Properties of Enzymes

(a) Solubility:

Enzymes as proteins are soluble in water or dilute salt solution

(b) Molecular weight

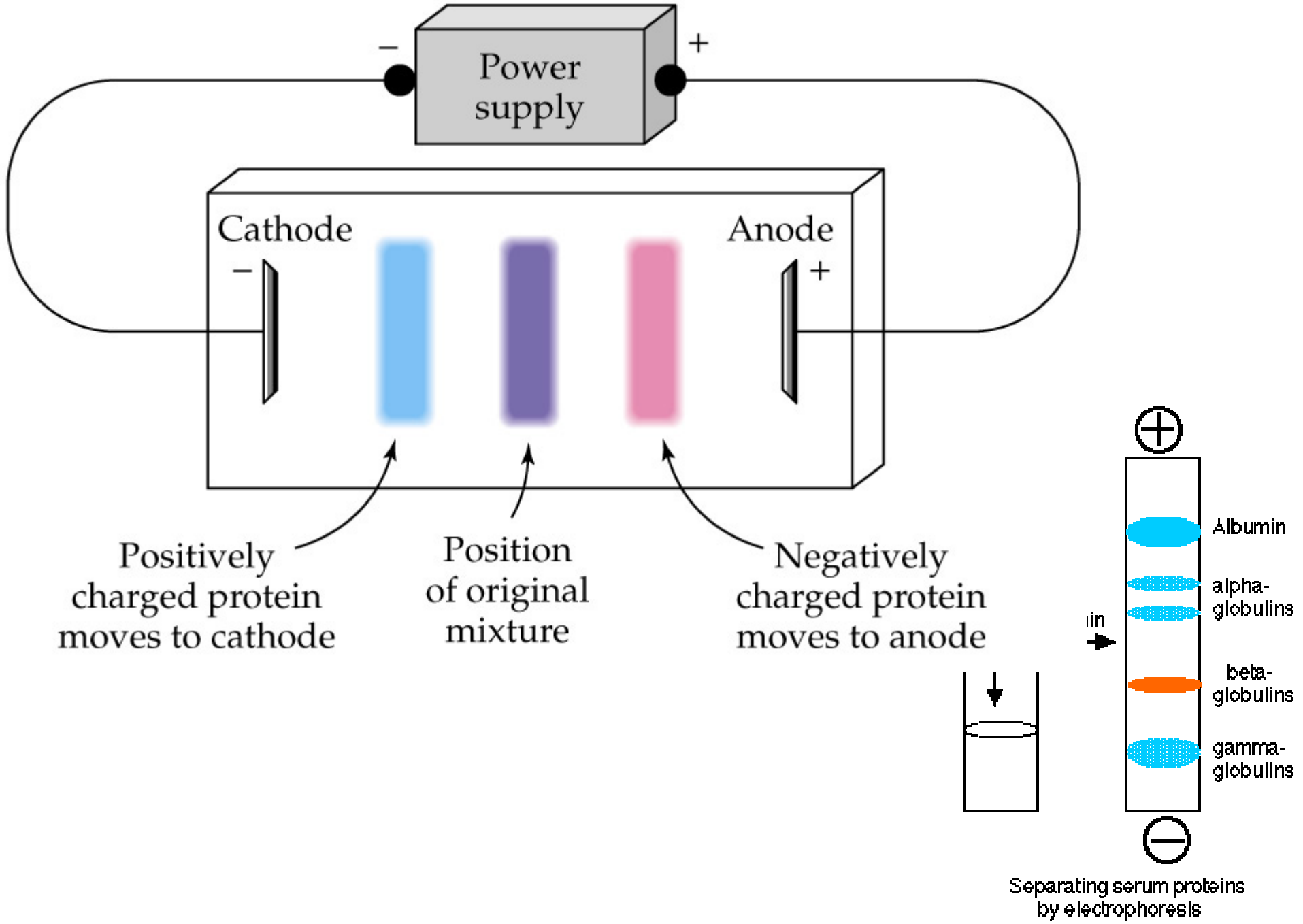
Enzymes have ↑ Mw (varying from 10000 - several thousands

(c) Enzymes are charged molecules:

Due to the presences of amino acids, each enzyme has a charge.

The charge depends on the pH of the solution.

At very low pH the amino acids are fully protonated and there is a positive charge on the proteins; as pH is increased, the protein losses a proton to neutralize the OH- group and becomes a zwitter ion (a charged molecule with equal number of +ve and –ve charges. As more alkali is added, the NH_3^+ group gives its H^+ and protein becomes positively charged.


Power supply
−
+
Cathode
−
Anode
+
Positively charged protein moves to cathode
Position of original mixture
Negatively charged protein moves to anode
in
Albumin
alpha-globulins
beta-globulins
gamma-globulins
Separating serum proteins by electrophoresis

(c) **Enzymes have buffering capacity (acid-base).** They are amphoteric molecules i.e behave both as acids and bases. (due to presence of both free amino group and free carboxyl group) - they act as buffer. At pKa they make the most efficient buffer.

(d) **Each enzyme has a specific Isoelectric pH: (PI)**

It is the pH at which the net charge on protein equal to zero – so they do not move in an electric field.

[It is the pH at which the protein molecule carries an equal positive and negative charges]

Above PI - negatively charged can move in an electric field

Below PI - positive charged and can move under an electric field.

(e) Denaturation: When proteins are heated , or subjected to extremes of temperature, high salt, organic solvents etc, the non-covalent bonds break, changing the native structure to random coil. This unfolding of protein is due to loss of secondary, tertiary and quaternary structure.

It does not affect primary structure.

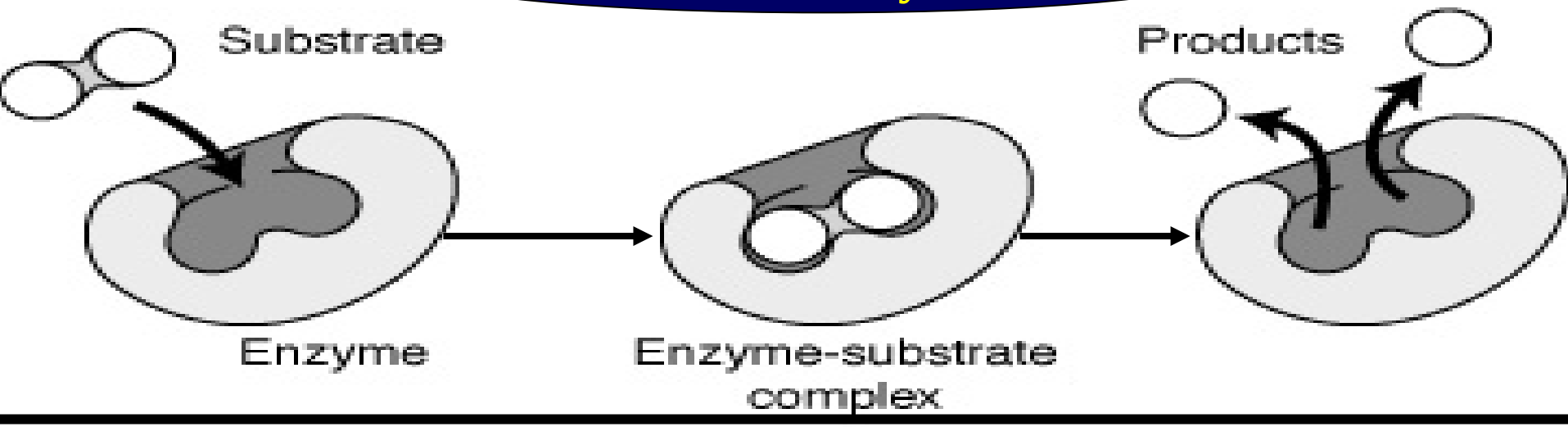
Effect of Denaturation: Loss of activity due to loss of shape and active site. (Protein become insoluble & precipitates).

Denaturing Factors:

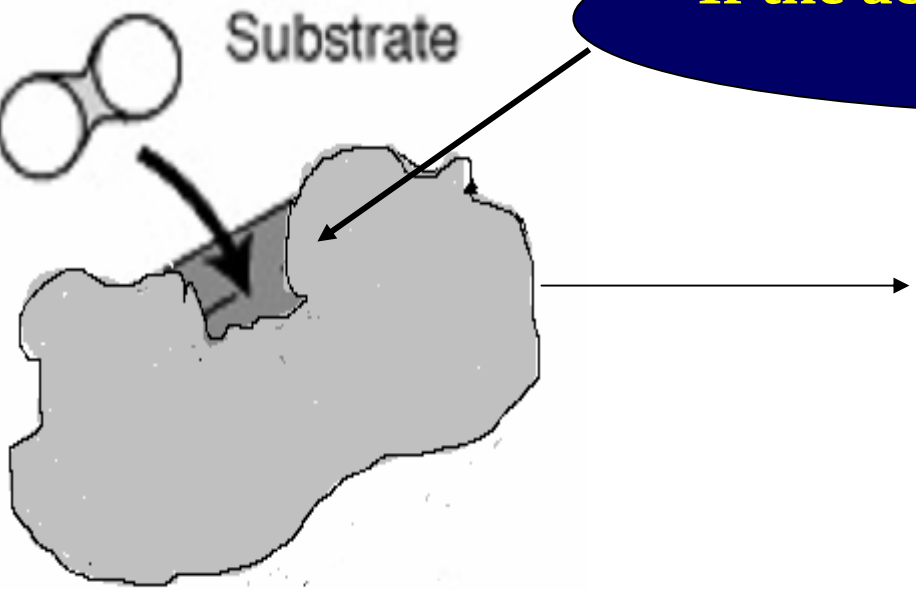
- Heat
- Change in pH (↑,↓)
- Radiation
- Heavy metals
- Detergents
- Digestive enzymes
- Urea (8M)
- Repeated freezing and thawing (which cause disruption of hydrogen or other weak bonds)

Mechanism of enzyme activity

Active Enzyme



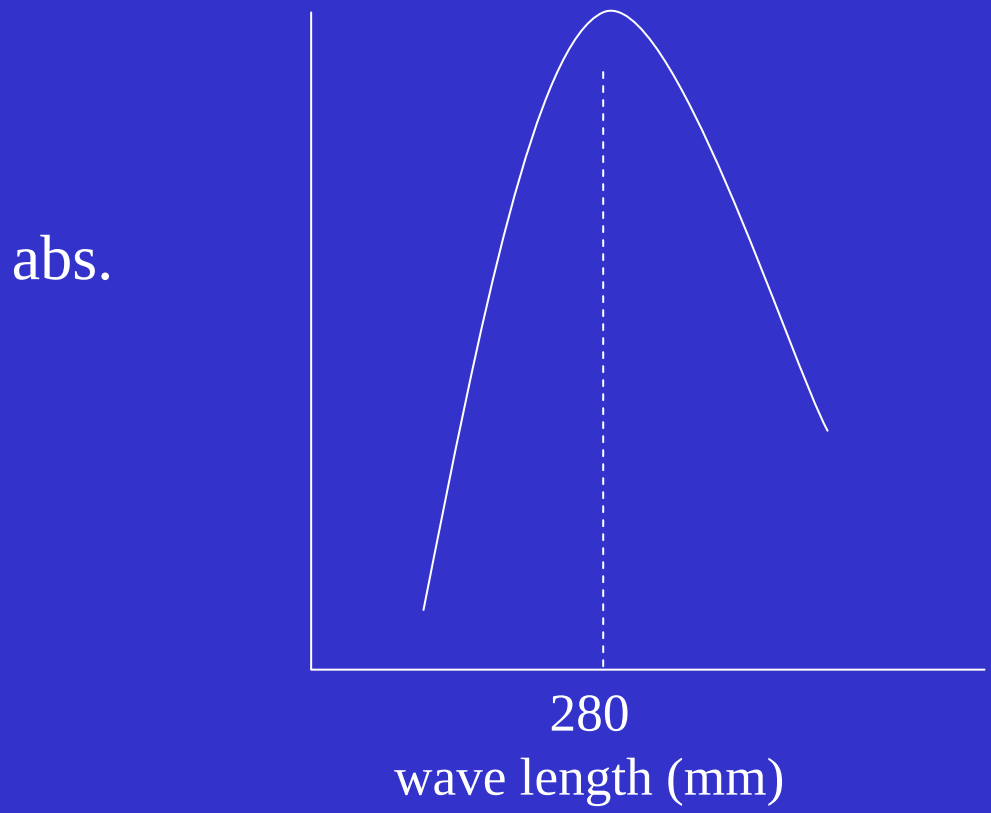
If the active site is altered E will lose activity



Change of shape causes loss of activity

(f) Absorption Spectra:

Enzymes are proteins give maximum absorption at 280mm. (due to their content of aromatic amino acids [trp, tyr, phe]



(g) Chemical reaction: (color reaction)

(i) Xanthroprotoic reaction:

with protein containing phenylalanine end tyrosine--- give orange colour.

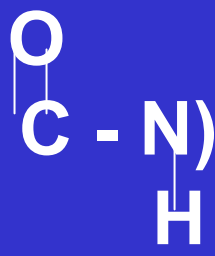
(ii) Millon's reaction:

Proteins containing phenolic (-OH) group of tyrosine--- give red colour

(iii) Sulfate reaction:

Proteins containing sulfur amino acids (cysteine) ----give black or grey colour

(iv) Biuret's Test:

- This is a general test for all proteins because it is given by peptide linkage ()

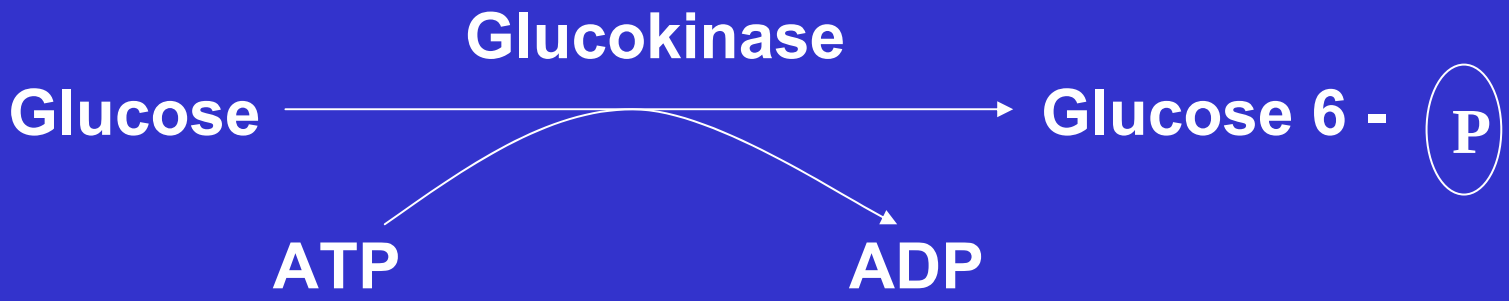
- The reaction occurs between protein, sodium hydroxide and copper sulfate giving violet complex.

Specificity:

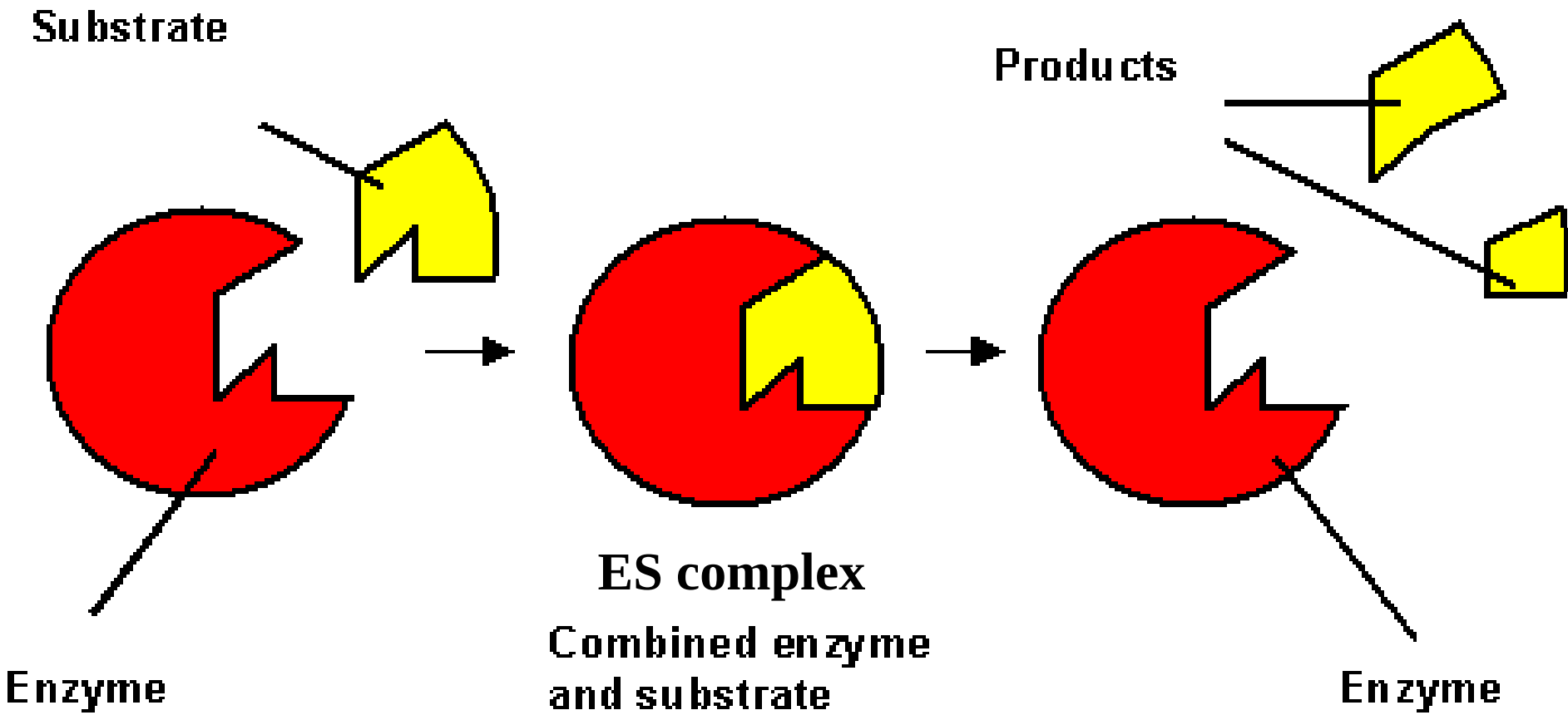
- Enzymes are highly specific both
- Very important characteristic of all enzymes.
- Enzymes are specific:
    - for the reactions they catalyze.
    - and in their course of reaction, which are called substrates.

(a) Absolute specificity:

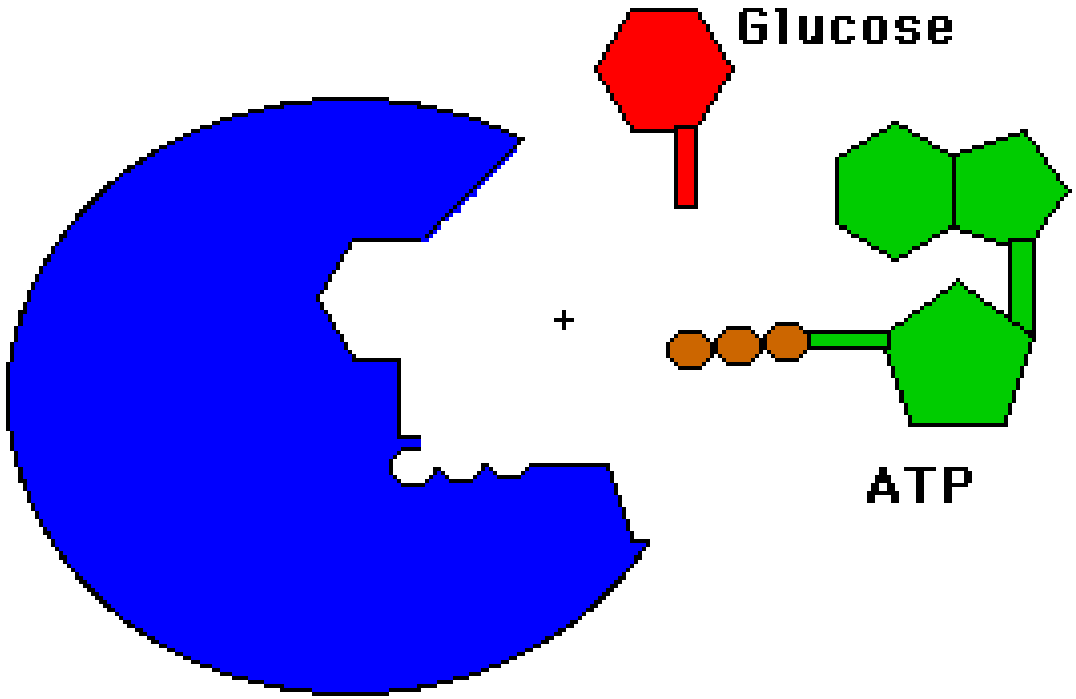
The enzyme can act only on one specific substrate.



Enzyme Reaction



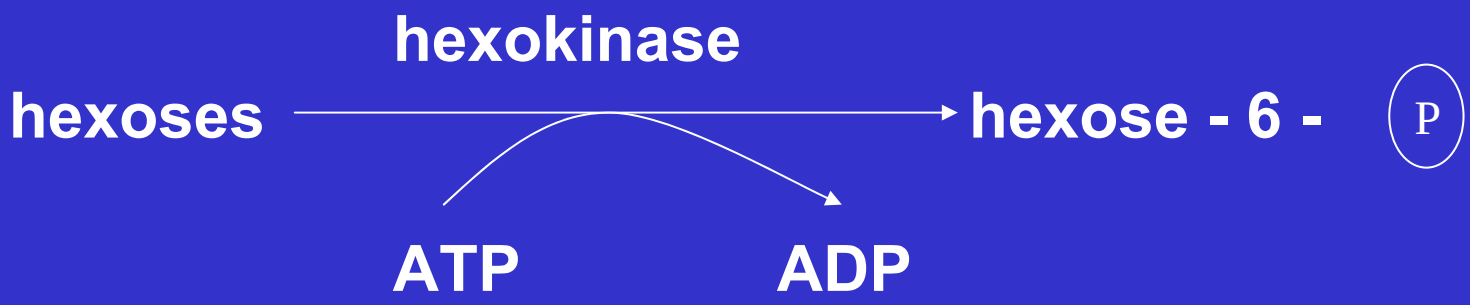
An enzyme catalysed reaction



(b) Group specificity:

- Broad specificity
- Enzyme act on a group of related substrates.
- The substrates have a common group on which the enzyme acts:

e.g. - esterase can act on different esters
- proteases can act on different protein
e.g. of proteases: chymotrypsin, trypsin, pepsin.



The specificity is due to substrate binding site (active site) which lies on the enzyme surface - in specificiting is due to the specific arrangement of a.a. in the active site that participate in the bond making and bond breaking (These residues are called catalytic groups)

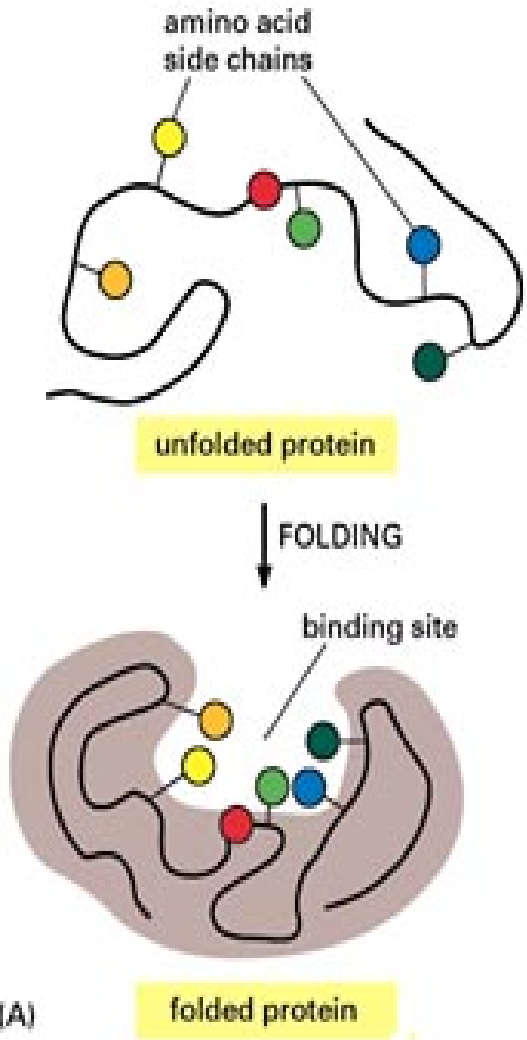
The specificiting of an enzyme is determined by:

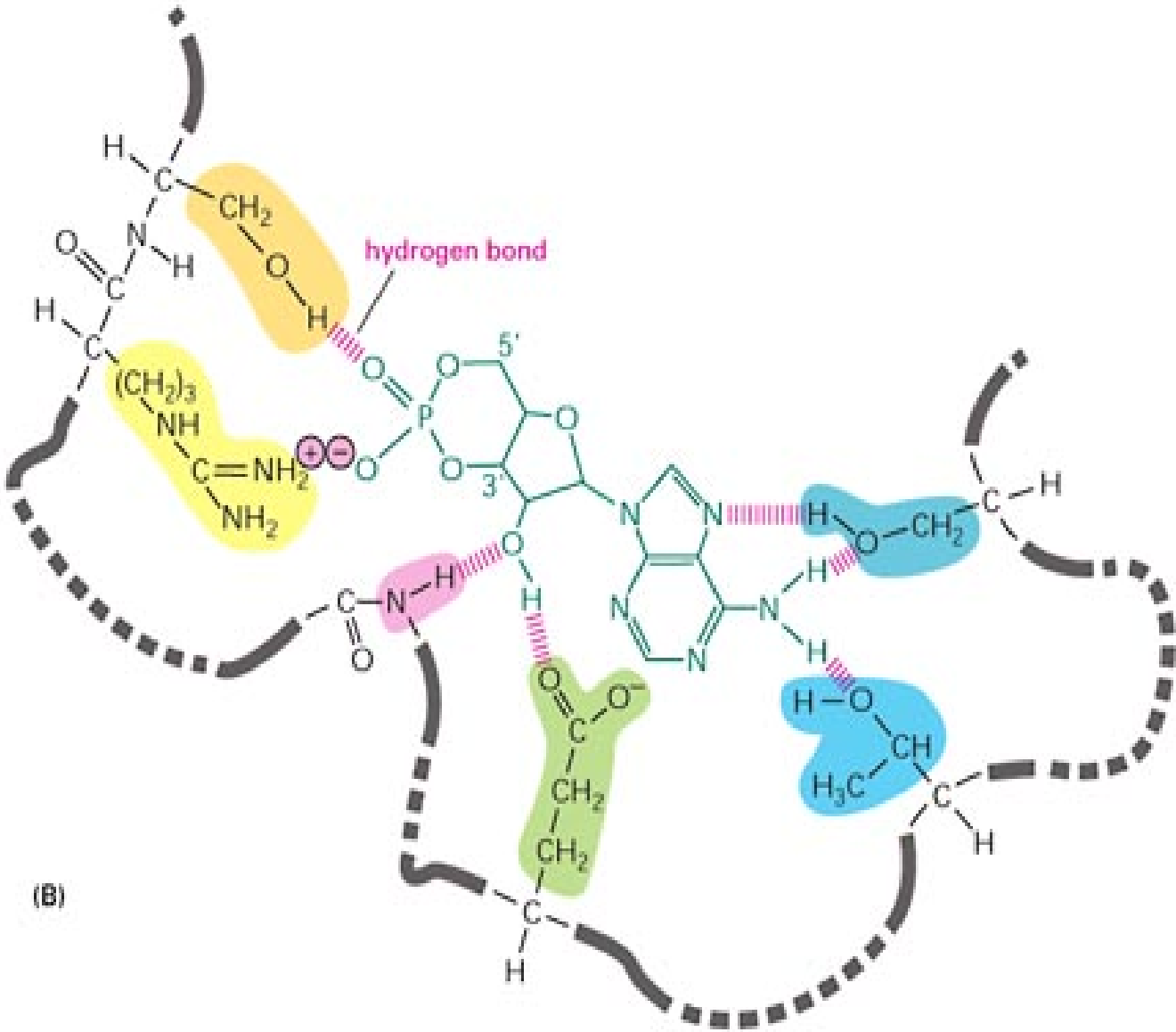
(a) Functional groups of enzyme (specific a.a. side chains, metal ions, and coenzymes)
(b) Functional groups of substrate (binding groups: bind to enzyme and position, the substrate molecule properly on the active side).

During enzyme action, there is a temporary combination between enzyme and its substrate forming enzyme by relatively weak forces (hydrogen bonds, bydrophobic, ionic and vander Waels bonds).
This occur at the active site of the enzyme (most substrates are bound to the enzyme by relatively weak forces (hydrogen bonds, hydrophobic, ionic and vander Waels bonds)

$$E + S \rightleftharpoons \text{ES complex}$$

Active site of enzymes





This is followed by dissociation of this complex into enzyme and product.

Models to explain substrate specificity of enzymes.

Lock and Key Model:

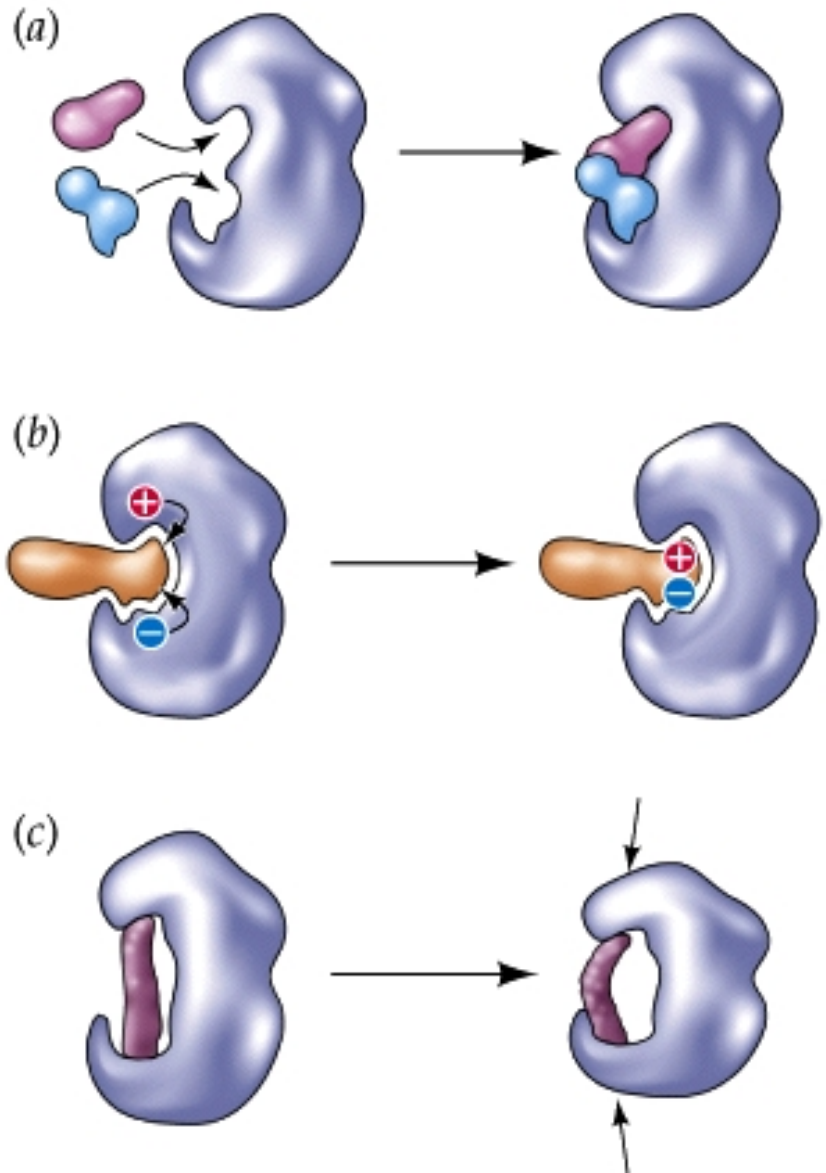
The active site of the unbound enzyme is complementary in shape to the substrate.

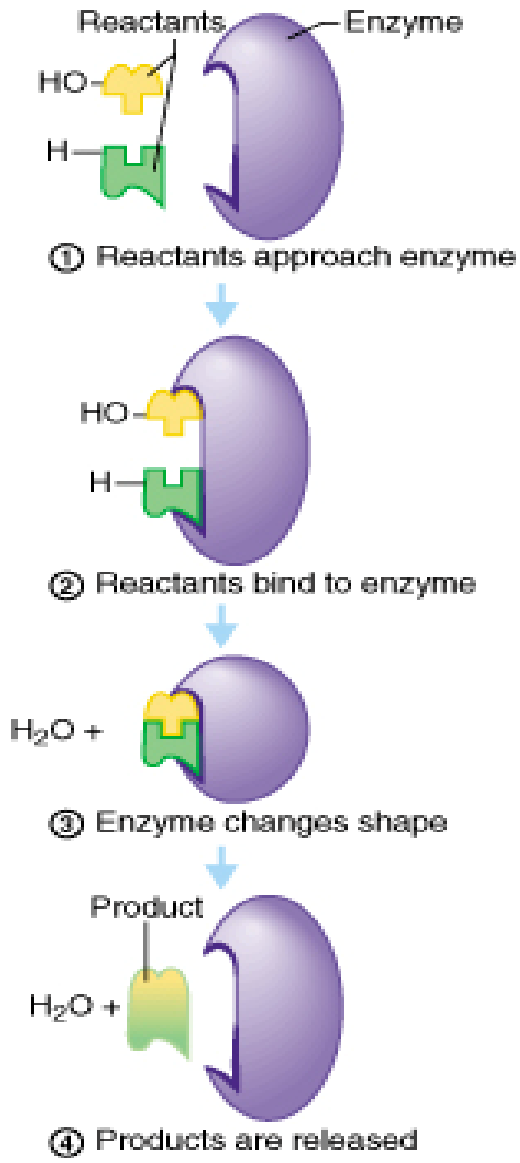
(The enzyme active site is rigid and fixed → does not change)

Induce Fit Model:

The enzyme change shape on the substrate binding. The active site forms a shape complementary to the substrate only *after* the substrate has been bound.

Lock and key and Induced fit model





Naming (Nomenclature) and Classification of enzymes

(i) Many enzymes have been named by adding the suffix "ase" to the name of the substrate or to a word describing the action or activity: e.g.protease, sucrase, lipase, amylase, dehydrogenase, oxidase, carboxylase etc

(ii) Some enzymes were given trivial names: e.g. trypsin, chymotrypsin, pepsin, thrombin etc.

Nomenclature of Enzymes

- When enzymes were first discovered there were only a few enzymes and so they were given trivial names.e.g. trypsin, chymotrypsin, pepsin, thrombin etc.
- These names did not give any idea about the source, reaction or function of enzymes.
- Soon a large number of enzymes were identified and it became necessary to have a system for naming enzymes, in order for everyone, in different parts of the world, to name them in the same way.
- The Normenclature [method of naming] was put forward by the International Union of Biochemistry

The International Union of Biochemical (IUB) developed a system of nomenclature:

(i) The enzyme name has 2 parts:
- The First names the substrate or substrates.
- The Second ending in -ase, indicates the type of reaction catalyzed.

(ii) The system give each enzyme a code number (EC) [called Enzyme Commission numerical code] and it contains 4 digits separated by points:

First digit:	Class
Second digit:	Sub-class
Third digit:	sub-sub-class
Fourth digit:	Serial number of the enzyme

Classification of enzymes

[on the basis of the nature of reaction catalysed]

This system divide enzymes into six major classes:

(1) **Oxido-reductases- catalyse oxidation and reduction reactions**

(2) **Transferases- transfer one group from donor to acceptor**

(3) **Hydrolases- break bonds by adding water**

(4) **Lyases- break bond but do not use water**

(5) **Isomerases- change one isomer to another**

(6) **Ligases- bring about formation of a bond (biosynthetic reactions)**

Number	Classification	Biochemical Properties
1.	**Oxidoreductases**	Act on many chemical groupings to add or remove hydrogen atoms or electrons [oxidation-reduction]
2.	**Transferases**	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules.
3.	**Hydrolases**	Cleavage of bond between C and other group by addition of water. [hydrolyzing it].
4.	**Lyases**	Nonhydrolytic cleavage of C-C, C-S, C-N bond. Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds.
5.	**Isomerases**	Convert on isomer to another, by transfering a group from one position to another within the same molecule: L to D isomerizations, mutase reactions (shifts of chemical groups) and others.
6.	**Ligases**	Catalyze reactions in which two chemical groups are joined (or **ligated)** with the use of energy from ATP. Formation of C-C, C-S, C-N bonds etc

Class 1. Oxidoreductases

- This class includes all those enzymes that catalyze oxidation - reduction reaction between two substrates:

$$A_{reduced} + B_{oxidized} \longleftrightarrow A_{oxidized} + B_{reduced}$$

alchohol — aldehyde

EC (1) 1.1.1.1

(EC (1) Class: oxidoreductase
EC 1.(I) Sub-class: acting on the CH-OH group of donors.
EC 1.1.(I) Sub-sub-class: with coenzyme (NAD^+) as acceptor.

Recommended Name: alcohol dehydrogenase
Systematic Name: alcohol: NAD^+ oxidoreductase

Class 2: Transferases

The enzymes in this group catalyze a transfer of a group (other than hydrogen) between two substrates.

$$A - X + B \longleftrightarrow A + B - X$$

- Enzymes catalyzing the transfer of groups:
  - one-carbon groups
  - aldehyde or ketone
  - acyl (acyl transferases)
  - aryl
  - glycosyl (glycosyl transferase)
  - phosphorus or sulfer-containing groups
  - nitrogenous group (amino transferase)
- They are further classified according to the group they transferred:
  - Phospho transferases = kinase
  - Amino transferases (Transaminases)
  - Transketolase
  - Methyl transferases (transmethylase)
  - Acyl transferases (transacylases)

Glutemate Pyruvate Transaminase

Alanine + α-keto gluterate ⟷ Pyruvate + L-Glutamate

Glutemate oxaloacetate Transaminase

Aspartate+ α-keto gluterate ⟶ Oxaloacetate+L-Glutamate

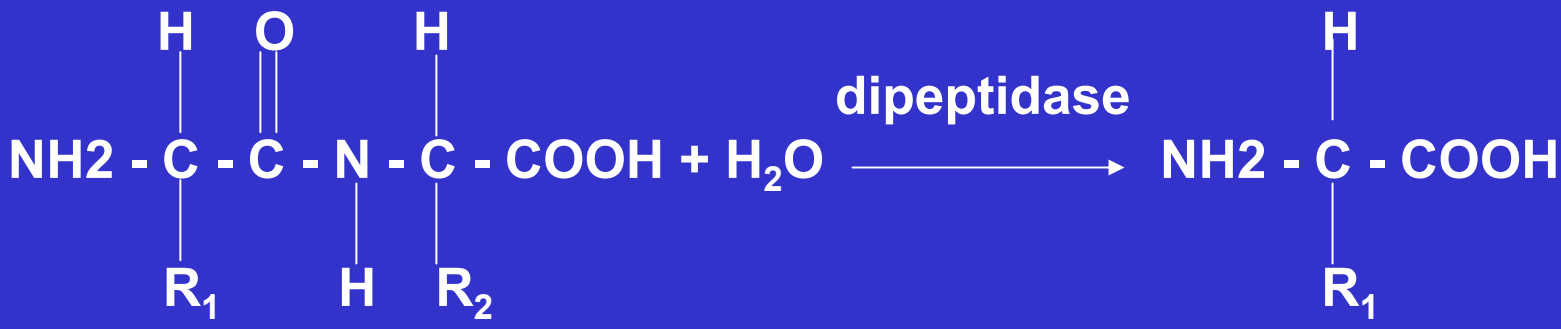
Glucokinase

Glucose+ ATP ⟷ Glucose-6-phosphate+ ADP

Note: Phosphotransferases = kinases (are phosphorylating enzyme that catalyze the transfer of the phosphoryl group from ATP to another nucleotide, phosphate, to alcohol or amino group acceptor).

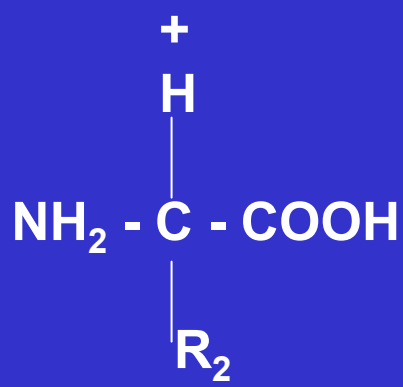
Class 3: Hydrolases

Enzymes in this class catalyze the breakdown (cleavage) of bonds by addition of water.



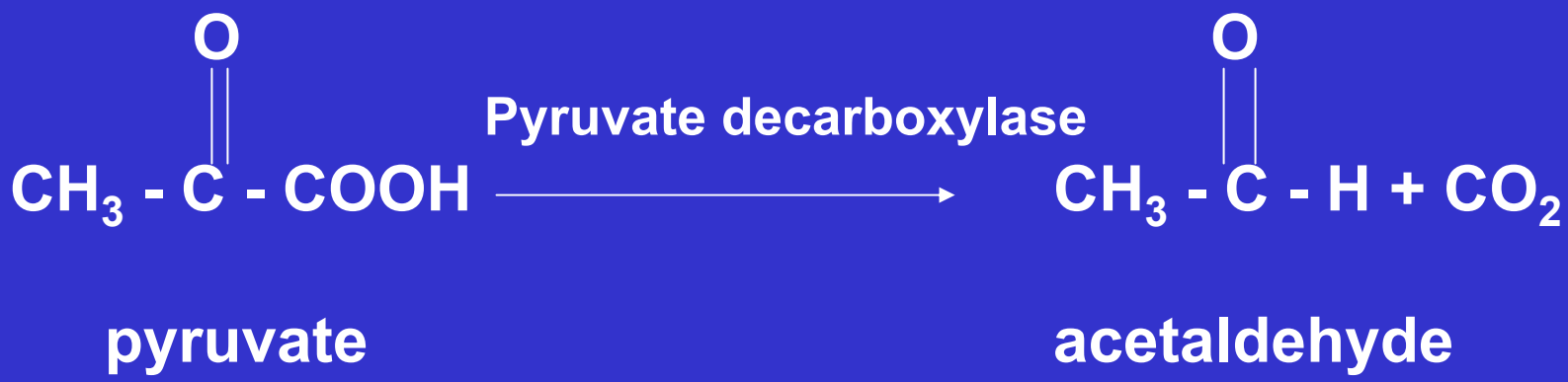
dipeptide

amino acid



Class 4 Lyases:

This group includes enzymes that catalyze removal of groups from substrates by mechanisms other than hydrolysis, leaving double bonds (in some cases):

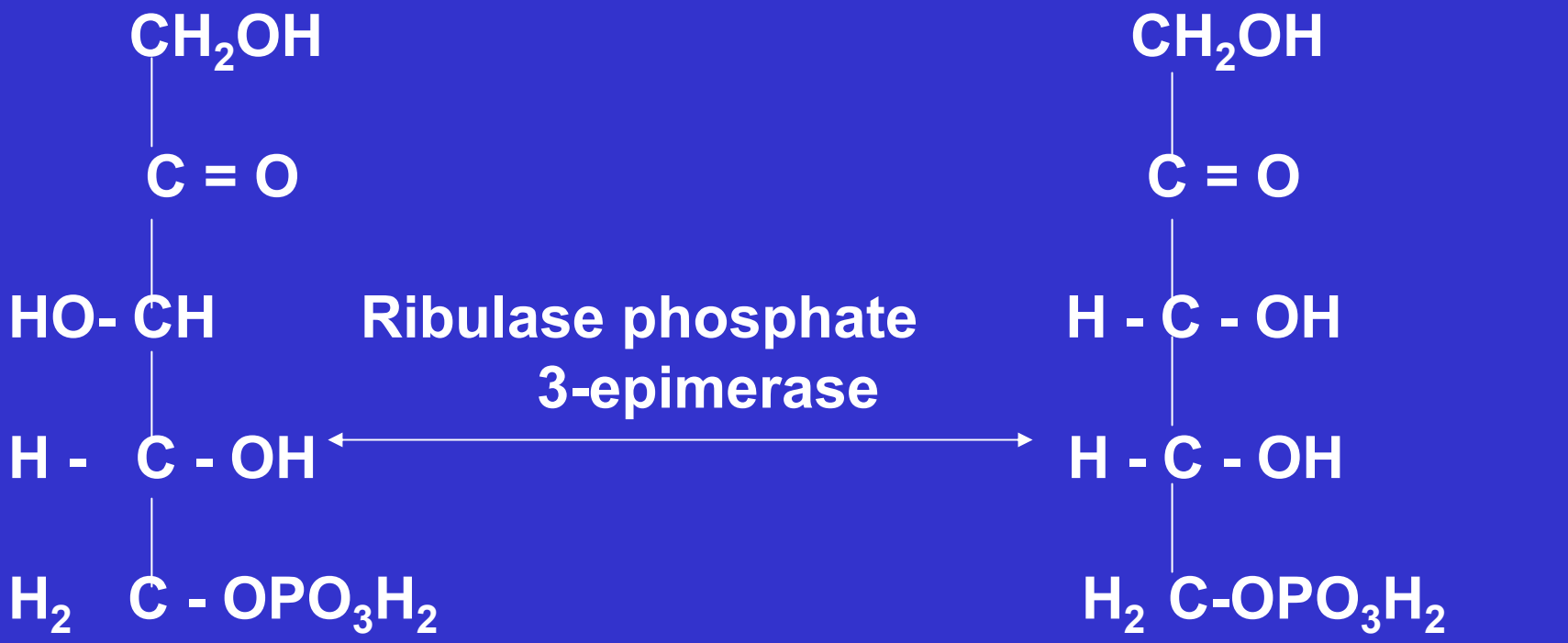


Class 5. Isomerases:

Enzymes in this class catalyze the interconversion of one isomer to another.

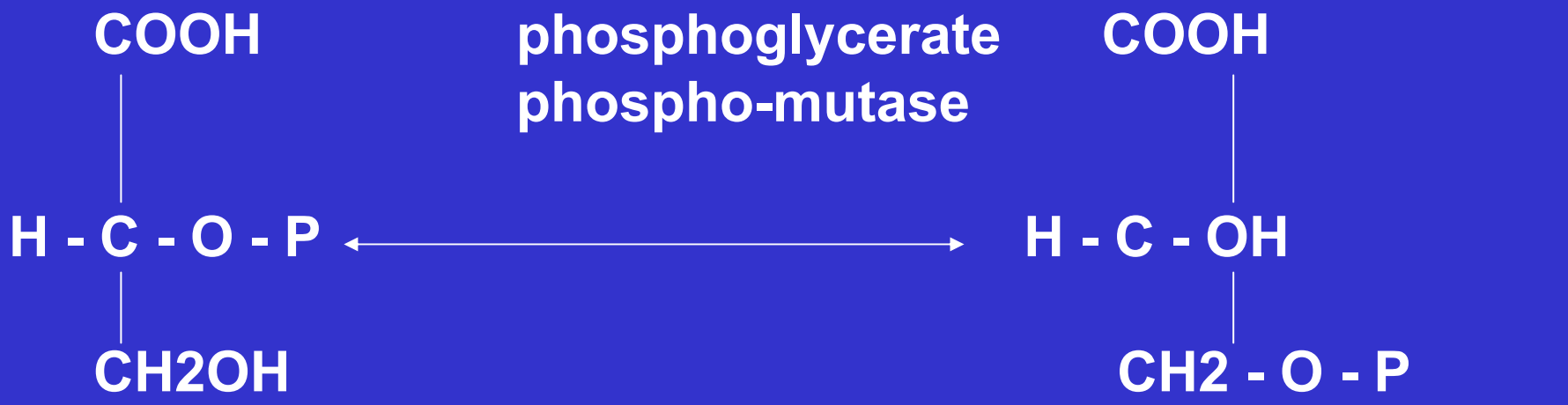
- These include: isomerase, mutase, i.e. epimerase, racemase
- i.e. interconversion include:
    - cis-trans interconversion
    - ketone enol "
    - aldose-ketose "

*Isomerases that catalyze the interconversion at asymmetric carbon are either epimerase or racemase.



D-xylulase 5-phosphate

D-Ribulase 5-phosphate



2-phosphoglycerate

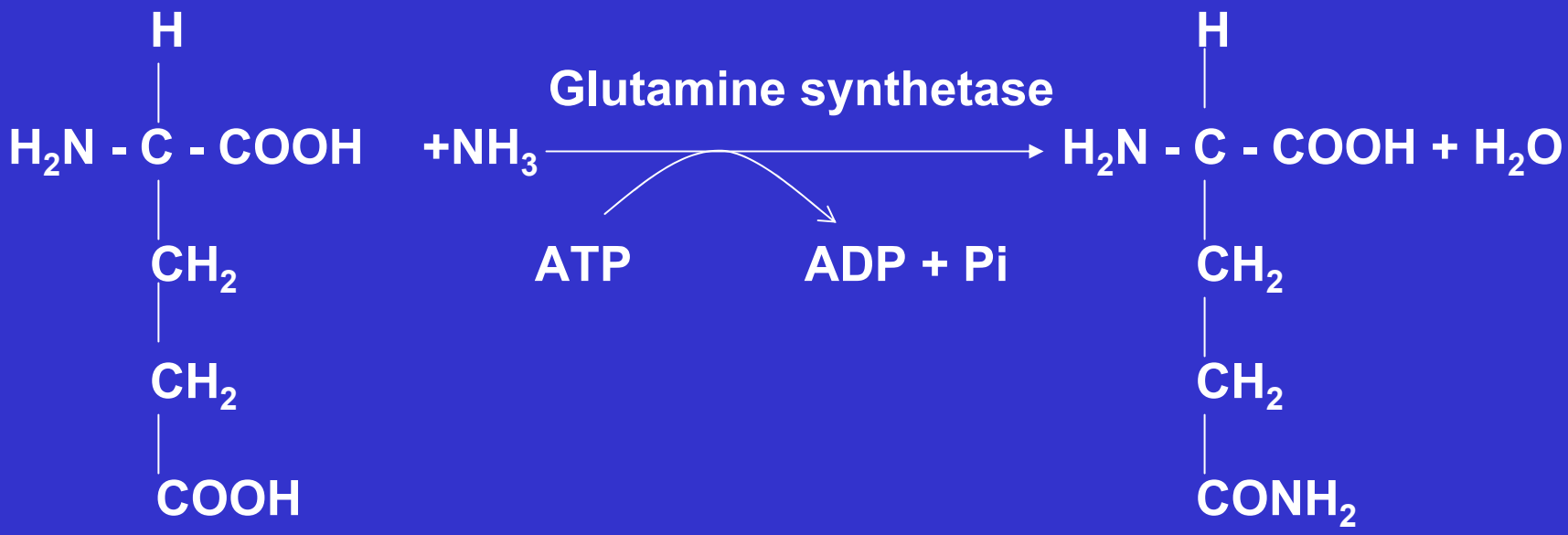
3-phosphoglycerate

*Mutase catalyze the intramolecular transfer of a group such as the phosphoryl group e.g. phosphoglycerate mutase catalyze the conversion of 2-phosphoglycerate to 3-phosphoglycerate.

Class 6. Ligases (synthetases):

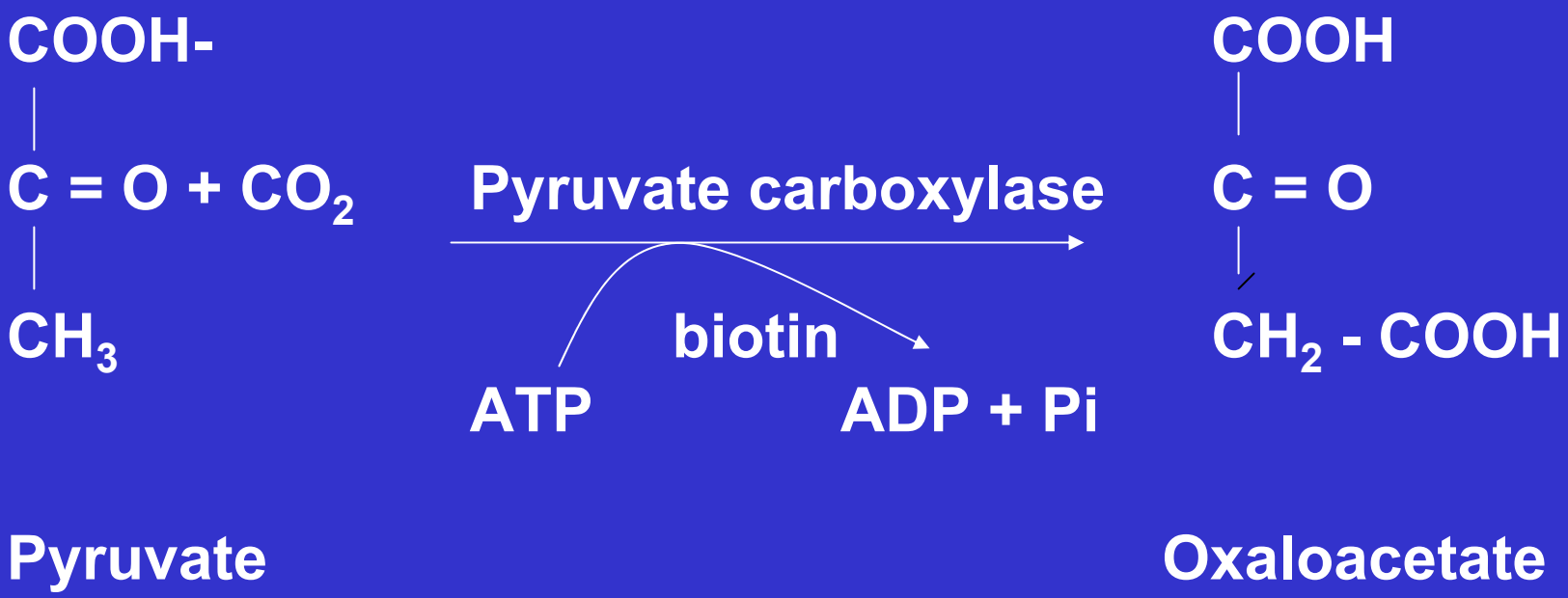
(Ligate = to bind)

The enzymes in this group catalyze joining the two molecules (or substrates) using the energy released by the hydrolysis of a high energy phosphate compound as ATP or GTP.



L-Glutumate

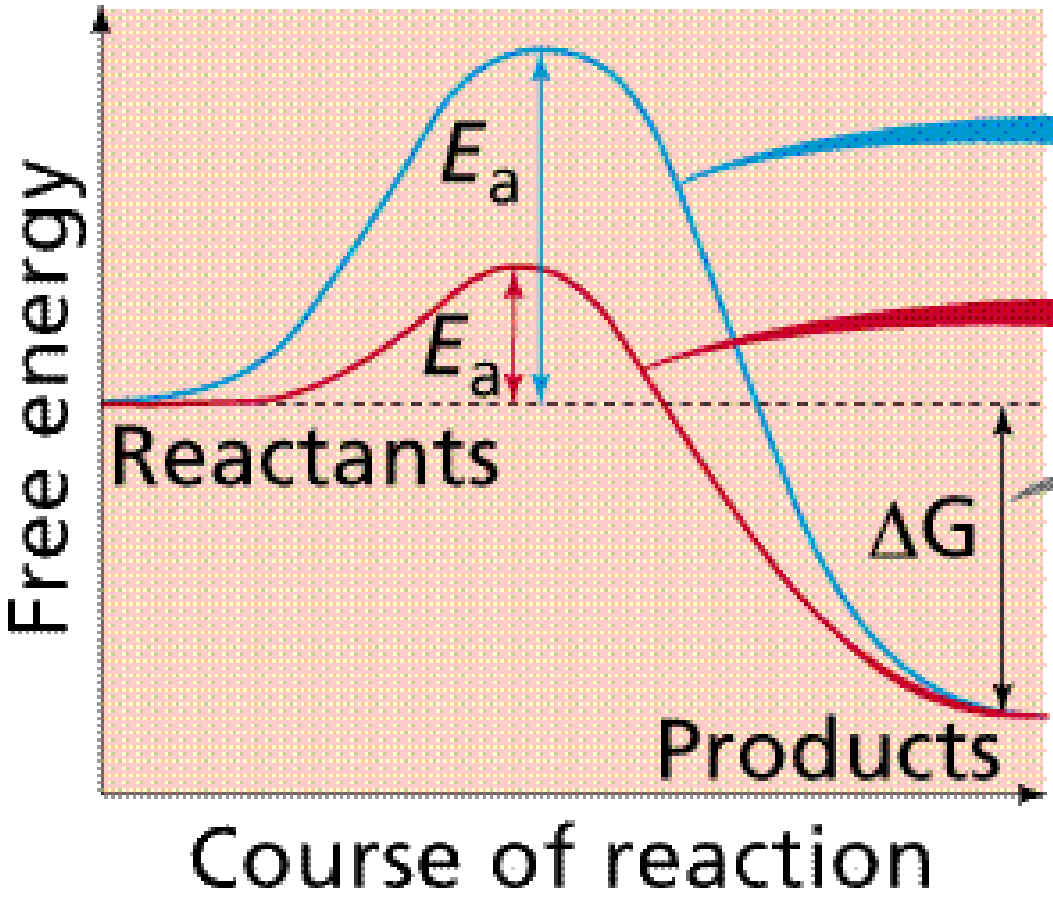
L-glutamine


COOH-
C = O + CO2
CH3
Pyruvate carboxylase
biotin
ATP
ADP + Pi
COOH
C = O
CH2 - COOH
Pyruvate
Oxaloacetate

Enzymes increase the rate of reaction

How?

How do enzymes increase the rate reaction



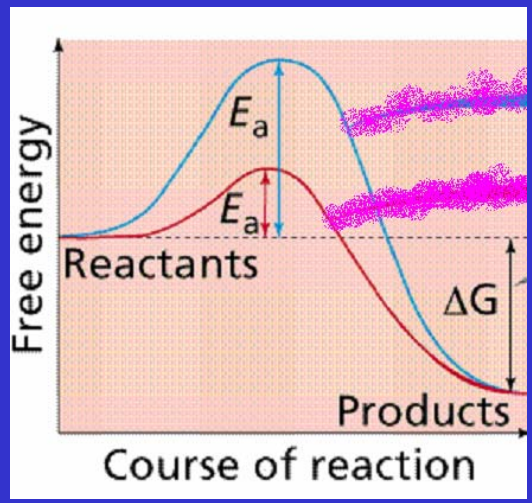
An uncatalyzed reaction requires a higher activation energy than does a catalyzed reaction

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

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 increase the rate of a reaction? Enzymes increase rate of reaction by decreasing energy of activation ($\Delta G^{\#}$)



$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

Such a reaction S $\longrightarrow$ P can be given by the above figure.

- This is picture of the energetic course of the reaction.
- The free energy of the system is plotted against the progress of the reaction.
- In its normal stable form or (ground state), any molecule (such as S or P) contains characteristic amount of free energy.
- Normal stable form of S or P contains a characteristic amount of free energy.

ΔG^o : (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 between substrate and product. $\Delta G^o = G_{product} - G_s$

ΔG^o expresses the amount of energy capable of doing work during a reaction at constant temp. and pressure.

The relationship between Keq and ΔG^o:-

$$\Delta G^o = -RT \ln Keq$$

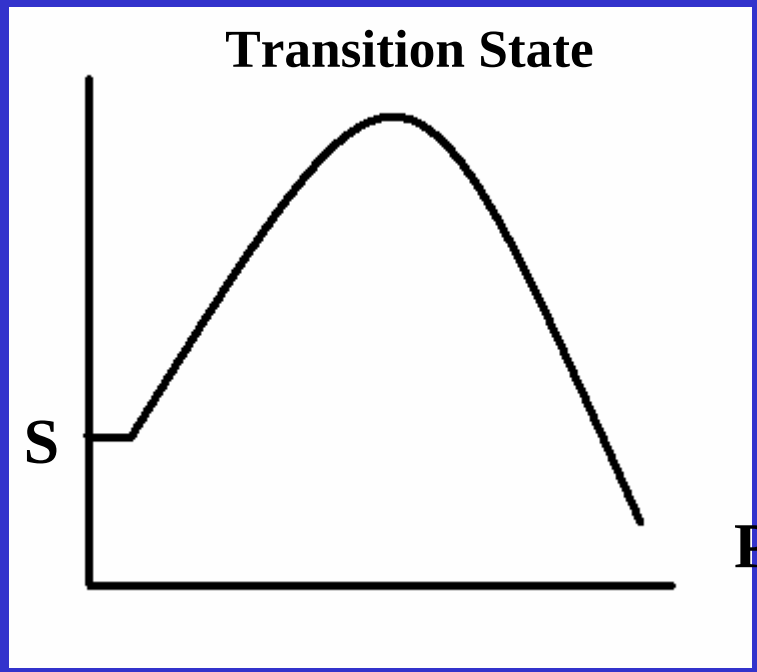
R: gas constant $\Delta G° = -2.303\ RT \log Keq$

T : absolute Temp (t + 273) → 298k
(c°)

$$Keq = \frac{[P]}{[S]}$$

Both $\Delta G°$ and Keq tell in which direction and how for a reaction will proceed when all substrates and products are 1M

Exothermic reaction:



e.g

if energy of:

S = 9

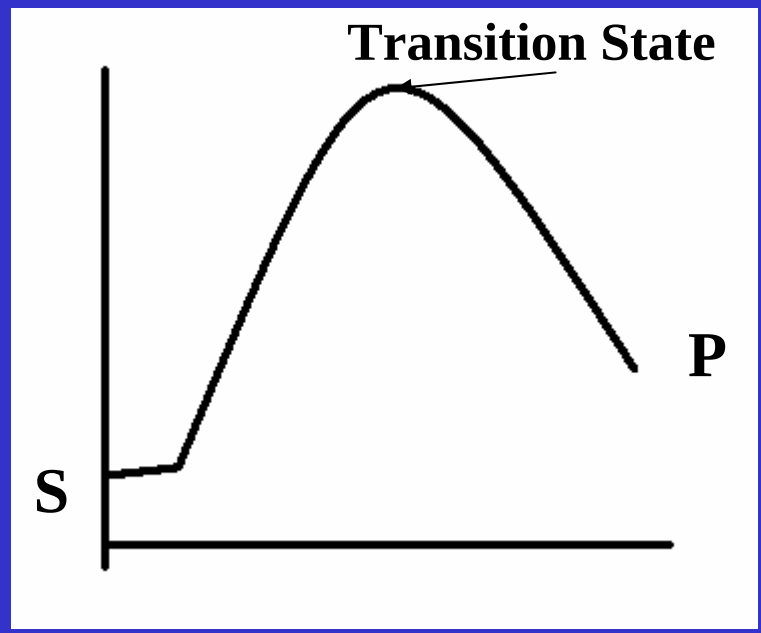
P = 5

$\Delta G° = 5-9$

$= -4$

$\Delta G°$ is negative → mean that the reaction will proceed from the left to right toward a state of minimum energy (spontaneous reaction)

Endothermic reaction:



e.g

if energy of:

S = 5

P = 10

ΔG° = 10-5 = 5

ΔG° is positive

- for exothermic reaction → ΔG° is negative

- for endothermic reaction → ΔG° is positive

The rate of reaction is dependent on entirely different parameters.

T : Transition State (represent half way point)

An unstable activated state in which new bonds are formed and old bonds are broken (not on intermediate) i.e. activated state.

*[The rate of reaction S → P depends on the number of molecules and S that enters the transition state per unit time]

Difference in the energy level of S (or P) and the transition state (high $\Delta G^{\#}$ → slower reaction)

(i.e. the rate of reaction reflect the activation energy).

At the top of the energy hill is a point at which decay to the S or P state is equally probable (It is down hill either way) → This is called Transition State)

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- 10+15 times faster than the same uncatalysed.

- Enzymes speed up the rate of which a reaction approaches equilibrium.

- Enzymes increase rate of reaction by decreasing energy of activation ($\Delta G^{\#}$).

*Enzyme increase rate of reaction by decrease $\Delta G^{\#}$ (energy of activation) but do not change $\Delta G°$.

*<u>Enzyme do not change $K_{equilibrium}$ (K_{eq}) of a reaction</u>

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)

$r_f \alpha [S]$
$r_f = k_f [S]$

[ ] : molar concentration

$r_b \alpha [P]$
$r_b = k_b [P]$

At equilibrium : rate of forward reaction is equal to rate of backward reaction.

$$r_f = r_b$$

$$k_f [S] = k_b [P]$$

$$\frac{K_f}{K_b} = \frac{[P]}{[S]}$$

$$\text{in } K_{eq} = \frac{[P]}{[S]}$$

e.g. for a reaction A $\rightleftharpoons$ P $\quad k_{eq} = \frac{[P]}{[S]}$

$$\text{or } K_{eq} = \frac{10^{-3}}{10^{-5}} = 100 \text{ (without enzyme)}$$

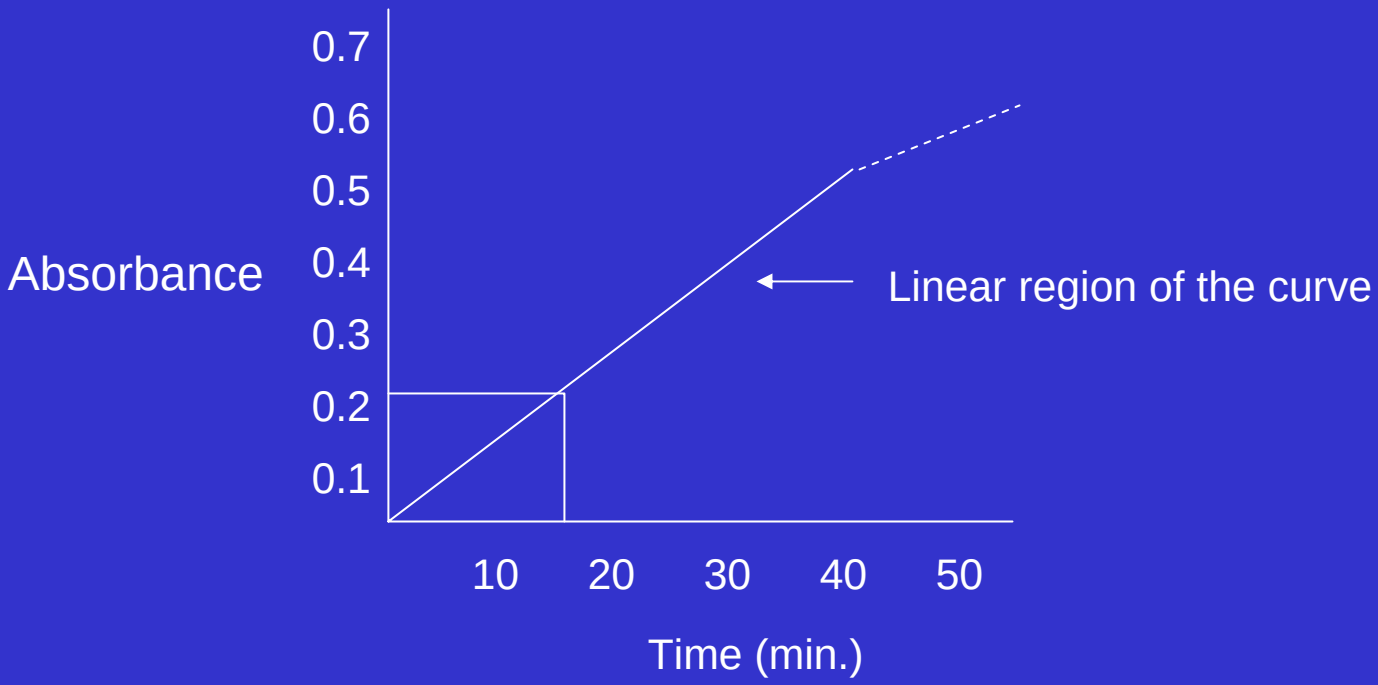
When enzyme is added both rate of forward reaction and rate of backward reaction are both increase.

(if Kf = 6 fold, Kb also increase 6 fold)

$$\text{in } K_{eq} = \frac{K_f}{K_b} = \frac{10^{-3}}{10^{-5}} = 100$$

A Chemical Reaction Progress Curve

A chemical reaction progress curve:



- **Enzymes affect the rate of chemical reaction.**
- **It is expressed in terms of change in concentration of the S or P per unit time.**
- **Refers to change in total quantity (moles or grams) per unit time.**

***Rate and velocity are the same.**

- The velocity of a reaction A $\rightarrow$ P is determined from a progress curve or velocity profile of a reaction.

- The progress curve can be determined by following the disappearance of the reactants or the appearance of the product of several different times.

$$V \text{ (or r)} = \frac{\text{amount of product formed}}{\text{unit time (minutes, seconds)}}$$

OR

$$= \frac{\text{amount of substrate used up}}{\text{unit time}}$$

Mathematically, the velocity expressed as:

$$V \text{ (or r)} = \frac{dP}{dt} \text{ or } \frac{-dS}{dt}$$

It 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 in enzymatic reaction.
- V is constant as rate and time.
- 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.
- Velocity decreases as time increases as;
  - (a) S may be used up.
  - (b) P may inhibit reaction (E)
  - (c) Change of pH may occur and decrease enzyme reaction
  - (d) Cofactor or coenzyme may be used up.
  - (e) Enzyme may lose activity.
- It is very important to measure initial velocity when studying enzyme reactions.

$T_{1/2}$:
It is the time required for the initial concentration of substrate to reduce to half its original concentration.

Reaction orders:

A. **First Order Reaction** : are reactions those proceed at a rate exactly proportional to the concentration of one reactant

e.g. $A \rightleftharpoons P$

$r \alpha [A]$

$r = k [A]$

k = is rate constant for first order reaction

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

$$\frac{-d[A]}{dt} = k[A]$$

$\frac{-d[A]}{dt}$ 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: (mainly useful for carrying out k calculation)

$$\log \frac{[A_o]}{[A]} = \frac{kt}{2.303}$$

$$kt = 2.303 \log \frac{[A_o]}{[A]}$$

$[A_o]$ is the concentration of A at zero time.

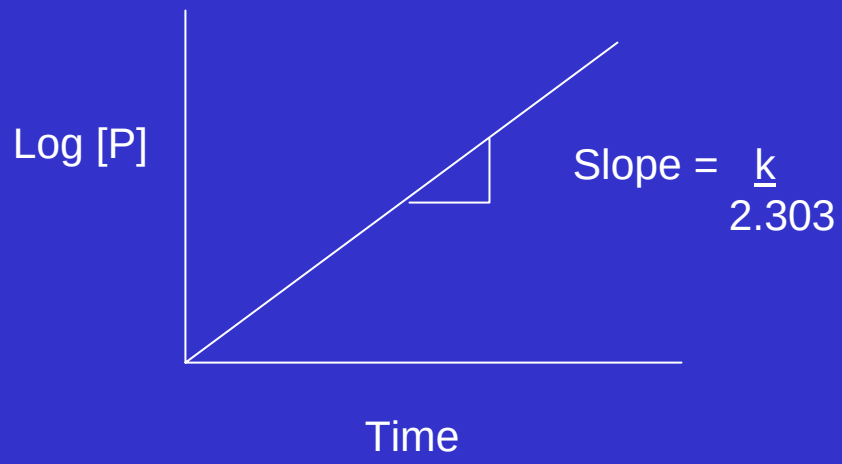
[A] the concentration at time t.

The half time of first order reaction is:

($t_{1/2}$: is the time required to convert half the substrate originally present to product.

$t_{1/2}$ = 0.693 ($t_{1/2}$ is constant for first order reaction and is related to k.

$t_{1/2}$: half time of the reaction.



2. Second Order Reactions

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 $A + B \rightarrow P$

$r \alpha [A][B]$

$r = k[A][B]$

k = second order rate constant

i.e. $= \frac{d[A]}{dt}$ or $\frac{-d[A]}{dt}$ or $\frac{dp}{dt} = k[A]\{B]$

(are relatively rare)

3. Third Order Reactions

are those in which the rate is proportional to the product of the concentration of three reactants.

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

$r \alpha [A\} [B\}^2$

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

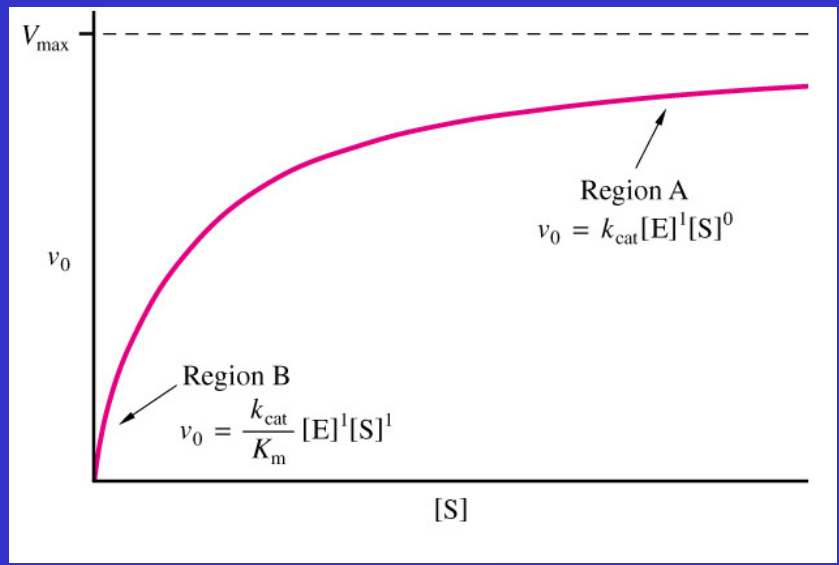
k = third order rate constant

4. Zero Order Reactions

- Rate of reaction is independent of concentration of the reactants.
- Change in concentration of [S] has no effect on rate

$$v = k_o$$

- This occurs in catalyzed reactions when they are saturated with the substrate.
- Under this conditions, the enzyme is operating at its maximum velocity.
- In addition of more reactions [S] has no effect on reaction rate → and rate does not change.



Enzyme activity

It is defined as the amount of enzyme that will convert a certain amount of S to P in a specified period of time under conditions of constant temperature and pH.

- **The international Enzyme Commission (IEC) have adapted a standard unit of enzyme activity called The International Unit (IU).**
- **It is defined as the amount of enzyme that can convert one µmole of substrate into product per minute at 25°C.**

(1 → µmole = 1 x 10^{-6} moles)

Katal

It is defined as the number of moles of substrate transformed into product per second at 25°C.

$$\text{I.U.} = \frac{10^{-6}\ \text{mole}/60\ \text{sec}}{\text{IU}} = 16.7 \times \frac{10^{-9}\ (\text{mole/sec})}{(\text{katal})}$$

Specific activity

It is defined as the number of enzyme units per milligram of protein (µmole/min/mg of protein)
($\mu mole.min^{-1}.mg\ of\ protein^{-1}$)

This is valuable during enzyme purification.
As enzyme become pure, specific activity increases.

Turnover number:
Can be used in two ways:
<u>One Way</u>: It is redefined as the number of moles of substrate transformed per minute per mole of enzyme (Units per µmole of active site or catalytic center under optimum conditions.

Turnover number

This tells how many S molecules are converted to product by each enzyme molecule.

It tells us how fast an enzyme work or turnovers S into P.

e.g. for catalase:

turnover number is 5×10^6
for α-amylase → it is 1.9×10^4

This indicates that catalase is ~ 250 times more active than amylase.

Example:

1 µg of pure enzyme (Mw = 92000) catalyzed a reaction at a rate of 0.5 µmole/min. under optimum conditions.

Calculate:

(a)The specific activity of the enzyme in term of units/mg protein.
(b)Turnover number

(a) $\frac{0.5 \text{ units}}{1 \text{ µg} \times 10^{-3}}$ = 500 units/mg protein

(b)Turnover number:

= moles of S/min./moles of enzyme

moles of S = 0.5×10^{-6} mole/min.

Moles of enzyme = $\frac{wt}{M_w} = \frac{(1\ \mu g \times 10^{6})\ g}{92000\ mole/g}$

$= 1.09 \times 10\text{-}11$

Turnover number = $\frac{0.5 \times 10^{-6} / min.}{1.09 \times 10^{-11}}$

$= 0.46 \times 10^{5}\ min^{-1}$

$= 4.6 \times 10^{4}\ min^{-1}$

Enzyme Assay methods

- Different enzymes require different estimation methods depending on the type of reation catalysed, the nature of S and P or coenzyme.

1. Spectrophotometric methods:

- Many substrates and products of enzyme reactions absorb light either in the visible region or in the u.v. region.
- Mostly the spectra of S and product are not the same and a wave length can be found at which the conversion of one into another is followed by a considerable change of absorption and by measuring this change → The progress of the reaction can be followed quantitatively.
- The enzyme is allowed to react with substrate and the decrease in the conc. of substrate or the increase of product produced will be followed spectrophotometrically.

- Easy
- Simple
- Sensitive
- Require small sample
- Whole progress curve can be obtained with one small sample
- Whole progress curve can be followed quantitatively.

(1) Cases in which product absorb but not the substrate: (include enzymes catalyze the addition of groups to double bonds)

Fumarate ⟶ Malate (This is easily followed)

Hypoxanthine ⟶ Urate

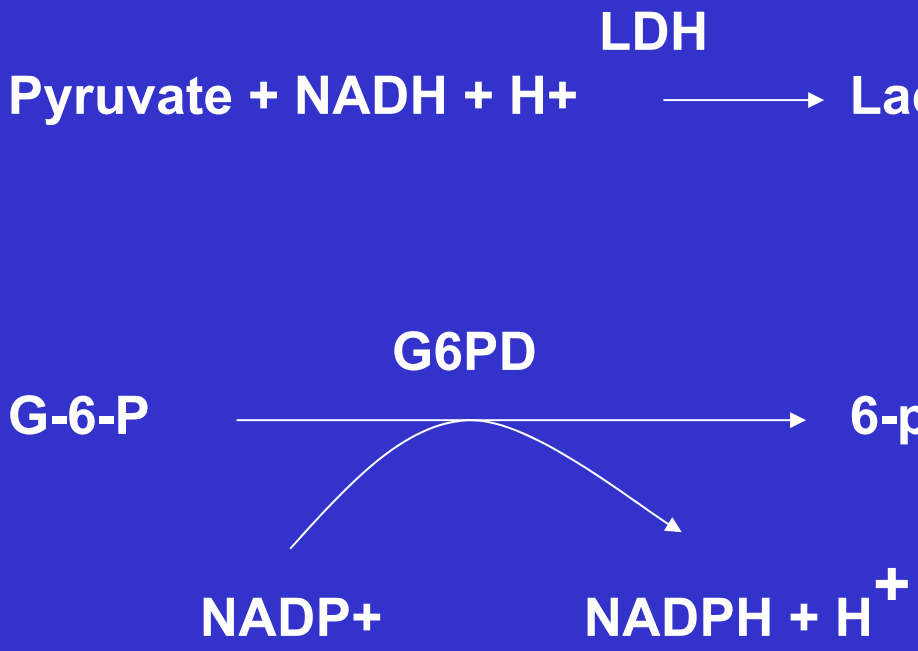
(2) The coenzyme undergoes change in absorption on reduction or oxidation by S.

NAD^+ ⟶ NADH

$NADP^+$ ⟶ NADPH

Oxidized form ⟶ Reduced form

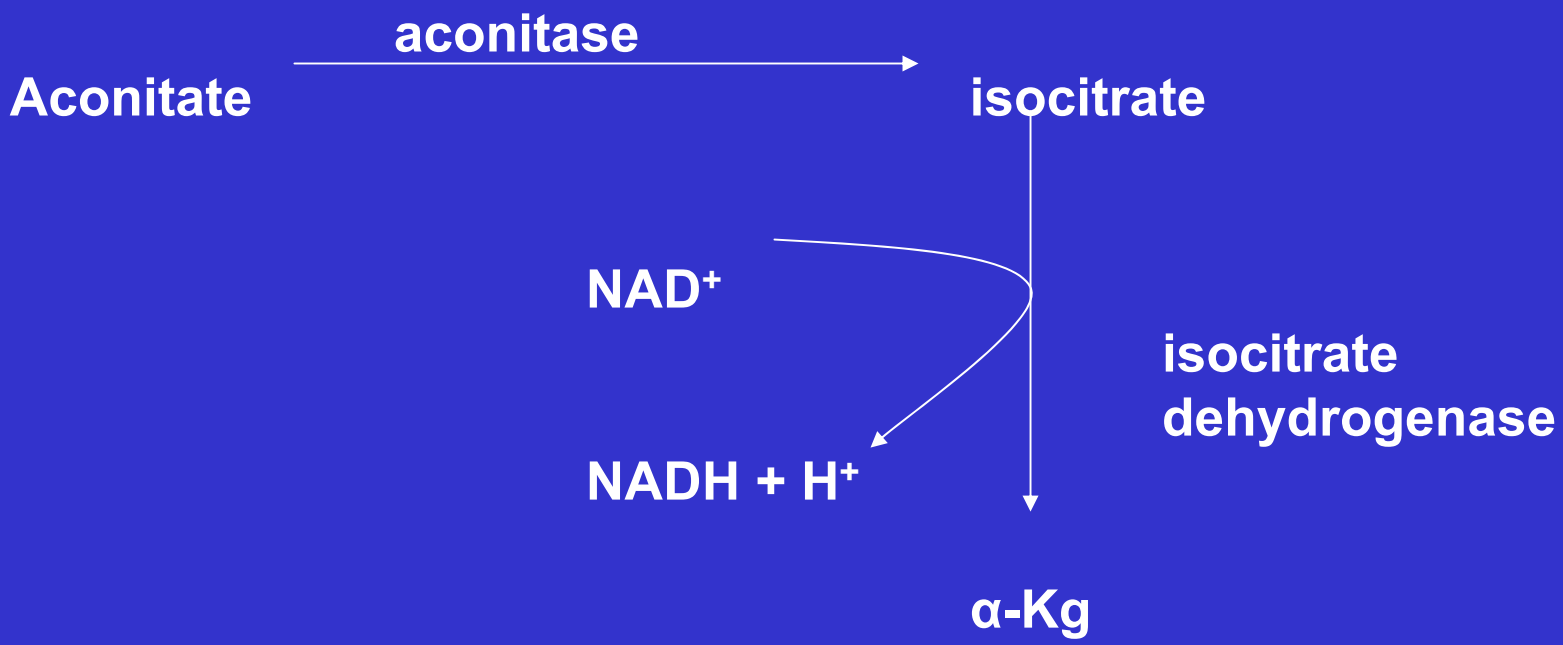
FAD ⟶ $FADH_2$



In decrease in abs./min can be used to measure rate of LDH activity.

Coupled

When the enzyme reaction being studied cannot be made to give an absorption change → spectrophotometric method will be used by adding another purified enzyme which acts on the product of the first reaction to cause such a change → so this help to follow the first enzymatic reaction.



In reaction can be followed by measuring the increase in abs. at 340 nm (specific for NADH.H+ but not NAD^+).

(2) Fluorescence Method: (Fluorometric method)

- Uses a fluorometer
- In some cases, measurements of fluorescence may be more sensitive than absorption measurements.
- Flarin compounds → fluoresce strongly in the oxidized form, and lose their fluorescence on reduction.

FAD	FADH2
Oxidized form (Fluorescence)	Reduced form (No fluorescence)

- NAD+ and NADP+ do not fluoresence in their oxidized forms, but the reduced form have a blue fluorescence.

NAD(P)	$NAD(P)H + H^+$
(No fluorescence)	(Blue Fluorescence)

This can be used for following enzymatic oxidation reduction reaction.

(3) Manometric Method:

- Use manometer
- These are convenient and accurate methods for following reactions in which one of the component is a gas.

These methods → for the study of:

(a)Oxidases (O_2 uptake)
(b)Decarboxylase (CO_2 output)

(4) Electrode Method: (To follows reactions which involve the production of acids.

- Use glass or platinum electrode.
- In this method pH meter is used to measure change in H^+ conc. During enzyme reactions. (i.e. measure change in pH as the reaction proceeds).

pH is kept constant by addition of alkali. The rate of additional alkali gives the reaction velocity and does not depend on the amount of buffer:

- Automatic apparatus
- Convenient
- Keeps pH constant by addition of acid or alkali
- And plots a curve of amount added against time.
- With this apparatus progress cures of many enzyme reactions can be obtained automatically.

(5) Polarimetric method:

- Use polarimeter.
- For isomerases that convert one isomer to another.
  - Or that convert optically active to inactive or vice versa.
  - It can be used if both S and P are optically active but different in specific rotation.

D-glucose $\rightleftharpoons$ L-glucose

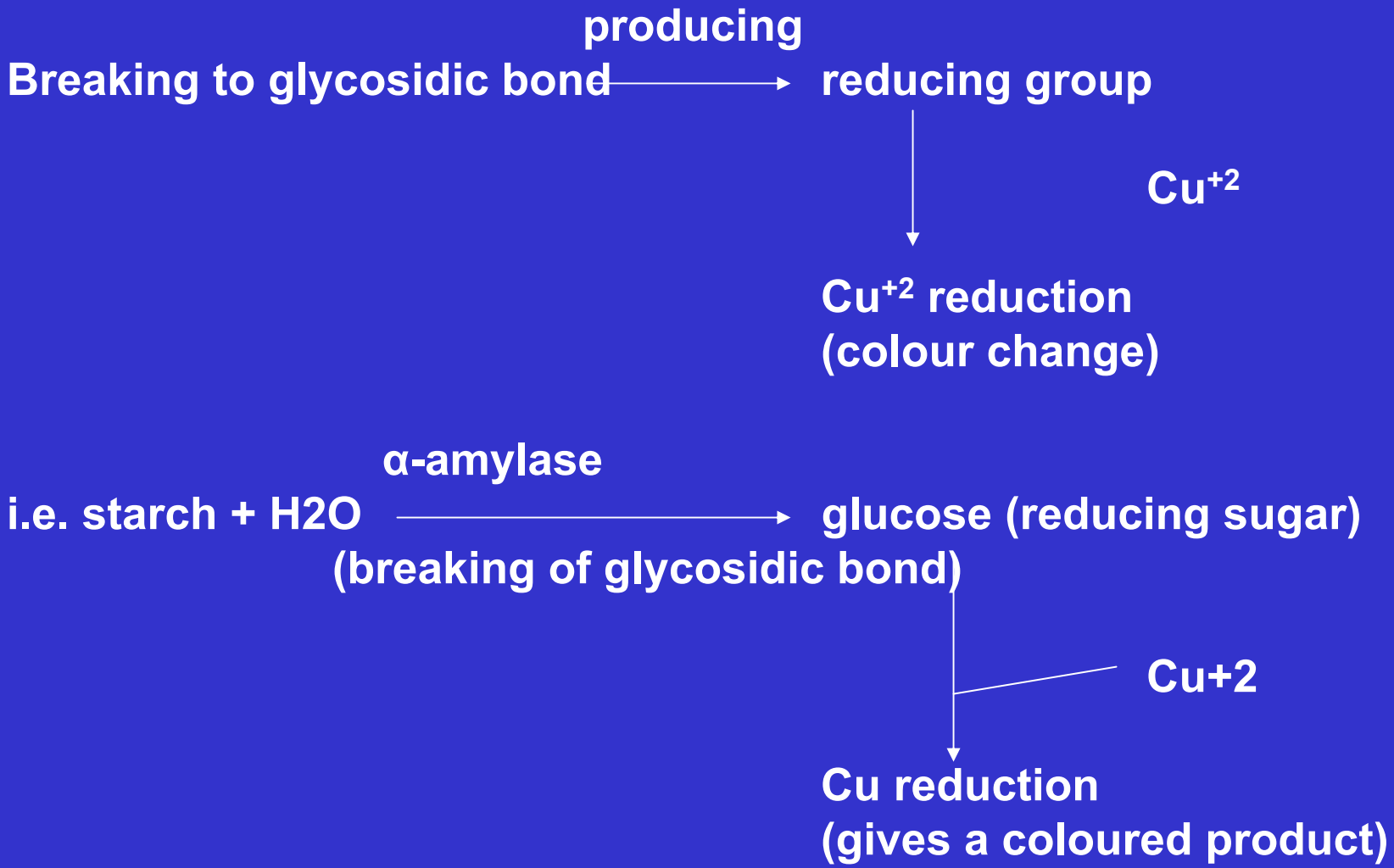
Sucrose $\longrightarrow$ D-glucose + D-fructose

(6) Sampling method:

- Many enzyme reactions are followed by withdrawing samples at intervals and estimating the substrate or product by chemical methods in different enzymes require different estimation methods.

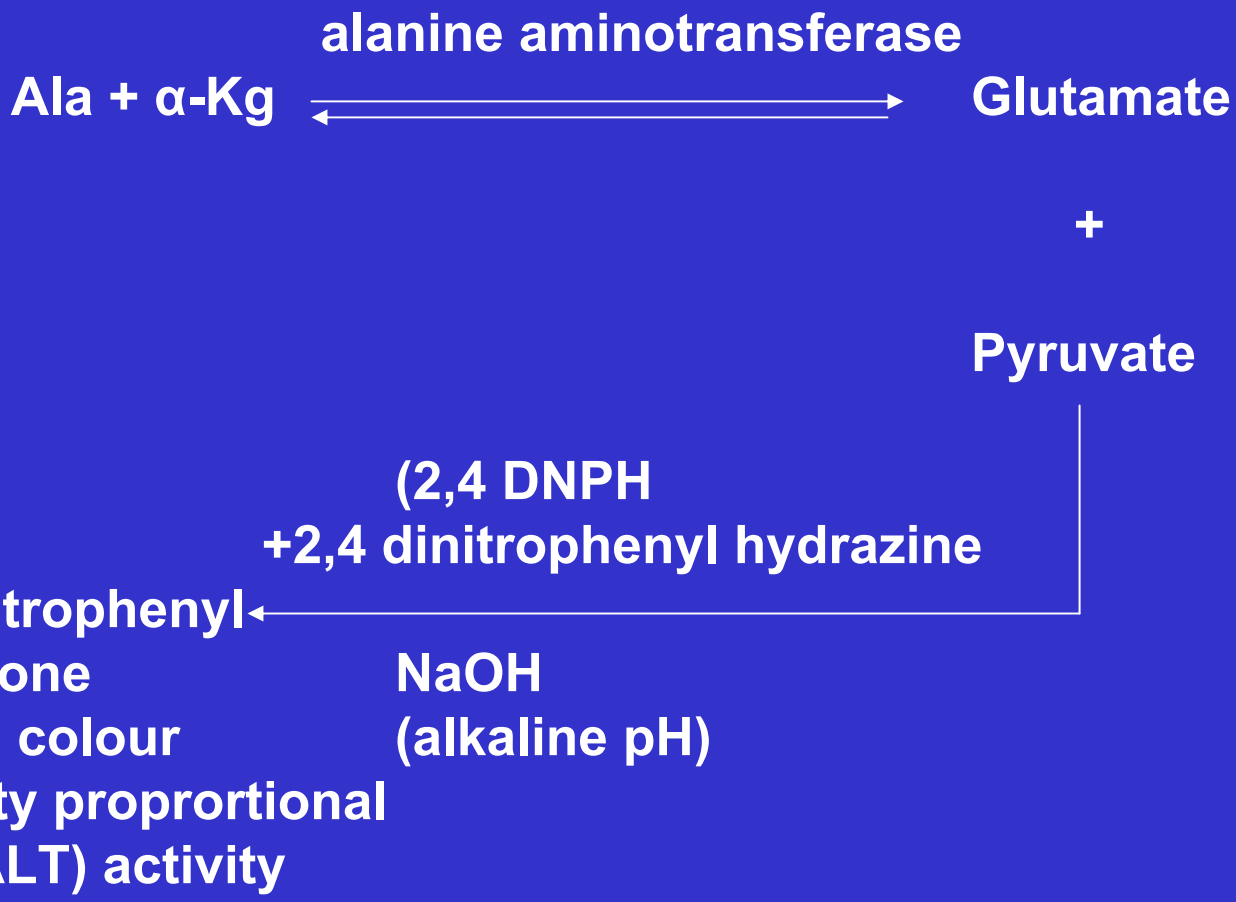
- It is for inorganic phosphate. It can be used for phosphatase, phosphorylase, nucleotides and all enzymes involving ATP or ADP including some kinase and synthetase.

Used for study of enzymes acting on carbohydrates (since the breaking of a glycosidic link produces a reducing group).



*Intensity of colour α E activity

e.g.


alanine aminotransferase
Ala + α-Kg
Glutamate
+
Pyruvate
(2,4 DNPH
+2,4 dinitrophenyl hydrazine
2,4 dinitrophenyl
Hydrazone
(Brown colour
Intensity proprortional
With (ALT) activity
NaOH
(alkaline pH)