### Frederich W. Kühne coined the term Enzyme.<sup>Q</sup>

### Classification of Enzymes<sup>Q</sup>

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Enzymes are divided into six major classes with several subclasses:

- a. Oxidoreductases are involved in oxidation and reduction.
- b. Transferases transfer functional groups (e.g. amino or phosphate groups).
- c. Hydrolases catalyze the hydrolysis of a substrate utilizing water.
- d. Lyases add or remove the elements of water, ammonia, or carbon dioxide (CO<sub>2</sub>), to (or from) double bonds.
- e. Isomerases catalyze rearrangements of atoms within a molecule.
- f. Ligases join two molecules.

Classification	Distinguishing feature	
Oxidoreductases [transfer of electron (hydride ions or H atom)]		
Oxidases	Use oxygen as an electron acceptor but do not incorporate it into the substrate	
Dehydrogenases	Use molecules other than oxygen as an electron acceptor	
Oxygenases	Directly incorporate oxygen into the substrate	
Peroxidases	Use $H_2O_2$ as an electron acceptor	
Transferases [group tran	nsfer reaction]	
Methyltransferases	Transfer one-carbon units between substrates	
Aminotransferases	Transfer NH <sub>2</sub> from amino acids to keto acids	
Kinases	Transfer $PO_4^-$ from ATP to a substrate	
Phosphorylases	Transfer $PO_4^-$ from inorganic phosphate ( $P_i$ ) to a substrate <sup>Q</sup>	
Hydrolases [transfer of f	unctional group to water]	
Phosphatases	Remove $PO_4^-$ from a substrate	
Phosphodiesterases	Cleave phosphodiester bonds such as those present in nucleic acids	
Proteases	Cleave amide bonds such as those in proteins	
Lyases [addition of grou	ip to double bond or formation of double bond by removal of groups]	
Decarboxylases	Produce CO <sub>2</sub> via elimination reactions	
Aldolase <sup>Q</sup>	Produce aldehydes via elimination reactions	

(*Contd...*)

1	Enzymes 17
(Contd)	
Classification	Distinguishing feature
Isomerases [transfer	of group within the same molecule to form isomer]
Racemase	Interconvert L and D stereoisomers
Mutase	Transfer groups between atoms within a molecule
Ligases [formation of C	C-C, C-N, C-O, C-S bond by condensation reaction coupled to hydrolysis of ATP or similar cofactor]
Carboxylases	Use $CO_2$ as a substrate
Synthetases	Link two molecules via an ATP-dependent reaction

### Cofactor and Coenzyme

Some enzymes require an additional chemical component for their activity, this additional component is known as cofactor.

Cofactor may be inorganic ions, such as Fe<sup>++</sup>, Mg<sup>++</sup>, Mn<sup>++</sup> or Zn<sup>++</sup>; or it may be a complex organic or metalloorganic molecule called a coenzyme (Tables 6.1 and 6.2).

Cu**   Superoxide dismutase (SOD)     Monoamino oxidase (MAO)   Lysyl oxidase     Lysyl oxidase   Cytochrome oxidase     ALA synthase   Tyrosinase     Fe**/Fe***   Cytochrome oxidase     Fe**/Fe***   Cytochrome oxidase     Reversidase   Peroxidase     Proline hydroxylase   Proline hydroxylase     K*   Pyruvate kinase     Mg**   Hexokinase     Glucose-6-phosphatase   Pyruvate kinase     Mn**   Arginase     Superoxide dismutase (SOD)   Ribonucleotide reductase     Se   Glutathione peroxidase (GPO)?     Deiodinase   Carbonic anhydrase     Alcohol dehydrogenase   Carboxypeptidase A and B     ALA synthase   Superoxide dismutase (SOD)     RNA polymerase   ALP     LDH   LDH     Mo° (Molybdenum)   Xanthine oxidase	Table 6.1: Inorganic elements as a cofactor for certain enzymes <sup>Q</sup>		
Lysyl oxidase Cytochrome oxidase ALA synthase TyrosinaseFe++/Fe+++Cytochrome oxidase Catalase Peroxidase Proline hydroxylaseK*Pyruvate kinaseMg++Hexokinase Glucose-6-phosphatase Pyruvate kinaseMn++Arginase Superoxide dismutase (SOD) Ribonucleotide reductaseSeGlutathione peroxidase (GPO) DeiodinaseZn++Carboxypeptidase A and B ALA synthase Superoxide dismutase (SOD) Ribonucleotide reductaseZn++Carboxypeptidase A and B ALA synthase Superoxide dismutase (SOD) RNA polymerase ALP LDHMo© (Molybdenum)Xanthine oxidase Sufite oxidase	Cu++	Superoxide dismutase (SOD)	
Cytochrome oxidase ALA synthase TyrosinaseFe++/Fe+++Cytochrome oxidase Catalase Peroxidase Peroxidase Proline hydroxylaseK+Pyruvate kinaseMg++Hexokinase Glucose-6-phosphatase Pyruvate kinaseMn++Arginase Superoxide dismutase (SOD) Ribonucleotide reductaseSeGlutathione peroxidase (GPO)Q DeiodinaseZn++Carbonic anhydrase Alcohol dehydrogenase Carboxypeptidase A and B ALA synthase Superoxide dismutase (SOD) Ribonucleotide reductaseMoQ (Molybdenum)Xanthine oxidase Suffite oxidase		Monoamino oxidase ( MAO)	
ALA synthase Tyrosinase     Fe+*/Fe+**   Cytochrome oxidase Catalase Peroxidase Proline hydroxylase     K*   Pyruvate kinase     Mg+*   Hexokinase Glucose-6-phosphatase Pyruvate kinase     Mn+*   Arginase Superoxide dismutase (SOD) Ribonucleotide reductase     Se   Glutathione peroxidase (GPO) <sup>Q</sup> Deiodinase     Zn+*   Carbonic anhydrase Alcohol dehydrogenase Carboxypeptidase A and B ALA synthase Superoxide dismutase (SOD) RNA polymerase ALP LDH     Mo <sup>Q</sup> (Molybdenum)   Xanthine oxidase Sulfite oxidase		Lysyl oxidase	
TyrosinaseFe+*/Fe+**Cytochrome oxidase Catalase Peroxidase Proline hydroxylaseK*Pyrourate kinaseMg+*Hexokinase Glucose-6-phosphatase Pyruvate kinaseMn+*Arginase Superoxide dismutase (SOD) Ribonucleotide reductaseSeGlutathione peroxidase (GPO) DeiodinaseZn+*Carbonic anhydrase Alcohol dehydrogenase Carboxypeptidase A and B ALA synthase Superoxide dismutase (SOD) RiNA polymerase ALP LDHMo <sup>o</sup> (Molybdenum)Xanthine oxidase Sulfite oxidase		Cytochrome oxidase	
Fe**/Fe***   Cytochrome oxidase     Catalase   Peroxidase     Proline hydroxylase   K*     Mg**   Hexokinase     Mg**   Arginase     Mn**   Arginase     Se   Glutathione peroxidase (GPO) <sup>Q</sup> Deiodinase   Carbonic anhydrase     Alcohol dehydrogenase   Carbonic anhydrase     Alcohol dehydrogenase   Carboxypeptidase A and B     ALA synthase   Superoxide dismutase (SOD)     RNA polymerase   ALP     LDH   Xanthine oxidase			
Catalase Peroxidase Proline hydroxylaseK*Pyruvate kinaseMg*+Hexokinase Glucose-6-phosphatase 		Tyrosinase	
Peroxidase     Proline hydroxylase     K*   Pyruvate kinase     Mg++   Hexokinase     Glucose-6-phosphatase     Pyruvate kinase     Mn++   Arginase     Superoxide dismutase (SOD)     Ribonucleotide reductase     Se   Glutathione peroxidase (GPO) <sup>Q</sup> Deiodinase     Zn++   Carbonic anhydrase     Alcohol dehydrogenase     Carboxypeptidase A and B     ALA synthase     Superoxide dismutase (SOD)     RNA polymerase     ALP     LDH     Mo <sup>Q</sup> (Molybdenum)   Xanthine oxidase	Fe <sup>++</sup> /Fe <sup>+++</sup>	Cytochrome oxidase	
Proline hydroxylase     K*   Pyruvate kinase     Mg**   Hexokinase     Glucose-6-phosphatase   Pyruvate kinase     Mn**   Arginase     Superoxide dismutase (SOD)   Ribonucleotide reductase     Se   Glutathione peroxidase (GPO) <sup>Q</sup> Deiodinase   Deiodinase     Zn**   Carbonic anhydrase     Alcohol dehydrogenase   Carboxypeptidase A and B     ALA synthase   Superoxide dismutase (SOD)     RNA polymerase   ALP     LDH   Xanthine oxidase     Mo <sup>Q</sup> (Molybdenum)   Xanthine oxidase			
K*   Pyruvate kinase     Mg**   Hexokinase     Glucose-6-phosphatase   Pyruvate kinase     Mn**   Arginase     Superoxide dismutase (SOD)   Ribonucleotide reductase     Se   Glutathione peroxidase (GPO) <sup>Q</sup> Deiodinase   Zn**     Zn**   Carbonic anhydrase     Alcohol dehydrogenase   Carboxypeptidase A and B     ALA synthase   Superoxide dismutase (SOD)     RNA polymerase   ALP     LDH   Mo <sup>Q</sup> (Molybdenum)     Xanthine oxidase   Sulfite oxidase			
Mg**   Hexokinase     Glucose-6-phosphatase   Pyruvate kinase     Mn**   Arginase     Superoxide dismutase (SOD)   Ribonucleotide reductase     Se   Glutathione peroxidase (GPO) <sup>Q</sup> Deiodinase   Deiodinase     Zn**   Carbonic anhydrase     Alcohol dehydrogenase   Carboxypeptidase A and B     ALA synthase   Superoxide dismutase (SOD)     RNA polymerase   ALP     LDH   Mo <sup>Q</sup> (Molybdenum)     Xanthine oxidase   Sulfite oxidase		Proline hydroxylase	
Glucose-6-phosphatase Pyruvate kinaseMn++Arginase Superoxide dismutase (SOD) Ribonucleotide reductaseSeGlutathione peroxidase (GPO)Q DeiodinaseZn++Carbonic anhydrase Alcohol dehydrogenase Carboxypeptidase A and B ALA synthase Superoxide dismutase (SOD) RNA polymerase ALP LDHMoQ (Molybdenum)Xanthine oxidase Sulfite oxidase	K <sup>+</sup>	Pyruvate kinase	
Pyruvate kinaseMn++Arginase Superoxide dismutase (SOD) Ribonucleotide reductaseSeGlutathione peroxidase (GPO) DeiodinaseZn++Carbonic anhydrase Alcohol dehydrogenase Carboxypeptidase A and B ALA synthase Superoxide dismutase (SOD) RNA polymerase ALP LDHMo <sup>Q</sup> (Molybdenum)Xanthine oxidase Sulfite oxidase	Mg <sup>++</sup>	Hexokinase	
Mn++   Arginase     Superoxide dismutase (SOD)   Ribonucleotide reductase     Se   Glutathione peroxidase (GPO)Q     Deiodinase   Deiodinase     Zn++   Carbonic anhydrase     Alcohol dehydrogenase   Carboxypeptidase A and B     ALA synthase   Superoxide dismutase (SOD)     RNA polymerase   ALP     LDH   MoQ (Molybdenum)     Xanthine oxidase   Sulfite oxidase		Glucose-6-phosphatase	
Superoxide dismutase (SOD) Ribonucleotide reductaseSeGlutathione peroxidase (GPO)Q DeiodinaseZn++Carbonic anhydrase Alcohol dehydrogenase Carboxypeptidase A and B ALA synthase Superoxide dismutase (SOD) RNA polymerase ALP LDHMoQ (Molybdenum)Xanthine oxidase Sulfite oxidase		Pyruvate kinase	
Ribonucleotide reductase     Se   Glutathione peroxidase (GPO)Q     Deiodinase     Zn <sup>++</sup> Carbonic anhydrase     Alcohol dehydrogenase     Carboxypeptidase A and B     ALA synthase     Superoxide dismutase (SOD)     RNA polymerase     ALP     LDH     Mo <sup>Q</sup> (Molybdenum)     Xanthine oxidase     Sulfite oxidase	Mn <sup>++</sup>	Arginase	
Se   Glutathione peroxidase (GPO)Q     Deiodinase     Zn <sup>++</sup> Carbonic anhydrase     Alcohol dehydrogenase     Carboxypeptidase A and B     ALA synthase     Superoxide dismutase (SOD)     RNA polymerase     ALP     LDH     Mo <sup>Q</sup> (Molybdenum)     Xanthine oxidase     Sulfite oxidase			
Zn++   Carbonic anhydrase     Alcohol dehydrogenase   Carboxypeptidase A and B     ALA synthase   Superoxide dismutase (SOD)     RNA polymerase   ALP     LDH   LDH     Mo <sup>Q</sup> (Molybdenum)   Xanthine oxidase     Sulfite oxidase   Sulfite oxidase		Ribonucleotide reductase	
Zn++   Carbonic anhydrase     Alcohol dehydrogenase   Carboxypeptidase A and B     ALA synthase   Superoxide dismutase (SOD)     RNA polymerase   ALP     LDH   LDH     Mo <sup>Q</sup> (Molybdenum)   Xanthine oxidase     Sulfite oxidase   Sulfite oxidase	Se	Glutathione peroxidase (GPO) <sup>Q</sup>	
Alcohol dehydrogenase Carboxypeptidase A and B ALA synthase Superoxide dismutase (SOD) RNA polymerase ALP LDH Mo <sup>Q</sup> (Molybdenum) Xanthine oxidase Sulfite oxidase		Deiodinase	
Carboxypeptidase A and B     ALA synthase     Superoxide dismutase (SOD)     RNA polymerase     ALP     LDH     Mo <sup>Q</sup> (Molybdenum)     Xanthine oxidase     Sulfite oxidase	Zn++	Carbonic anhydrase	
ALA synthase Superoxide dismutase (SOD) RNA polymerase ALP LDH Mo <sup>Q</sup> (Molybdenum) Xanthine oxidase Sulfite oxidase		Alcohol dehydrogenase	
Superoxide dismutase (SOD)     RNA polymerase     ALP     LDH     Mo <sup>Q</sup> (Molybdenum)     Xanthine oxidase     Sulfite oxidase			
RNA polymerase     ALP     LDH     Mo <sup>Q</sup> (Molybdenum)     Xanthine oxidase     Sulfite oxidase			
ALP LDH Mo <sup>Q</sup> (Molybdenum) Xanthine oxidase Sulfite oxidase			
LDH   Mo <sup>Q</sup> (Molybdenum) Xanthine oxidase   Sulfite oxidase			
Mo <sup>Q</sup> (Molybdenum) Xanthine oxidase Sulfite oxidase			
Sulfite oxidase		LDH	
	Mo <sup>Q</sup> (Molybdenum)	Xanthine oxidase	
Aldehyde oxidase		Sulfite oxidase	
		Aldehyde oxidase	

Table 6.2: Coenzymes and the group they transfer<sup>Q</sup>

Coenzyme	Group they transfer	Dietary precursor
Biocytin	CO <sub>2</sub>	Biotin
Coenzyme A	Acyl group	Pantothenic acid and other compound
FAD	Electron	Riboflavin (vit B <sub>2</sub> )
Lipoate	Electron and acyl group	Not required in diet
NÂD	Hydride ion (H <sup>-</sup> )	Nicotinic acid (niacin)
Pyridoxal phosphate (PLP)	Amino group	Pyridoxine (B <sub>6</sub> )
THF	1 Carbon groups	Folate
TPP	Aldehyde	Thiamine
Coenzyme B <sub>12</sub>	H atom and alkyl group	Vit B <sub>12</sub>
(5'-Deoxyadenosylcobalamin)		

Coenzyme may be covalently or noncovalently linked.

### Prosthetic Group Denotes Covalently Bound Coenzyme<sup>Q</sup>

### Serine Proteases

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These are proteolytic enzymes with **serine** residue at its active center. **Trypsin**, **chymotrypsin**, **thrombin** and **elastin** are examples of such enzymes.

### Mechanism of Action of Enzyme

### Lowering the Activation Energy<sup>Q</sup>

Activation energy is required to sufficiently energize a substrate molecule to reach a transition state in which there is a high probability that a chemical bond will be made or broken to form the product.

Enzymes increase the rate of reaction by **decreasing the energy of activation**<sup>Q</sup> (Diagram 6.1).

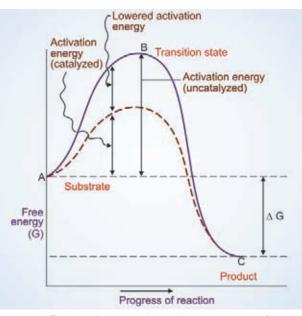


Diagram 6.1: Enzyme lowering the activation energy of a reaction

Enzymes

### **Factors Affecting Reaction Velocity**

- a. Substrate concentration
- b. Temperature
- c. pH

### **Enzyme Kinetics**

### Michaelis-Menten Equation

The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration

$$v_0 = \frac{V_{\max}[S]}{K_{\max} + [S]}$$

where,  $v_0$  = Initial reaction velocity

 $V_{\rm max}$  = Maximal velocity

 $K_{\rm m}$  = Michaelis constant =  $(k_1 + k_2)/k_1$ 

[*S*] = Substrate concentration

*Characteristics of*  $K_m$ : The Michaelis constant is characteristic of an enzyme and a particular substrate. It reflects the affinity of the enzyme for that substrate.

 $K_{\rm m}$  is numerically equal to the substrate concentration at which the reaction velocity is equal to  $\frac{1}{2} V_{\rm max}$ .  ${}^{Q} K_{\rm m}$  does not vary with the concentration of enzyme.

- a. *Small*  $K_m$ : A numerically small (low)  $K_m$  reflects a high affinity of the enzyme for substrate because a low concentration of substrate is needed to half-saturate the enzyme—that is, reach a velocity that is  $\frac{1}{2} V_{max}$ .
- b. *Large*  $K_m$ : A numerically large (high)  $K_m$  reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme—that is, reach a velocity that is  $\frac{1}{2} V_{max}$ .

**Lineweaver-Burke plot:** When the reaction velocity,  $v_0$ , is plotted against the substrate concentration [*S*], it is not always possible to determine when  $V_{\text{max}}$  has been achieved, because of the gradual upward slope of the hyperbolic curve at high substrate concentration. However, if  $1/v_0$  is plotted vs. 1/[S], a straight line is obtained. This plot is called the **Lineweaver-Burke plot (also called a double-reciprocal plot)** and can be used to calculate  $K_{\text{m}}$  and  $V_{\text{max}}$  as well as to determine the mechanism of action of enzyme inhibitors.

The equation describing the Lineweaver-Burke plot is:

$$\frac{1}{v_0} = \frac{K_{\rm m}}{V_{\rm max}[S]} + \frac{1}{V_{\rm max}}$$

The intercept on the *x*-axis is equal to  $-1/K_{\rm m}$ . The intercept on the *y*-axis is equal to  $1/V_{\rm max}$ .

### Inhibition of Enzyme Activity

**A.** *Competitive inhibition*: This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy, and therefore, competes with the substrate for that site.

Enzymes

- 1. *Effect on*  $V_{\text{max}}$ <sup>Q</sup> The effect of a competitive inhibitor is reversed by increasing [S]. At a sufficiently high substrate concentration, the reaction velocity reaches the  $V_{\text{max}}$  observed in the absence of inhibitor.
- 2. *Effect on*  $K_m$ :<sup>Q</sup> A competitive inhibitor increases the apparent  $K_m$  for a given substrate. This means that in the presence of a competitive inhibitor more substrate is needed to achieve  $\frac{1}{2} V_{max}$ .
- 3. *Effect on Lineweaver-Burke plot*:<sup>Q</sup> Competitive inhibition shows a characteristic Lineweaver-Burke plot in which the plots of the inhibited and uninhibited reactions intersect on the *y*-axis at  $1/V_{max}$  ( $V_{max}$  is unchanged). The inhibited and uninhibited reactions show different *x*-axis intercepts, indicating that the apparent  $K_m$  is increased in the presence of the competitive inhibitor (Diagram 6.2).

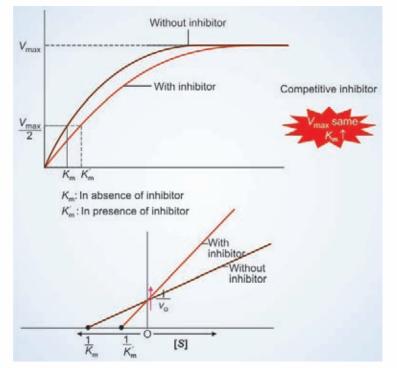


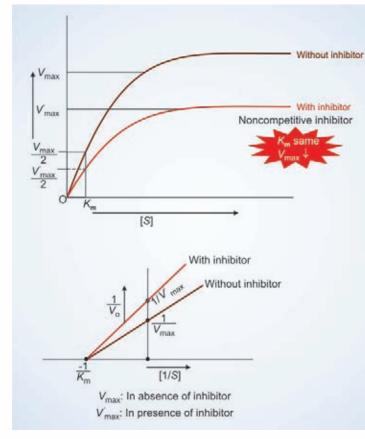
Diagram 6.2: Competitive inhibitor

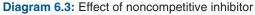
### **Example of competitive inhibitors**

- 1. Sulphonamide as PABA analogue
- 2. Methotrexate as folate reductase inhibitor
- 3. Dicumarol as vitamin K analogue
- 4. INH as vitamin B<sub>6</sub> analogue
- 5. Statins as HMG CoA reductase analogue
- **B.** *Uncompetitive inhibition:* This type of inhibition is recognized by decrease of both  $V_{\text{max}}$  and  $K_{\text{m}}$  value. It is a very rare type of inhibition.
- **Example:** Inhibition of placental ALP by phenylalanine.

**C.** *Noncompetitive inhibition (mixed type of inhibition)*: This type of inhibition is recognized by its characteristic effect on  $V_{\text{max}}$  and occurs when the inhibitor and substrate bind at different sites on the enzyme. The noncompetitive inhibitor can bind either free enzyme or the ES complex, thereby preventing the reaction from occurring.

- 1. *Effect on*  $V_{\text{max}}$ :<sup>Q</sup> Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate. Thus, noncompetitive inhibitors decrease the  $V_{\text{max}}$  of the reaction.
- 2. *Effect on*  $K_m$ :<sup>Q</sup> Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme. Thus, the enzyme shows the same  $K_m$  in the presence or absence of the noncompetitive inhibitor.
- 3. *Effect on Lineweaver-Burke plot*<sup>Q</sup>: Noncompetitive inhibition is readily differentiated from competitive inhibition by plotting  $1/v_0$  vs. 1/[S] and noting that  $V_{\text{max}}$  decreases in the presence of a noncompetitive inhibitor, whereas  $K_{\text{m}}$  is unchanged (Diagram 6.3).





#### **Example of noncompetitive inhibitors**

- 1. Cyanide as cytochrome oxidase inhibitor
- 2. Fluoride as enolase inhibitor

# Enzymes

- 3. Iodoacetate as inhibitor of glyceraldehydes-3-phosphate dehydrogenase
- 4. BAL as antidote of heavy metal poisoning
- 5. Organophosphorus poisoning as inhibitor of acetylcholinesterase.

### **Covalent Modification**

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There are various varieties of covalent modification. Most common type of covalent modification is the phosphorylation/dephosphorylation type.

### Effect on Enzyme Activity

In certain enzymes, the **addition of a phosphate group** to a specific amino acid residue [usually **serine (Ser), tyrosine (Tyr), or threonine (Thr)**<sup>Q</sup>] by specific protein kinases dramatically enhances or depresses activity.

### Allosteric Regulation of Metabolic Pathways

The activity of enzymes that catalyzes key regulatory reactions (committed steps) of metabolic pathways are often subject to allosteric regulation. Their activity can be modulated by the binding of allosteric effectors to a site on the enzyme that is distinct from the active site (i.e. allosteric site). Effectors are positive if they enhance the rate of a reaction (i.e. activators) and negative if they decrease the rate of reaction (i.e. inhibitors).

**Feedback inhibition** is negative modulation of the committed step of a metabolic pathway by its end product.

### **ISOENZYMES**

*Definition*:<sup>Q</sup> Isozymes are different molecular forms of enzymes that may be isolated from the same or different tissues.

Isoenzymes are physically distinct and separable forms of given enzyme.

### Types of Isoenzymes<sup>Q</sup>

- a. *True isoenzymes*: Here the genes of isoenzymes are different which may be located on same or different chromosomes.
  - **Malate dehydrogenase** isoenzymes (cytosolic and mitochondrial) are derived from different gene located on the **same chromosome**.
  - Salivary and pancreatic amylase are derived from different gene located on different chromosome.
- b. *Hybrid isoenzymes*: Here isoenzymes are made up of more than two subunits which are different. It is varied subunit combination which gives rise to different isoenzymes, e.g. LDH isoenzymes, CPK isoenzymes. (LDH is made up of 4 subunits either of all H, or all M or HM in varied combination.)

E	n	Z	vr	n	e	s
_		·	y -	•••	~	~

LDH <sub>1</sub>	HHHH
LDH <sub>2</sub>	HHHM
LDH <sub>3</sub>	HHMM
LDH <sub>4</sub>	HMMM
LDH <sub>5</sub>	MMMM

СРК-1	BB
СРК-2	MB
СРК-3	MM

- c. *Allozymes/allelozymes:* Here isoenzymes are derived from different alleles of the same gene, e.g. G6PD. There are more than 300 alleles known for G6PD of human species.
- d. *Isoforms:* These isoforms are derived after different post-translational modification, e.g. sialic acid content of ALP in various isoenzymes is different.

### Isoenzymes have following characteristics

- 1. Same function/biochemical role
- 2. Same EC number

For example 2.7.1.1. for glucokinase

- D-glucose + ATP = D-glucose-6-phosphate + ADP
- 2: Class (transferase)
- 7: Subclass (phosphotransferase)
- 1: Phosphotransferase with phosphoryl group transfer to hydroxyl group
- 1: D-glucose as phosphoryl group acceptor
- 3. Different structure
- 4. Different electrophoretic mobility
- 5. Different immunological characteristic
- 6. Different affinity to substrate
- 7. Different  $K_{\rm m}$  value.

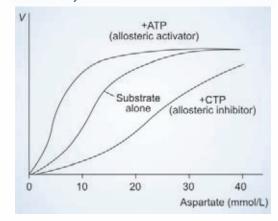
Enzymes

#### **MULTIPLE CHOICE QUESTIONS**

### Q 1. The substrate saturation curve given below is the characteristic of allosteric enzyme. True statement is

### (May 2016 AIIMS)

- a. Allosteric modifier binds in a concentration dependent manner
- b. Modifier can affect the catalytic site by binding to the allosteric site
- c. Adding more substrate to the enzyme can displace the allosteric modifier
- d. Allosteric modifiers changes the binding constant of the enzyme but not the velocity of reaction



### **Ans. b:** Modifier can affect the catalytic site by binding to the allosteric site

Ref: Harper's Illustrated Biochemistry, 30/e, p. 80, 91.

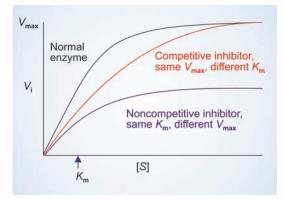
- Allosteric enzyme does not bind the modifier in concentration dependent manner as exemplified by sigmoidal shape of such curve.
- Allosteric modifier binds the allosteric site and addition of more substrate as such does not displaces the allosteric modifier from allosteric sites.
- Allosteric modifier changes both the binding constant of the enzyme and velocity of reaction.

### Q 2. True statement(s) regarding enzyme inhibitors: (PGI)

- a. Competitive inhibitors have same  $V_{max}$
- b. Competitive inhibitors have increased  $K_m$
- c. Non-competitive inhibitors have same  $V_{max}$
- d. Non-competitive inhibitors have increased K<sub>m</sub>
- e. Non-competitive inhibitors have decreased  $V_{max}$

### Ans. a, b, e

### Explanation



### Q 3. Which among the following is a feature of noncompetitive inhibition?

### (PGI)

a. Increased $V_{\text{max}}$	b. Decreased $V_{\text{max}}$
c. Increased $K_{\rm m}$	d. Decreased $K_{\rm m}$

### Ans. b: Decreased $V_{\text{max}}$

- A noncompetitive inhibitor has no effect on *K*<sub>m</sub> but decrease *V*<sub>max</sub>
- *V*<sub>max</sub>: Maximum velocity
- $K_{\rm m}$ : The substrate concentration at which the enzyme attains half of the  $V_{\rm max}$

# Enzymes

	Competitive inhibition	Noncompetitive inhibition
Acting on	Active site	May or may not
Structure of inhibitor	Substrate analogue	Unrelated
Inhibition	Reversible	Generally irreversible
Excess substrate	Inhibition relieved	No effect
K <sub>m</sub>	Increased	No change
$V_{\rm max}$	No change	Decreased
Significance	Drug action	Toxicological

# Q 4. Which of the following does not undergo phosphorylation by protein kinases?

- a. Threonine
- b. Tyrosine
- c. Asparagine
- d. Serine

#### Ans. c: Asparagine

Phosphorylation occurs at the hydroxyl groups of **serine**, **threonine and tyrosine**.

A protein kinase is a kinase enzyme that modifies other proteins by chemically adding phosphate groups to them (phosphorylation). Phosphorylation usually results in a functional change of the target protein (substrate) by changing enzyme activity, cellular location, or association with other proteins.

The chemical activity of a kinase involves transferring a phosphate group from a nucleoside triphosphate (usually ATP) and covalently attaching it to one of **three amino acids** that have a free **hydroxyl group. Most kinases act on both serine and threonine, others act on tyrosine, and a number (dual-specificity kinases) acts on all three**. There are also protein kinases that phosphorylate other amino acids, including **histidine kinases** that phosphorylate histidine residues.

### Q 5. Which amino acid in a protein acts as a potential O-glycosylation site for attachment of an oligosaccharide unit?

- a. Glutamine
- b. Cysteine
- c. Serine
- d. Asparagine

### Ans. c: Serine

*Ref: Harper's 25/e, p. 682.* 

- Glycosylation occurs between OH group of the amino acid and oligosaccharide, while N-glycosylation occurs between amino group of the amino acid and a oligosaccharide chain.
- Tyrosine, serine and threonine are OH group containing amino acid which gets attached with the oligosaccharide chain with the help of O-glycosidic linkage.
- **Glutamine and asparagine** are amide group containing amino acid which gets attached with the oligosaccharide chain with the help of **N-glycosidic linkage**.

#### Q 6. Activator of sulfite oxidase is:

- a. Molybdenum
- b. Copper
- c. Selenium
- d. Zinc

#### Ans. a: Molybdenum

## Q 7. The type of enzyme known as a phosphoribosyltransferase is involved in all of the following *except*:

- a. Salvage of pyrimidine bases.
- b. The *de novo* synthesis of pyrimidine nucleotides.
- c. The *de novo* synthesis of purine nucleotides.
- d. Salvage of purine bases.

Ans. c: The *de novo* synthesis of purine nucleotides

Enzymes

In purine nucleotide synthesis, the purine ring is built up stepwise on ribose-5phosphate and not transferred to it.

## Q8. In which one of the following conditions would the serum acid phosphatase be elevated?

a. Hurler's syndrome

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- b. Carcinoma of the rectum
- c. Primary hyperparathyroidism
- d. Gaucher's disease

### Ans. d: Gaucher's disease

Acid phosphatase is found in the **prostate gland**, **platelets**, **red cells and Gaucher's** cells. Abnormal reticuloendothelial cells seen in Gaucher's disease.

### Q 9. True about oxygenase enzymes are all *except*:

- a. Incorporates one oxygen atom in the substrate
- b. Incorporates two oxygen atoms in the substrate
- c. Involved in hydroxylation reaction
- d. Involved in carboxylation of drugs

### Ans. d: Involved in carboxylation of drugs

- Oxygenases are oxidoreductase class of enzymes where, oxygen is incorporated into the substrate.
- **Monooxygenase incorporates** one atom of the oxygen into the substrate.
- Addition of hydroxyl group is catalysed by monooxygenase enzymes.
- **Dioxygenase incorporates** two atoms of the oxygen into the substrate.
- **Carboxylation is catalysed** by carboxylase group of enzyme which incorporates CO<sub>2</sub> into the substrate.

### Q 10. Chymotrypsinogen is a:

(AIIMS Nov 2006)

a. Zymogen

- b. Carboxypeptidase
- c. Transaminase
- d. Clot lysing protein

### Ans. a: Zymogen

The proteases are secreted as inactive **zymogens**; the active site of the enzyme is masked by a small region of the peptide chain that is removed by hydrolysis of a specific peptide bond. Pepsinogen is activated to pepsin by gastric acid and by activated pepsin (autocatalysis). In the small intestine, trypsinogen, the precursor of trypsin, is activated by enteropeptidase, which is secreted by the duodenal epithelial cells; trypsin can then activate chymotrypsinogen to chymotrypsin, proelastase to elastase, procarboxypeptidase to carboxypeptidase, and proaminopeptidase to aminopeptidase.

Chymotrypsinogen is an endopeptidase, breaking the peptide bond formed by alpha carboxyl group of amino acids: Phenylalanine, tyrosine, tryptophan, valine or leucine.

# Q 11. Transfer of an amino group from an amino acid to an alpha-keto acid is done by: (Al 2012)

- a. Transaminase
- b. Oxidase
- c. Transketolase
- d. Deaminase

#### Ans. a: Transaminase

Transaminase is vitamin  $B_6$  (PLP) dependent enzyme.

### Q 12. Which of the following liver enzymes is predominantly mitochondrial?

a. SGOT (AST)	b. SGPT (ALT)
c. GGT	d. 5'-nucleotidase

Ans. a: SGOT (AST)

SGPT, GGT, 5'-nucleotidase are cytosolic enzymes.

### Q 13. All of the following are trypsin inhibitors *except*: (Al 2008)

- a.  $\alpha_1$ -antitrypsin
- b.  $\alpha_1$ -antiproteinase
- c. Enterokinase
- d. Egg white

#### Ans. c: Enterokinase

Enterokinase is rather an activator of the trypsin. It is a glycoprotein calcium and trypsin itself is also an activator of the trypsin.

### Following are the Inhibitors of Trypsin

- Alpha antiproteinase/α<sub>1</sub>-antitrypsin
- Egg white
- DFP (Diisofluorophosphate)
- Raw soya bean

### Q 14. Treatment of multiple carboxylase deficiency is: (Al 2007)

a. Biotin	b. Pyridoxine
c. Thiamine	d. Folic acid

### Ans. a: Biotin

Biotin is a water-soluble vitamin and acts as a coenzyme for carboxylase group of enzymes.

### Q 15. Which of the following enzymes is stable at acid pH? (Al 2007)

- a. Pepsin
- b. Trypsin
- c. Chymotrypsin
- d. Carboxypeptidase

### Ans. a: Pepsin

pH optima for the pepsin is 1.6–3.2 and it is fully active at this acidic medium. Trypsin, chymotrypsin, carboxypeptidases are active in alkaline medium.

### Q 16. Basement membrane degradation is mediated by: (Al 2007)

- a. Metalloproteinase
- b. Oxidase
- c. Elastase
- d. Hydroxylase

#### Ans. a: Metalloproteinase

Matrix metalloproteinases (MMPs) are collagenases which are needing Zn for their activity and are responsible for basement membrane degradation.

### Q 17. All of the following enzymes are regulated by calcium or calmodulin *except*: (Al 2006)

- a. Adenylate cyclase
- b. Glycogen synthase
- c. Guanylyl cyclase
- d. Hexokinase

### Ans. d: Hexokinase

Following is the list of enzymes which are regulated by calcium or calmodulin:

- 1. Adenylyl cyclase
- 2. Guanylyl cyclase
- 3. Glycogen synthase
- 4. Phospholipase A<sub>2</sub>
- 5. Pyruvate carboxylase
- 6. Pyruvate dehydrogenase
- 7. Pyruvate kinase
- 8. Phosphodiesterase
- 9. Glycerol-3-phosphate dehydrogenase

### Q 18. A common feature of all serine proteases is: (Al 2006)

- a. Autocatalytic activation of zymogen receptor
- b. Tight binding of pancreatic trypsin inhibitor
- c. Cleavage of protein on the carboxyl site of serine residue
- d. Presence of Ser-His-Asp catalytic triad at the active site

Enzymes

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### **Ans. d:** Presence of Ser-His-Asp catalytic triad at the active site

Active site of all the serine protease contains histidine-aspartate-serine (HAS). These three amino acids together are called catalytic triad. Chymotrypsin, elastase, trypsin are examples of serine protease.

### Q 19. Which is true about isoenzymes? (AIIMS Nov 2011)

- a. They have same action with similar kinetics
- b. They have similar quarternary structure
- c. They have same name, number and classification
- d. They are found in the same organ or tissue

### **Ans. c:** They have same name, number and classification

(see the explanation, page 155)

### Q 20. True about isoenzyme(s) is/are:

(PGI 01)

- a. Different  $K_{\rm m}$  value
- b. Acts on different substrate
- c. Consist of multimeric complex
- d. Same electrophoretic mobility
- e. Have different physical properties

#### Ans. a, c, e

Isoenzymes act on 'same substrate' and catalyse same biochemical reaction. Due to difference in the physical property, they move differently during electrophoresis. Hybrid isoenzymes have multi-subunits and it is the different combinations of various subunits which give various isoenzymes.

### Q 21. The predominant isoenzyme of LDH in the cardiac muscle is: (Al 2005)

a. LDH-1	b. LDH-2
c. LDH-3	d. LDH-5

### Ans. a: LDH-1

In normal plasma LDH-2 is more in concentration than LDH-1.

In myocardial infarction level of LDH-1 increases and this leads to altered ratio of LDH isoenzymes. It means LDH-1 becomes more than LDH-2 (LDH-1 > LDH-2).

This altered ratio of the LDH is known as **flipped pattern.** 

### Q 22. All are true about oxygenase enzyme *except*.

### (AI 2007, AIIMS Nov 2011)

- a. Incorporates one oxygen atom in the substrate
- b. Incorporates two oxygen atoms in the substrate
- c. Involved in hydroxylation reaction
- d. Involved in carboxylation of drugs

#### Ans. d: Involved in carboxylation of drugs

Oxygenases are oxidoreductase class of enzymes, where oxygen is incorporated into the substrate.

Monooxygenase incorporates one atom of the oxygen into the substrate. Addition of hydroxyl group is catalysed by monooxygenase enzymes.

Dioxygenase incorporates two atoms of the oxygen into the substrate.

Carboxylation is catalysed by carboxylase group of enzyme which incorporates  $CO_2$  into the substrate. Carboxylase enzymes belong to 'ligases'.

### Q 23. All of the following enzymes are involved in oxidation-reduction reactions *except*: (Al 2009)

- a. Dehydrogenase
- b. Peroxidase
- c. Hydrolase
- d. Oxygenase

# Enzymes

### Ans. c: Hydrolase

Dehydrogenases, peroxidases, and oxygenases are examples of oxidoreductase class of enzymes.

### Q 24. All are true about glutathione except: (AllMS Nov 08)

- a. It is a tripeptide
- b. It converts hemoglobin to methemoglobin
- c. It conjugates with xenobiotics
- d. It is a cofactor of various enzymes

### Ans. b: It converts hemoglobin to methemoglobin

### Q 25. All known effects of cAMP in eukaryotic cell results from:

- a. Activation of catalytic subunit of adenylyl cyclase
- b. Activation of synthetase
- c. Activation of protein kinase
- d. Activation of phosphorylation of G protein

### Ans. c: Activation of protein kinase

### Q 26. Aspirin inhibits which of the following enzymes? (Delhi PG 09)

- a. Lipoprotein lipase
- b. Lipooxygenase
- c. Cyclooxygenase
- d. Phospholipase

### Ans. c: Cyclooxygenase

### Q 27. True about ETC are all: (PGI)

- a. FADH<sub>2</sub> gives 1.5 ATPs
- b. NADH gives 2.5 ATPs
- c. FADH<sub>2</sub> gives 2.5 ATPs
- d. Entry point of NADH is at complex II
- e. NADH enters the step I of the cycle

#### Ans. a, b and e

### Q 28. Transport of fatty acid through mitochondrial membrane is by:

- a. Active transport
- b. Facilitated transport
- c. Diffusion
- d. Lipase

### Ans. b: Facilitated transport

#### Q 29. Tyrosinase is:

- a. Oxidase
- b. Transferase
- c. Lyase
- d. Isomerase

### Ans. a: Oxidase

### Q 30. Fumarase is:

- a. Oxidoreductase
- b. Transferase
- c. Lyase
- d. Isomerase
- Ans. d: Isomerase

### Q 31. Dehydrogenases use as coenzymes all of the following *except*:

- a. FMN
- b. FAD
- c. NADP
- d. NAD
- e. Ferriprotoporphyrin

### Ans. e: Ferriprotoporphyrin

# Q 32. Enzymes that move a molecular group from one molecule to another are known as:

- a. Ligases
- b. Oxidoreductase
- c. Transferases
- d. Dipeptidase

### Ans. c: Transferases

# Enzymes

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(AIIMS 96, UP 01)

(PGI 92, AMU 03)

### Q 33. Coenzyme, in an enzymatic reaction, usually functions to: (AIIMS 96)

a. Activate the substrate

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- b. Increase the active sites of apoenzyme
- c. Enhance the specificity
- d. Acceptor of the cleavage products

Ans. d: Acceptor of the cleavage products

### Q 34. Coenzymes are ...... organic compound. (PGI 94)

a. Lipoprotein	b. Proteinaceous
c. Nonprotein	d. All of the above

### Ans. c: Nonprotein

Holoenzyme = Apoenzyme + cofactor

Apoenzyme is protein portion and

cofactor is nonprotein portion of the enzyme Cofactor may be organic (coenzyme) or

inorganic (metallic).

### Q 35. Lactate dehydrogenase is:

a.	Isozyme	b.	Coenzyme
c.	Antienzyme	d.	Zymogen

Ans. a: Isozyme

### Q 36. Hexokinase is a:

a. Transferase	b. Reductase
c. Oxidoreductase	d. Oxidase

### Ans. a: Transferase

### Q 37. Forms of coenzymes are correctly matched *except*:

- a. Biotin-carboxylated
- b. Vitamin B<sub>12</sub>—ATP
- c. Niacin-NAD<sup>+</sup> NADP
- d. Vitamin B<sub>2</sub>—FMN<sup>+</sup> FAD

Ans. b: Vitamin B<sub>12</sub>—ATP

Q 38. Which is true about enzymes specificity? (PGI 01)

- a. Amount of enzyme required per second, per mole of product formation
- b. Number of sites per substrate
- c. Amount of enzyme binding with various substrates
- d. Number of enzyme units per milligram of enzyme protein
- e. Amount of enzyme causing transformation of 1 µmol of substrate per minute under standard condition

Ans. e: Amount of enzyme causing transformation of 1 µmol of substrate per minute under standard condition.

**Specificity (enzyme activity):** 1 micromol of substrate converted to product under specified assay condition.

**Specific activity:** Number of enzyme units presents per mg of protein. It is the measurement of purity of preparation.

### Q 39. Enzyme marker for Golgi apparatus:

- a. Peroxidase
- b. Galactosidase
- c. Galactosyl transferase
- d. Catalase
- e. Acid phosphatase

### Ans. c: Galactosyl transferase

Organelle and their enzyme marker

### Q 40. The buffering capacity of buffer is maximum at pH equal to:

a. 0.5 pKa	b. pKa
c. pKa + 1	d. 2 pKa

#### Ans. b: pKa

### Q41. Which of the following is an example of a reverse transcriptase?

a. Gyrase	b. Helicase
c. Telomerase	d. RNA polymerase

### Ans. c: Telomerase

Telomerase has got reverse transcriptase activity.

This enzyme activity is present in its protein factors (not in its RNA template) and hence it is a normal enzyme (not the ribozyme).<sup>Q</sup>

### Q 42. The adenylate cyclase system is mediated by:

- a. cAMP
- b. Phosphodiesterase
- c. GTP regulating protein
- d. Nuclear receptors

### Ans. a: cAMP

### Q 43. Non-functional enzymes are all except: (AllMS Nov 2008)

- a. Alkaline phosphatase
- b. Acid phosphatase
- c. Lipoprotein lipase
- d. Gamma glutamyl transpeptidase

### Ans. c: Lipoprotein lipase

Non-functional plasma enzymes are those which normally do not function/reside in the plasma, rather they come to plasma only due to damage of respective cell where they are normally reside.

#### **Example:** Lipoprotein lipase

- Clotting factor
- 5'-nucleotidase

### Q 44. Refsum's disease is due to deficiency of which of the following enzymes?

- a. Malonate dehydrogenase
- b. Thiophorase
- c. Succinate thiokinase
- d. Phytanic alpha oxidase

### Ans. d: Phytanic alpha oxidase

# Q 45. The specific activity of an enzyme would be reported in which of the following units of measures? (*PGI Dec 06*)

- a. Millimoles per liter
- b. Units of activity per milligram of protein
- c. Micromoles per minute
- d. Units of activity per minute

**Ans. b: Units of activity per milligram of protein.** 

### Q 46. Which of the following enzymes is active in dephosphorylated state?

(PGI 2009)

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- a. HMG-CoA reductase
- b. Glycogen phosphorylase
- c. Glycogen phosphorylase kinase
- d. Citrate lyase
- e. Glycogen synthases

#### Ans. a and e

### Enzyme active in dephosphorylated state

- Glycogen synthase
- Glucokinase
- Phosphofructokinase
- Pyruvate kinase
- HMG-CoA reductase

### Enzyme active in phosphorylated state

- Glycogen phosphorylase
- Phosphorylase kinase
- HMG-CoA reductase kinase

### Q 47. Zinc is a cofactor for: (AIIMS Nov 09)

- a. Pyruvate dehydrogenase
- b. Pyruvate decarboxylase
- c.  $\alpha$ -ketoglutarate dehydrogenase
- d. Alcohol dehydrogenase

#### Ans. d: Alcohol dehydrogenase

Enzymes requiring Zn are:

- a. Carbonic anhydrase
- b. Alcohol dehydrogenase
- c. Carboxypeptidase A&B

Enzymes

- d. ALA synthase
- e. Superoxide dismutase (SOD)
- f. RNA polymerase
- g. ALP

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h. LDH

### Q 48. The enzyme marker of electron transport system is:

- a. Cytochrome reductase
- b. Fumarase
- c. Pyruvate
- d. Malate dehydrogenase

### Ans. a: Cytochrome reductase

# Q 49. Which of the following enzymes does not participate in oxidation-reduction reactions? (AIIMS May 2013)

- a. Dehydrogenases
- b. Hydroperoxidases
- c. Oxygenases
- d. Peroxidases

### Ans. b: Hydroperoxidases

Oxidoreductases are enzymes responsible for oxidation of one substrate with simultaneous reduction of another substrate. Under this class of enzyme, there are 4 subclasses.

- a. Dehydrogenase
- b. Oxidases
- c. Oxygenases
- d. Peroxidases

This subclassification depends on the fate of hydrogen removed from the substrate.

- Dehydrogenase donates the hydrogen to some coenzyme (NAD, NADP, FMN, FAD)
- Oxidase donates hydrogen to molecular oxygen to form water
- Oxygenase incorporates oxygen to the substrate atom with the help of hydrogen

• Peroxidase utilises hydrogen to detoxify hydrogen peroxide.

Hydroperoxidase consists of two enzymes (peroxidase and catalase). Catalase is never mentioned to be a oxidoreductase, making option 'd' as the answer.

### Q 50. Which of the following is known as 'suicide enzyme'?

- a. 5-Lipo-oxygenase
- b. Cyclo-oxygenase
- c. 5'-nucleotidase
- d. Thromboxane synthase

### Ans. b: Cyclo-oxygenase

*Ref: Harper's Illustrated Biochemistry; 27/e, p. 204–205.* 

### Cyclo-oxygenase is a 'Suicide Enzyme'

'Switching off' of prostaglandin activity is partly achieved by a remarkable property of cyclo-oxygenase—that of self-catalyzed destruction, i.e. it is a suicide enzyme.

Furthermore, the inactivation of prostaglandins by 15-hydroxyprostaglandin dehydrogenase is rapid.

**Note:** The question asks about suicide enzyme; not suicide inhibition, which is a different property.

Suicide enzyme is one which undergoes self-destruction in order to terminate its own activity, e.g. cyclo-oxygenase.

Suicide inhibition, on the other hand, refers to conversion of a substrate by the enzyme into a metabolite, which is a potent inhibitor of the enzyme, e.g. xanthine oxidase converts allopurinol to alloxanthine (oxypurinol) which is a more potent inhibitor of allopurinol.

Q 51. Assertion (A): Aldolase is an example of hydrolase class of enzyme.

Enzymes

Reasoning (R): Aldolase cleaves the bond in the fructose-1,6-bisphosphate to form glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, and water is not required for this reaction.

- a. A and R both are right but R is not the correct explanation of A
- b. A as well as R are right and R is a correct explanation of A
- c. A is wrong but R is a right statement
- d. A and R both are wrong

**Ans. c:** A is wrong but R is a right statement **Aldolase is an example of lyase** which cleaves the bond without the need of water molecule.

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