

Enzymes

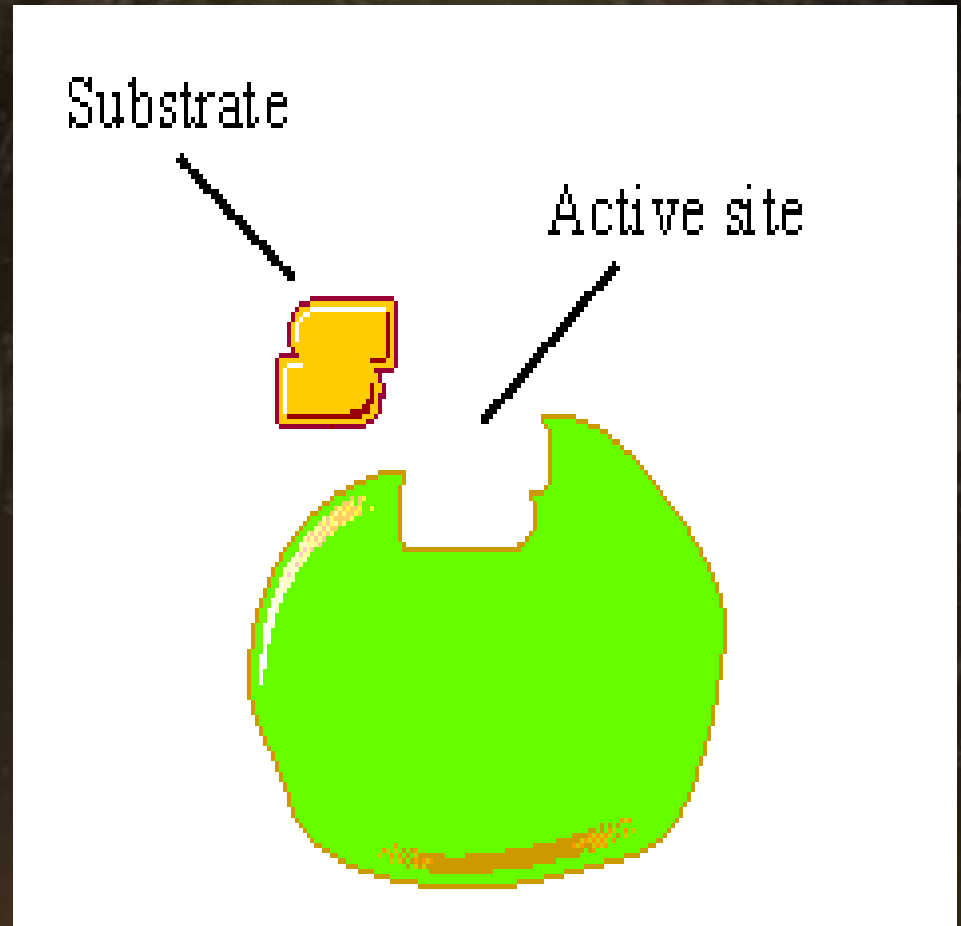
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What Are Enzymes?

- Most enzymes are **Proteins** (tertiary and quaternary structures)
- **except for a class of RNA modifying catalysts known as ribozymes**
- Act as Bio-**Catalyst** to accelerates a reaction
- **Not permanently** changed in the process

Enzymes

- Are specific for what they will **catalyze**
- Are **Reusable**
- **Enzymes are found in all tissues and fluids of the body.**
- **Enzymes bind to substrates at the active site.**



Properties of enzymes

- 1. Solubility: water soluble
- 2. Molecular weights range from 10000-several hundred thousand
- 3. Absorb light in the UV range. Maximum absorption at 280nm due to aromatic a.a
- 4. Enzymes are charged molecules. Charge on enzyme depends on pH of the solution. At low pH charge is positive at high pH charge is negative.
- 5. Enzymes have buffering capacity. They are amphoteric molecules, can behave both as acid and as base.
- 6. Have a specific Isoelectric pH [pI]. Positively charged below pI, and negatively charged above pI

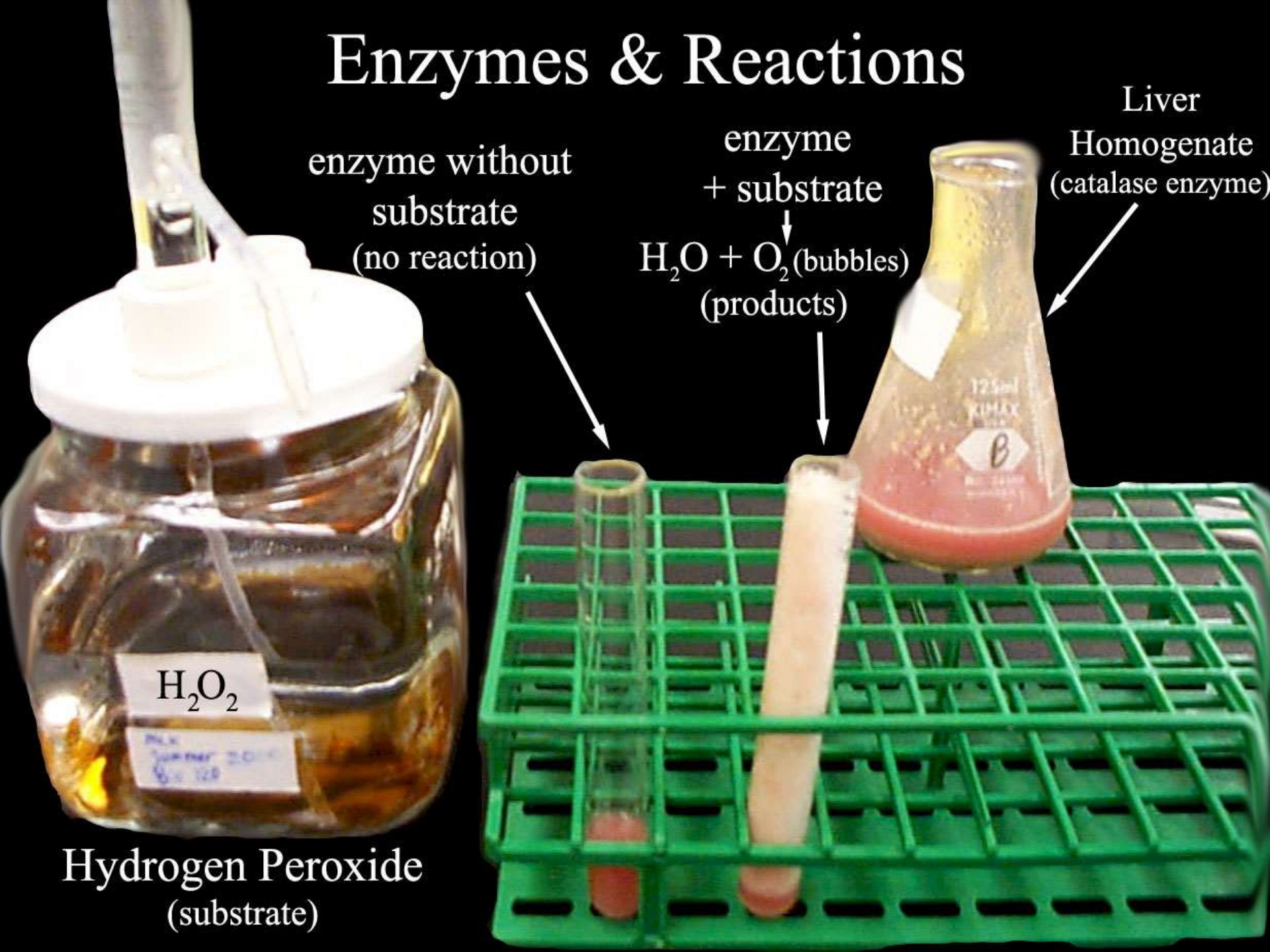
Enzyme Denaturation

- Enzymes (proteins) when exposed to heat or to certain denaturing agents loses its secondary ,tertiary and quaternary structures and changes its native structure to random coil . However the primary structure is not affected.
- Effect of denaturation: Enzyme loses its activity.
- Denaturing agent include:
 - Extreme change in pH
 - 8M urea
 - Heavy metals
 - Radiations
 - Detergents

Chemical reactions of Proteins(enzymes)

- Ninhydrin test- is a test for amino acids
- Biuret test-is a test for proteins
- Xanthoproteic test- is a test for aromatic amino acids
- Millon's test- is a test for tyrosine (has phenol group)
- Nitroprusside test-is a test for cysteine (sulfhydryl group)
- Hopkins-cole test- is a test for tryptophan (Indole group)

Enzymes & Reactions



Enzyme-Substrate Complex

The substance
(reactant) an
enzyme acts on
is the **substrate**

Substrate



Active Site

- A **restricted region** of an enzyme molecule which **binds** to the substrate.

Active Site

Substrate

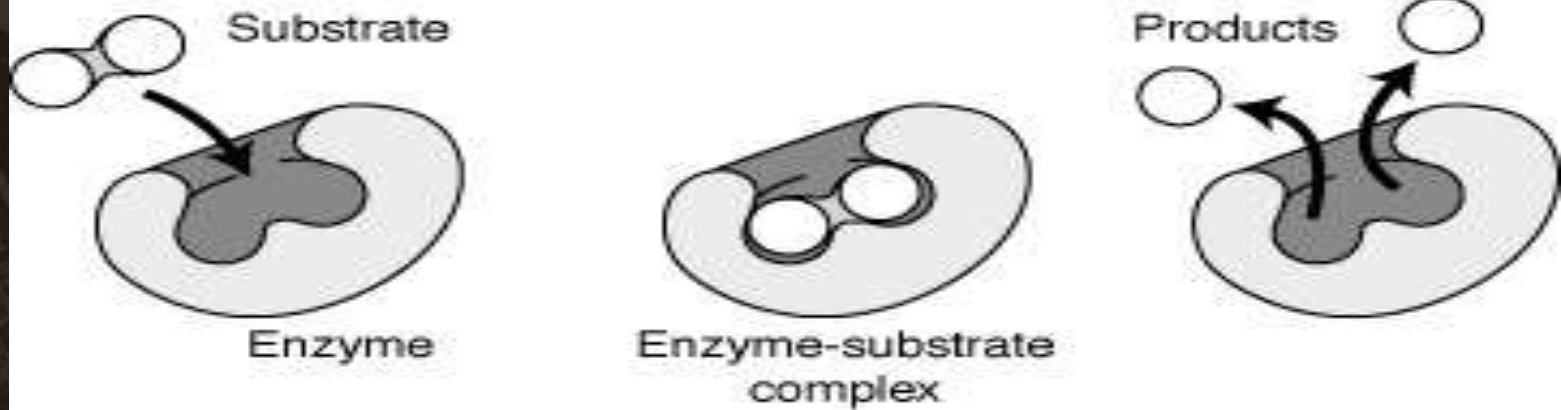
Enzyme



Active Site

- It is a pocket or cleft in an enzyme molecule. It contains amino acid that create a three dimensional surface.
- The amino acids present in the active site (catalytic amino acids) may be far away from each other in the primary structure of the enzyme.
- Active site contains a machinery involved in catalyzing a reaction.
- Substrates bind to active site making an enzyme substrate complex. After the reaction the product molecules are released from the active site.

Mechanism of enzyme activity



Cofactor & Coenzyme

- Some enzymes depend for activity only their proteins structure [simple proteins], while others require one or more non-protein component [conjugated proteins]
- Cofactor may be metal ion or an organic molecule called Coenzyme.
- Apoenzyme + Cofactor → Holoenzyme
(Catalytically inactive) (active enzyme)

Examples of Metal as cofactor

- Alcohol dehydrogenase-Zn⁺⁺
- Kinases(phosphotransformer)-Mg⁺⁺
- Cytochromes-Fe⁺⁺or Fe⁺⁺⁺
- Cytochromeoxidase-Cu⁺⁺

Coenzyme

- Organic molecules, heat stable
- Derived from water soluble vitamins.
- Usually function as intermediate carriers of functional groups of specific molecules

Pyridoxalphosphate - Amino transferase

NAD⁺, NADP - in H⁺ transfer

FAD, FMN - in H⁺ transfer

COQ - in H⁺ transfer

Coenzyme A - Acylgroup transfer

Biotin - Addition of CO₂

Thiamine pyrophosphate-Removal of CO₂

- If the coenzyme is tightly bond to the enzyme molecule, it is called a prosthetic group.

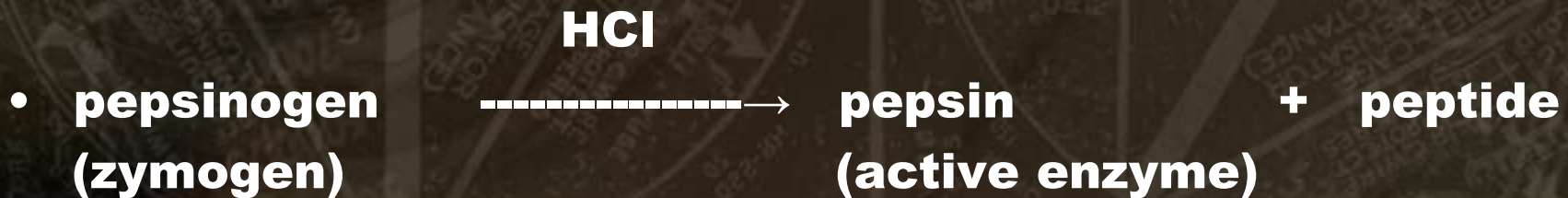
Enzyme Inhibitor

- **They are molecules that bind to enzyme ,either at its active site or else where, and cause reduction in enzyme activity.**
- **The inhibitor may bind to enzyme reversibly or irreversibly**

Zymogens

- **These are inactive precursors of enzymes. They are activated by removal of a small peptide by action of a protease or some other chemical such as HCl**

- **Example:**



Isoenzyme

- **These are enzymes produced by different genes at different sites but catalyze the same reaction.**
- **Example :**
There are five isoenzymes of lactate dehydrogenase .

Enzyme Specificity

- **Enzyme are specific for the reaction the catalyse.**
- **Specificity is due to a specific arrangement of catalytic groups that participate in bond making and bond breaking.**
- **Specificity is determined not only by the functional groups of amino acids in enzyme active site but also by the functional groups of substrate.**
- **There are various degree of enzyme specificity:**
 - 1. Absolute specificity**
 - 2. Group specificity**
 - 3. Reaction specificity**
 - 4. Optical specificity**

Absolute specificity

- **Is the highest degree of specificity.**
- **The enzyme active site is recognized by a single substrate.**
- **Example: Glucokinase catalyzes the conversion of glucose to glucose -6-phosphate**

Group specificity

- **Enzyme active site can recognize many substrates , all belonging to same group of compounds.**
- **Example:**
- **Trypsin catalyzes the hydrolysis of peptide bond in several proteins.**
- **Hexokinases act on six carbon sugars.**

Reaction specificity

- **The enzyme catalyzes only one type of reaction**
- **Example:**
- **Oxidoreductases catalyze oxidation –reduction reactions**

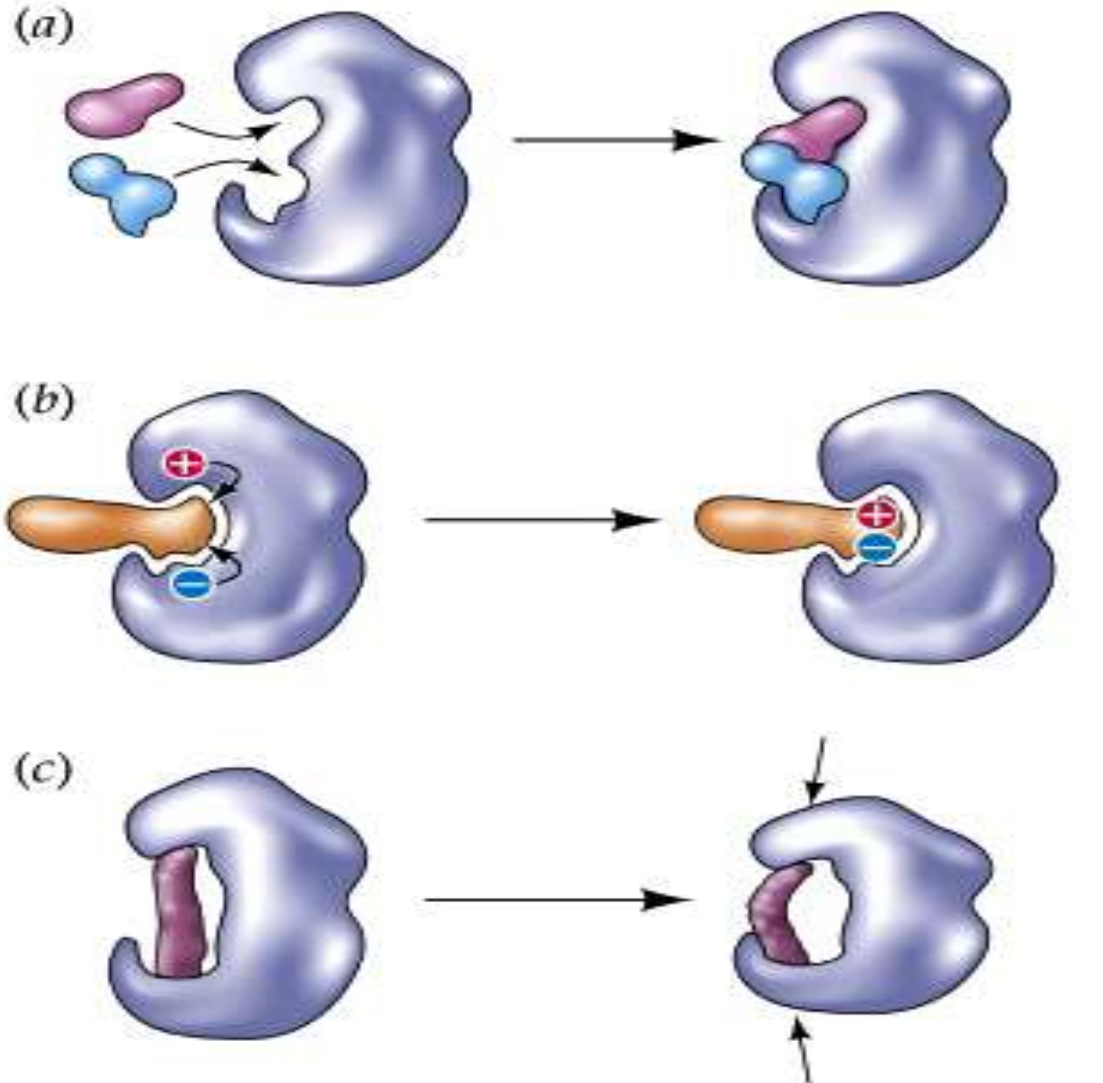
Optical specificity

- **Enzyme is stereospecific. Is capable to differentiate between L- and D- isomers of a compound.**

Theories to explain specificity of enzyme action

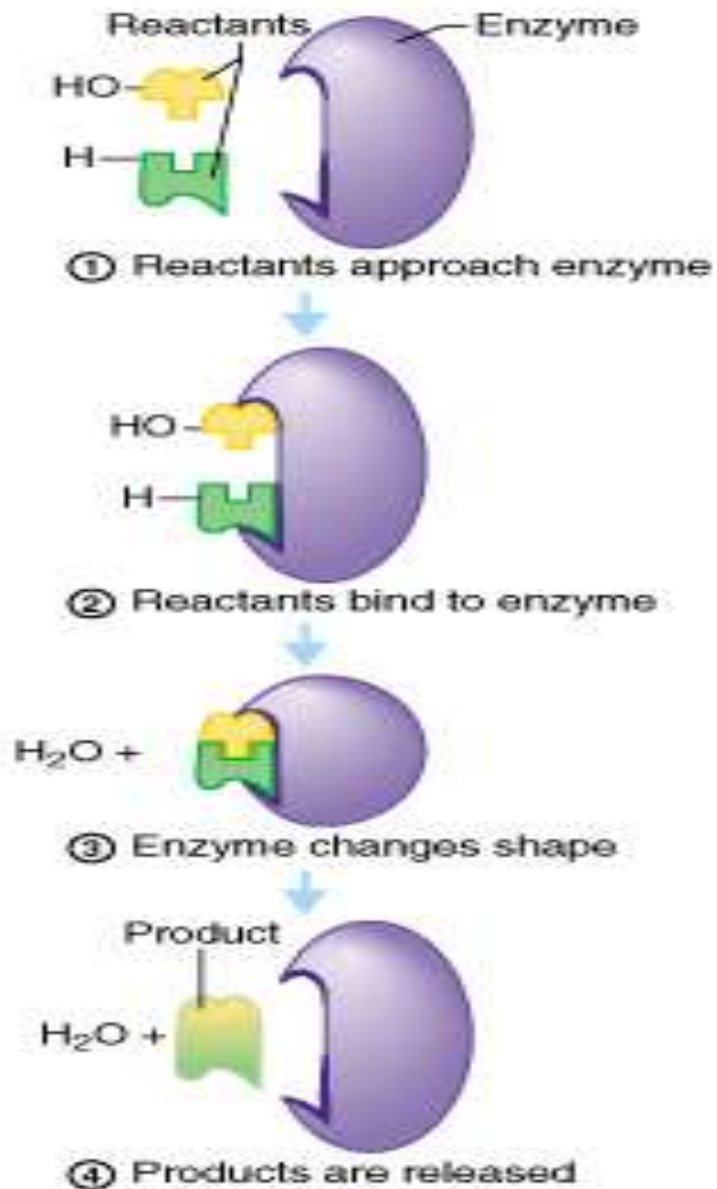
- **Lock and Key theory: the enzyme active site is complementary in conformation to the substrate, so that enzyme and substrate recognize each other.**
- **Induced-fit theory: The enzyme changes shape on binding to the substrate, so that the conformation of substrate and enzyme active site is complementary only after binding.**

Lock and key

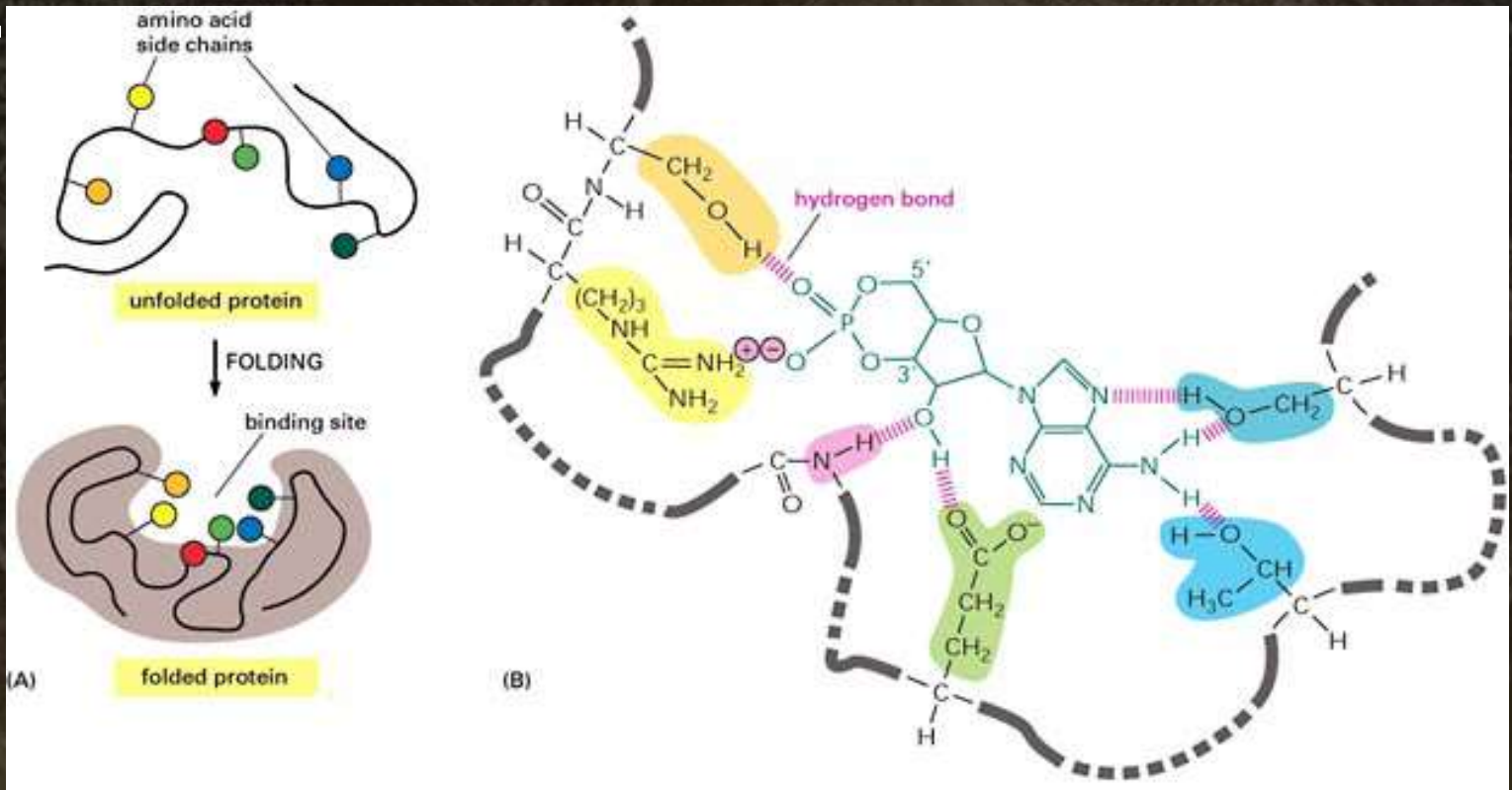


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Induced fit model



The enzyme change shape on the substrate binding.



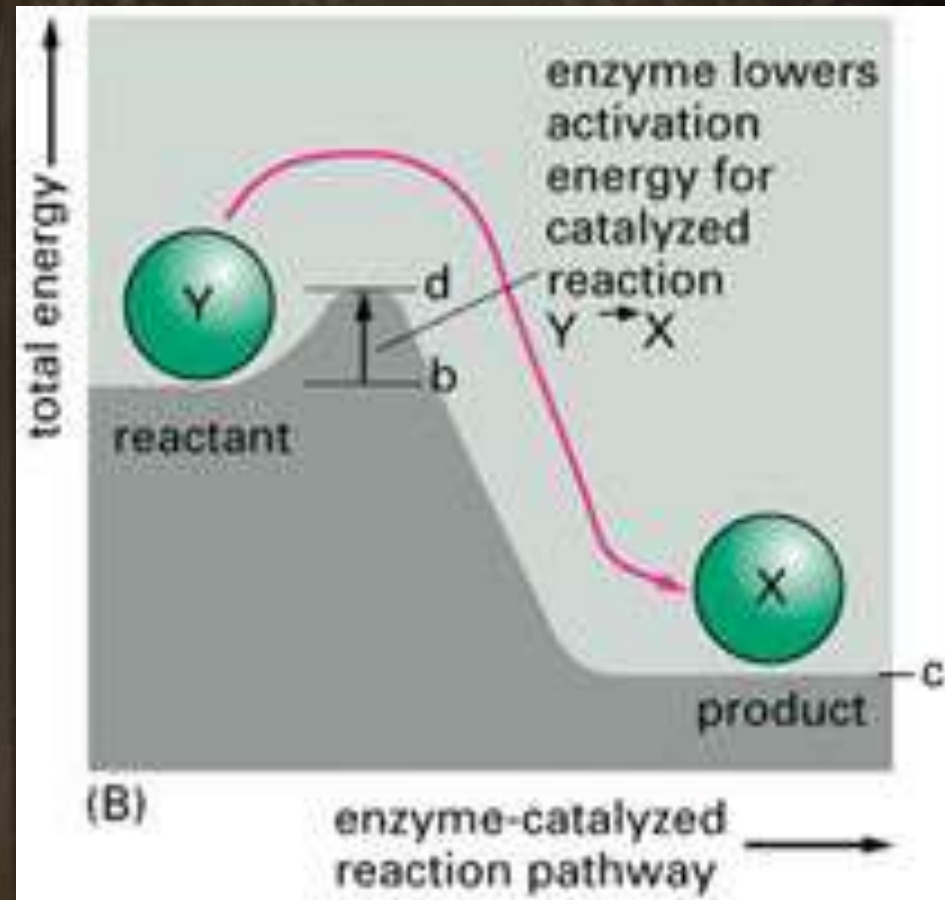
Mechanism of enzyme catalysis

How do enzymes increase the rate of reaction?

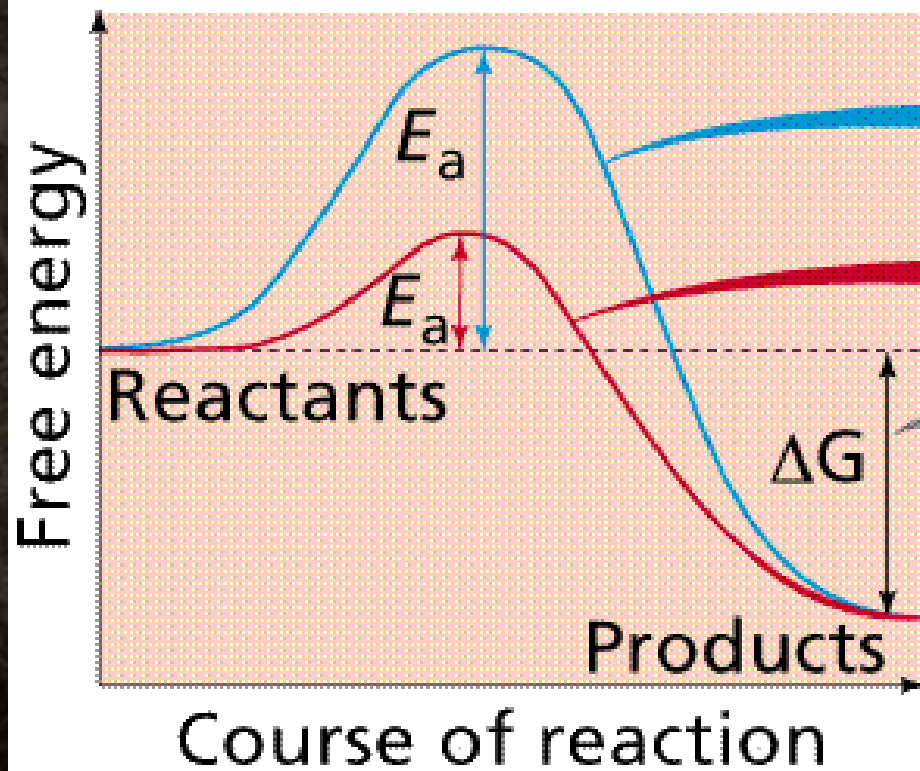
- **Enzymes increase reaction rates by decreasing the amount of energy required to form a complex of reactants that is competent to produce reaction products. This complex is known as the activated state or transition state complex for the reaction.**
- **Enzymes and other catalysts accelerate reactions by lowering the energy of the transition state.**

How do enzymes Work?

Enzymes work by **weakening bonds** which **lowers activation energy**



How do enzymes increase the rate of the reaction



An **uncatalyzed reaction** requires a higher activation energy than does a **catalyzed reaction**

There is no difference in free energy between catalyzed and uncatalyzed reactions

Free energy change

- ΔG° : (Standard free energy change (Gibbs free energy) → it is ΔG under standard state conditions.
- Standard free energy change (Gibbs free energy) It is energy difference in free energy between substrate and product.
- $\Delta G^\circ = G_{\text{product}} - G_{\text{substrate}}$
- ΔG° expresses the amount of energy capable of doing work during a reaction at constant temp. and pressure.
- The relationship between K_{eq} and ΔG° :-
- $\Delta G^\circ = -RT \ln K_{\text{eq}}$
- R : gas constant , $\Delta G^\circ = -2.303 RT \log K_{\text{eq}}$
- T : absolute Temp ($t + 273$) → 298k(c°)
- $K_{\text{eq}} = \frac{[P]}{[S]}$
- Both ΔG° and K_{eq} tell in which direction and how far a reaction will proceed
- when all substrates and products are 1M

For exothermic reaction

- **Exothermic reaction:**
- **Free energy of substrate(S) is more than free energy of product(P), therefore value of ΔG° is negative**
- **e.g**
- **if energy of : S = 9 and of P = 5**
- **$\Delta G^\circ = 5 - 9 = -4$ is negative**
- **→ mean that the reaction will proceed from the left to right toward a state of minimum energy (spontaneous reaction)**

For endothermic reaction

- **Endothermic reaction:**
- **Free energy of substrate(S) is less than free energy of product(P), therefore value of ΔG° is positive**
- **e.g**
- **if energy of : S = 5 and of P = 10**
- **$\Delta G^\circ = 10 - 5 = +5$**
- **ΔG° is positive**
- **→ mean that the reaction will not proceed from the left to right toward a state of minimum energy (nonspontaneous reaction) . An input of free energy is required to drive such a reaction.**

equilibrium reaction

- **If free energy of substrate and product is same then ΔG° is zero. The reaction is said to be at equilibrium.**

The rate of reaction is dependent on entirely different parameters

- Transition state : At the top of the energy hill is a point at which decay to the S or P state can occur. It is an unstable activated state in which new bonds are formed and old bonds are broken i.e. activated state of the substrate.
- *[The rate of reaction $S \rightarrow P$ depends on the number of molecules of S that enters the transition state per unit time]

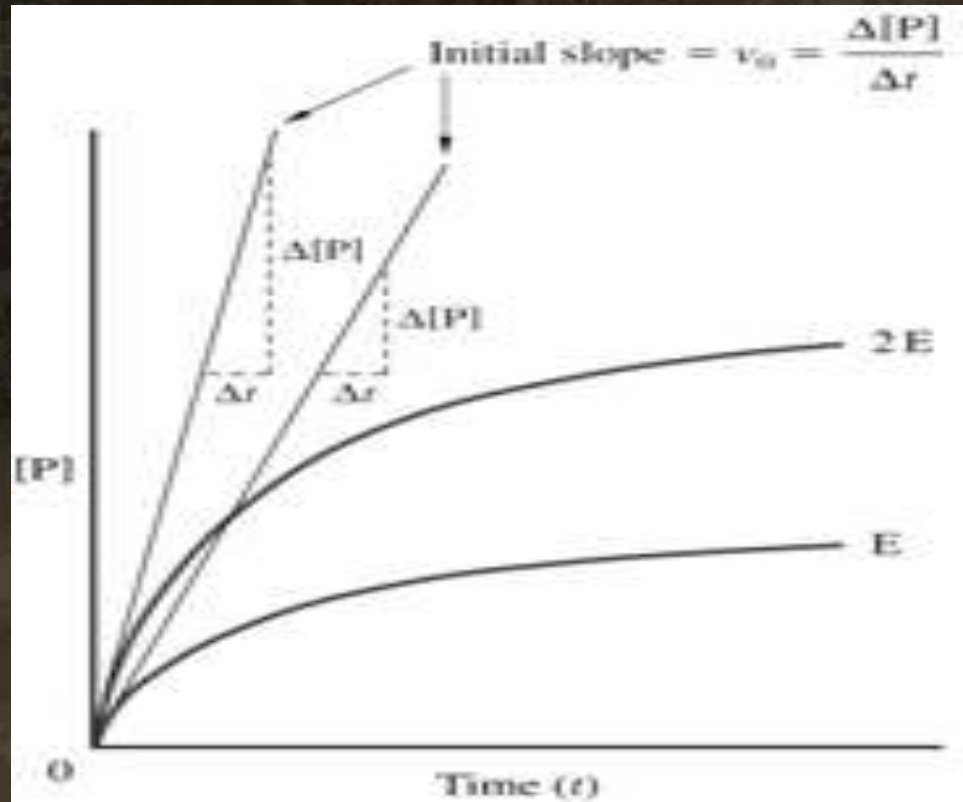
The rate of reaction is dependent on entirely different parameters

- Reaction rates can be increase by:
- •↑Temperature•
- ↓activation energy
- (1)Raising the temp. resulting in →increasing the number of molecules with sufficient energy to overcome this energy barrier.
- (2)Catalysts (enzymes) → 10^3 - 10^{15} times faster than the same uncatalyzed.
- -Enzymes speed up the rate at which a reaction approaches equilibrium.
- -Enzymes increase rate of reaction by decreasing energy of
- activation (ΔG^\ddagger).

- *Enzyme increase rate of reaction by decrease ΔG^\ddagger (energy of activation) but do not change ΔG° .
- *Enzyme do not change Kequilibrium(K_{eq}) of a reaction
- $S \leftrightarrow P$
- r_f : rate of forward reaction
- r_b : rate of backward reaction
- According to Laws of mass action : rate of reaction is proportional to molar concentration of reactants
- $r_f \propto [S]$ [] : molar concentration
- $r_f = k_f[S]$
- $r_b \propto [P]$
- $r_b = k_b[P]$

- At equilibrium : rate of forward reaction is equal to rate of backward reaction
- $r_f = r_b$
- $k_f[S] = k_b[P]$
- $K_f / K_b = [P] / [S]$
- $K_{eq} = [P] / [S]$
- e.g. for a reaction $A \leftrightarrow P$: $k_{eq} = [P] / [S]$
- or $K_{eq} = 10^{-3} / 10^{-5} = 100$ (without enzyme)
- When enzyme is added both rate of forward reaction and rate of backward reaction are both increase. (if $K_f = 6$ fold, K_b also increase 6 fold) in $K_{eq} = K_f / K_b = 10^{-3} / 10^{-5} = 100$

Progress curve of an enzymatic reaction

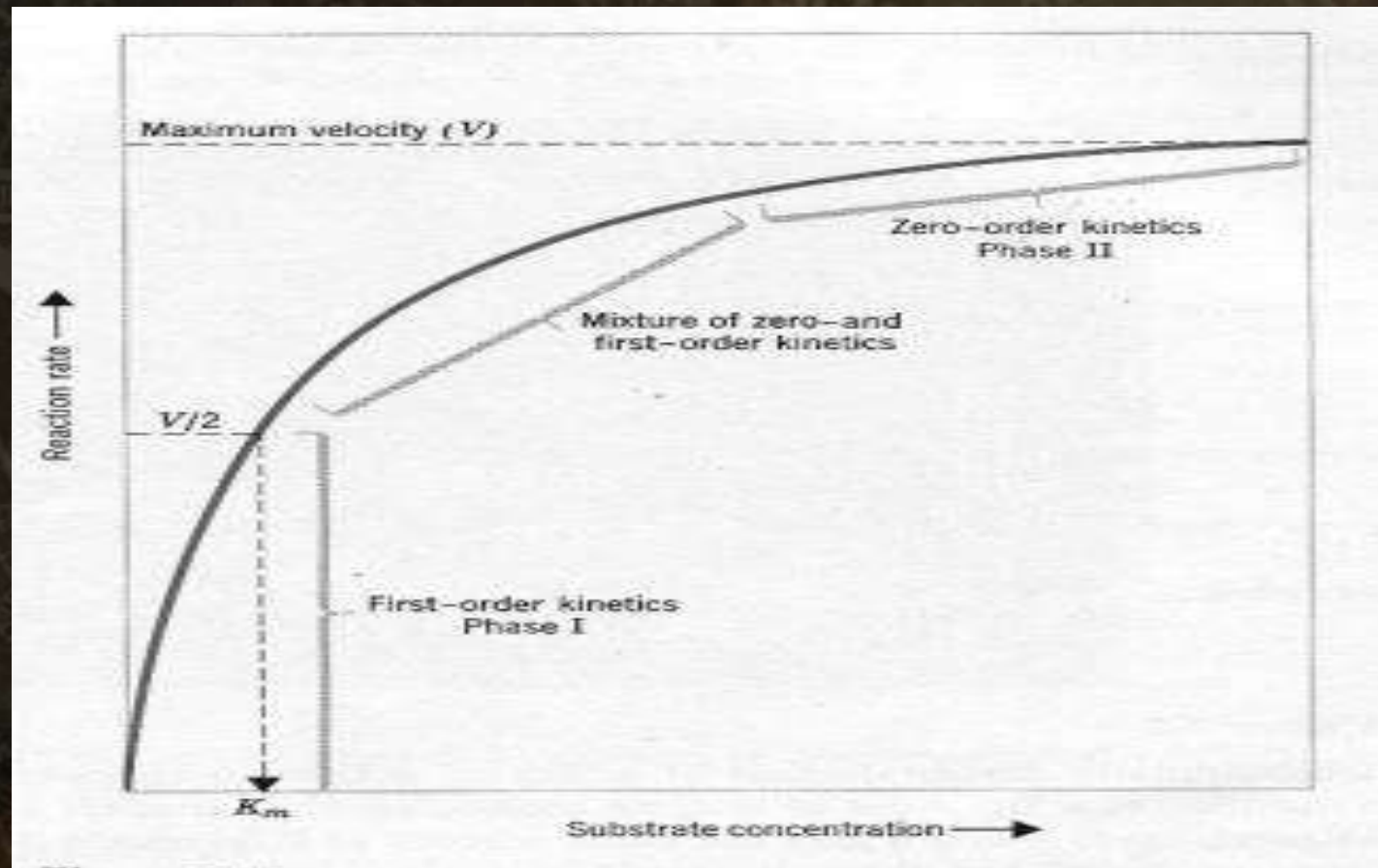


Initial velocity

- Initial velocity : is velocity at the beginning of the reaction (linear part) e.g. as soon as [S] or [E] are mixed.
- It is very important parameter to measure when studying enzymatic reaction.
- It is determined from the slope of the progress curve at the beginning of the reaction (The velocity constantly changes as the reaction proceed to equilibrium and become zero at equilibrium.)
- Rate of reaction doubles when twice as much enzyme is used

- Velocity decreases with time because :
 - (a) S may be used up.
 - (b) P may inhibit reaction (E)
 - (c) Change of pH
 - (d) Cofactor or coenzyme may be used up.
 - (e) Enzyme may lose activity
- $T_{1/2}$: It is the time required for the initial concentration of substrate to reduce to half its original concentration.

Reaction order kinetics



Reaction Orders

A. First Order Reaction : are reactions those proceed at a rate exactly proportional to the concentration of one reactant. e.g. $A \leftrightarrow P$: $r \propto [A]$,

$$r = k [A]$$

k = is rate constant for first order reaction

The reaction at any time t is given by first-order rate equation:

$$-d[A]/dt = k [A]$$

$-d[A]/dt$ is the rate at which the conc. Of A decreases.

$[A]$: is molar conc. Of A

k : rate constant•

The integrated form of this equation:

$$\log [A_0]/ [A] = kt \cdot 2.303$$

$$kt = 2.303 \log [A_0]/[A]$$

$[A_0]$ is the concentration of A at zero time., $[A]$ the concentration at time t .

Half time of first order reaction

- The half time of first order reaction
- $t_{1/2}$: is the time required to convert half the substrate originally present to product.
- $t_{1/2} = 0.693 / k$ ($t_{1/2}$ is constant for first order reaction and is related to k .)

Reaction Orders

B. Second Order reaction: are those in which the rate is proportional to the product of the concentration of two reactants (or to the second power at single reactant ($2A \rightarrow p$))

- In a reaction $A + B \rightarrow P$
- $r \propto [A][B]$
- $r = k [A][B]$
- $k =$ second order rate constant . i.e. $= d[A]/dt$ or $-d[A]/dt$ or $d p/dt = k [A][B]$

C. Third Order reaction : (are relatively rare) are those in which the rate is proportional to the product of the concentration of three reactants.

In a reaction $A + 2B \rightarrow P$

$$r \propto [A][B]^2$$

$$r = k [A][B]^2$$

$k =$ third order rate constant

Reaction order

- **D.** Zero order reaction :Rate of reaction is independent of any concentration.
- Change in concentration of Substrate has no effect on rate
- $v = k_0$
- This occurs in catalyzed reactions.
- Under this conditions, the enzyme is operating at its maximum velocity.