B. PHARMACY 2ND SEMESTER - BIOCHEMISTRY

UNIT – 5 🧬 ENZYMES

POINTS TO BE COVERED IN THIS TOPIC

- ➤ INTRODUCTION 🔬
- ➤ PROPERTIES OF ENZYMES ⚡
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INTRODUCTION 🔬

Enzymes are highly specialized protein molecules that act as biological catalysts in living organisms. These remarkable macromolecules accelerate the rate of biochemical reactions by lowering the activation energy required for the reaction to proceed. Without enzymes, the metabolic processes essential for life would occur too slowly to sustain cellular functions.

Key Features of Enzymes:

- They are primarily composed of proteins, though some RNA molecules also exhibit catalytic activity (ribozymes)
- They remain unchanged after catalyzing a reaction and can be reused multiple times
- They exhibit remarkable specificity for their substrates
- Their activity is influenced by factors such as temperature, pH, and the presence of inhibitors or activators
- They play crucial roles in virtually all biological processes including digestion, metabolism, DNA replication, and cellular signaling

Historical Perspective: The study of enzymes began in the 19th century with the observation of fermentation processes. The term "enzyme" was coined from the Greek words "en" (in) and "zyme" (leaven), meaning "in leaven." The understanding of enzyme structure and function has evolved dramatically, leading to modern enzyme technology and therapeutic applications.

PROPERTIES OF ENZYMES ⚡

Enzymes possess several distinctive properties that make them highly efficient biological catalysts:

🔹 CATALYTIC EFFICIENCY

- Enzymes can increase reaction rates by factors of 10^6 to 10^17 compared to uncatalyzed reactions
- They achieve this by providing alternative reaction pathways with lower activation energy

- The catalytic efficiency is measured by the ratio kcat/Km, where kcat is the turnover number and Km is the Michaelis constant

SPECIFICITY

- **Substrate Specificity:** Each enzyme typically catalyzes only one type of reaction or acts on closely related substrates
- **Reaction Specificity:** Enzymes are specific for the type of chemical reaction they catalyze
- **Stereochemical Specificity:** Enzymes can distinguish between different stereoisomers of the same compound

REGULATION

- Enzyme activity can be controlled through various mechanisms including allosteric regulation, covalent modification, and enzyme induction/repression
- This regulation allows cells to control metabolic pathways according to their needs
- Feedback inhibition prevents overproduction of metabolites

MILD REACTION CONDITIONS

- Enzymes function optimally under physiological conditions (body temperature, near-neutral pH, aqueous environment)
- This contrasts with industrial catalysts that often require extreme conditions
- The mild conditions preserve the integrity of other cellular components

🔹 THERMODYNAMIC LIMITATIONS

- Enzymes do not change the equilibrium position of a reaction; they only accelerate the attainment of equilibrium
- They cannot make thermodynamically unfavorable reactions occur spontaneously
- The overall free energy change (ΔG) of the reaction remains unchanged

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NOMENCLATURE AND IUB CLASSIFICATION 📝

The **International Union of Biochemistry (IUB)** established a systematic approach to enzyme nomenclature and classification to bring order to the rapidly expanding field of enzymology.

🔹 ENZYME NOMENCLATURE

Common Names vs. Systematic Names:

- **Common Names:** Often reflect the substrate or the source of the enzyme
- **Systematic Names:** Provide detailed information about the reaction catalyzed
- **EC Numbers:** Each enzyme is assigned a unique Enzyme Commission number

Naming Conventions:

- Most enzyme names end with the suffix "-ase"

- The root name often indicates the substrate or type of reaction
- Systematic names describe both the substrate and the type of reaction

🔹 IUB CLASSIFICATION SYSTEM

The IUB system classifies enzymes into **six major classes** based on the type of reaction they catalyze:

EC Class	Enzyme Type	Reaction Catalyzed	Examples
EC 1	**Oxidoreductases**	Oxidation-reduction reactions	Dehydrogenases, Oxidases
EC 2	**Transferases**	Transfer of functional groups	Aminotransferases, Kinases
EC 3	**Hydrolases**	Hydrolysis reactions	Lipases, Proteases
EC 4	**Lyases**	Addition/removal of groups to form double bonds	Decarboxylases, Aldolases
EC 5	**Isomerases**	Intramolecular rearrangements	Mutases, Racemases
EC 6	**Ligases**	Formation of bonds with ATP hydrolysis	Synthetases, Carboxylases

🔹 EC NUMBER SYSTEM

The EC number consists of four digits separated by periods:

- **First digit:** Indicates the main class (1-6)
- **Second digit:** Indicates the subclass
- **Third digit:** Indicates the sub-subclass

- **Fourth digit:** Indicates the specific enzyme

ENZYME KINETICS 📊

Enzyme kinetics is the study of the rates of enzyme-catalyzed reactions and the factors that affect these rates. Understanding enzyme kinetics is crucial for comprehending how enzymes function and how they can be regulated.

🔹 MICHAELIS-MENTEN KINETICS

The **Michaelis-Menten equation** describes the relationship between enzyme concentration, substrate concentration, and reaction rate:

v = (Vmax × [S]) / (Km + [S])

Where:

- **v** = initial reaction rate
- **Vmax** = maximum reaction rate
- **[S]** = substrate concentration
- **Km** = Michaelis constant

🔹 MICHAELIS CONSTANT (Km)

- Km represents the substrate concentration at which the reaction rate is half of Vmax
- It is a measure of the enzyme's affinity for its substrate

- Lower Km values indicate higher affinity between enzyme and substrate
- Km is expressed in units of concentration (typically mM or μM)

◆ MAXIMUM VELOCITY (Vmax)

- Vmax is the maximum rate of reaction when the enzyme is saturated with substrate
- It is directly proportional to the enzyme concentration
- Vmax = kcat × [E]total, where kcat is the turnover number

◆ MICHAELIS PLOT

The **Michaelis plot** is a graph of reaction rate (v) versus substrate concentration ([S]). This plot shows:

- **Initial linear region:** Reaction is first-order with respect to substrate
- **Transition region:** Mixed-order kinetics
- **Plateau region:** Zero-order kinetics (enzyme saturation)

◆ LINEWEAVER-BURK PLOT

The **Lineweaver-Burk plot** (also called the double reciprocal plot) is a linear transformation of the Michaelis-Menten equation:

1/v = (Km/Vmax) × (1/[S]) + 1/Vmax

Advantages of Lineweaver-Burk Plot:

- Converts hyperbolic curve to straight line
- Easy determination of Km and Vmax from intercepts

- Useful for analyzing enzyme inhibition patterns
- Y-intercept = 1/Vmax
- X-intercept = -1/Km
- Slope = Km/Vmax

ENZYME INHIBITORS 🚫

Enzyme inhibitors are molecules that decrease or completely abolish enzyme activity. Understanding enzyme inhibition is crucial for drug development, metabolic regulation, and biochemical research.

🔹 CLASSIFICATION OF ENZYME INHIBITORS

Type of Inhibition	Binding Site	Effect on Km	Effect on Vmax
Competitive	Active site	Increases	No change
Non-competitive	Allosteric site	No change	Decreases
Uncompetitive	Enzyme-substrate complex	Decreases	Decreases

🔹 COMPETITIVE INHIBITION

- **Mechanism:** Inhibitor competes with substrate for binding to the active site
- **Characteristics:** Inhibition can be overcome by increasing substrate concentration
- **Kinetic Effects:** Km appears to increase, but Vmax remains unchanged

- **Lineweaver-Burk Plot:** Lines intersect at y-axis (1/Vmax unchanged)

NON-COMPETITIVE INHIBITION

- **Mechanism:** Inhibitor binds to a site other than the active site (allosteric site)
- **Characteristics:** Inhibition cannot be overcome by increasing substrate concentration
- **Kinetic Effects:** Vmax decreases, but Km remains unchanged
- **Lineweaver-Burk Plot:** Lines intersect at x-axis (Km unchanged)

UNCOMPETITIVE INHIBITION

- **Mechanism:** Inhibitor binds only to the enzyme-substrate complex
- **Characteristics:** Rare type of inhibition, often seen in multi-substrate reactions
- **Kinetic Effects:** Both Km and Vmax decrease proportionally
- **Lineweaver-Burk Plot:** Parallel lines (same slope)

IRREVERSIBLE INHIBITION

- **Covalent Modification:** Inhibitor forms covalent bonds with enzyme
- **Permanent Inactivation:** Enzyme cannot regain activity without synthesis of new enzyme molecules
- **Time-Dependent:** Progressive loss of enzyme activity over time

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REGULATION OF ENZYMES 🎛️

Enzyme regulation is essential for controlling metabolic pathways and maintaining cellular homeostasis. Cells employ multiple mechanisms to regulate enzyme activity according to their metabolic needs.

🔹 ENZYME INDUCTION

- **Definition:** Increase in enzyme synthesis in response to specific stimuli
- **Mechanism:** Enhanced transcription of genes encoding the enzyme
- **Time Course:** Relatively slow process (hours to days)
- **Physiological Significance:** Allows adaptation to changing metabolic demands
- **Control Level:** Primarily at the transcriptional level

Characteristics of Enzyme Induction:

- Results in increased enzyme concentration
- Often involves regulatory proteins that bind to gene promoters
- Can be induced by substrates, hormones, or environmental factors
- Provides long-term metabolic adaptation

🔹 ENZYME REPRESSION

- **Definition:** Decrease in enzyme synthesis when the enzyme product is abundant
- **Mechanism:** Reduced transcription of genes encoding the enzyme
- **Feedback Control:** End product acts as a corepressor

- **Metabolic Logic:** Prevents wasteful synthesis of enzymes when their products are readily available

Characteristics of Enzyme Repression:

- Results in decreased enzyme concentration
- Often involves repressor proteins
- Provides metabolic economy
- Complementary to enzyme induction

🔹 ALLOSTERIC ENZYME REGULATION

Allosteric enzymes are regulatory enzymes that can exist in multiple conformational states with different catalytic activities.

Key Features of Allosteric Enzymes:

- Possess allosteric sites separate from the active site
- Show sigmoidal (S-shaped) kinetic curves rather than hyperbolic Michaelis-Menten curves
- Often exist as oligomers (multiple subunits)
- Play key roles in metabolic pathway regulation

Types of Allosteric Regulation:

- **Positive Allosterism:** Binding of effector increases enzyme activity
- **Negative Allosterism:** Binding of effector decreases enzyme activity
- **Homotropic Effects:** Substrate binding affects subsequent substrate binding (cooperativity)

- **Heterotropic Effects:** Binding of molecules other than substrate affects activity

Cooperative Binding:

- **Positive Cooperativity:** Binding of one substrate molecule increases affinity for subsequent binding
- **Negative Cooperativity:** Binding of one substrate molecule decreases affinity for subsequent binding
- **Hill Coefficient:** Quantifies the degree of cooperativity

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THERAPEUTIC AND DIAGNOSTIC APPLICATIONS

Enzymes have revolutionized both therapeutic interventions and diagnostic procedures in medicine. Their specificity and efficiency make them valuable tools in healthcare.

THERAPEUTIC APPLICATIONS OF ENZYMES

Enzyme Replacement Therapy:

- Treatment of genetic enzyme deficiencies
- Administration of purified enzymes to supplement missing or defective enzymes
- Challenges include enzyme stability, delivery, and immunogenicity

Therapeutic Uses:

- **Digestive Disorders:** Pancreatic enzymes for pancreatic insufficiency

- **Blood Clot Dissolution:** Streptokinase and tissue plasminogen activator (tPA)
- **Wound Healing:** Collagenase for debridement
- **Cancer Treatment:** L-asparaginase for acute lymphoblastic leukemia
- **Anti-inflammatory:** Hyaluronidase to enhance drug penetration

🔹 DIAGNOSTIC APPLICATIONS OF ENZYMES

Clinical Enzyme Assays:

- Measurement of enzyme activity or concentration in biological samples
- Provides information about tissue damage, metabolic status, or disease progression

Commonly Measured Enzymes:

- **Cardiac Markers:** Creatine kinase-MB (CK-MB), troponins for myocardial infarction
- **Liver Function:** Alanine aminotransferase (ALT), aspartate aminotransferase (AST)
- **Bone Metabolism:** Alkaline phosphatase (ALP)
- **Pancreatic Function:** Amylase and lipase
- **Prostate Health:** Prostate-specific antigen (PSA)

🔹 ISOENZYMES IN DIAGNOSIS

Isoenzymes are different molecular forms of the same enzyme that catalyze the same reaction but have different physical, chemical, or

immunological properties.

Characteristics of Isoenzymes:

- Same catalytic function but different structure
- Different tissue distribution
- Different kinetic properties
- Useful for tissue-specific diagnosis

Diagnostic Significance:

- **Tissue-Specific Markers:** Different isoenzymes are predominant in different tissues
- **Disease Localization:** Helps identify the source of tissue damage
- **Prognosis:** Changes in isoenzyme patterns can indicate disease progression

Enzyme	Isoenzymes	Primary Location	Clinical Significance
Lactate Dehydrogenase	LDH1-LDH5	Heart, Liver, Muscle	Myocardial infarction, liver disease
Creatine Kinase	CK-MM, CK-MB, CK-BB	Muscle, Heart, Brain	Heart attack, muscle disorders
Alkaline Phosphatase	Liver, Bone, Intestinal	Liver, Bone, Intestine	Liver disease, bone disorders

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COENZYMES 🔗

Coenzymes are organic, non-protein molecules that are essential for the catalytic activity of many enzymes. They act as cofactors, assisting enzymes in carrying out their biological functions.

🔹 DEFINITION AND CHARACTERISTICS

Coenzymes are small organic molecules that:

- Bind temporarily to enzymes during catalysis
- Are essential for enzyme activity
- Often derived from vitamins
- Participate directly in the chemical reaction
- Are regenerated and can be reused

Distinction from Prosthetic Groups:

- **Coenzymes:** Loosely bound, can dissociate from enzyme
- **Prosthetic Groups:** Tightly bound, permanent part of enzyme structure

🔹 CLASSIFICATION OF COENZYMES

Based on Binding:

- **Loosely Bound:** Can be separated from enzyme relatively easily
- **Tightly Bound:** Require harsh conditions for separation

Based on Function:

- **Oxidation-Reduction:** Involved in electron transfer reactions
- **Group Transfer:** Facilitate transfer of functional groups
- **Rearrangement:** Assist in molecular rearrangements

🔹 MAJOR COENZYMES AND THEIR FUNCTIONS

Coenzyme	Vitamin Source	Primary Function	Key Reactions
NAD+/NADH	Niacin (B3)	Electron transfer	Glycolysis, TCA cycle
FAD/FADH2	Riboflavin (B2)	Electron transfer	β-oxidation, TCA cycle
Coenzyme A	Pantothenic acid (B5)	Acyl group transfer	Fatty acid metabolism
TPP	Thiamine (B1)	Aldehyde group transfer	Decarboxylation reactions
Biotin	Biotin (B7)	Carboxyl group transfer	Carboxylation reactions

🔹 STRUCTURE AND BIOCHEMICAL FUNCTIONS

NAD+ (Nicotinamide Adenine Dinucleotide):

- **Structure:** Contains nicotinamide ring, ribose sugar, phosphate groups, and adenine
- **Function:** Primary electron acceptor in catabolic pathways
- **Mechanism:** Reversible reduction to NADH + H+
- **Metabolic Role:** Essential for glycolysis, TCA cycle, and electron transport

FAD (Flavin Adenine Dinucleotide):

- **Structure:** Contains isoalloxazine ring system, ribose, phosphate, and adenine
- **Function:** Electron acceptor, particularly in oxidative reactions
- **Mechanism:** Reversible reduction to FADH2
- **Metabolic Role:** β-oxidation, TCA cycle, amino acid catabolism

Coenzyme A (CoA-SH):

- **Structure:** Contains adenine, ribose, phosphate groups, and pantothenic acid derivative
- **Function:** Acyl group carrier and activator
- **Mechanism:** Forms thioester bonds with carboxylic acids
- **Metabolic Role:** Central to fatty acid synthesis and oxidation, TCA cycle

Thiamine Pyrophosphate (TPP):

- **Structure:** Thiamine molecule with two phosphate groups
- **Function:** Aldehyde group transfer and decarboxylation
- **Mechanism:** Forms carbanion intermediates
- **Metabolic Role:** Pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase

Biotin:

- **Structure:** Imidazolidine and tetrahydrothiophene rings
- **Function:** CO2 carrier in carboxylation reactions
- **Mechanism:** Forms carboxybiotin intermediate

- **Metabolic Role:** Fatty acid synthesis, gluconeogenesis

🔹 COENZYME RECYCLING

- Coenzymes must be continuously recycled between their oxidized and reduced forms
- This recycling is essential for maintaining metabolic flux
- Disruption of coenzyme recycling can lead to metabolic dysfunction
- Some coenzymes require specific regeneration pathways

🔹 CLINICAL SIGNIFICANCE

- **Vitamin Deficiencies:** Lead to coenzyme deficiencies and metabolic disorders
- **Drug Interactions:** Some drugs can interfere with coenzyme function
- **Therapeutic Applications:** Coenzyme supplementation in certain disease states
- **Diagnostic Markers:** Coenzyme levels can indicate nutritional or metabolic status