Enzymology: General Concepts and Enzyme Kinetics

Enzymology: General Concepts and Enzyme Kinetics

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**.

2. Transferases

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

3. Hydrolases

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

4. Lyases

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

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**.

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., ?-galactosidase).

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 B?)**

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

• **Biotin (Vitamin B?)**

• 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 B??)**

• Required for **methionine synthase** and **methylmalonyl-CoA mutase**.

• **Thiamine Pyrophosphate – TPP (from Vitamin B?)**

• Coenzyme in **oxidative decarboxylation** (PDH, ?-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 **?-ketoglutarate dehydrogenase**.

Enzyme Prosthetic Groups vs Coenzymes

• **Coenzymes** ? loosely bound, dissociable.
• **Prosthetic groups** ? tightly or covalently attached.

Clinically Important Points

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

Mode of Action of Enzymes

1. Lowering Activation Energy

• Enzymes speed up reactions by lowering **activation energy (Ea)**.
• They do NOT change **?G (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

E + S ? ES ? EP ? E + P
• 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
• **Vmax** = maximum velocity when enzyme is saturated
• **Km** = substrate concentration at ½ **Vmax**

Assumptions

• ES complex formation is reversible.
• Steady-state concentration of ES.
• Substrate >> enzyme concentration.

Interpretation

• At **low [S]** ? reaction first-order (rate ? [S]).
• At **high [S]** ? reaction zero-order (rate independent of [S]).
• Vmax depends on **enzyme concentration**.
• Km is independent of enzyme concentration.

Michaelis Constant (Km)

Definition

• Km is the substrate concentration at which the reaction velocity is **half of Vmax**.

Significance

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

Clinical Uses

• Hexokinase has **low Km** ? high affinity ? active even at low glucose.
• Glucokinase has **higher Km** ? 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

• **Vmax** ? *unchanged*
• **Km** ? *increased* (lower affinity, more substrate needed)
• **Lineweaver–Burk Plot:**

– Lines intersect on the **y-axis** (same Vmax).
– 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

• **Vmax** ? *decreased*
• **Km** ? *unchanged* (affinity same, but active enzyme molecules fewer)
• **Lineweaver–Burk Plot:**
– Lines intersect on the **x-axis** (same Km).
– 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

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**.

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

• **Vmax** ? decreased
• **Km** ? 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 **Km** and **Vmax** and to differentiate types of inhibition.

Equation

1/v = (Km/Vmax) × (1/[S]) + 1/Vmax
• Straight line where:
– **Y-intercept** = 1/Vmax
– **X-intercept** = –1/Km

– **Slope** = Km/Vmax

Interpretation in Inhibition

1. Competitive Inhibition
• Vmax same
• Km increases
• Lines intersect at **y-axis**

2. Noncompetitive Inhibition
• Vmax decreases
• Km unchanged
• Lines intersect at **x-axis**

3. Uncompetitive Inhibition
• Vmax decreases
• Km 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 **?-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 (~37°C).
• High temperature ? denaturation.

2. pH

• Each enzyme has an **optimum pH**.
• Extreme pH ? denatures enzyme.

3. Substrate Concentration

• Activity increases until **Vmax** is reached (enzyme saturation).
• Follows the Michaelis–Menten curve.

4. Enzyme Concentration

• Rate ? 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.

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.

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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).

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4. What is the purpose of enzyme engineering?

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

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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**.

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9. What is an isoenzyme?

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

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10. Why are isoenzymes clinically important?

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

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11. Which LDH isoenzyme indicates myocardial infarction?

LDH-1 > LDH-2 (“flipped pattern”).

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12. Which CK isoenzyme is specific for heart muscle?

CK-MB.

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

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

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14. Which enzymes rise in acute pancreatitis?

Amylase and lipase, with lipase being more specific.

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15. What enzyme pattern suggests liver cell damage?

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

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16. What enzyme pattern suggests biliary obstruction?

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

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17. What does increased bone ALP indicate?

Rickets, osteomalacia, Paget disease.

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18. Which isoenzyme increases in skeletal muscle injury?

CK-MM.

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19. How are isoenzymes separated?

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

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20. Which amylase isoenzyme rises in acute pancreatitis?

Pancreatic amylase.

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21. What is repression in enzyme regulation?

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

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22. What is induction?

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

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23. Give an example of an induced enzyme.

Cytochrome P450 enzymes induced by **barbiturates**.

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24. Which metal ion activates most kinases?

Mg^{2}?.

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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

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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

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5. International Unit of enzyme activity means:

A. 1 mmol/min
B. 1 ?mol/min
C. 1 mol/sec
D. 1 ?mol/sec
Answer: B

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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

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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

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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

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15. CK-BB is mainly found in:

A. Heart
B. Skeletal muscle
C. Brain
D. Liver
Answer: C

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16. Isoenzymes differ in:

A. Function
B. Activation energy
C. Amino acid sequence
D. Reaction catalyzed
Answer: C
(Reaction catalyzed is same.)

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17. Isoenzymes are best separated by:

A. Simple centrifugation
B. Electrophoresis
C. Precipitation
D. Dialysis
Answer: B

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

A. Km increases
B. Km decreases
C. Vmax decreases
D. Vmax 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

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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.

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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.

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4. What is stereospecificity?

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

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5. What is reaction specificity?

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

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6. What is enzyme engineering?

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

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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 ?mol 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.

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14. Why are isoenzymes important clinically?

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

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15. Which LDH isoenzyme rises in myocardial infarction?

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

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16. Which CK isoenzyme is specific for cardiac muscle?

CK-MB.

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17. When does CK-MB appear after MI?

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

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18. Which enzyme is most specific for acute pancreatitis?

Lipase.

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19. Which enzyme rises in cholestasis?

ALP, along with **GGT**.

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20. Which ALP isoenzyme rises in bone disease?

Bone ALP.

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21. What does elevated CK-MM indicate?

Skeletal muscle injury or rhabdomyolysis.

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22. What is enzyme electrophoresis?

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

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23. Which technique gives best isoenzyme separation?

Isoelectric focusing, based on pI differences.

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24. What is feedback inhibition?

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

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25. What is repression?

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

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26. What is induction?

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

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27. What type of regulation do allosteric enzymes show?

Sigmoidal kinetics and rapid, reversible control.

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

ATP.

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29. Which metal ion activates most kinases?

Mg^{2}?.

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30. What does high GGT with high ALP indicate?

Obstructive or alcoholic liver disease.