1.

Unit-V

10-15 Marks Questions

Q.1. Define introduction, Nomenclature & IUB Classification of Enzymes?

⇨ **ANSWER** : Introduction

- Enzymes are biological catalysts - mostly protein molecules - that accelerate metabolic reactions by lowering activation energy without being consumed.
- They act under mild physiological conditions and display high specificity for substrates and reactions.
- Enzymes activity is regulated by environmental factors (pH, temperature), cofactors / coenzymes and regulatory molecules.
- Definition: An enzyme is a protein (sometimes RNA-ribozyme) that increases the rate of a specific chemical reaction by providing an alternate lower-energy reaction pathway.

- Characteristics:

1. Specificity:
   - Substrate specificity: enzymes bind to particular substrates (lock and key / induced fit concepts).
   - Reaction specificity: one enzyme catalyzes a single type of chemical transformation.
2. Catalytic Efficiency:
   - High catalytic rates (Kcat): one enzyme molecule can process thousands of substrate molecules per minute.
3. Regulation:
   - Activity is modulated by pH, temperature, substrate concentration, allosteric effectors, covalent modification (e.g. phosphorylation) and proteolytic activation (zymogens).
4. Reusability:
   - Enzymes are not consumed they are regenerated after reactions.

5. Saturation Kinetics: At high substrate conc. the enzyme active sites become saturated → V_{max} reached (Michaelis-Meten kinetics).

6. Requirement for Cofactors / Coenzymes:

- Many enzymes require inorganic ions (metal cofactors) or organic molecules (coenzymes derived from vitamins) for activity.
- To systematically name and categorize enzymes, the IUB / Nomenclature committee developed rules and the EC classification).

Nomenclature of Enzymes

- Enzyme names fall into two major systems - trivial/common names and systematic names plus the EC (Enzyme Commission) number system.

A] Trivial / Common names

- These are historical or practical names often ending with '-ase' (eg. lipase, amylase, pepsin).
- Advantages: simple and widely used.
- Disadvantages: not systematic; many names give no information about reaction type or specificity.

B] Systematic names (IUB/IUPAC recommended)

- Systematic names describe the chemical reaction: substrate : acceptor reaction-type (for ex. L-lactate : NAD+ oxidoreductase for lactate dehydrogenase).
- These names are more informative but long; therefore common names remain in everyday use.

C] EC Numbering (Enzyme Commission Number).

- The EC number provides a concise, unambiguous code reflecting the enzymatic reaction type. It has four parts : EC x.x.x.x.
  - 1st digit : Major class (type of reaction).
  - 2nd digit : Subclass (group transferred or type of bond acted on).
  - 3rd digit : Sub-subclass (specifies about the reaction).
  - 4th digit : Serial number identifying the specific enzyme.
- Example : EC.1.1.1.27 = Alcohol dehydrogenase
- EC1 = oxidoreductases,
- .1 = acting on the CH-OH group.

- .1 = with NAD^+ or $NADP^+$ as acceptor,
- .27 = enzyme serial number in this group.

IUB (IUBMB) Classification of Enzymes

- The International Union of Biochemistry and Molecular Biology classifies enzymes into six major classes based on type of chemical reaction catalyzed.
- For each class given a definition, reaction & e.g.

Class 1: Oxidoreductases (EC1)

- Catalyze oxidation-reduction reactions (transfer of electrons or hydrogen).
- Dehydrogenation, oxidations, reductions: Typical Reactions.
- Example: Lactate dehydrogenase (LDH) converts lactate ⇌ pyruvate using NAD^+ / NADH.

(EC.1.1.1.27 as an illustration).

- **Class 2 : Transferases (EC 2)**
- Transfer functional groups (e.g. Methyl, phosphoryl, glycosyl) from one molecule to another.
- Typical reactions : Aminotransfer, kinase reactions.
- Example : Alanine transaminase (ALT; transfers amino groups). Hexokinase (ATP ⟶ glucose-6-phosphate, EC 2.7.1.1).

- **Class 3 : Hydrolases (EC 3)**
- Catalyze hydrolytic cleavage of C-O, C-N, C-C and other bonds by addition of water.
- Typical reactions: Proteolysis, ester hydrolysis.
- Example: Trypsin (protease), Lipase, Amylase (EC 3.2.1.x for glycosidases).

- **Class 4 : Lyases (EC 4)**
- Catalyze addition or removal of groups to form double bonds or the reverse, without hydrolysis or oxidation.
- Typical reactions: Decarboxylation, aldol cleavage.

- Example: Pyruvate decarboxylase or Aldolase (EC. 4.1.x.x).

- Class 5 : Isomerases (EC5)

- Catalyze intramolecular rearrangements (isomerization).

- Typical reactions : Epimerization, racemization, intramolecular transfer.

- Example : Triose phosphate isomerase (EC.5.3.1.1).

- Class 6 : Ligases (EC6)

- Join two molecules with concomitant hydrolysis of a diphosphate bond in ATP (or similar).

- Typical Reactions : formation of C-C, C-N, C-S bonds using ATP.

- Example : DNA Ligase (EC6.5.1.1) joins DNA strands breaks.

- Conclusion :

- Enzymes are indispensable biological catalyst that ensure metabolic reactions proceed rapidly and specifically under physiological conditions.

Q.2. Therapeutic and Diagnotis Applications of Enzymes and Isoenzymes.

⇨ **ANSWER**: Introduction

- Enzymes and isoenzymes play a critical role in ~~medical~~ modern medicine both as therapeutic agents and diagnostic biomarkers. Therapeutic enzymes are administered to treat various diseases by replacing deficient enzymes, dissolving clots or assisting digestion.
- Diagnostic enzyme measurements help detect tissue damage, organ dysfunction and metabolic disorders.
- Isoenzymes provide tissue-specific patterns that increase diagnostic accuracy.
- Therapeutic Applications of Enzymes
- Therapeutic use of enzymes is based on their ability to catalyze biological reactions efficiently and specifically, with minimal side effects.
- The following categories explain major uses in clinical practice :

1. Thrombolytic (Clot-dissolving) Enzymes.

- Used in emergency treatment of myocardial, infarction, pulmonary embolism and stroke.

a] Streptokinase

- Obtained from Streptococcus bacteria
- Activates plasminogen → plasmin → dissolves fibrin clots.
- Rapidly restores blood flow to heart muscles.

b] Urokinase

- Human urinary enzyme; directly converts plasminogen to plasmin.
- Used in deep vein thrombosis and catheter clearance.

c] Tissue Plasminogen Activator (tPA)

- Recombinant enzyme.
- Highly specific for fibrin-bound plasminogen → minimizes bleeding.

2. Digestive Enzymes.

- Used in conditions where natural enzyme secretion is deficient.

a] Pancreatic Enzyme Preparations (Pancreatin).

- Contains amylase, lipase, trypsin.
- Used in: Chronic pancreatitis
  Cystic fibrosis
  After pancreatectomy

b] Lactase

- Given orally to treat lactose intolerance.

3. Anti-inflammatory Enzymes

a) Serratiopeptidase
b) Trypsin- Chymotrypsin combinations.

- Used to reduce swelling, edema and tissue inflammation

4. Enzyme Replacement Therapy (ERT)

- Used in inherited metabolic disorders where a specific enzyme is deficient.
- L-Asparaginase
- β-Glucocerebrosidase

- α- Galactosidases
- Adenosine deaminase (ADA)

5. **Wound Debridement & Tissue Healing Enzymes.**

- These enzymes remove necrotic tissue and promote new tissue formation.
- Examples :
  - Papain
  - Chymotrypsin
  - Collagenase
- Used in bedsores, burns, diabetic foot ulcers.

6. **Anti- coagulant Enzymes.**

- Heparin
- Activates antithrombin III → prevents clot formation.
- Used in thrombosis, heart surgery, dialysis.

7. **Miscellaneous Uses**

a. Hyaluronidase

- Increases tissue permeability.
- Used to enhance absorption & diffusion of injected drugs.

b. DNAase (Dornase alfa)

- Used in cystic fibrosis to reduce mucus viscosity.

B. Diagnostic Applications of Enzymes and Isoenzymes

- Enzymes leak into the Blood when specific tissues are damaged.
- Measuring their levels helps diagnose diseases.
- Isoenzymes (molecular variants) help identify the exact tissue involved.

1. Diagnosis of Myocardial Infarction (Heart attack)

- The most important clinical use of enzymes.

a] Creatine Kinase

- High CK-MB strongly indicates acute MI.

b] Lactate Dehydrogenase (LDH) Isoenzymes.

- Used for delayed diagnosis.

c] AST (Aspartate Transaminase).

- Increased in heart and liver damage.
- Used historically but less specific.

2. **Diagnosis of liver Diseases**.

- Different enzymes indicate different hepatic problems.

a. ALT (Alanine Transaminase) - Liver specific.

- Elevated in hepatocellular injury (hepatitis, cirrhosis).

b. AST (Aspartate Transaminase)

- Also in heart & muscles; helps differential diagnosis.

c. ALP (Alkaline Phosphatase)

- Elevated in obstructive jaundice, bone diseases (Paget's fractures).

d. GGT (Gamma- Glutamyl Transferase).

- Highly elevated in alcoholic liver disease and bile duct obstruction.

3. **Diagnosis of Pancreatitis**.

a. Serum Amylase

- Rises rapidly in acute pancreatitis.
- Returns to normal within 48-72 hours.

b. Serum Lipase

- More specific than amylase.
- High lipase confirms pancreatic inflammation.

4. **Diagnosis of Bone Disorders**.

a. Bone-specific ALP enzyme

- Increased in rickets, bone tumours, osteomalacia.

5. **Diagnosis of Renal function**.

a. Serum Aldolase

- Elevated in renal tubular injury.

Conclusion :

- Enzymes and isoenzymes play a crucial role in clinical biochemistry, serving both as potent therapeutic agents and as sensitive diagnostic markers. Therapeutic enzymes help manage life-threatening conditions such as Myocardial Infraction, Leukemia and metabolic disorders, while diagnostic enzyme measurements allow early and specific detection of organ damage. Thus, understanding enzyme behaviour is essential for effective medical diagnosis and treatment.

5 marks / 7 marks Questions

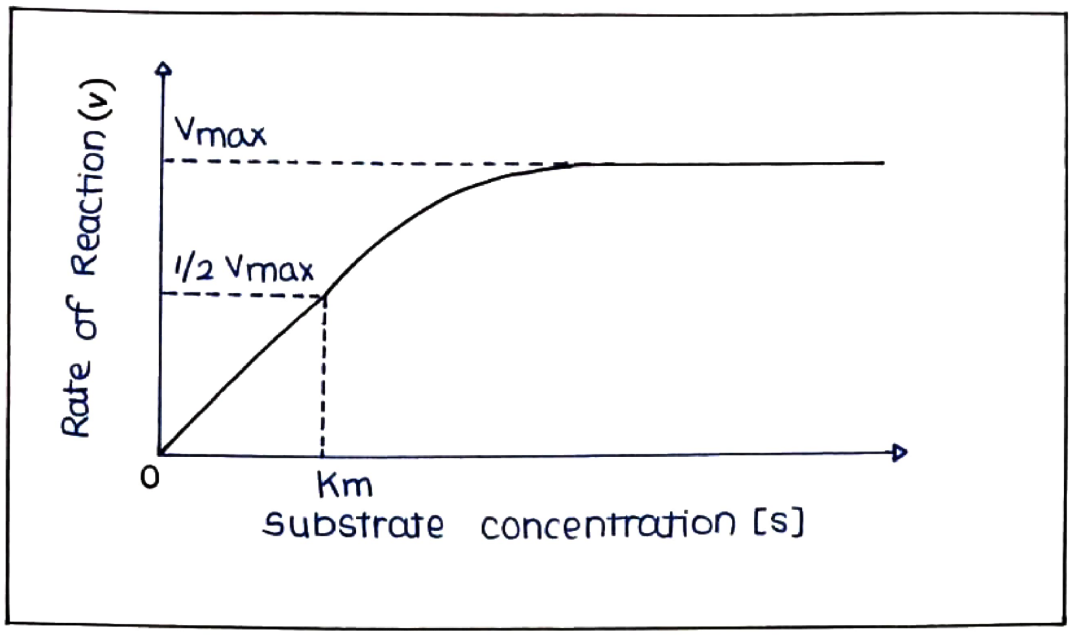
Q.1 Michaelis Plot (Michaelis-Menten Curve)

⇨ **ANSWER** : Introduction :

- The Michaelis-Meten plot describes the relationship between substrate concentration (S) and initial reaction velocity (V) of an enzyme-catalyzed reaction.
- It helps understand enzyme kinetics, affinity (Km), and maximum velocity (V_{max}).
- Definition of Michaelis Plot
- A Michaelis plot is a graph obtained by plotting reaction velocity (V) on the Y-axis against substrate concentration (S) on the x-axis.
- This plot is based on the Michaelis-Menten equation:

$$V = \frac{V_{max}\,[S]}{K_m + [S]}$$

- It produces a rectangular hyperbola, which is a characteristic feature of enzyme kinetics.



1. At low substrate concentration :

- The reaction rate increases almost linearly with increasing substrate concentration.
- This region shows first-order kinetics (rate depends on S).

2. At moderate substrate concentration :

- Enzyme active sites begin to fill.
- The rate increases but starts slowing down.

3. At high substrate concentration :

- Enzymes become saturated ~~concentrat~~ with substrate.

- Increasing substrate concentration does not increase velocity.
- Reaction reaches V_{max} (maximum velocity).
- Enzyme operates under zero-order kinetics.

- **Michaelis Constant (K_m)**

- K_m is the substrate concentration at which reaction velocity is half of V_{max}.
- It indicates enzyme-substrate affinity.
- Low K_m → high affinity
- High K_m → low affinity
- K_m is a constant for a given enzyme at a fixed pH and temperature.

- **Significance of Michaelis Plot**

1. Helps determine V_{max}, K_m and enzyme efficiency.
2. Indicates how enzyme velocity changes with substrate availability.

3. Useful to understand enzyme saturation.
4. Helps in studying enzyme inhibition (competitive / noncompetitive).
5. Provides visualization of enzyme-substrate interaction.

- **Conclusion**
- The Michaelis plot is a fundamental tool in enzymology, illustrating how substrate concentration influences enzyme velocity. It helps in determining kinetic constants and understanding enzyme behaviour under different physiological conditions.

Q.2 Enzyme Inhibitors with examples

⇨ **ANSWER** : Introduction

- Enzyme inhibitors are substances that reduce or completely block enzyme activity.
- They play an important role in regulating metabolic pathways and are widely used as therapeutic drugs.
- Inhibition can be reversible or irreversible based on the nature of inhibitor binding.

- Types of Enzyme Inhibition

1. Competitive Inhibition

- Inhibitor resembles the substrate structurally.
- Competes for the active site.
- Increasing substrate concentration can overcome inhibition.
- K_m increases, V_{max} unchanged.

Examples :

- Malonate inhibits succinate dehydrogenase.
- Statins inhibit HMG-CoA reductase in cholesterol synthesis.
- Methotrexate inhibits dihydrofolate reductase.

2. Noncompetitive Inhibition

- Inhibition binds to a site other than active site (allosteric site).
- Binding reduces enzyme activity regardless of substrate level.
- Vmax decreases, K_m remains same.
- Cannot be overcome by adding substrate.

- **Examples**:
- Heavy metals (Ag^+, Hg^{2+}).
- Cyanide inhibits cytochrome oxidase.

3. Uncompetitive Inhibition

- Inhibitor binds ONLY to the enzyme-substrate complex.
- Both K_m and V_{max} decrease.
- Occurs in multi-substrate enzyme systems.

Example:

- Lithium inhibiting inositol monophosphatase.

4. Irreversible Inhibition

- Inhibitor binds covalently → permanently inactivates enzyme.

- Enzyme must be resynthesized for activity to return.

- **Examples**:
  - Organophosphates (nerve gases) inhibit acetylcholinesterase.
  - Aspirin irreversibly inhibits cyclooxygenase (cox).
  - Penicillin inhibits bacterial transpeptidase.

- **Importance of Enzyme Inhibitors**

- Used in many life-saving drugs.
- Help study enzyme mechanisms.
- Regulate metabolic pathways.
- Used in treatment of cancer, hypercholesterolemia, hypertension, infections.

- **Conclusion**

- Enzyme inhibitors are crucial in controlling enzyme activity and serve as important therapeutic agents.
- Understanding the types of inhibition helps in drug design and clinical biochemistry.

Q.3 Coenzymes - Structure and Biochemical Functions.

⇨ **ANSWER** : **Introduction**:

- Coenzymes are small organic molecules that bind to enzymes and are essential for catalytic activity.
- They act as transient carriers of atoms, electrons, or chemical groups during biochemical reactions.
- Most coenzymes are derived from B-complex vitamins.

- **Definitions**:
- A coenzyme is a non-protein, organic, diffusible molecule that binds temporarily to the apoenzyme & participates directly in enzyme catalysis by transferring groups or electrons.

- **Structure of Coenzyme**
- Most coenzymes have three structural features:

1. Vitamin-derived component:

- eg. niacin in NAD^+, riboflavin in FAD, pantothetic acid in CoA.

2. Adenine nucleotide moiety

- Many coenzymes contain AMP or ADP (e.g. NAD^+, FAD, CoA).
- Helps in enzyme recognition and binding.

3. Reaction group / site

- Accepts or donates electrons, hydrogen atoms, acyl groups, amino groups, etc.

- **Examples of Coenzyme Structures**
- NAD^+ = Nicotinamide + Ribose + Adenine + 2 Phosphate groups.
- FAD = Riboflavin + AMP.
- Coenzyme A = Pantothenic acid + Adenine + Phosphate + Sulfhydryl group (-SH).

- **Biochemical Functions of Coenzymes.**

1. Coenzymes as Electron Carriers

NAD^+ / $NADP^+$ (Niacin-derived)

- Accept/donate electrons in dehydrogenase reactions.
- Used in glycolysis, TCA cycle, fatty acid synthesis.

- FAD / FMN (Riboflavin-derived)
  - carry hydrogen atoms.
  - Essential in ETC and oxidative metabolism.

2. Coenzymes in Acyl Group Transfer.

Coenzyme A (CoA)

- Transfers acyl groups.
- Key role in β-oxidation, TCA cycle, synthesis of fatty acids.

3. Coenzyme in Decarboxylation

Thiamine Pyrophosphate (TPP).

- From Vitamin B_1.
- Required for oxidative decarboxylation (Pyruvate → acetyl-CoA).

4. Coenzymes in Carboxylation

- Biotin
- CO_2 carrier.
- Required in gluconeogenesis and fatty acid synthesis.

5. Coenzymes as Methyl Group Donors.

- Tetrahydrofolate (THF)

- Transfers one-carbon units.
- Important in purine, thymidine and DNA synthesis.

- **Conclusion**
- Coenzymes are vital molecules that assist enzymes in catalyzing a wide range of biochemical reactions.
- Their structures allow them to act as carriers of electrons or groups, making them indispensable in metabolism.

UNIT V

2 marks / 3 marks / MCQ

Q.1 Define enzyme

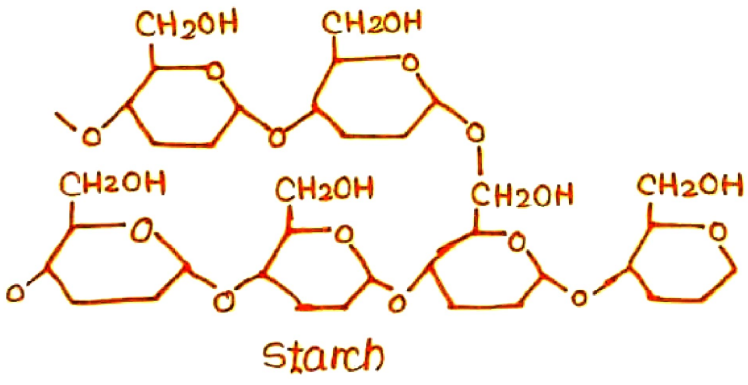
ANSWER : ENZYME

Definition : An enzyme is a biological catalyst, usually a protein, which speeds up the rate of biochemical reactions in living organisms without being consumed or permanently changed during the reaction.

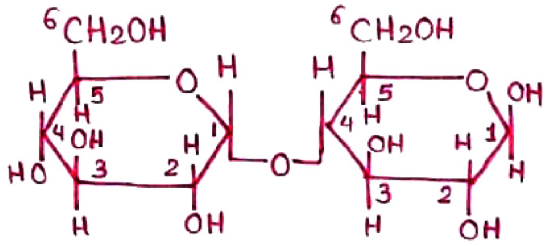
- Enzymes are highly specific for their substrates and work by lowering the activation energy needed for a reaction to occur.

- **Example :** Amylase : It is a carbohydrate digesting enzyme, produced by salivary glands and pancreatic acinar cells, that hydrolyzes starch into maltose (disaccharides) :

- Starch + H_2O $\xrightarrow{\text{Amylase}}$ maltose (disaccharides).



Starch


Maltose

Q.2 Define enzyme inhibitors and its example.

ANSWER : ENZYME INHIBITORS

- **Definition** : Enzyme inhibitors are substances that decreases or completely stop the activity of enzymes by binding to them. They may bind reversibly or irreversibly to the enzyme and interfere with the binding of substrate or the catalytic process.

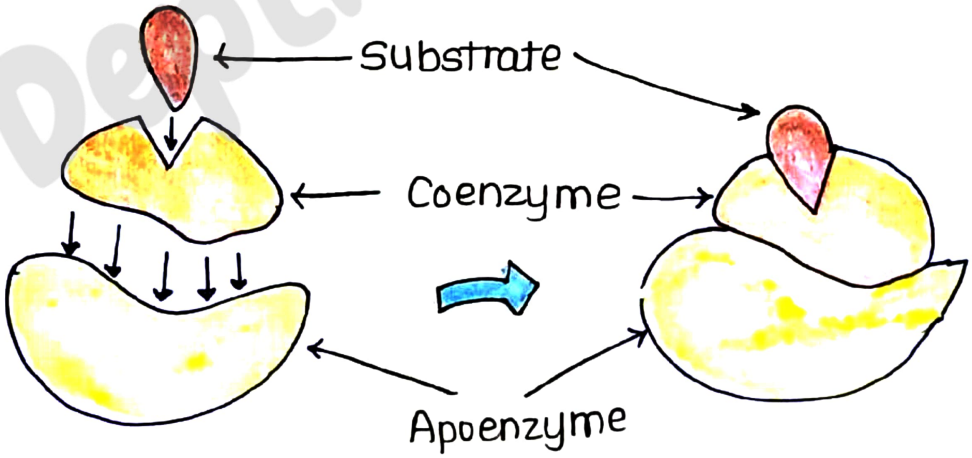
- **Types with examples**:

- Competitive inhibitors : Compete with substrate for the enzyme's active site.
- Example : Methotrexate inhibits dihydrofolate reductase.
- Non-competitive inhibitors : Bind to a site other than the active site and change enzyme shape.
- Example : Cyanide inhibits cytochrome oxidase.
- Uncompetitive inhibitors : Bind only to the enzyme-substrate complex.
- Example : Lithium inhibits inositol monophosphate.
- These inhibitors play important roles in regulating metabolic pathways and are also used in drug design.

Q.3. What is coenzyme

ANSWER : COENZYME

- **Definition** : A coenzyme is a small organic non-protein molecule that binds temporarily or permanently with an enzyme and is essential for its catalytic activity.
- Co-enzymes often acts as carriers of electrons, atoms, or functional groups transferred in the enzymatic reaction.
- They are usually derived from vitamins.



- Example : NAD+ (Nicotinamide adenine dinucleotide).

Q.4. Define isoenzyme

ANSWER: ISOENZYMES

- **Definition:** Isoenzymes (also called isozymes) are different molecular forms of the same enzyme that catalyze the same biochemical reaction, but differ in their physical properties, such as structure, amino acid sequence, electrophoretic mobility, and kinetic properties.

- They usually occur in different tissues or developmental stages, allowing fine regulation of metabolism.
- **Example:**
  - Lactate dehydrogenase (LDH) exists as five isoenzymes (LDH1 to LDH5) found in heart, liver, muscle, etc.