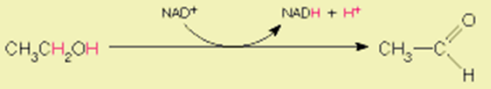
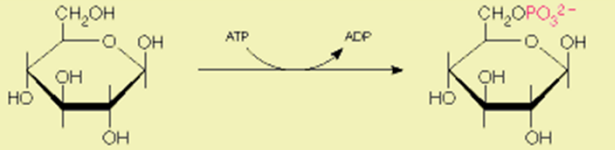
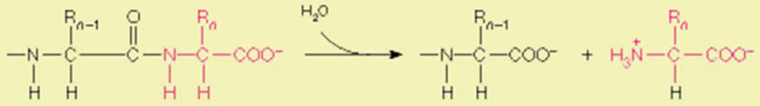
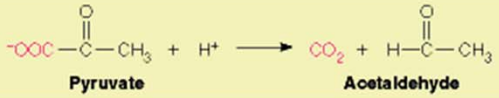
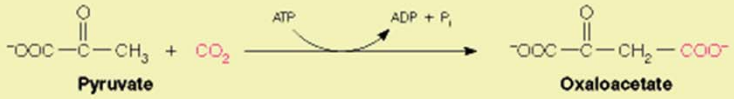
ENZYMES

Enzymes:

- **act as biological catalysts**
- **increase the rate of reaction**
- **are not chemically altered at the completion of the reaction**
- **are mostly specific for the reactions that are catalyzed**

Classification of enzymes

1. **Oxido-reductases = catalyze oxidation-reduction reactions**
2. **Transferases = catalyze transfer of functional groups from one molecule to another**
3. **Hydrolases = break C-N, C-O and C-C bonds with water as a substrate**
4. **Lyases = break C-N, C-O and C-C bonds without water as a substrate**
5. **Isomerases = catalyze intramolecular rearrangement without changing their molecular formula**
6. **Ligases = catalyze reactions in which two molecules are joined by making C-N, C-O and C-C bonds**

Class	Example (reaction type)	Reaction Catalyzed
1. Oxidoreductases	Alcohol dehydrogenase (EC 1.1.1.1) (oxidation with NAD^+)	 Ethanol — Acetaldehyde
2. Transferases	Hexokinase (EC 2.7.1.2) (phosphorylation)	 D-Glucose — D-Glucose-6-phosphate
3. Hydrolases	Carboxypeptidase A (EC 3.4.17.1) (peptide bond cleavage)	 C-terminus of polypeptide — Shortened polypeptide — C-terminal residue
4. Lyases X	Pyruvate decarboxylase (EC 4.1.1.1) (decarboxylation)	 Pyruvate — Acetaldehyde (ATP:Citrate lyase)
5. Isomerases X	Maleate isomerase (EC 5.2.1.1) (*cis–trans* isomerization)	 Maleate — Fumarate (Phosphoglycerate mutase)
6. Ligases	Pyruvate carboxylase (EC 6.4.1.1) (carboxylation)	 Pyruvate — Oxaloacetate

Cofactors/Coenzymes/Prosthetic groups

Cofactors/coenzymes/prosthetic groups are organic or inorganic molecules that are required for the activity of certain enzymes

- **Prosthetic group - Bound to enzyme very tightly (e.g., heme, FAD, TPP)**
- **Apoenzyme = enzyme without the cofactor**
- **Holoenzyme = enzyme with the cofactor**
- **Cofactors – Inorganic ions like Mg^{2+} are not attached to enzymes but are needed for their maximal activity**
- **Coenzymes - Organic molecules that help in the transfer of functional groups (e.g., S-adenosylmethionine, CoASH)**

Vitamin	Coenzyme	Deficiency
Niacin (B_3)	NAD^+, $NADP^+$	Pellagra
Riboflavin (B_2)	FAD	
Thiamin (B_1)	Thiamin-pyrophosphate	Beriberi
Pyridoxal (B_6)	Pyridoxal phosphate	
Pantothenate (B_5)	Coenzyme A (CoASH)	

Active site of an enzyme

- **Represents a small part of the enzyme**
- **Contains specific amino acids depending on the type of reaction catalyzed and the nature of the substrates and products**
- **Active site amino acids are not located next to each other in the primary structure; they can be present anywhere in the primary structure, but they come close to form the active site because of the secondary and tertiary structure of the enzyme**
- **Substrates interact with the active site of the enzyme by various mechanisms: electrostatic interaction, hydrophobic interaction, hydrogen-binding, etc.**
- **Mutations in the active site amino acids might affect the K_m (i.e., affinity) or V (i.e., velocity) or both**

Enzyme Kinetics

$$S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} P$$

Law of mass action: Velocity of forward reaction α [S]

Velocity of reverse reaction α [P]

Velocity of the forward reaction: $V_F = k_1 [S]$

Velocity of the reverse reaction: $V_R = k_2 [P]$

k_1 = rate constant for the forward reaction

k_2 = rate constant for the reverse reaction

Therefore, at equilibrium, $V_F = V_R$ {remember [S]≠[P]}

i.e., $k_1 [S] = k_2 [P]$

The equilibrium constant (K_{eq}) is expressed as: $K_{eq} = k_1/k_2 = [P]/[S]$

The equilibrium constant (K_{eq}) is a constant for a given chemical reaction

Change in Free Energy

$$S \rightleftharpoons P$$
$$(G_S) \qquad (G_P)$$

Free energy (G) - amount of energy present in a substance that is available to do work

$$\Delta G = G_P - G_S$$

ΔG is not a constant for a given reaction; it changes depending on the concentrations of the substrate and the product at the beginning of the reaction.

Standard free energy change (ΔG^0): Free energy change for a reaction when the concentrations of S and P are kept at 1 M at the beginning of the reaction

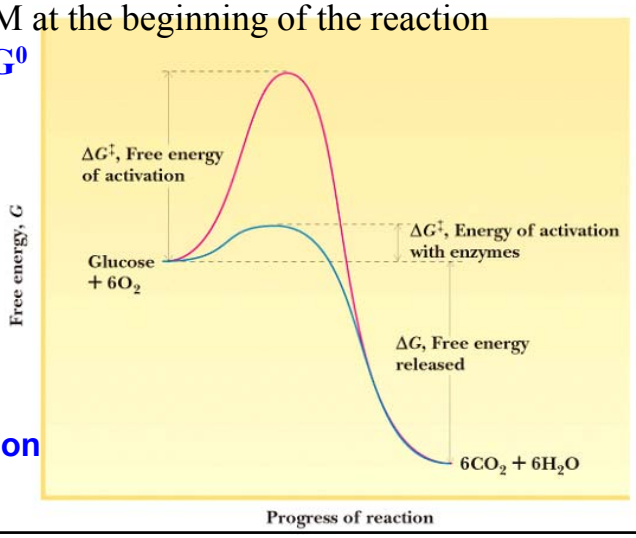
Enzymes DO NOT affect K_{eq}, ΔG and ΔG^0

Spontaneous reaction: ΔG is negative, but ΔG^0 does not have to be negative

Free energy of activation:

$\Delta G^‡ = G_S^‡ - G_S$ (always positive) where $G_S^‡$ is the free energy of the substrate at the transition state and G_S is the free energy of the substrate at the ground state.

Enzymes activate chemical reactions by decreasing the free energy of activation (activation energy) $\Delta G^‡$

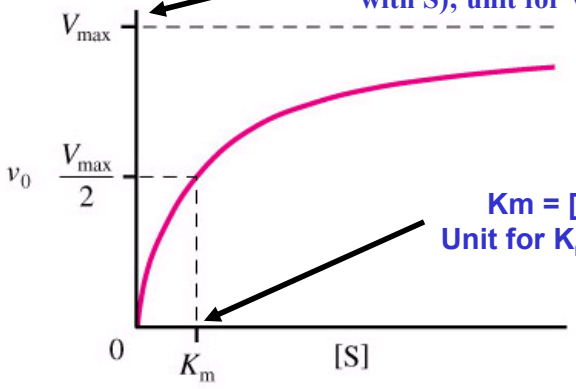


Factors affecting enzyme activity

- pH
- Temperature
- Enzyme concentration
- Substrate concentration

Effect of [S] on enzymatic reactions

V_{max} = velocity of the reaction at infinite concentration of S (all of the enzyme is bound to substrate; i.e., (enzyme is saturated with S); unit for V_{max} is moles/time



Km = [S] when v is equal to ½ V_{max}
Unit for K_m is concentration (moles/L = M)

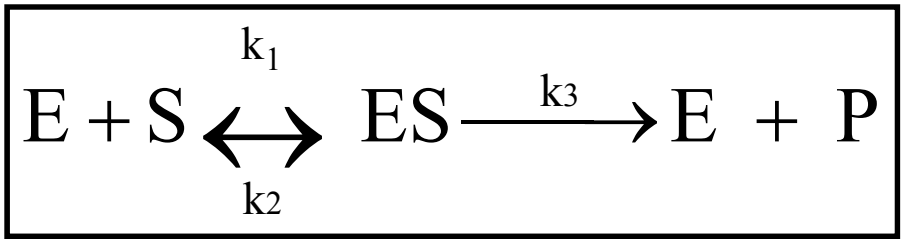
First order kinetics
(E is not saturated)
[ES] is proportional to [S]

Michaelis-Menton Equation

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

Zero order kinetics
(E is saturated with S)
[ES] = [E]

Michaelis-Menten Equation



E = Enzyme S = Substrate P = Product

ES = Enzyme-Substrate complex

k_1 = rate constant for the association of E with S to form ES

k_2 = rate constant for the dissociation of ES to E and S

k_3 = rate constant for the conversion of ES to E and the product

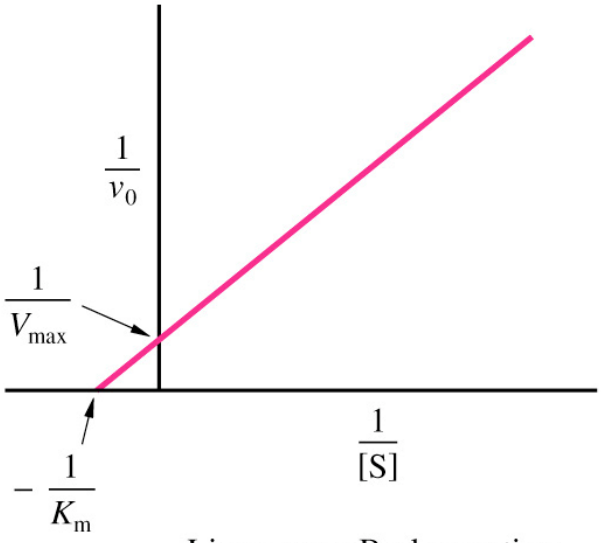
Michaelis constant $K_m = (k_2+k_3)/k_1$ $(k_2>>>>k_3)$

So, $K_m = k_2/k_1$

K_m is inversely proportional to the affinity of the substrate to the enzyme

(i.e., higher the K_m, lower the affinity; lower the K_m, higher the affinity

Lineweaver-Burk Plot
(double-reciprocal plot)



- Plot $1/[S]$ vs $1/V_o$
- x-intercept = $-1/K_m$
- y-intercept = $1/V_{max}$

Lineweaver-Burk equation:

$$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Effect of [E] on the reaction rate

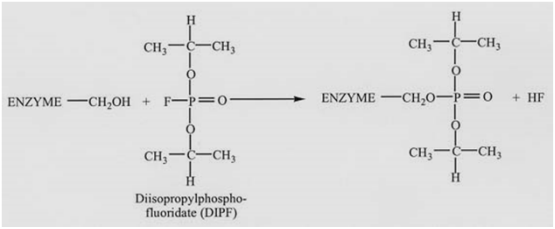
- The velocity of the reaction is directly proportional to [E]
- $V \alpha [E]$ $V_{max} \alpha [E]$
- Double the [E], V is doubled and V_{max} is doubled
- But the concentration of [S] that gives one-half of the maximal velocity (i.e., one-half of V_{max}) is not changed
- The Michaelis constant (K_m) (i.e., the affinity of the substrate for the enzyme) is independent of the enzyme concentration
- Altering the [E] does not affect K_m

Enzyme Inhibition

- **Irreversible**
- **Reversible**
  - **Competitive**
  - **Non-competitive**

Irreversible Inhibitors

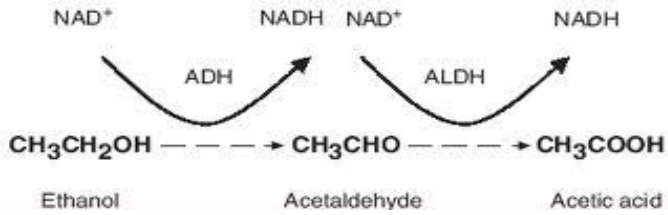
Acetylcholine esterase



Potent inhibitors of acetylcholine esterase cause muscle paralysis because they cause sustained activation of the acetylcholine receptor at the neuromuscular junction (Nerve gases; DIPF is a nerve gas)

Mild inhibitors of the enzyme have potential for the treatment of Alzheimer's disease because this disease is caused by decreased cholinergic activity in the brain

Aldehyde dehydrogenase

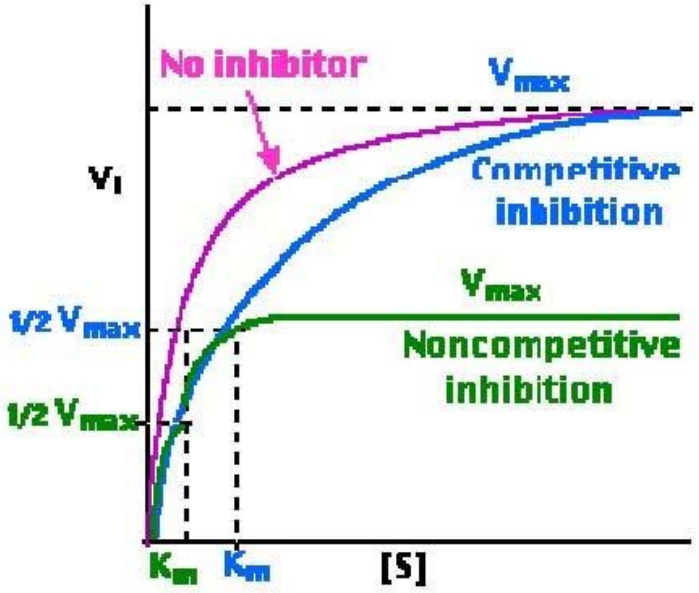


Inhibitors of aldehyde dehydrogenase Increase the levels of aldehyde in the Brain and cause unpleasant side effects (hangover; flushing, nausea, headache, dyspnea, tachycardia, hypotension, virtigo).

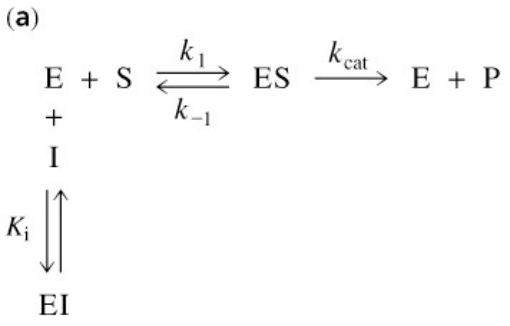
Such inhibitors have use for the treatment of alcoholism (e.g., Disulfiram - Antabuse)

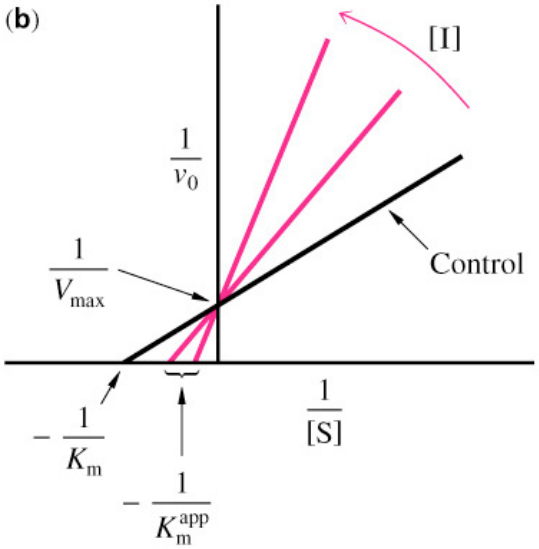
Mutations in the active site of aldehyde dehydrogenase that decrease the catalytic activity protect against alcoholism

Effect of Inhibitors on Michaelis Menten curve



Competitive Inhibition (CI)

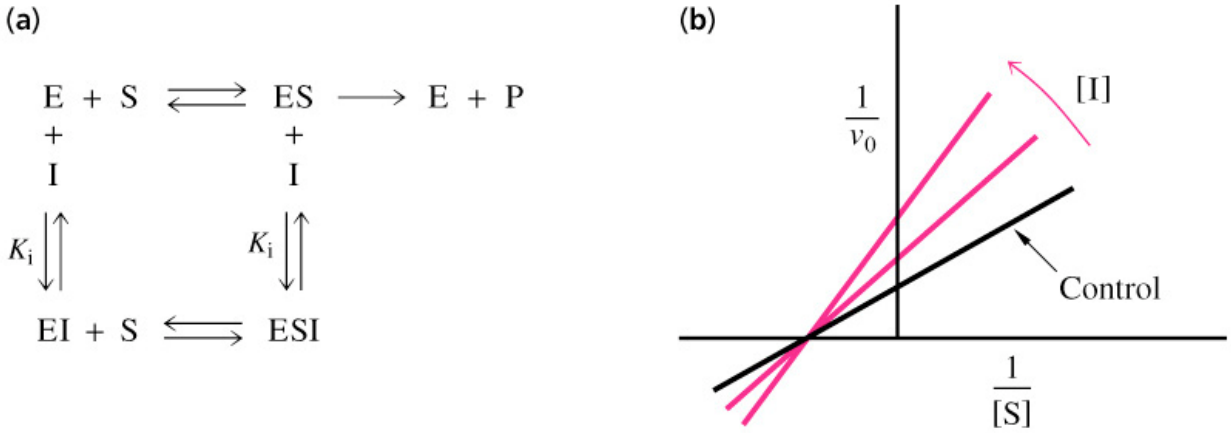




$$K_m^{app} = K_m \left(1 + \frac{[I]}{K_i}\right)$$

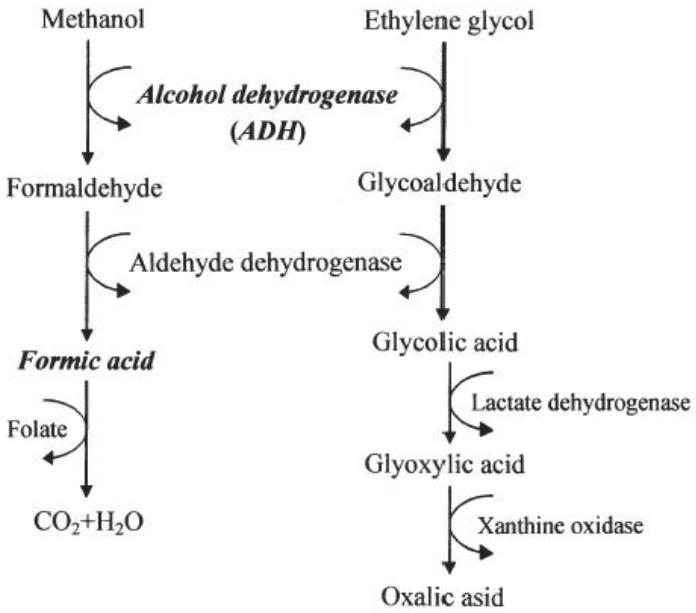
- CI binds free enzyme
- CI is structurally similar to substrate
- It competes with substrate for binding to the enzyme at the same site (active site).
- The inhibition by CI can be relieved with more [S] and hence V_{max} is not altered
- CI increases K_m without affecting V_{max} ↑ ↓ ↑ ↓

Non-competitive Inhibition (NCI)

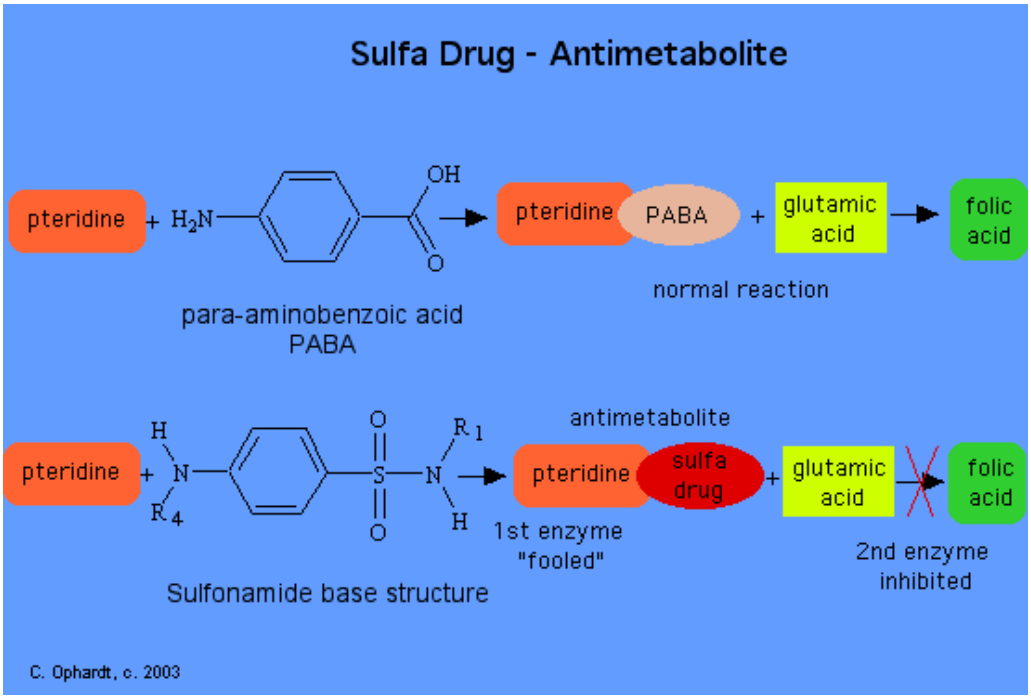


- NCI binds free E or ES complex
- NCI lowers V_{max}, but K_m remains the same
- NCI does not bind to the active site, and hence does not affect K_m
- NCI alters conformation of enzyme and affects catalysis but not substrate binding

Clinical correlations for CI



Ethanol competes with ethylene glycol and methanol for cellular metabolism and prevents the formation of toxic metabolites, thereby preventing ethylene glycol and methanol toxicity



Sulfa drugs are used in the treatment of bacterial infections to serve as inhibitors for folic acid synthesis by competing with PABA; this process occurs only in bacteria, not in humans; only bacteria are capable of endogenous synthesis of folic acid

Regulation of Enzyme Activity

Enzyme quantity – regulation of gene expression (Response time = minutes to hours)

a) Transcription

b) Translation

Enzyme activity (Response time = seconds, rapid)

a) Allosteric enzyme effectors – Inhibition and activation

b) Covalent modification – acetylation, methylation, phosphorylation

c) Feedback Inhibition

d) Proteolytic cleavage of proenzyme

Allosteric Regulation

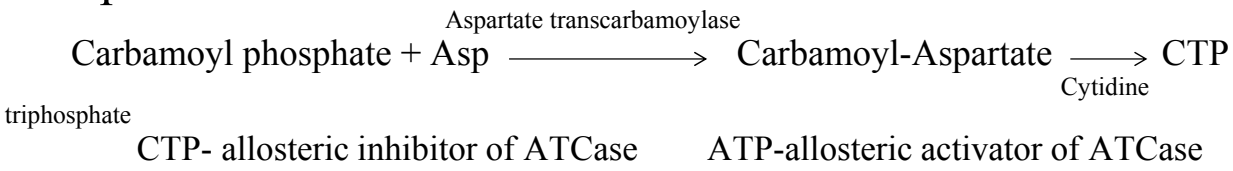
Allosteric modulators bind to a site other than the active site

Allosteric enzymes must have multi-subunits with quaternary structure

V vs [S] plots give sigmoidal curve (instead of an hyperbola); $V = V_{max} \ S^n/(K_m^n + S^n)$

n (or h) is called the Hill Coefficient; it is 1 for normal enzymes but is greater than 1 (2 or 3) for allosteric enzymes

End products are often inhibitors - Feedback inhibition



CTP- allosteric inhibitor of ATCase ATP-allosteric activator of ATCase

K-class and V-class effectors

Mechanisms of enzyme-mediated catalysis

Catalysis by proximity: Binding of the substrates to the active site brings the substrates close to each other so that the reaction can occur.

Catalysis by strain: Substrate binds to the active-site amino acids in a sterically constrained manner so that the target bond can be broken to generate the products.

Acid-base catalysis: Active site amino acids serve as donors and acceptors of protons/electrons to facilitate the transfer of protons/electrons in between substrates to promote the reaction. e.g., in the reaction catalyzed by pepsin, the active site contains two aspartate residues which function as an acid and a base in catalysis.

Covalent catalysis: This involves a transient formation of covalent bonding between one of the active site amino acids and the substrate; the bond is subsequently broken to generate the products. E.g., in the reaction catalyzed by chymotrypsin, the peptide substrate A-B is hydrolyzed into the products A and B, but during the reaction, one of the products is bound transiently to the active site amino acid serine by covalent bond, which is then broken to release the product. The active site also contains histidine and aspartate, that facilitate this process.

E-Ser + A-B → E-Ser-A + B → E-Ser + A + B

Isoenzymes

- **Multiple forms of the same enzyme.**
- **Catalyse the same reaction. Act on the same S and give the same P.**
- **Differ in molecular weight or structure or charge. Can be separated by electrophoresis.**
- **Have different Km for the same S.**
- **Important in diagnosis of disease.**

Lactate Dehydrogenase (LDH)

Lactate + NAD^+ $\leftrightarrow$ Pyruvate + NADH + H^+

- It is a tetramer. (4 subunits)
- Composed of 2 types of polypeptide chains (M & H).
- Has 5 isoenzymes, due to different combination of M & H chains.

M_4	LDH5	$\rightarrow$	In skeletal muscles and liver
M_3H	LDH4	$\rightarrow$	In many tissues
M_2H_2	LDH3	$\rightarrow$	In lungs
MH_3	LDH2	$\rightarrow$	In Heart
H_4	LDH1	$\rightarrow$	In Heart muscles

- Useful in differential diagnosis.

e.g. $\uparrow$ LDH 1 and 2 ..... Myocardial infarction (MI)

Isoenzymes

Isoenzymes:

Catalyze the same reaction

Do not affect the equilibrium constant (K_{eq}), ΔG, or ΔG^0

But isoenzymes will have different values for ΔG^* (i.e., activation energy)

Isoenzymes will have different K_m values and different V_{max} values

Isoenzymes will have different regulatory features (inhibitors, allosteric modulators)