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BIOCHEMISTRY

UNIT 5

TOPIC :

- **Enzymes**

  Introduction, properties, nomenclature and IUB classification of enzymes

  Enzyme kinetics (Michaelis plot, Line Weaver Burke plot)

  Enzyme inhibitors with examples

  Regulation of enzymes: enzyme induction and repression, allosteric enzymes regulation

  Therapeutic and diagnostic applications of enzymes and isoenzymes Coenzymes–Structure and biochemical functions

Enzymes

- Enzymes are biological catalysts that speed up chemical reactions in living organisms without being consumed in the process.
- They are mostly proteins (some RNA molecules also act as enzymes, e.g., ribozymes).
- Enzymes are essential for metabolic reactions, such as digestion, respiration, DNA replication, etc.

Properties of Enzymes

1. **Catalytic Nature**
   - Enzymes increase the rate of reactions by lowering the activation energy.
2. **Specificity**
   - Enzymes are highly specific to the type of reaction or substrate.
3. **Efficiency**
   - A small amount of enzyme can catalyze thousands of reactions per second.
4. **Reversible Action**
   - Some enzyme-catalyzed reactions are reversible, depending on substrate/product concentration.
5. **Affected by pH and Temperature**
   - Enzymes work best at an optimal pH and temperature. Extreme conditions can denature them.
6. **Do Not Alter Equilibrium**
   - Enzymes speed up the reaction but do not change the equilibrium of the reaction.
7. **Regulated Activity**
   - Enzyme activity can be regulated by activators or inhibitors.
8. **Can Be Saturated**
   - When all active sites are occupied by substrates, increasing substrate concentration won't increase the rate.

Nomenclature of Enzymes

- Traditionally, enzymes were named by **adding** "**-ase**" to the name of the **substrate** or the **type of reaction**.
    - Example:
        - **Urease** – acts on urea
        - **Lactase** – acts on lactose
        - **Oxidase** – catalyzes oxidation
- Some enzymes have **common/traditional names** like **pepsin**, **trypsin**, etc.

IUB classification of enzymes

→ The International Union of Biochemistry and Molecular Biology (IUBMB) classified enzymes into six major classes based on the type of reaction they catalyze.

→ Each enzyme is given a unique EC number (Enzyme Commission number).

6 Major Classes of Enzymes

EC Class	Enzyme Type	Reaction Catalyzed	Examples
EC 1	**Oxidoreductases**	Oxidation–reduction reactions	Dehydrogenase, Oxidase
EC 2	**Transferases**	Transfer of functional groups	Transaminase, Kinase
EC 3	**Hydrolases**	Hydrolysis reactions (add water to break bonds)	Protease, Lipase, Amylase
EC 4	**Lyases**	Removal or addition of groups without hydrolysis	Decarboxylase, Aldolase
EC 5	**Isomerases**	Isomerization (rearrangement within molecule)	Isomerase, Mutase
EC 6	**Ligases (Synthetases)**	Joining of two molecules using ATP	DNA ligase, Carboxylase

Mechanism of Action of Enzymes

Step 1 : The enzyme attracts the substrate to its active site and becomes Enzyme - Substrate comple .

$$E + S \rightarrow ES \text{ complex}$$

Step 2 : a process called catalysis occurs

Step 3 : Now The enzyme release the Substrate and now substrate is called Product (p) .

$$ES \rightarrow E + P$$

There are two models which explained the Mechanism of action of enzyme :

1. Lock and Key Hypothesis (Emil Fisher)
2. Induced fit Hypothesis (Koshland)

Factors Affecting Enzymes Activity

- **Temperature :** Enzymes have optimal temperatures at which they function best. An increase in temperature can increase enzyme activity, but excessive heat can denature the enzyme, rendering it inactive.
- **pH :** Enzymes have optimal pH levels at which they function best. A change in pH can affect the shape of the enzyme, making it unable to bind to its substrate, and therefore less active.
- **Substrate concentration :** As the concentration of substrate increases, the rate of enzymecatalyzed reactions also increases, up to a point. Beyond that point, the enzymes become saturated and the reaction rate levels off.
- **Enzyme concentration :** Increasing the amount of enzyme present will increase the rate of the reaction, up to a point. Beyond that point, the reaction rate levels off.
- **Inhibitors :** Compounds that bind to enzymes and prevent them from functioning properly are called inhibitors. They can be competitive or non-competitive, and can be reversible or irreversible.
- **Co-factors :** Many enzymes require small, non-protein molecules, called cofactors, to function. Cofactors can be metal ions or organic molecules, and if they are not present, the enzyme will not function

Enzyme Kinetics

- Enzyme kinetics is the study of the rate of chemical reactions that are catalyzed by enzymes.
  It involves measuring how fast a reaction proceeds and how different conditions (like temperature, pH, substrate concentration) affect the reaction.
- By studying enzyme kinetics, scientists can understand the catalytic mechanism—how enzymes work to speed up reactions.
- Enzymes are usually protein molecules.
- They act on specific molecules called substrates.
- Substrates bind to the active site of the enzyme.
- The enzyme then converts the substrate into product(s) through a series of steps, known as the enzymatic mechanism.

Types of Enzyme Mechanisms

1. **Single-Substrate Mechanisms**
   - The enzyme acts on **only one substrate**.
   - Example: **Triose phosphate isomerase**
   - Kinetic studies aim to determine:
     - **Affinity** of enzyme for substrate (Km)
     - **Turnover rate** or speed of conversion (Vmax)
2. **Multiple-Substrate Mechanisms**
   - The enzyme binds **to two or more substrates** in a specific sequence.
   - More complex and require additional kinetic models.

Michaelis-Menten Equation

- The Michaelis-Menten equation describes how the rate of an enzyme-catalyzed reaction changes with increasing substrate concentration.
- It is one of the most important formulas in enzyme kinetics.

Basic Reaction Model

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

Where:

- **E** = Free enzyme
- **S** = Substrate
- **ES** = Enzyme-substrate complex
- **P** = Product
- k_1, k_{-1} and k_2 are rate constants for formation and breakdown of ES

Michaelis-Menten Equation

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

Where:

- V = Initial velocity of the reaction
- V_{max} = Maximum velocity (when enzyme is saturated with substrate)
- [S] = Substrate concentration
- K_m = Michaelis constant (substrate concentration at which the reaction rate is **half of Vmax)**

Uses of Michaelis-Menten Equation

- Calculate **enzyme efficiency**
- Understand **enzyme-substrate interactions**
- Analyze the effect of **inhibitors**
- Used in **drug development** and clinical enzyme assays

LINE WEAVER-BURKE PLOT

→ The Line weaver–Burke plot is a graphical representation of the Line weaver– Burke equation of enzyme kinetics, described by Hans Line weaver and Dean Burke in 1934. The plot provides a useful graphical method for analysis of the Michaelis-Menten

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

Equation : Taking the reciprocal gives

$$\frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

Where,

Km is the Michaelis—Menten constant

[S] is the substrate concentration

V is the reaction velocity (the reaction rate)

V_{max} the maximum reaction velocity

Uses of Lineweaver-Burk Plot

- To find **Km and Vmax** from experimental data
- To study **types of enzyme inhibition**:
  - **Competitive**: Lines intersect at Y-axis (Vmax same, Km increases)
  - **Non-competitive**: Lines intersect at X-axis (Km same, Vmax decreases)
  - **Uncompetitive**: Parallel lines (both Km and Vmax decrease)

Enzyme Inhibitors

- Enzyme inhibitors are chemical substances that reduce or completely stop the activity of enzymes.
  They do this by binding to the enzyme and interfering with its ability to bind the substrate or perform its catalytic function.
- Inhibitors can be reversible or irreversible depending on how they interact with the enzyme.

Types of Enzyme Inhibitors

1. Competitive Inhibition

- The inhibitor competes with the substrate for binding at the active site of the enzyme.
- It resembles the substrate in structure.
- Can be reversed by increasing substrate concentration.

Effect on Kinetics:

- **Km increases** (lower affinity)
- **Vmax remains unchanged**

Examples:

- **Malonate** inhibits **succinate dehydrogenase**
- **Methotrexate** inhibits **dihydrofolate reductase**
- **Statins** (e.g., Atorvastatin) inhibit **HMG-CoA reductase** (cholesterol synthesis)

2. Non-Competitive Inhibition

- The inhibitor binds to a site other than the active site (allosteric site).
- It does not compete with the substrate.
- Binding changes enzyme shape, making it less active or inactive.
- Increasing substrate concentration does not overcome inhibition.

Effect on Kinetics:

- **Vmax decreases**
- **Km remains unchanged**

Examples:

- **Heavy metals** (like lead, mercury) inhibit many enzymes.
- **Fluoride** inhibits **enolase** in glycolysis.
- **Alanine** inhibits **pyruvate kinase**

3. Uncompetitive Inhibition

- Inhibitor binds only to the enzyme-substrate complex (ES), not to free enzyme.
- Forms an inactive ESI complex.

Effect on Kinetics:

- **Both Km and Vmax decrease**

Examples:

- **Lithium** inhibits **inositol monophosphatase**
- Some **cancer drugs** act as uncompetitive inhibitors

4. Irreversible Inhibition

- Inhibitor forms a covalent bond with the enzyme.
- Permanently inactivates the enzyme.
- Cannot be reversed by removing the inhibitor.

Examples:

- **Aspirin** irreversibly inhibits **cyclooxygenase (COX)** enzyme.
- **Penicillin** inhibits **transpeptidase** (used in bacterial cell wall synthesis).

- **Organophosphates** inhibit **acetylcholinesterase** (nerve agent effect).

Regulation of Enzymes

- Enzyme regulation ensures that metabolic reactions occur efficiently, only when needed, and in proper balance.
- There are three major types of enzyme regulation:
  1. Enzyme Induction
  2. Enzyme Repression
  3. Allosteric Regulation

1. Enzyme Induction

→ Enzyme induction is the process by which a cell increases the synthesis of specific enzymes in response to certain stimuli or the presence of a substrate.

How it works:

- A **signal molecule** (often the substrate or drug) activates a gene.
- This leads to **increased transcription and translation** of the enzyme.
- As a result, the **enzyme concentration increases**, enhancing the reaction rate.

Example:

- **Cytochrome P450 enzymes** are induced by **barbiturates and rifampin** (important in drug metabolism).
- **Lactose** induces the enzyme **β-galactosidase** in *E. coli*.

Importance:

- Allows cells to adapt to new environmental conditions.

- Common in **drug metabolism, xenobiotic clearance**, and **hormone action.**

2. Enzyme Repression

→ Enzyme repression is the inhibition of enzyme synthesis at the genetic level, often in response to the accumulation of the end product of a metabolic pathway.

How it works:

- The end product acts as a **repressor**, binding to a regulatory protein or DNA operator.
- This **prevents transcription** of the enzyme's gene.
- As a result, **enzyme synthesis is reduced or stopped.**

Example:

- In *E. coli*, **tryptophan** acts as a **corepressor** to shut down the **tryptophan synthetase** gene.
- **Glucose** represses the synthesis of enzymes involved in **lactose metabolism** (catabolite repression).

Importance:

- Prevents **wasteful synthesis** of enzymes when not needed.
- Maintains **homeostasis** in metabolic pathways.

3. Allosteric Enzyme Regulation

→ Allosteric regulation occurs when an enzyme's activity is modified by binding of a molecule (effector) at a site other than the active site (called the allosteric site).

Types of Effectors:

1. **Activators** – increase enzyme activity.
2. **Inhibitors** – decrease enzyme activity.

Key Features:

- Common in enzymes with **quaternary structure** (multiple subunits).
- Causes a **conformational change** in enzyme shape and function.
- Shows **sigmoidal (S-shaped) kinetics**, unlike Michaelis-Menten enzymes.

Examples:

- **Phosphofructokinase-1 (PFK-1)** is inhibited by **ATP** and activated by **AMP** in glycolysis.
- **Aspartate transcarbamoylase (ATCase)** is regulated by **CTP (inhibitor)** and **ATP (activator)** in nucleotide synthesis.

Importance:

- Provides **fine control** over enzyme activity.
- Allows **rapid, reversible** regulation (not involving gene expression).

Therapeutic and Diagnostic Applications of Enzymes

Therapeutic Applications of Enzymes

- Therapeutic enzymes are used to treat diseases by either replacing deficient enzymes, dissolving clots, or helping with digestion and inflammation. These enzymes are either naturally occurring or recombinantly produced.

Common Therapeutic Enzymes:

Enzyme	Medical Use	Description
Streptokinase / Urokinase	Dissolving blood clots (thrombolysis)	Converts plasminogen to plasmin to break fibrin clots in heart attack or stroke
Asparaginase	Cancer therapy (leukemia)	Breaks down asparagine, starving cancer cells (esp. in acute lymphoblastic leukemia)
Pancreatin / Trypsin / Amylase / Lipase	Digestive disorders	Helps in digestion for patients with pancreatic insufficiency
Hyaluronidase	Enhancing drug diffusion	Breaks down hyaluronic acid in connective tissue to enhance absorption of drugs
DNase (Dornase alfa)	Cystic fibrosis	Breaks down DNA in mucus to reduce viscosity and improve breathing
Collagenase	Wound healing / debridement	Breaks down collagen in damaged tissues
Glucocerebrosidase	Gaucher's disease	Replaces deficient enzyme in lysosomal storage disorder

Pegademase (PEG-ADA)	SCID (Severe Combined Immunodeficiency)	Replaces deficient adenosine deaminase enzyme

Advantages:

- Highly specific in action
- Fast-acting with fewer side effects
- Can be produced using biotechnology (recombinant enzymes)

Diagnostic Applications of Enzymes

→ Certain enzymes and isoenzymes (enzyme variants) are used as biomarkers to diagnose diseases, especially organ-specific damage, because they leak into the blood when cells are damaged.

Enzymes in Clinical Diagnosis:

Enzyme / Isoenzyme	Diagnostic Use	Source
Amylase & Lipase	Acute pancreatitis	Pancreas
Alkaline Phosphatase (ALP)	Liver or bone disorders	Liver, bones
Acid Phosphatase (ACP)	Prostate cancer	Prostate
Lactate Dehydrogenase (LDH)	Heart attack, liver disease, cancer	Heart, liver, muscles
Creatine Kinase (CK / CPK)	Muscle damage, heart attack	Heart, skeletal muscles
CK-MB (isoenzyme of CK)	Myocardial infarction (heart attack)	Heart muscle
SGOT (AST)	Liver & heart damage	Liver, heart
SGPT (ALT)	Liver disease	Liver
Gamma Glutamyl Transferase (GGT)	Alcoholic liver disease	Liver
Troponins (T, I)	Highly specific marker of myocardial infarction	Heart muscle

Isoenzymes (Isozymes)

Isoenzymes, or **isozymes**, are **physically distinct forms of the same enzyme** that perform the **same catalytic function** but **differ in structure** and are often produced in **different tissues**.

They help in the **identification of diseases** in specific organs because the same enzyme may appear in **different forms** in the **heart, liver, muscles, or other tissues**.

Isoenzymes are **multiple molecular forms** of an enzyme that:

- **Catalyze the same chemical reaction**
- Differ in **structure, charge, or amino acid sequence**
- Are coded by **different genes** or **same gene with modifications**
- Occur in **different tissues or cell types**

Isoenzymes Are Formed:

1. **True Isoenzymes** – Produced by **different genes (different loci)**
   Example: **Salivary and pancreatic amylase** (same function, different genes)
2. **Isoenzymes from the Same Individual** – Different forms coexist in one person
   Example: **Lactate Dehydrogenase (LDH)** has **5 isoenzymes**, all present in the same body.

Clinical Importance:

- Isoenzymes are organ-specific, so measuring them helps to identify the affected organ.
- Used as biomarkers for heart attacks, liver damage, muscle disorders, etc.

Coenzymes

- Coenzymes are non-protein organic molecules that bind to enzymes and help in catalyzing biochemical reactions.
  They act as transient carriers of specific atoms or functional groups (like electrons, acyl groups, or hydrogen atoms).
- They are essential for the activity of many enzymes, especially in oxidation-reduction and group transfer reactions.

Examples of Coenzymes – Structure & Function

Coenzyme	Derived from	Structure Highlights	Biochemical Function
NAD^+ (Nicotinamide adenine dinucleotide)	Vitamin B_3 (Niacin)	Contains nicotinamide ring and adenine	Accepts and donates electrons (oxidation-reduction reactions)
FAD (Flavin adenine dinucleotide)	Vitamin B_2 (Riboflavin)	Flavin ring attached to AMP	Transfers electrons in redox reactions
Coenzyme A (CoA)	Vitamin B_5 (Pantothenic acid)	Contains a thiol (-SH) group	Transfers acyl groups (e.g., in fatty acid metabolism)
TPP (Thiamine pyrophosphate)	Vitamin B_1 (Thiamine)	Contains thiazolium ring	Transfers aldehyde groups (in decarboxylation)
PLP (Pyridoxal phosphate)	Vitamin B_6 (Pyridoxine)	Aldehyde group and pyridine ring	Involved in amino acid metabolism (transamination, decarboxylation)
Biotin	Vitamin B_7 (Biotin)	Imidazole-like ring with valeric acid side chain	Transfers carboxyl groups (in carboxylation reactions)

Cobalamin (Vitamin B_{12})	Vitamin B_{12}	Corrin ring with cobalt center	Methyl group transfers and rearrangement of hydrogen
Lipoic Acid	Not a vitamin	Dithiol group in ring	Electron and acyl group transfer in oxidative decarboxylation

Functions of Coenzymes in Biochemical Reactions

1. **Electron Carriers**
   - NAD^+, FAD transfer **electrons** during oxidation-reduction reactions (e.g., in respiration)
2. **Group Transfer**
   - CoA transfers **acyl groups** (e.g., in fatty acid metabolism)
   - PLP transfers **amino groups** (e.g., in amino acid metabolism)
3. **Carbon Transfer**
   - Biotin transfers **CO_2** (e.g., in pyruvate carboxylase)
   - TPP transfers **aldehydes** (e.g., in pyruvate decarboxylation)
4. **Methylation Reactions**
   - Vitamin B_{12} involved in **methyl group transfers**