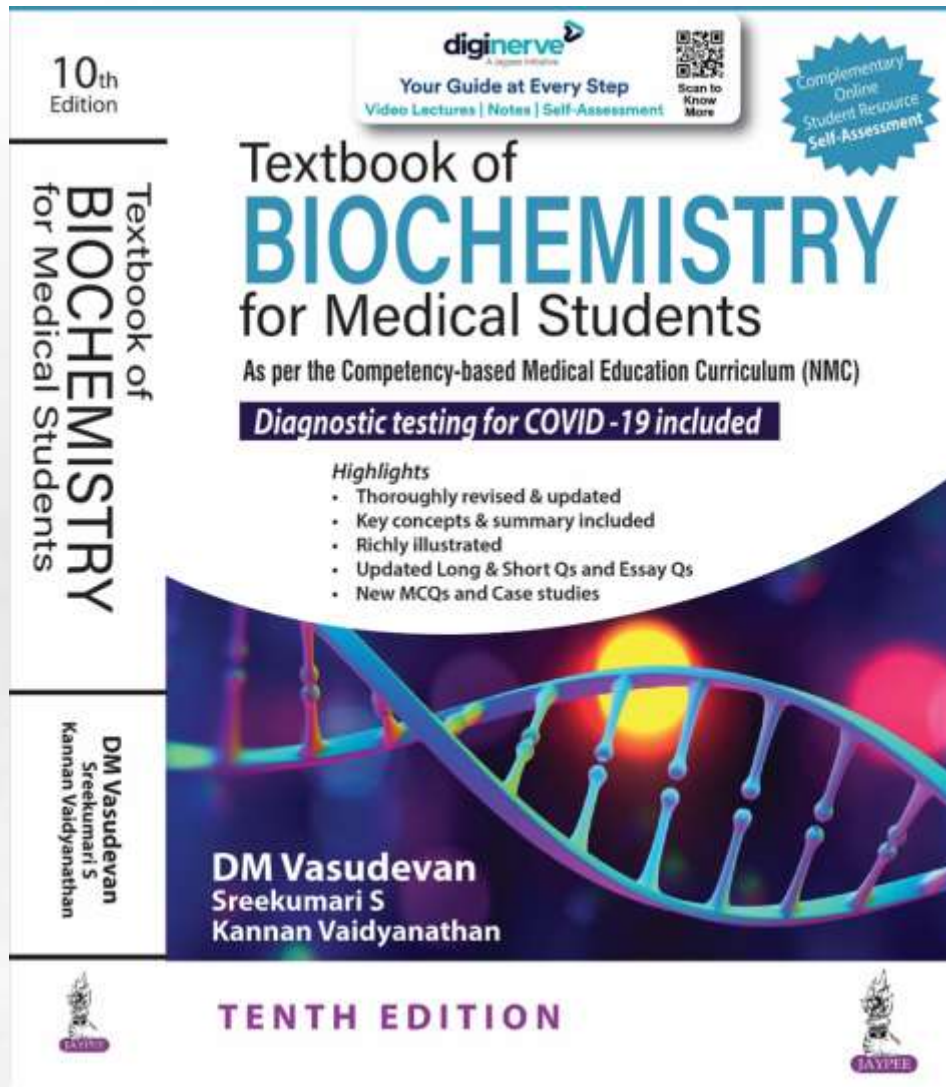


Chapter 4A

Enzymology



Textbook of
BIOCHEMISTRY
for Medical Students
By DM Vasudevan, *et al.*

TENTH EDITION

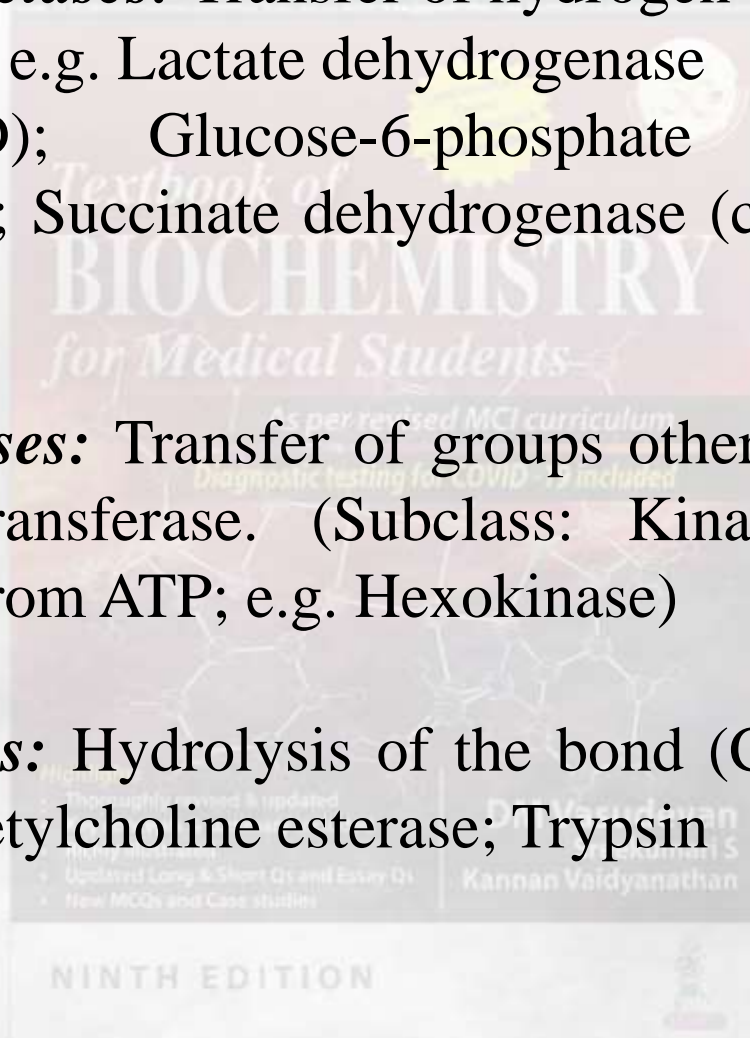
Classification of Enzymes



Class 1. Oxidoreductases: Transfer of hydrogen or addition of oxygen; e.g. Lactate dehydrogenase (coenzyme NAD); Glucose-6-phosphate dehydrogenase (coenzyme NADP); Succinate dehydrogenase (coenzyme FAD); dioxygenases.

Class 2. Transferases: Transfer of groups other than hydrogen. Example, Aminotransferase. (Subclass: Kinase, transfer of phosphoryl group from ATP; e.g. Hexokinase)

Class 3. Hydrolases: Hydrolysis of the bond (Cleave bond and add water); e.g. Acetylcholine esterase; Trypsin



Classification of Enzymes



Class 4. Lyases: Cleave without adding water, e.g. Aldolase; HMG CoA lyase; ATP Citrate lyase.

Class 5. Isomerases: Intramolecular transfers. They include racemases and epimerases. Example, Triose phosphate isomerase.

Class 6. Ligases: ATP dependent condensation of two molecules, e.g. Acetyl-CoA carboxylase; Glutamine synthetase; PRPP synthetase

Class 7: Translocases

A new class of enzymes have been added in 2018. **Translocases**, catalyze the translocation of ions and other molecules across membranes Eg. There are specific translocases for transferring hydrogen ions, inorganic anions, cations, amino acids

Classification of Enzymes



Class	Name	Function	Example
1	Oxido-reductases	Transfer of hydrogen	Alcohol dehydrogenase
2	Transferases	Transfer of groups other than hydrogen.	Subclass: Kinase, transfer of phosphoryl group from ATP; e.g., hexokinase
3	Hydrolases	Cleave bond; adds water	acetylcholine esterase
4	Lyases	Cleave without adding water	Aldolase. (Subclass: Hydratase; add water to double bond)

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Classification of Enzymes

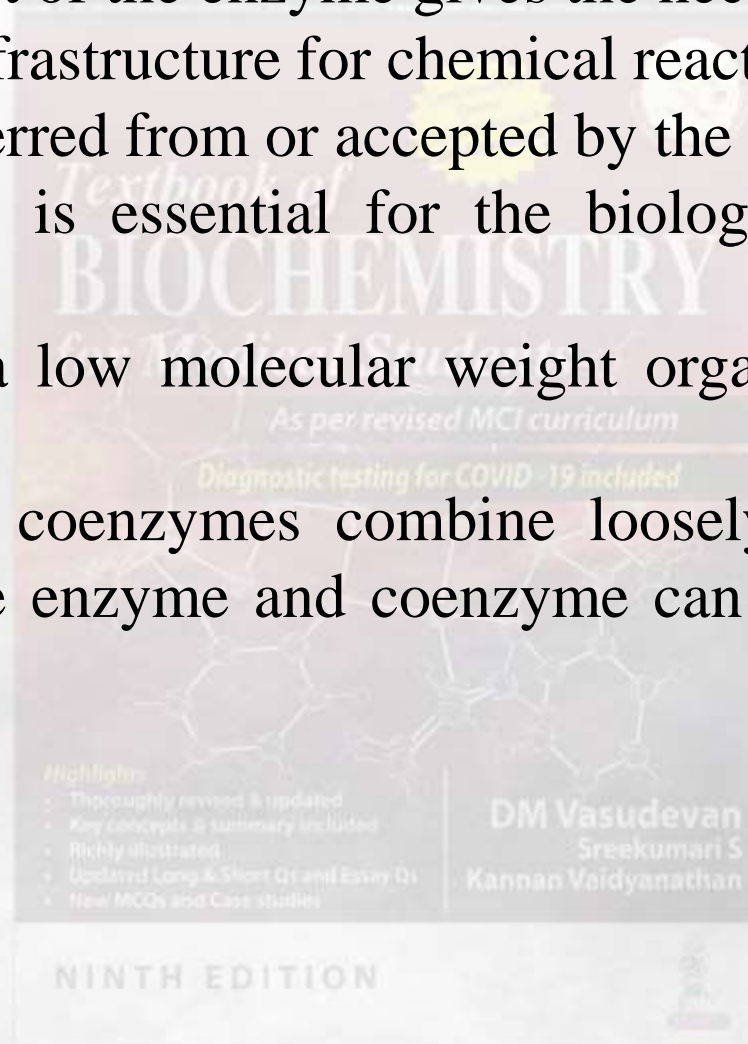


Class	Name	Function	Example
5	Isomerases	Intramolecular transfers	This class includes racemases and epimerases. Example, triose phosphate isomerase.
6	Ligases	ATP dependent condensation of two molecules	Acetyl-CoA carboxylase
7	Translocases	Translocation of ions across membranes	Transfer of hydrogen ions, amino acids etc.

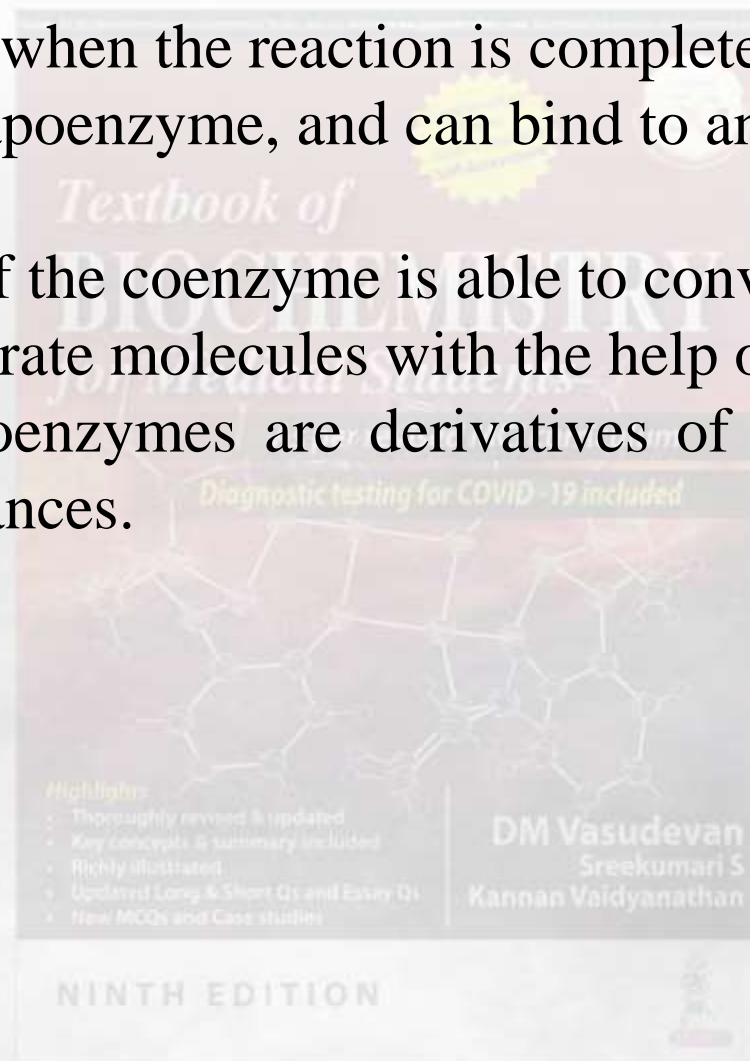
Salient features of Coenzymes



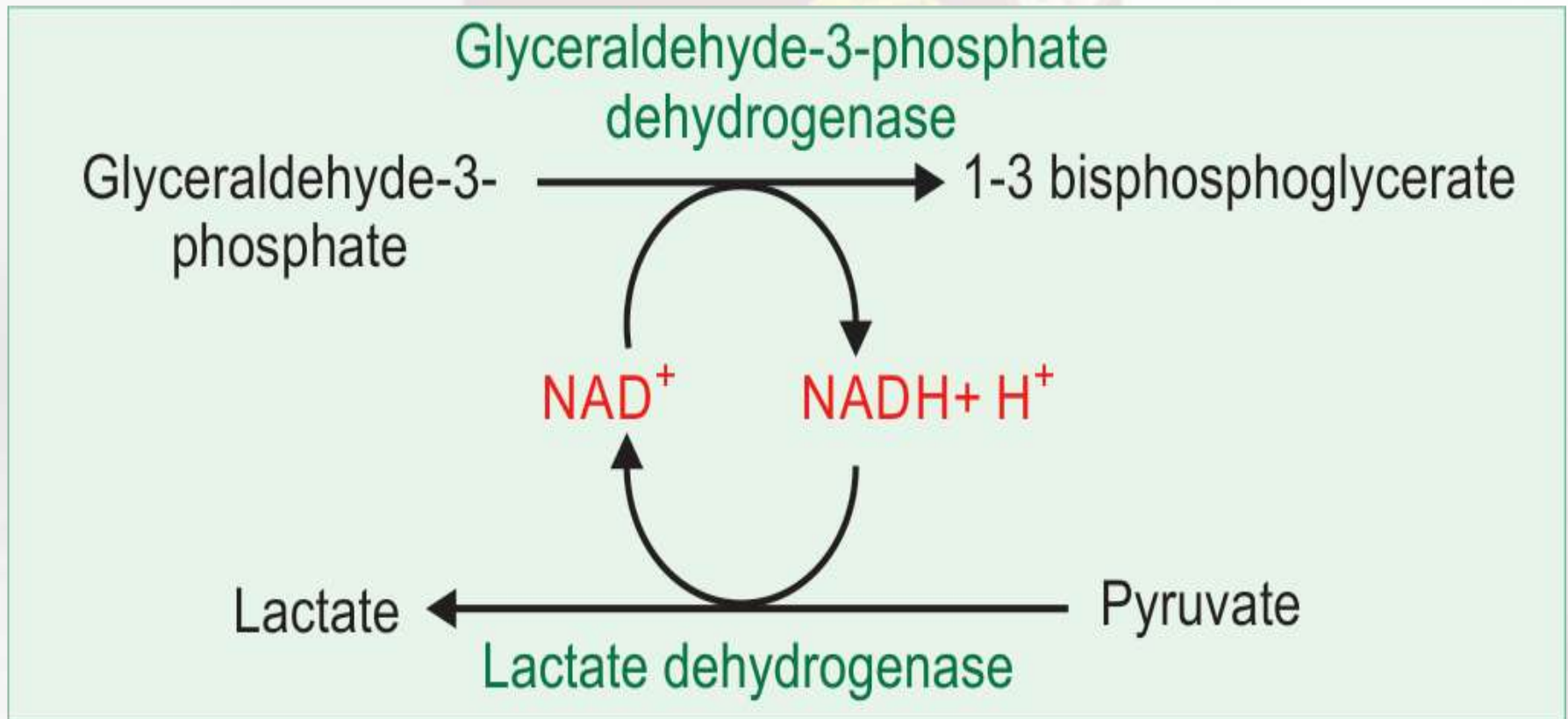
1. The protein part of the enzyme gives the necessary three dimensional infrastructure for chemical reaction; but the group is transferred from or accepted by the coenzyme.
2. The coenzyme is essential for the biological activity of the enzyme.
3. Coenzyme is a low molecular weight organic substance. It is heat stable.
4. Generally, the coenzymes combine loosely with the enzyme molecules. The enzyme and coenzyme can be separated easily by dialysis.



5. Inside the body, when the reaction is completed, the coenzyme is released from the apoenzyme, and can bind to another enzyme molecule.
6. One molecule of the coenzyme is able to convert a large number of substrate molecules with the help of enzyme.
7. Most of the coenzymes are derivatives of vitamin B complex group of substances.



One coenzyme molecule can work with different enzymes.



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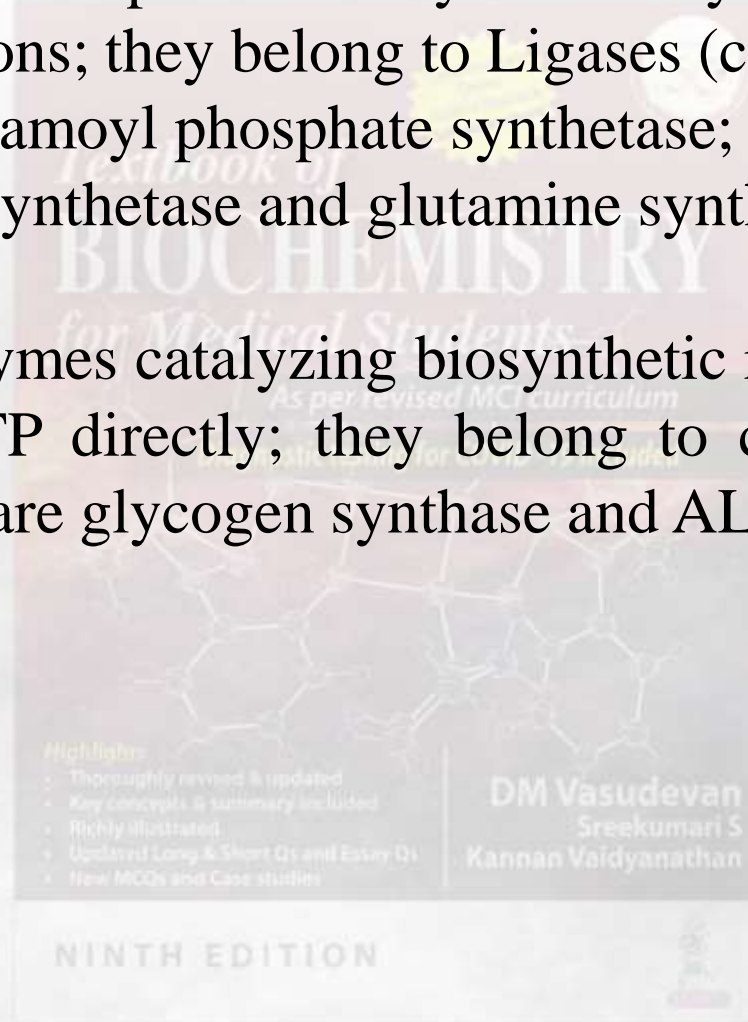
Synthetase and Synthase are Different

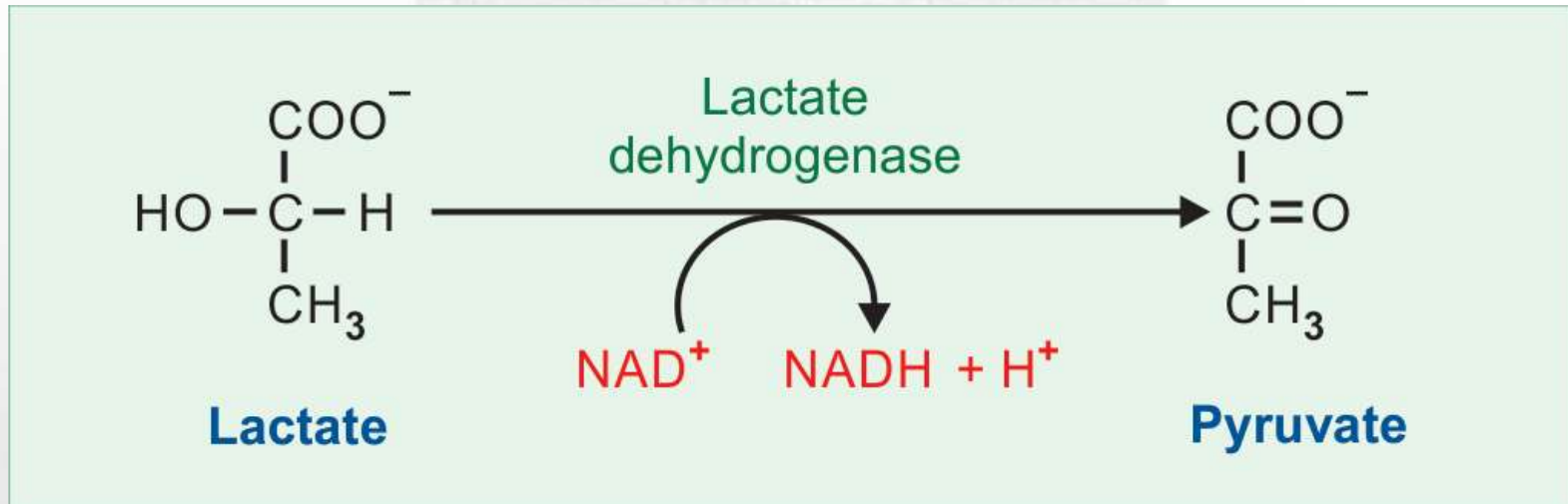


Synthetases are ATP dependent enzymes catalyzing biosynthetic reactions; they belong to Ligases (class 6).

Examples are Carbamoyl phosphate synthetase; Argininosuccinate synthetase; PRPP synthetase and glutamine synthetase.

Synthases are enzymes catalyzing biosynthetic reactions; but they do not require ATP directly; they belong to classes other than ligases. Examples are glycogen synthase and ALA synthase.





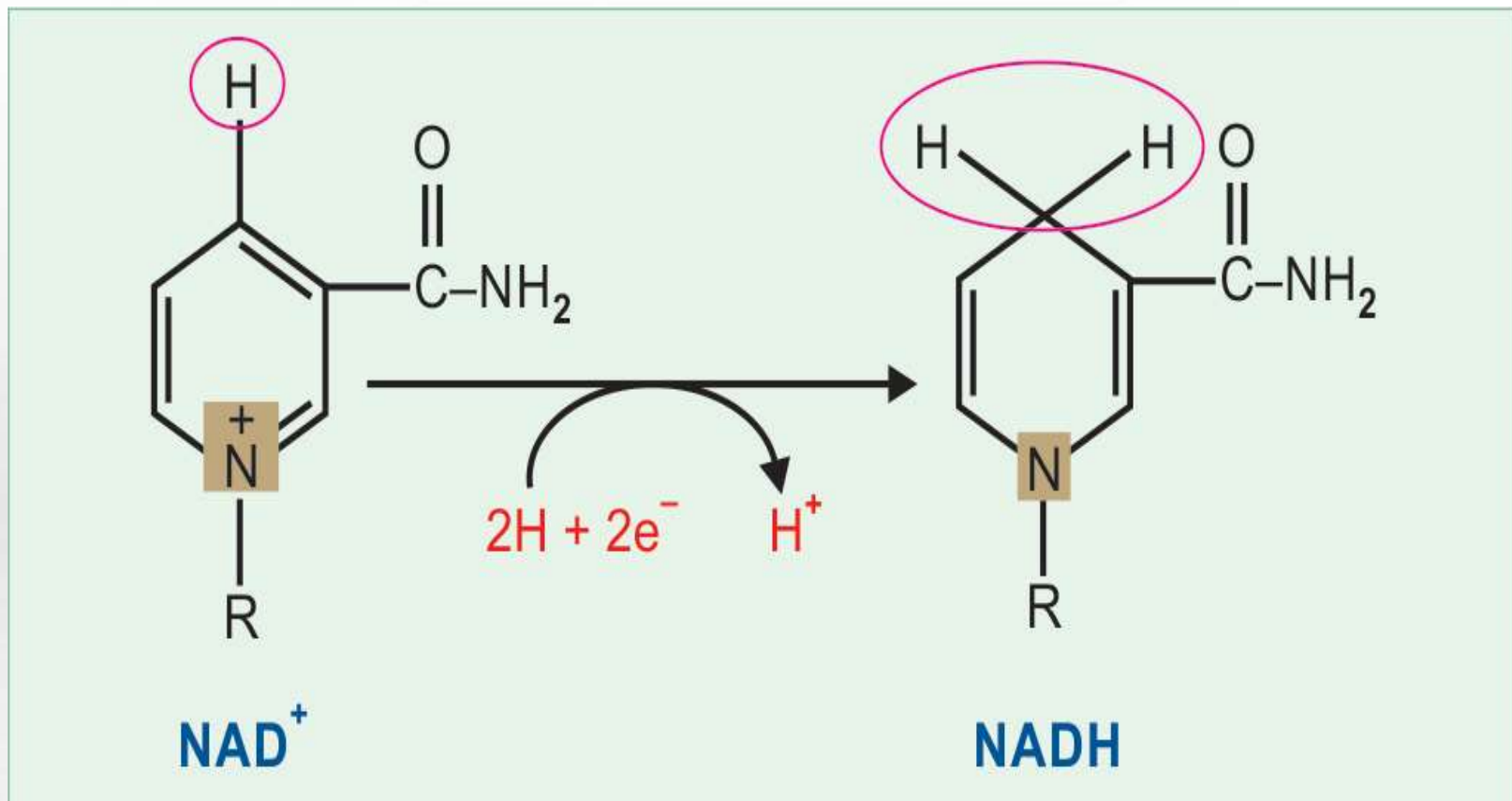
Reaction of lactate dehydrogenase needs the help of NAD⁺

Highlights

- Thoroughly revised & updated
- Key concepts & summary included
- Richly illustrated
- Updated Long & Short Qs and Essay Qs
- New MCQs and Case studies

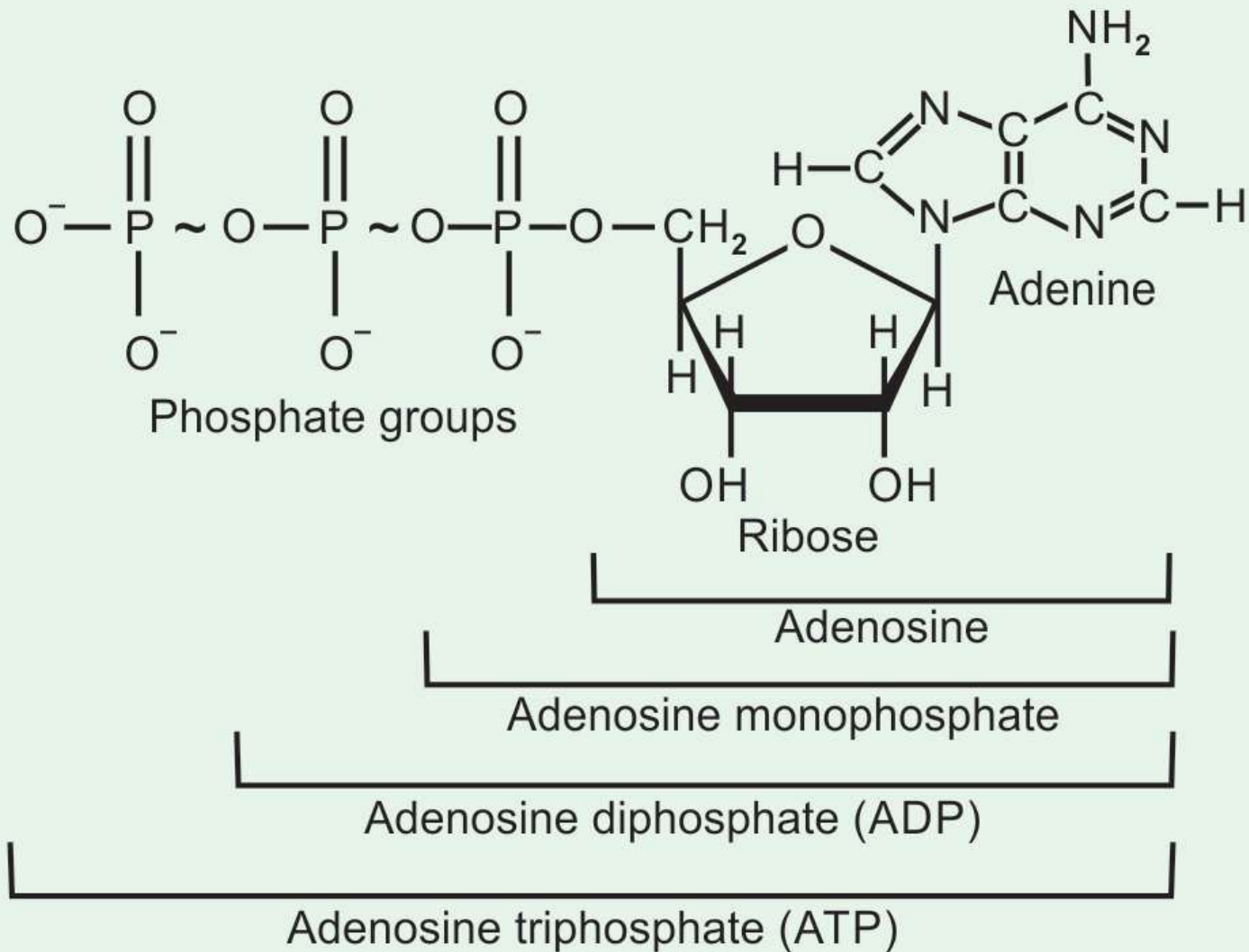
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NAD⁺ accepts hydrogen

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Examples of Coenzymes



<i>Coenzyme</i>	<i>Group transferred</i>
Thiamine pyrophosphate (TPP)	Hydroxy ethyl
Pyridoxal phosphate (PLP)	Amino group
Biotin	Carbon dioxide
Coenzyme A (Co A)	Acyl groups
Tetrahydrofolate (FH ₄)	One carbon group
Adenosine triphosphate (ATP)	Phosphate

Metalloenzymes

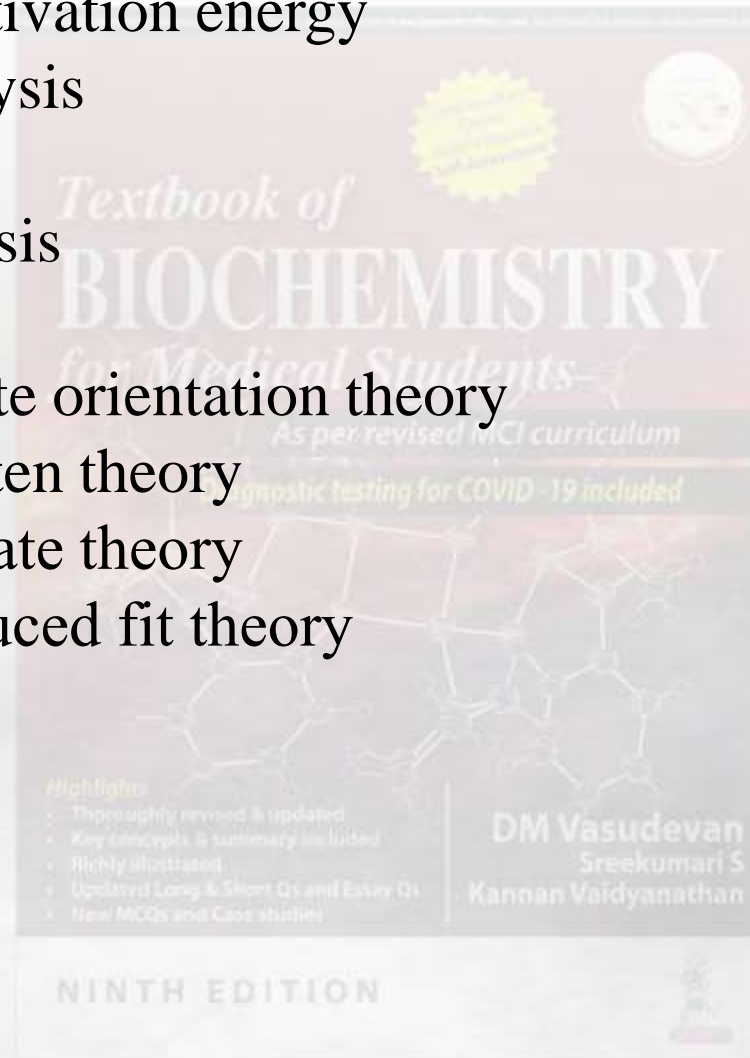


<i>Metal</i>	<i>Enzyme containing the metal</i>
Zinc	Carbonic anhydrase, carboxy peptidase, alcohol dehydrogenase
Magnesium	Hexokinase, phosphofructokinase, enolase, pyruvate kinase
Manganese	Phosphoglucomutase, glycosyl transferases
Copper	Tyrosinase, cytochrome oxidase, lysyl oxidase, superoxide dismutase
Iron	Cytochrome oxidase, catalase, peroxidase, xanthine oxidase
Calcium	Phospholipase, lipase
Molybdenum	Xanthine oxidase

Theories Explaining the Mode of Action of Enzymes



1. Lowering of activation energy
2. Acid base catalysis
3. Substrate strain
4. Covalent catalysis
5. Entropy effect
6. Product substrate orientation theory
7. Michaelis-Menten theory
8. Fischer's template theory
9. Koshland's induced fit theory

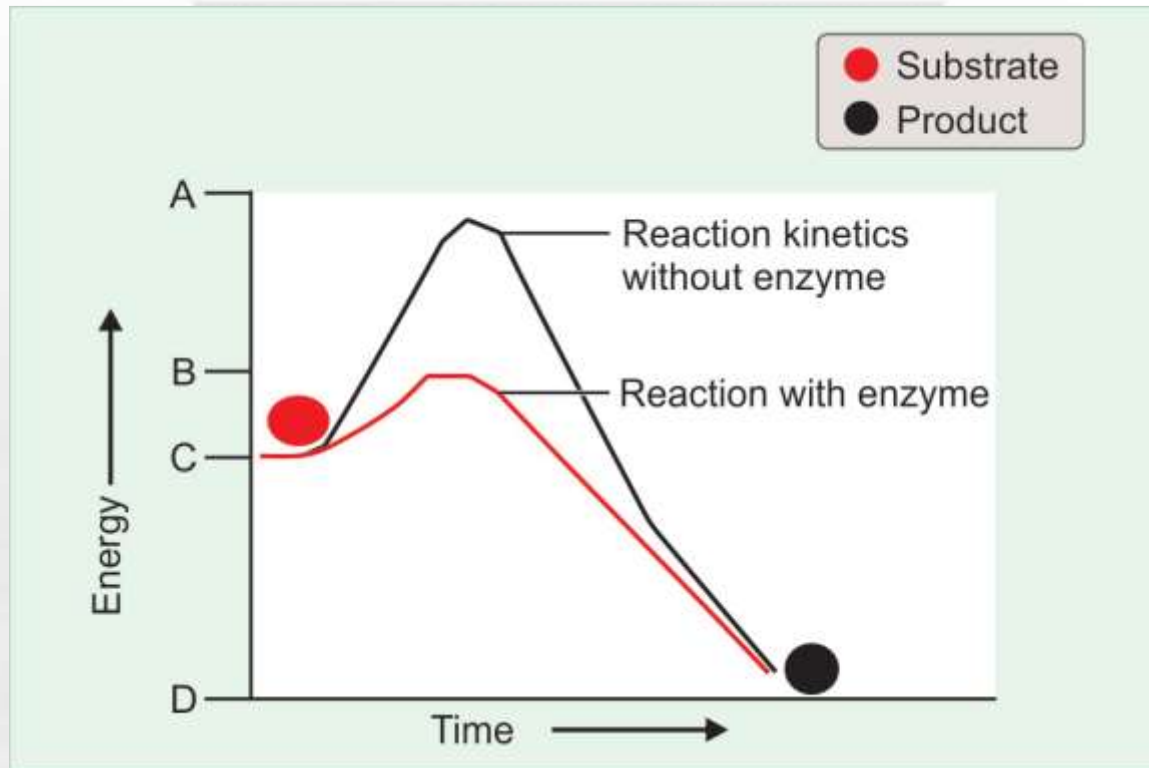


Highlights

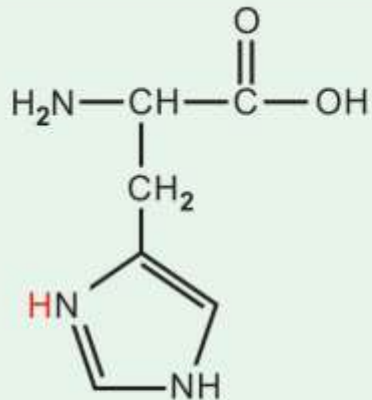
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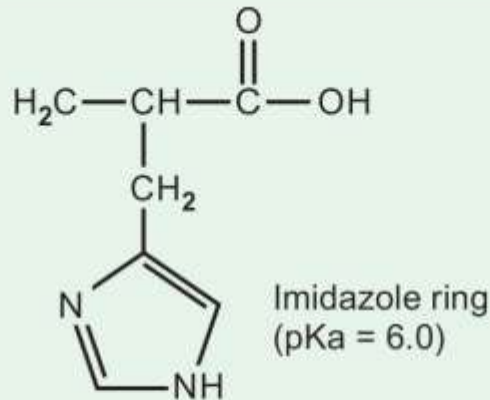
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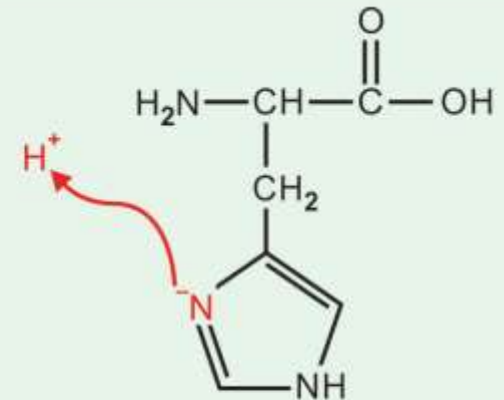
Lowering of activation energy by enzymes. Red circle = substrate; D = energy level of product. C to A = activation energy in the absence of enzyme; C to B is activation energy in presence of enzyme; B to A = lowering of activation energy by enzyme.



Below a pH of 6.0, histidine acts as a proton acceptor (the imidazole ring is mostly protonated)



L-Histidine



Above a pH of 6.0, histidine acts as a proton donor

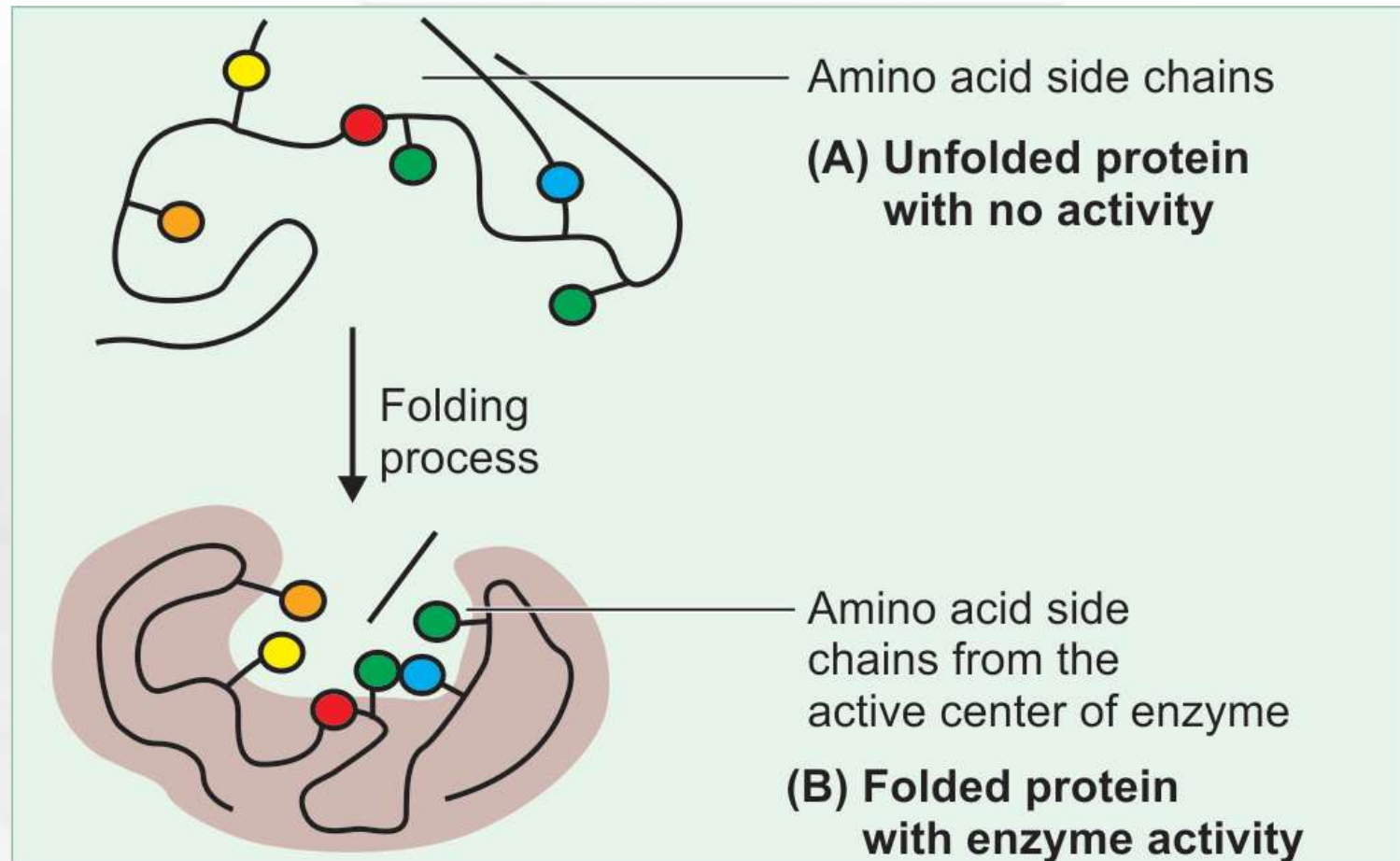
Acid base catalysis with the help of histidine

Highlights

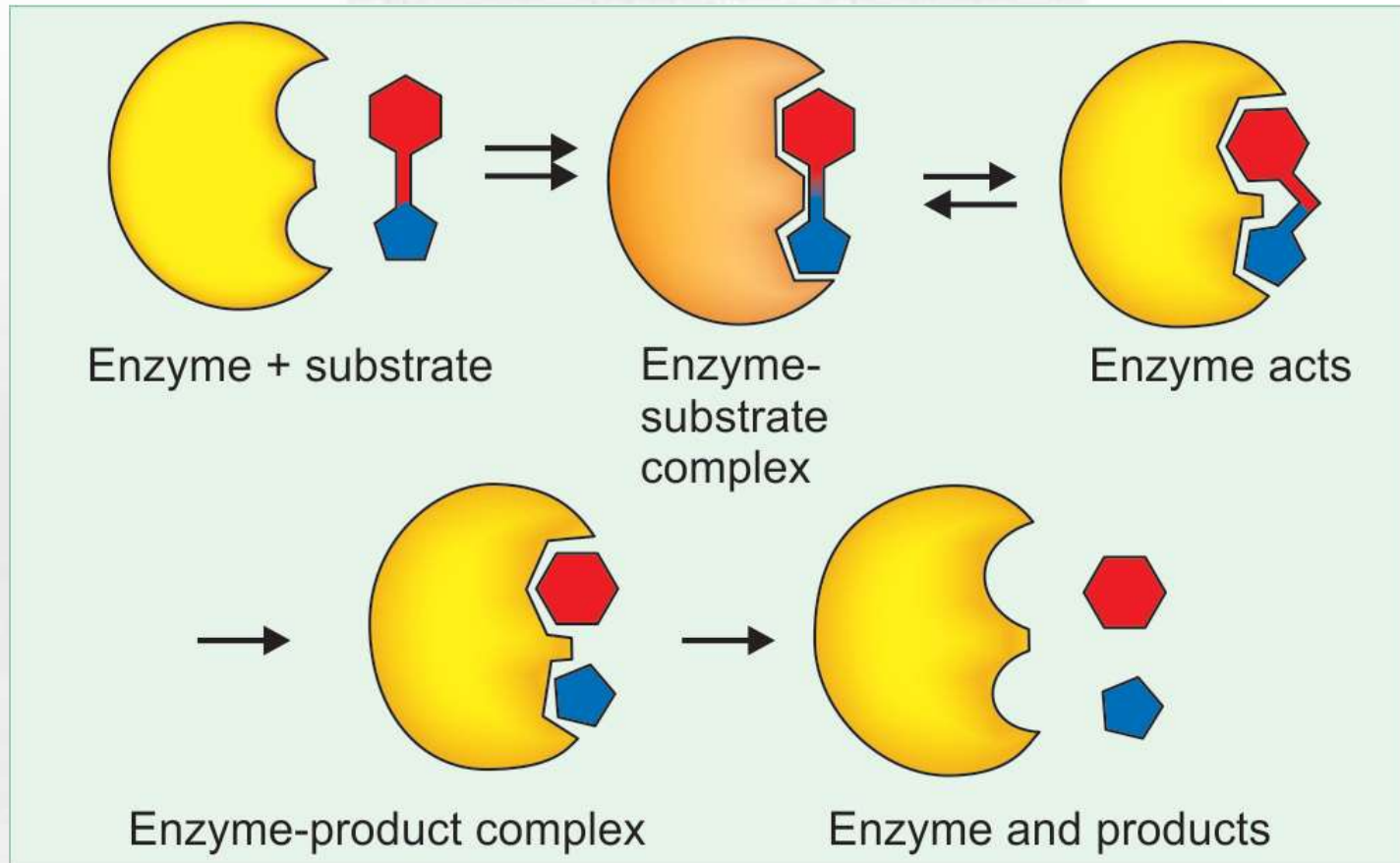
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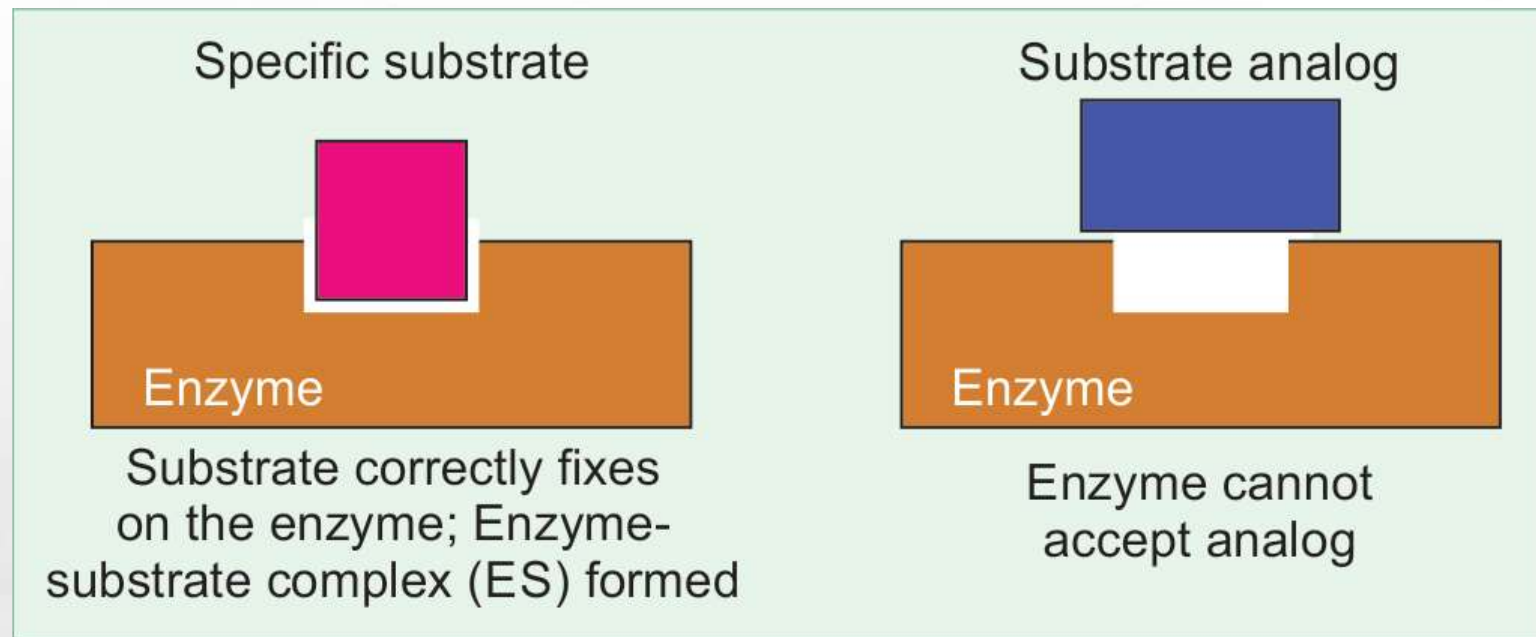
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Correct alignment of amino acids in the active center of the enzyme.

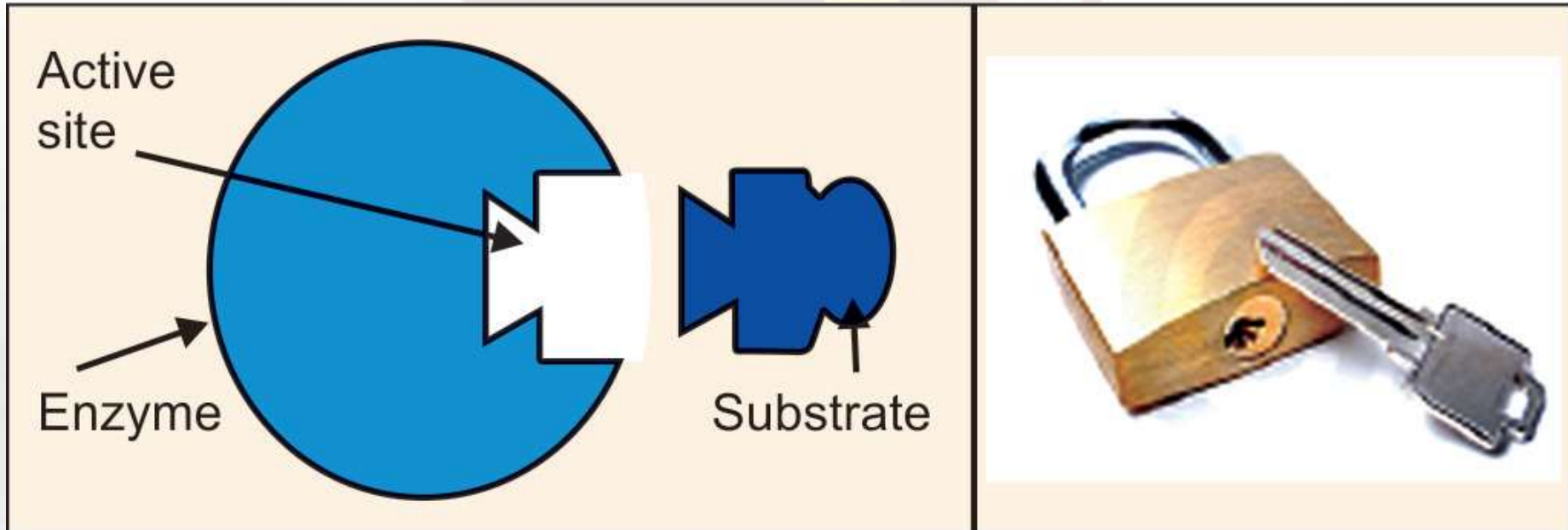


Enzyme substrate complex



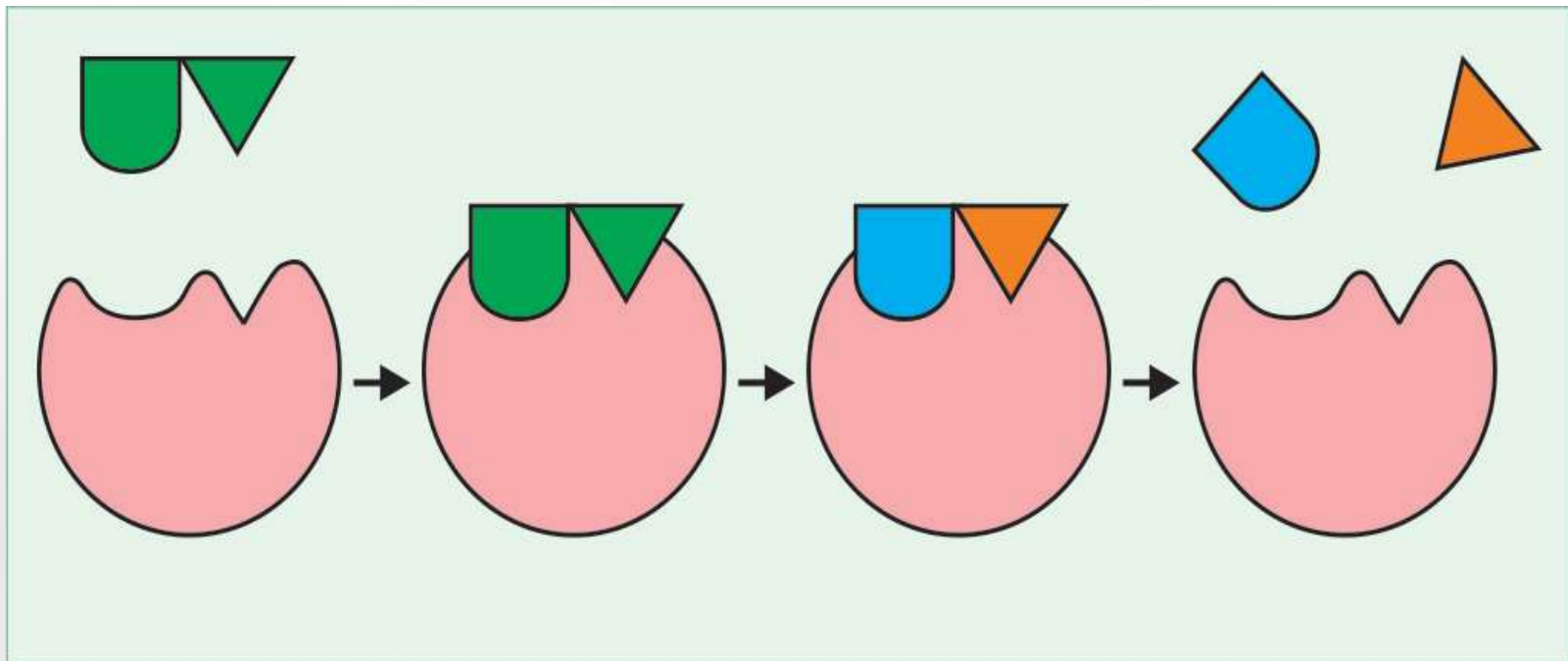
Fischer's Template Theory

This theory states that the three dimensional structure of the active site of the enzyme is complementary to the substrate. Thus **enzyme and substrate fit each other**. Substrate fits on the enzyme, similar to **lock and key**. The lock can be opened only by its specific key. However, Fischer envisaged a rigid structure for enzymes, which could not explain the flexibility shown by enzymes.



Enzyme and substrate are specific to each other. This is similar to key and lock (Fischer's theory)

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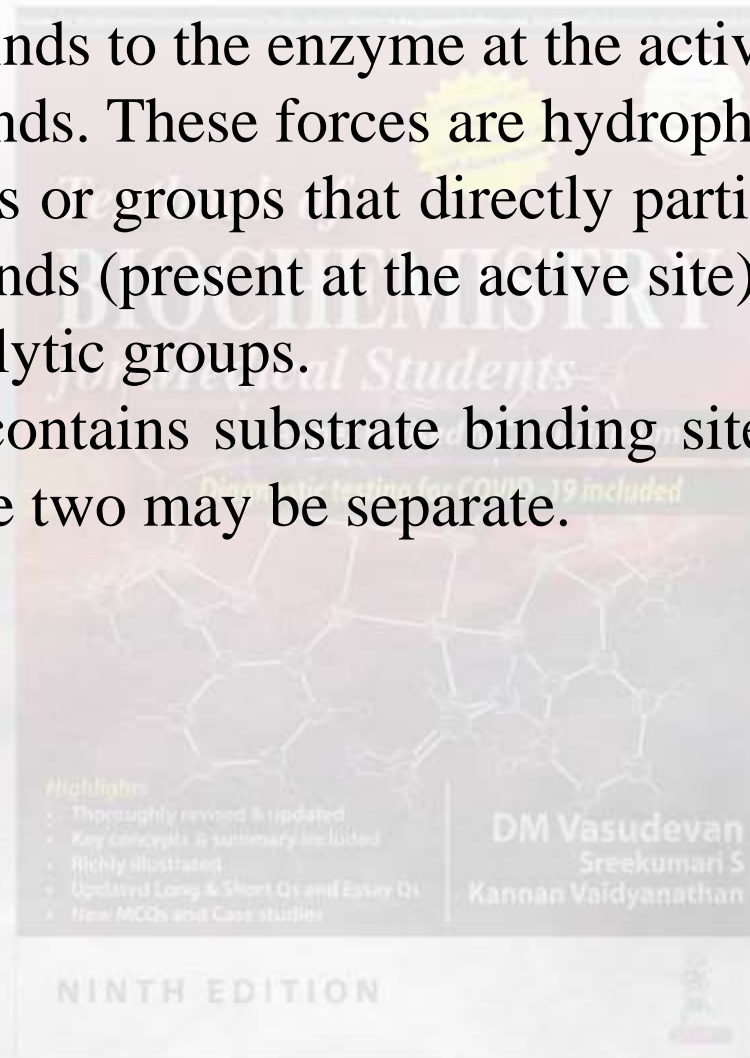
Koshland's induced fit theory.

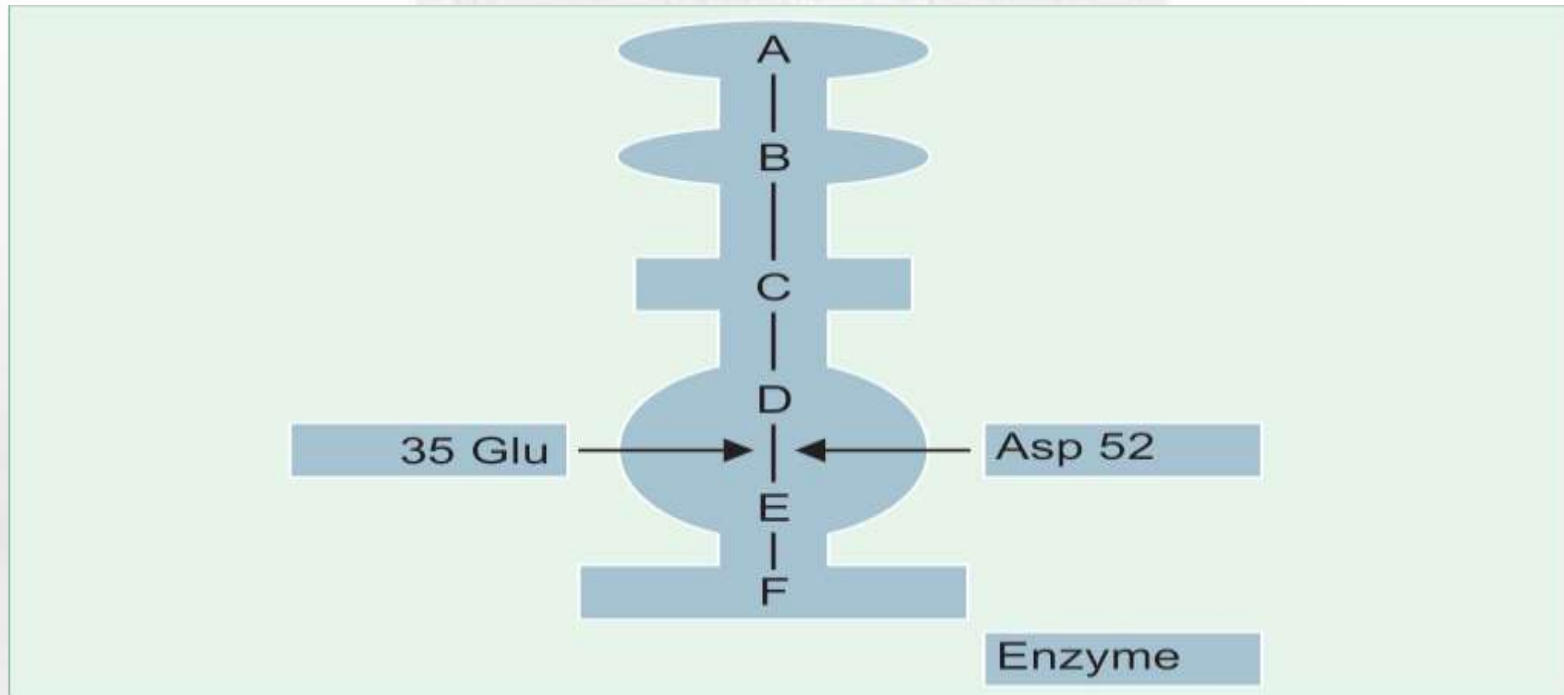
- (A) Enzyme has shallow grooves; substrate alignment is not correct.
- (B) Fixing of substrate induces structural changes in enzyme.
- (C) Now substrate correctly fits into the active site of enzyme.
- (D) Substrate is cleaved into two products

Active center of enzyme

1. The region of the enzyme where substrate binding and catalysis occurs is referred to as active site or active center
2. Although all parts are required for keeping the exact three dimensional structure of the enzyme, the reaction is taking place at the active site. The active site occupies only a small portion of the whole enzyme.
3. Generally active site is situated in a crevice or cleft of the enzyme molecule. To the active site, the specific substrate is bound. The binding of substrate to active site depends on the alignment of specific groups or atoms at active site.
4. During the binding, these groups may realign themselves to provide the unique conformational orientation so as to promote exact fitting of substrate to the active site.

5. The substrate binds to the enzyme at the active site by noncovalent bonds. These forces are hydrophobic in nature.
6. The amino acids or groups that directly participate in making or breaking the bonds (present at the active site) are called catalytic residues or catalytic groups.
7. The active site contains substrate binding site and catalytic site; sometimes these two may be separate.





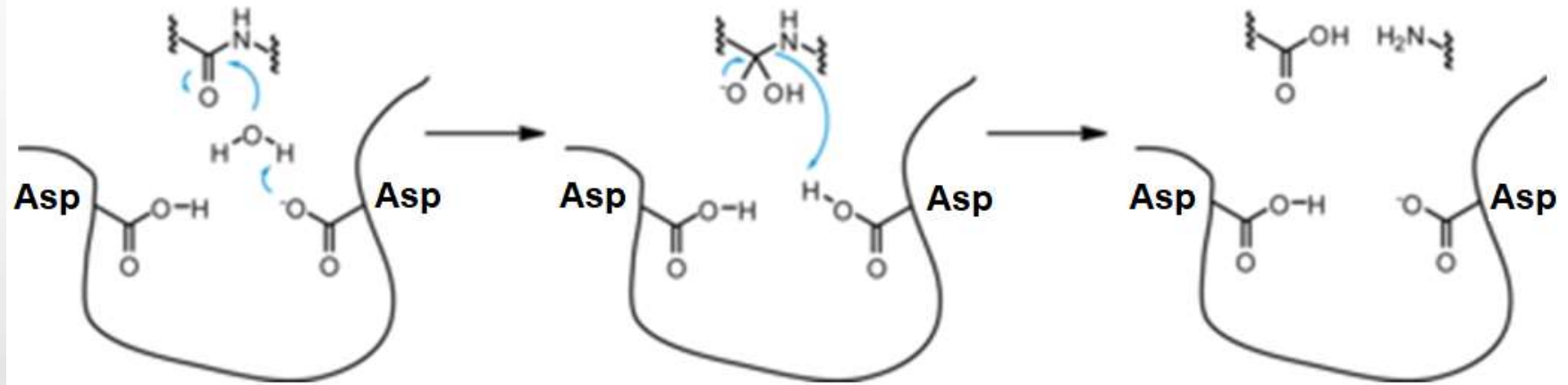
Active center of Lysozyme. A, B, C, D, E, F are carbohydrate units (substrate). D = N-acetyl muramic acid; E= N-acetyl glucosamine. Bond is broken between D and E, with the help of Glu and Asp residues in the enzyme, which are opposite to each other

Name of enzyme	Important amino acid at the catalytic site
Chymotrypsin	His (57), Asp (102), Ser (195)
Trypsin	Serine, Histidine
Thrombin	Serine, Histidine
Phosphoglucomutase	Serine
Alkaline phosphatase	Serine
Acetyl cholinesterase	Serine
Carbonic anhydrase	Cysteine
Hexokinase	Histidine
Carboxypeptidase	Histidine, Arginine, Tyrosine
Aldolase	Lysine

Proteases



Type	Example
Serine proteases	Trypsin, chymotrypsin, clotting factors
Aspartic proteases	Pepsin
Cysteinylnl aspartic proteases	Caspases in apoptosis
Metalloproteases	Carboxy peptidases
Protease inhibitors used as drugs	ACE inhibitor (Captopril), HIV protease inhibitor (Retonavir)



Mechanism of aspartate protease

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1. Exergonic or Exothermic Reaction

Energy is released from the reaction, and therefore reaction goes to completion, e.g. urease enzyme:



2. Isothermic Reaction

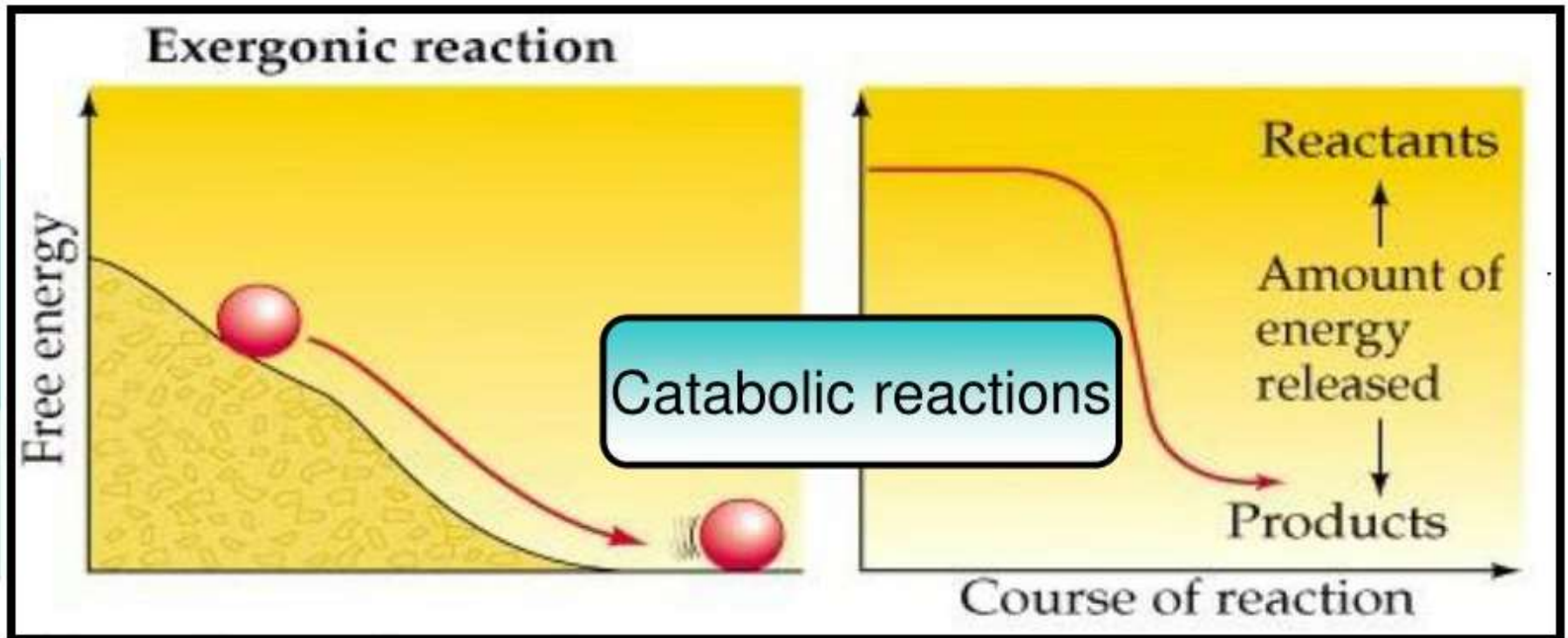
When energy exchange is negligible, and the reaction is easily reversible, e.g.



3. Endergonic or Endothermic Reaction

Energy is consumed and external energy is to be supplied for these reactions for these reactions. In the body this is usually accomplished by coupling the endergonic reaction with an exergonic reaction, e.g. Hexokinase catalyzes the following reaction:

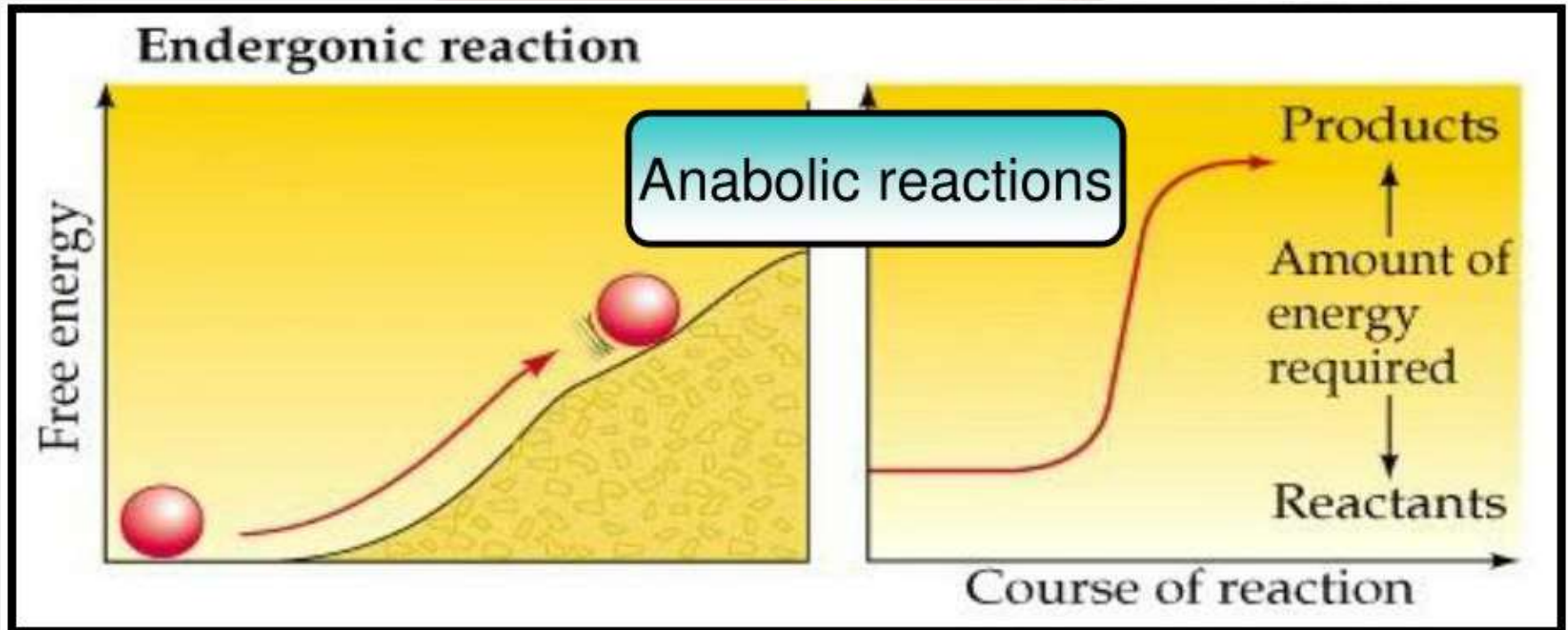




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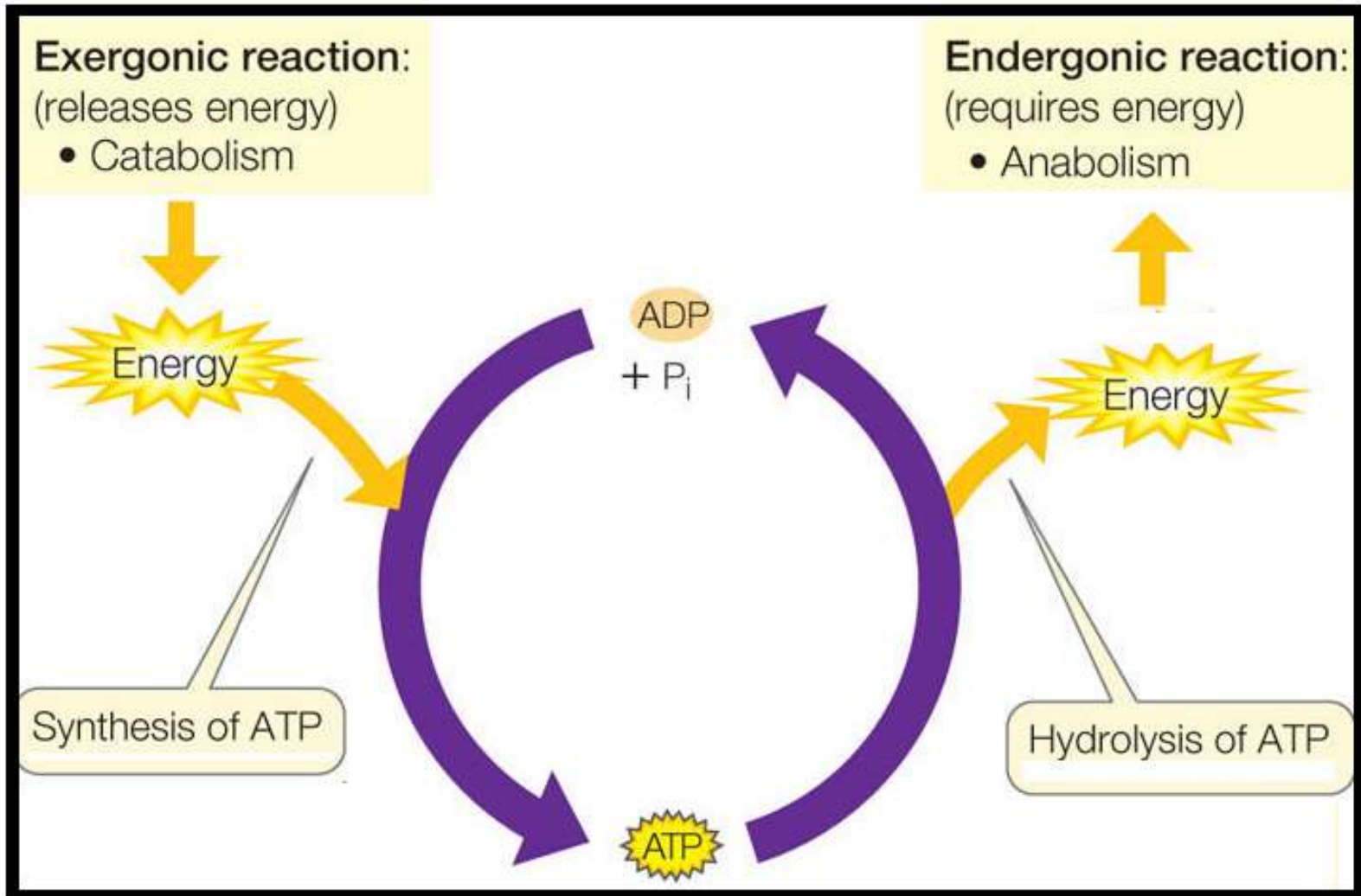
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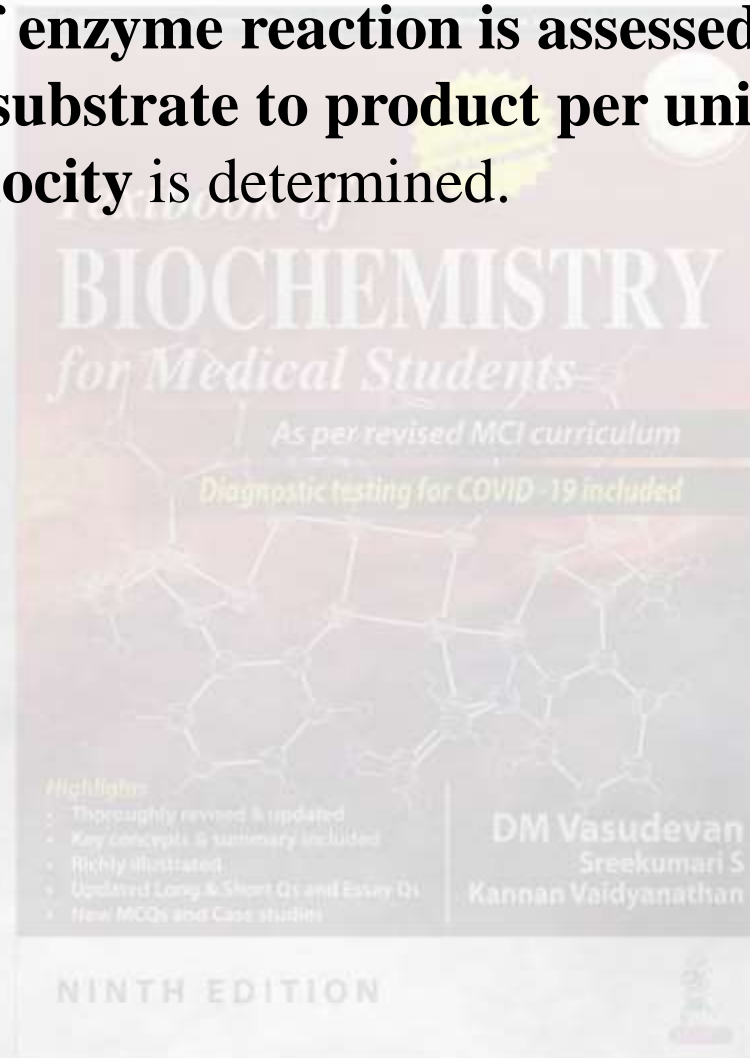
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Body couples exergonic and endergonic reactions for synthetic reactions.

Velocity or rate of enzyme reaction is assessed by the rate of change of substrate to product per unit time. In practice, initial velocity is determined.



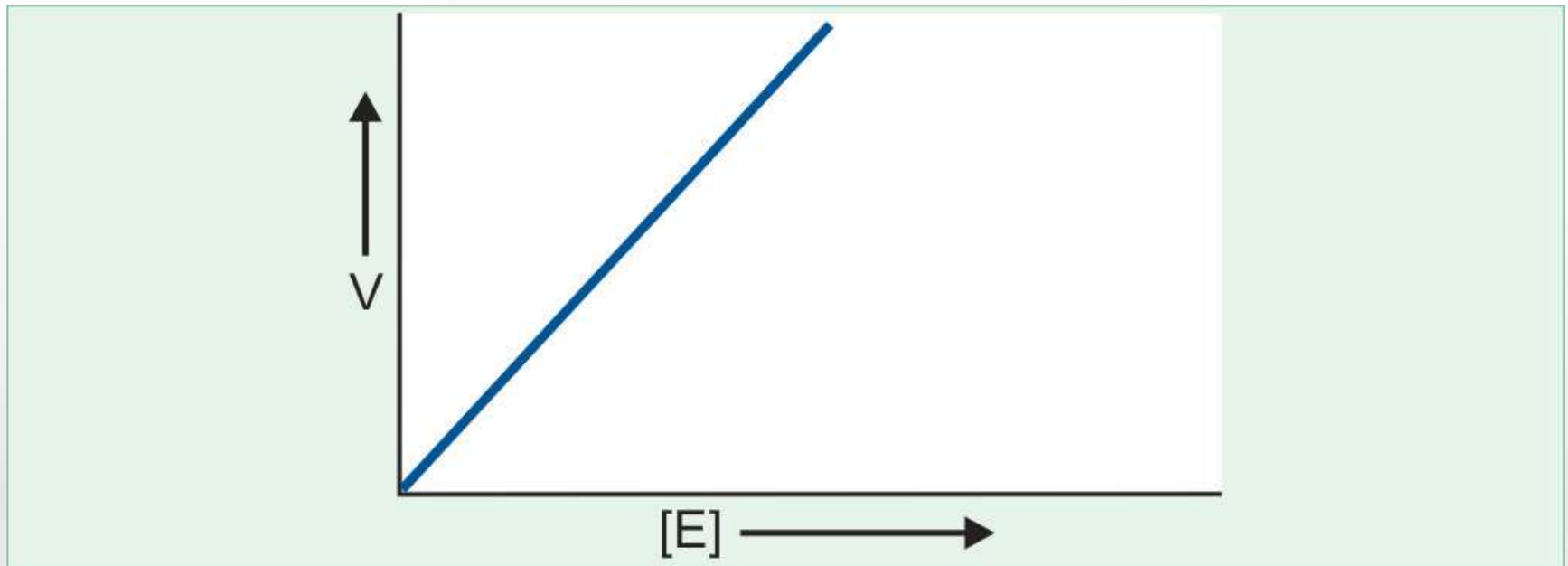
1. The **equilibrium constant** of the reaction is the ratio of reaction rate constants of forward and backward reactions.
2. At equilibrium, forward and backward reactions are equal. Equilibrium is a dynamic state. Even though no net change in concentrations of substrate and product occurs, molecules are always interconverted.
3. Numerical value of the constant can be calculated by finding the concentrations of substrates and products.
4. If K_{eq} is more than 1, the forward reaction is favored. In such instances, the reaction is spontaneous and exothermic.
5. **Concentration of enzyme does not affect the K_{eq} .** Concentration of enzyme certainly increases the rate of reaction; but not the K_{eq} or the ultimate state. In other words, enzyme makes it quicker to reach the equilibrium. **Catalysts increase the rate of reaction, but do not alter the equilibrium.**

Factors Affecting Enzyme Activity

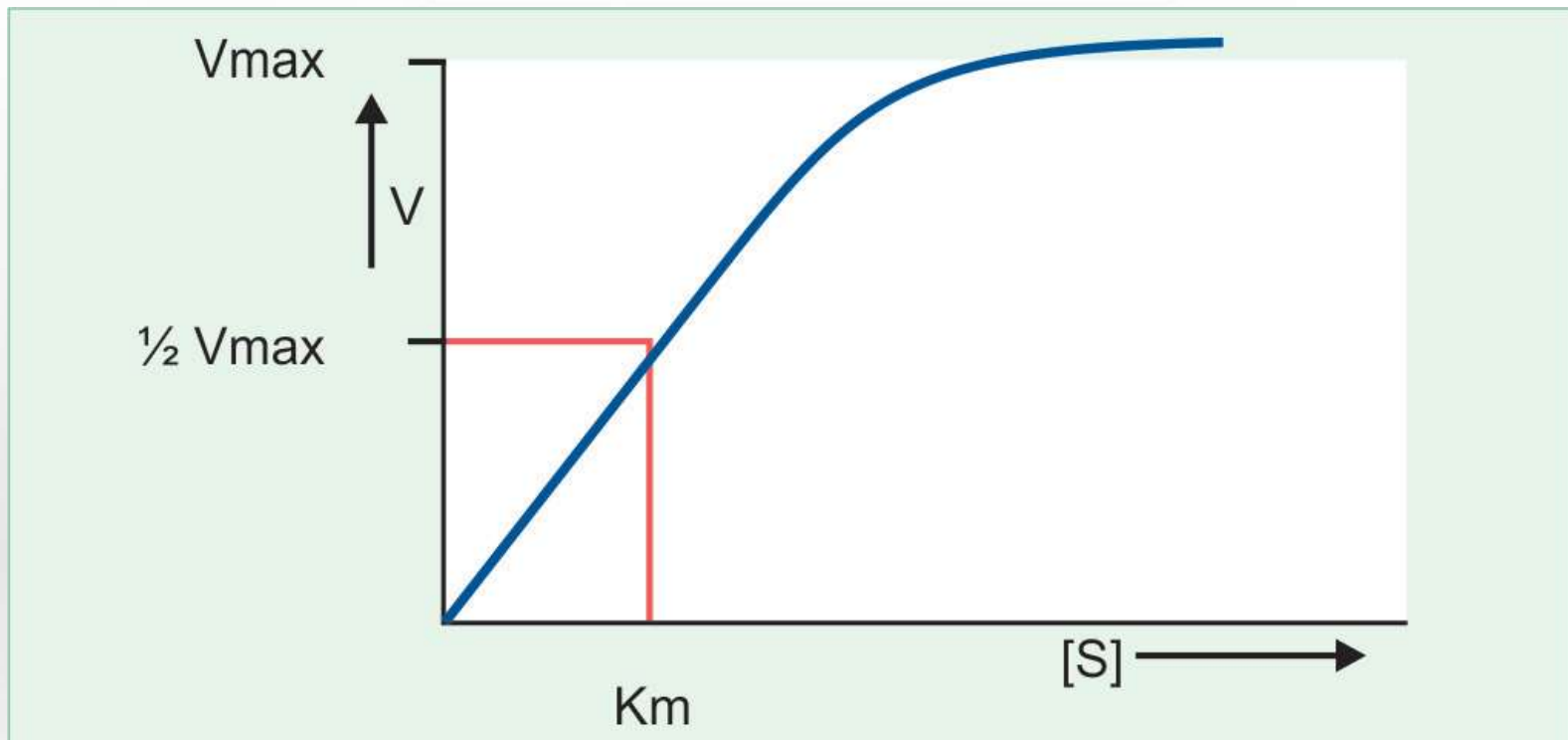


1. Enzyme concentration
2. Substrate concentration
3. Product concentration
4. Temperature
5. Hydrogen ion concentration (pH)
6. Presence of activators
7. Presence of inhibitors
8. Presence of repressor or derepressor
9. Covalent modification



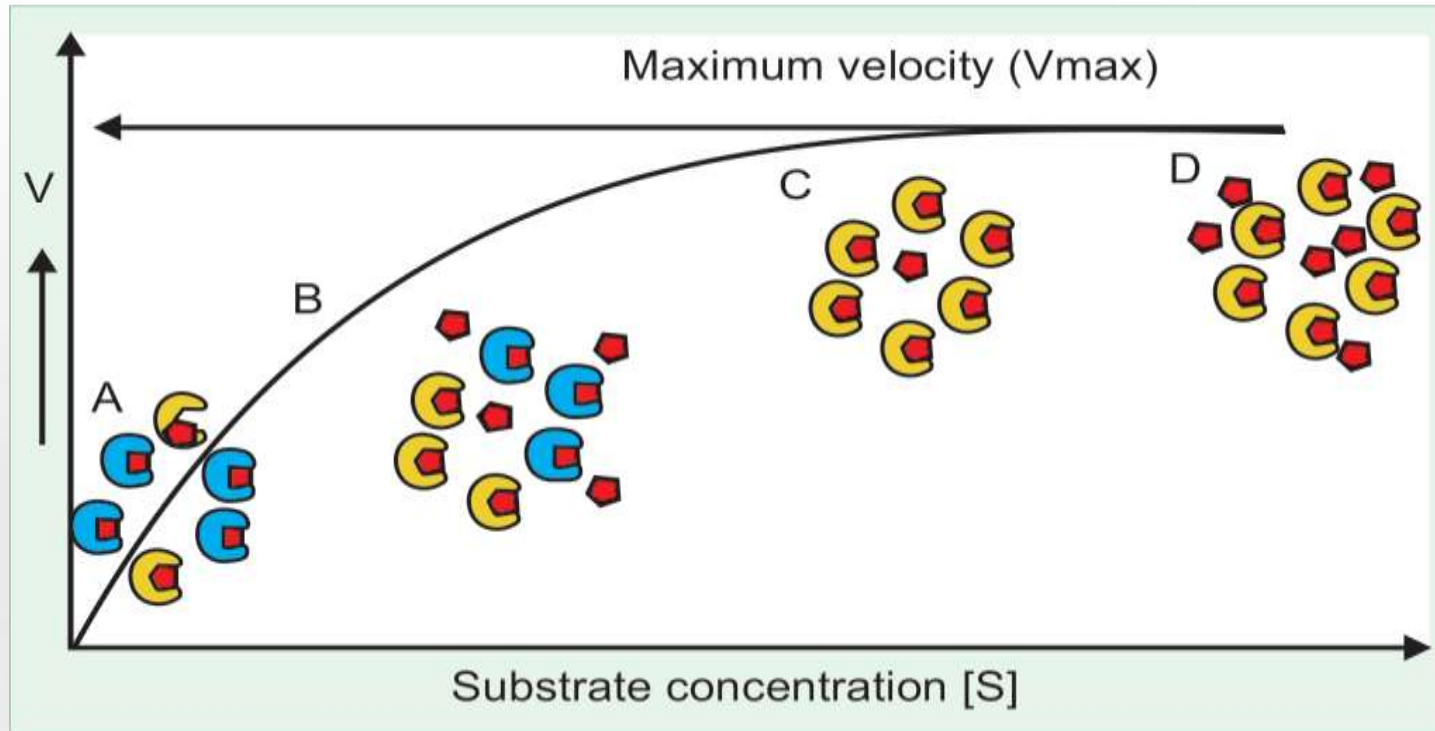


Rate of a reaction or velocity (V) is directly proportional to the enzyme concentration, when sufficient substrate is present. Velocity of reaction increases proportionately with the concentration of enzyme, provided substrate concentration is unlimited.



Effect of substrate concentration (substrate saturation curve).

As substrate concentration is increased, the velocity is also correspondingly increased in the initial phases; but the curve flattens afterwards.



Effect of substrate concentration on enzyme activity.

Enzyme molecules are shown as half-circles. Substrate molecules are red dots. (A) Substrate molecules are low; so only a few enzyme molecules are working and velocity is less. (B) At half-maximal velocity (K_m). 50% enzyme molecules are bound with substrate. (C) As a lot of substrate molecules are available, all enzyme molecules are bound. (D) Further increases in the substrate will not increase the velocity further.

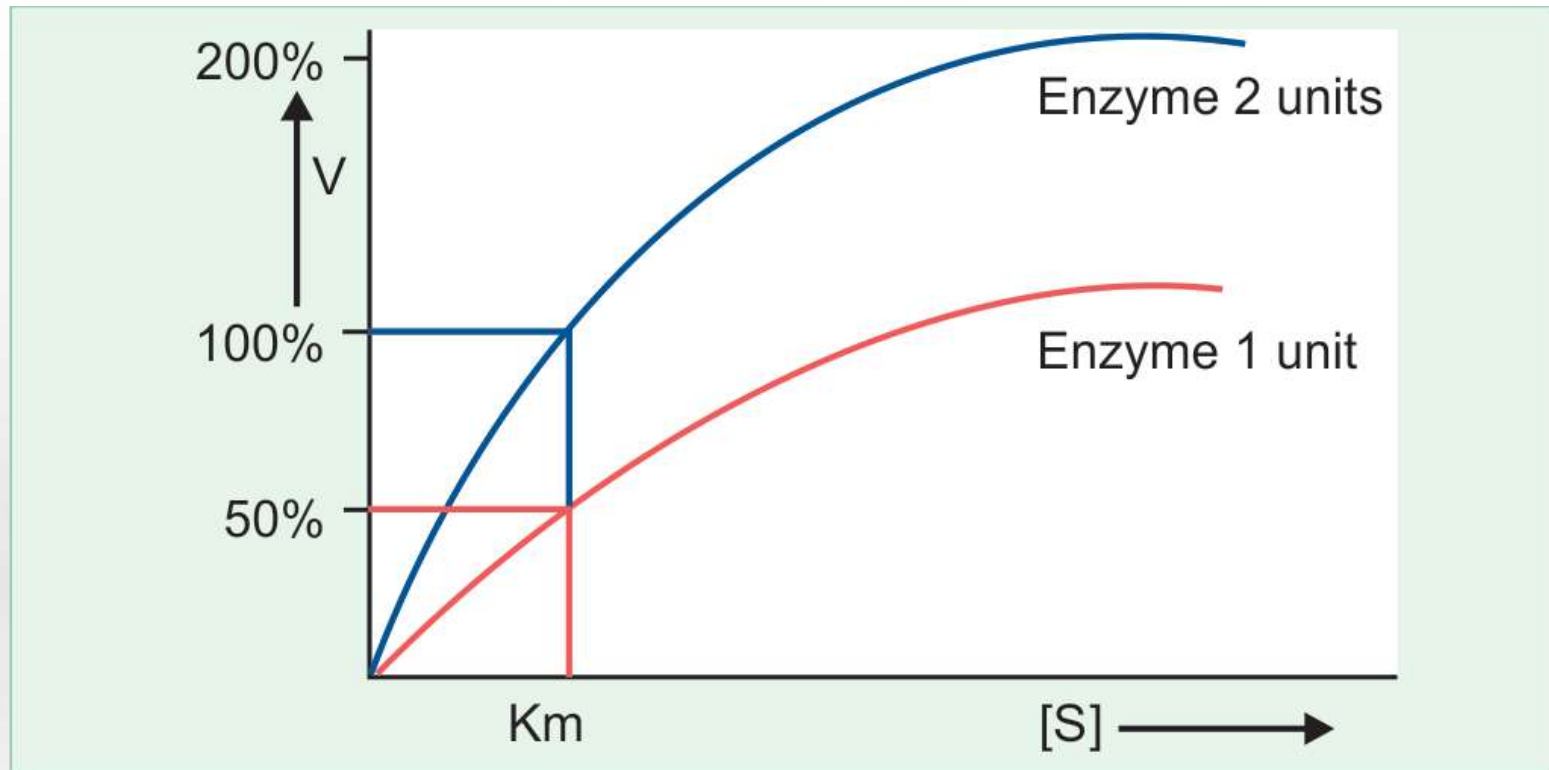
50% velocity in Y axis is extrapolated to the corresponding point on X-axis, which gives the numerical value of K_m . **K_m is independent of enzyme concentration.** If enzyme concentration is doubled, the V_{max} will be double. But the K_m will remain exactly same. Thus, irrespective of enzyme concentration, 50% molecules are bound to substrate at that particular substrate concentration.

The lesser the numerical value of K_m , the affinity of the enzyme for the substrate is more. To cite an example, K_m of glucokinase is 10 mmol/L and that of hexokinase is 0.05 mmol/L. Therefore, 50% molecules of hexokinase are saturated even at a lower concentration of glucose. In other words, hexokinase has more affinity for glucose than glucokinase.

Salient Features of K_m

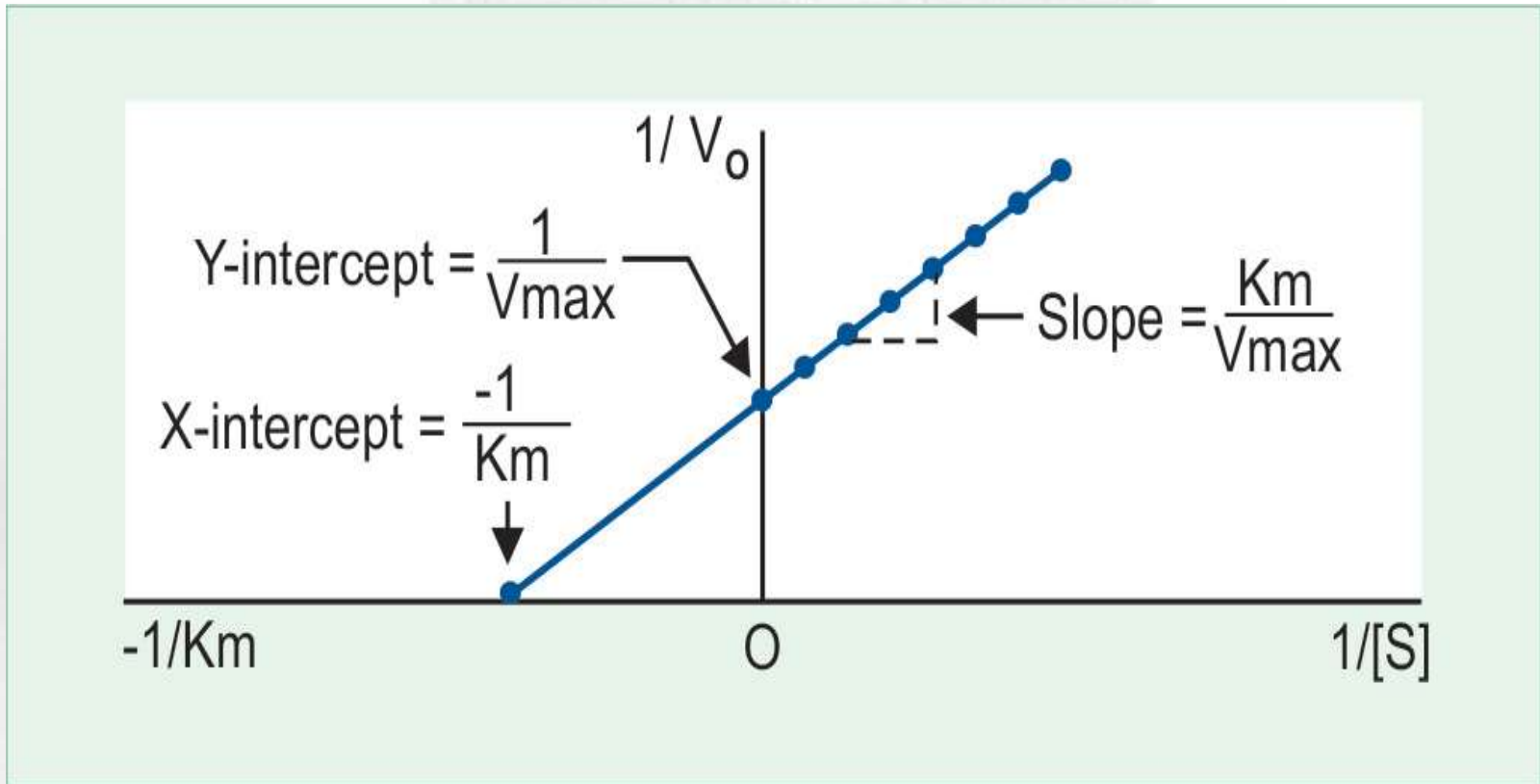


1. **K_m value is substrate concentration** (expressed in moles/L) **at half-maximal velocity.**
2. It denotes that **50% of enzyme molecules are bound with substrate molecules** at that particular substrate concentration.
3. **K_m is independent of enzyme concentration.** If enzyme concentration is doubled, the V_{max} will be double. But the K_m will remain exactly same. In other words, irrespective of enzyme concentration, 50% molecules are bound to substrate at that particular substrate concentration.
4. K_m is the signature of the enzyme. K_m value is thus a constant for an enzyme. It is the **characteristic feature of a particular enzyme** for a specific substrate.
5. **K_m denotes the affinity of enzyme for substrate.** The lesser the numerical value of K_m , the affinity of the enzyme for the substrate is more



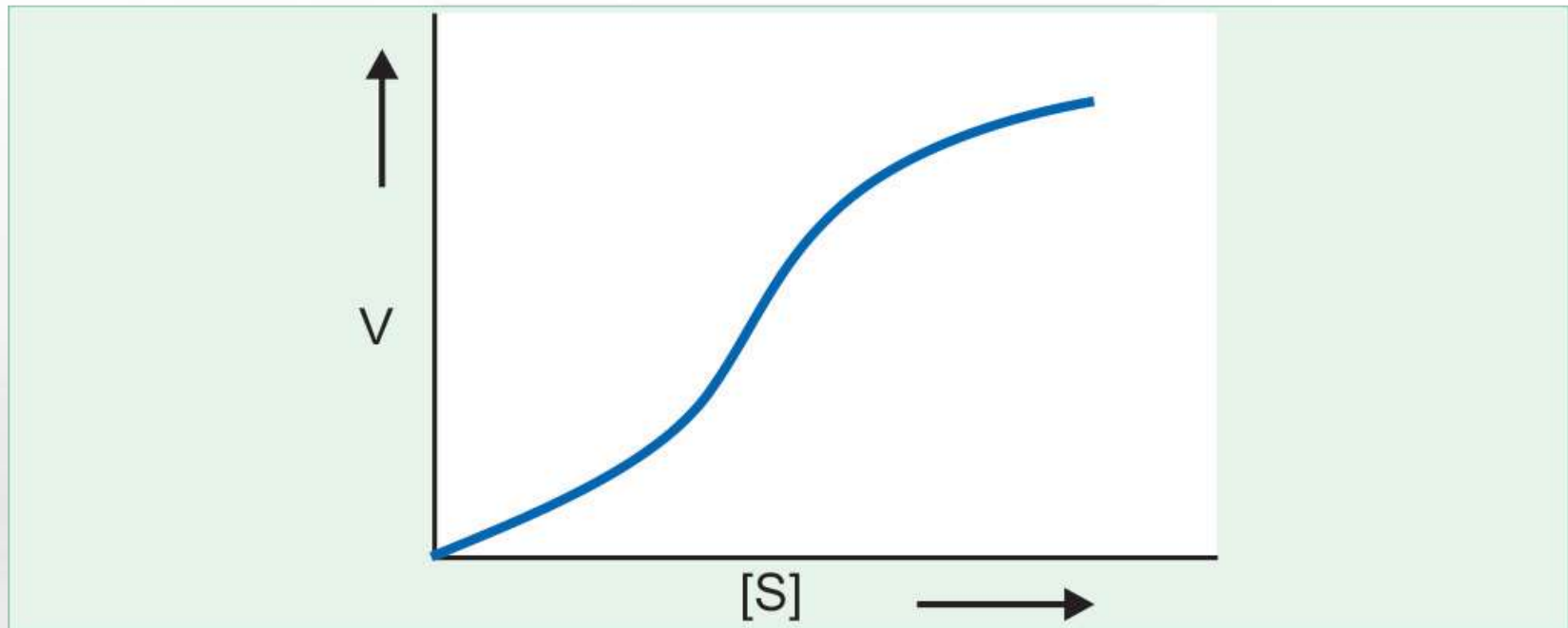
Effect of enzyme concentration on K_m .

K_m is independent of enzyme concentration. If enzyme concentration is doubled, the V_{max} will be double. But the K_m will remain exactly same.



Lineweaver-Burk Plot or Double Reciprocal Plot

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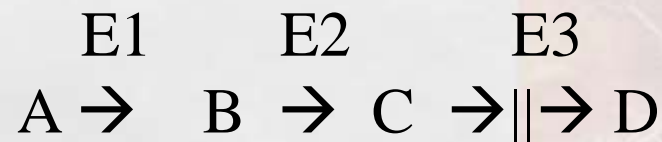
Sigmoid substrate-saturation kinetics of cooperative binding. When the enzyme has many subunits, the **binding of substrate to one unit enhances the affinity for binding to other subunits**. This is called co-operative binding. In such cases, the saturation curve will be sigmoid shaped. Oxygen binding to hemoglobin is a good example.

Effect of Concentration of Products

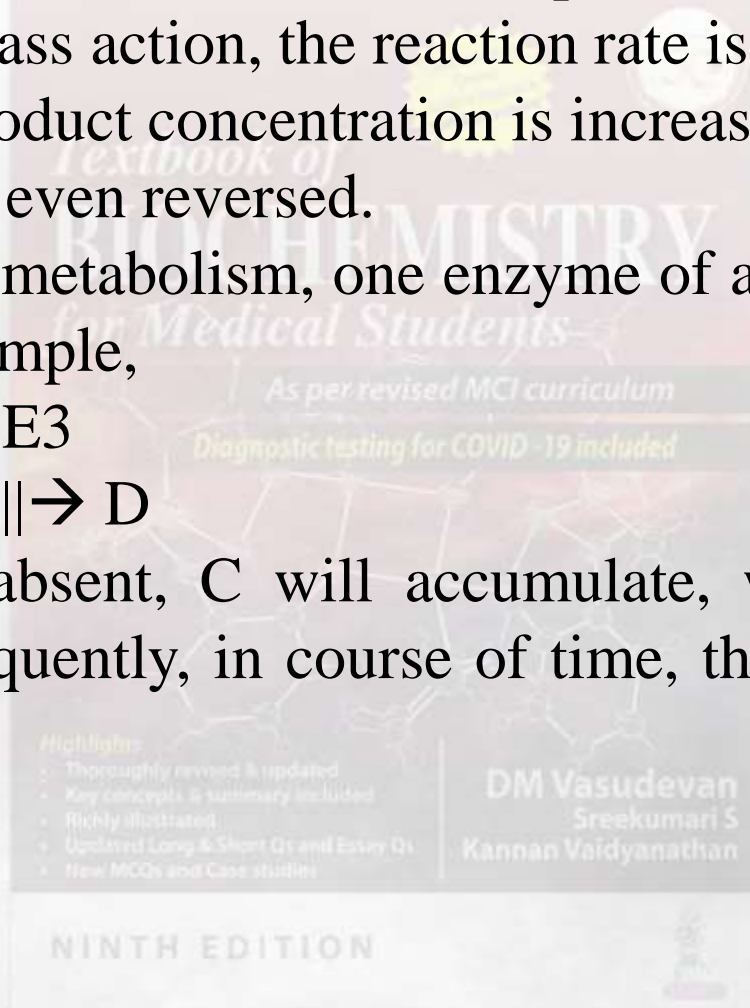


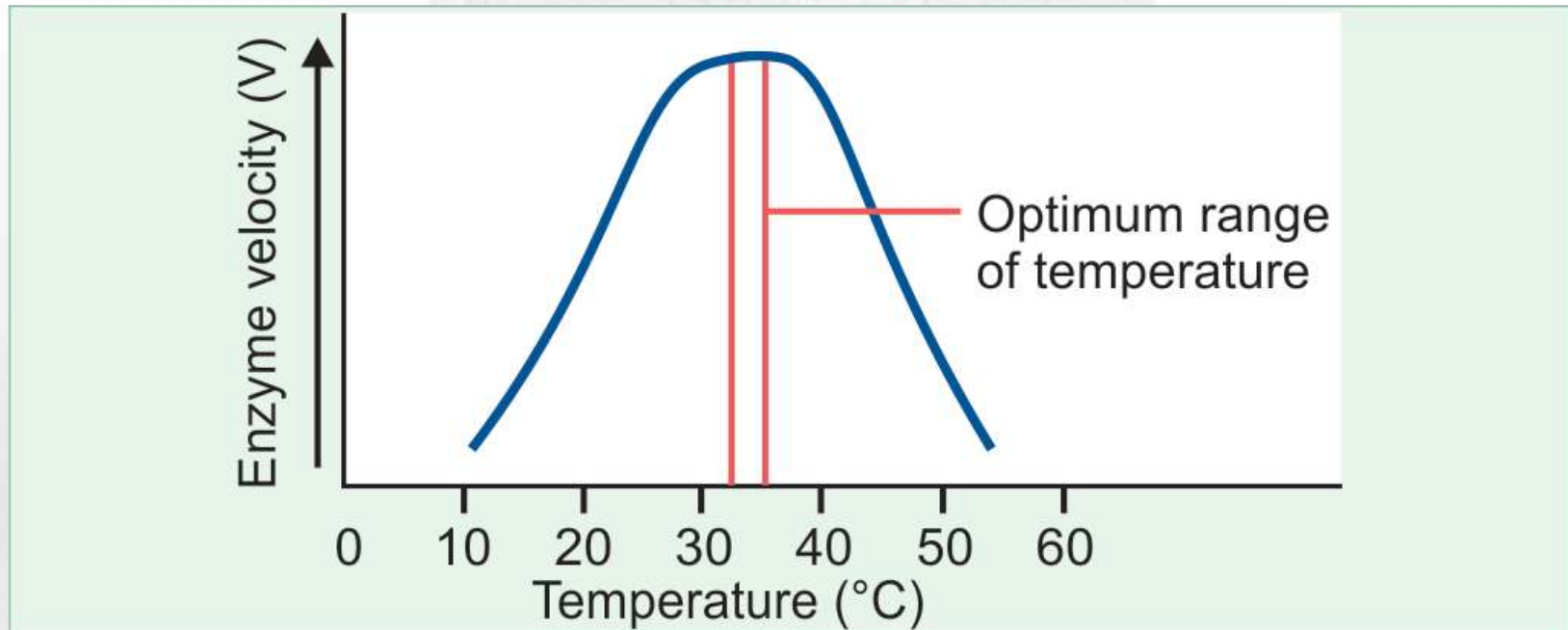
In a reversible reaction, $S \rightleftharpoons P$, when equilibrium is reached, as per the law of mass action, the reaction rate is slowed down. So, when product concentration is increased, the reaction is slowed, stopped or even reversed.

In inborn errors of metabolism, one enzyme of a metabolic pathway is blocked. For example,



If E3 enzyme is absent, C will accumulate, which in turn, will inhibit E2. Consequently, in course of time, the whole pathway is blocked.



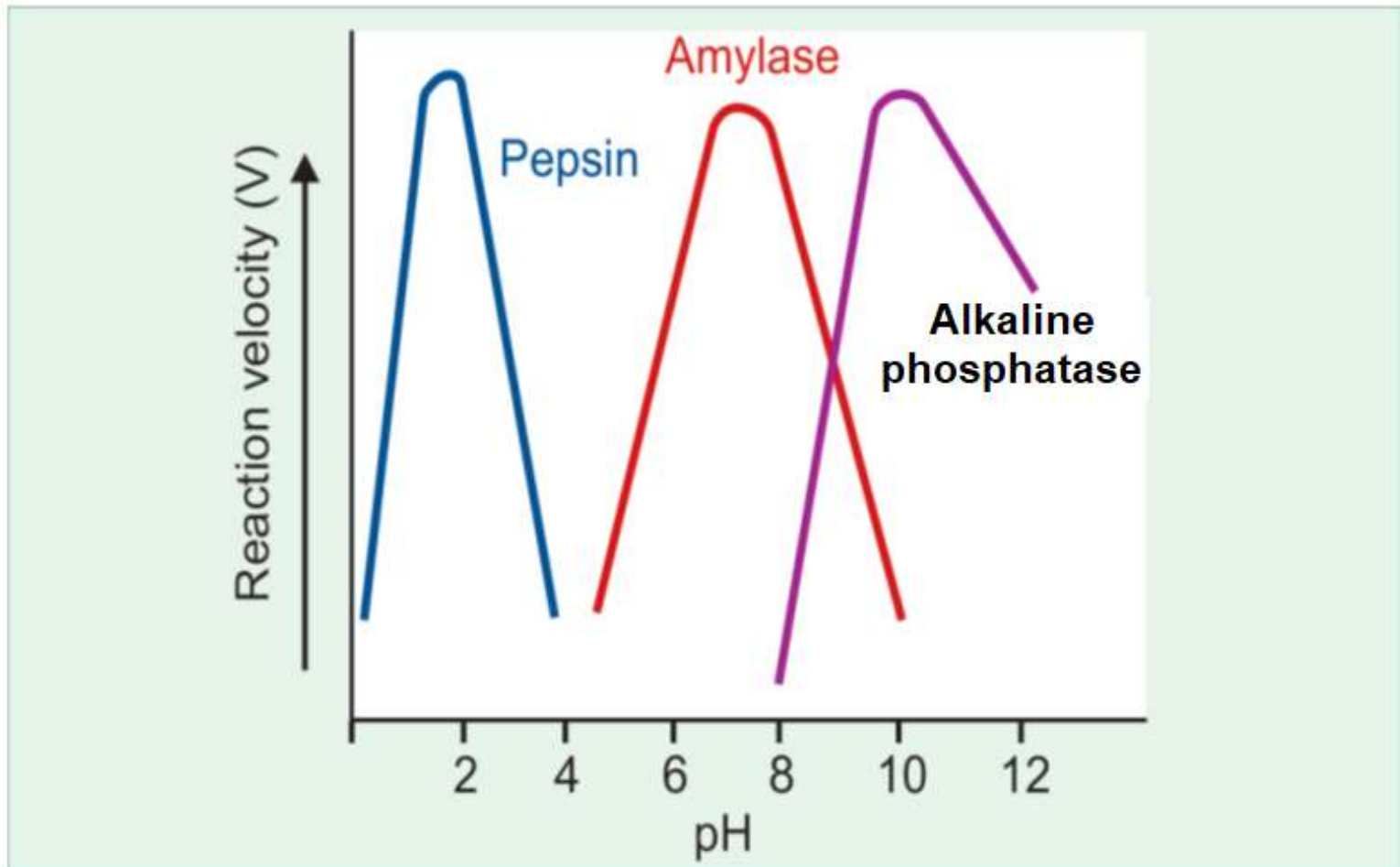


Effect of temperature on velocity.

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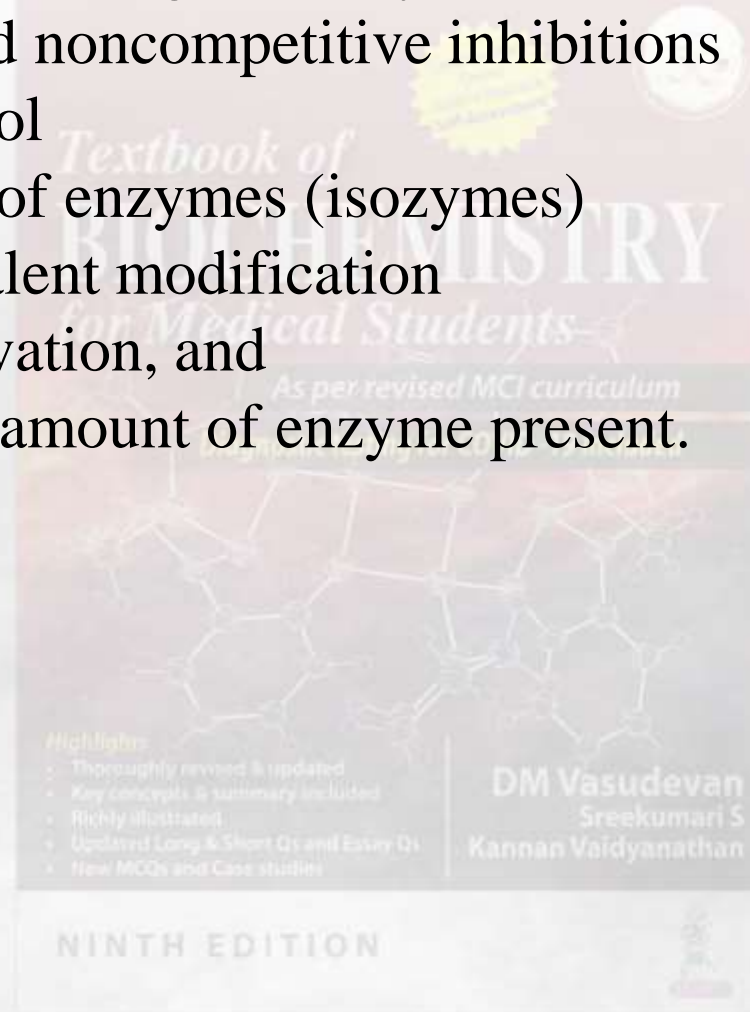
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Effect of pH on enzyme velocity.

Enzyme activity can be regulated by the following methods:

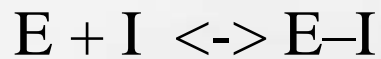
1. Competitive and noncompetitive inhibitions
2. Allosteric control
3. Multiple forms of enzymes (isozymes)
4. Reversible covalent modification
5. Proteolytic activation, and
6. Controlling the amount of enzyme present.



Competitive Inhibition

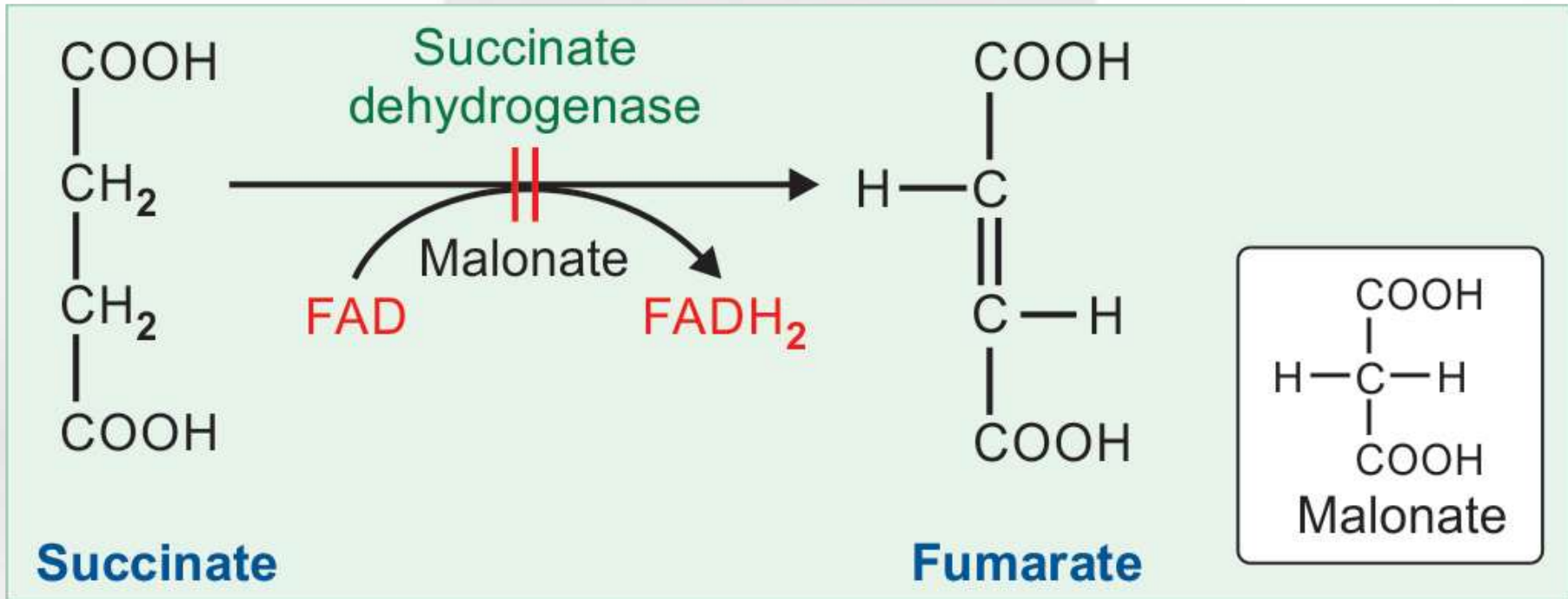


Inhibitor molecules are competing with the normal substrate molecules for binding to the active site of the enzyme, because the inhibitor is a structural analog of the substrate.

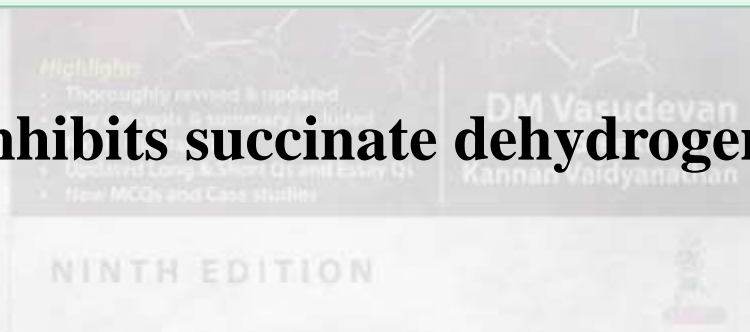


Since E-I (enzyme–inhibitor complex) can react only to reform the enzyme and inhibitor, the number of enzyme molecules available for E-S formation is reduced. Suppose 100 molecules of substrate and 100 molecules of inhibitor are competing for 100 molecules of the enzyme. So, half of enzyme molecules are trapped by the inhibitor and only half the molecules are available for catalysis to form the product. Since effective concentration of enzyme is reduced, the reaction **velocity is decreased**.

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Malonate inhibits succinate dehydrogenase enzyme

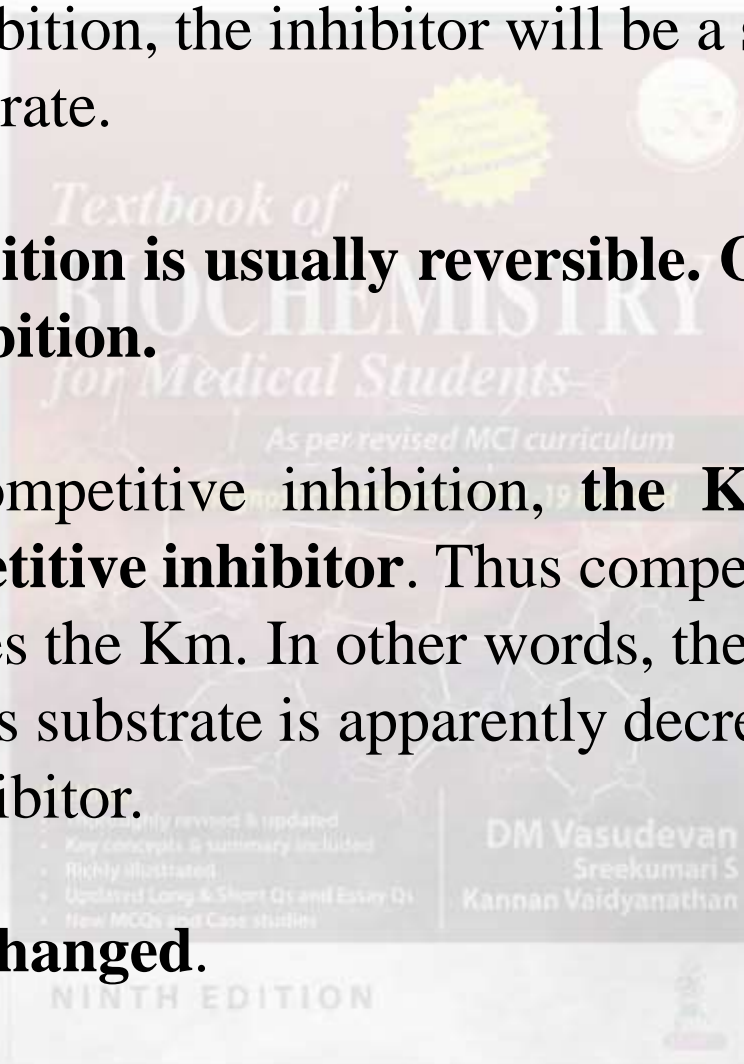


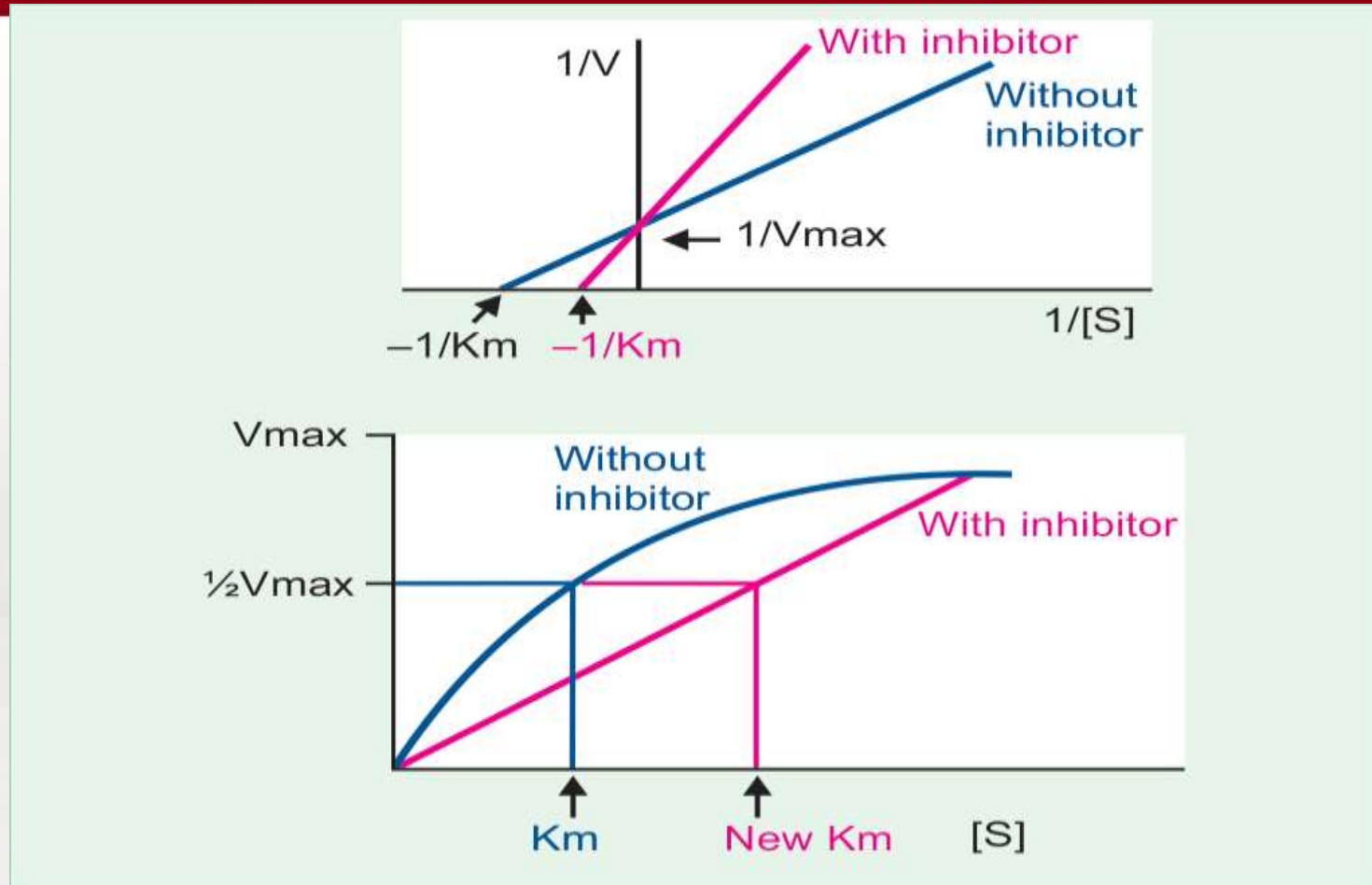
In competitive inhibition, the inhibitor will be a **structural analog** of the substrate.

Competitive inhibition is usually reversible. Or, excess substrate abolishes the inhibition.

In the case of competitive inhibition, **the K_m is increased in presence of competitive inhibitor**. Thus competitive inhibitor apparently increases the K_m . In other words, the affinity of the enzyme towards substrate is apparently decreased in presence of the inhibitor.

But V_{max} is not changed.





Substrate saturation curve in presence and absence of competitive inhibitor. (A) Lineweaver-Burk Plot; (B) Michaelis Menten Saturation Curve.

Clinical importance of competitive inhibition

- 1. Sulfonamides:** They are commonly employed antibacterial agents. Bacteria synthesize folic acid by combining PABA with pteroyl glutamic acid. Sulfa drugs, being structural analog of PABA, will inhibit the folic acid synthesis in bacteria, and they die.
- 2. Methotrexate** is a structural analogue of folic acid, and so can competitively inhibit folate reductase enzyme. Therefore, methotrexate is used as an anticancer drug.
- 3. Dicoumarol:** It is structurally similar to vitamin K and can act as an anticoagulant by competitively inhibiting the vitamin K activity.
- 4. Isonicotinic acid hydrazide (INH)** is an antituberculous drug. It is structurally similar to pyridoxal, and prolonged use of INH may cause pyridoxal deficiency and peripheral neuropathy.

Clinically Useful Competitive Inhibitors



<i>Drug</i>	<i>Enzyme inhibited</i>	<i>Clinical use</i>
Allopurinol	Xanthine oxidase	Gout
Dicoumarol	Vitamin K epoxide reductase	Anticoagulant
Penicillin	Transpeptidase	Antibacterial
Sulfonamide	Pteroid synthetase	Antibacterial
Trimethoprim	FH ₂ -reductase	Antibacterial
Pyrimethamine	FH ₂ -reductase	Antimalarial
Methotrexate	FH ₂ -reductase	Anticancer
6-mercaptopurine	Adenylosuccinate synthetase	Anticancer
5-fluorouracil	Thymidylate synthase	Anticancer

Clinically Useful Competitive Inhibitors



<i>Drug</i>	<i>Enzyme inhibited</i>	<i>Clinical use</i>
Azaserine	Phosphoribosylamidotransferase	Anticancer
Cytosine arabinoside	DNA polymerase	Anticancer
Acyclovir	DNAP of virus	Antiviral
Neostigmine	ACh-esterase	Myasthenia
Alpha-methyldopa	Dopa decarboxylase	Hypertension
Lovastatin	HMGCoA reductase	Cholesterol lowering
Oseltamiver (Tamiflu)	Neuraminidase	Influenza

Noncompetitive Inhibition (Irreversible)



There is no competition between substrate and inhibitor. The inhibitor usually binds to a different domain on the enzyme, other than the substrate binding site.

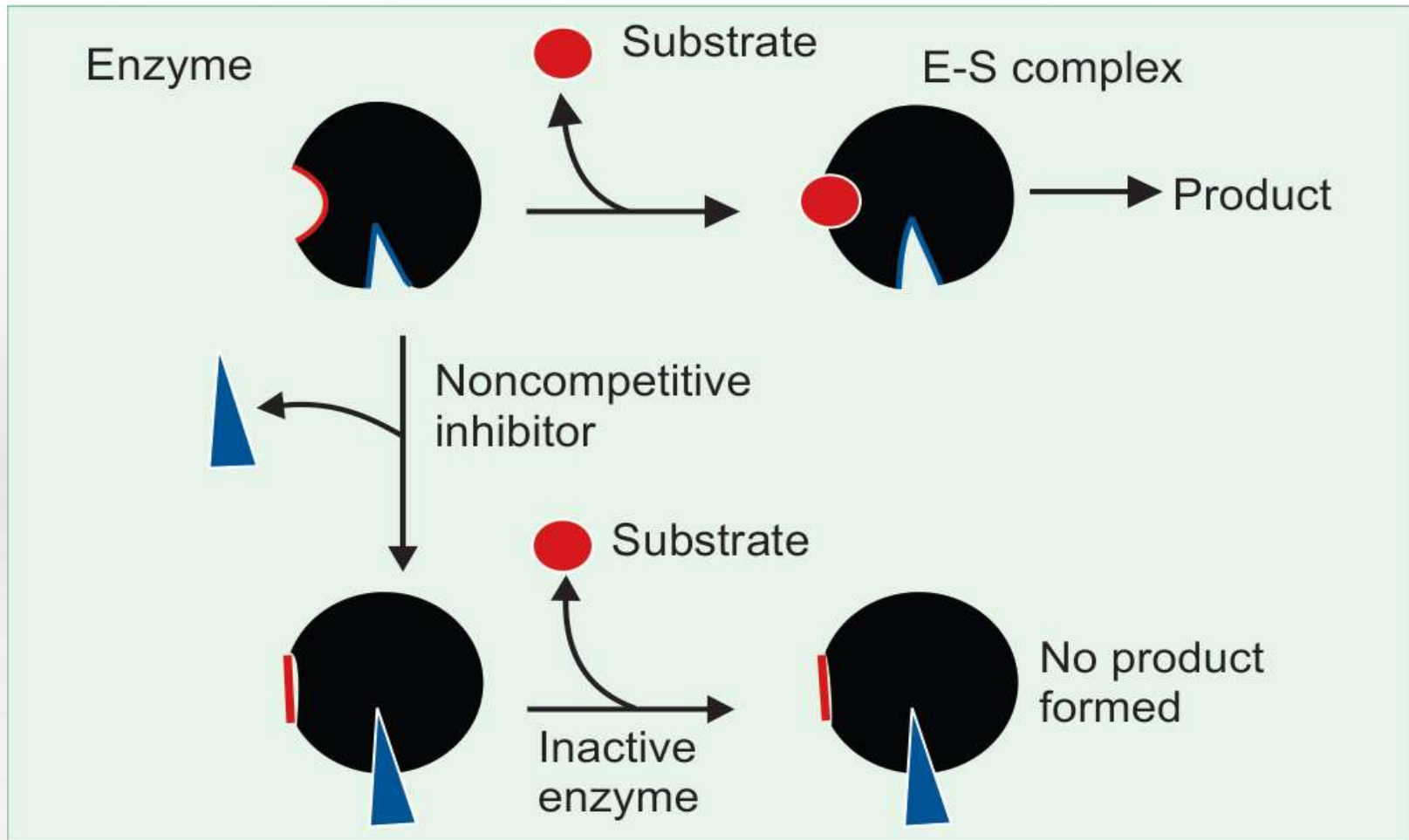
Since these inhibitors have no structural resemblance to the substrate, an **increase in the substrate concentration generally does not relieve this inhibition**. Examples are:

(a) **Cyanide** inhibits cytochrome oxidase.

(b) **Fluoride** will remove magnesium and manganese ions and so will inhibit the enzyme, enolase, and consequently the glycolysis.

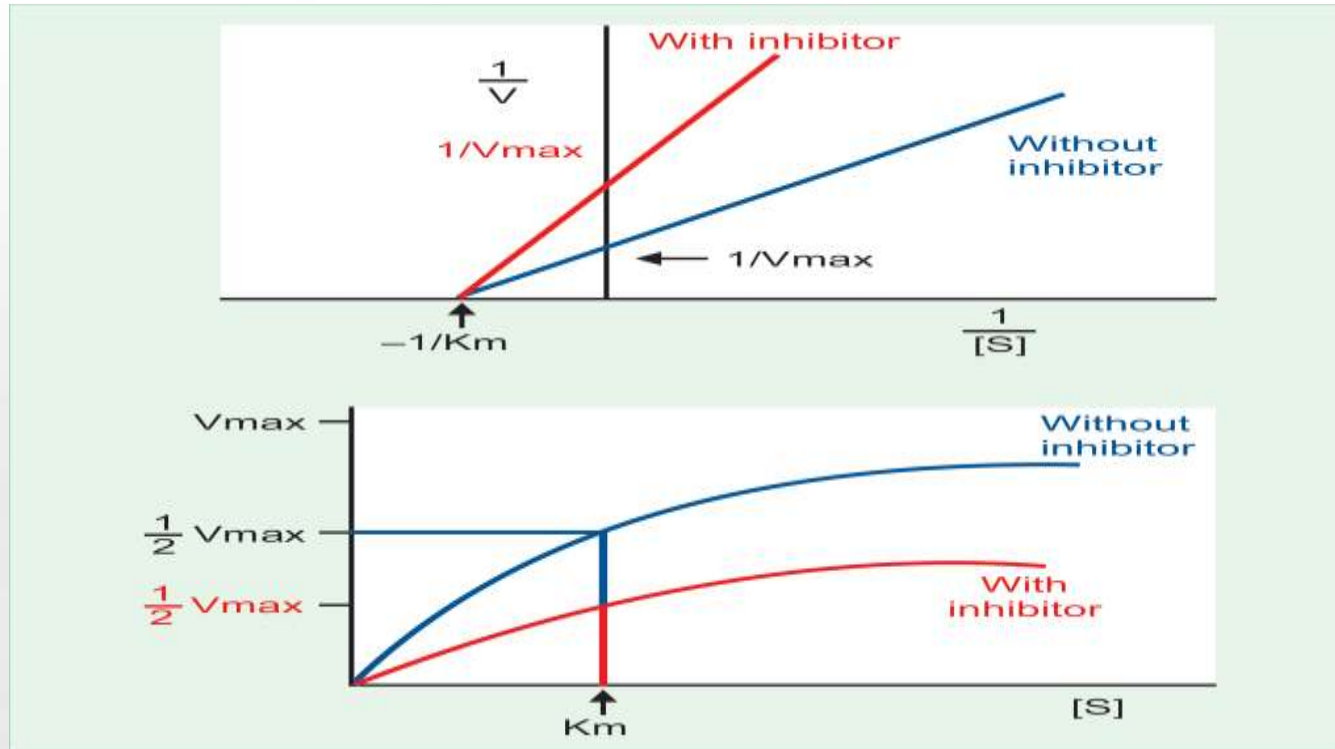
(c) **Iodoacetate** would inhibit enzymes having -SH group in their active centers.

(d) **BAL** (British Anti Lewisite; dimercaprol) is used as an antidote for heavy metal poisoning.



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Non-competitive inhibition



Saturation curve of non-competitive inhibition. (A) Lineweaver-Burk Plot; (B) Michaelis-Menten Saturation Curve. The velocity (V_{max}) is reduced. But **K_m value is not changed**, because the remaining enzyme molecules have the same affinity for the substrate.

Comparison of Two Types of Inhibition



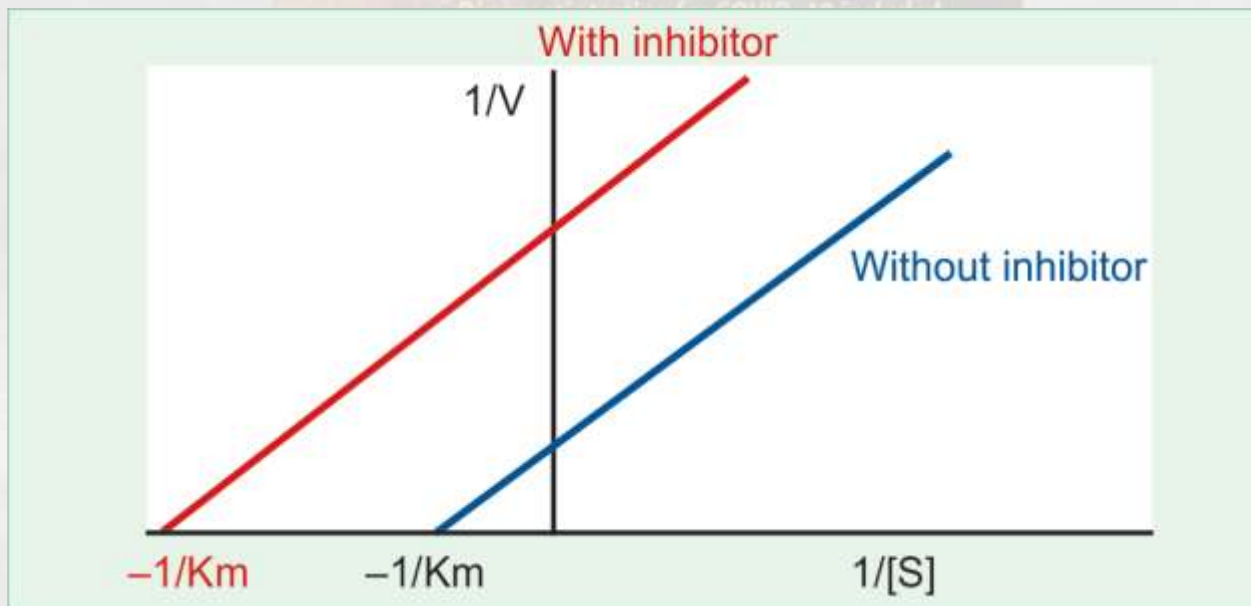
	<i>Competitive inhibition</i>	<i>Noncompetitive inhibition</i>
Acting on	Active site	May or may not
Structure of inhibitor	Substrate analog	Unrelated molecule
Inhibition is	Reversible	Generally irreversible
Excess substrate	Inhibition relieved	No effect
K _m	Increased	No change
V _{max}	No change	Decreased
Significance	Drug action	Toxicological

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Uncompetitive Inhibition



Inhibitor does not have any affinity for free enzyme. Inhibitor binds to enzyme–substrate complex; but not to the free enzyme. In such cases both V_{max} and K_m are decreased. Inhibition of placental alkaline phosphatase (Regan isoenzyme) by phenylalanine is an example of uncompetitive inhibition.



Suicide Inhibition



It is also known as **mechanism-based inactivation**. The inhibitor makes use of the enzyme's own reaction mechanism to inactivate it. In suicide inhibition, the structural analog is converted to a more effective inhibitor with the help of the enzyme to be inhibited.

For example, **ornithine decarboxylase** (ODC) catalyzes the conversion of ornithine to putrescine which is necessary for polyamine synthesis. When the ODC in trypanosoma is inhibited multiplication of the parasite is arrested. Therefore, inhibitors of ODC enzyme such as difluoro methyl ornithine (DFMO) have been found to be effective against **trypanosomiasis** (sleeping sickness).

A similar mechanism is observed in the case of **Allopurinol** which is oxidized by xanthine oxidase to alloxanthine that is a strong inhibitor of xanthine oxidase.

The anti-inflammatory action of **Aspirin** is also based on the suicide inhibition. Arachidonic acid is converted to prostaglandin by the enzyme **Cyclo-oxygenase**. Aspirin acetylates a serine residue in the active center of cyclo-oxygenase, thus prostaglandin synthesis is inhibited, and so inflammation subsides.

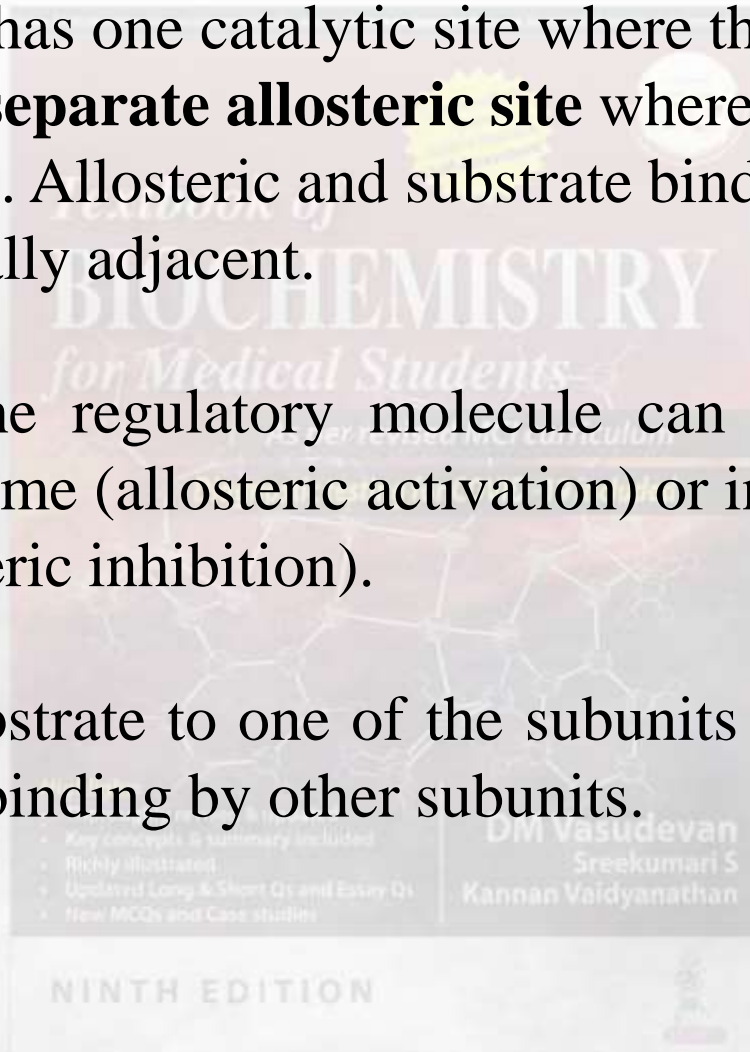
Allosteric Regulation



Allosteric enzyme has one catalytic site where the substrate binds and another **separate allosteric site** where the modifier binds (allo = other). Allosteric and substrate binding sites may or may not be physically adjacent.

The binding of the regulatory molecule can either enhance the activity of the enzyme (allosteric activation) or inhibit the activity of the enzyme (allosteric inhibition).

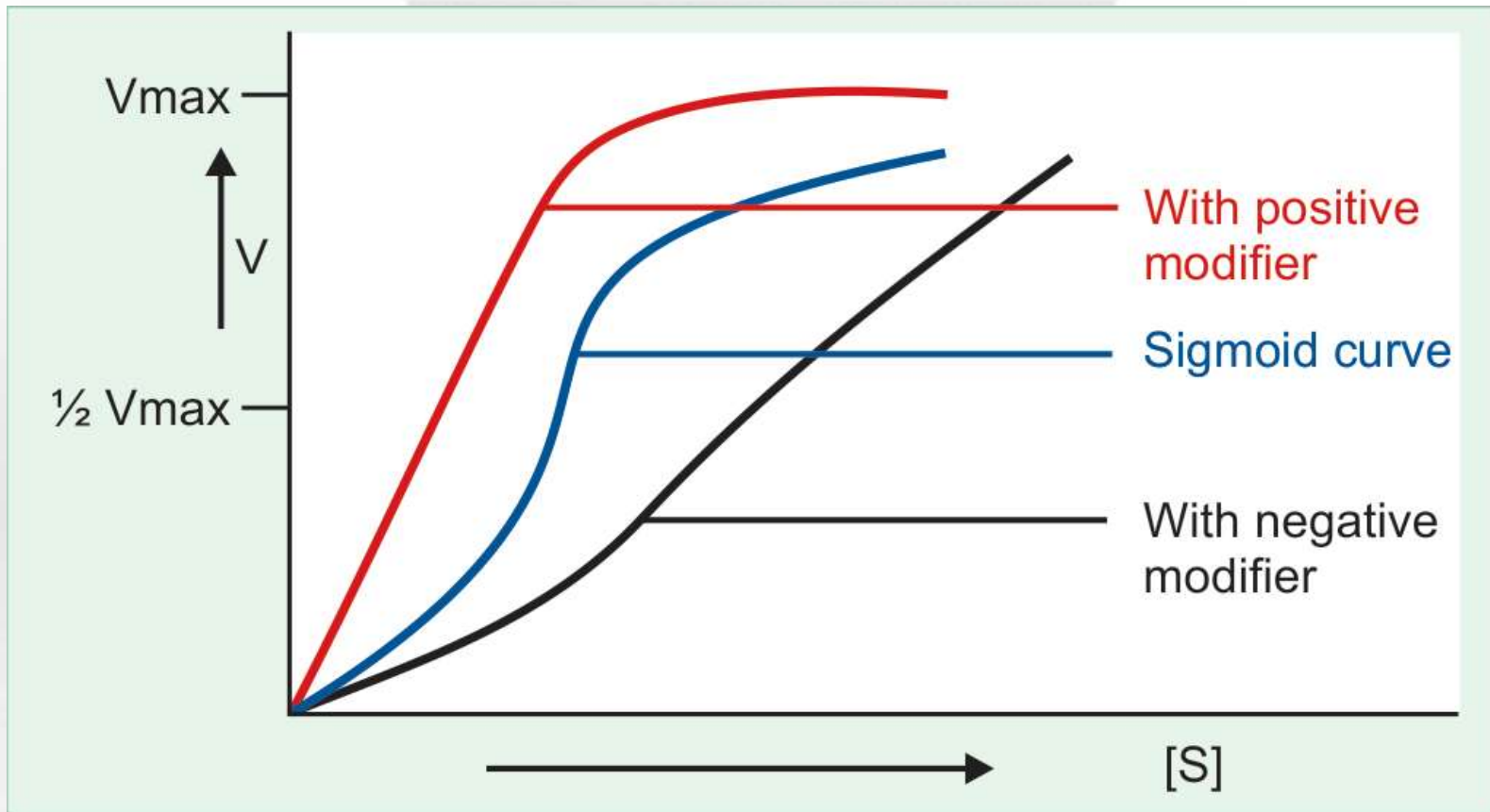
The binding of substrate to one of the subunits of the enzyme may enhance substrate binding by other subunits.





Action of allosteric enzymes. The enzyme has separate active site and allosteric site. Figure 1 depicts that the activator is fixed at the allosteric site. When active site has correct conformation, and the substrate is correctly fixed. Figure 2 shows that the inhibitor is fixed at the allosteric site when active site is deformed and the substrate could not fix.

(A: active site; Allo: allosteric; S: substrate; AA: allosteric activator; In: inhibitor site; Aln: allosteric inhibitor).

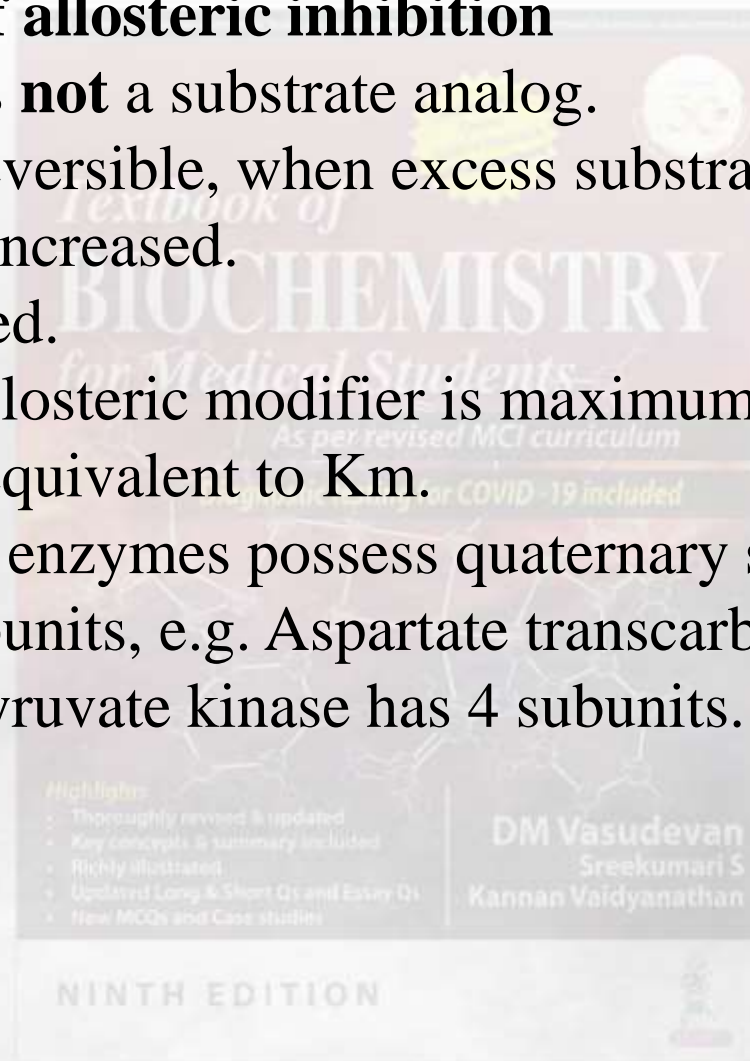


Saturation curve of allosteric inhibition

Updated Long & Short Q1 and Essay Q1
New MCQs and Case studies
Kannan Vaidyanathan
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Salient features of allosteric inhibition

1. The inhibitor is **not** a substrate analog.
2. It is partially reversible, when excess substrate is added.
3. K_m is usually increased.
4. V_{max} is reduced.
5. The effect of allosteric modifier is maximum at or near substrate concentration equivalent to K_m .
6. Most allosteric enzymes possess quaternary structure. They are made up of subunits, e.g. Aspartate transcarbamoylase has 6 subunits and pyruvate kinase has 4 subunits.



Examples of allosteric enzymes

<i>Enzyme</i>	<i>Allosteric inhibitor</i>	<i>Allosteric activator</i>
ALA synthase	Heme	--
Aspartate transcarbamoylase	CTP	ATP
HMGCoA reductase	Cholesterol	--
Phosphofructokinase	ATP, citrate	AMP, F-2,6-P
Pyruvate carboxylase	ADP	Acetyl CoA
Acetyl CoA carboxylase	Acyl CoA	Citrate
Citrate synthase	ATP	
Carbamoyl phosphate synthetase I	NAG	
Carbamoyl phosphate synthetase II	UTP	

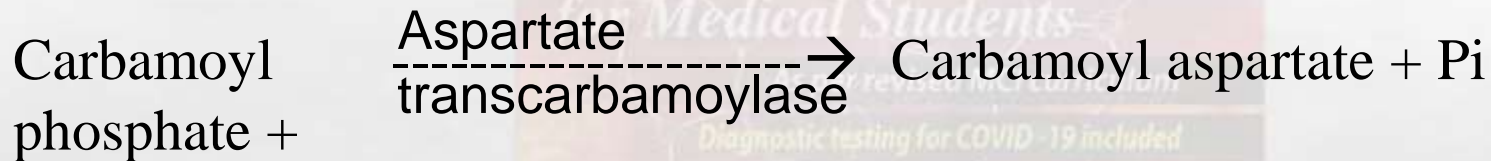
Feedback Inhibition



The term feedback inhibition means that the activity of the enzyme is inhibited by the product of the reaction. Inhibition of hexokinase by glucose-6-phosphate is an example.



End product inhibition



Aspartate

(CTP, the end product will allosterically inhibit aspartate transcarbamoylase)



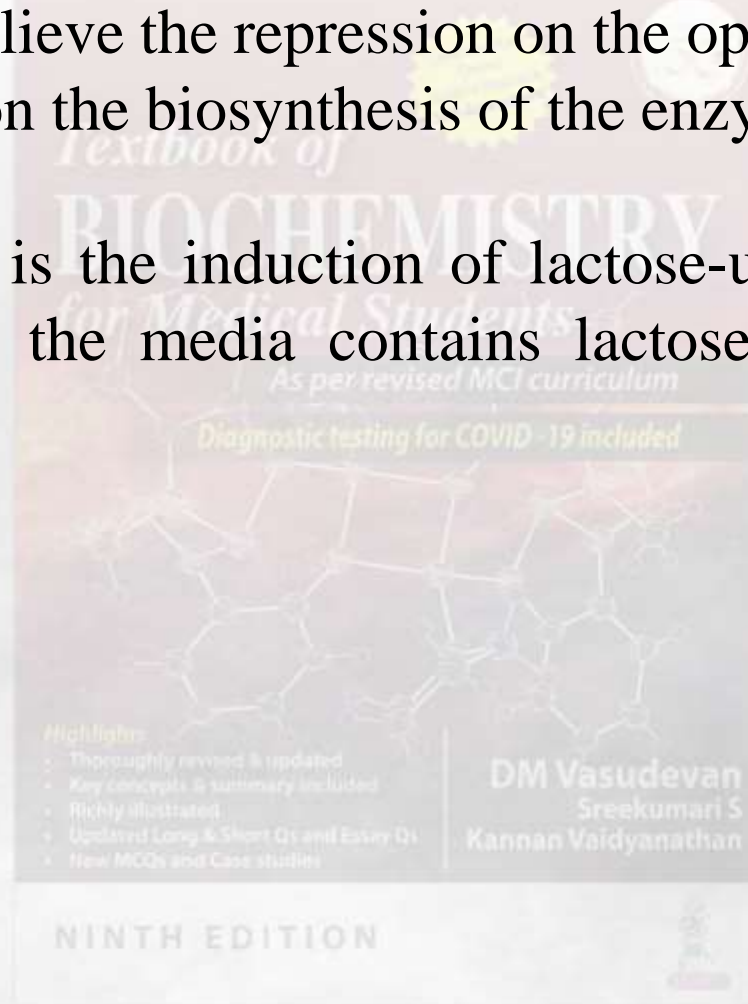
(The end product, heme will allosterically inhibit the ALA synthase).

Induction



Induction is effected through the process of derepression. The inducer will relieve the repression on the operator site and will remove the block on the biosynthesis of the enzyme molecules.

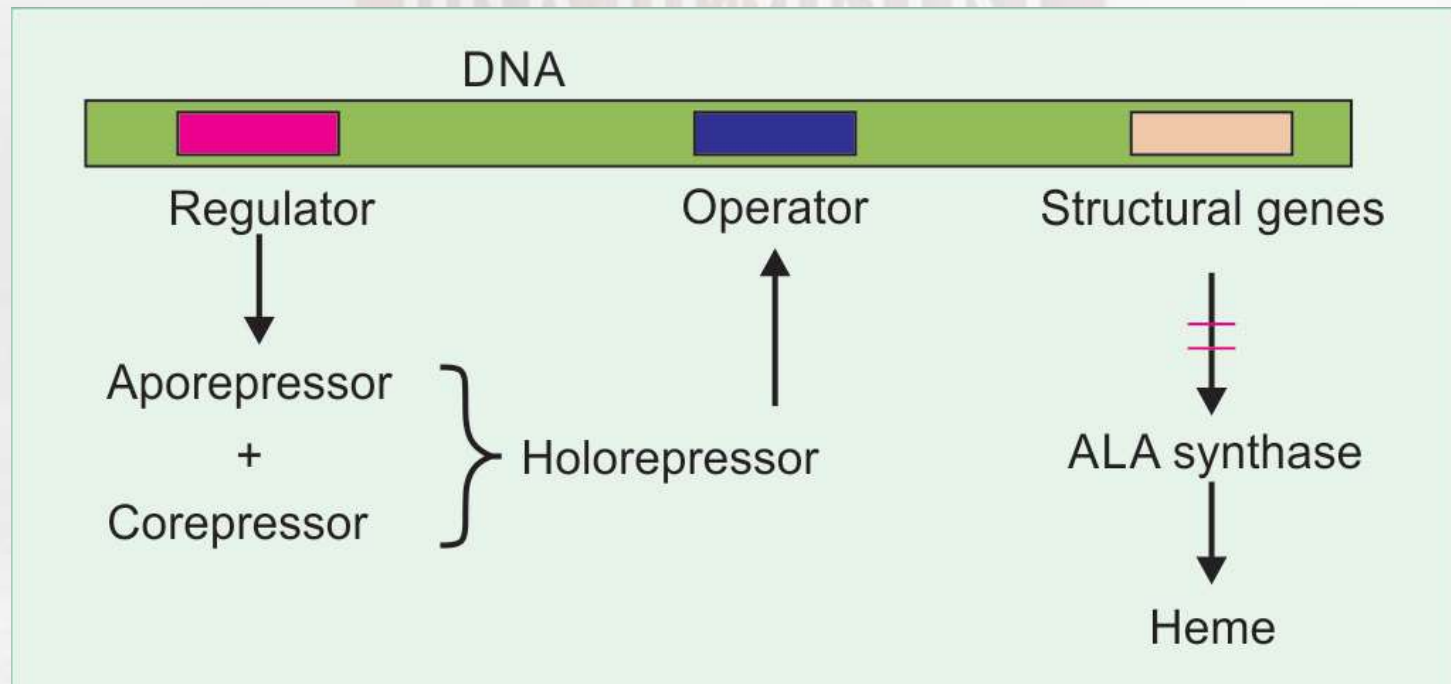
Classical example is the induction of lactose-utilizing enzymes in the bacteria when the media contains lactose in the absence of glucose.



Repression



Repressor acts at the gene level; the effect is noticeable only after a lag period of hours or days; and the number of enzyme molecules is reduced in the presence of repressor molecule.



Covalent Modification



The activity of enzymes may be increased or decreased by covalent modification. It means, either addition of a group to the enzyme protein by a covalent bond; or removal of a group by cleaving a covalent bond.

Zymogen activation by partial proteolysis is an example of covalent activation.

Commonest type of covalent modification is the reversible **protein phosphorylation**. The phosphate group may be attached to serine, threonine or tyrosine residues.

When hormone binds to the membrane bound receptor, hormone-receptor complex (HR) is formed. The enzyme adenylate cyclase is activated. This activation is mediated through a G protein.

Examples of Covalent Modification



<i>Enzyme</i>	<i>Phosphorylated enzyme</i>
Acetyl-CoA carboxylase	Inactive
Glycogen synthase	Inactive
Pyruvate dehydrogenase	Inactive
HMG-CoA reductase	Inactive
Pyruvate kinase	Inactive
PFK2	Inactive
Glycogen phosphorylase	Active
Citrate lyase	Active
Phosphorylase b kinase	Active
HMG-CoA reductase kinase	Active
Fructose-2,6-bisphosphatase	Active

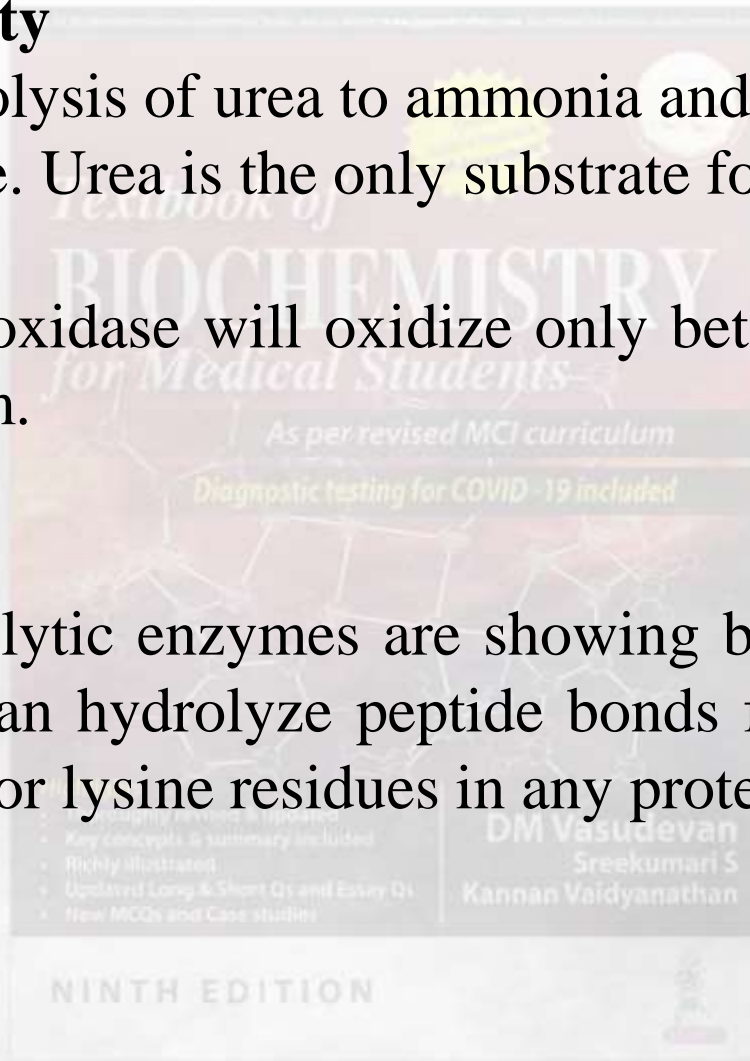
Absolute Specificity

For example, hydrolysis of urea to ammonia and carbon dioxide is catalyzed by urease. Urea is the only substrate for **urease**.

Similarly glucose oxidase will oxidize only beta-D-glucose and no other isomeric form.

Bond Specificity

Most of the proteolytic enzymes are showing bond specificity. For example, trypsin can hydrolyze peptide bonds formed by carboxyl groups of arginine or lysine residues in any proteins.



Group Specificity

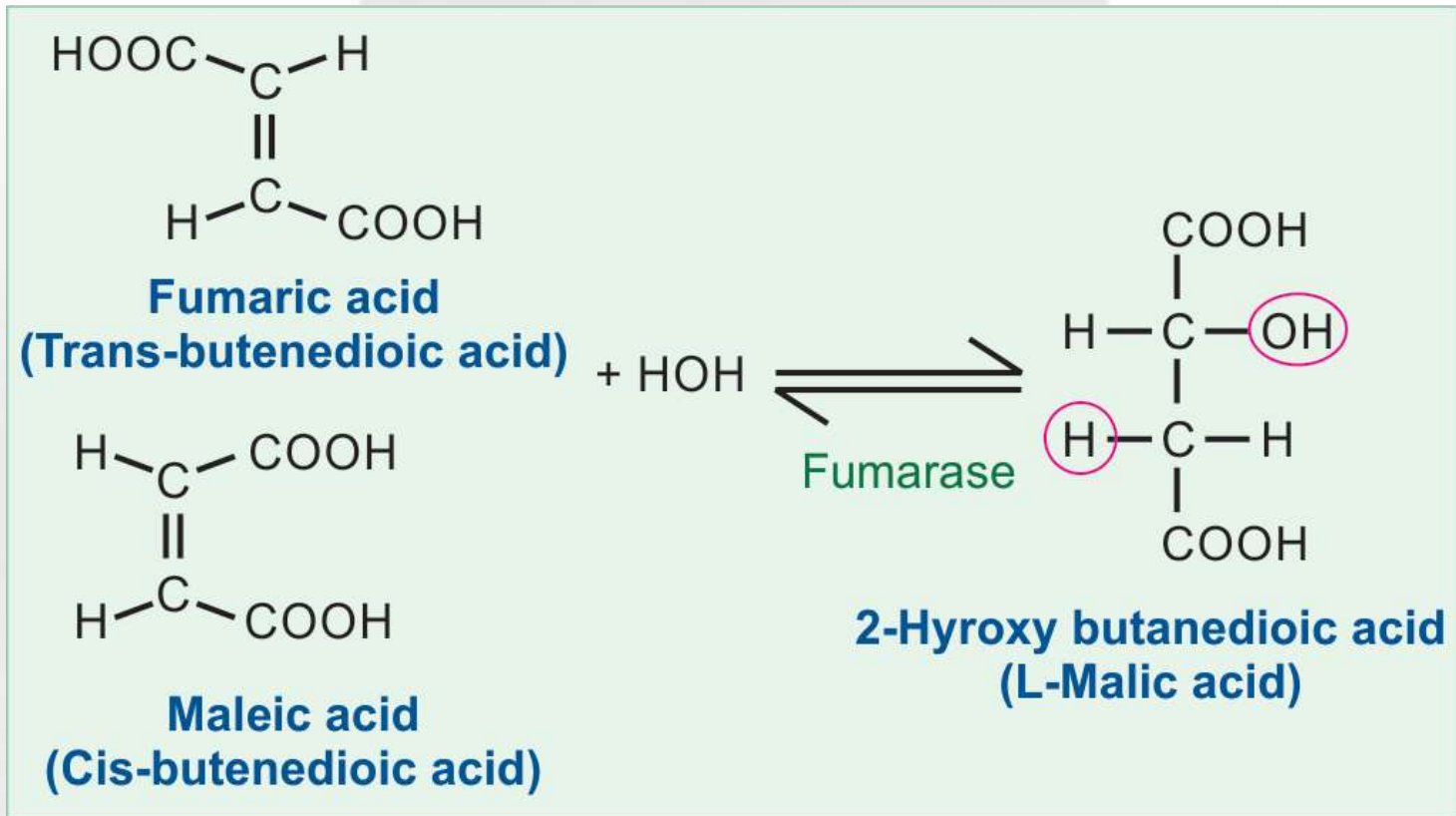
One enzyme can catalyze the same reaction on a group of structurally similar compounds, e.g. hexokinase can catalyze phosphorylation of glucose, galactose and mannose.

Stereospecificity

Human enzymes are specific for L amino acids and D carbohydrates.

Fumarase will hydrate fumaric acid (transform) to malic acid; but the corresponding cis form, maleic acid will not be acted upon

Lactate dehydrogenase, acting on pyruvate will form only L lactate, but not the D variety.



Example of stereospecificity.

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