

Enzymology: General Concepts and Enzyme Kinetics

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Topics: Classification, Co-enzymes

(No figures, clean text-only style.)

Classification of Enzymes

Enzymes are classified by the **International Union of Biochemistry (IUB)** into **six major classes** based on the reactions they catalyze.

1. Oxidoreductases

- Catalyze **oxidation–reduction** reactions.
 - Transfer of electrons or hydrogen atoms.
 - Examples: **Dehydrogenases, oxidases, reductases.**
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2. Transferases

- Transfer **functional groups** (methyl, amino, phosphate).
 - Examples: **Transaminases, kinases, methyltransferases.**
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3. Hydrolases

- Catalyze **hydrolysis** of bonds (using water).
 - Examples: **Proteases, lipases, amylases, phosphatases.**
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4. Lyases

- Break bonds **without hydrolysis or oxidation**, forming double bonds.
 - Examples: **Decarboxylases, aldolases.**
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5. Isomerases

- Catalyze **intramolecular rearrangements**.
 - Examples: **Racemases, epimerases, mutases**.
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6. Ligases (Synthetases)

- Join two molecules together using **ATP**.
 - Examples: **Carboxylase, DNA ligase**.
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Mnemonic

O-T-H-L-I-L ? *"Only Tigers Hunt Lions In Laos"*

Additional Enzyme Classification Concepts

Based on Composition

- **Simple enzymes** ? made of protein only.
 - **Conjugated enzymes** ? protein (apoenzyme) + non-protein part (cofactor).
- Apoenzyme + cofactor ? **holoenzyme**.

Based on Location

- **Intracellular enzymes** ? metabolic enzymes.
- **Extracellular enzymes** ? digestive enzymes (amylase, lipase).

Based on Reaction Rate

- **Constitutive enzymes** ? always present.
 - **Inducible enzymes** ? upregulated when substrate appears (e.g., β -galactosidase).
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Co-enzymes

Co-enzymes are **organic, non-protein molecules** required by some enzymes for catalytic activity.

Most are derived from **vitamins**.

1. Coenzymes Derived from B-Complex Vitamins

- **NAD⁺ / NADP⁺ (from Niacin)**

- Participate in **oxidation–reduction** reactions.
- Accept **hydride ions (H⁻)**.
- Used by dehydrogenases (e.g., lactate dehydrogenase).

- **FAD / FMN (from Riboflavin)**

- Accept **two hydrogens** in redox reactions.
- Cofactor for **succinate dehydrogenase**.

- **Coenzyme A (from Pantothenic Acid)**

- Carries **acyl groups**.
- Essential for **fatty acid oxidation**, Krebs cycle, cholesterol synthesis.

- **Pyridoxal Phosphate – PLP (from Vitamin B₆)**

- Coenzyme for **transamination, decarboxylation, deamination**.
- Used by aminotransferases (ALT, AST).

- **Biotin (Vitamin B₇)**

- Cofactor for **carboxylation** reactions.
- Enzymes: **pyruvate carboxylase, acetyl-CoA carboxylase**.

- **Tetrahydrofolate – THF (from Folate)**

- Transfers **one-carbon units** (methyl, formyl).
- Essential for **nucleotide synthesis**.

- **Cobalamin Coenzymes (Vitamin B₁₂)**

- Required for **methionine synthase** and **methylmalonyl-CoA mutase**.
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- **Thiamine Pyrophosphate – TPP (from Vitamin B₁)**

- Coenzyme in **oxidative decarboxylation** (PDH, α -ketoglutarate dehydrogenase).
 - Also for **transketolase** in HMP shunt.
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2. Non-Vitamin Coenzymes

- **Coenzyme Q (Ubiquinone)**

- Electron carrier in the electron transport chain.

- **Heme**

- Cofactor in cytochromes & peroxidases.

- **Lipoic Acid**

- Coenzyme for **pyruvate dehydrogenase** and α -ketoglutarate dehydrogenase.
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Enzyme Prosthetic Groups vs Coenzymes

- **Coenzymes** ? loosely bound, dissociable.
 - **Prosthetic groups** ? tightly or covalently attached.
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Clinically Important Points

- B-complex vitamin deficiency ? enzyme dysfunction ? metabolic disorders.
- ALT/AST require **PLP**; deficiency ? defective amino-acid metabolism.
- B₁ deficiency ? PDH dysfunction ? lactic acidosis.

Mode of Action of Enzymes

1. Lowering Activation Energy

- Enzymes speed up reactions by lowering **activation energy (E_a)**.
- They do NOT change **ΔG (free energy)** or **equilibrium constant**.

2. Formation of Enzyme–Substrate (ES) Complex

- Substrate binds to the enzyme's **active site** → forms ES complex.
- ES complex stabilizes the transition state → faster product formation.

3. Models of Enzyme Action

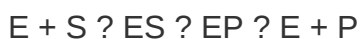
• Lock and Key Model

- Active site is rigid and fits substrate exactly.

• Induced Fit Model

- Active site is flexible; binding induces conformational change.
- More accurate for most enzymes.

4. Reaction Pathway



- Enzyme remains unchanged after reaction.

Active Center (Active Site)

Definition

- The region of the enzyme where **substrate binding** and **catalysis** occur.

Characteristics

- Occupies only a **small portion** of the enzyme.
- Formed by **specific amino acids** (Ser, His, Asp, Cys, Lys, Glu).
- 3D orientation determines specificity.

Types of Active Site Residues

- **Binding residues** → hold the substrate.
- **Catalytic residues** → perform bond-breaking/bond-making.

Microenvironment

- Hydrophobic pocket
- Correct orientation for catalysis
- Stabilizes transition state

Substrate Specificity

- Absolute (urease acts only on urea)
- Group-specific (hexokinase phosphorylates many hexoses)
- Stereo-specific (L-amino acid oxidase)

Enzyme Kinetics (Michaelis–Menten)

Basic Equation

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

Definitions

- **v** = reaction velocity
- **V_{max}** = maximum velocity when enzyme is saturated
- **K_m** = substrate concentration at $\frac{1}{2} V_{\max}$

Assumptions

- ES complex formation is reversible.
- Steady-state concentration of ES.
- Substrate $\gg$ enzyme concentration.

Interpretation

- At **low [S]** ? reaction first-order (rate $\propto$ [S]).
- At **high [S]** ? reaction zero-order (rate independent of [S]).
- V_{max} depends on **enzyme concentration**.
- K_m is independent of enzyme concentration.

Michaelis Constant (K_m)

Definition

- K_m is the substrate concentration at which the reaction velocity is **half of V_{max}** .

Significance

- Measures **enzyme affinity** for substrate.
 - **Low K_m** ? high affinity ? enzyme saturates quickly
 - **High K_m** ? low affinity
- GIVES a quantitative measure of how strongly an enzyme binds its substrate.

Clinical Uses

- Hexokinase has **low K_m** ? high affinity ? active even at low glucose.
- Glucokinase has **higher K_m** ? active only after meals ? prevents hypoglycemia.
- Useful in diagnosing genetic enzyme defects.

Enzyme Activation

1. Zymogen Activation

- Enzymes synthesized in **inactive precursor** forms (zymogens).
- Activated by **proteolytic cleavage**.

Examples:

- Pepsinogen ? pepsin
- Trypsinogen ? trypsin

2. Allosteric Activation

- Activator binds to **allosteric site** ? increases enzyme activity.

Example:

- ATP activates phosphofructokinase-1 (PFK-1) in glycolysis (at high energy states).

3. Covalent Modification

- **Phosphorylation/dephosphorylation** alters enzyme activity.

Examples:

- Glycogen phosphorylase active when phosphorylated.

- Acetyl-CoA carboxylase active when dephosphorylated.

4. Metal Ion Activation

- Some enzymes require metal ions as activators.

Examples:

- Mg^{2+} ? kinases
- Zn^{2+} ? carbonic anhydrase
- Ca^{2+} ? clotting enzymes

5. pH and Temperature Activation

- Each enzyme has optimum pH & temperature.
- Small changes can enhance activity until denaturation occurs.

Competitive Inhibition

Definition

- In competitive inhibition, the **inhibitor resembles the substrate** and **competes for the active site** of the enzyme.

Key Mechanism

- Inhibitor binds **only to the active site** of free enzyme (E).
- Prevents ES complex formation.

Reversibility

- Reversible by **increasing substrate concentration**.

Effect on Kinetics

- **V_{max} ? unchanged**
- **K_m ? increased** (lower affinity, more substrate needed)
- **Lineweaver–Burk Plot:**

- Lines intersect on the **y-axis** (same V_{max}).
- Slope increases.

Examples

- **Malonate** inhibits succinate dehydrogenase.
- **Statins** competitively inhibit HMG-CoA reductase.
- **Methotrexate** inhibits dihydrofolate reductase.

Clinical Relevance

- Increasing substrate (e.g., high-dose folate) can overcome methotrexate toxicity.

Noncompetitive Inhibition

Definition

- Inhibitor binds to a **site other than the active site** (allosteric site).
- Binding distorts enzyme conformation ? reduces activity.

Key Mechanism

- Inhibitor can bind to **E or ES** complex.
- Does **not compete** with substrate.

Reversibility

- Cannot be reversed by increasing substrate concentration.

Effect on Kinetics

- **V_{max}** ? *decreased*
- **K_m** ? *unchanged* (affinity same, but active enzyme molecules fewer)
- **Lineweaver–Burk Plot:**
 - Lines intersect on the **x-axis** (same K_m).
 - Slope increases, y-intercept increases.

Examples

- **Cyanide** inhibits cytochrome oxidase.
- **Heavy metals** (Hg^{2+} , Ag^+) inhibit SH-containing enzymes.
- **Alanine** noncompetitively inhibits pyruvate kinase.

Clinical Relevance

- Removal of inhibitor or chelation of metal ions can restore activity (e.g., BAL for arsenic poisoning).

Allosteric Inhibition

Definition

- Allosteric inhibition occurs when an inhibitor binds to an **allosteric (regulatory) site**, not the active site.
- Binding causes a **conformational change** → decreased enzyme activity.

Characteristics

- Does **not resemble the substrate**.
- Can act rapidly and reversibly.
- Often occurs in **regulatory enzymes** of metabolic pathways.
- Shows **sigmoidal (S-shaped) kinetics**, not Michaelis–Menten.

Example

- ATP inhibits **phosphofructokinase-1 (PFK-1)** in glycolysis.
- CTP inhibits **aspartate transcarbamoylase**.

Key (Regulatory) Enzymes

Definition

- Enzymes that catalyze **rate-limiting steps** of metabolic pathways.

Properties

- Usually **allosteric enzymes**.
- Irreversible, early in the pathway.
- Highly regulated by activators/inhibitors.

Important Examples

- **PFK-1** – rate-limiting enzyme of glycolysis
 - **Glutamate dehydrogenase** – amino-acid metabolism
 - **HMG-CoA reductase** – cholesterol synthesis
 - **Glycogen phosphorylase** – glycogen breakdown
 - **Carbamoyl phosphate synthetase I** – urea cycle
 - **Acetyl-CoA carboxylase** – fatty acid synthesis
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Feedback Inhibition

Definition

- End-product of a metabolic pathway **inhibits the first committed step** ? prevents overproduction.

Mechanism

- End-product binds to an **allosteric site** of the initial enzyme.
- Reduces enzyme activity by conformational change.

Importance

- Maintains metabolic balance.
- Prevents waste of energy and substrates.
- Quick and reversible control mechanism.

Examples

- **Isoleucine inhibits threonine dehydratase.**
 - **Cholesterol inhibits HMG-CoA reductase.**
 - **ATP inhibits PFK-1.**
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Uncompetitive Inhibition

Definition

- Inhibitor binds **only to the ES complex**, not to free enzyme.
- Prevents formation of product ? ES becomes ESI (inactive).

Effect on Kinetics

- **V_{max}** ? decreased
- **K_m** ? decreased

(Because inhibitor locks ES complex, making enzyme appear to have higher affinity)

Reversibility

- Cannot be reversed by increasing substrate concentration.

Lineweaver–Burk Characteristics

- Lines are **parallel** (same slope).
- Y-intercept increases; x-intercept shifts.

Example

- Lithium inhibits **inositol monophosphatase** uncompetitively.

Lineweaver–Burk Plot

Purpose

- Double reciprocal plot used to determine **K_m** and **V_{max}** and to differentiate types of inhibition.

Equation

$$1/v = (K_m/V_{max}) \times (1/[S]) + 1/V_{max}$$

- Straight line where:
 - **Y-intercept** = $1/V_{max}$
 - **X-intercept** = $-1/K_m$

– **Slope** = K_m/V_{max}

Interpretation in Inhibition

1. Competitive Inhibition

- V_{max} same
- K_m increases
- Lines intersect at **y-axis**

2. Noncompetitive Inhibition

- V_{max} decreases
- K_m unchanged
- Lines intersect at **x-axis**

3. Uncompetitive Inhibition

- V_{max} decreases
- K_m decreases
- Lines are **parallel**

Advantages

- Easy comparison of inhibition patterns.

Disadvantages

- Distorts error at low substrate concentrations; Eadie–Hofstee plot is more accurate.

Covalent Modification

Definition

- Regulation of enzyme activity through **reversible covalent addition or removal** of a chemical group.

Most Common: Phosphorylation / Dephosphorylation

- **Kinases** add phosphate (ATP → ADP).
- **Phosphatases** remove phosphate.

Effects

- Can **activate or inhibit** depending on the enzyme.

Examples

- **Glycogen phosphorylase** ? active when phosphorylated.
- **Glycogen synthase** ? inactive when phosphorylated.
- **Acetyl-CoA carboxylase** ? active when dephosphorylated.

Other Covalent Modifications

- Adenylation
- Methylation
- ADP-ribosylation
- Ubiquitination (? marks proteins for degradation)

Repression

Definition

- Long-term regulation where synthesis of an enzyme is **suppressed at the gene level** when its product is abundant.

Characteristics

- Slower, affects **amount** of enzyme, not immediate activity.
- Seen in bacteria and human metabolic pathways.

Example

- High cholesterol represses **HMG-CoA reductase** gene expression.

Induction

Definition

- Increased **gene expression** ? increased enzyme synthesis in response to a metabolite or drug.

Examples

- High carbohydrate diet induces **glucokinase**.
- Barbiturates induce **cytochrome P450** enzymes.
- Lactose induces **β -galactosidase** in bacteria.

Importance

- Allows metabolic adaptation to environmental or dietary conditions.

Factors Affecting Enzyme Activity

1. Temperature

- Activity increases with temperature up to optimum ($\sim 37^{\circ}\text{C}$).
- High temperature $\rightarrow$ denaturation.

2. pH

- Each enzyme has an **optimum pH**.
- Extreme pH $\rightarrow$ denatures enzyme.

3. Substrate Concentration

- Activity increases until **V_{max}** is reached (enzyme saturation).
- Follows the Michaelis–Menten curve.

4. Enzyme Concentration

- Rate $\propto$ enzyme concentration (when substrate is in excess).

5. Product Concentration

- Accumulation of product slows reaction (product inhibition).

6. Activators

- Metal ions (Mg^{2+} , Zn^{2+} , Ca^{2+}) often essential.
- Example: kinases need **Mg^{2+}** .

7. Inhibitors

- Competitive, noncompetitive, uncompetitive, allosteric inhibitors decrease activity.

Isoenzymes (Isozymes)

Definition

- Different molecular forms of the **same enzyme** that catalyze the same reaction but differ in structure, kinetics, and tissue distribution.

Clinical Significance

- Useful in diagnosing **tissue damage** because each isoenzyme is tissue-specific.

Lactate Dehydrogenase (LDH) Isoenzymes

LDH has **five isoenzymes** (tetramers of H and M subunits):

1. **LDH-1 (H₄)** – Heart, RBC
2. **LDH-2 (H₃M₁)** – Reticuloendothelial system
3. **LDH-3 (H₂M₂)** – Lungs
4. **LDH-4 (H₁M₃)** – Kidneys, pancreas
5. **LDH-5 (M₄)** – Liver, skeletal muscle

Clinical Patterns

- **MI (heart attack)** ? LDH-1 ? above LDH-2 (flipped pattern).
- **Liver disease / muscle injury** ? LDH-5 ?.
- **Hemolysis** ? LDH-1 ? (released from RBCs).

Creatine Kinase (CK) Isoenzymes

CK exists in **three isoforms**:

1. **CK-BB (CK-1)**

- Brain, smooth muscle
- Increased in CNS injury

2. **CK-MB (CK-2)**

- Heart muscle
- **Most specific marker for myocardial infarction**
- Rises 4–6 hours after MI, peaks at 24 hours, normal in 48 hours

3. **CK-MM (CK-3)**

- Skeletal muscle
- Increased in muscular dystrophy, rhabdomyolysis, trauma

Clinical Use

- LDH isoenzymes ? differentiate liver, heart, lung, muscle diseases.
- CK-MB ? early diagnosis of **acute myocardial infarction**.
- CK-BB ? stroke, CNS tumors.
- CK-MM ? muscle injury.

Specificity of Enzymes

Enzymes show **high specificity** toward substrates and reactions.

1. Absolute Specificity

- Enzyme acts only on **one substrate**.
- Example: **Urease** ? only urea.

2. Group Specificity

- Acts on substrates with **similar functional groups**.
- Example: **Hexokinase** ? phosphorylates many hexoses.

3. Bond Specificity

- Acts only on a particular type of **bond**.
- Example: **Esterases** ? hydrolyze ester bonds.

4. Stereospecificity

- Distinguish between **D- and L-forms**.
- Example: **L-amino acid oxidase**, **D-lactate dehydrogenase**.

5. Reaction Specificity

- One type of chemical transformation only.
- Example: **Oxidoreductases** ? redox reactions only.

Enzyme Engineering

Definition

Modification of enzymes through biochemical, genetic, or structural changes to improve function.

Methods

- **Site-directed mutagenesis** ? change specific amino acids.
- **Directed evolution** ? repeated mutation + selection.
- **Fusion proteins** ? catalytic domain + tag (His-tag).

Applications

- Improved stability (heat-stable enzymes).
 - Reduced inhibition.
 - Faster industrial biocatalysis (detergent enzymes).
 - Design of **insulin analogs**, engineered proteases, and enzyme replacement therapies.
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Enzyme Units

1. International Unit (IU)

- Amount of enzyme that converts **1 micromole of substrate per minute** under defined conditions.

2. Katal

- SI unit.
- Amount converting **1 mole of substrate per second**.
- (1 katal = 60,000 IU)

3. Specific Activity

- Units of enzyme **per mg of protein**.
- Indicates enzyme **purity**.

4. Turnover Number (kcat)

- Number of substrate molecules converted to product **per enzyme molecule per second**.

Isoenzymes (Isozymes)

(Already partly covered earlier, expanded here)

Definition

Different molecular forms of the **same enzyme** with:

- same catalytic action,
- different amino acid sequences,
- different tissue distribution.

Example Families

- **LDH** (LDH-1 ? LDH-5)
- **Creatine Kinase** (CK-BB, CK-MB, CK-MM)
- **Alkaline phosphatase (ALP)** isoenzymes – liver, bone, placenta
- **Amylase** – pancreatic vs salivary

Diagnostic Enzymes (Clinical Enzymology)

Enzymes used as **biomarkers** for tissue injury.

1. Cardiac Enzymes

- **CK-MB** ? MI (rises 4–6 h, normal in 48 h)
- **LDH-1** ? MI (LDH1 > LDH2 = flipped pattern)
- **Troponin** (not an enzyme but key marker)

2. Liver Enzymes

- **ALT (SGPT)** ? hepatocellular damage
- **AST (SGOT)** ? liver & muscle
- **ALP** ? cholestasis, bone disease
- **GGT** ? alcoholism, biliary obstruction

3. Pancreatic Enzymes

- **Amylase**
- **Lipase** ? more specific for acute pancreatitis

4. Muscle Enzymes

- **CK-MM** ? muscle injury, rhabdomyolysis
- **Aldolase** ? muscle diseases

5. Bone/Placenta

- **Bone ALP** ? rickets, Paget disease
- **Placental ALP** ? pregnancy, germ cell tumors

Isoenzyme Electrophoresis

Definition

Separation of isoenzymes based on differences in **charge, mobility, and size**.

Methods

- Agarose gel electrophoresis
- Cellulose acetate electrophoresis
- Isoelectric focusing

LDH Example

LDH isoforms migrate differently:

- LDH-1 (H₄) ? fastest, most negative, moves furthest
- LDH-5 (M₄) ? slowest, least negative

Clinical Uses

- Diagnosing **myocardial infarction** (LDH₁ > LDH₂).
- Differentiating **liver vs bone ALP**.
- Identifying cancer-related isoenzyme patterns.
- Confirming **salivary vs pancreatic** amylase.

Summary (Exam-Ready One-Liners)

- Enzymes show **absolute, group, stereo, bond** specificity.
- Enzyme engineering modifies catalytic efficiency and stability.
- IU = amount of enzyme converting **1 ?mol/min**.
- Isoenzymes differ in structure but catalyze **same reaction**.
- LDH-1 elevation ? myocardial infarction.
- CK-MB ? most specific enzymatic marker for MI.
- ALP high with GGT normal ? **bone disease**.
- Electrophoresis separates isoenzymes based on charge differences.

Frequently Asked Questions (FAQs)

1. What determines enzyme specificity?

The **3D structure of the active site**, which recognizes the substrate based on shape, charge, and stereochemistry.

2. What is absolute specificity?

Enzyme acts on **only one substrate**. Example: **Urease ? Urea**.

3. What is group specificity?

Enzyme acts on substrates with **similar functional groups** (e.g., hexokinase).

4. What is the purpose of enzyme engineering?

To improve enzyme **stability, activity, or specificity** using genetic or chemical modifications.

5. What is site-directed mutagenesis?

A technique to modify **specific amino acids** in an enzyme to alter function.

6. What is an international unit (IU) of enzyme?

Amount of enzyme that converts **1 micromole of substrate per minute**.

7. What is a Katal?

SI unit of enzyme activity = **1 mole of product per second**.

8. What is specific activity?

Units of enzyme per **mg of protein**—an indicator of enzyme **purity**.

9. What is an isoenzyme?

Different molecular forms of an enzyme, with same function but **different structure and tissue distribution**.

10. Why are isoenzymes clinically important?

They help identify **which tissue is damaged** during disease.

11. Which LDH isoenzyme indicates myocardial infarction?

LDH-1 > LDH-2 ("flipped pattern").

12. Which CK isoenzyme is specific for heart muscle?

CK-MB.

13. When does CK-MB rise after an MI?

Rises at **4–6 hours**, peaks at 24 hours, normal in 48 hours.

14. Which enzymes rise in acute pancreatitis?

Amylase and lipase, with lipase being more specific.

15. What enzyme pattern suggests liver cell damage?

? **ALT**, ? **AST** (AST may rise higher in alcohol-related damage).

16. What enzyme pattern suggests biliary obstruction?

? **ALP** and ? **GGT**.

17. What does increased bone ALP indicate?

Rickets, osteomalacia, Paget disease.

18. Which isoenzyme increases in skeletal muscle injury?

CK-MM.

19. How are isoenzymes separated?

By **electrophoresis** (agarose gel, cellulose acetate) or **isoelectric focusing**.

20. Which amylase isoenzyme rises in acute pancreatitis?

Pancreatic amylase.

21. What is repression in enzyme regulation?

Reduction in **enzyme synthesis at gene level** when product is abundant.

22. What is induction?

Increased **enzyme synthesis** in response to a substrate, hormone, or drug.

23. Give an example of an induced enzyme.

Cytochrome P450 enzymes induced by **barbiturates**.

24. Which metal ion activates most kinases?

Mg²⁺.

25. What is the advantage of isoenzyme electrophoresis?

It distinguishes tissue-specific enzyme forms, aiding **diagnosis** (heart vs liver vs bone pathology).

MCQs

1. Absolute specificity is seen in which enzyme?

- A. Hexokinase
- B. Trypsin
- C. Urease
- D. Lipase

Answer: C

2. Hexokinase shows which type of specificity?

- A. Absolute
- B. Group
- C. Bond
- D. Reaction

Answer: B

3. Stereospecificity is shown by:

- A. Pepsin
- B. D-amino acid oxidase
- C. Amylase
- D. Catalase

Answer: B

4. Site-directed mutagenesis is used in:

- A. Enzyme repression
- B. Enzyme engineering
- C. Feedback inhibition
- D. Zymogen activation

Answer: B

5. International Unit of enzyme activity means:

- A. 1 mmol/min
- B. 1 μ mol/min
- C. 1 mol/sec
- D. 1 μ mol/sec

Answer: B

6. Specific activity indicates:

- A. Purity of enzyme
- B. pH of enzyme
- C. Amount of substrate
- D. Temperature stability

Answer: A

7. Which isoenzyme is elevated in myocardial infarction?

- A. LDH-5
- B. LDH-3
- C. LDH-1
- D. LDH-4

Answer: C

8. LDH-1 > LDH-2 pattern is called:

- A. Forward pattern
- B. Flipped pattern
- C. Reverse pattern
- D. Saturation pattern

Answer: B

9. CK-MB is a marker for:

- A. Liver failure
- B. Acute pancreatitis
- C. Skeletal muscle injury
- D. Myocardial infarction

Answer: D

10. Which enzyme rises earliest after MI?

- A. LDH
- B. CK-MB
- C. Troponin I

D. AST

Answer: B

11. Which enzyme rises highest in obstructive jaundice?

A. ALT

B. AST

C. ALP

D. LDH

Answer: C

12. GGT elevation indicates:

A. Acute bone disease

B. Alcoholic liver disease

C. Muscle injury

D. Rickets

Answer: B

13. Which enzyme is most specific for acute pancreatitis?

A. Amylase

B. Trypsin

C. Lipase

D. Elastase

Answer: C

14. Bone ALP is elevated in:

A. Cirrhosis

B. Paget disease

C. Myocardial infarction

D. Cushing syndrome

Answer: B

15. CK-BB is mainly found in:

- A. Heart
- B. Skeletal muscle
- C. Brain
- D. Liver

Answer: C

16. Isoenzymes differ in:

- A. Function
- B. Activation energy
- C. Amino acid sequence
- D. Reaction catalyzed

Answer: C

(Reaction catalyzed is same.)

17. Isoenzymes are best separated by:

- A. Simple centrifugation
- B. Electrophoresis
- C. Precipitation
- D. Dialysis

Answer: B

18. In non-competitive inhibition, which parameter changes?

- A. K_m increases
- B. K_m decreases
- C. V_{max} decreases
- D. V_{max} increases

Answer: C

19. An enzyme showing sigmoidal kinetics is usually:

- A. A simple enzyme
- B. An allosteric enzyme
- C. A hydrolase
- D. A zymogen

Answer: B

20. Feedback inhibition usually acts on:

- A. The last enzyme of the pathway
- B. Any random enzyme
- C. The rate-limiting enzyme
- D. The fastest enzyme

Answer: C

21. An inhibitor that binds only to the ES complex is:

- A. Competitive
- B. Non-competitive
- C. Uncompetitive
- D. Allosteric

Answer: C

22. Which enzyme requires Mg^{2+} for activation?

- A. Pepsin
- B. Kinases
- C. Urease
- D. Lipase

Answer: B

23. Enzyme induction means:

- A. Increase in substrate concentration
- B. Increase in enzyme activity
- C. Increase in enzyme synthesis

D. Decrease in enzyme affinity

Answer: C

24. Cytochrome P450 enzymes are induced by:

A. Vitamin C

B. Barbiturates

C. Insulin

D. Iron deficiency

Answer: B

25. Enzyme repression occurs when:

A. Substrate is in excess

B. Product accumulates

C. Temperature increases

D. pH increases

Answer: B

Viva Voce

1. What is enzyme specificity?

It is the ability of an enzyme to choose a **single substrate or group of substrates** based on its active-site structure.

2. What is absolute specificity?

The enzyme acts on **only one specific substrate**.

Example: Urease ? urea.

3. What is group specificity?

The enzyme catalyzes reactions of substrates with **similar functional groups**.

Example: Hexokinase.

4. What is stereospecificity?

The enzyme distinguishes between **D- and L-forms** of molecules.

5. What is reaction specificity?

The enzyme catalyzes only **one type of chemical reaction**, regardless of substrate variety.

6. What is enzyme engineering?

Modification of enzyme structure by **genetic or chemical methods** to improve activity, stability, or specificity.

7. Give an example of enzyme engineering.

Site-directed mutagenesis to create **heat-stable enzymes**.

8. What is site-directed mutagenesis?

Technique to change a **specific amino acid** in a protein to alter its function.

9. What is an International Unit (IU)?

Amount of enzyme that converts **1 μmol of substrate per minute**.

10. What is a Katal?

SI unit of enzyme activity (1 mole of substrate converted per second).

11. What is specific activity?

Enzyme units **per mg of protein**—indicator of enzyme purity.

12. What is turnover number (kcat)?

The number of **substrate molecules converted per enzyme molecule per second**.

13. What are isoenzymes?

Different molecular forms of the **same enzyme** with different structures but identical catalytic function.

14. Why are isoenzymes important clinically?

They help identify **which tissue is damaged**, since each isoenzyme is tissue-specific.

15. Which LDH isoenzyme rises in myocardial infarction?

LDH-1, showing the *flipped pattern* (LDH-1 > LDH-2).

16. Which CK isoenzyme is specific for cardiac muscle?

CK-MB.

17. When does CK-MB appear after MI?

Rises in 4–6 hours, peaks at 24 hours, normal after 48 hours.

18. Which enzyme is most specific for acute pancreatitis?

Lipase.

19. Which enzyme rises in cholestasis?

ALP, along with **GGT**.

20. Which ALP isoenzyme rises in bone disease?

Bone ALP.

21. What does elevated CK-MM indicate?

Skeletal muscle injury or rhabdomyolysis.

22. What is enzyme electrophoresis?

A technique to separate isoenzymes based on **charge and mobility**.

23. Which technique gives best isoenzyme separation?

Isoelectric focusing, based on pI differences.

24. What is feedback inhibition?

The end-product inhibits the **rate-limiting enzyme** of its own pathway.

25. What is repression?

Decreased **enzyme synthesis** at the gene level due to excess product.

26. What is induction?

Increased **enzyme synthesis** in response to a metabolite, hormone, or drug.

27. What type of regulation do allosteric enzymes show?

Sigmoidal kinetics and rapid, reversible control.

28. What is the major allosteric inhibitor of PFK-1?

ATP.

29. Which metal ion activates most kinases?

Mg²⁺.

30. What does high GGT with high ALP indicate?

Obstructive or alcoholic liver disease.