AL- MUSTAQBAL UNIVERSITY
College Of Health And Medical Techniques
Prosthetic Dental Techniques Department
Second Grade
Second Semester

Advanced chemistry

Lecture 17 (The theoretical part)

(Enzymes)

By:
Prof.Dr. Hussein Kadhem AL-Hakeim

Giving the lecture

Assist.Prof.Dr.Ahmed A. AL-Khafagi

Enzymes

Enzymes: basics

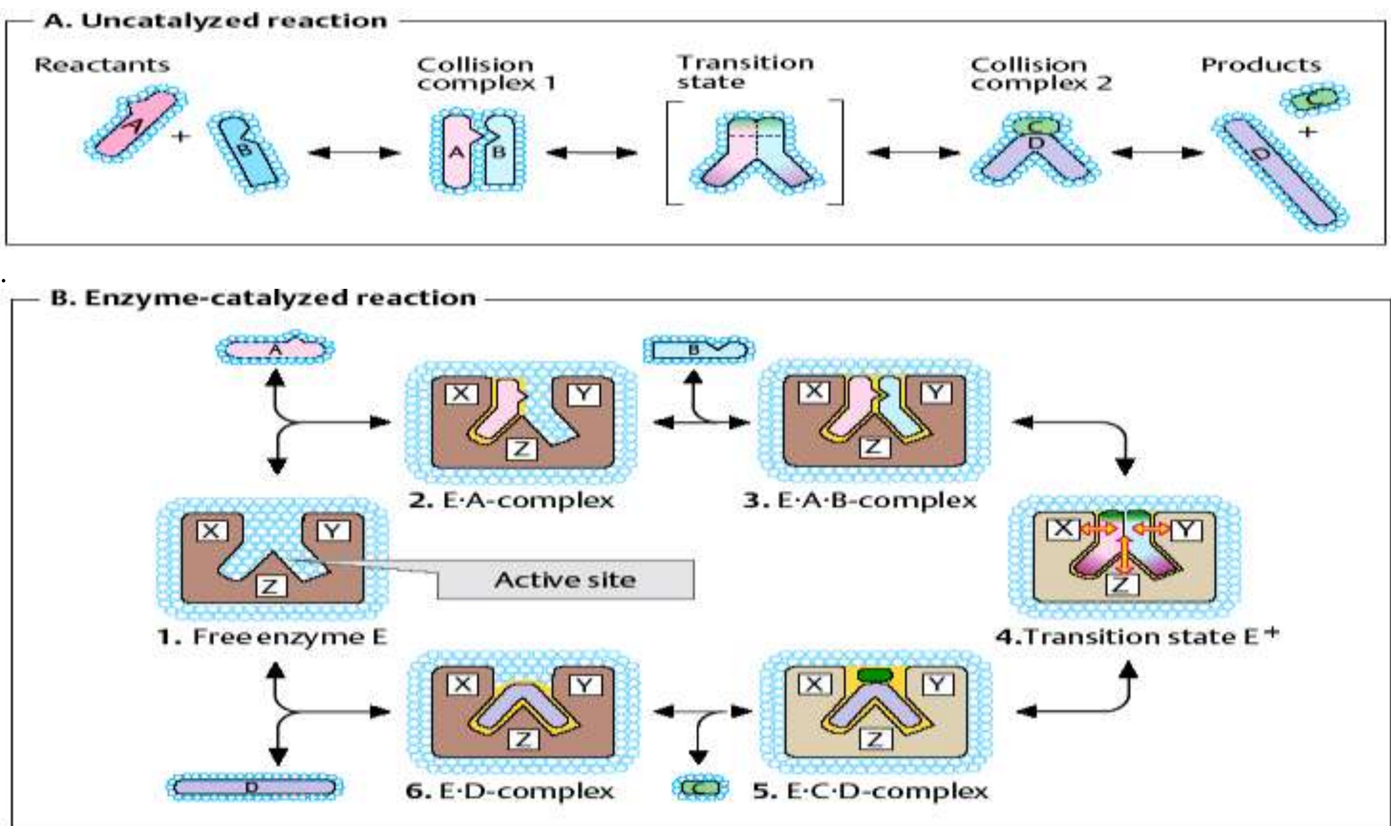
- More than 2000 different enzymes are currently known.
- The function of enzymes and other catalysts is to lower the activation energy, $\Delta G‡$, for a reaction and thereby enhance the reaction rate. The equilibrium of a reaction is unaffected by the enzyme.

Enzymes: are mostly soluble colloid protein compound acts as biological catalysts—i. e., substances of biological origin that accelerate chemical reactions. Each cell is equipped with its own genetically determined set of enzymes.

Q/What are the most important properties of enzymes?

1. Almost all enzymes are **proteins** (With the exception of a few catalytically active RNAs, the *"ribozymes"*.
2. Water soluble-nondialyzable-amphoteric compounds
3. Enzymes are active outside the cells (enzymes are active outside the cells)
4. Life depends on the existence of powerful and specific catalysts (the enzymes). Almost every biochemical reaction is catalyzed by an enzyme.
5. Enzymes are involved in **metabolic pathways &** many regulatory mechanisms that allow the metabolism to adapt to changing conditions.
6. Enzymes are highly effective **biological** catalysts, commonly enhancing reaction rates by a factor of 10^5 to 10^{17}. E.g. each *Catalase* enzyme molecule can break 40 millions molecules of H_2O_2.

Lysozyme: Globular proteins with deep cleft across part of its surface. It present in tears & degrade polysaccharides of bacterial cells.



Purification of Enzymes:

The intracellular enzymes should be liberated from the cell by:

1-Grinding the tissue with sand

2-Using homogenizer

3-By rupturing the cell wall by dehydration with acetone and then filtration to dissolve lipid membrane.

4-protein extraction by:1- dialysis 2-suitable buffer 3-Adsorption on different surface active substances 4-By glycerol.
5-Recently the most important methods are electrophoresis & chromatography.
Q/What are the types of enzymes?
A/ 1-digestive enzymes: Secreted by stomach, pancreas, & salivary glands
2-Food enzymes: exist in food
3-Exist in all cells &organs. Act to maintain normal function of cells.

Q/Why all enzymes molecules have large size?
A/ A significant part of the energy used for enzymatic rate enhancements is derived from weak interactions (hydrogen bonds and hydrophobic and ionic interactions) between substrate and enzyme. The enzyme active site is structured so that some of these weak interactions occur preferentially in the reaction transition state, thus stabilizing the transition state. The need for multiple interactions is one reason for the large size of enzymes.
Q/What are the advantages of binding energy between substrate & active sites?
A/ The binding energy can be used to 1-lower substrate entropy or to cause a conformational change in the enzyme (induced fit). 2-Binding energy also accounts for the fine specificity of enzymes for their substrates.
Q/What are the important facts about Regulatory Enzymes
A/
■ The activities of metabolic pathways in cells are regulated by control of the activities of certain enzymes.
■ In feedback inhibition, the end product of a pathway inhibits the first enzyme of that pathway.
■ The activity of allosteric enzymes is adjusted by reversible binding of a specific modulator to a regulatory site. Modulators may be the substrate itself or some other metabolite, and the effect of the modulator may be inhibitory or stimulatory. The kinetic behavior of allosteric enzymes reflects cooperative interactions among enzyme subunits.
■ Other regulatory enzymes are modulated by covalent modification of a specific functional group necessary for activity. The phosphorylation of specific amino acid residues is a particularly common way to regulate enzyme activity.
■ Many proteolytic enzymes are synthesized as inactive precursors called zymogens, which are activated by cleavage of small peptide fragments.
■ Enzymes at important metabolic intersections may be regulated by complex combinations of effectors, allowing coordination of the activities of interconnected pathways.

Q/What are the additional catalytic mechanisms employed by enzymes?
A/Catalysis includes:
1- Catalysis involves general acid-base catalysis: Chymotrypsin is a serine protease with a well understood mechanism, featuring general acid-base catalysis, covalent catalysis, and transition-state stabilization.,
2-covalent catalysis (modification), and metal ion catalysis: The enolase reaction proceeds via metal ion catalysis.
3-Catalysis often involves transient covalent interactions between the substrate and the enzyme, or group transfers to and from the enzyme: Lysozyme makes use of covalent catalysis and general acid catalysis as it promotes two successive nucleophilic displacement reactions.
4- Provide a new, lower-energy reaction path: Hexokinase provides an excellent example of induced fit as a means of using substrate binding energy.

A. Enzymatic activity: _

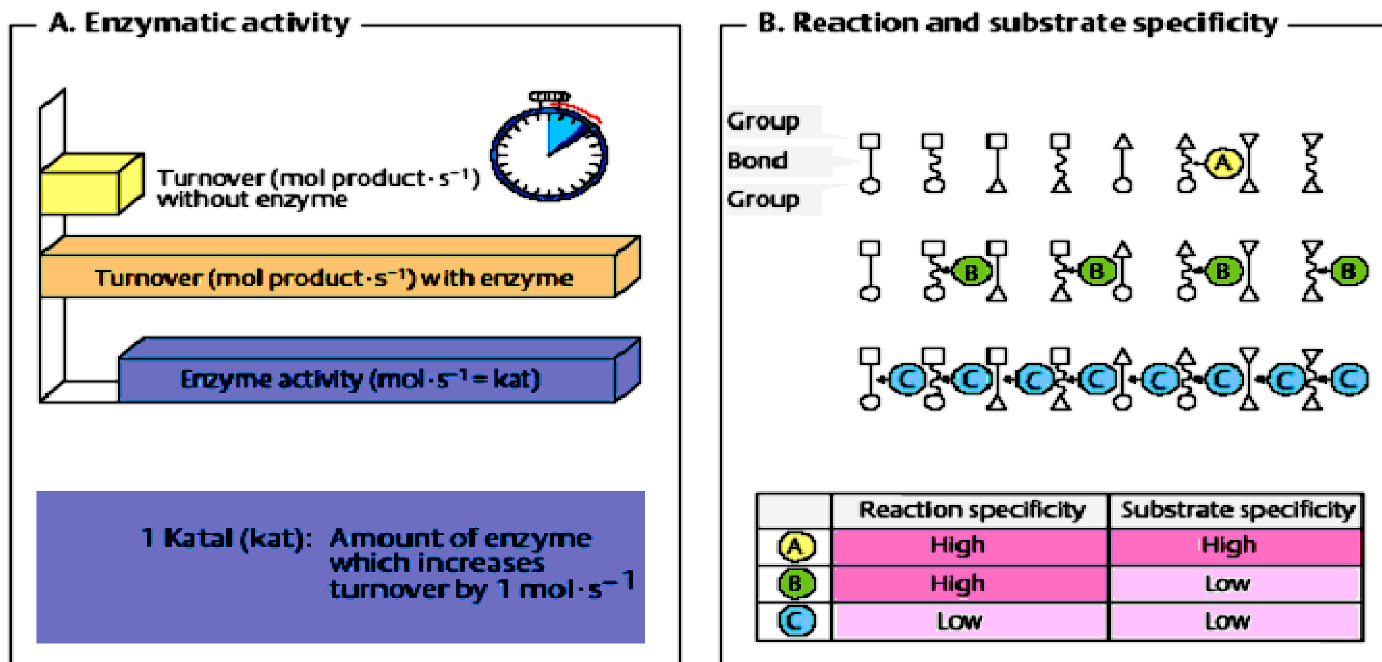
The catalytic action of an enzyme, its **activity**, is measured by determining the **increase in the reaction rate** under precisely defined conditions.

- International unit (IU) for enzymes activity: can be defined as: (The amount of enzymes which, under given assay conditions, will catalyze the conversion of (1 mmol of substrate per minute).

Note: other unit called (katal)= mol /second.

- Reaction rates: are expressed as the *change in concentration per unit of time* (mol. $L^{-1}.S^{-1}$).

Since the catalytic activity of an enzyme is independent of the volume, the unit used for enzymes is usually *turnover per unit time*, expressed in **katal** (katal= mol S^{-1}). However, the **international unit U** is still more commonly used (µmol turnover min^{-1}; 1 U =16.7 nanokatal).



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Q/What are the mechanisms for the binding between active site of an enzyme & substrate?

A/There are two main theories for the explanation of binding between active site of an enzyme & substrate:

1-Lock & Key theory:

To fit the interaction between substrate & enzyme, the enzyme has amatching shpe for substrate at the active site:

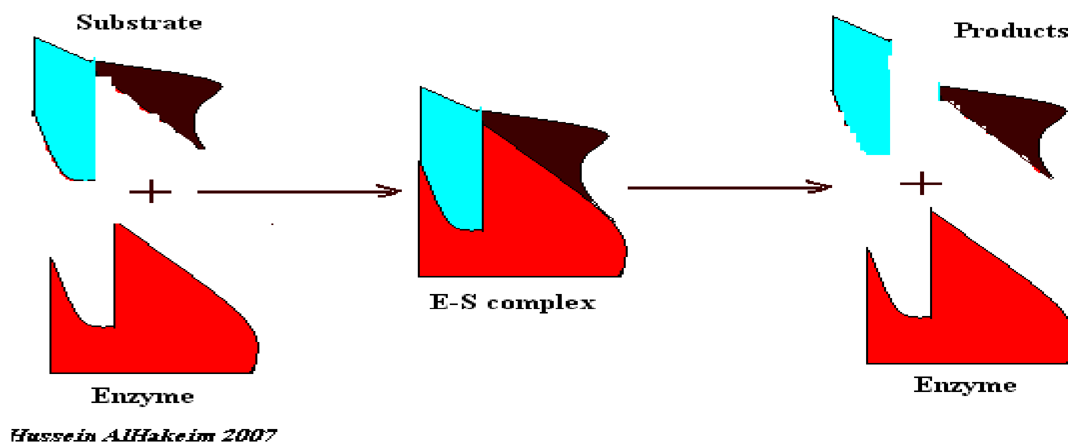


Figure: The interaction between substrate & enzyme according to key & lock theory

2-Induced Fit Theory (Hand &Glove):

The active site of the enzyme changing to be complementary to that of the substrate only after the substrate is bound.

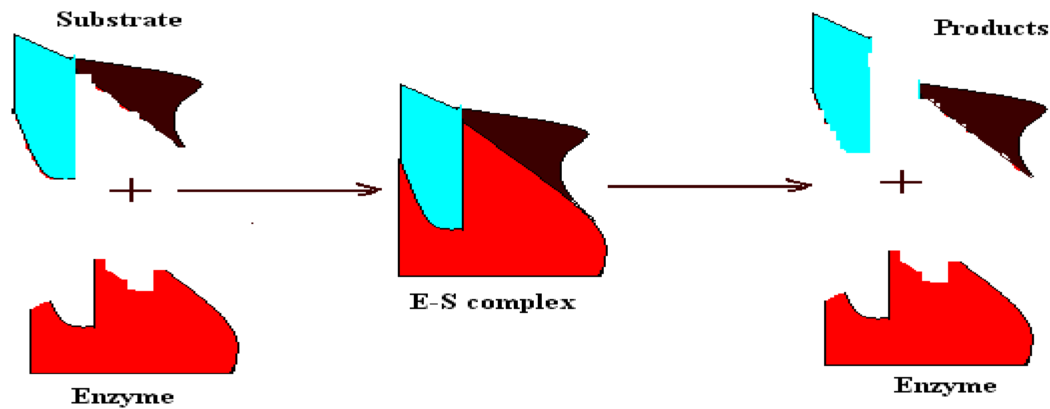


Figure: The interaction between substrate & enzyme according to induced fit theory

In 1993 M. Britt introduced the *(Shifting specificity)* model for enzyme catalysis. This theory is a mixture of these theories with modifications.

B. Reaction and substrate specificity: _

The multiplicity of enzymes, their specificity (the ability to discriminate between reactants), and their susceptibility to regulation give cells the capacity to lower activation barriers selectively.

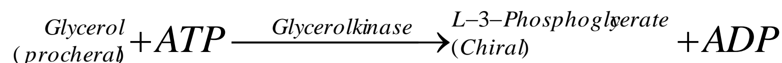
- The action of enzymes is usually very *specific*. It's the most significant property

This applies not only to the type of reaction being catalyzed, but also to the nature of the reactants ("substrates") that are involved (**substrate specificity**).

(**Reaction specificity**): The ability of an enzyme to catalyze one specific reaction & essentially no others.

(**Substrate specificity):** A particular enzyme acts only on particular chemical grouping e.g., Alcohol dehydrogenase acts on alcohol group (C-OH).

(Optical specificity): The complementary of substrate & enzyme must include the attachment at 3 different sites of attachment to distinguish between identical group on substrate



$$\underset{(procheral)}{Glycerol} + ATP \xrightarrow{Glycerolkinase} \underset{(Chiral)}{L-3-Phosphoglycerate} + ADP$$

In the Figure **B**, this is illustrated schematically using a bond-breaking enzyme as an example.

Type A (top): Highly specific enzymes catalyze the cleavage of only *one* type of bond, and only when the structure of the substrate is the correct one.

Type B (middle): Other enzymes have narrow reaction specificity, but broad substrate specificity.

Type C: enzymes (with low reaction specificity *and* low substrate specificity, bottom) are very rare.

C. Classification & Nomenclature of Enzymes:

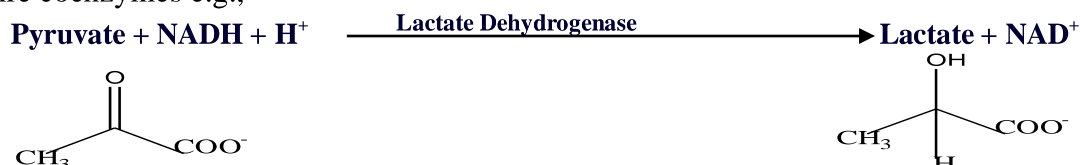
1. By using the suffex (-ase) after the reactant name. e.g. Urease, Arginase….etc.
2. Arbitrary (traditional) names. e.g. trypsin, & chemotrypsin…etc.
3. Enzymes are classified according to the type of reaction they catalyze (six types). All enzymes have the *Enzyme Catalogue* with a four-digit Enzyme Commission number (**EC number**). The first digit indicates membership of one of the six **major classes**. The next two indicate subclasses and subsubclasses. The last digit indicates where the enzyme belongs in the subsubclass.

For example:-lactate dehydrogenase has the EC number *1.1.1.27*(class 1, oxidoreductases; subclass 1.1, CH–OH group as electron *donor*; sub-subclass 1.1.1, $NAD(P)^+$ as electron *acceptor*).

#A system of *classification* has been developed that takes into account both their:1- *reaction specificity* and their 2-*substrate specificity*.

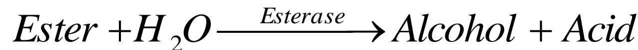
Enzymes with similar reaction specificities are grouped into each of the six major classes:

(***Class 1***)=**Oxidoreductases** catalyze the transfer of reducing equivalents from one redox system to another. They require coenzymes e.g.,



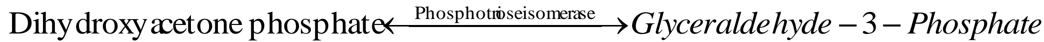
(***Class 2***)=**Transferases** catalyze the transfer of other groups from one molecule to another. Transferases generally require coenzymes.e.g.,

(*Class 3*)=**Hydrolases** are also involved in group transfer, but the acceptor is always a *water molecule.e.g.,*

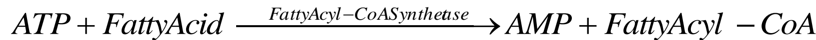


(*Class 4)*=**Lyases**, often also referred to as "synthases" catalyze reactions involving either the cleavage or formation of chemical bonds, with double bonds either arising or disappearing. e.g.,

(*Class 5*)=**Isomerases** move groups within a molecule, without changing the gross composition of the substrate.e.g.,



(Class 6)=**Ligases (Synthetases):** The ligation reactions catalyzed by are energy-dependent and are therefore always coupled to the hydrolysis of nucleoside triphosphates.e.g.,



In addition to the enzyme name, we also usually give its Enzyme Catalogue (Commission (EC) number:

Class 1: Oxidoreductases (catalyze reduction-oxidation reactions)
Subclass 1.n: What is the electron *donor?*
Sub-subclass 1.n.n: What is the electron *acceptor*?
1.1 A –CH–OH group is the donor
1.1.1 $NAD(P)_+$ is the acceptor (*dehydrogenases, reductases*): 1.1.1.8 Glycerol 3-phosphate dehydrogenase (NAD^+)
1.1.3 Molecular oxygen is the acceptor (*oxidases*): 1.1.99.5 Glycerol-3-phosphate dehydrogenase (FAD)
1.2.1 $NAD(P)^+$ is the acceptor *(dehydrogenases):* 1.2.1.3 Aldehyde dehydrogenase (NAD_+)
1.2.4 A disulfide is the acceptor: 1.2.4.1 Pyruvate dehydrogenase (lipoamide) [TPP]
1.6 $NAD(P)H^+$ is the donor: 16.4.2 Glutathione reductase (NADPH) [FAD]
1.9 A heme group is the donor: 1.9.3.1 Cytochrome *c* oxidase [heme, Cu, Zn]
1.11 A peroxide is the acceptor (*peroxidases*): 1.11.1.9 Glutathione peroxidase [Se]
1.18 Reduced ferredoxin is the donor:1.18.6.1 Nitrogenase [Fe, Mo, Fe_4S_4]

Class 2: Transferases (catalyze the transfer of groups from one molecule to another)
Subclass 2.n: *Which group* is transferred?
2.1 A C_1 group is transferred.
2.1.1 A methyl group: 2.1.1.45 Thymidylate synthase
2.7.1 With –CH–OH as acceptor: 2.7.1.1 Hexokinase

Class 3: Hydrolases (catalyze bond cleavage by hydrolysis)
Subclass 3.n: *What kind of bond* is hydrolyzed?
3.1 An ester bond is hydrolyzed (*esterases*)
3.1.3 In phosphoric acid monoesters (*phosphatases*): 3.1.3.1 Alkaline phosphatase [Zn_{2+}]
3.7 A C–C bond is hydrolyzed: 3.7.1.2 Fumarylacetoacetase

Class 4: Lyases (cleave or form bonds without oxidative or hydrolytic steps)
Subclass 4.n: *What kind of bond* is formed or cleaved?
4.1 A C–C bond is formed or cleaved
4.1.1 Carboxy-lyases (*carboxylases, decarboxylases*):4.1.1.1 Pyruvate decarboxylase [TPP]
4.1.2 Acting on aldehydes or ketones:4.1.3.7 Citrate synthase
4.1.99 Other C–C lyases:4.1.99.3 Deoxyribodipyrimidine photolyase [FAD]—"photolyase"
4.6 A P–O bond is formed or cleaved: 4.6.1.1 Adenylate cyclase

Class 5: Isomerases (catalyze changes within one molecule)
Subclass 5.n: *What kind of isomerization* is taking place?
5.1 A racemization or epimerization (*epimerases*): 5.1.3.1 Ribulose phosphate 3-epimerase
5.2 A *cis–trans* isomerization: 5.2.1.2 Maleylacetoacetate isomerase
5.3 An intramolecular electron transfer: 5.3.1.9 Glucose 6-phosphate isomerase

Class 6: Ligases (join two molecules with hydrolysis of an "energy-rich" bond)
Subclass 6.n: *What kind of bond* is formed?
6.1 A C–O bond is formed: 6.1.1.n (Amino acid)-tRNA ligases (*aminoacyl-tRNA synthetases*)
6.2 A C–S bond is formed : 6.2.1.4 Succinate-CoA ligase (GDP-forming)—"thiokinase"

6.3 A C–N bond is formed: 6.3.4.5 Argininosuccinate synthase.

C. The enzyme classes

Class	Reaction type	Important subclasses
1 Oxidoreductases	○ = Reduction equivalent A red + B ox ⇄ A ox + B red	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	A–B + C ⇄ A + B–C	C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	A–B + H_2O ⇄ A–H + B–OH	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	A + B ⇄ A–B	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	A ⇄ Iso-A	Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")	B + A + XTP (X=A,G,U,C) ⇄ A–B + XDP + P	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

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Allosteric Enzymes:

An **allosteric enzyme** is one in which the binding of a ligand to one site affects the binding properties of another site on the same protein.

Q/What are the properties of allosteric enzymes:

A/

1. Allosteric enzymes are usually present in oligomeric form (2–12 subunits). Some are catalytic subunits and some are regulatory subunits.
   - Catalytic subunits bind with substrate
   - Regulatory subunits bind the allosteric effectors CTP and ATP.
2. In most allosteric enzymes, the affinity of allosteric enzymes is not constant, but depends on the type and concentration of the substrate.
3. Allosteric enzymes have multiple substrate-binding sites of a cooperative effect. (If the interacted molecule is the same substrate =*Homotropic interaction* (always cooperative). If another molecule= *Heterotropic interaction(may be* cooperative or antagonistic)
4. Allosteric enzymes can occur in various *conformations*, which have different catalytic properties and whose proportion of the total number of enzyme molecules is influenced by substrates and other ligands (inhibitors or activators).
5. Plot of Vo against [S] is distinctly S-shaped (*sigmoidal*), which cannot be described using the Michaelis model.
6. Allosteric enzymes can be present in two conformations: the less active **T state** (for “tense”) and the more active **R state** (for “relaxed”). Substrates and effectors influence the equilibrium between the two states, and thereby give rise to sigmoidal saturation behavior.

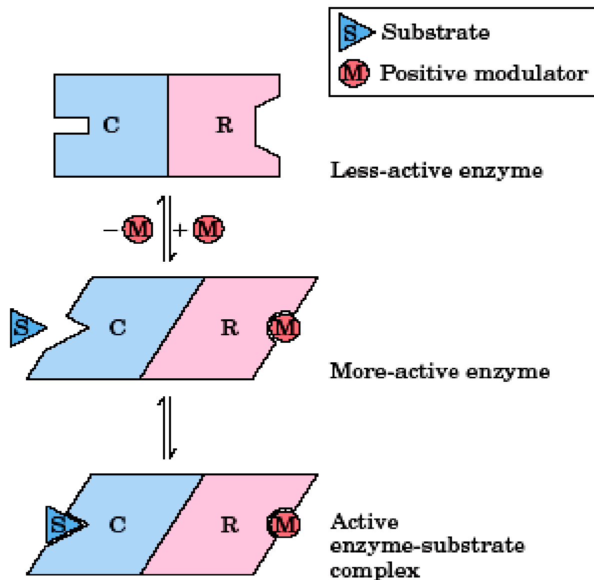


Figure: Catalytic sites (C) will be ready to bind to substrate after parts (R) with a positive modulator

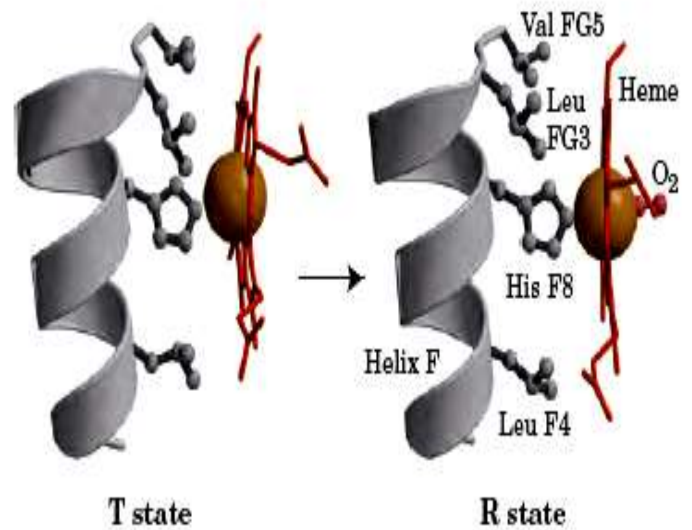
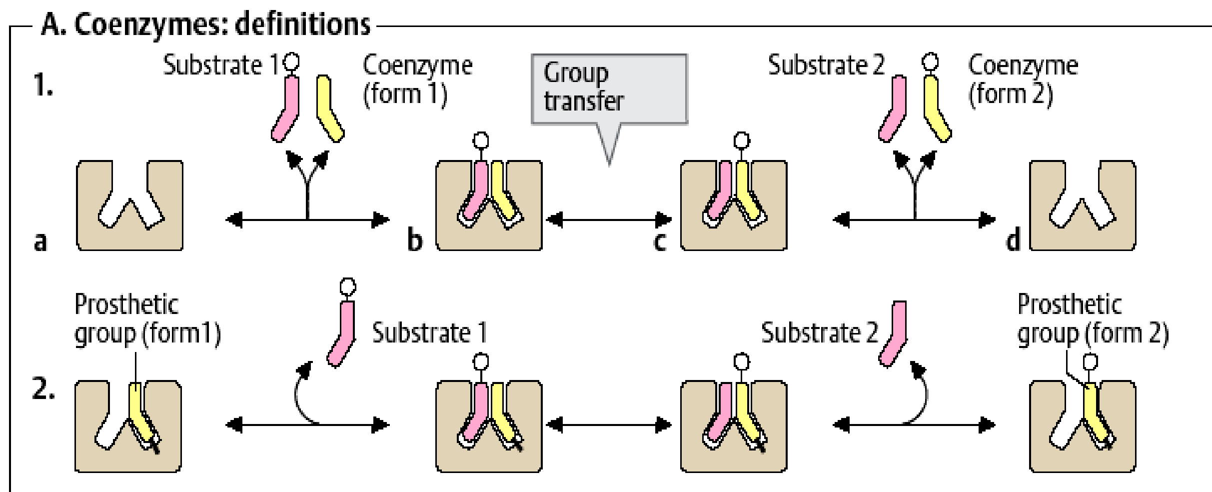


Figure: Changes in conformation near heme on O_2 binding binding of regulatory to deoxyhemoglobin

PROSTHETIC GROUPS, COFACTORS, & COENZYMES:

Many enzymes contain small nonprotein molecules and metal ions that participate directly in substrate binding or catalysis. Termed prosthetic groups, cofactors, and coenzymes



Class	Prosthetic group	Example
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin

Coenzyme	Examples of chemical groups transferred
Biocytin	CO_2
Coenzyme A	Acyl groups
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups
Flavin adenine dinucleotide	Electrons
Lipoate	Electrons and acyl groups
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)
Pyridoxal phosphate	Amino groups
Tetrahydrofolate	One-carbon groups
Thiamine pyrophosphate	Aldehydes

Prosthetic group: The non–amino acid part (A coenzyme or metal ion) of a conjugated protein that contributes to the protein's function**.** Prosthetic groups are distinguished by their tight, stable incorporation into a protein's structure by covalent or noncovalent forces. A number of proteins contain more than one prosthetic group.

E.g., pyridoxal phosphate, flavin mononucleotide (FMN), flavin dinucleotide (FAD), thiamin pyrophosphate, biotin, and the metal ions of Co, Cu, Mg, Mn, Se, and Zn.

e.g., Hemoglobin is a tetrameric protein containing four heme prosthetic groups,

e.g., succinate dehydrogenase is bound covalently to the flavin nucleotide in most flavoproteins protein, Metals are the most common prosthetic groups (termed **metalloenzymes).**

Cofactors: serve as recyclable shuttles—or group transfer reagents—that transport many substrates from their point of generation to their point of utilization. It binds in a transient (cofactors associate reversibly with enzymes or substrates), dissociable manner either to the enzyme or to a substrate such as ATP. Unlike the stably associated prosthetic groups, cofactors therefore must be present in the medium surrounding the enzyme for catalysis to occur.
Chemical moieties transported by coenzymes include methyl groups (folates), acyl groups (coenzyme A), and oligosaccharides.
Note: Many Coenzymes, Cofactors, & Prosthetic Groups Are Derivatives of B Vitamins such as Nicotinamide and riboflavin.
Q/what is the difference between coenzymes and prosthetic group?
A/ the coenzymes need to move from enzyme to enzyme to carry out their function. While prosthetic group bind to enzymes. To be active for example: ATP is converted to ADP during hexokinase action but another phosphate transfer (catalyzed by another enzymes.) is required to convert ADP back to ATP.

Apoenzyme + Prosthetic group or Cofactor $\longrightarrow$ **HoloEnzyme**
(Inactive) (Active)

THE ACTIVE SITE:

The **active site is** a cleft or pocket of three dimensional small portion of enzyme molecule at which the substrate contact with. The properties of the active sites:

1. Take small size of the enzyme molecules.
2. It's a group of sequences of a.a. folded by three dimensional structure.
3. It represents the catalytic site of the enzyme.
4. substrate binds with the enzyme by multiple weak attractions at the active site.
5. Active sites are cleft or cervices at which the substrate binds with.

Enzyme Kinetics As an Approach to Understanding Mechanism

- Michaelis –Menten Model:

To measure the activity of an enzymes, it is therefore necessary to measure the initial velocity of the enzymes –catalyzed reaction so that interferences by all factors will be minimal.

Q/ why one should measure the initial velocity of an enzymes.-catalyzed reaction.

A/ the best method to estimate the enzymes. Activity is to measure the initial velocity due to the following reasons:

1-The product many inhibit the activity of the enzymes.

2-The enzymes may be labile (unstable)

3-If the reaction is reversible, the velocity of the back reaction will increase as the conc of the product arises.

4- If the substrate conc. is low, its conc. will decrease during of the course of the reaction so causing a progressive (falling off).
Q/Why Vmax & Km determination is important in the enzyme catalyzed reaction?
A/ Since Vmax & Km define the quantitative relationship between *Vo* & [S] for a simple enzyme catalyzed reaction, their values are distinctive for each reaction (reflecting the magnitudes of rate constant involved in the reaction mechanism.

Q/What are the assumptions of the Michaelis–Menten model?

A/The assumptions of the Michaelis–Menten model are:

1. The substrate-binding step and formation of the ES complex are fast relative to the breakdown rate. This leads to the approximation that the substrate binding reaction is at equilibrium.

2. The concentration of substrate remains essentially constant during the time course of the reaction ($[S_0] \approx [S_t]$). This is due partly to the fact that initial velocities are used and that $[S_0] >>> [E_T]$.

3. The conversion of product back to substrate is negligible, since very little product has had time to accumulate during the time course of the reaction.

Q/What are the assumptions of the Michaelis–Menten model are based on:

A/ The assumptions of the Michaelis–Menten model are based on the following conditions:

1. The enzyme is stable during the time course of the measurements used to determine the reaction velocities.
2. Initial rates are used as reaction velocities.
3. The reaction velocity is directly proportional to the total enzyme concentration.

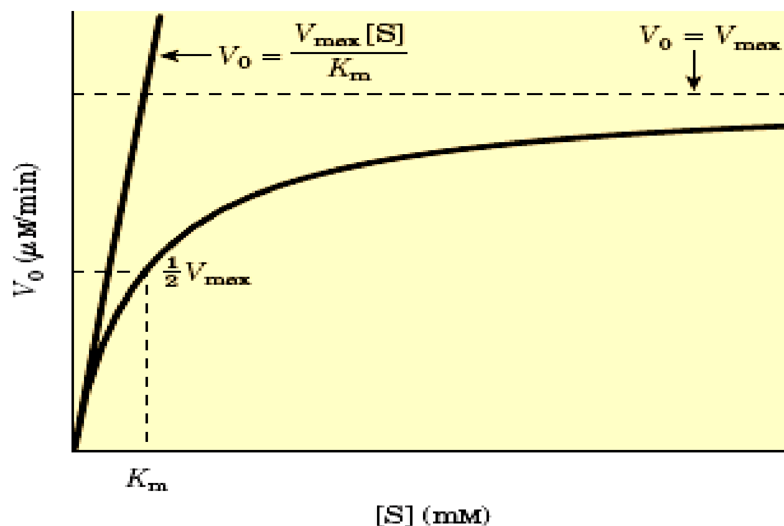


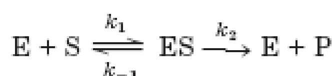
FIGURE: Dependence of initial velocity on substrate concentration.

A modern derivation of the Michaelis-Menten equation

Michaelis-Menten kinetics is also called steady-state kinetics because it includes the steady-state assumption. Enzyme-catalyzed reactions are characterized by the formation of a complex between substrate and enzyme (an ES complex). Substrate binding occurs in a pocket on the enzyme called the active site.

The derivation starts with the two basic steps of the formation and breakdown of ES

The overall reaction then reduces to



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Initial velocity (V_o) is determined by the breakdown of ES to form product, which is determined by [ES]:

$$V_0 = k_2[ES] \quad \text{---} \quad (1)$$

Using **steady-state assumption; t**he rates of formation and breakdown of ES are determined by the steps governed by the rate constants $k1$ (formation) and k-1 & $k2$ (breakdown), according to the expressions:

$$\text{Rate of ES formation} = k_1([E_t] - [ES])[S]$$

$$\text{Rate of ES breakdown} = k_{-1}[ES] + k_2[ES]$$

At steady state, rate of formation = rate of breakdown of ES complex:

$$k_1([E_t] - [ES])[S] = k_{-1}[ES] + k_2[ES]$$

$$k_1[E_t][S] - k_1[ES][S] = (k_{-1} + k_2)[ES]$$

$$k_1[E_t][S] = (k_1[S] + k_{-1} + k_2)[ES]$$

$$[ES] = \frac{k_1[E_t][S]}{k_1[S] + k_{-1} + k_2}$$

This can now be simplified further, combining the rate constants into one expression:

$$[ES] = \frac{[E_t][S]}{[S] + (k_2 + k_{-1})/k_1}$$

The term ($k2$+ k-1)/$k1$ is defined as the **Michaelis constant, *K*m.** Then:

$$[ES] = \frac{[E_t][S]}{K_m + [S]}$$ -- (2)

Substituting the right side of Equation 2 for [ES] in Equation 1 gives

$$V_0 = \frac{k_2[E_t][S]}{K_m + [S]}$$ ---*(3)*

Because the maximum velocity occurs when the enzyme is saturated (that is, with [ES]=[Et]), *Vmax*=*k*2[Et]. Substituting this in Equation (30) gives:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

This is the **Michaelis-Menten equation,** the rate equation for a one-substrate enzyme-catalyzed reaction.
Note that *K*m

Q/What is the meaning (or importance) of Vmax & Km?

A/ The kinetic parameters (Vmax and Km) defined as follows:

1- Vmax: maximum initial velocity when the substrate concentration is very high ie., [S]→∞
*Vmax units = quantity transformed/unit time for a given conc. of enzymes.

2- Km: Michaelis constant: Experimental term equal the value of [S] at which the reaction rate equal to the half of its maximum value ie., v=½ Vmax .
* Km units values between (10^{-5} mal/L 10^{-2} mol/L)

Q/What is the rate of enzymes catalyzed reaction at 1-very low conc. of [s] 2-very high conc. Of [s]

A/ 1/ At very low conc. of [s]:

$$v = \frac{V\max[S]}{Km + [S]}$$ approximately; km +[s] ~ Km

Then $$v = \frac{V\max[S]}{Km}$$

i.e. initial velocity directly proportional to substrate concentration (v depends on [s])

2/ At very high conc. of [s]

$$v = \frac{V\max[S]}{Km + [S]}$$ approximately; km +[s]≈[s]

$$v = \frac{V\max[S]}{[S]} \rightarrow v = V\max$$

i.e. the velocity reaches the maximum velocity.

Transformations of the Michaelis-Menten Equation: The Double-Reciprocal Plot

The Michaelis-Menten equation:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

can be algebraically transformed into equations that are more useful in plotting experimental data. One common transformation is derived simply by taking the reciprocal of both sides of the Michaelis-Menten equation:

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]}$$

Separating the components of the numerator on the right side of the equation gives

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$ This simplifies to $$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

This form of the Michaelis-Menten equation is called the **Lineweaver-Burk equation.** For enzymes obeying the Michaelis-Menten relationship, a plot of 1/Vo versus 1/[S] (the "double reciprocal" of the *V*o versus [S] plot we have been using to this point) yields a straight line (Fig. 1).

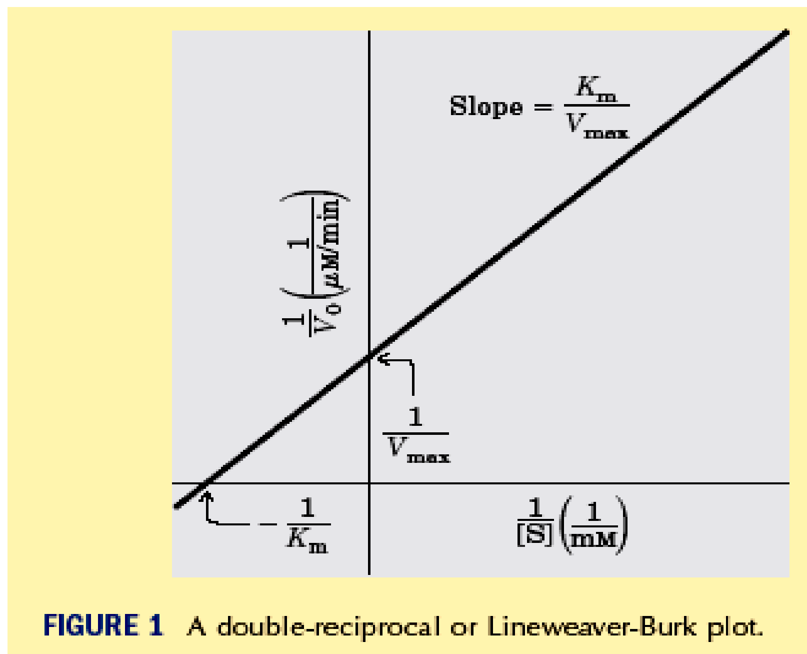


FIGURE 1 A double-reciprocal or Lineweaver-Burk plot.

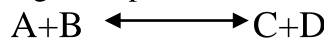
Thermodynamic of enzyme catalyzed reaction (Bioenergetics):

- Thermodynamic of enzyme catalyzed reaction; is a study of overall energy changes in chemical reaction.
- The most important thermodynamic function in biochemical reactions is the free energy change(ΔG)

@ ΔG^o=is the standard free energy change of reactants and products when they occur at (1mol/L)

@ $\Delta G^{o\prime}$=is the standard free energy change of reactants and products when they occur at (1mol/L) &pH=7

- Exergonic reactions=Energy yielding spontaneous reactions.
- Endergonic reactions=Energy requiring non-spontaneous reactions.



$$A+B \longleftrightarrow C+D$$

Equilibrium constant Keq = $\frac{[product]}{[reactant]} = \frac{[C][D]}{[A][B]}$

$\Delta G = \Delta G^0 + 2.303\ RT \log \frac{[product]}{[reactant]}$

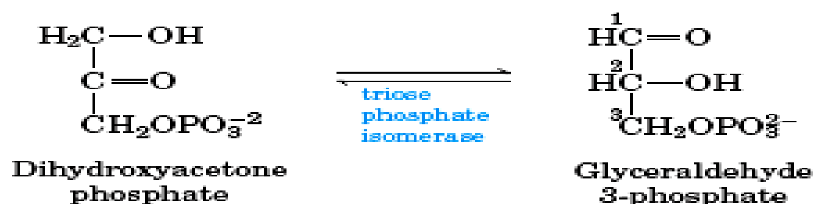
NOTE: At equilibrium ΔG =0

$$0 = \Delta G'^{o} + RT \ln \frac{[C][D]}{[A][B]} \Longrightarrow \Delta G'^{o} = -RT \ln \frac{[C][D]}{[A][B]}$$

$\Delta G'^{o}$ =-RT ln keq $\Longrightarrow$ ΔG =-2.303RT logkeq

Keq =$10^{-\Delta G/2.303RT}$

- Example: calculate $\Delta G'^{o}$ & ΔG^{o} for the following reaction:



Knowing that: Keq= 0.0475 at PH=7, T=25 C^0, and R=1.987cal.$mol^{-1}.K^{-1}$

The conc. of the reactant = $2*10^{-4}$M, Product=$3*10^{-6}$ M.

- Solution: 1/ $\Delta G'^{o}$ =-2.303 RT log Keq

=-2.303*298* 1.987*10^{-3} log0.0475

=+1.8 kcal/mal.

2/ $\Delta G = \Delta G^0 + 2.303\ RT \log \frac{[product]}{[reactant]}$

$\Delta G = 1.8 + 2.303 * 1.987*10^{-3}*298*\log \frac{3*10-6}{2*10-4}$

= -0.7 kcal/mol.

Note: the ΔG for this reaction is negative although the ΔGó is positive

Note: the mast important factor affecting ΔG & $\Delta G^{ó}$ equality is the conc. of reactant.

Note: the criterion of spontaneity for a reaction is ΔG, not ΔGó.

Note: Enzymes accelerate the forward & reverse by same factor hence, the enzymes cannot alter equilibrium constant.

i.e. enzymes attained the equilibrium state faster than the state if it is absent.

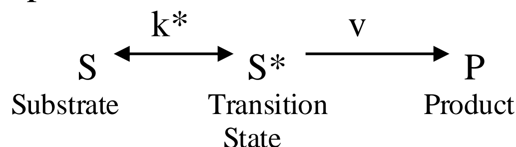
Note: ΔH: the change in enthalpy (heat content) of the system

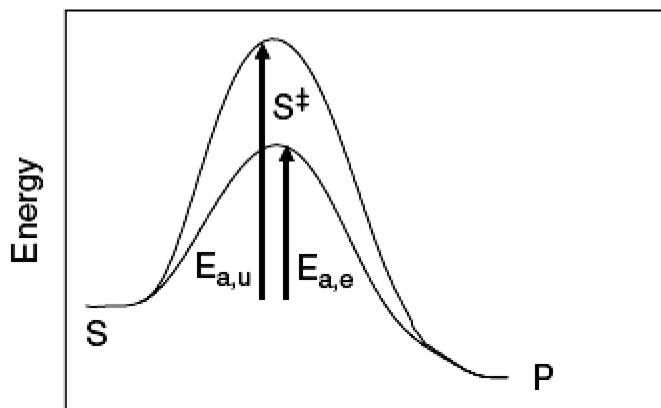
ΔS: degree of randomness or disorder of the system.

Note: the Gibbs equation introduced the free energy change by combining the first & the second laws of thermodynamics:

$$\Delta G = \Delta H - T\Delta S.$$

*Enzymes Accelerate reactions by stabilizing transition state which is an intermediate state between products & reactants:





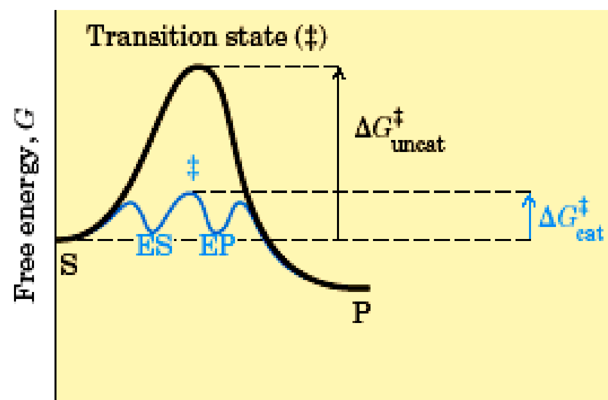


FIGURE:Reaction coordinate diagram comparing enzymecatalyzed and uncatalyzed reactions. In the reaction S→P, the ES and EP intermediates occupy minima in the energy progress curve of the enzyme-catalyzed reaction. The activation energy is lower when the enzyme catalyzes the reaction.

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Factors influencing Enzyme catalyzed reactions:

*Enzymes, being proteins, are usually denaturized by high temperature or by pH values more than a few units away from neutrality. This denaturation is in most cases irreversible

1/ Effect of temperature:

There is an optimal temperature at which the reaction is most rapid. Above or below this temperature, the reaction rate decreases sharply, generally due to heat denaturation of the enzymes.

- The optimal temperature approximates those of the environment of the cell. the enzymes of human has the optimal temperature of body =37.5°C
- When temperature increases, there is consequently loss of secondary, tertiary structure & parallel loss of biological activity.

Note: IF a cell extract having catalytic activity loses this activity when boiled (denaturized), the catalyst probably was an enzymes.

2/PH: Every enzyme has an optimum pH (or pH range) at which it has maximal activity.
pH changes affects the ionic state of the enzymes & substrate. Optimal activity of enzymes is generally observed between pH values of (5-9). However, a few enzymes e.g. pepsin are active at pH values outside this range. The shape of pH activity shape (Figure) determined by these factors
1/ Enzymes denaturation at high or low pH values
2/ Effects on the charged state of the substrate or enzymes.
The charge changes may affect by changing the structure or by changing the functional groups in substrate binding or catalysis.

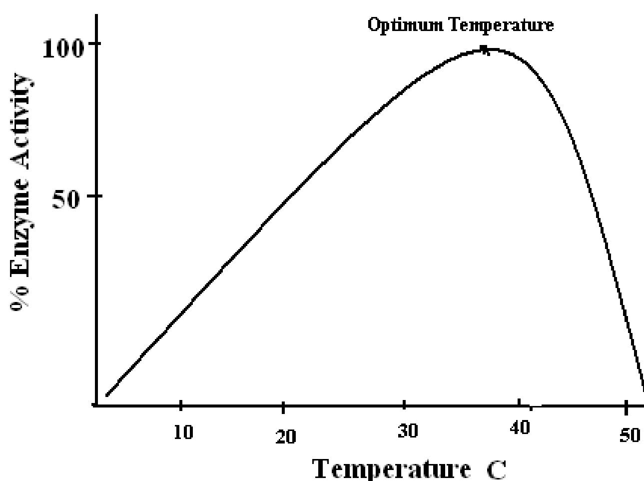


Figure: Effect of temperature on the enzymes activity

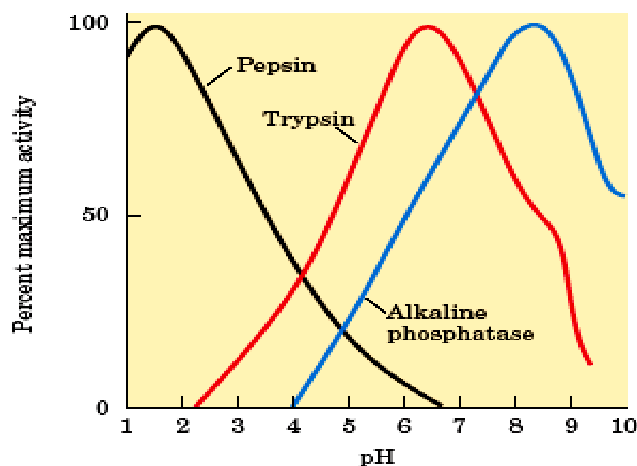


Figure: The pH optima of some enzymes.

3/ Enzymes concentration: the initial velocity of an enzymes catalyzed reaction is directly proportional to the enzyme concentration.
Since enzymes concentration is not changed and only affect rates of the forward and back reaction, not rate constants, they cannot affect equilibrium constants (Keq), as follow:

$$E + S \longleftrightarrow ES \longleftrightarrow E + P$$

$$Rate1 = k1[E][S] \quad , \quad Rate-1 = k-1[E][P]$$

At equilibrium: $Keq = \frac{K1}{K-1} = \frac{[E][P]}{[E][S]} = \frac{[P]}{[S]}$

4/ Substrate conc: the velocity increases as the substrate conc. is increased up to a point where the enzymes. Is said to be saturated. For many enzymes, the rate of catalysis (v) varies with the substrate conc. [s] (v) is defined as the number of moles of product formed per second. (v) varies with the substrate conc. in a manner shown in the following figure:
The relationship between velocities, Vmax substrate conc. was discussed previously.

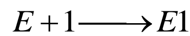
5/ Oxidation: sulfhydryl (-SH) groups of many enzymes are essential for enzymes activity. Oxidation of these (SH) groups forming disulfide linkages (S-S) leads to conformational changes.
e.g: Dehydrogenates enzymes are active when (-SH) present in reduced form and inactive in (S-S) from (oxidized form).
e.g. Ribonuclease are active when (-S-S-) present & inactive when (-SH) present in the enzymes.

6/Radiation: Enzymes are highly sensitive to short wavelength (high energy) such as (UV, X, β, or γ – rays). This due to oxidation of the enzymes by peroxides formed by high energy radiation.

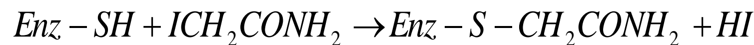
Inhibition of enzyme Catalyzed reaction:

- Inhibitors are chemicals that act by combining with S, cofactor or various forms of the one enzyme. There are two types of inhibitors:

1/ Irreversible inhibitors: inhibitor binds with substrate to produce undissociable complex and so inactivate enzymes.

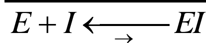


e.g.: iodoacetamide bind with essential (- SH) group and in actirate enzymes



E.g. organophosphorous compounds inhibits cholinesterase by binding with (-OH) group of serine side chain.

2/ Reversible inhibitors: the reaction between E and I is reversible



There are three types of reversible inhibitors:

a-Competitive inhibition: inhibitors bind with the active site. A competitive inhibitor competes with the substrate for the active site of an enzyme.

In the presence of a competitive inhibitor, the Michaelis-Menten equation becomes:

$V_0 = \frac{V_{max}[S]}{\alpha K_m + [S]}$ where $\alpha = 1 + \frac{[I]}{K_I}$ and $K_I = \frac{[E][I]}{[EI]}$

- In Linweaver-Burk plot: alter its slop but do not change intercept

e.g: malonate is a competitive inhibitor of succinate dehydrogenase

b-Mixed inhibitor: also binds at a site distinct from the substrate active site, but it binds to either E or ES. The rate equation describing mixed inhibition is

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + \alpha'[S]}$$ where α and ά are defined as above.

A mixed inhibitor usually affects both *K*m and *Vmax*. The special case of α =ά, rarely encountered in experiments, classically has been defined as **noncompetitive inhibition.**

inhibitors bind with other side but not with the active site

- In linweaver-Burk plot: alter both slop and intercept

e.g: cytdine triphosphate inhibit (*Aspartate Transcarbamoylase*)

Note: this type of inhibition also called (allosteric) because the inhibition usually is the product.

c-Uncompetitive inhibition: inhibitors bind with the complex (ES). In the presence of an uncompetitive inhibitor, the Michaelis-Menten equation is altered to

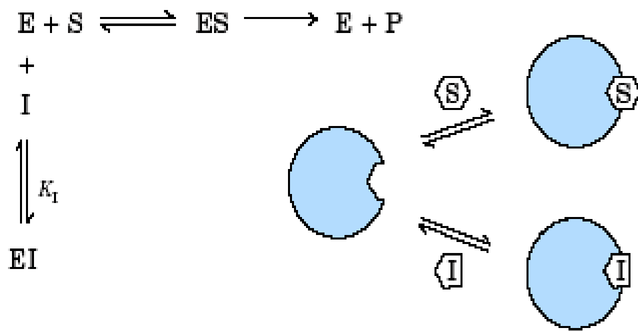
$$V_0 = \frac{V_{max}[S]}{K_m + \alpha'[S]}$$ Where $\alpha' = 1 + \frac{[I]}{K'_I}$ and $K'_I = \frac{[ES][I]}{[ESI]}$

- In lineweaver-Burk plot: alter intercept and not affect the slope.

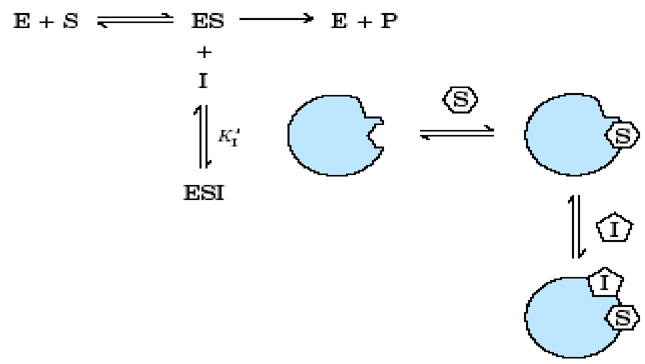
Note: In practice, uncompetitive and mixed inhibition are observed only for enzymes with two or more substrates— say, S1 and S2—and are very important in the experimental analysis of such enzymes.

- The experimentally determined variable α *K*m, the *K*m observed in the presence of the inhibitor, is often called the “apparent” *K*m.

(a) Competitive inhibition



(b) Uncompetitive inhibition



(c) Mixed inhibition

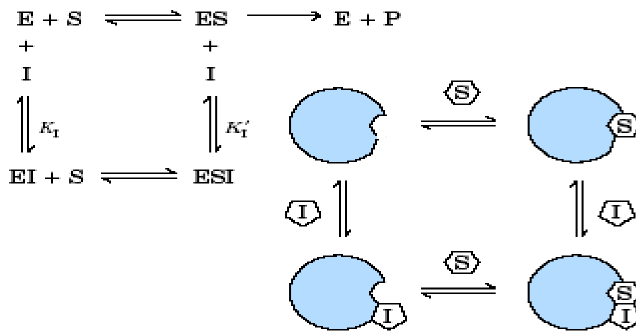


FIGURE : Three types of reversible inhibition. (a) Competitive inhibitors bind to the enzyme's active site. (b) Uncompetitive inhibitors bind at a separate site, but bind only to the ES complex.(c) Mixed inhibitors bind at a separate site, but may bind to either E or ES.

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

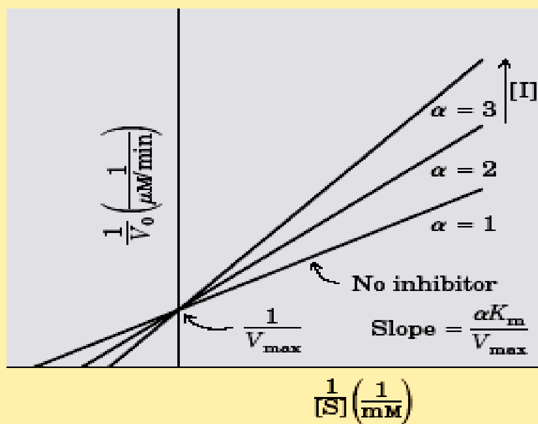


FIGURE 1 Competitive inhibition.

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

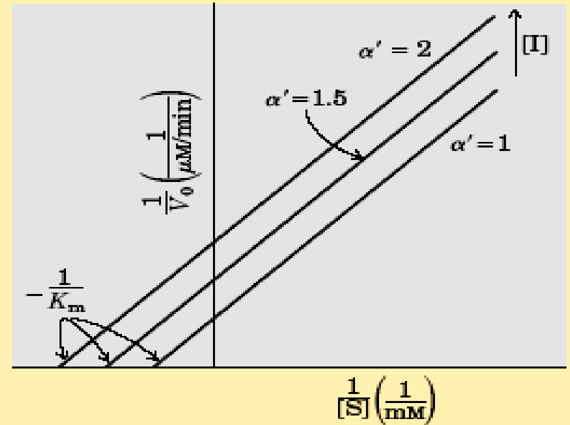


FIGURE 2 Uncompetitive inhibition.

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

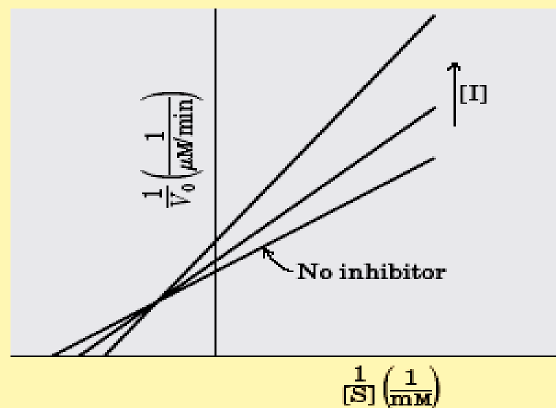


FIGURE 3 Mixed inhibition.