Biochemistry and clinical enzymology

Medical Biochemistry Department

Objectives

- Define enzymes & describe structural components of the enzyme system.
- Discuss enzyme nomenclature, and classification
- Discuss mechanism of enzyme action
- Explain types of enzyme specificity
- Enumerate factors affecting enzyme activity
- Describe kinetics of enzyme reaction

What is an enzyme?

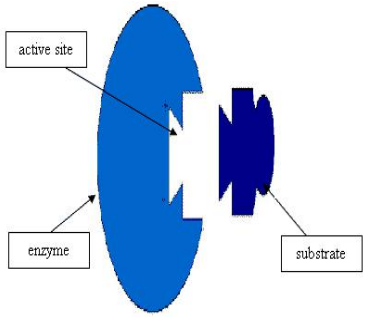
Enzymes are biological catalysts that enable the cell to carryout its chemical activities with maximum speed and efficiency under conditions that are compatible with life.

Substrate: It is a substance or substances (reactants) upon which an enzyme acts, e.g., sucrose is a substrate for sucrase enzyme that converts it into glucose and fructose.

Functional (or active) sites in the enzymes system:

AEnzymesAareAcomposedAofAlongAchainsAofAaminoAacidsAthatA haveAfoldedAintoAaAveryAspecificAthree-dimensionalAshapeAwhichA containsAanAactiveAsites.

a) Substrate binding-site.
b) Catalytic site.
c) Allosteric site.



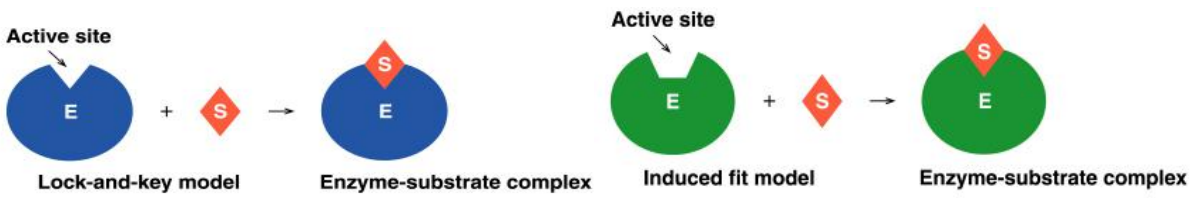
A)Substrate-binding site:

-site at which substrate specifically binds and carries out the chemical action.

-site may be rigid or flexible.

- In the rigid model, they have rigid tertiary structure and do not change their shape after combination with substrate. the lock and key model.

-In the flexible model, the substrate induces a conformational change in the enzyme tertiary structure to fit the substrate. This is described as the induced fitting model.



b) *Catalytic site*:

-It is the site on the enzyme surface that catalyzes the chemical reaction

-It may be separated from the substrate-binding site by a large or a small distant or they may be combined into one site.

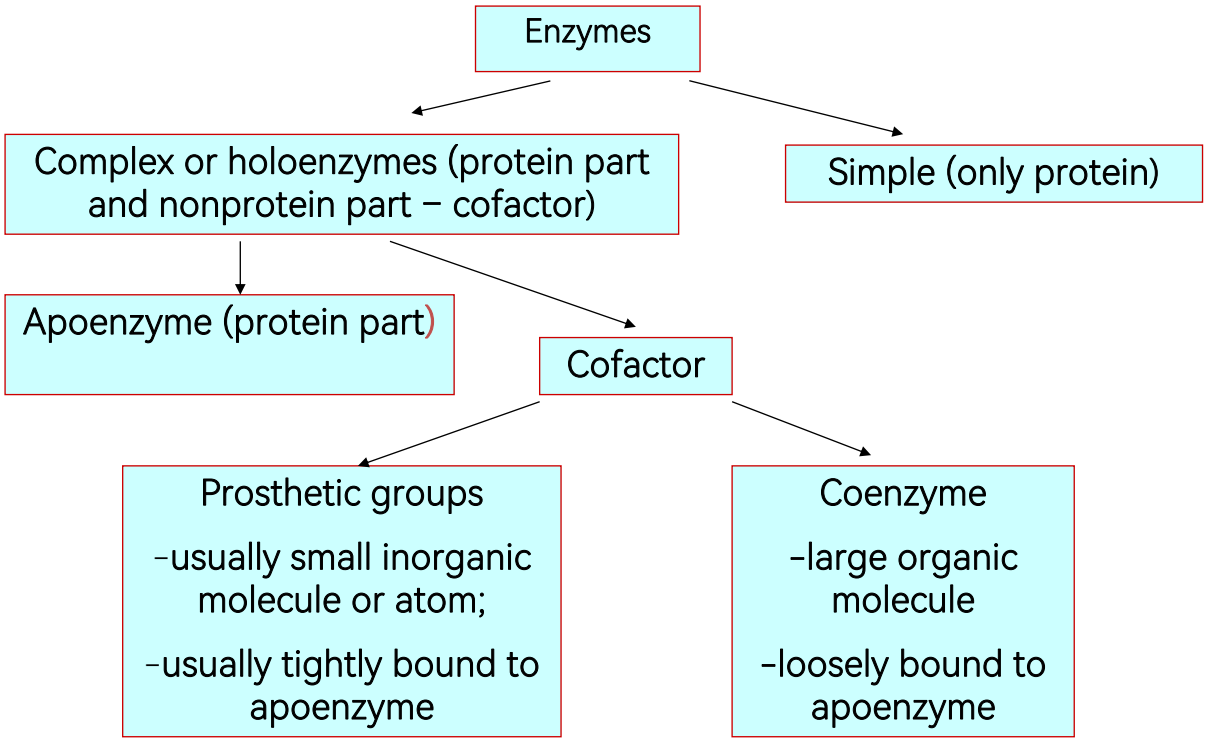
c) Allosteric site:

-The term allosteric means change in shape".

-The allosteric site is usually far from the catalytic site(s)

-*Allosteric effectors* are substances of low molecular weight having little or no structural similarity to substrate.

Structure of enzymes

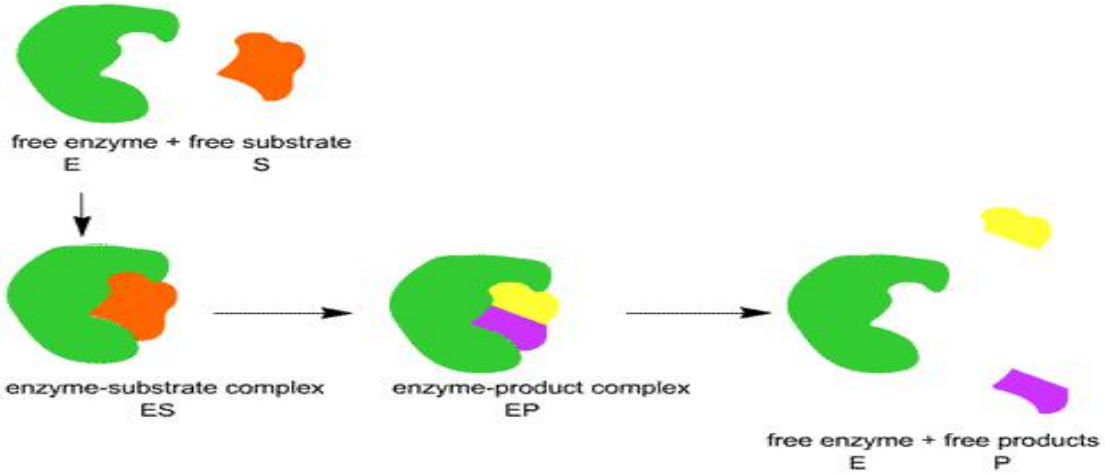


	Apoenzyme	*Coenzyme*	*Prosthetic Group*	*Cofactor*
Nature	Protein	Organic, non-protein	Organic and inorganic	Inorganic
Source	Specific gene	Vitamins or nucleotides	Heme and inorganic metals, Selenium in GSHPX, Cu^{2+} in SOD	Inorganic elements
Examples	All enzymes	NAD, FAD, TPP, ATP, UTP	FMN, iron-Heme	Mg^{2+}, Ca^{2+}, Cu^{2+}, Mn^{2+}
Attachment to the apoenzyme		Loose (non-covalent)	Very tight (covalent)	Loose
Heat stability	labile	Fairly stable	Stable	Very stable
MW	largest	Smaller	Smaller	Smallest
Determine specificity	Yes	No	No	No
Determine chemical nature of the reaction	Yes	Yes	Yes	Yes & NO

Mechanism of enzyme action

- Enzyme-catalyzed reactions have three basic steps:

(1) Binding of substrate (reactant) : E + S → ES
(2) Conversion of bound substrate to bound product: ES ↔ EP
(3) Release of product: EP↔E + P.



Substrate specificity of enzymes

1. Absolute Specificity:

- the enzyme acts on only one substrate
- uricase enzyme acts on uric acid
- arginase enzyme acts on arginine.

2- Dual specificity:

- There are 2 types of dual specificity:
- An enzyme acting on 2 different substrates but catalyzes one type of reaction, e.g., xanthine oxidase acting on hypoxanthine and xanthine causes oxidation of both substrates into uric acid.
- Hypoxanthine Xanthine Uric acid
- An enzyme acting on one substrate but catalyzes 2 different reactions, e.g., isocitrate dehydrogenase acts on isocitrate causing dehydrogenation and decarboxylation into – ketoglutarate.
- Isocitrate + NAD CO_2 + $NADH.H^+$ + -ketoglutarate

3-Stereo-specificity:

- the enzyme is specific to a specific isomer of a substrate and does not act on other isomers.
- L-amino acid oxidase acting on L-AAs only and D-amino acid oxidase acting on D-AAs only.

4- Relative specificity:

- enzyme acts on a group of compounds related to each other in having the same type of bond
- Lipase catalyzes the process of hydrolysis of ester linkage present in triglycerides containing different types of fatty acids.
- Amylase catalyzes the process of hydrolysis of glycosidic linkages present in starch, dextrin or glycogen.

5. Structural specificity: group specificity

- the enzyme is specific to the bond like the relative specificity but it requires chemical groups or atoms around this bond.
- Pepsin hydrolyzes the middle or terminal peptide linkages formed by the amino groups of phenylalanine or tyrosine.
- Trypsin attacks the peptide linkage containing the carboxyl group of arginine or lysine.

Systems of enzyme nomenclature

Substrate-dependent naming:

The name is derived from the substrate with -ase as a suffix, e.g., urease (for urea), lipase (for lipids) and protease (for protein).

Chemical nature of the reaction-dependent naming:

The name depends on nature of chemical reaction with -ase as a suffix, e.g., hydrolase (for hydrolysis) and dehydrogenase (for removal of hydrogen).

Both systems,1&2 are recommended for naming enzymes.

Systemic naming:

The International Biochemical Union have subdivided enzymes into classes, subclasses and sub-subclasses According to this system there are 6 classes of enzymes.

1) The **oxidoreductases** (class 1) catalyze the transfer of reducing equivalents(Hydrogen and electrons)from one redox system to another.

2) The **transferases** (class 2) catalyze the transfer of other groups from one molecule to another. Oxidoreductases and transferases generally require coenzymes

3) The **hydrolases** (class 3) hydrolases cause cleavage of bond using water

4) **Lyases** (class 4, often also referred to as"synthases") catalyze reactions involving either the cleavage or formation of chemical bonds, with double bonds either arising or disappearing.(See figure- reversible reaction is shown). Cleavage of bond does not require water.

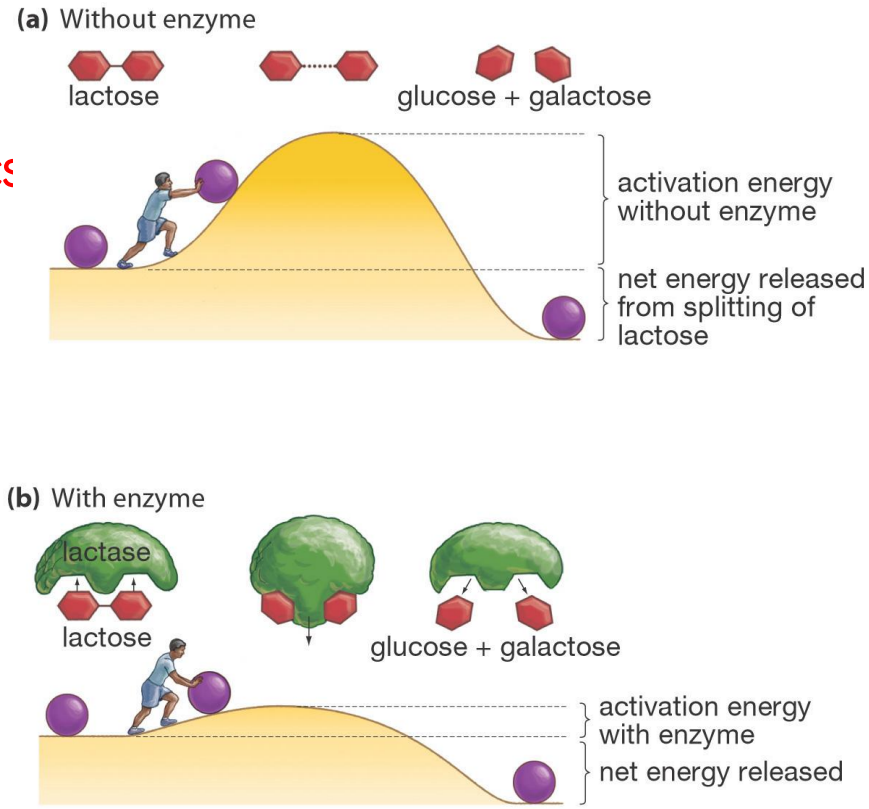
5) The **isomerases** (class 5) move groups within a molecule, without changing the gross composition of the substrate.

6) The ligation reactions catalyzed by **ligases** ("synthetases," class 6) are energy-dependent and are therefore always coupled to the hydrolysis of nucleoside triphosphates(See figure)

Class	Reaction type	Important subclasses
1 Oxidoreductases	○ = Reduction equivalent $A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}$	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	$A\text{–}B + C \rightleftharpoons A + B\text{–}C$	C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	$A\text{–}B + H_2O \rightleftharpoons A\text{–}H + B\text{–}OH$	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	$A + B \rightleftharpoons A\text{–}B$	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	$A \rightleftharpoons Iso\text{-}A$	Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")	$A + B + XTP \rightleftharpoons A\text{–}B + XDP$	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

Enzyme kinetics

The presence of enzymes lowers the energy required for the reaction to occur *(activation energy* , (G)} .



Enzyme kinetics terminology

[S] – substrate concentration

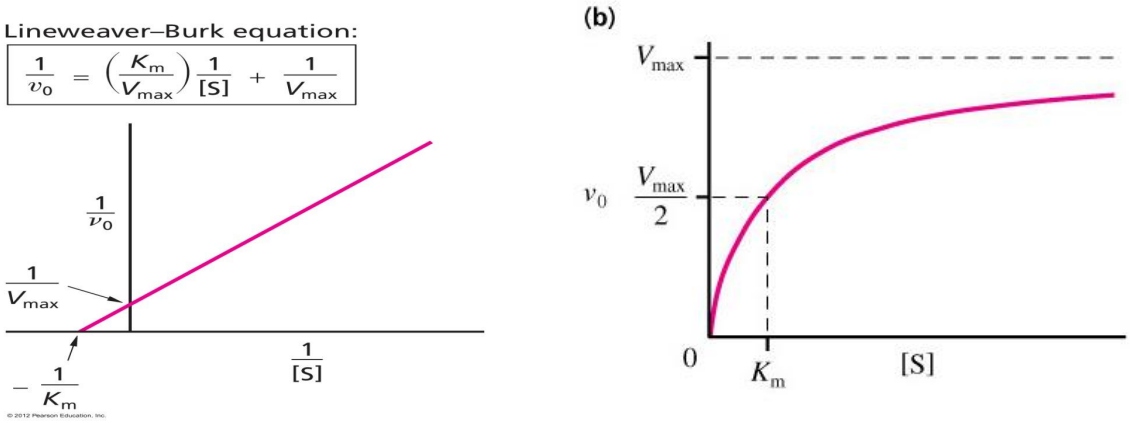
V_o – initial velocity of a reaction. A significant amount c substrate has not yet been converted to product.

V_{max} – maximal velocity of a reaction. Addition of more substrate will not increase the rate of the reaction

K_m – The concentration of substrate at which the rate of the reaction is half-maximal

Michaelis-Menten equation

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



By increasing the substrate concentration [S], the value of the reaction(Km) velocity increases until reaching a plateau, where the velocity is maximum (V_{max}) due to the business of all the enzyme molecules.

Significance of Km value:

1. Substrates are usually present in physiological fluids at a concentration ranging around the Km values.

2. Km reflects the affinity of the enzyme for the substrate. The lower the Km value, the higher the enzyme affinity to substrate i.e. smaller concentration of the substrate needed to reach 1/2 V max . The higher Km reflects a low affinity of the enzyme for substrate i.e. higher concentration of the substrate needed to reach 1/2 V max.

3- It reflects effect of allosteric regulators on the rate of enzyme catalyzed reaction

- Positive allosteric effector→ decrease Km.
- Negative allosteric effector→ increase Km.

4-It reflects presence or absence of enzyme inhibitor and it's nature:

Competitive enzyme inhibitor → increase Km .

Non-competitive enzyme inhibitor→ has no effect on Km.

Un-competitive enzyme inhibitor→ equally reduce both Km and Vmax.

Factors affecting the rate of enzyme catalyzed reaction(Regulation of enzyme activity

1- Temperature:

- There is optimal temperature at which the enzyme attains its maximal activity (37C-40C) .

2- PH:

- There is optimal PH at which the enzyme acts maximally .For example, blood enzymes act at 7.4.

3-Concentration of the enzyme:

- Increase the enzyme concentration leads to increase the rate of enzyme catalyzed reaction till certain point , after which increase in the enzyme concentration is not associated with further increase in the rate of the enzyme catalyzed reaction due to inhibition of the enzyme by the accumulated products.

4- Concentration of the substrate

• Increase the substrate concentration leads to increase the rate of enzyme catalyzed reaction till certain point , after which increase in the substrate concentration is not associated with further increase in the rate of the enzyme catalyzed reaction due to saturation of all the active sites of the enzyme

5-Protecting the enzymes from light and radiation which lead to their denaturation.

6- Protecting the enzymes from oxidation or inhibitors.

7-Concentration of cofactors or coenzymes:

• In the conjugated protein enzymes that need coenzymes or cofactors , the increase in the coenzymes or cofactors concentration causes an increase in the rate of the enzyme action.

8- Effect of end products or feedback regulation:

Allosteric enzymes:-

There is a site on the enzyme surface other than the catalytic and substrate binding site to which allosteric effector binds.

• Allosteric effector(regulatory molecule) is a low molecular weight substance with no or little structural similarities to the substrate and may be end product of enzyme catalyzed reaction & leads to one of the following effects:-

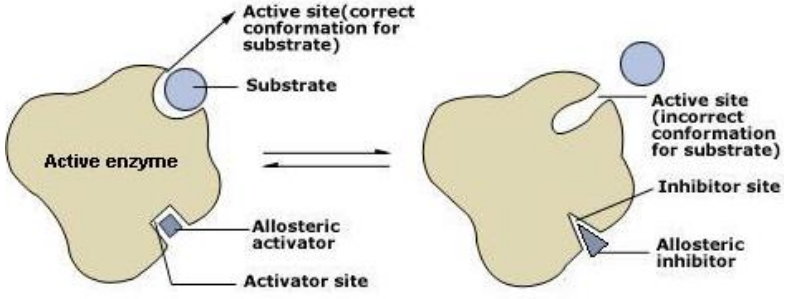
A)- Conformational changes mainly in catalytic site , leading to increase the rate of enzyme catalyzed reaction i.e. increase enzyme activity through:-

1- Increase enzyme substrate affinity.
2- Decrease Km.
3-And called *Allosteric activator* or " *feed back activator*" or " *positive allosteric effector*"

• B)- Conformational changes mainly in catalytic site , leading to decrease the rate of
enzyme catalyzed reaction i.e. decrease enzyme activity through:-

1- Decrease enzyme substrate affinity.
2- Increase km.
• And called *Allosteric inhibitor* or " *feed back inhibitor* " or " *negative allosteric effector*" . They are non-competitive inhibitors

Allosteric inhibitor may be one of the following types:-
- *Cumulative :-* in which single product presents in enough amount inhibit the enzyme.
- *Concerted*:- in which two or more products have to be present simultaneous and in enough amount to inhibit the enzyme.
- *Co-operative*: has the feature of the previous two types.
- *Enzyme multiplicity*: multiple enzymes having different catalytic actions , all are inhibited by single product.



Schematic representation of allosteric enzyme activity

Objectives

- Define enzymes & describe structural components of the enzyme system.
- Discuss enzyme nomenclature, and classification
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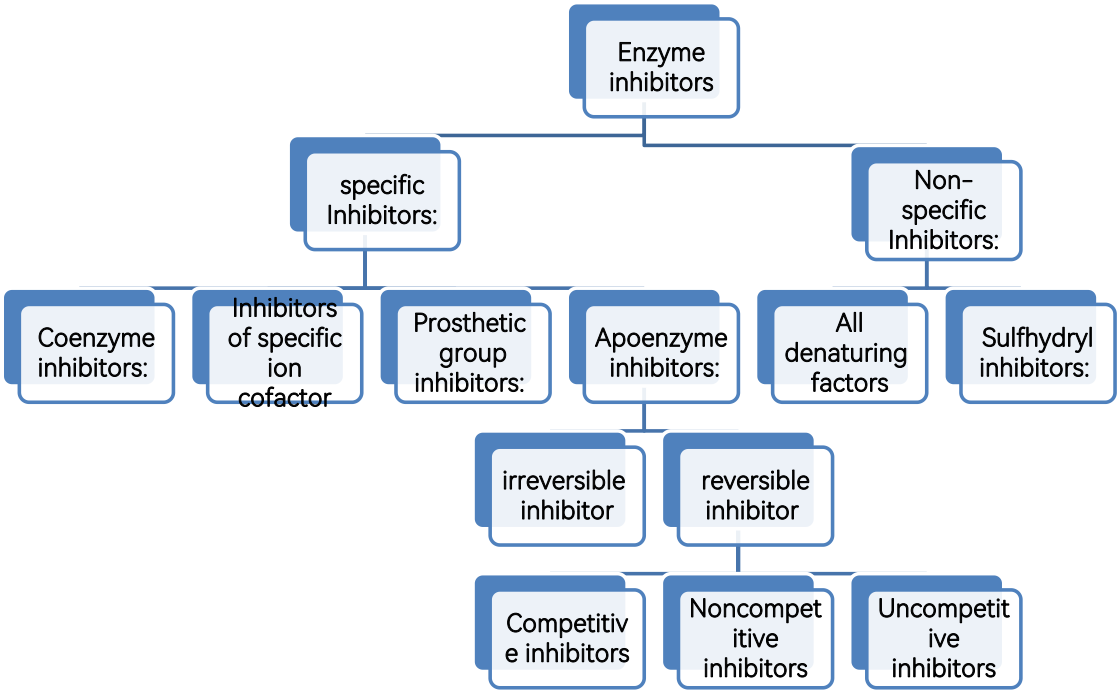
- Describe kinetics of enzyme reaction

Objectives:

- List different types of enzyme Inhibition
- Explain with examples mechanisms of enzyme inhibition
- Enumerate different modes of regulation of enzyme activity
- Discuss regulation of enzyme activity via enzyme activation
- Discuss allosteric enzymes
- Define isoenzymes & explain its clinical application

Enzyme inhibitors

•Enzyme inhibitors are substances that inhibit the enzyme activity and divided into.



1. Competitive enzyme inhibitors:-

• The inhibitor is structurally similar to the substrate and binds to substrate binding site on the enzyme surface in competition with the substrate.

Equation description:

• This type of inhibition is reversible i.e. enzyme inhibition can be overcame by increase the substrate concentration or decrease enzyme inhibitor.

• It can be summarized as follows:

• Without inhibitor:

• E + S ES E + P.

• With inhibitor:

• E + I EI No product

Examples:

- Allopurinol is a competitive inhibitor for xanthine oxidase that synthesizes uric acid from purines. Therefore, allopurinol is used for treating Gout.
- Methotrexate is competitive inhibitor of dihydrofolate reductase that convert dihydrofolate to tetrahydrofolate which is essential for cell division. Methotrexate is structural analogue of dihydrofolate, used as anticancer agent.
- Sulfonamides are competitive inhibitors for conversion of p-aminobenzoic acid into folic acid in bacteria. Therefore, sulfonamides are used as bacteriostatic antibiotics. Sulfonamide is structural analogueof p-aminobenzoic acid.
- inhibition of succinic acid dehydrogenase (that oxidizes succinate into malate) by malonic acid. Malonic acid is structural analogue for succinic acid That prevents energy production and so malonic acid is toxic to

2- Non-competitive enzyme inhibitors:-

• The inhibitor has no structural similarity to the substrate and doesn't bind to substrate binding site on enzyme surface but binds to different site on it . It binds either free enzyme or enzyme-substrate complex as it not inhibit binding of the enzyme to substrate but it decrease enzyme catalytic activity.

• *Equation description:*

–This type of inhibition is irreversible i.e. enzyme inhibition can't be overcame by increase the substrate concentration .

–It can be summarized as follows:

• Without inhibitor:

• E + S ES E + P.

• With inhibitor:

• E + S + I EI or ESI no product.

Examples:

• Allosteric inhibitors: see functional sites of the enzyme.

• Inhibition of the enzyme by hydrogen ions at the acidic side and by hydroxyl ions at the basic side of its optimal PH.

3- Uncompetitive inhibitors

- This inhibitor binds at a site that only becomes available after the substrate has bound to the enzyme. This is encountered in multi-substrate enzymes, where the inhibitor competes with one substrate (e.g. S_2) of them and is uncompetitive for the other (e.g. S_1). The inhibitor has some structural similarity to such substrate, is overcome only by special measures such as dialysis. The reaction is summarized as follows:

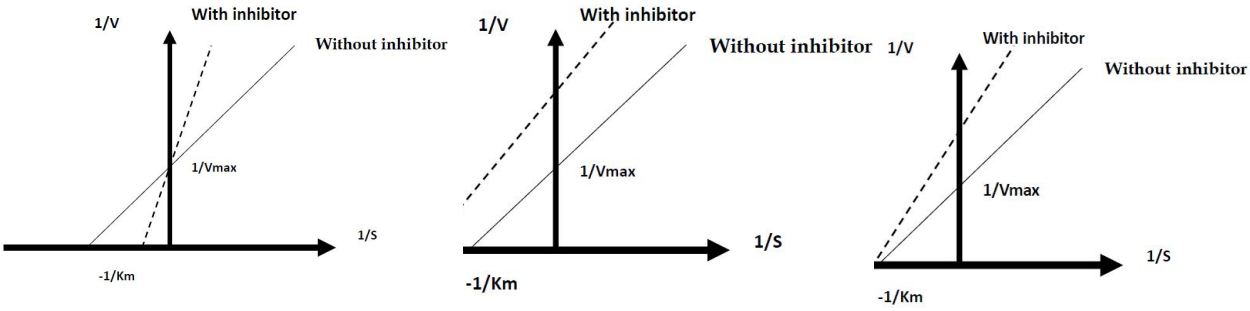
- Without inhibitor: $E + S_1 \quad ES_1 + S2 \quad ES_1S_2 \quad E + Ps.$
- With inhibitor: $E + S_1 \quad ES_1 + I \quad ES_1I \quad$ no product.

-The change in K_m, V_{max} and Lineweaver-Burke plot:

- The increase in substrate concentration (e.g. S_1) cannot overcome the inhibition that equally reduces both V_{max} and K_m leading to double reciprocal plots (see below).

Table 5.3 Effects of reversible inhibitors on kinetic constants

Type of inhibitor	Effect
Competitive (I binds to E only)	Raises K_m V_{max} remains unchanged
Uncompetitive (I binds to ES only)	Lowers V_{max} and K_m Ratio of V_{max}/K_m remains unchanged
Noncompetitive (I binds to E or ES)	Lowers V_{max} K_m remains unchanged



. Regulation of enzyme activity (within the cell)

1-By Covalent Modification:

- The addition by a covalent bond of a foreign group, e.g. phosphate group, into the enzyme or removal of such group.
- Some enzymes such as glycogen synthase (that polymerizes glucose into glycogen) is inactivated upon phosphorylation, whereas, glycogen phosphorylase (that breaks glycogen into glucose) is activated by phosphorylation.
- Phosphorylation is done by protein kinases.
- dephosphorylation is done by protein phosphatases

2. Compartmentation of the enzyme Location within the cell:

- Many enzymes are localized in specific organelles within the cell due to presence of the substrate and appropriate controls in this site.

3-Hormonal control:

- altering the conc of an allosteric effector, e.g., cAMP.
- by covalent modification of the enzyme.
- by changing enzyme concentration through controlling its rate of synthesis and degradation.

4-Allosteric inhibitors:

- Allosteric enzymes are regulated by molecules called effectors (modifiers) that binds nonconvalently at a site other than the active site.

5. Zymogen Cleavage *(or proenzymes):*

- Definition:
- Most enzymes are synthesized in an inactive form called zymogens or proenzymes)
- This is due to
  - Presence of an inhibitory extra-polypeptide chain.
  - Presence of conformational changes.
  - Presence of inhibitory subunit.
  - Presence of regulatory covalent modification.
  - The enzyme require for it's activity cofactor or allosteric activator or activating protein or coenzyme.
- Its significance is:
- To protect the secretory cells and transporting duct system.
- To keep the enzyme in a storable form till time of use.
- To provide mechanisms for regulating the enzymes activity.

• Zymogens are activated by:

• Release of inhibitory polypeptide by cleavage or conformational change:

I. By a specific enzyme such as blood clotting enzymes by each others.
II. By a non-specific proteolytic action such as trypsin activates chymotrypsinogen into chymotrypsin
III. By auto-activation such as pepsin activate pepsinogen into pepsin
IV. By acid hydrolysis such as HCl activates pepsinogen into pepsin.

• Association with a cofactor such as activation by metal ions, e.g., kinases by Mg^{2+}.

• Association with another protein such as activation of pancreatic lipase with co-lipase.

- Association with an allosteric activator such as activation of glycogen synthase by accumulation of glucose-6-phosphate (its substrate)
- Dissociation of a subunit of the enzyme such as cAMP-dependent protein kinase.
- Covalent modification, e.g., by phosphorylation and dephosphorylation mechanisms.

6-Induction and repression of enzyme sysnthesis:
by altering the rate of enzyme synthesis.

a) Enzyme induction:

Increase of substrate conc will increase rate of synthesis of certain enzyme that utilizes that substrate.

Many toxins that enter the body induced the rate of synthesis of enzymes that detoxify them

b) Enzyme Repression

Accumulation of a product of certain enzyme leads to decrease rate of synthesis of that enzyme, enzyme repression or negative feedback regulation. The inhibitory product is called co-repressor.

Ø Cholesterol and its derivatives are strong repressors for the expression of the key regulatory enzymes for cholesterol synthesis

c) Constitutional Enzymes:

Ø Housekeeping enzymes are constitutive enzymes

Ø their rate of synthesis in a cell is constant and does not depend on an inducer.

Ø The level of the enzyme is only controlled by its rate of degradation.

Isozyme (isoenzyme)

- Isoenzymes are isomers of the same enzyme.
- They are physically (structurally, electrophoretically and immunologically) distinct forms of the same enzyme
- theyt catalyze the same chemical reaction(s) and differ in their catalytic activity and in distribution between different tissues and subcellular compartments.
- The physical differences between isoenzymes are due to different genes (alleles), different subunits and/or modified subunits used in synthesizing isoenzyme forms.
- Can be separated by:
  .Electrophoresis.
  .Ion exchange chromatography techniques

Biochemical significance of isoenzymes:

1- They explain metabolic differences :

A- subcellular organelles of a cell, mitochondrial and cytostolar and isocitratate dehydrogenase

B-different tissues.

C-individuals.

2- They explain differences in drug metabolism between different individuals.

3-They have laboratory clinical diagnostic application.

4-They explain structure-function relationship of proteins.

LACTATE DEHYDROGENASE (LDH)

Lactate + NAD^+ *lactate dehydrogenase* pyruvate + $NADH+H^+$

§ It is a tetrameric protein and made of two types of subunits namely H = Heart, M = skeletal muscle

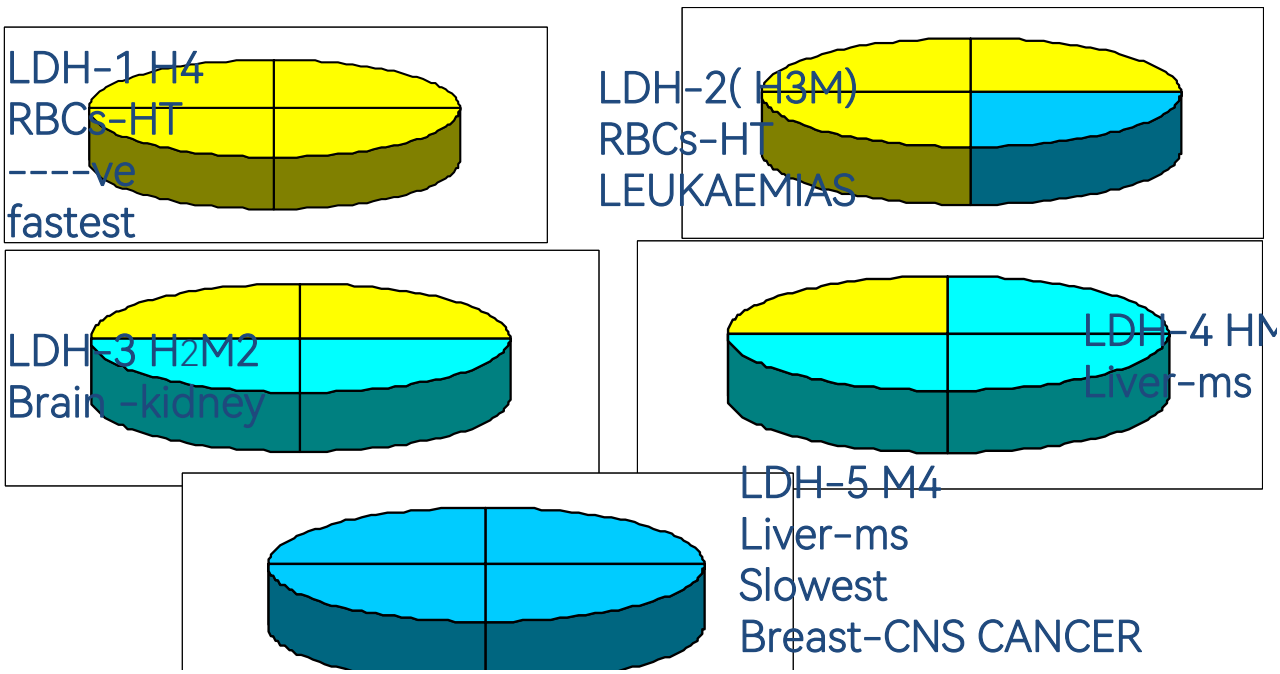
there are 5 physically distinct isoenzymes of this enzyme with various combinations of H and M subunits, known as LDH-1, LDH-2, LDH-3, LDH-4 and LDH-5.

The different isozymes may catalyze the same reaction at different rates. –

The isozymes have different pH optima and Km values

Isoenzymes

Structure of LDH isoenzymes:



Isoenzyme name	Composition	Present in	Elevated in
LDH1	(H_4)	Myocardium, RBC	myocardial infarction
LDH2	(H_3M_1)	Myocardium, RBC	
LDH3	(H_2M_2)	Kidney, brain	
LDH4	(H_1M_3)	Liver, Skeletal muscle	
LDH5	(M_4)	Liver, Skeletal muscle	Skeletal muscle and liver diseases

Clinical significance:

-Damage to either of myocardium or liver causes increase in total serum LDH and the type of specific isozyme is easily identifiable.
-In normal serum, LDH_2 (H_3M) is the most prominent isozyme, and LDH-5 is rarely seen.
- After myocardial infarction, LDH-1 and LDH-2 predominate
- In acute viral hepatitis, LDH-5 and LDH-4 predominate.
- In malignancy:- Total serum LDH is frequently elevated in neoplastic diseases. there is increase in LDH-3, LDH-4 and LDH-5. An increase in LDH-5 is seen in breast carcinoma

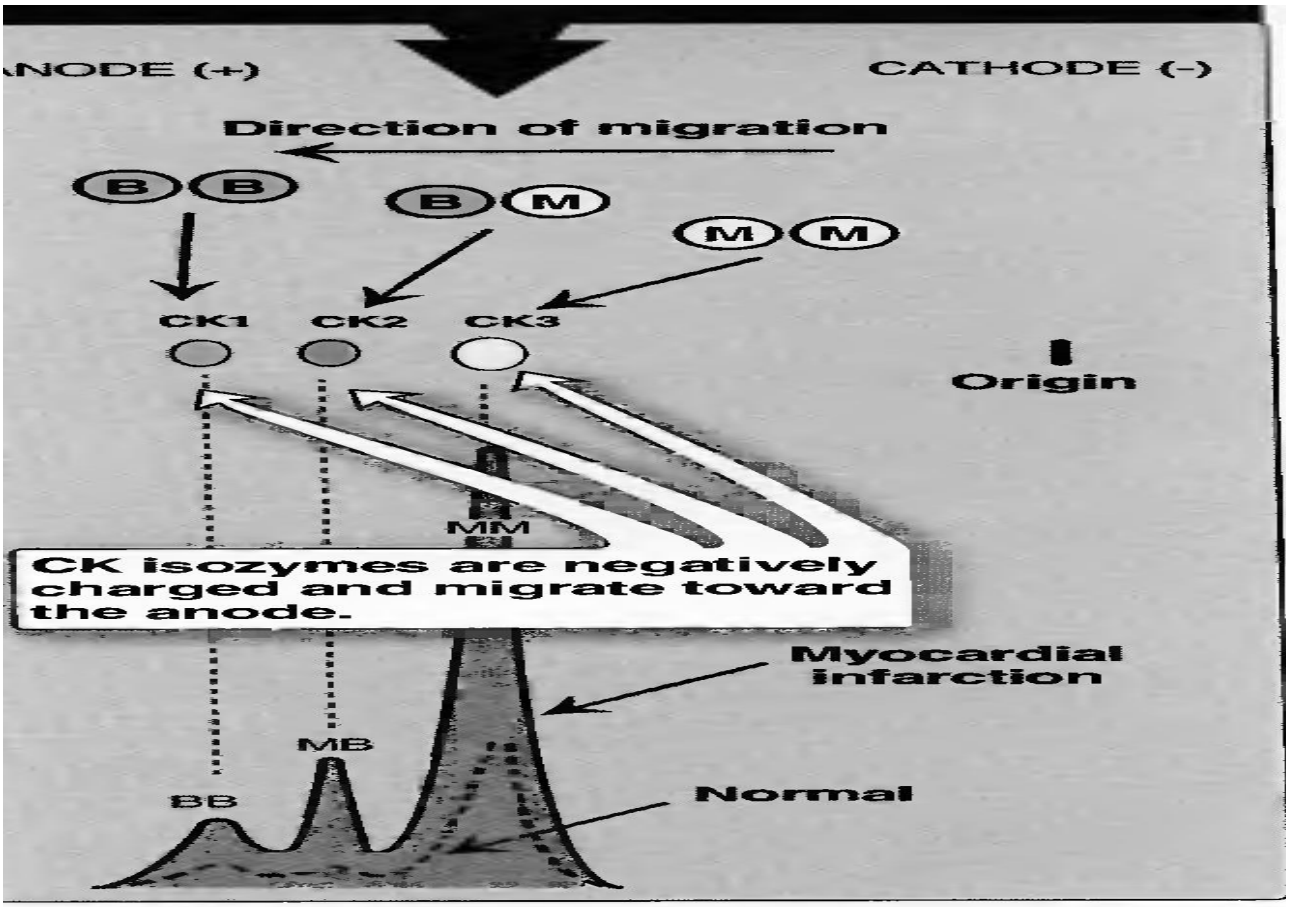
CREATINE KINASE (CK)

Creatine + ATP ⇌ phosphocreatine + ADP

(Phosphocreatine – serves as energy reserve during muscle contraction).

§ Creatine kinase is a dimer made of 2 monomers“M” (for muscle) and “B” (for brain).

§ Three different isoenzymes are formed:CPK-1, CPK-2, CPK-3.



Type	Structure	Electrophoretic mobility	Tissues distribution
CPK-1	BB	Fastest (highly -ve)	Brain
CPK-2	MB	Follows	Myocardium
CPK-3	MM	Slowest (highly +ve)	Skeletal muscle

Remarks:

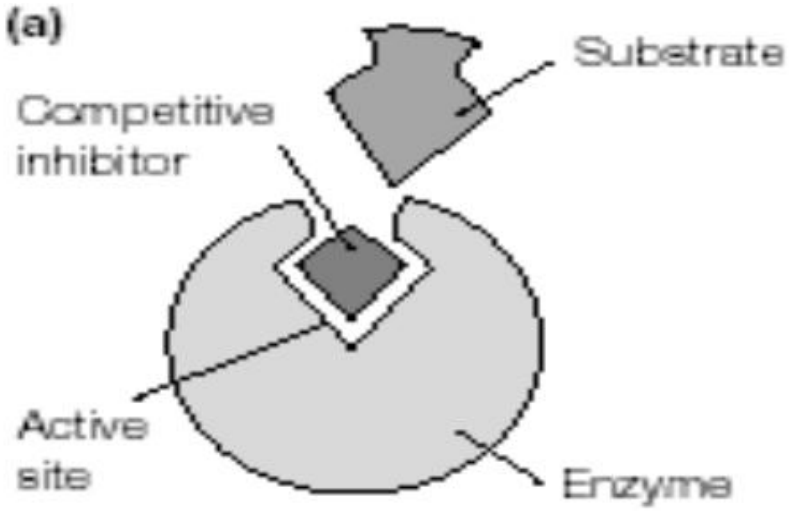
Ø Normally, CPK-2 (MB) isozyme is very few, accounting for about 2% of total CPK activity of plasma, and almost undetectable.

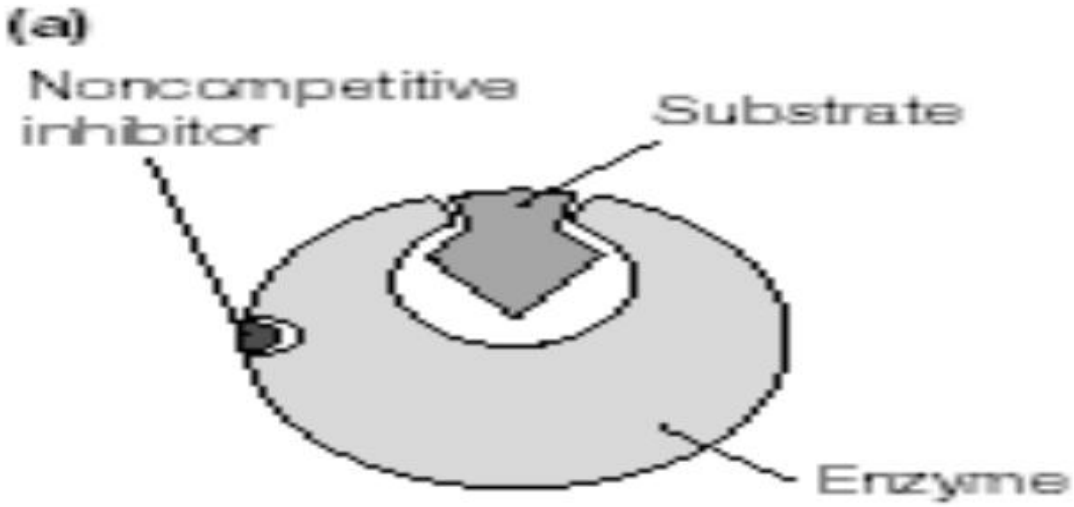
Ø In myocardial infarction, increase of CPK-2 (MB) level occurs within 4 hours maximum in 24 hours, then falls rapidly (up to 20 fold above normal).

Ø MB accounts for 4.5 to 20% of the total CPK activity in plasma of patients with a recent myocardial infarction

ALKALINE PHOSPHATASE (ALP)

- High levels of ALP is indicative of extrahepatic obstruction rather than intrahepatic obstruction
- In bones, the enzyme is derived from osteoblasts. Hence increased in bone diseases like rickets, osteomalacia, neoplastic diseases with bone metastates and healing fractures





1. *A zymogen is:*
   A. Isoenzyme
   B. Anti-enzyme
   C. Enzyme precursor
   D. Enzyme inhibitor
   E. Enzyme activator

C

4. *In competitive inhibition:*

A. The concentration of active enzyme molecules is reduced.
B. Vmax is increased
C. Vmax is decreased
D. Km is increase
E. Km is decreased

D

6. *All of the following are characters of enzymes EXCEPT:*

A. Specific carbohydrate catalysts
B. They do not affect the equilibrium constant
C. Highly specific
D. Enzyme structures are not affected by entering the reaction
E. May be synthesized as zymogen

- A

7. *Isoenzyme is:*
   A. Inactive form of enzyme
   B. Enzymes that need the presence of certain group to act
   C. Unit of enzyme activity
   D. Fractions of enzyme, having the same catalytic activity but differ in chemical and immunological structure
   E. Inorganic substances that accelerate the rate of chemical reactions.

D

8. *Zymogen is:*
   A. Inactive form of enzyme
   B. Enzymes that need the presence of certain group to act
   C. Unit of enzyme activity
   D. Fractions of enzyme, having the same catalytic activity but differ in chemical and immunological structure
   E. Inorganic substances that accelerate the rate of chemical reactions.

A

10. A substrate concentration that produces half maximum velocity:

A. Km
B. Katal
C. Vmax
D. Km/Vmax
E. 1/Vmax

A

12. The Michaelis constant (km) is:

A. A substrate concentration that produces maximum velocity.
B. A substrate concentration that produces half maximum velocity.
C. A measure of equilibrium between active and inactive enzymes
D. A fixed true constant that is not changed from one substrate to another

- B

Substance which can alter or speed up chemical reaction without itself being chemically unchanged is

- Saliva
- Pathology
- Catalyst
- Influenza

Substances on which enzymes act are called as

- prostrate
- hydrate
- glucate
- substrate

In lock and key hypothesis, Enzyme is the

- key
- Lock
- Both A and B
- magenta molecule