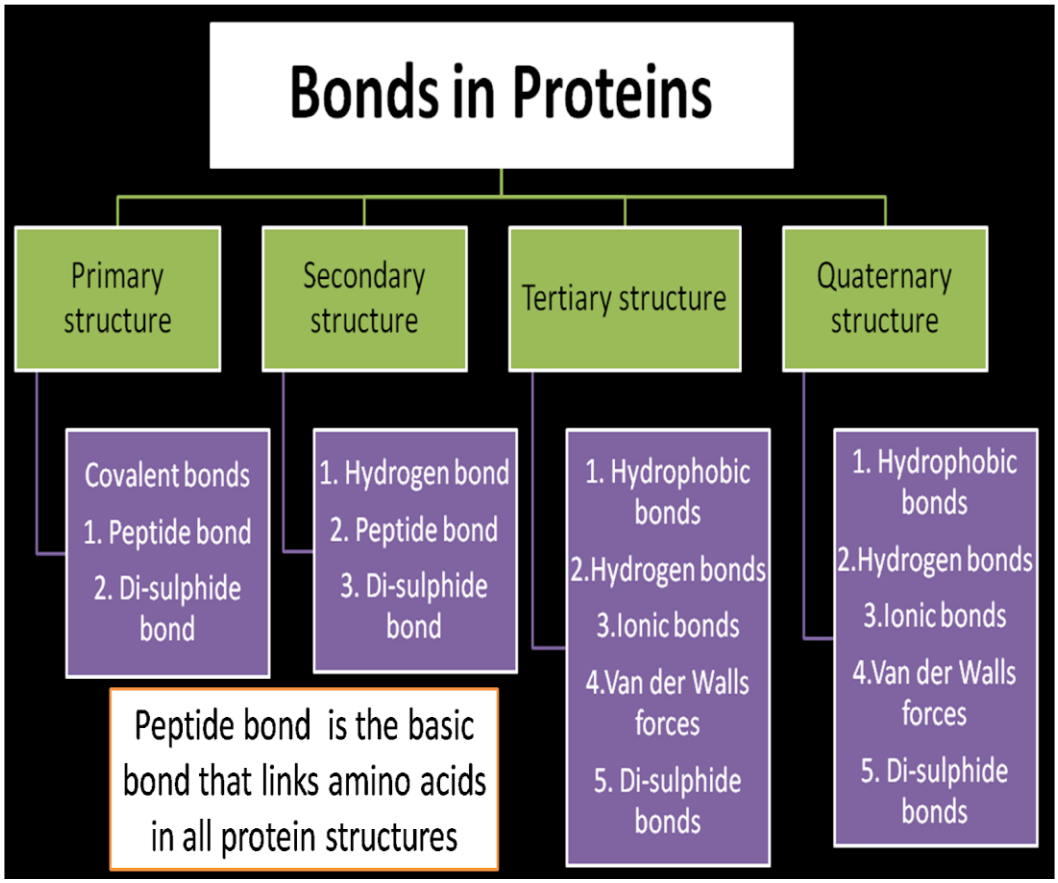


Fig 4.1 Bonds in protein

Peptide Bonds:

- A covalent bond established amid the carboxylic group of 1 amino acid and the amino group of another amino acid.
- They are robust covalent bonds characterized by high bond dissociation energy.
- They form during protein synthesis by the linkage of 2 amino acid residues.
- The carboxylic group (-COOH) of 1 amino acid combines with the amino group (-NH2) of another amino acid to create the peptide bond.
- the bond formation exemplifies a condensation or elimination reaction, where the formation process expels one molecule of water.
- The resultant compound after peptide bond formation is termed a dipeptide.

Ionic Bonds:

- A chemical bond established between two ions of conflicting charges.
- Within proteins, ionic bonds arise between ionized acidic
- Certain amino acids' basic (-NH3+) groups R groups (side chains) contain additional acidic (-COO-) or, which can ionize to yield charged groups at specific pH levels.
- Acidic R groups release H+ ions so they become negatively charged.

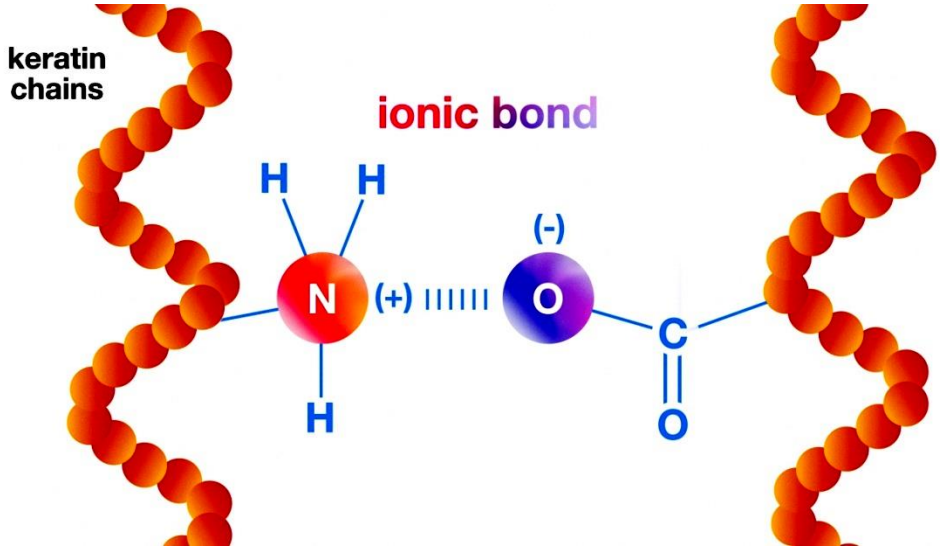


Fig 4.2 Ionic Bond in protein

Disulfide Bonds:

- A covalent bond formed between the thiol groups of two cysteine residues in a protein.
- The sulfhydryl group is both polar and reactive.
- When two cysteine molecules align, adjacent sulfhydryl groups can undergo oxidation, leading to the formation of a permanent covalent connection termed a disulfide bond.
- In protein chemistry, disulfide bonds are commonly referred to as disulfide bridges or S-S bonds.

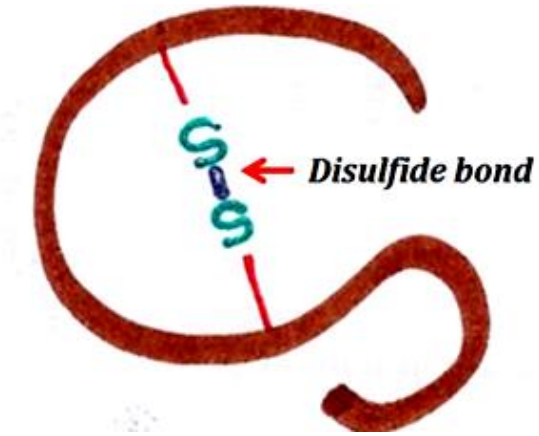


Fig 4.3 Disulfide bond in protein

Hydrogen Bonds:

- The term "hydrogen bond" refers to the electrostatic attraction between an electronegative atom that is covalently bound to a highly electronegative atom (such as nitrogen or oxygen) and another electronegative atom that is nearby in the same or other molecules.
- Hydrogens in the -OH group of -NH2 amino acids exhibit slight electropositivity due to the high electronegativity of oxygen and nitrogen compared to hydrogen.
- This results in the attraction of shared electrons towards oxygen and nitrogen, causing hydrogen attached to these electronegative atoms to develop a partial positive charge (**δ**+), while the electronegative atoms acquire a partial negative charge (**δ**-).

Hydrophobic Interactions:

- Certain R groups within amino acids exhibit non-polar characteristics.
- Examples include isoleucine alanine, valine, methionineleucine.
- These hydrophobic R groups have an aversion to water.
- Within a lengthy polypeptide chain, numerous non-polar amino acids may be adjacent to each other or interspersed with polar R groups.
- In an aqueous environment, such as inside a cell, the linear polypeptide adopts a configuration where hydrophobic amino acids cluster together, actively excluding water due to their hydrophobic nature.
- In the aqueous medium, this clustering mechanism leads the globular protein's peptide chain to fold into a spherical shape.

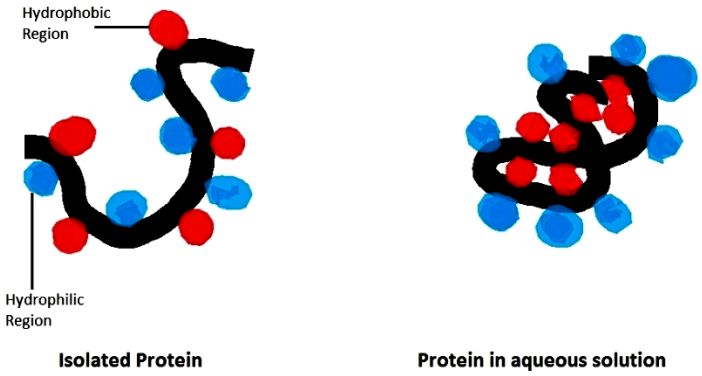


Fig 4.4 Hydrophobic interaction in protein

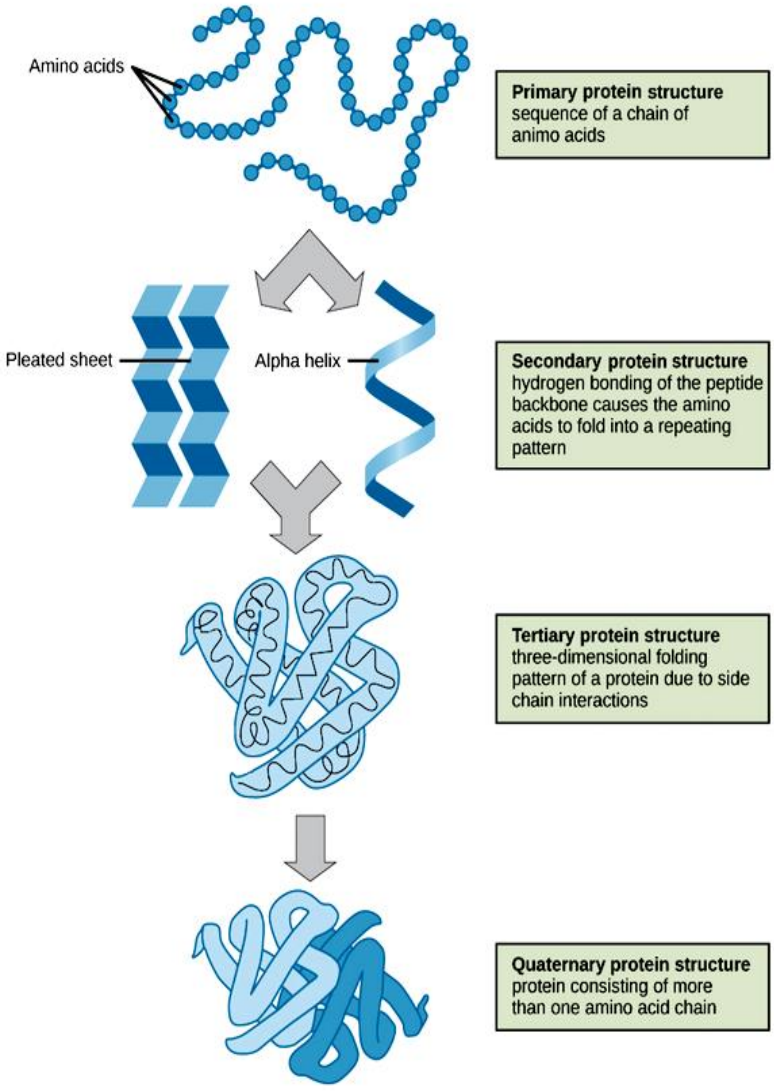


Fig 4.5 Structure of protein

4.3 LEVELS OF PROTEIN STRUCTURE:

Proteins' structural features are typically categorized into four levels of complexity:

1. **Primary Structure:**

- refers to its unique sequence of amino acids and the location of disulfide bonds.
- Amino acids, linked by peptide bonds, are termed residues, with short chains often referred to as (oligo-) peptides.
- Essentially, the primary structure is the protein's genetic blueprint encoded in DNA, dictating all its properties directly or indirectly.

2. **Secondary Structure**:

- Based on their secondary structure—the regular, local organization of the protein backbone—protein structures are further categorized.
- Intramolecular and occasionally intermolecular forces stabilize secondary structures.
- Hydrogen bonding between amide groups.
- The alpha helix and beta strand are two typical forms of secondary structure.

3. **Tertiary Structure:**

- It arises from the spatial arrangement of secondary structure elements, forming the folded shape
- Examples of tertiary structures include single-domain globular proteins, which are roughly spherical and soluble in aqueous solutions.

4. **Quaternary Structure:**

- Some proteins assemble with other molecules, constituting the quaternary structure.
- Examples include hemoglobin, composed of four globular proteins, and actin microfilaments, comprising numerous actin molecules.

4.4 DENATURATION OF PROTEINS:

Denaturation entails the loss of a protein's tertiary quaternary, and secondary structures present in its native state-owned due to exterior stress or compounds such as concentrated inorganic salts, strong acids or bases, radiation,organic solvents, , or heat.

- Denaturation disrupts cellular activity, potentially leading to cell death.
- Denatured proteins may exhibit characteristics ranging from conformational changes and loss of solubility to aggregation due to the exposure of hydrophobic groups.
- Protein folding, critical for proper functioning, is susceptible to disruption by various stressors, underscoring the necessity of physiological homeostasis in many organisms.

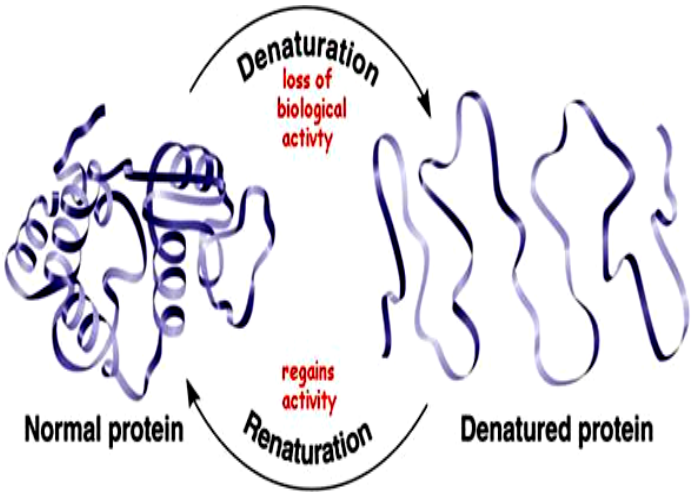


Fig 4.6 Denaturation of protein

4.5 SIMPLE AND CONJUGATE PROTEIN

(I) SIMPLE PROTEINS:

It undergo hydrolysis to yield only amino acids. They are categorized constructed on their solubility in various solvents.

Albumins:

- They are readily solvable in water, alkalies and dilute acids, but coagulate upon heating.
- They are found in lesser quantities in seed proteins.
- Precipitation of albumins from solution can be achieved using increased salt concentration, a process known as 'salting out'.
- Albumins are lacking in glycine.
- Examples include BSA and ovalbumin (found in egg white).

Globulins:

- They are insoluble or frugally soluble in water but become more soluble in neutral salts like sodium chloride.

- They coagulate upon exposure to heat and are deficient in methionine.
- Examples encompass serum globulin, fibrinogen, myosin (found in muscle), and globulins present in pulses.

Prolamins:

- they are insoluble in water but soluble in 70-80% aqueous alcohol.
- Upon hydrolysis, they produce a significant amount of proline and amide nitrogen, hence the name "prolamin".
- They lack lysine and are found in substances like gliadin (wheat) and zein (corn).

Glutelins:

- They areunsolvable in water and absolute alcohol but soluble in dilute acids and alkalies.
- Examples include glutenin found in wheat.

Histones:

- they are small, stable, and basic proteins containing a substantial amount of histidine.
- They are soluble in water but insoluble in ammonium hydroxide.
- Unlike many other proteins, histones are not readily coagulated by heat and are found in globin (part of hemoglobin) and nucleoproteins.

Protamines:

- they are basic proteins soluble in water and resistant to heat-induced coagulation.
- They are rich in arginine and are associated with nucleic acids in the sperm cells of certain fish.
- Typically, protamines lack tyrosine and tryptophan.

Albuminoids:

- They are highly stable and insoluble in water and salt solutions.
- They closely resemble albumins and globulins, exhibiting resistance to proteolytic enzymes.

- These fibrous proteins form the bulk of supporting structures in animals, such as hair, horn, and nails.

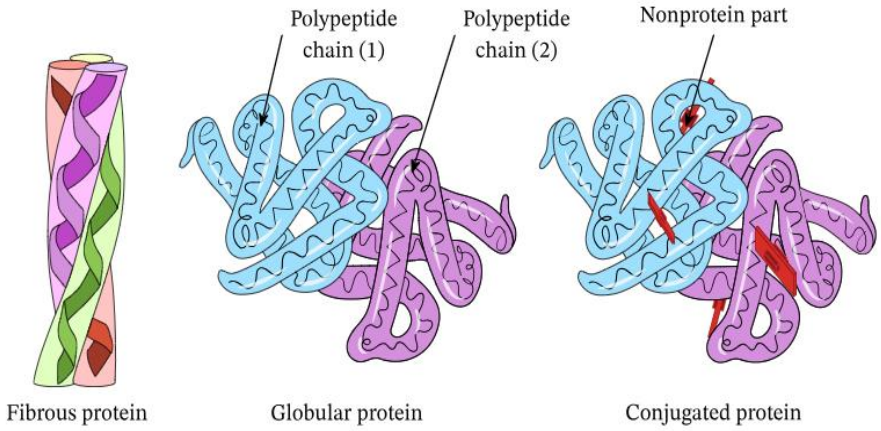


Fig 4.7 Conjugated protein

Conjugate Proteins:

- They interact with non-polypeptide chemical groups through covalent bonding or weak interactions.
- Unlike simple proteins, they yield additional chemical components upon hydrolysis. The non-amino part of a conjugated protein is referred to as its prosthetic group, often derived from vitamins.
- Examples include lipoproteins, glycoproteins, phosphoproteins, hemoproteins (e.g., hemoglobin), flavoproteins, metalloproteins, and others.
- Hemoglobin, for instance, contains heme as its prosthetic group, facilitating oxygen transport in the bloodstream.
- Glycoproteins, being the largest and most abundant group of conjugated proteins, are noteworthy for their diverse biological functions.

SELF ASSESSMENT

- Describe various bonds in proteins
- Explain structure of protein
- What is denaturation?
- Differentiate between simple and conjugate protein

UNIT 5
IMMUNOGLOBULINS

LEARNING OBJECTIVES

- Understand of the structure, function, and diverse roles of immunoglobulins in the immune system,
- Emphasize their significance in pathogen recognition,
- Describe immune response modulation, and therapeutic applications."

Antibodies, also called immunoglobulins, are vital glycoprotein molecules crafted by plasma cells, a type of WBC. They play a pivotal role in the immune system by specifically recognizing and attaching to particular antigens, like viruses or bacteria, assisting in their eradication.

The antibody response is intricate and highly specific, with different classes and subclasses displaying varying biological characteristics, structures, target specificities, and distributions.

Understanding the diversity of immunoglobulin isotypes provides valuable insights into the complex humoral immune response.

There are two primary forms of immunoglobulins: soluble antibodies and membrane-bound antibodies, with the latter containing a hydrophobic transmembrane region.

Initially, B cells express IgM and IgD as their first antigen receptors, prototypes of the antibodies they are primed to produce. The B cell receptor (BCR) binds only to antigens, with Ig alpha and Ig beta heterodimers facilitating signal transduction and B cell activation upon antigen recognition.

Antibodies are classified into various isotypes, each differing in function and antigen responses primarily due to structural variability.

Placental mammals produce five major antibody classes: IgA, IgD, IgE, IgG, and IgM.

This classification stems from disparities in the aa sequence in the constant region (Fc) of the antibody heavy chains. Subclasses exist within IgG and IgA, such as IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2 in humans, distinguished by minor differences in heavy chain sequences.

Further subclassification is possible based on alterations in the aa sequence in the constant region of the light chain, determining the type of light chain (kappa or lambda).

A light chain comprises 2 consecutive domains: that is constant and variable. While the ratio of kappa to lambda light chains varies among species, they are always homogenous within an individual, either both kappa or both lambda.

Identifying individual subclasses is crucial in evaluating primary immunodeficiencies, particularly when total IgG or IgA concentration remains unaltered or raised.

This knowledge is also pertinent in selecting and preparing antibodies for immunoassays and other detection applications.

Human immunoglobulins and their properties.

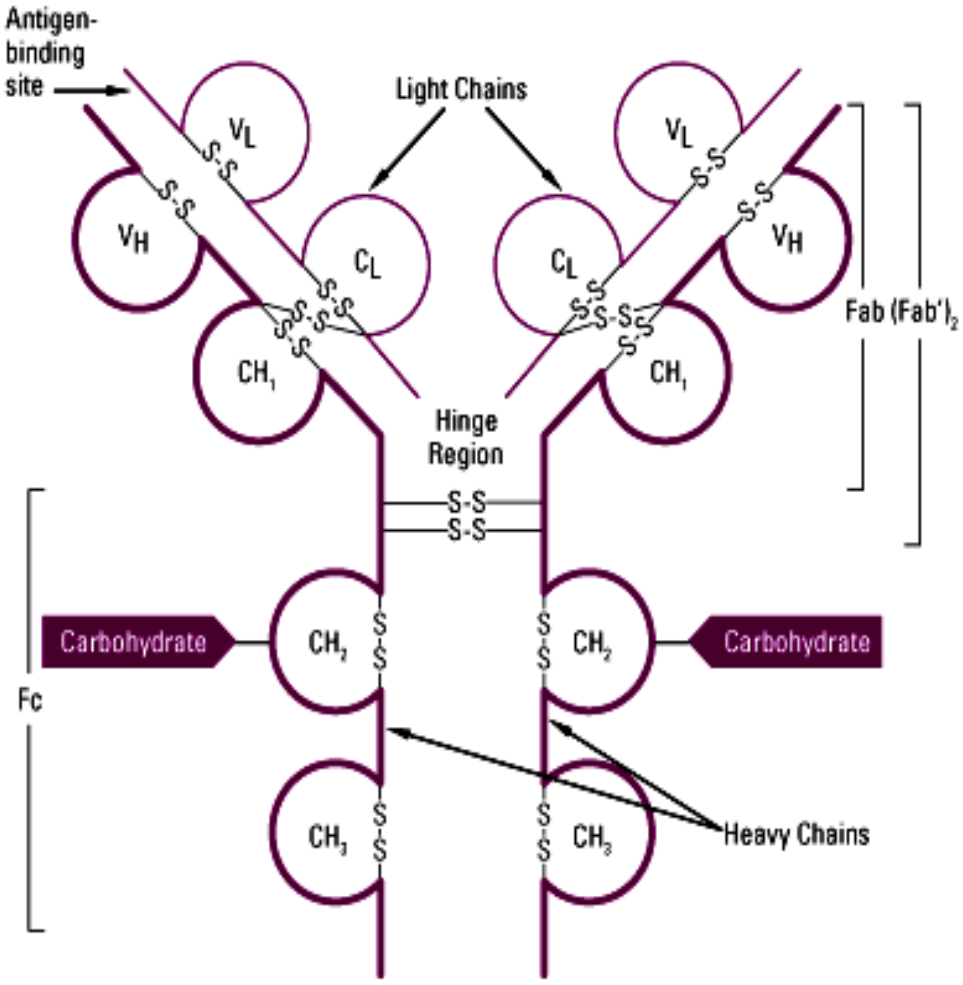
	Immunoglobulin	Serum level (mg/mL)	Molecular mass (kD)	Activation/Binding of complement	Half-life (days)
Human	IgA1	0.6–3	160 (monomer)	-	5.5
Human	IgA2	0.06–0.6	160 (monomer)	-	5.5
Human	IgM	1.5	970 (pentamer)	+++	5–10
Human	IgE	5×10^{-5}	188	-	2
Human	IgG1	3.8–11.4	146	++	23
Human	IgG2	1.5–6.9	146	+	23
Human	IgG3	0.2–1.7	165	+++	7
Human	IgG4	0.08–1.4	146	-	23

+++ = Very strong affinity; ++ = Strong affinity; + = Moderate affinity; - = No affinity

5.1 IMMUNOGLOBULINS

Commonly referred to as antibodies, are glycoprotein molecules synthesized by plasma cells or white blood cells.

They possess the remarkable ability to identify and attach to specific antigens.



5.2 STRUCTURE OF IMMUNOGLOBULINS

The architecture of antibody molecules, called asimmunoglobulins, consists of glycoproteins comprising multiple or single unit, each containing 4 polypeptide chains: 2 heavy (H) and 2 light chains (L).

Variability in aa composition distinguishes the amino terminal ends of the polypeptide chains, termed the variable (V) regions, from the relatively constant (C) regions.

light chain comprises a variable domain (VL) and a constant domain (CL), while each heavy chain encompasses a single variable domain (VH) and 3 constant domains (CH1, CH2, and CH3).

Heavy chains, with about twice the number of aa and molecular weight (~50 kDa) as light chains (~25 kDa), contribute to the overall molecular weight of approximately 150kDa for the immunoglobulin monomer.

Non-covalent interactions and covalent interchaindisulfide bonds unite heavy and light chains, making a symmetric structure.

The V regions of both heavy and light chains house the antigen-binding sites of the immunoglobulin molecules, rendering each Ig monomer bivalent, with two antigen-binding sites. The hinge region, present in IgG, IgA, and IgD (but absent in IgM and IgE), lies between the first and second C region domains of the heavy chains, interconnected by disulfide bonds. This flexible hinge enables variation in the distance between the two antigen-binding sites.

Immunoglobulins are classified as: IgG, IgM, IgA, IgD, and IgE, distinguished by the heavy chain type.

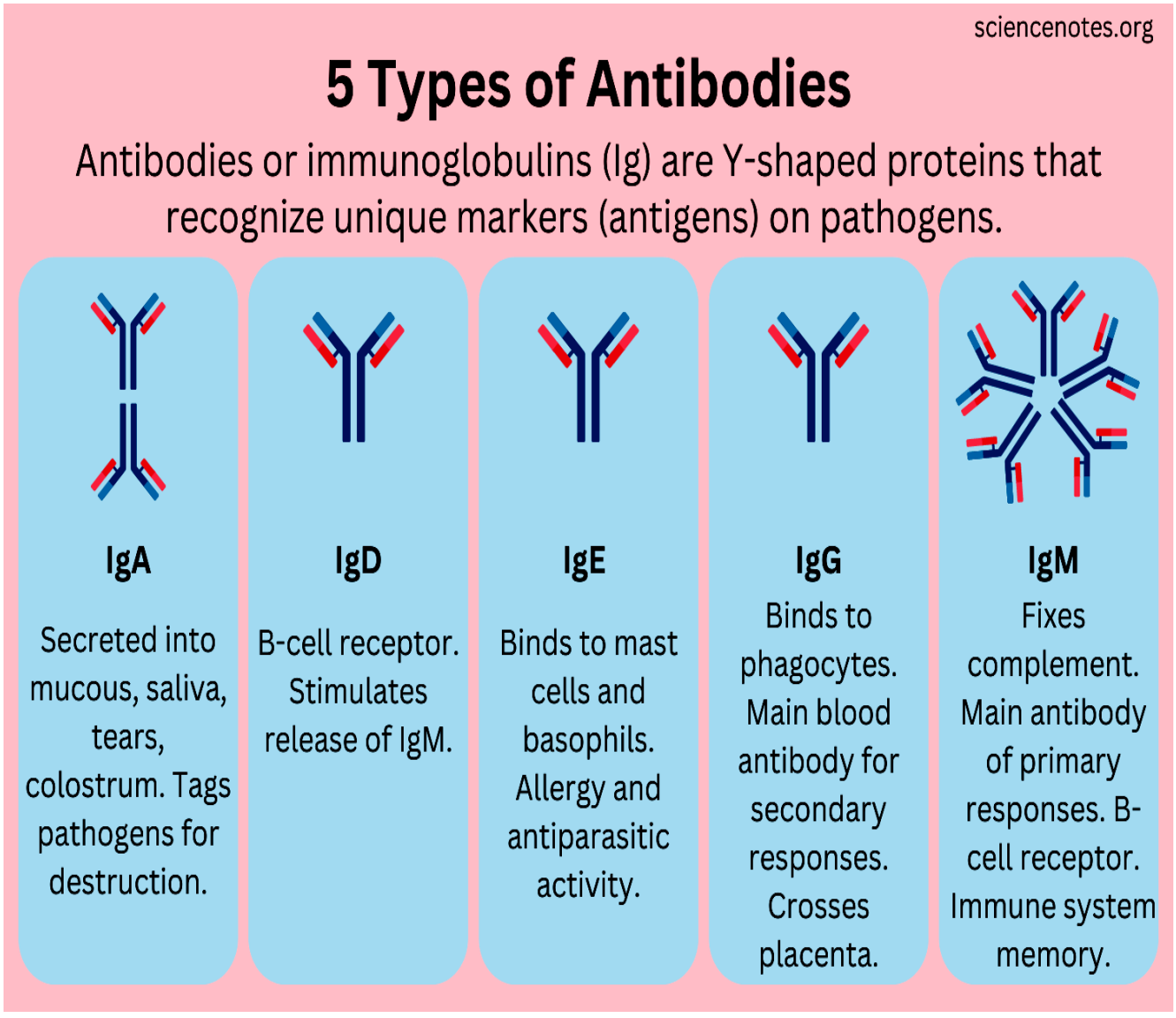
Gamma-chains characterize IgG, mu-chains signify IgM, alpha-chains denote IgA, epsilon-chains represent IgE, and delta-chains typify IgD.

	IgM	IgG	IgA	IgE	IgD
Heavy Chain	μ	γ	α	ε	δ
MW (Da)	900 K	150 K	385 K	200 K	180 K
% of total antibody in serum	6%	80%	13%	0.00%	1%
Fixes complement	Yes	Yes	No	No	No
Function	Primary response, fixes complement monomer serves as B-cell receptor	Main blood antibody, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva	Antibody of allergy and anti-parasitic activity	B-cell receptor

Variations polypeptides of heavy chain allow these immunoglobulins (IGs) to participate in diverse immune responses and stages of the immune reaction, with distinctive protein sequences primarily located in the Fc fragment. While 5 different types of heavy chains exist, only 2 main types of light chains—kappa (κ) and lambda (λ)—are present.

Antibody classes vary in valence due to differences in the number of Y-shaped units (monomers) forming the complete protein. For instance, functional IgM antibodies comprise five pentameric Y-shaped units, totaling ten light chains, ten heavy chains, and ten antigen-binding sites in humans.

5.3 TYPES OF IgG

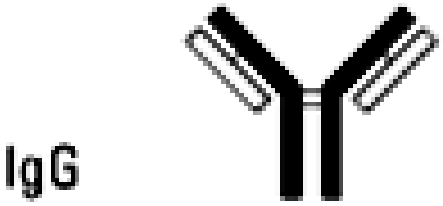


IgG class

Characteristics of IgG:

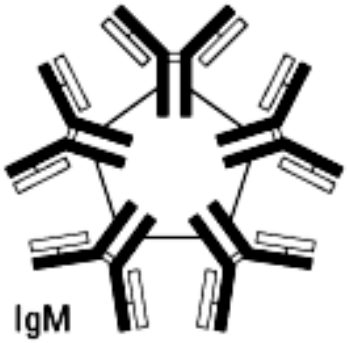
- MW: 15KDa
- Serum conc: Ranges from 10 to 16 mg/mL

- Percentage of total immunoglobulin: Comprises approximately 75%
- Glycosylation (by weight): Constitutes about 3%
- Distribution: Found intra- and extravascularly
- Function: Primarily involved in secondary immune responses



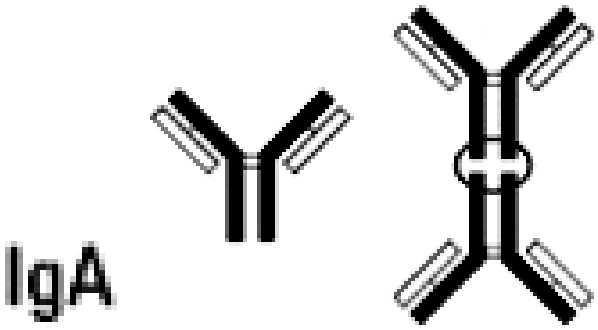
Characteristics of IgM:

- MW: 900 kDa
- Serum concentration: Typically ranges from 0.5 to 2 mg/mL
- % of total immunoglobulin: 10%
- Glycosylation (by weight): Accounts for approximately 12%
- Distribution: Predominantly found intravascularly
- Function: Primarily associated with primary immune responses



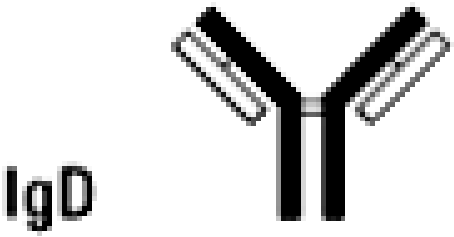
Characteristics of IgA:

- MW: 320kDa
- Serum conc: Typically ranges from 1 to 4 mg/mL
- Percentage of total immunoglobulin: Accounts for approximately 15%
- Glycosylation (by weight): Represents about 10%
- Distribution: Found in intravascular compartments and secretions
- Function: Primarily tasked with protecting mucous membrane



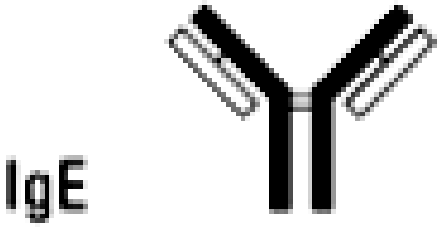
Characteristics of IgD:

- MW: 180 kDa
- Serum concentration: Typically ranges from 0 to 0.4 mg/mL
- Percentage of total immunoglobulin: 0.2%
- Glycosylation (by weight): Constitutes about 13%
- Distribution: Predominantly found on lymphocyte surfaces
- Function: Currently unknown



Characteristics of IgE:

- MW: 200 kDa
- Serum concentration: Ranges from 10 to 400 ng/mL
- Percentage of total immunoglobulin: Accounts for approximately 0.002%
- Glycosylation (by weight): Represents about 12%
- Distribution: Located on basophils and mast cells in saliva and nasal secretions
- Function: Primarily involved in protection against parasites



Self Assessment:

1. Define Immunoglobulin
2. Write the charattistics of IgG and IgA
3. Draw the structure of Immunoglobulin

Unit- 6

DNA and RNA

Learning Objectives:

- To understand the difference between DNA and RNA.
- To determine the characteristic of nucleotide and nucleosides.
- To understand the different types of DNA and RNA.

6.1 Structure of Purine and Pyrimidine

6.1.1 Purine

- These are substances that include two nitrogen atoms in a six-membered carbon ring. An imidazole ring is bonded to this ring.
- The most prevalent heterocyclic rings in nature containing nitrogen are purines.
- Meat products are often contaminated with purines.
- DNA and RNA are the fundamental components of genetic material.
- With four tautomers, each containing a hydrogen bond to a distinct nitrogen atom, purine is an aromatic compound. The designations for them are 1-H, 3-H, 7-H, and 9-H.
- The most fundamental structure in purine biochemistry is the nucleotide, which consists of phosphoric base, purine base, and either ribose or deoxyribose.
- The four most significant purine bases are xanthine, hypoxanthine, guanine, and adenine (fig 3.1).
- These bases' ribosides are produced during metabolism.
- It is well known that xanthine and free hypoxanthine function as purine breakdown intermediates.
- Water and bodily fluids contain trace amounts of purines.
- Purine exhibits low acidity (pKa 8.93) and low base weakness (pKa 2.39).[6] The pH is in the middle of these two pKa values when dissolved in clean water
- Peter and Van Slyke have computed the maximal solubility in plasma to be 6.6g/100ml.
- They can dissolve in water to generate uric acid.
- Purines not only create nucleic acids but also biomolecules including coenzyme A, ATP, NAD, GTP, and cyclic AMP.

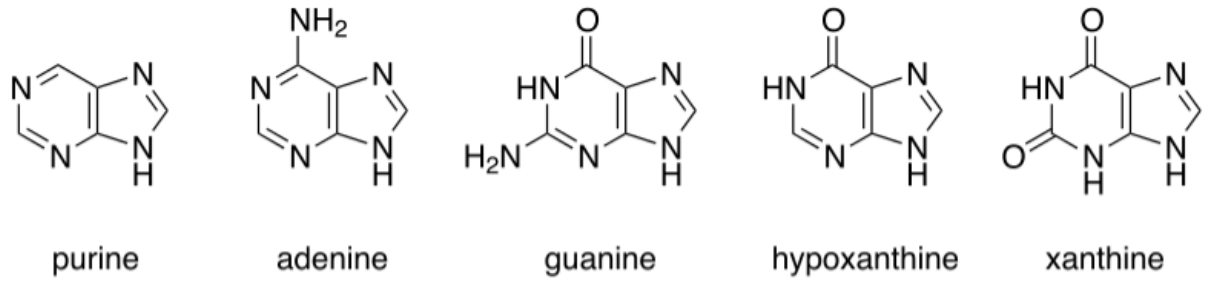


Fig 6.1 various types of purines

Source: https://en.wikipedia.org/wiki/Purine

6.1.1.1 Types of Purine

1. Adenine

- It is that is white and crystalline and is found in both DNA and RNA. An amine group is attached to the carbon at number 6.
- Adenine and thymine are complementary in DNA and RNA, respectively.
- Adenine differs from guanine due to the presence of an amine group at position 6 and an extra double bond in the heterocyclic aromatic (pyrimidine) ring between N-1 and C-6.
- Some examples of adenine derivatives that are involved in many aspects of biochemistry, such as cellular respiration, are ATP and the cofactors FAD, NAD, and coenzyme A.

2. Guanine

- It is a crystalline, colorless and non- soluble substance.
- In both DNA and RNA, it constitutes 2-aminopurine with a 6-oxo substituent. and guanine and cytosine complement each other.
- It was initially isolated from guano (bird feces). Thus, called as guanine.
- Spiders, scorpions, and a few other amphibians convert NH4, a result of protein breakdown in cells, to guanine that expelled with little loss of water.

6.1.2 Pyrimidine

- These are heterocyclic substances with two nitrogen atoms and six-membered rings.
- Their ring structure is comparable to the purine ring structure.
- A nucleobase ring structure has three isomerizing diazene structures.

- In pyridazine, nitrogen atoms are located in regions one and two of the heterocyclic ring.
- In the heterocyclic ring of pyrimidine, nitrogen atoms are located at positions one and three.
- Salvage and de novo synthesis are the methods used to create pyrimidines.(fig 6.2)
- Among the well-known pyrimidine compounds are thiamine (vitamin B1), cytosine, thymine, and uracil are found in nucleic acids and sulfadiazine, sulfamerazine, and sulfamethazine are medications used to treat bacterial and viral infections.

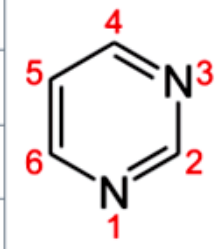
Formula	Name	Structure	C2	C4	C5	C6
$C_4H_5N_3O$	cytosine		=O	$-NH_2$	–H	–H
$C_4H_4N_2O_2$	uracil		=O	=O	–H	–H
$C_4H_3FN_2O_2$	fluorouracil		=O	=O	–F	–H
$C_5H_6N_2O_2$	thymine		=O	=O	$-CH_3$	–H
$C_4H_4N_2O_3$	barbituric acid		=O	=O	–H	=O
$C_5H_4N_2O_4$	orotic acid		=O	=O	–H	-COOH

Fig 6.2 Derivatives of different types of pyrimidines

Source:https://en.wikipedia.org/wiki/Pyrimidine

6.1.2.1 Types of Pyrimidine(fig 6.3)

1. Cytosine:They are found in nucleic acids, which regulate genetic material in all living cells, and in some coenzymes, which function in tandem with enzymes to catalyze chemical reactions within the body.
2. Thymine: A member of the pyrimidine class of chemical compounds, thymine is a component of DNA. All living cells have their genetic traits regulated by DNA and RNA (ribonucleic acid).
   - Thymine, like the other nitrogenous elements of nucleic acids, is chemically bound to the sugar deoxyribose to form thymidine.
3. It is also a phosphate ester of thymidine and a component of thymidylic acid.

4. Uracil: A member of the pyrimidine family of chemical compounds, uracil is a colorless, crystalline molecule that is a part of ribonucleic acid (RNA), a molecule that helps pass on genetic traits.

- Each nucleotide in the RNA molecule has three components: a nitrogenous base, a phosphate group, and a five-carbon sugar called ribose.
- The RNA contains four nitrogenous bases: uracil, cytosine, adenine, and guanine. All of these bases are also present in DNA, with the exception that uracil is replaced with thymine. Only uracil and guanine pair with one another during transcription, the process by which an RNA strand is synthesized from a DNA template.

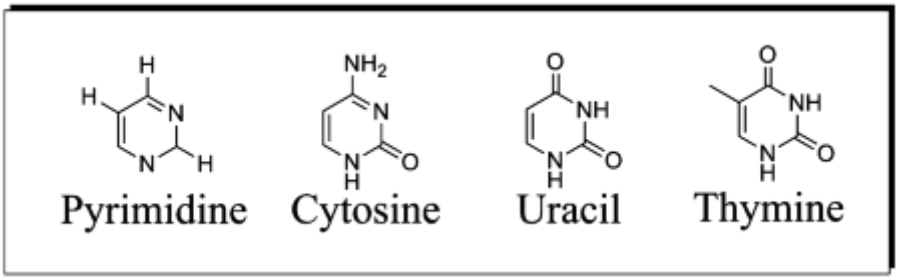


Fig 6.3 Types of Pyrimidine

Source:https://study.com/learn/lesson/pyrimidine-bases-structures.html

6.2 Nucleotides and Nucleosides(Fig 6.4)

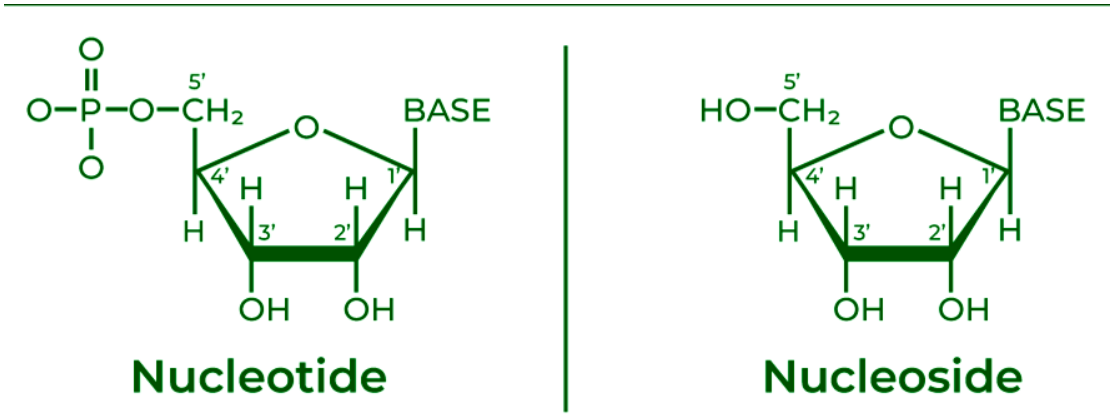


Fig 6.4 Difference between Nucleotide and Nucleoside

Source:https://www.geeksforgeeks.org/difference-between-nucleotide-and-nucleoside/

6.2.1 **Nucleotides:**

- A chemical molecule known as a nucleotide are the building block of DNA and RNA. It also play roles in metabolism, cell signaling, and enzyme functions. The nucleotide has a nitrogenous base, a phosphate group, and a 5-carbon sugar.
- They are made up of a phosphate group and a nitrogenous base that are joined to a 5 Carbon sugar that can be either ribose or deoxyribose.
- The pentose sugar with carbon-5 may contain 1 to 3 phosphate groups.
- .Thymine,cytosine and uracilare pyrimidine, whereas guanine and adenine are purine (Fig 6.5).

Nitrogenous Bases	Corresponding Examples
Guanine	GMP, GDP, GTP, dGMP, dGDP, dGTP, and dGTP
Adenine	AMP, ADP, ATP, dAMP, dADP, dATP, and ddATP
Cytosine	CMP, CDP, CTP, dCMP, dCDP, dCTP, and ddCTP
Thymine	TMP, TDP, TTP, dTMP, dTDP, dTTP, and ddTTP

Fig 6.5 Example of nucleotides that corresponds to their nitrogenous base

Source: https://www.vedantu.com/biology/difference-between-nucleotide-and-nucleoside

6.2.2 Nucleoside:

- Any nucleotide attached to the pentose sugar's 5' carbon that lacks a phosphate group is referred to as a nucleoside.
- A nucleoside that joins the one to three phosphate groups is always present in a nucleotide.

- Ribo or Deoxyribonucleoside depends on the 5 Carbon sugar molecule.
- A nucleoside that has the ribose sugar component present is called a ribonucleoside.
- Conversely, a nucleoside containing deoxyribose sugar is called a deoxyribonucleoside.
- Inosine,uridine, Cytidine, adenosine, thymidine, and guanosine are a few examples of nucleosides. The pentose sugar's 3' position is connected to the nitrogenous base by a beta-glycosidic link.
- Nucleosides have antiviral and anticancer properties.
- Various types of nucleosides are :
    a. Adenosine:Adenine is glycosidically bound to a ribose sugar to form adenosine, a purine nucleoside. It is a structural element of important macromolecules that may be present in all living things. It is a crucial molecular component of ATP, AMP, and ADP. Consequently, it contributes to physiological functions like as energy transfer (ATP, for example) and signal transduction (cAMP, for example). Additionally, it helps promote sleep by acting as an inhibitory neurotransmitter.
    b. Guanosine: Composed of guanine and ribose sugar, guanosine is a purine nucleoside. Nucleotides like GMP, cGMP, GDP, and GTP can be produced from it by phosphorylation. Among the many biological functions that these nucleotides perform are intracellular transmission, protein synthesis, and nucleic acid synthesis, photosynthesis, and muscle contraction.
    c. Cytidine: pyrimidine nucleoside havingC sugar ribose connected to it.
    d. Uridine: With uracil and a 5C ring attached, uridine is a ribonucleoside. It's a white powder with no smell that helps with the metabolism of glucose.

6.3 Nucleic acid

- Nucleic acid can be broken down to produce phosphoric acid, a combination purines and pyrimidinesand sugars.
- Nucleic acids, the main information-carrying molecules in cells, regulate the synthesis of proteins and, consequently, specify the hereditary characteristics of all organisms.
    - The two main forms of nucleic acids are RNA and DNA.
    - DNA is the genetic substance and the ultimate blueprint for life; it makes up all

free-living organisms and most viruses. RNA is a component of all living cells and is essential to numerous biological processes, such as the creation of proteins, in addition to being the DNA of certain viruses.

Types of Nucleic acid:

a. Deoxyribonucleic Acid (DNA):

- Chemically, phosphoric acid, pentose sugar, and some cyclic bases (N2 containing) make up DNA. DNA molecules include the sugar component known as β-D-2-deoxyribose.
- The cyclic bases are adenine (A), cytosine (C), Guanine (G), and thymine (T).
- Information is stored in DNA molecules and is passed through generations mostly depending on the arrangement of these bases. The strands in DNA's double-strand helical helix are complementary to one another.
- It is structurally and chemically stable hence a perfect genetic material..

b. Ribonucleic Acid (RNA)

- B-D-ribose is the sugar moiety found in RNA.
- It contains the heterocyclic bases guanine (G),uracil (U), cytosine (C), and adenine (A).
- RNA is made up of a single strand that occasionally folds back to form a double helix.

Types of RNA (Fig 3.6)

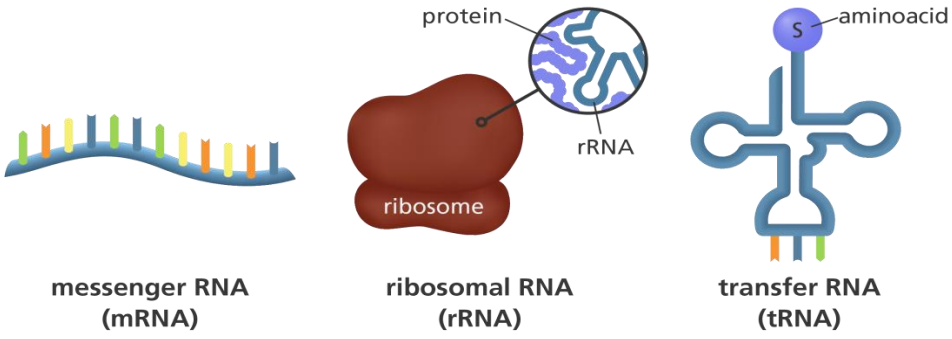


Fig 3.6 Different types of RNA

Source:https://www.yourgenome.org/theme/what-is-rna/

(i) Messenger RNA (mRNA):

- It carries data from DNA to an organelle's or specialized structure's ribosome, where it is translated into a protein.
- This kind of information is present in one or more genes. Prokaryotes contain mRNAs, which are precisely transcribed versions of the DNA sequence having terminal 5′-triphosphate group and a 3′-hydroxyl residue.
- In eukaryotes, the mRNA molecules are intricate.
- A structure called a cap is shaped by the 5′-triphosphate residue following further esterification. Long polyA lines of adenosine residues are typically seen at the 3′ ends of eukaryotic mRNAs. and are generated by cleaving and reassembling an original gene, and are frequently composed of brief segments of the original

(ii) Ribosomal RNA (rRNA):

- rRNA molecules are the fundamental units of the ribosome's structure.
- The rRNAs, which also form sizable subordinate structures, actively recognize the conserved areas of mRNAs and tRNAs.
- They aid in the catalysis of protein synthesis as well.
- In eukaryotes, these rRNA genes loop out of the primary chromosomal fibers and combine to form the nucleolus, an organelle that arises in the presence of proteins. The nucleolus is where the transcription of the early rRNA genes takes place.

(iii) Transfer RNA (tRNA):

- tRNA carries individual amino acids into the ribosome where they are synthesized into the growing polypeptide chain.
- The tRNA molecules have 70–80 nucleotides and fold into a unique cloverleaf shape.
- specifictRNA for each of the 20 amino acids needed for protein synthesis, and in many cases, there is a second tRNA for every amino acid.
- To construct a protein sequence from a nucleotide sequence, each codon—a three-base sequence—is translated with a different protein.
- Less than 61 distinct tRNAs are able to read the 61 codons required for amino acid coding.

3.4 Cot Curves and DNA Renaturation Kinetics

The Cot Curve can be used to analyze DNA renaturation.

Cot value and study of the Cot Curve:

- It is a method for calculating the genome's or DNA's complexity (size). In 1960, Roy Britten and Eric Davidson invented the approach.
- The method is predicated on the kinetics of DNA renaturation.

  *Principle:*The number of times a sequence appears in the genome is closely correlated with the rate of renaturation. In a given DNA sample, all denatured DNA will reassociate, or reanneal, given enough time.
- The amount of time needed for renaturation will decrease with increasing sequence repetition.
- The following variables affect renaturation: viscosity, cation concentration, reassociation temperature, and DNA concentration.

  Cot Value= Concentration of DNA (mol/L) *Renaturation time (in sec)* Buffer factor
- A low cot value shows a higher frequency of recurring sequences.
- A high cot value denotes a higher proportion of unique sequences and a lower proportion of repetitive sequences (Fig 3.7)

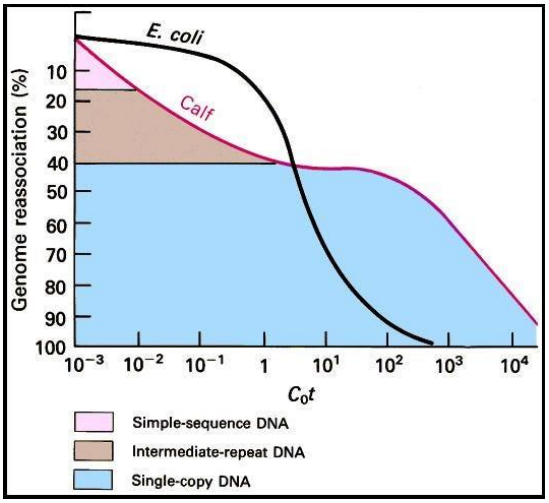


Fig 3.7 Graph showing Genomic reassociation v/s Cot values of Calf and E. coli

Source: https://medium.com/@tapashigupta/what-is-cot-curve-how-does-it-helps-in-analysis-of-renaturation-of-the-dna-7c8727145191

Steps in Cot Analysis:

a. After being mixed thoroughly in a blender with an antioxidant media, the organism's tissue is extracted. Plasmids are selectively lysed, the homogenate is filtered, and centrifugation is used to pellet the nuclei.
b. Phase-contrast microscopy is used to check whether the pellet contains any contaminated organelles. Using phenol/chloroform extractions combined with proteinase and RNase digestions, DNA is extracted from purified nuclei.
c. By high-speed mixing or sonication, the DNA is divided into fragments of roughly 450 bp.
d. Agarose gel electrophoresis is used to measure fragment size. After precipitating sheared DNA, aliquots of the material are dissolved in sodium phosphate buffer (SPB) at concentrations of 0.03 M, 0.12 M, and 0.50 M to create solutions with known contents.
e. The DNA concentrations are divided among glass ampoules or microcapillary tubes, with each one holding 100 µg of DNA. The tubes' or ampoules' ends are sealed.
f. A tube with a predetermined amount of DNA is chosen.
g. To denature DNA duplexes, the tube is submerged in hot water.
h. After that, the tube was put in a water bath that was heated in sample or Temperature at 25ºC. Renaturation is permitted to continue until the sample reaches a predetermined Cot value, which is determined by multiplying the nucleotide concentration by the reassociation time and adding a suitable buffer factor based on the concentration of cations in the sample.
i. The sample's end is broken off from the tube or ampoule once it reaches the required Cot value, and the solution is blown into a 100-fold excess of 0.03 M SPB.

Interpretation of Cot curve:

- The Y-axis of a DNA renaturation graph shows the proportion of single-stranded DNA that still exists. The single-stranded DNA concentration (C) is divided by the concentration of all DNA (C0) to get this percentage.
- The result of the overall DNA concentration (C0) and the reaction time (t), expressed in seconds, is shown on the X-axis. The C0t curve gets its name from this value, which is known as C0t. To account for the influence of cation, a buffer factor is also taken into consideration in the calculation.

Applications of Cot Curves:

A sample DNA strand's cot value curve can be used to assess the level of complexity in a given gene sequence. This curve aids in figuring out how many repeating sequences are there in a DNA strand. When DNA bases are extremely repeated, the curve displays a high growth rate; when repeating sequences are moderate, the re-anneal graph displays a moderate pace. The graph offers a low pick that aids in the analysis of the sample DNA strand's complexity when there are few repeated sequences. The Cot curve's value aids in both simplifying the DNA sample and figuring out the genome's structure.

6.4 Types of DNA *(Fig 3.8)*:

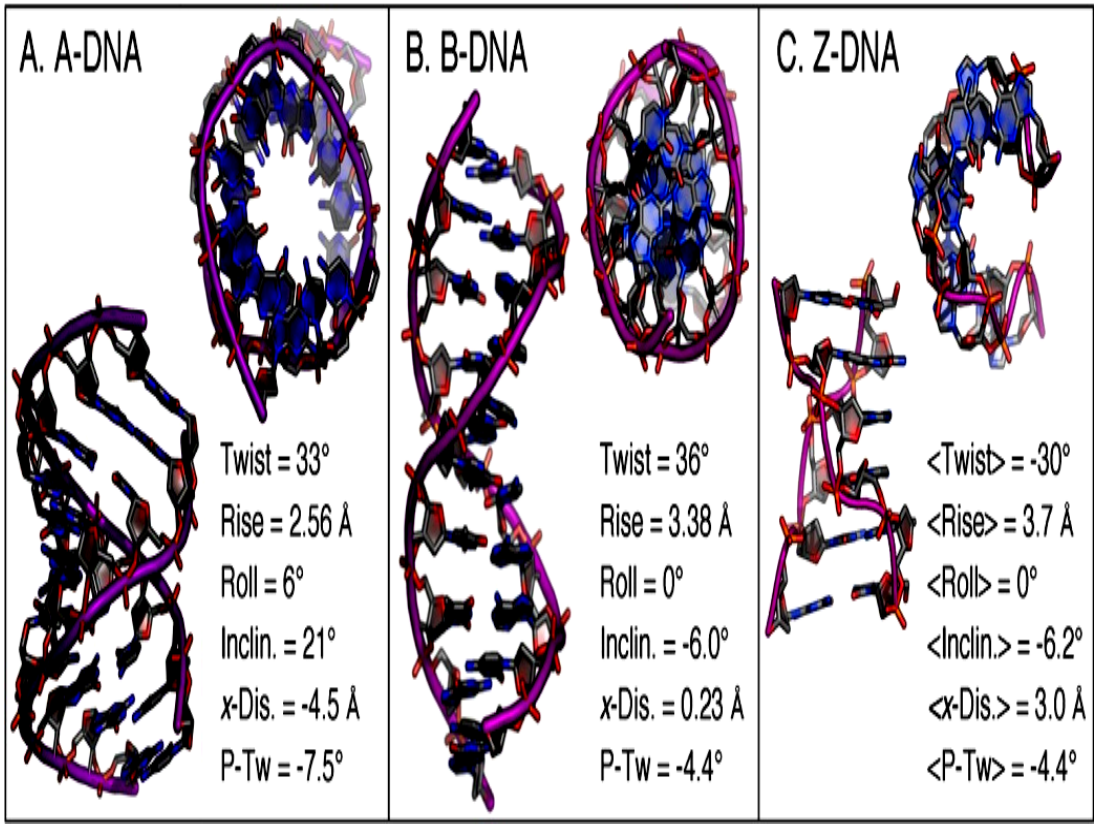


Fig 3.8 Different types of DNA

Source:https://www.intechopen.com/chapters/16928

1. **B form DNA**- Watson and Crick suggested that if two DNA strands coiled around the same axis, each in a right-hand helix. H-bonding between the bases that binds the two strands together.

- The duplex consists of two strands that are plectonemically coiled and oriented antiparallel. The nucleotides of one strand, arranged from 5′ to 3′, align with complementary nucleotides on the opposite strand, arranged from 3′ to 5′.
- In pyrimidine one strand pairs with a purine on the other, the bases fit the double helical structure. According to Chargaff's rules, the two strands pair A with T and G with C.
- Each complete turn of the helix contains ten bases, with the helix having a diameter of 1.9 nm.
- 11 bp per turn
- 20° base-pair tilt
- 33° twist angle
- 2.3 nm helix diameter
- 0.26 nm axial rise
- 28° helix pitch
- Minor and Major Groove: In DNA, the major groove is larger than the minor groove and serves as a site of interaction for numerous sequence-specific proteins. Purines' and pyrimidines' C4 and C5 groups and purines' N7 and C6 groups face into the main groove, allowing them to specifically interact with amino acids in DNA-binding proteins. As a result, certain amino acids work as donors and acceptors of H-bonds to create H-bonds with particular DNA nucleotides. The minor groove contains H-bond donors and acceptors as well; in fact, certain proteins bind there exclusively. Base pairs rotate slightly among themselves as they stack (fig 3.9).

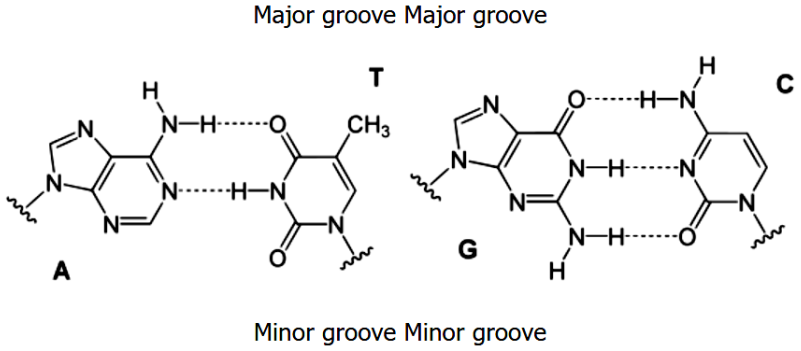


Fig 3.9 B form DNA

Source:https://bio.libretexts.org/Bookshelves_

2. A form DNA:

- A- and B-form nucleic acids vary primarily in that the deoxyribose sugar ring confirmation is present.
- InB-form, the conformation is C2′ endo and in the A-form it is C3′ endo.
    - A significant distinction between nucleic acids in A- and B-forms is where base pairs are positioned inside the double side.
- In A-form, the basepairs gets shifted far from the medial axis towards the major groove, but in the B-form it gets aligned with the helix.
- This results in an A-shaped helix that resembles a ribbon with a more exposed cylindrical core.
- Helix in the right hand.
- It possess 11 bases for every full turn, and the helix's width is 2.3 nm.

3. Z form DNA:

- Z-DNA has a distinctly different duplex structure, with the two strands coiling in left-handed helical configurations and a visible zig-zag pattern in the phosphodiester backbone.
- The zigzag pattern of GCGCGC, an alternating purine-pyrimidine sequence, is caused by the G and C nucleotides being in different forms, which might result in Z-DNA.
- The main distinction is seen in the G nucleotide.
- This reverses the typical anticonformation seen in A- and B-form nucleic acid, placing the guanine back onto the sugar ring. • Unlike B-form DNA, it has the sugar in the C3′ endoconformation and the guanine base is in the synconformation.
- Discovered in 1984 by Rich, Nordheim, and Wang.
- Like B-DNA, it has antiparallel strands.
- In comparison to B-DNA, it is slender and lengthy.
- 0.45 nm axial rise ,1.8 nm helix diameter, 45 o helix pitch ,12 bp per turn, 7 o base-pair tilt; 30 oC twist angle.

Conditions that Favors the formation of different types of DNA:

- A minimum of three requirements must be met for a DNA sequence to be in the A-, B-, or Z-DNA types.
- The hydrating environment is the first, as it can help in the transfers of various helical forms.

- Z-DNA may be favored by large concentration of salt, but A-DNA has been favored by low hydration.
- The second need relates to the DNA sequence: specific purine (or pyrimidine) sequences are preferred for A-DNA, while purine-pyrimidine steps that alternate are most effective for Z-DNA formation.
- The third requirement is the existence of proteins, like that bind to B form DNA and push it to either the Z or A forms which can attach to DNA on one helix and compel the DNA to switch to a different helix.
- DNA in living cells are A and B-DNA configurations, with a few tiny areas having the ability to create Z-DNA.

6.5 Complementary DNA

- A complimentary copy of the mRNA called double-stranded DNA is generated by the enzyme reverse transcriptase.
- This cDNA is modified by adding restriction site-containing linker sequences at the end, and then treating the resulting cDNA preparation with a restriction enzyme to create cohesive ends that are prepared for integration into a vector.
- The genetic cloning of genes from eukaryotes is frequently unfeasible due to the presence of multiple large introns.
- Plasmid vectors have a practical width limit of under ten kilobase pairs (kbp), and PCR becomes challenging beyond 10 k. Therefore, genes from eukaryotes (with large introns) cannot be cloned in plasmid.
- Prokaryotes frequently employ cDNA to clone eukaryotic genes.
- Scientists insert cDNA into a vector and transfer it as a recombinant DNA molecule into the host cell when they wish to express a particular protein in a cell that doesn't ordinarily make that protein. The receiving bacterial cell expresses the desired gene, which is transported by the cDNA, to make the targeted protein.
- While gene products can be readily identified using cDNA libraries, these libraries are not as comprehensive as genomic DNA libraries when it comes to information regarding enhancers, introns, and other regulatory elements.

cDNA Libraries:

- A group of cloned fragments of cDNA into different host cells is called a cDNA library. They are often used to identify the fraction of eukaryotic genes that code for proteins that are expressed.

cDNA Synthesis:(fig 6.10)

1. The mature messenger RNA strands are taken out of the cell during the mRNA extraction process.
2. After being isolated from the other RNAs, the mRNA is extracted. There are numerous techniques for purifying RNA, including column purification and trizol extraction.
3. There is an addition of deoxyribonucleotide triphosphates (A, T, G, and C) and enzyme reverse transcriptase. By doing this, a single complementary DNA strand hybridized with the primary mRNA strand is created.
4. Following treatment with alkali or an enzyme (RNase), the resulting mRNA-cDNA hybrid is hydrolyzed to yield single-stranded cDNA.
5. The loop is subsequently broken by an enzyme known as S1 nuclease.
6. In order to add the single-stranded tail to the cDNA strands at the 3' end, the double stranded cDNA is finally treated with the enzyme terminals transferase.
7. The double-stranded cDNA molecule is now prepared for insertion into the proper cloning vector in order to be amplified.

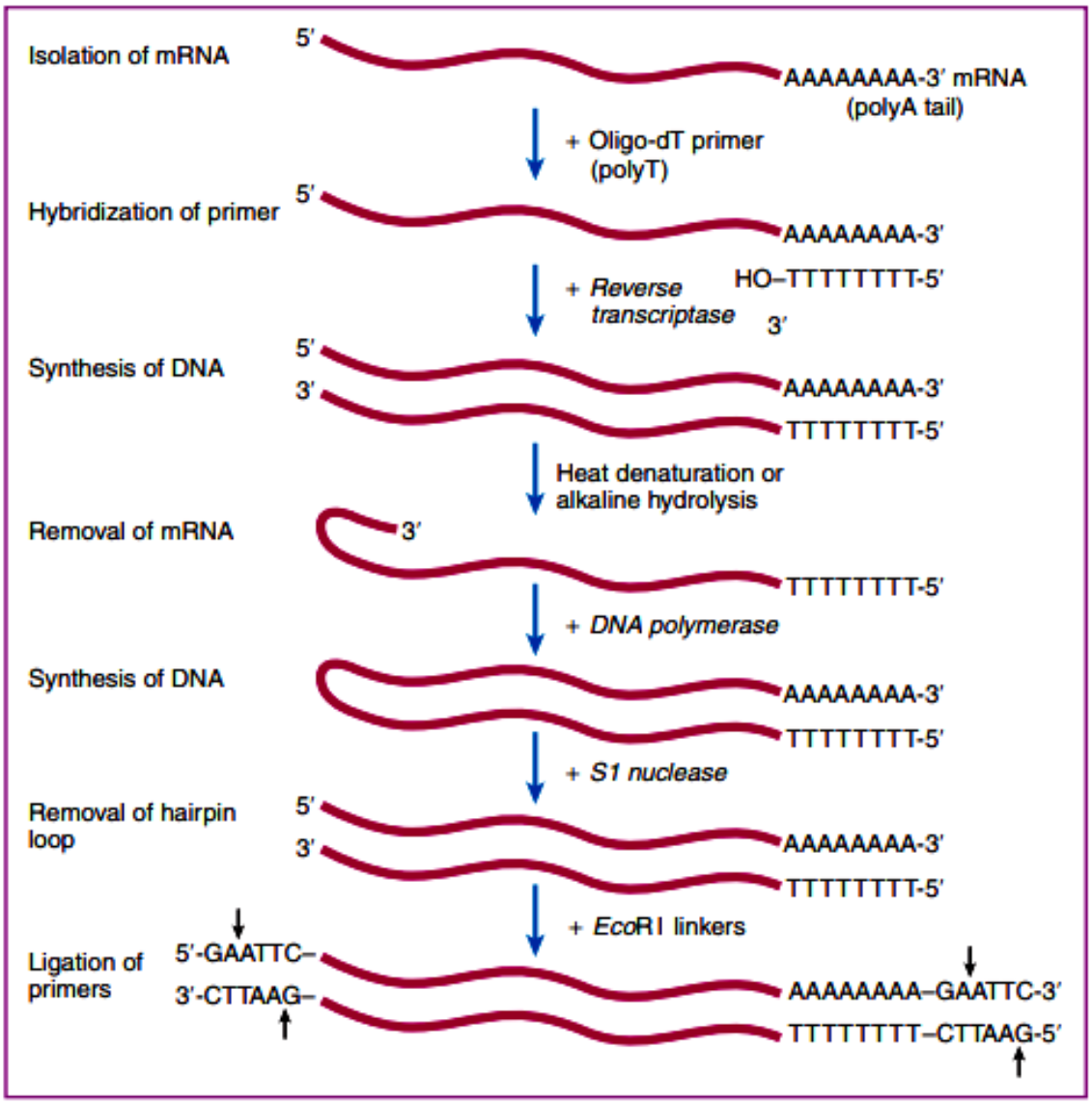


Fig 6.10 cDNA preparation from mRNA

Source:https://sci-hub.se/https://doi.org/10.1016/B978-0-323-03410-4.50024-9

Applications of cDNA library:

a. A potent and practical approch in the field of biotechnology is the cDNA library.
b. It facilitates the process of transcription of prokaryotes by helping to express eukaryotic genes in prokaryotes.
c. In order to code mRNA, it is employed to separate DNA sequences.
d. A cDNA library's ability to separate homologous genes is beneficial.

e. Additionally, it is employed in the screening of genomic libraries to separate certain cDNA.

f. Protein cDNA can aid in the production of monoclonal and antibody synthesis.

g. The investigation of mRNA expression is the primary use of cDNA libraries.

6.6 Hypochromicity and Hyperchromicity

6.6.1 Hypochromicity

- The term "hypochromicity" refers to a material's declining light-absorbing capacity. The material's increased capacity to absorb light is known as hyperchromicity.
- The decrease in UV light absorption in double-stranded DNA relative to single-stranded DNA is known as the hypochromic effect.
- A double stranded DNA has stacked bases as opposed to a single-strand DNA, which adds to the stability and hypochromicity of DNA (Fig 6.11).
- The stacked nucleotides in a double stranded DNA fragment and become less stable as the DNA is denatured. Since the bases are free to absorb light and no longer form hydrogen bonds, it also absorbs more UV light. Raising the pH level, adding denaturant, and heating DNA are methods for denaturing it.

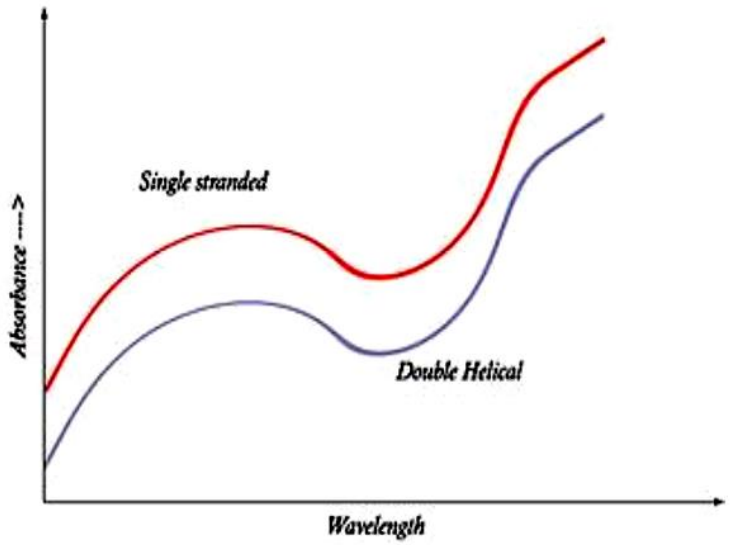


Fig 6.11 Wavelength difference of absorbance of UV in single and double stranded DNA
Source:https://dhingcollegeonline.co.in/attendence/classnotes/files/1602344266.pdf

Applications of Hypochromicity:

Measuring the amount of light absorbed is crucial for tracking the process of melting and maturation of DNA. Half of the DNA is double stranded and half denatured at the melting temperature (Tm). A temperature drop below the melting point (Tm) would cause the denatured strands of DNA to anneal again and form double stranded DNA. The DNA becomes denatured when the temperature rises above the Tm.

Since melting happens very immediately at a particular temperature, the melting temperature can be determined by tracking the absorbance of DNA at different temperatures. Researchers are able to separate and anneal DNA strands with each other by determining the temperature where the DNA melted and became annealed. This is crucial for generating hybrid DNAs, which are made up of two distinct DNA strands. The annealing of DNA strands requires similarity, hence the formation of hybrids DNAs can reveal commonalities between the genomes of other animals.

6.6.2Hyperchromicity

- The increase in absorbance (optical density) is known as hyperchromicity.
- As both individual DNA strands have been separated, either by heat, denaturant addition, or pH increase, the UV absorbance increases. Hypochromicity is the opposite, a reduction in absorbance.
- Single-stranded DNA is created when heat denaturation, also known as melting, forces the double helix arrangement to unravel.
- Double-stranded DNA in solution unwinds to create single-stranded DNA when heated over its melting point, which is typically more than 80 °C. As a result, the bases become less packed and can take in more light. Light in the wavelength range of 260 nm is absorbed by DNA bases in their natural state.
- The amount absorbed rises by 37% while the wavelength of the highest absorption remains unchanged as the bases get unstacked. A sudden cooperative transition is created when a double-stranded DNA strand splits into two single strands.
- The state of DNA can be monitored as temperature changes using hyperchromicity. The temperature at which 50% of the DNA is denatured, or where the absorbance of UV radiation is halfway between the maximum and minimum, is known as the

transition/melting temperature (Tm). Temperature increases by 16.6 °C when the concentration of monovalent cations is ten times higher.

- The dramatically increased absorbance of DNA upon denaturation is known as the hyperchromic effect. The hydrophobic effect between the complementary bases, stacking interactions, and hydrogen bonds are the primary forces holding the two strands of DNA together (Fig 6.12).

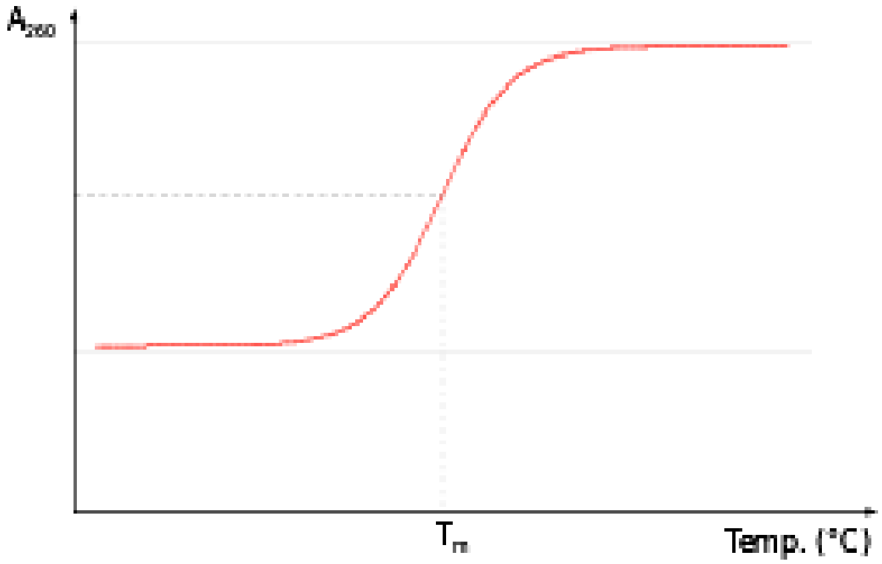


Fig 6.12 Melting curve of Nucleic acid showing hyperchromicity as a dunction of temperature

Source:https://dhingcollegeonline.co.in/attendence/classnotes/files/1602344266.pdf

Questions:

1. Write the difference between DNA and RNA.
2. Write an importance of cDNA library.
3. Explain the types of DNA and RNA.
4. Elaborate about hypochromicity and hyperchromicity of DNA.
5. Explain the process of creating a cDNA library.
6. Write the process of cot Analysis.

UNIT 7

Enzymes

Learning Objective

At the end of the unit student will be able to understand

- The types of enzymes, explain cofactors, and explain how enzymes reduce activation energy in linked processes.
- Mechanism of the enzymes work.
- Talk about how the temperature, pH, and concentration of the enzyme affect the rate at which an enzyme acts.

In all living things, enzymes function as catalysts and have an impact on the rate of reaction. In actuality, enzymes catalyze almost every chemical reaction in living things. Every enzyme that has been discovered to date is a protein with a distinct three-dimensional structure that serves as an active site for the surface binding of other molecules, or substrates. Each enzyme can catalyze a variety of reactions, but most of the time it can only catalyze one kind of reaction. They must be present in minuscule amounts or concentrations in order to transform substrates into products. At the conclusion of the reaction, the enzyme is unaffected. The size of the enzyme's molecule is significantly greater than that of its substrate. Enzymes have molecular weights that vary from thousands to millions, although the molecular weight of the enzymes range in hundreds.

7.1 Activation Energy

An energy barrier exists in every chemical reaction in a biological system, preventing reactions from happening uncontrollably or spontaneously. Activation energy is the amount of energy needed to initiate a reaction or to break over this energy barrier. For instance, until a mixture of hydrogen and oxygen receives sufficient energy from a heat source to reach the activation energy, they will not react with one another. You may have observed that, absent a spark, a tin of gasoline or kerosene oil left out in the open at room temperature would not catch fire. A few molecules can react with the activation energy provided by this little spark. The quantity of energy released during the transformation of first few molecules.

Activation energy is also needed for chemical processes that occur in living things. Enzymes play a crucial role in biological systems by facilitating chemical reactions at a rate that suits the needs of the cell and by reducing the activation energy of those reactions. Enzymes reduce activation energy, which accelerates the processes. When enzymes interact with various substrates, the amount of energy needed for a given process is decreased (Fig. 7.1). If your stomach did not have digestive enzymes, it would take years rather than just a few hours for your last meal to be broken down. The following sections contain information on the mechanism of action of enzymes.

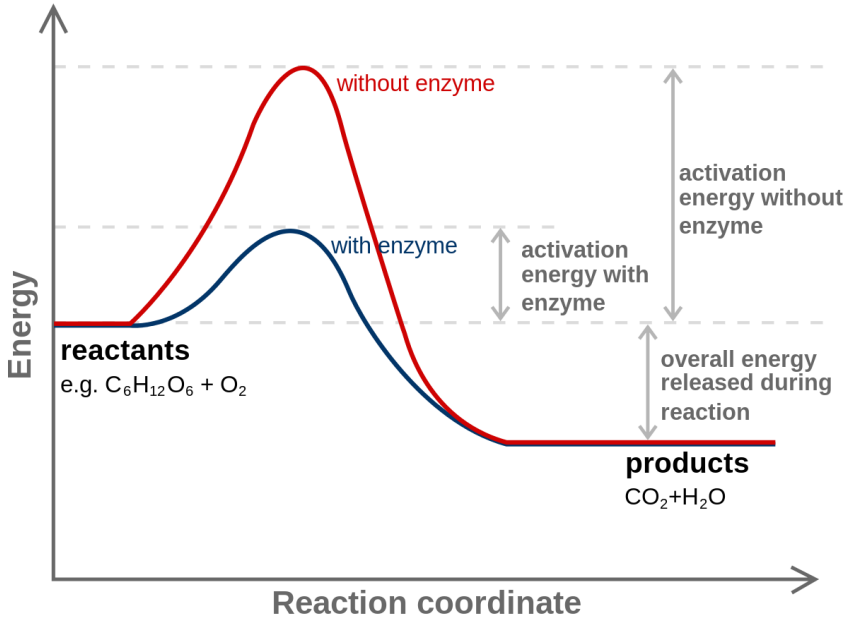


Fig 7.1: Activation energy graph in the presence and absence of enzymes

7.2 Coupled Reaction

The process of burning food releases energy into the cells. These foods are broken down in a complex and controlled manner through a series of events known as catabolic reactions. The cell uses this energy to power synthetic processes that need energy, such as endergonic anabolic reactions. Thus, anabolic chemical events are linked to catabolic reactions, which are exergonic in nature and provide energy. The living systems' equilibrium is preserved by this linking mechanism.

Enzyme-directed reaction pathways facilitate the coupling of various processes, which is a crucial aspect of living organisms. This coupling mechanism can be comprehended by looking at

a straightforward comparison wherein stones falling from a hilltop symbolize an energy-releasing catabolic chemical reaction(Fig 7.2). Typically, the heat produced when falling stones strike the ground wastes their kinetic energy. However, assome of this kinetic energy can be captured and utilized to power a paddle wheel that raises a pail of water. Thus, we can conclude that there is a clear correlation between the energy produced by a stone dropping and lifting the bucket of water.

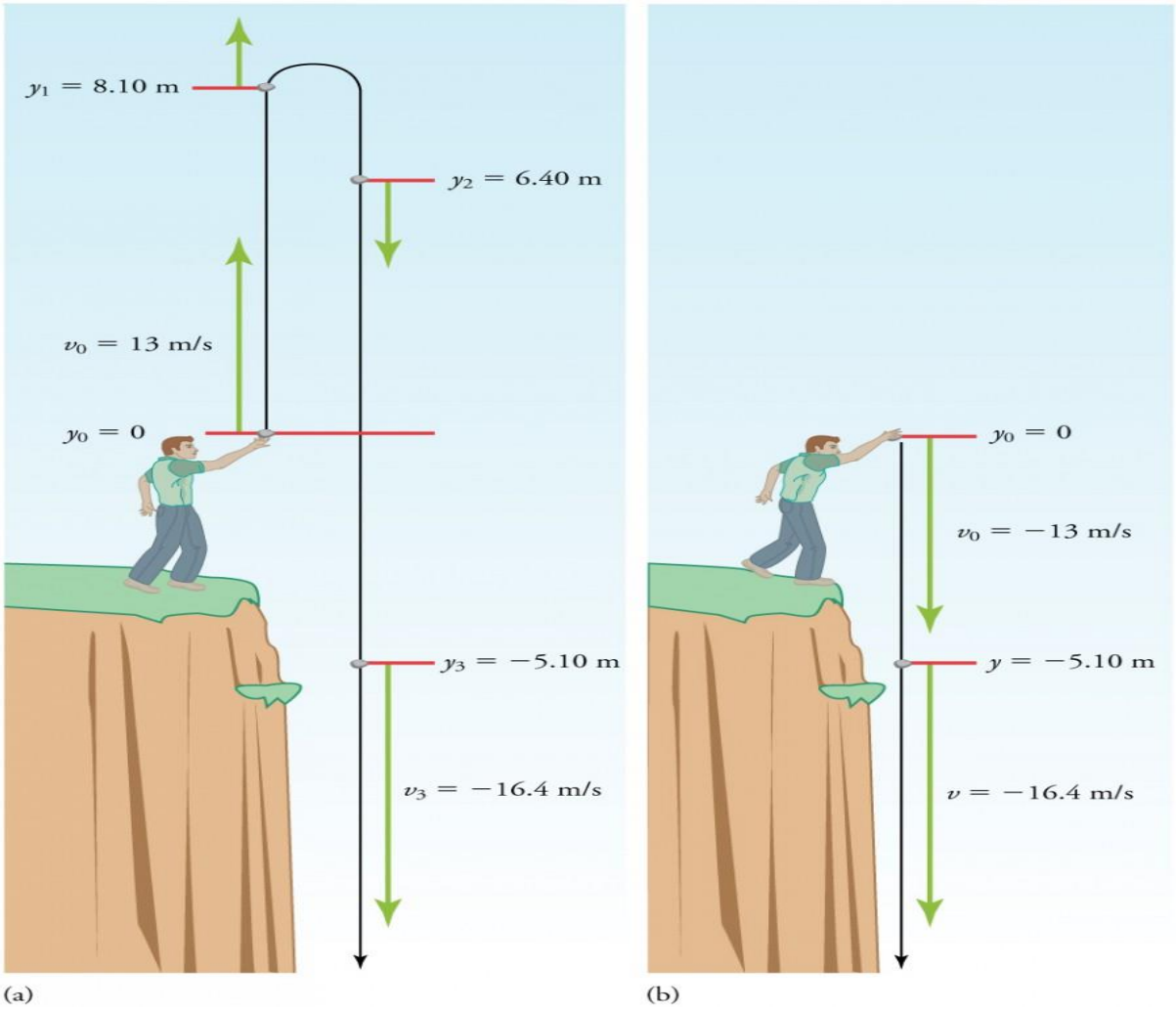


Fig 7.2: Schematic diagram of a mechanical model indicating the coupling of reactions.

Enzymes produce and store energy in the form of high energy phosphate bonds of adenosine triphosphate (ATP), acting as the paddle wheels of living things. The ATP that is therefore produced is referred to as the cell's energy currency and is used as a source of energy for numerous energy-dependent functions. In neutral aqueous solutions, ATP hydrolyzes and

releases energy. 7.3 Kcal of energy are released per molecule during the initial cleavage of ATP to create ADP and inorganic phosphate (see Fig. 7.3).

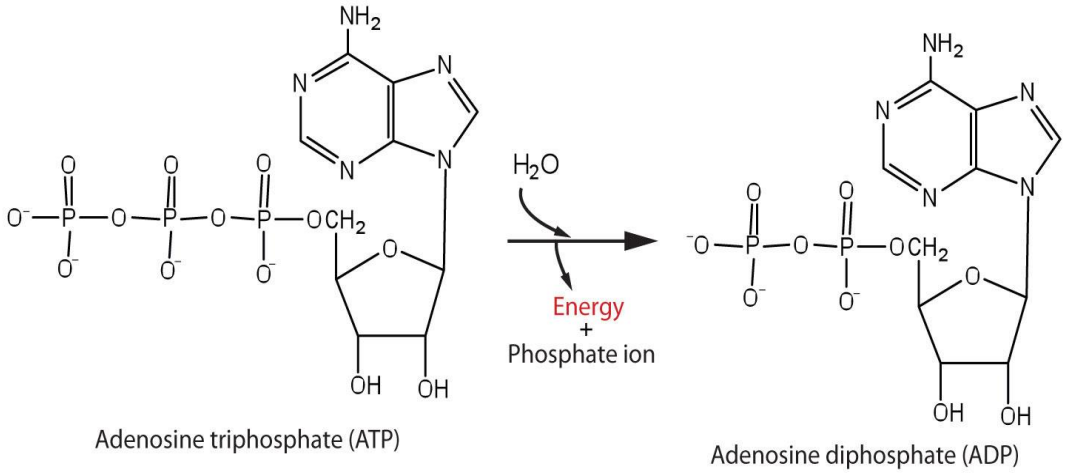


Fig 7.3: ATP and its Activation

7.3: Types of Enzymes

Based on their functional specificity, the International Union of Biochemists (IUB) Enzyme Commission has divided enzymes into six groups. Every category is further separated into specific groups based on the quantity of enzymes (Table 7.1). The majority of enzymes terminate in -ase. The substrate that an enzyme operates on is typically indicated by the first part of its name; for example, the enzyme amylase acts on starch or amylose. Some enzymes, such as pepsin, trypsin, chymotrypsin, and so on, are still referred to by their former names.

Nomenclature:

Enzyme nomenclature involves suffix –ase after substrate or the type of reaction catalyzed. Standard procedure for enzyme nomenclature were decided by International Union of Biochemistry, enzymes are classified according to their substrate and the type of reaction enzyme catalyses and are further sub-classified as per the characteristics and diversity under different classes. Example: Uric acid oxidizing enzyme (trivial name: Uricase) is named as urate:O2oxidoreductase that demonstrates urate and O2 as substrate and redox reaction as the catalyzed reaction.

Enzymes are also represented as numbers. Example: alcohol dehydrogenase is represented as 1.1.1.1. First number here represents class 1 of oxidoreductases, second number represents sub class 1 of action on CH-OH group of donor alcohols, third number represents sub-sub-class 1 of utilization of NAD^+/$NADP^+$ as electron acceptor molecule and fourth number represents sub-sub- sub-class 1 of NAD^+ as electron acceptor.

Example: alcohol dehydrogenase is represented as 1.1.1.1. First number here represents class 1 of oxidoreductases, second number represents sub class 1 of action on primary alcohols, third number represents sub- sub-class and fourth number represents sub-sub- sub-class

There are 7 enzyme classes that are defined on the basis of types of reactions catalysed:

1.Oxidoreductase catalyse redox reactions. Example: Alcohol:NADoxidoreductase.

2. Transferases catalyse reactions involving transfer of functional groups. Ex: L-Aspartate:2-oxglutarate aminotransferase.

3. Hydrolases catalyses addition or removal of water from substrate. Ex: Acetylcholinesesterase

4. Lyases catalyse a C=C bond splitting by addition of groups or C=C bond formation. Ex:Argininosuccinatelyase

5. Isomerases catalyse an intramolecularstrcuctural rearrangement. Ex: Peptidylprolylisomerase.

6. Synthases catalyse synthesis of a molecule without utilizing energy molecules of high energy phosphate bonds (ATP). Ex: Thymidylate synthase.

(Note: Synthetases are a separate class that involves molecular synthesis utilizing energy of high energy phosphate bonds (from ATP, GTP etc.). Ex: Argininosuccinatesynthetase.

Table 1. Enzyme classes

	Name	Reaction catalysed
1	Oxidoreductases	*$AH_2 + B = A + BH_2$
2	Transferases	AX + B = BX + A
3	Hydrolases	$A\text{-}B + H_2O = AH + BOH$
4	Lyases	A=B + X-Y = A-B (with X and Y attached below A and B)
5	Isomerases	A = B
6	Ligases	†A + B + NTP = A-B + NDP + P (or NMP + PP)
7	Translocases	AX + B‖ = A + X + ‖B (side 1) (side 2)

*Where nicotinamide-adenine dinucleotides are the acceptors, NAD^+ and $NADH + H^+$ are used, by convention.

†NTP = nucleoside triphosphate.

7.4: Cofactors

All of the enzymes are proteinaceous in nature, as you have just read. The majority of enzymes are made entirely of polypeptide chains. However, certain enzymes cannot perform their catalytic function without the inclusion of another chemical component. These components, also referred to as cofactors, are not proteins. A holoenzyme is a full, catalytically active enzyme that functions in tandem with a cofactor. Apoenzyme is the term for the protein component of holoenzyme. Coenzymes, metal ions, and prosthetic groups are the three types of cofactors.

Prosthetic Enzymes

Organic substances known as prosthetic groups are affixed to apoenzymes permanently. Heme, for instance, is the prosthetic group and a permanent component of the active site of the enzymes peroxidase and catalase, which catalyze the conversion of hydrogen peroxide to water and oxygen(Fig 7.4).

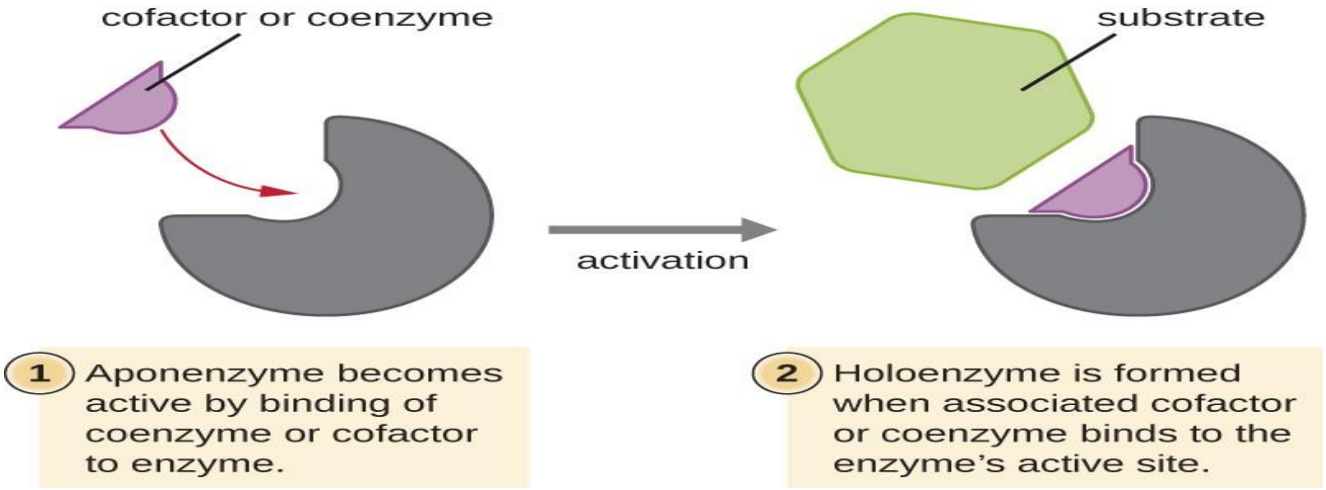


Fig 7.4: Apoenzymes and holoenzyme

Coenzymes

Coenzymes are organic substances as well, however they only bind to apoenzymes momentarily during catalysis. Different enzyme-catalyzed processes can use the same coenzyme molecule as a cofactor. These coenzymes serve as temporary acceptors for particular atoms or functional groups in addition to assisting enzymes in their catalytic activity. A lot of coenzymes require vitamins as necessary components. For instance, the coenzymes nicotinamide adenine dinucleotide phosphate (NADP) and nicotinamide adenine dinucleotide (NAD) function as

electron carriers and include vitamin niacin. Pantothenic acid is a vitamin that is present in Coenzyme A (CoA), which also carries (transfers) functional groups like acetyl groups

Metal ions

Certain enzymes need metal ions in order to function catalytically. Certain side chains at the active site form bonds with metal ions like Mg^{2+}, Mn^{2+}, or Zn^{2+}, which also bind to the substrate simultaneously.It breaks down into products with the help of metal ions binding to thiolate. For instance, carboxypeptidase, a proteolytic enzyme, requires zinc as a cofactor. It forms bonds with the a-carboxyl group of the substrate amino acid as well as side chains of amino acid residues in the enzyme's active site. This is where the enzyme breaks the peptide bonds in the substrate.

7.5 Mechanism of Action enzymes

Despite the wealth of knowledge that has amassed over the past few years regarding the structural, chemical, and physical properties of enzymes, there is still much that is unknown about how they work. Nonetheless, much knowledge on the processes behind the catalysis of reactions by ribonuclease, chymotrypsin, lysozyme, and other enzymes is currently accessible.

Three steps can be identified in the overall process of enzyme function. Prior to the creation of the enzyme substrate complex (ES), which ultimately modifies the substrate and creates the product, the enzyme first binds to the substrate. The equation depicts the events in an enzyme-catalyzed reaction.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

An enzyme reduces the activation energy of both forward and reverse reactions, or the energy barrier, to the same degree. It only quickens the rate at which a reaction approaches equilibrium—it doesn't change the equilibrium state. A catalyst, like an enzyme, also emerges from a reaction exactly as it began and is prepared to catalyze the reaction once more because it is not permanently altered by involvement in the reaction. You will read more about the specificity of enzymes in the next subsection. This is one of the variables that drives their action.

7.6 Enzyme Specificity

The selectivity of enzymes with regard to the type of reaction they catalyze as well as the reactant itself, or substrate molecule, is one of their most important characteristics. For instance, proteolytic enzymes catalyze protein-related processes, although succinic dehydrogenase and urease only function on urea and succinate, respectively. Even within a specific category of enzymes, there are variations in enzyme specificity. Let's use proteolytic enzymes as an example. The hydrolysis of peptide bonds is the reaction that these enzymes catalyze. Whereas the enzyme thrombin catalyzes the hydrolysis of peptide bonds solely between arginine and glycine, subtilisin cleaves any peptide bond connecting the amino acids of the substrate.

However, the degree of specificity exhibited by other proteolytic enzymes varies. Trypsin, for instance, is limited to breaking peptide bonds on the carboxyl side, which is the C-terminal region of amino acids such as arginine and lysine.

The biological roles of different proteolytic enzymes account for the variations in specificity. Bacteria utilize any kind of protein as a source of carbon and nitrogen, hence any kind of protein can be acted upon by the subtilisin enzyme that is present in them. In the mammalian digestive system, the enzymes trypsin and chymotrypsin fragment proteins into pieces ranging in length from five to twenty amino acids, so their specificity is restricted.

Because it only breaks one peptide link in fibrinogen, thrombin exhibits absolute specificity when it comes to blood clotting. This allows fibrin monomer to be released, aiding in the development of fibrin clots. Hydrolase enzymes are the least specialized class of enzymes.

Certain enzymes are stereospecific, meaning they only work on distinct stereoisomers of the same molecule. For example, kidney D-amino acid-oxidase only works on amino acid D-isomers. Nonetheless, the interconversion of D and Lforms is catalyzed by a tiny class of enzymes known as racemases. Certain enzymes only function on cisortransisomers, exhibiting geometric specificity.

When it comes to the double bond that joins the two carbon atoms, the constituent atoms and groups of the trans and trans isomers are arranged differently, making them geometric isomers. Maleic acid and fumaric acid, for instance, share the same chemical formula.

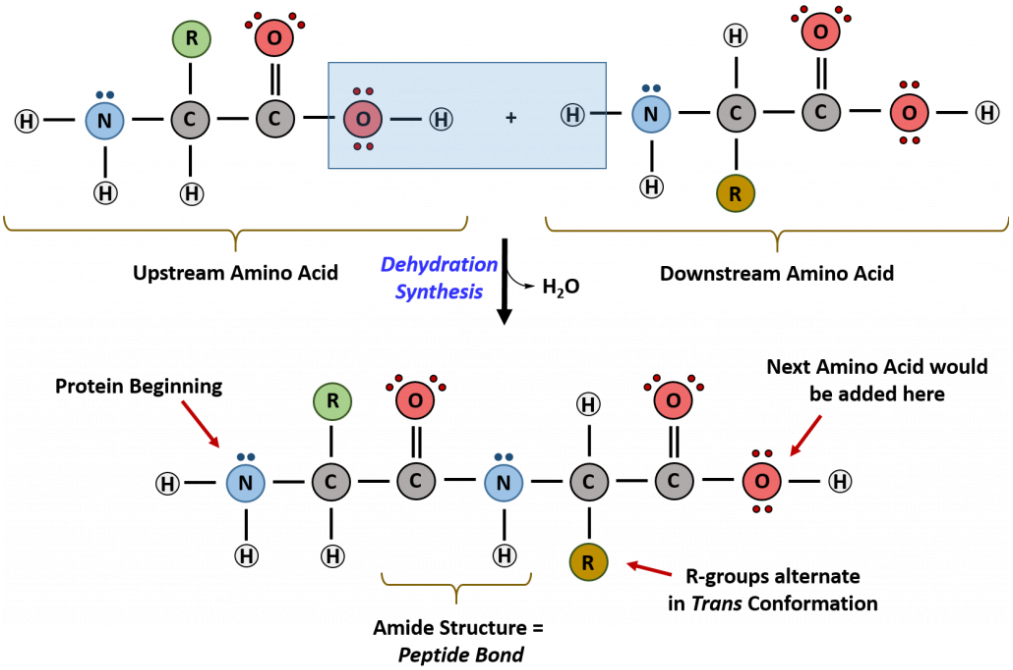


Fig 7.5: Enzyme and substrate reaction

7.7: Lock and Key Hypothesis

Emil Fischer proposed the lock and key theory in 1984 in light of the specificity shown by enzymes in binding to their substrates. This theory states that the substrate molecules fit into the enzymes' active regions similarly to how a key fits into a lock. As a result, temporary EScomplexes are formed, which subsequently disintegrate into an enzyme and a product (Fig. 7.6). It is possible to separate the enzyme substrate complex from the slower acting enzymes, providing concrete proof of the ES-complexes' creation.

Even while the lock and key model explains a large portion of the substrate specificity of enzymes, it is unable to explain other data on the behavior of enzymes. For instance, occasionally substances different than the real substrate bind to the enzyme but are unable to create the results of the reaction. The induced-fit hypothesis was put forth by Daniel Koshland in the 1960s. It states that the enzyme's active site is induced by the substrate to take on the complimentary shape in order to bind the substrate molecule, rather than existing in an initial

state that is complementary to it. An enzyme's active site is therefore thought to be flexible by nature. However, not all enzyme-catalyzed reactions require a flexible active site, as some can be sufficiently explained by the lock and key Model.

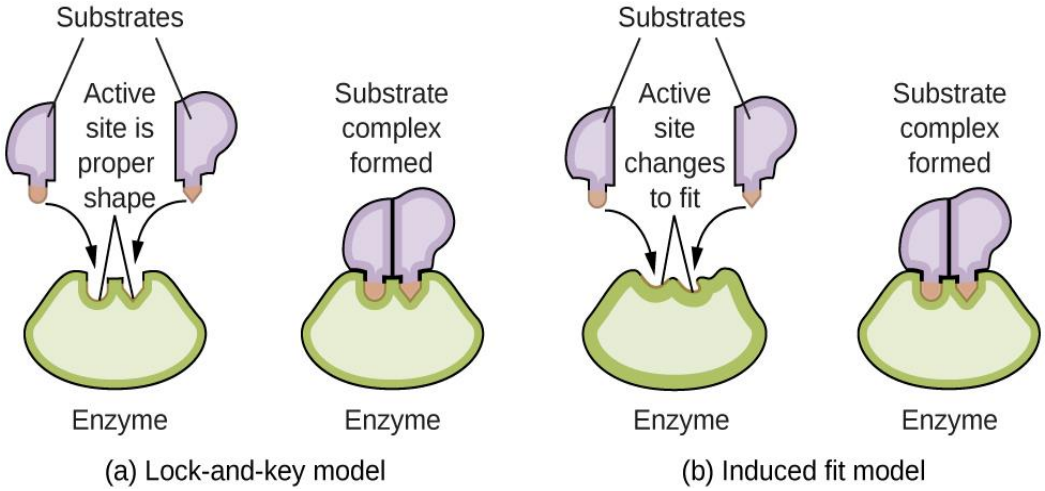


Fig 7.6: Enzyme substrate reaction (A) Lock and Key Model (B) Induced fit model

Various mechanisms function at the active site of enzymes, each contributing to the lowering of activation energy, despite the significant variations in the structure, specificity, and catalytic mode of each type of enzyme. The following is a generalization of these mechanisms:

- Reacting molecules are brought closer together by binding, which raises the likelihood of a reaction.
- The way the substrates and the enzyme are attached causes a redistribution of charges inside the substrates as a result of the creation of several transient non-covalent connections between them. Certain covalent bonds in the substrates are strained as a result of this redistribution, which breaks the bond.
- Water molecules are removed from the active site by hydrophobic amino acids, which leaves a water-free zone where nonpolar groups can react.

7.8: Self-Assessment

1. Define Enzyme? State all classes according to IUCBN nomenclature.
2. Give a model of Enzyme Substrate Reaction.
3. What is Activation Energy
4. What is the difference between Co-enzyme and Co-Factor

UNIT 8

Enzyme Action

Learning Objective

At the end of the unit student will be able to understand

- Enzyme kinetics
- List the key characteristics of allosteric and isoenzymes, inhibitors, and enzyme activity assays.
- Draw the curve for the Michaelis - Mentenequation and the Lineweaver - Burkplot to illustrate the enzyme kinetics.

In all living things, enzymes function as catalysts and have an impact on the rate of reaction. In actuality, enzymes catalyze almost every chemical reaction in living things. Every enzyme that has been discovered to date is a protein with a distinct three-dimensional structure that serves as an active site for the surface binding of other molecules, or substrates. Each enzyme can catalyze a variety of reactions, but most of the time it can only catalyze one kind of reaction. They must be present in minuscule amounts or concentrations in order to transform substrates into products. At the conclusion of the reaction, the enzyme is unaffected. The size of the enzyme's molecule is significantly greater than that of its substrate. Enzymes have molecular weights that vary from thousands to millions, although the molecular weight of substrate is in few hundred.

8.1 Activation Energy

An energy barrier exists in every chemical reaction in a biological system, preventing reactions from happening uncontrollably or spontaneously. Activation energy is the amount of energy needed to initiate a reaction or to break over this energy barrier. For instance, until a mixture of hydrogen and oxygen receives sufficient energy from a heat source to reach the activation energy, they will not react with one another. You may have observed that, absent a spark, a tin of gasoline or kerosene oil left out in the open at room temperature would not catch fire. A few molecules can react with the activation energy provided by this little spark. The quantity of energy emitted following the conversion of first few molecules of the reactant to product in sufficient to activate other molecules.

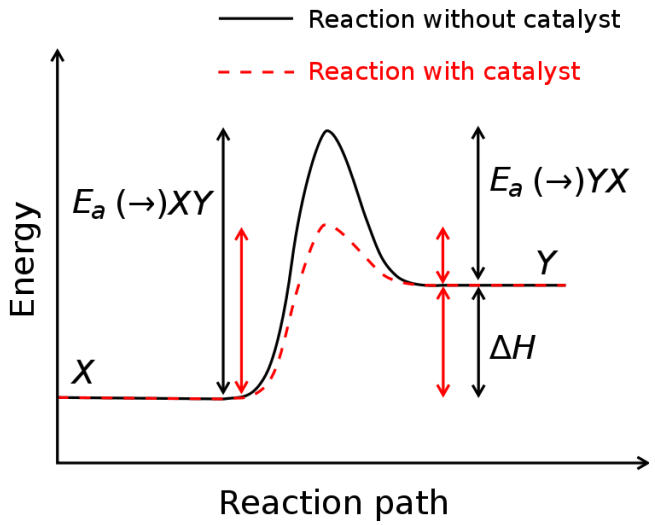


Fig 8.1: Role of enzymes for the activation

8.2: Enzyme Kinetics

The rate of an enzyme-catalyzed reaction is also influenced by the concentration of substrate. The number of substrate molecules is too small to occupy all of the active sites of the enzyme molecules at low substrate concentrations, therefore the substrate concentration directly affects the reaction's starting velocity. More and more molecules of substrate are occupying active sites as the concentration of substrate rises. When the concentration of substrate reaches a point where all of the enzymes' active sites are occupied, a saturation will eventually be obtained. .

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

The reaction's maximum velocity, or Vmax, is reached at this saturation point. The maximum velocity is unaffected by additional increases in the concentration of substrate. When comparing the rate of enzyme reaction to the concentration of substrate, the curve for enzymes made up of single polypeptide chain with one active site.

For enzymes consisting of a single polypeptide chain with a single active site, the shape of the rate of enzyme reaction curve vs substrate concentration is a rectangular hyperbola (see Fig. 8.2). The reaction's velocity [V] is half of the maximum velocity [Vmax/2] at a specific substrate

concentration, when half of the enzyme's molecules are saturated with substrate. The Michaelis constant, abbreviated as Km, is the derivative of the concentration at half the maximum velocity.

Michaelis-Menten derivation using above assumptions:

Rate of [ES] formation = $k_1[E][S] + k_{-2}[E][P]$

Assumption #1 says we can ignore the k_{-2} reaction, therefore:

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

Assumption #5 says $[E] = [E]_{total} - [ES]$, therefore:

Rate of ES formation = $k_1([E]_{total} - [ES])[S]$

The rate of [ES] breakdown is given by the rate

Rate of ES breakdown = $k_{-1}[ES] + k_2[ES]$

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

Assumption #2 says the rate of ES formation equals the rate of breakdown:

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

Rearrangement to define in terms of rate constants:

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

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

The new constant, $K_m = (k_{-1} + k_2) / k_1$

$([E]_{total}\ [S] / [ES]) - [S] = K_m$

Prove for the [ES] term

$[ES] = [E]_{total}\ [S] / (K_m + [S])$

The true reaction velocity measured at any given moment is given by:

$V = k_2[ES]$

Multiple both sides of the above equation by k_2:

$k_2[ES] = k_2[E]_{total}\ [S] / (K_m + [S])$

thus

$V = k_2[]_{total}\ [S] / (K_m + [S])$

The maximum possible velocity (Vmax) occurs when all the enzyme molecules are bound with substrate $[ES] = [E]_{total}$, thus:

$V_{max} = k_2[E]_{total}$

Substituting this into the prior expression gives:

$V = V_{max}\ [S] / (K_m + [S])$

The standard way to express reaction rates is as moles (or micromoles) of product produced per unit of time (sec or min) per mole (or micromole) of enzyme.

For an extensive range of substrate concentrations, the experiment is repeated.

The datapoints for [S] vs V are gathered into a table.

Plotting these datapoints (V vs S) should result in a curve that fits the Michaelis-Menten equation.

The words Vmax and Km refer to inherent characteristics of the specific enzyme/substrate combination under investigation.

The characteristics of the V versus S plot Vmax will be used to determine them.

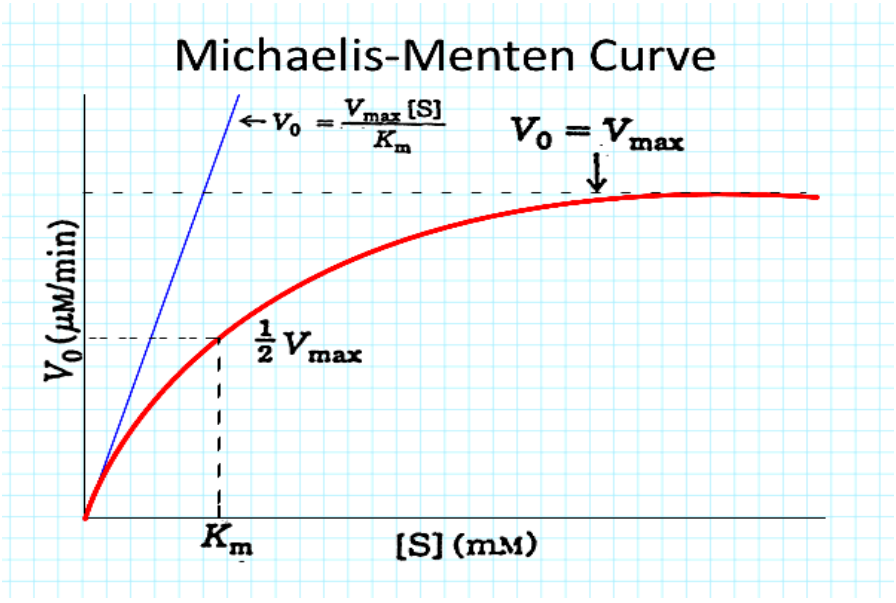


Fig 8.2: Relationship between substrate concentration [S] and reaction rate [V].

8.3: ALLOSTERIC ENZYMES

Certain enzymes may have two binding sites, each with a distinct function. The catalyst for the process is one of the sites, the active site, which binds the substrate. The other kind of site, referred to as an allosteric or regulatory site, attaches itself to an effector or modulator molecule. Allosteric enzymes are the name given to such enzymes. These enzymes possess many subunits, making them oligomeric in nature. The conformational changes brought about by binding are transferred between subunits of the enzyme, and the active and allosteric sites may be found on the same subunit or on separate subunits. Positive effectors, or activators that increase enzyme activation, and negative effectors, or inhibitors that decrease enzyme activity, are the two categories of effector molecules. Allosteric enzyme conformational changes are brought about by effector binding, and these can effect catalytic activity(Fig 8.3).

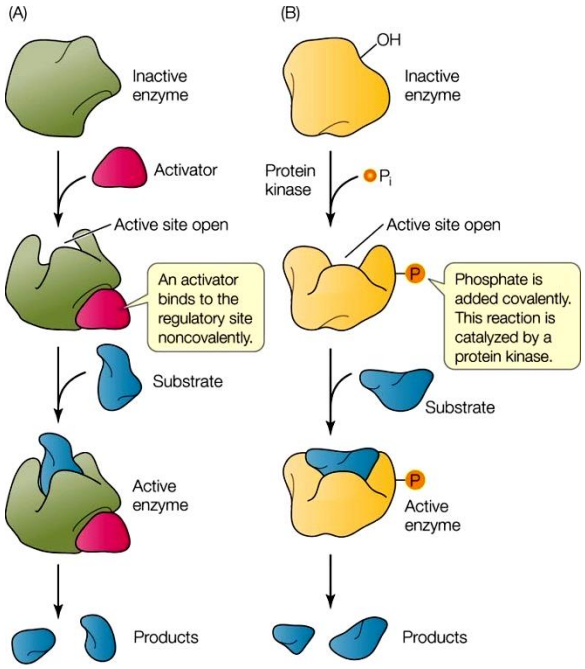


Fig 8.3: Schematic diagram showing the functioning of allosteric enzymes

When a substrate binds to one active site of an enzyme that has many active sites, it may affect subsequent binds to other active sites. Enzyme behavior like this is referred to as cooperativity. Positive cooperativity occurs when the binding of one substrate molecule encourages the binding of other substrate molecules. When subsequent substrate binding happens less frequently after the initial substrate molecule has bound, this is known as negative cooperativity. Enzymes that exhibit cooperativity and allostery are related to Michaelis-Mentenkinetics.Plotting such enzymes against substrate concentration, as seen in Fig. 8.4, reveals a sigmoidal curve formed of many subunits and active sites.

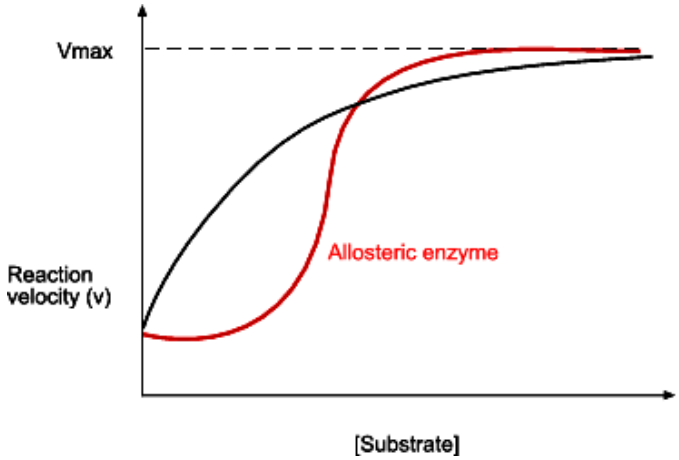


Fig. 8.4: Allosteric enzymes show sigmoidal curve

8.4 ISOENZYMES

Isomers, also known as isoenzymes, are several kinds of enzymes that exist. There is no isomer in these isoenzymes. Although they are chemically different molecules, all isoenzyme variants of a given enzyme catalyze the same process. Their molecular weight, charge, content, and sequence of amino acids can all vary. Their substrates typically differ in one or more kinetic characteristics, such as Km and Vmax.

Allvertebrates, insects, plants, and unicellular creatures include isoenzymes. It is possible for various tissues to contain isaenzymes with varying substrate affinities.

Lactate dehydrogenase is one of the first and best-known examples of a universally occurring isoenzyme. It appears in five distinct forms: M4, M3H, M2H2, MH3 and H4. These isozymes consist of four polypeptide subunits each, forming a tetramer. These subunits come in two different types and are found in various combinations within the isozymes.

8.5 INHIBITORS

Certain chemicals known as inhibitors have the ability to impede the action of enzymes. The characteristics of the functional groups at the active site, the substrate selectivity of enzymes, and the mechanisms underlying catalytic activity have all been elucidated by enzyme inhibitors. Certain medicines that are thought to be helpful in medicine work by blocking certain enzymes; for instance, blocking bacterial enzymes influences the metabolism of the bacteria and, consequently, their development and multiplication.

8.5.1 Irreversible inhibitors

These inhibitors change or eliminate a functional group on the molecules of the enzymes through covalent binding, which results in the enzymes' permanent loss of catalytic activity. Certain pesticides, arsenic, lead, and mercury are examples of irreversible inhibitors. The enzymes precipitate even at low concentrations of these substances.

8.5.2 Reversible inhibitors

These inhibitors have reversible effects and attach noncovalently to enzymes. Reversible inhibitors have provided valuable insights into the architecture of the active sites of many

different enzymes. Competitive and noncompetitive inhibitors are the two primary categories of reversible inhibitors.

(a) Competitive inhibitors:

Because they mimic the molecules of the substrate, competitive inhibitors compete with the actual substrate to bind to the enzyme's active site. This binding reduces the substrate's capacity to attach to the enzyme but has no effect on the enzyme's catalytic ability. These inhibitors, however, are not able to be converted into products. All it takes to reverse competitive inhibition is to raise the concentration of the substrate.

(b) Non-competitive inhibitors:

These inhibitors bind to the enzyme at a location other than the catalytic sites—the substrate binding site, for example—changing the molecule's conformation and causing the catalytic site to become distorted and inactive. To create the inactive complexes, non-competitive inhibitors bind reversibly to the ES complex as well as the free enzyme. Unlike competing inhibitors, extra substrate will not bring the rate of reaction back to its prior level. The naturally occurring metabolic intermediates, such as L-isoleucine, which inhibits L-threonine dehydratase, are important noncompetitive inhibitors. Inhibitors that are both competitive and noncompetitive are not always beneficial. Cells employ both extensively to regulate metabolism.

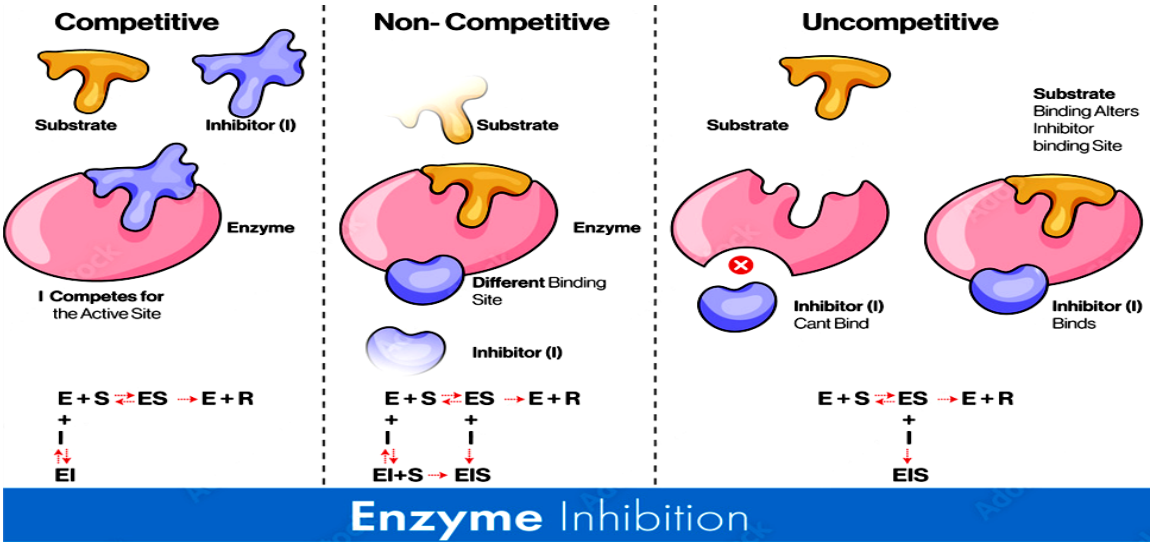


Fig. 8.5: Types of inhibition

Lineweaver-Burke

The Michaelis-Menten equation can be rearranged by taking the reciprocal, to yield:

1/V= km/Vmax[S]+1/Vmax

If X = 1/[S] and Y=1/***V*** then this is a linear equation with a slope of $K_m/\boldsymbol{V}_{max}$ and a Y intercept of $1/\boldsymbol{V}_{max}$

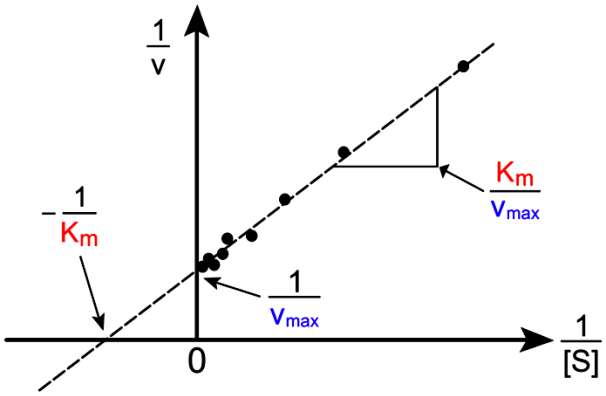


Fig 8.6: Lineweaver-Burke between 1/[S] and 1/V

It is simpler to fit a linear function to the data in this form since the plot of 1/[S] versus 1/V data should be a straight line, and Vmax and Km may be easily found from the plot.

Reversible Inhibition

Reversible inhibitors fall into two main categories: those that are competitive and those that are noncompetitive.

Competitive inhibitors

The substrate [S] and the inhibitor [I] compete for the enzyme's active site, also referred to as the S-binding site. It is mutually exclusive for either of these compounds to bind to the active site.

There is a great deal of structural similarity between the inhibitor and substrate. The inhibitor, however, is unable to allow the reaction to continue and result in product.

Raising the substrate concentration will cause the inhibitor to lose ground when it comes to binding to the active site of the enzyme.

The distinctive effects on kinetic data can be used to identify a competitive reversible inhibitor.

In the presence of a reversible competitive inhibitor, the expression for the Michaelis-Menten expression is:

$V = V_{max} [S] / (K_m(1+[I]/K_i) + [S])$

where Ki is the real dissociation constant of the EI complex.

The following are the kinetic effects of the reversible competitive inhibitor:

If [I] = 0 (i.e., there is no inhibitor present), then the equations are identical.

The impact of adding inhibitor is to change the apparent value of Km. Specifically, there will be an increase in apparent Km equal to (1 + [I]/KI). The reaction velocity, v, will decrease as Km increases.

Keep in mind that the denominator's value effectively equals [S] and Vmax when [S] grows very large. The reaction velocity can therefore be increased to reach Vmax when the substrate concentration is high enough.

Non-Competitive Inhibition

I, a non-competitive inhibitor, can attach to the ES complex as well as the unoccupied enzyme E (Figure 8.8). S can be bound by the EI complex, but EIS cannot produce products.

The equilibrium constant Ki controls the number of complexes that form, EI and EIS. Less enzyme is available to create a productive ES complex if more EI or EIS develops. High quantities of substrate are ineffective against the inhibitor since I can also bind to ES. Thus, this inhibition is referred to as non-competitive. It is uncommon for Ki to be the same in both stages for there to be true non-competitive inhibition. Mixed inhibition will occur if the Ki for I binding to empty E differs from that for I binding to occupied ES.

If inhibitor concentration [I] is set equal to K_i, this causes the V'_{max} observed to be halved relative to uninhibited enzyme.

Characteristics of a non-competitive inhibitor: V'_{max} decreases, K_M is unchanged.

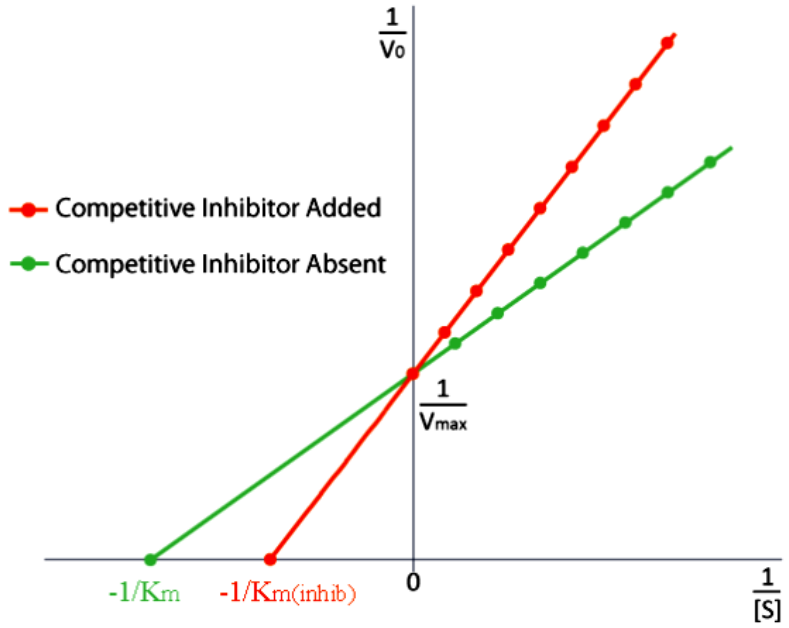


Fig 8.7: Competitive inhibitors

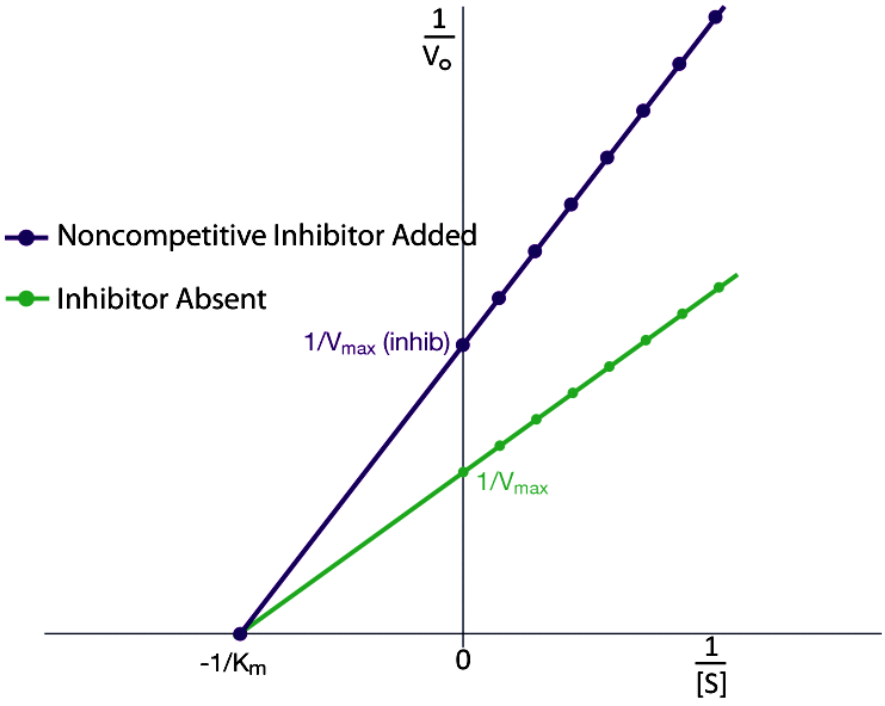


Fig 8.8: Non-Competitive inhibitors

8.6 Regulation of Enzyme Activity

The metabolic pathways that convert low molecular weight precursors into more complex molecules, such as proteins, lipids, carbohydrates, and nucleic acids, resulting in the synthesis of new cellular components and overall growth, are referred to as anabolism. Contrarily, the act of breaking down basic and complex materials in other metabolic pathways to create energy—either in the form of heat or higher energy compounds—is known as catabolism. There is connectivity between the anabolic and catabolic processes.

It is obvious that in order to steer metabolism in the right direction, concentrations of specific important metabolites must be carefully regulated in both space and time within such a complicated system of biochemical reactions and not allowed to drift into ultimate products of aerobics metabolism is driven by enzymes.

8.6.1 Regulation by Substrate or Product

Sometimes, the amount of a substrate present inside cells can control how that substrate's related enzyme functions. When the Km value of the enzyme is significantly more than the intracellular concentration of the substrate, indicating that the enzyme activity is first order relative to substrate concentration, this kind of regulation is achievable.

When an enzymatic reaction produces a product with a structure similar to the substrate, the reaction may be inhibited when the product builds up to a high enough concentration. However, as one would anticipate the product to build up to a high concentration before meaningful inhibition is accomplished, the significance of such product inhibition in enzyme regulation cannot be very great.

Furthermore, it is challenging to understand how a metabolic pathway is controlled if an enzyme's product inhibits that enzyme without regard to the requirements of the entire route.

Allostery is a crucial biological mechanism that regulates the activity of enzymes. By changing the activity of a crucial enzyme, this interaction may result in either stimulation or inhibition of activity. Enzymes' interactions with the compounds the cell produces make this possible. Let's go over this kind of regulation in more detail.

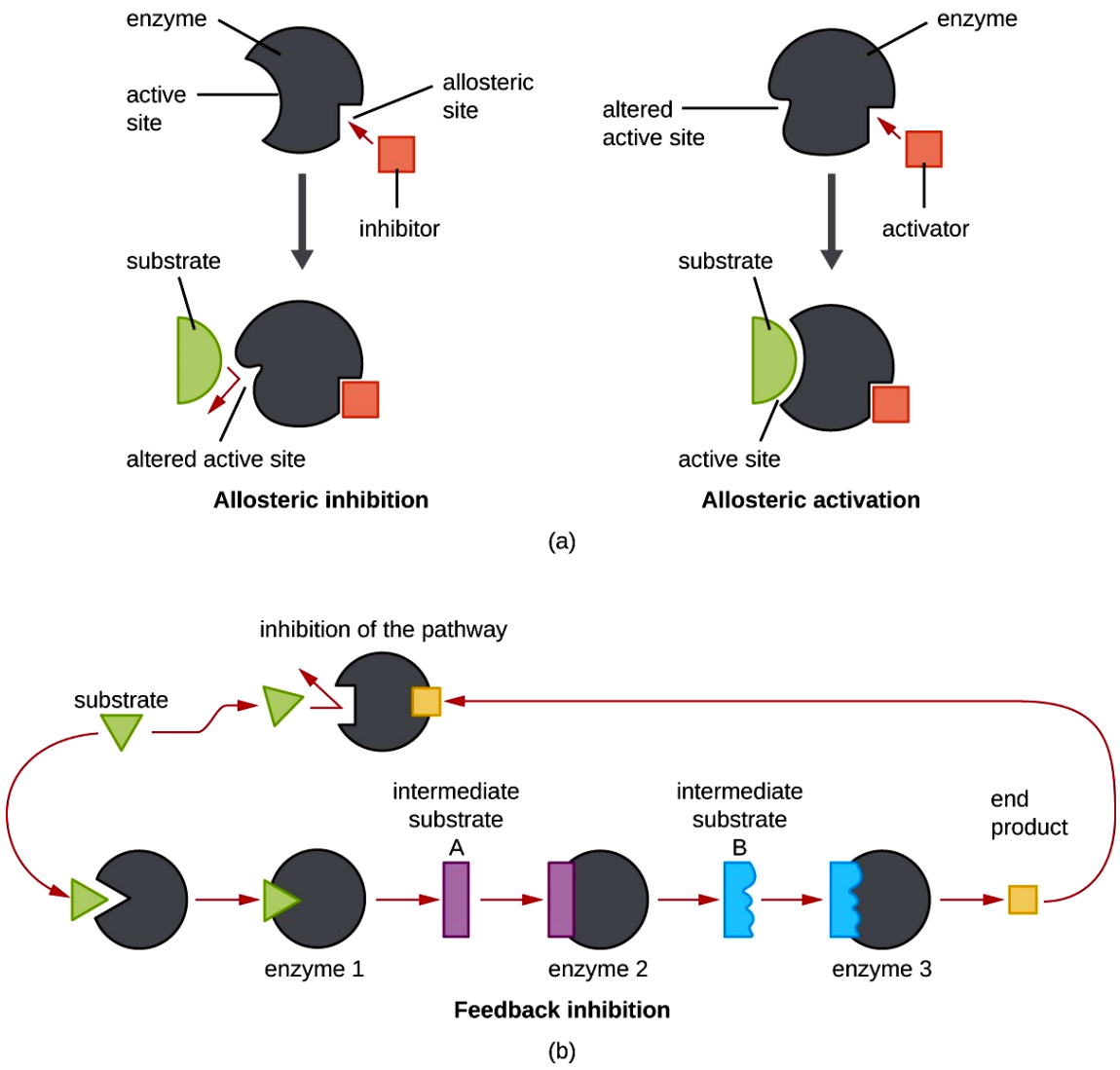


Fig 8.9: Allosteric inhibition and Feedback inhibition

8.6.2 Allosteric Regulation

This mode regulates a few of the metabolic pathways. In these situations, a biosynthetic pathway's final product severely inhibits the pathway's first enzyme (also termed feedback inhibition). For instance, the end result of this pathway, CTP, a pyrimidine nucleotide, inhibits aspartate carbamoyltransferase, the enzyme that catalyzes the initial step in pyrimidine biosynthesis (Fig 8.10)in*E. coli*.

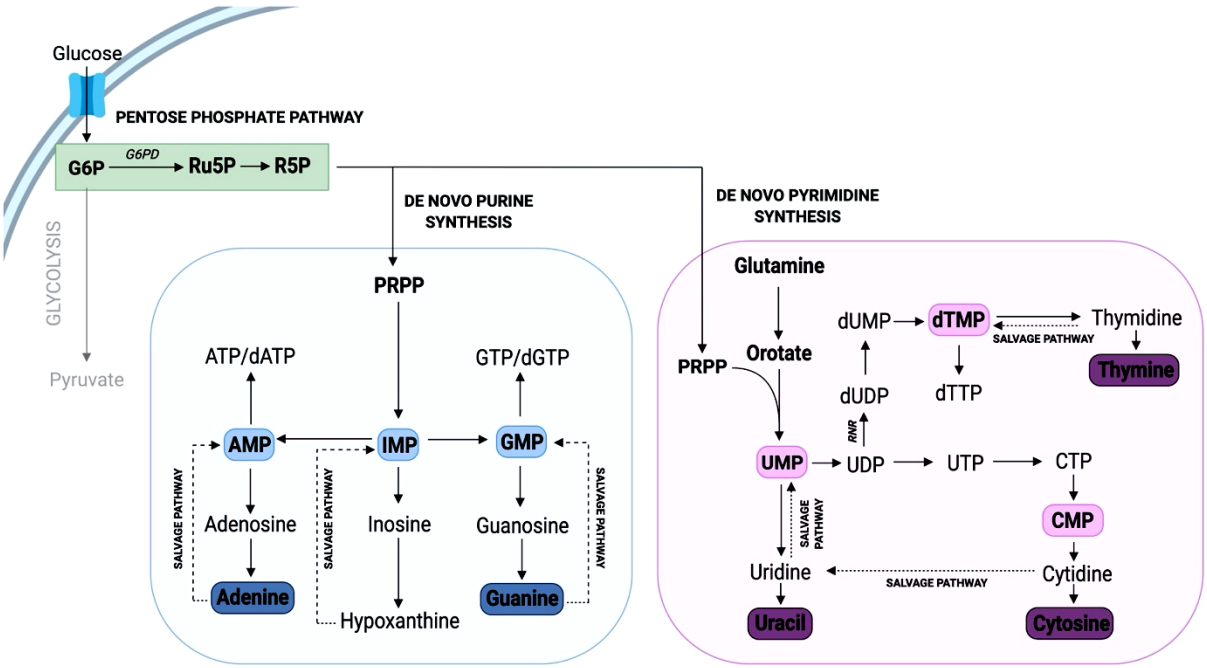


Fig 8.10: Feedback inhibition

Based on research conducted on multiple different pathways, the following broad conclusions about this kind of control have been drawn.

- Due to feedback inhibition by the system's ultimate product, only the first enzyme in the biosynthetic pathway is impacted.
- This metabolic pathway's end product has no structural similarities to the first enzyme's substrate or product, allowing for an independent method of regulating the enzyme regardless of substrate or product
- Because they differ structurally from the substrate or product of the first reaction, the regulatory molecules, also known as effectors, do not bind to the active site of the first enzyme. Instead, they carry out their regulatory function by binding to a different position on the allosteric enzyme site. the binding is reversible.
- These allosteric enzymes don't have hyperbolic or typical Michaelis-Menton kinetics. In these situations, sigmoidal behavior is seen in the velocity versus substrate concentration plot.
- The behavior suggests that, at some substrate concentrations, changes in substrate concentration have a greater impact on the activity of the enzyme than they would on an

enzyme with typical kinetic behavior. A regulating enzyme can be treated chemically or physically to convert its sigmoidal kinetics to hyperbolic kinetics.

- Regulatory enzymes often have an oligomeric shape, similar to that of blood's oxygen-transporting protein, hemoglobin. These enzymes' oligomeric structure, made up of many subunits, is held together by weak noncovalent interactions. When a regulator molecule binds to an enzyme at its allosteric site, the enzyme's subunits undergo a reversible conformational change that alters the structure of the active site and causes an oscillation in the activity of the enzyme.

Self-Assessment

1. What distinguishes a transferase from an oxidoreductase? Describe your response with an example.
2. Explain an enzyme's active site.
3. Go over the necessity of controlling the activity of enzymes.
4. Summarize the key components of allosteric regulation.
5. Describe the "transition state" of a response.
6. In what way does an enzyme aid in reducing the energy required to initiate a chemical reaction?
7. Explain the history of the lactate dehydrogenase isoenzymes.
8. What is a low value of Km and what does it mean?

GLOSSARY

1. **Carbohydrates**: Biomolecules consisting of carbon, hydrogen, and oxygen atoms, including sugars and starches, which serve as important sources of energy and structural components in living organisms.
2. **Monosaccharides**: Simplest form of carbohydrates, such as glucose and fructose, consisting of a single sugar molecule.
3. **Disaccharides**: Carbohydrates composed of two monosaccharide units linked together, like sucrose and lactose.
4. **Glycoconjugates**: Carbohydrates covalently linked to proteins or lipids, playing essential roles in cell recognition, signaling, and immunity.
5. **Lipids**: Diverse group of biomolecules, including fats, oils, and steroids, characterized by their insolubility in water and roles in energy storage, insulation, and cell membrane structure.
6. **Triglycerides**: Lipids composed of three fatty acid molecules linked to a glycerol molecule, serving as a major form of energy storage in animals.
7. **Phospholipids**: Lipids containing a phosphate group, integral to cell membrane structure and function due to their amphipathic nature.
8. **Glycolipids**: Lipids containing a carbohydrate moiety, found predominantly in cell membranes and involved in cell-cell recognition and signaling.
9. **Steroids**: Lipids characterized by a four-ring structure, serving as important signaling molecules (e.g., hormones) and components of cell membranes (e.g., cholesterol).
10. **Proteins**: Complex biomolecules composed of amino acid chains folded into specific three-dimensional structures, performing diverse functions in cells, including enzyme catalysis, structural support, and signaling.
11. **Immunoglobulins**: Antibodies produced by the immune system in response to foreign substances (antigens), comprising specific protein chains that recognize and neutralize pathogens.
12. **Nucleic acids**: Biomolecules, including DNA and RNA, responsible for storing and transmitting genetic information in cells.
13. **Purines and pyrimidines**: Nitrogenous bases found in nucleic acids, serving as the building blocks of DNA and RNA.

14. **Nucleosides and nucleotides**: Molecules composed of a nitrogenous base (purine or pyrimidine), a sugar (ribose or deoxyribose), and one or more phosphate groups, forming the monomeric units of nucleic acids.
15. **Cofactors**: Non-protein molecules or ions required for the proper functioning of enzymes, often assisting in catalysis or substrate binding.
16. **Enzyme kinetics**: Study of the rates of enzyme-catalyzed reactions and the factors influencing their velocity, including substrate concentration and temperature.
17. **Allosteric regulation**: Regulation of enzyme activity by the binding of a molecule to a site other than the active site, altering the enzyme's conformation and activity.

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