ENZYMES

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LEARNING OUTCOMES

- Properties of Enzymes
- Nomenclature and Classification of enzymes
- Structure and composition of enzymes
- Enzyme -Substrate complex, Active site, Allosteric site
- Enzyme Kinetics
- Catalytic mechanisms employed by enzymes
- Regulation of Enzyme activity
- Enzyme Inhibition

ENZYMES

- Enzymes catalyze hundreds of stepwise biochemical reactions.
- About a quarter of the genes in the human genome encode enzymes.
- Deficiency/ total absence or excessive activity of an enzyme may be the cause of inheritable genetic disorders.
- Measurements of the activities of enzymes is important in diagnosing certain illnesses.
- Many drugs exert their biological effects through interactions with enzymes.
- Important in chemical industry, food processing, and agriculture.

PROPERTIES OF ENZYMES

- Enzymes are biological catalysts. Almost every biochemical reaction is catalyzed by an enzyme.
- With the exception of a few catalytic RNAs, all known enzymes are proteins. Many require nonprotein coenzymes or cofactors for their catalytic function.
- Enzymes, like other proteins, have molecular weights ranging from about 12,000 to more than 1 million.

PROPERTIES OF ENZYMES

- Enzymes have high degree of specificity for their substrates and stabilize transition states.
- Enzymes accelerate specific chemical reactions without the formation of by-products.
- Enzymes function in dilute aqueous solutions under very mild conditions of temperature and pH.

PROPERTIES OF ENZYMES

- Enzymes get denatured (loses its catalytic activity) when subjected to elevated temperature or extremes of pH or non-physiological concentrations of salt, organic solvents, urea or other chemical agents.

PROPERTIES OF ENZYMES

Their catalytic activity depends on the integrity of their native protein conformation.

If an enzyme is denatured or dissociated into its subunits, catalytic activity is usually lost.

Thus the primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity.

PROPERTIES OF ENZYMES

- Enzymes catalyse reactions in both directions (forward and reverse) when free energy change in reaction is small.
- During enzymatic catalysis, substrate binds at the active site of the enzyme protein transiently (for a brief time) and after catalysis, product is released.
- Enzymes increase the rate of reaction but do not alter the equilibrium.

NOMENCLATURE AND CLASSIFICATION OF ENZYMES

According to **International Union of Biochemistry and Molecular Biology (IUBM)**, nomenclature and classification of enzymes is based on the

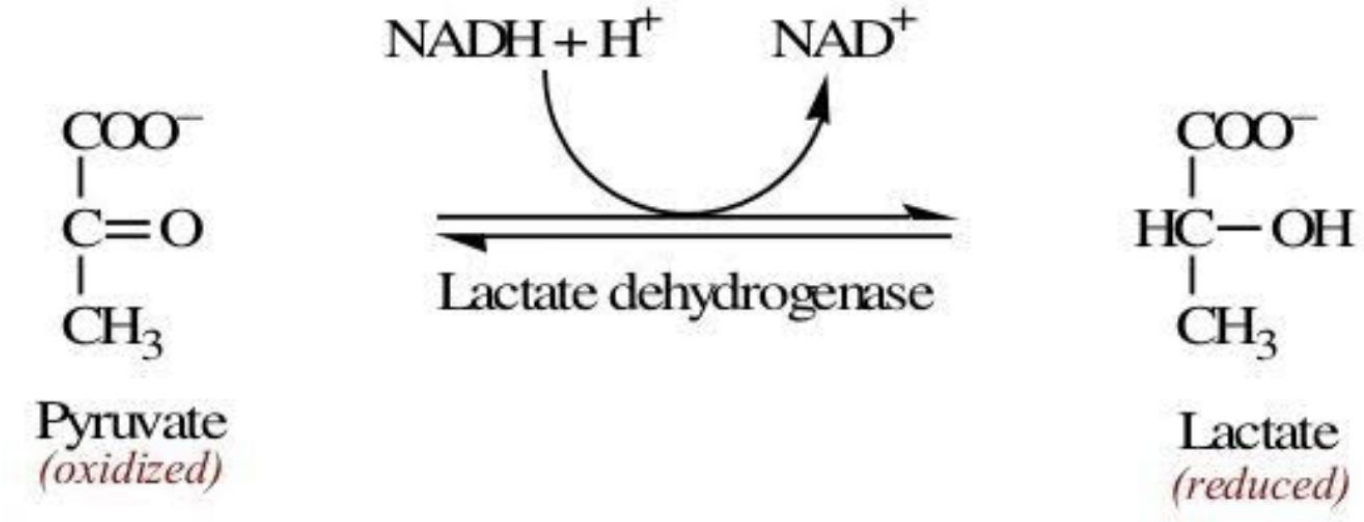
- Reactions they catalyse
- Substrates transformed &
- The products formed by the enzyme

NOMENCLATURE AND CLASSIFICATION OF ENZYMES

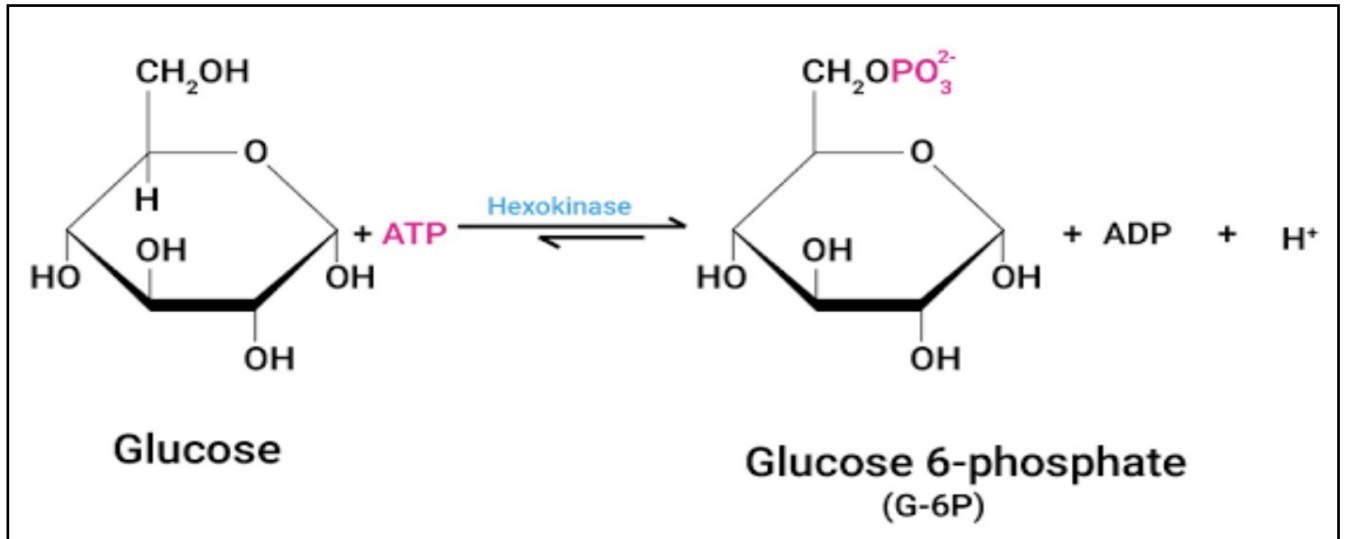
- Enzymes were divided into 6 major classes according to type of reaction catalysed and a **seventh class Translocases was added in 2018.**
- Currently there are **6476 enzymes** assigned to seven classes and the number keeps updating.
- Enzymes are identified by 4 digit EC (Enzyme Commission) number.

Enzyme class	Reaction type	Description	Enzyme count
EC 1 Oxidoreductases	$A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}$	Catalyze redox reaction (transfer of electrons) and can be categorized into oxidase and reductase.	1908
EC 2 Transferases	$A\text{-}B + C \longrightarrow A + B\text{-}C$	Group transfer reactions	1929
EC 3 Hydrolases	$A\text{-}B + H_2O \longrightarrow A\text{-}H + B\text{-}OH$	Hydrolysis reactions (transfer of functional groups to water)	1314
EC 4 Lyases	$A\text{-}B \rightleftharpoons A + B$ (reverse reaction: synthase)	Addition of groups to double bonds, or formation of double bonds by removal of groups	708
EC 5 Isomerases	$A\text{-}B\text{-}C \rightleftharpoons A\text{-}C\text{-}B$	Transfer of groups within molecules to yield isomeric forms	304
EC 6 Ligases	$A + B + ATP \longrightarrow A\text{-}B + ADP + P_i$	Formation of C- C, C- S, C- O, and C- N bonds by condensation reactions coupled to ATP cleavage (release of energy)	223
EC 7 Translocases		Catalyze the movement of ions or molecules across membranes or their separation within membranes	90
All classes			6476

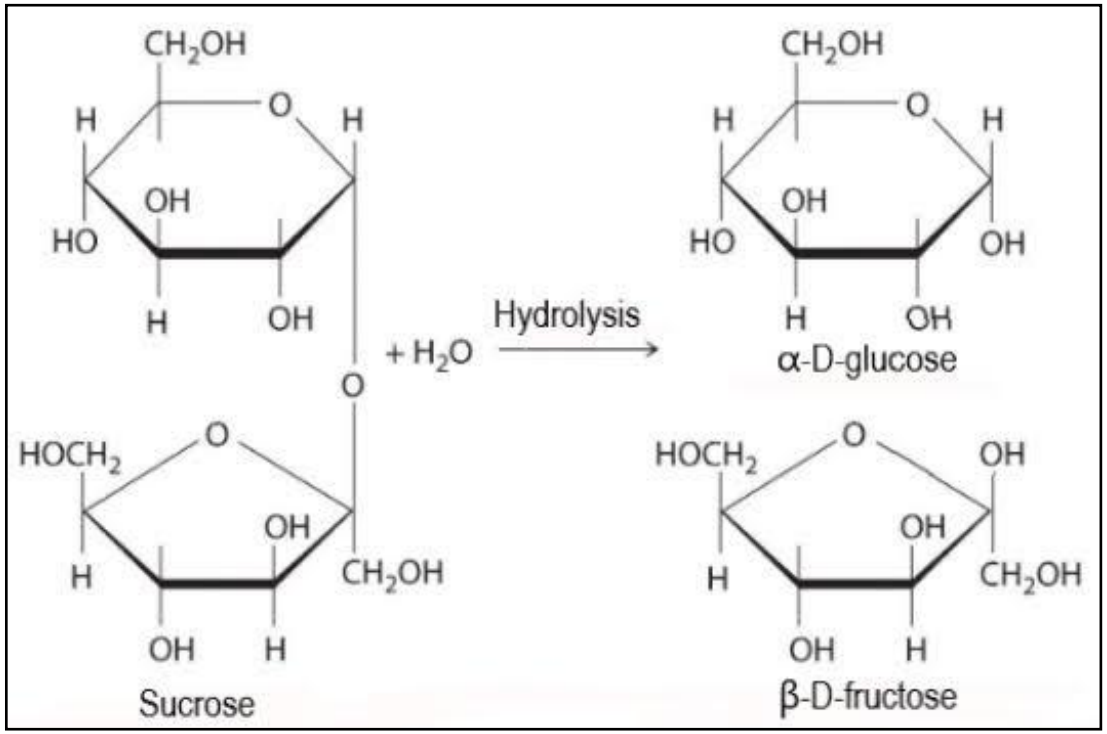
1.**OXIDOREDUCTASE**



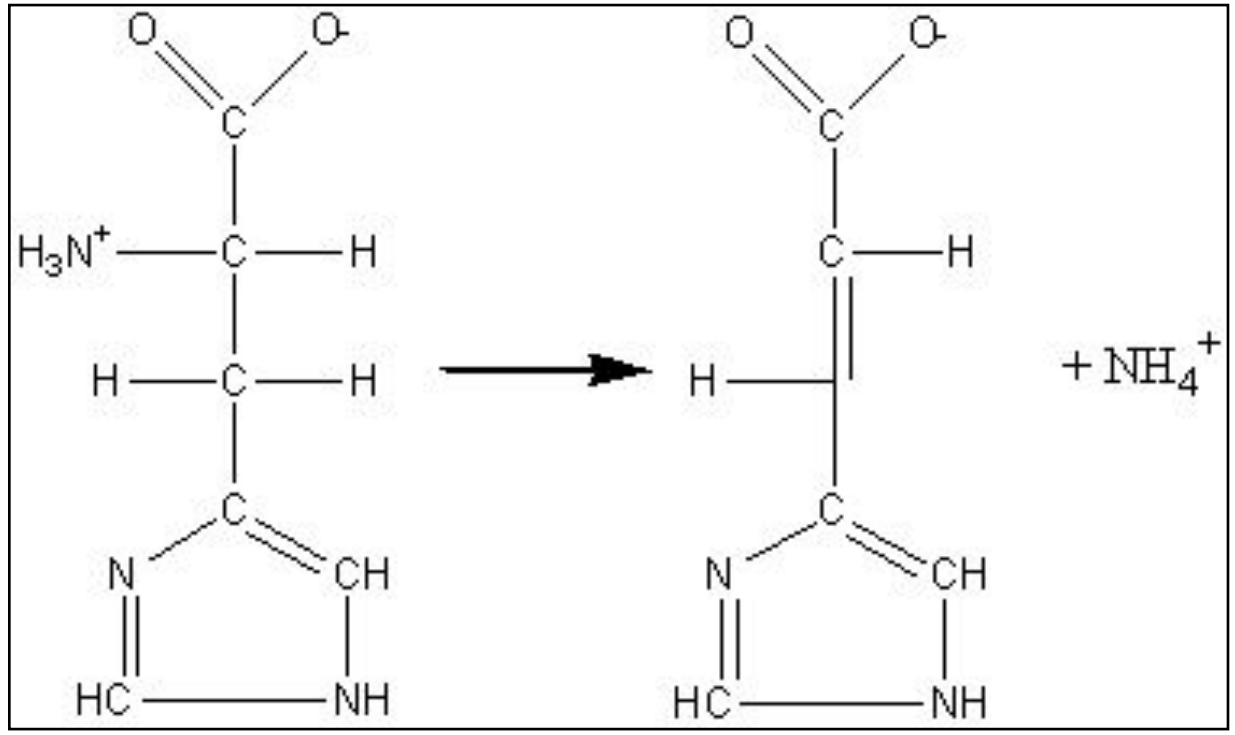
2. **TRANSFERASES**



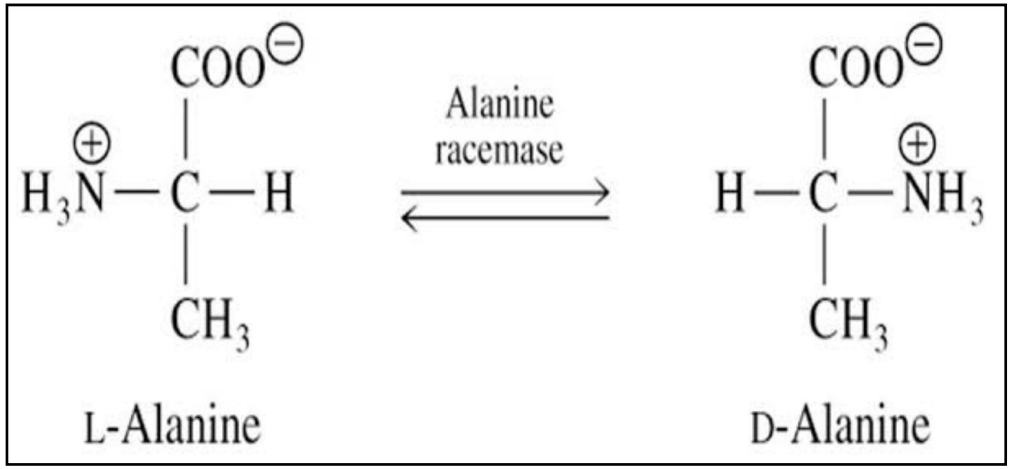
3. **HYDROLASES**



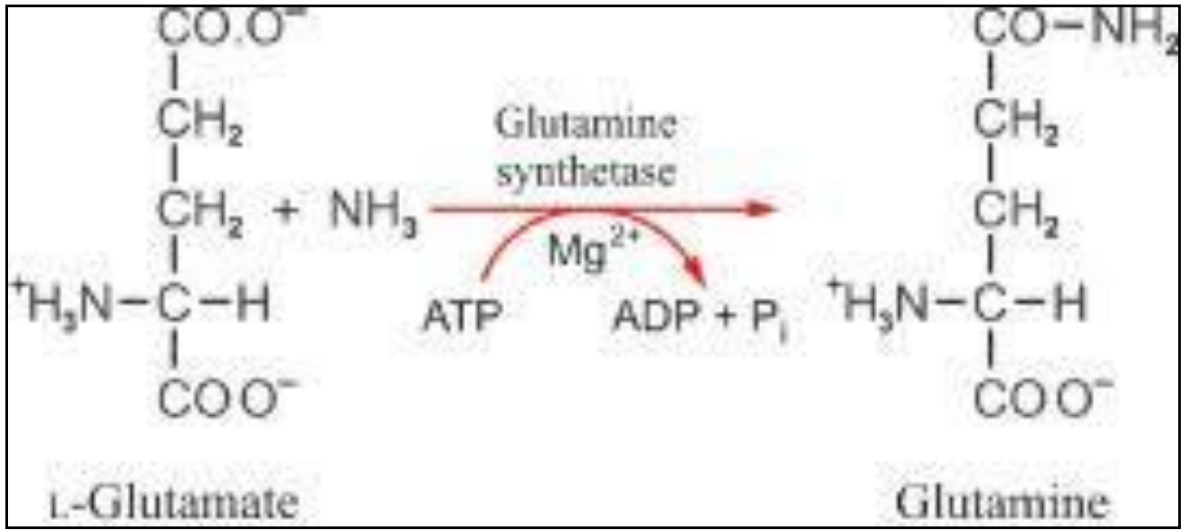
4. **LYASES**



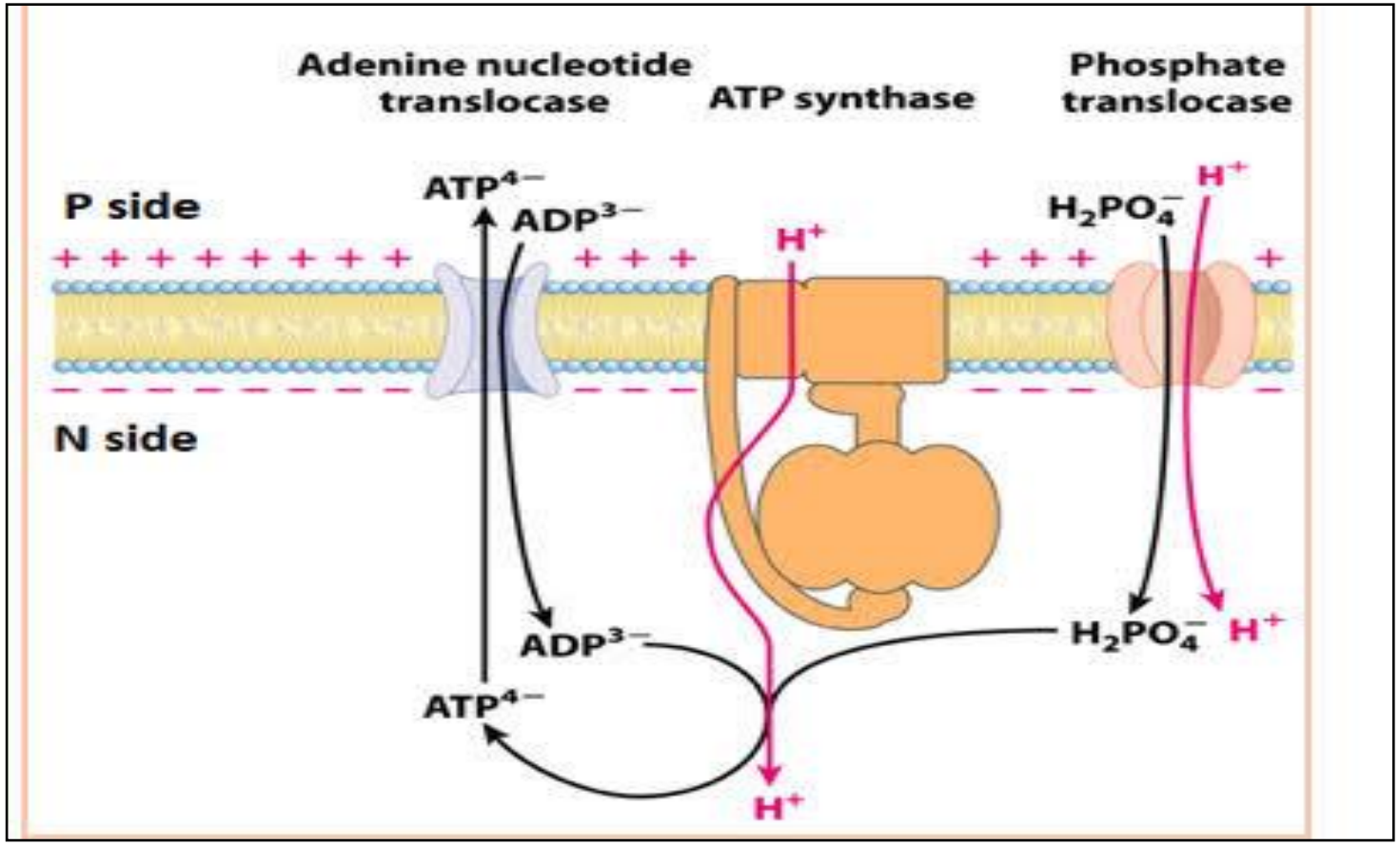
5. **ISOMERASES**



6. **LIGASES/ SYNTHETASES**



7. **TRANSLOCASES**



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NOMENCLATURE OF ENZYMES

Trivial name: **Hexokinase**

Formal systematic name: **ATP:glucose phosphotransferase**, (which indicates that it catalyzes the transfer of a phosphoryl group from ATP to glucose).

Its Enzyme Commission number (**E.C. number**) : **2.7.1.1**.

2.7.1.1 denotes the class name (transferase)

2.**7**.1.1 denotes subclass (phosphotransferase)

2.7.**1**.1 a phosphotransferase with a hydroxyl group as acceptor; and

2.7.1.**1** D-glucose as the phosphoryl group acceptor.

Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (www.chem.qmul.ac.uk/iubmb/enzyme) maintains a complete list and description of the thousands of known enzymes.

STRUCTURE AND COMPOSITION OF ENZYMES

On the basis of chemical nature, enzymes are of 2 types:

- **(i) Simple enzymes:**

They consist of only proteins, e.g. urease, lysozyme, pepsin, trypsin etc.

- **(ii) Holoenzyme or Conjugate enzyme:**

Holoenzyme = Apoenzyme (proteinaceous part) + Prosthetic group/ Cofactor / Coenzyme (non- protein part)

COFACTORS, COENZYMES, PROSTHETIC GROUPS

- According to IUPAC (International Union of Pure and Applied Chemistry),

  Cofactors are organic molecules (coenzymes) or ions (usually metal ions) that are required by an enzyme for its activity and are attached loosely to the enzyme. If attached tightly to enzyme protein it is called a prosthetic group.

  A cofactor binds with its associated protein (apoenzymes), which is functionally inactive, to form the active enzyme (holoenzyme).

Cofactors	Enzymes
Cu^{++}	Cytochrome oxidase
Fe^{++}or Fe^{+++}	Cytochrome oxidase, Catalase, Peroxidase
K^{+}	Pyruvate kinase
Mg^{++}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{++}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{++}	Urease
Se	Glutathione peroxidase
Zn^{++}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Coenzymes as Transient Carriers of Specific Atoms or Functional Groups:

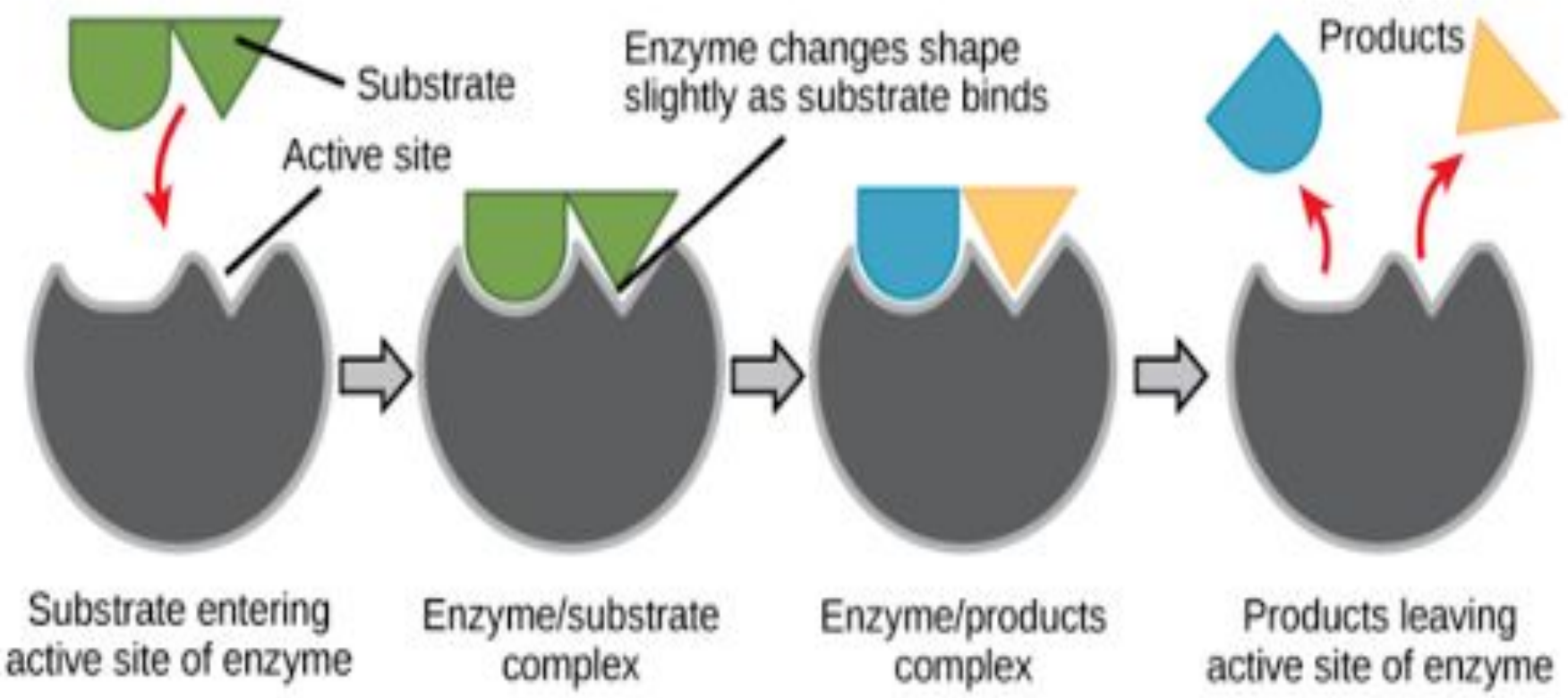
Biocytin	CO_2
Coenzyme A	Acyl groups
Coenzyme B12	H atoms and alkyl groups
Flavin adenine dinucleotide	Electrons
Lipoate	Electrons and acyl groups
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)
Pyridoxal phosphate	Amino groups
Tetrahydrofolate	One-carbon groups
Thiamine pyrophosphate	Aldehydes

ENZYME – SUBSTRATE COMPLEX

- The existence of enzyme-substrate complex was first proposed by **Charles-Adolphe Wurtz (1880)**.

- **Michaelis and Menten** defined the mechanism of action and kinetic behavior of enzyme-catalyzed reactions on the basis of ES complex formation .

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



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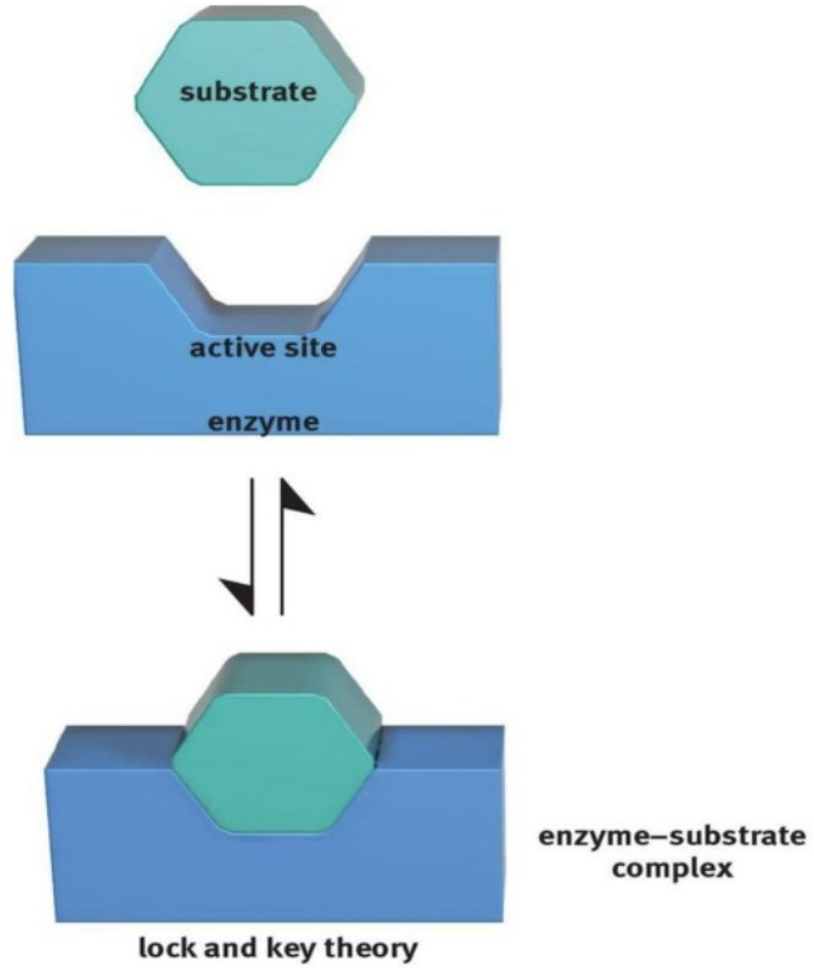
LOCK AND KEY MODEL/ TEMPLATE MODEL :

This model was proposed by **Emil Fischer (1898)**.

- Union between enzyme and substrate takes place at the active site in a manner in which a key fits a lock.
- This hypothesis is also known as "concept of intermolecular fit".
- In Emil Fischer's model , the active site is presumed to be rigid and pre- shaped to fit the substrate.



https://schoolbag.info/chemistry/mcat_biochemistry

Modern view of enzymatic catalysis

Proposed by **Michael Polanyi (1921)** and **Haldane (1930)**, elaborated by **Linus Pauling (1946)**:

In order to catalyze reactions, an **enzyme must be complementary to the reaction transition state**.

The essence of catalysis is selective **stabilization of the transition state.** Hence, an **enzyme binds the transition state more tightly than it binds the substrate.**

Linus Pauling (1948) proposed that compounds resembling the transition state (called transition-state analogs) of a catalyzed reaction should be very effective inhibitors of enzymes.

INDUCED FIT MODEL :

Daniel Koshland (1958) presumed that

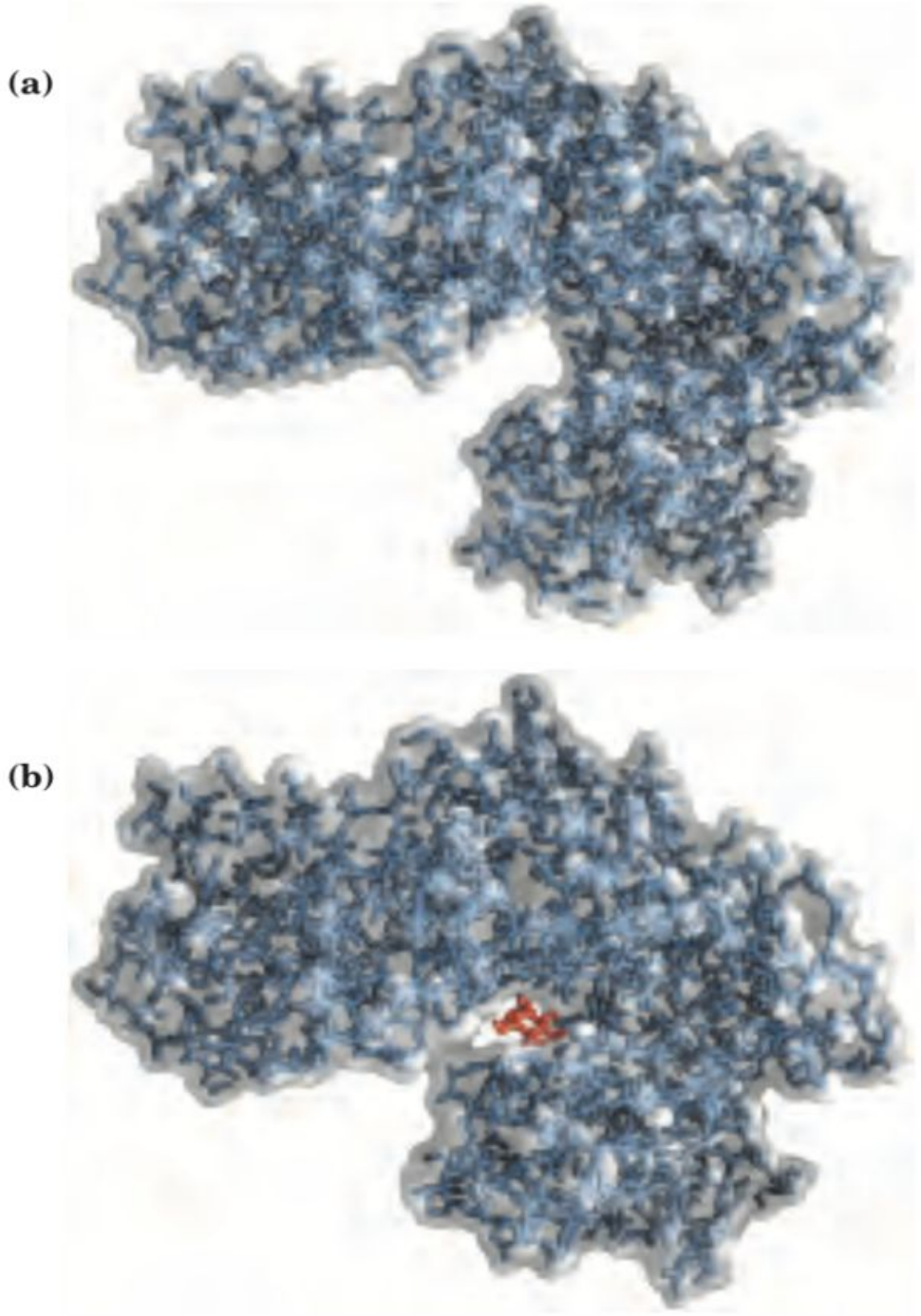
- The contact of the substrate induces some configurational or geometrical changes in the active site of the enzyme molecule and consequently the enzyme molds itself to the shape of the substrate molecule.
- Koshland's model has gained experimental support with various enzymes such as Hexokinase, Phosphoglucomutase, Creatine kinase, Carboxypeptidase etc.

Induced fit in hexokinase

(a) Hexokinase has a U-shaped structure

(b) The ends pinch toward each other in a conformational change induced by binding of D-glucose (red).

Ref: Lehninger



ACTIVE OR CATALYTIC SITE

- The **active or catalytic site** is the region (groove/ pocket) of an enzyme where substrate molecules bind.

- The surface of the active site is lined with amino acid residues with substituent groups that bind the substrate and catalyze its chemical transformation.

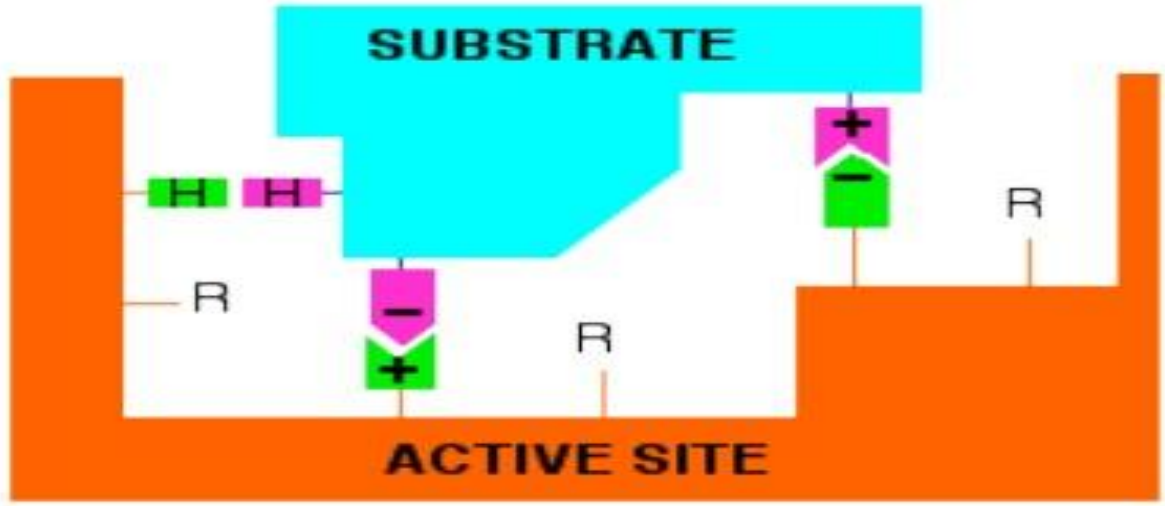
ACTIVE OR CATALYTIC SITE

- The specificity of active site is determined by the arrangement of amino acids within the active site and the structure of the substrates.

- The interaction between the active site and the substrate is non-covalent and transient.

  - hydrogen bonds,
  - van der Waals interactions,
  - hydrophobic interactions and
  - electrostatic force interactions.

Active Site



- The substrate is held in the active site by a variety of bonds, such as hydrogen bonds and electrostatic interactions

https://www.chemguide.co.uk

REGULATORY OR ALLOSTERIC SITE

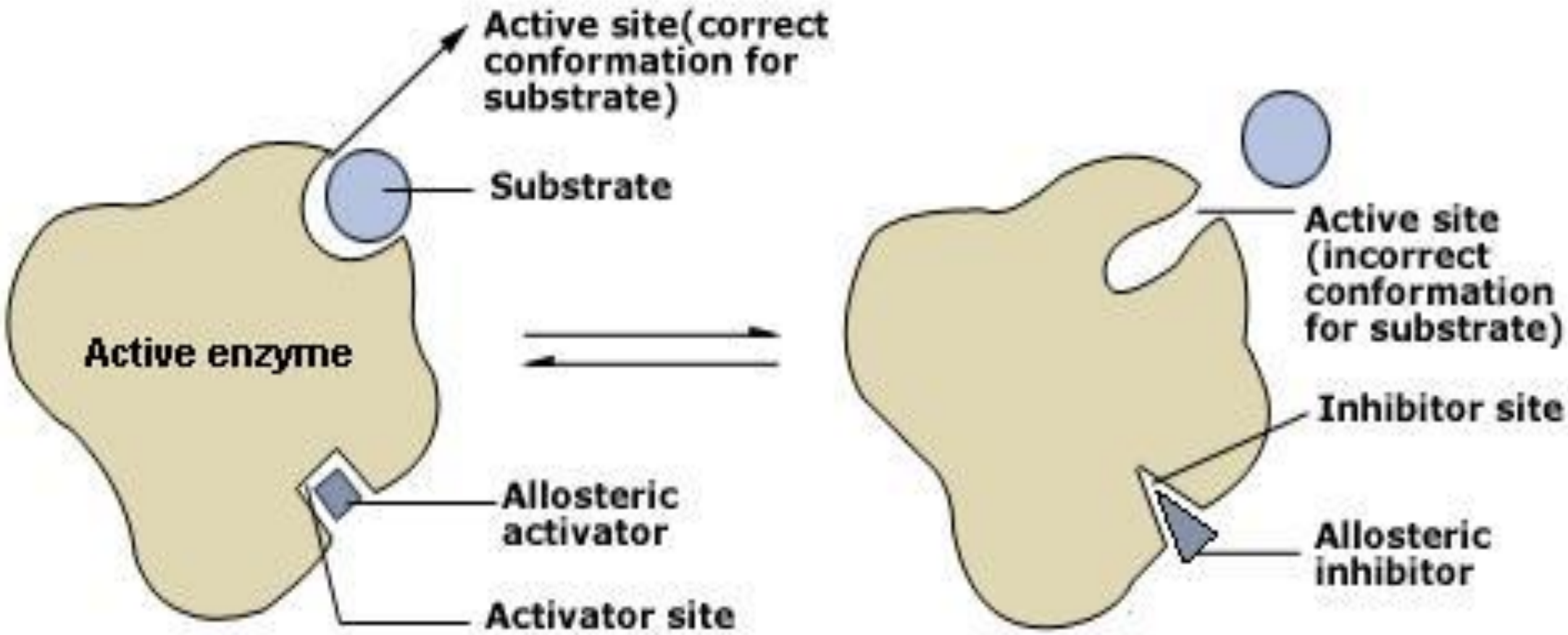
- Besides the presence of an active or catalytic site, the allosteric enzyme have one or more **regulatory or allosteric site** (allos = other, stereos = space/ site) for the binding of modulator/ effector.

- Binding of modulator changes the physical configuration of the enzyme molecule.

- The changes affect the catalytic properties of the enzyme, either inhibiting or stimulating the rate of the reaction.

REGULATORY OR ALLOSTERIC SITE

- A stimulator is often the substrate itself.
- The regulatory enzymes for which substrate and modulator are identical are called homotropic. When the modulator has a structure different then the substrate, the enzyme is called heterotropic.
- Allosteric inhibitors generally are the end products of the metabolic pathway in which that particular enzyme is participating. This kind of enzyme inhibition is also called **feedback/ end product/retro inhibition**.
- The effect of allosteric modulators is reversible, when they are withdrawn, the enzyme resumes the original activity.



Schematic representation of allosteric enzyme activity

https://biochem4you.files.wordpress.com/2013/04/allosteric-inhibition-

ENZYME KINETICS

Free Energy Change

1. A reaction can take place spontaneously, if Δ G is negative (exergonic reactions).

2. A system is at equilibrium and no net change can take place if Δ G is zero.

3. A reaction cannot take place spontaneously if ΔG is positive. An input of free energy is required to drive such a reaction (endergonic reactions).

4. The Δ G of a reaction depends only on the free energy of the products (the final state) minus the free energy of the reactants (the initial state).

5. The Δ G provides no information about the rate of a reaction.

The rate of a reaction depends on the free energy of activation (Δ G‡).

Reaction coordinate diagram

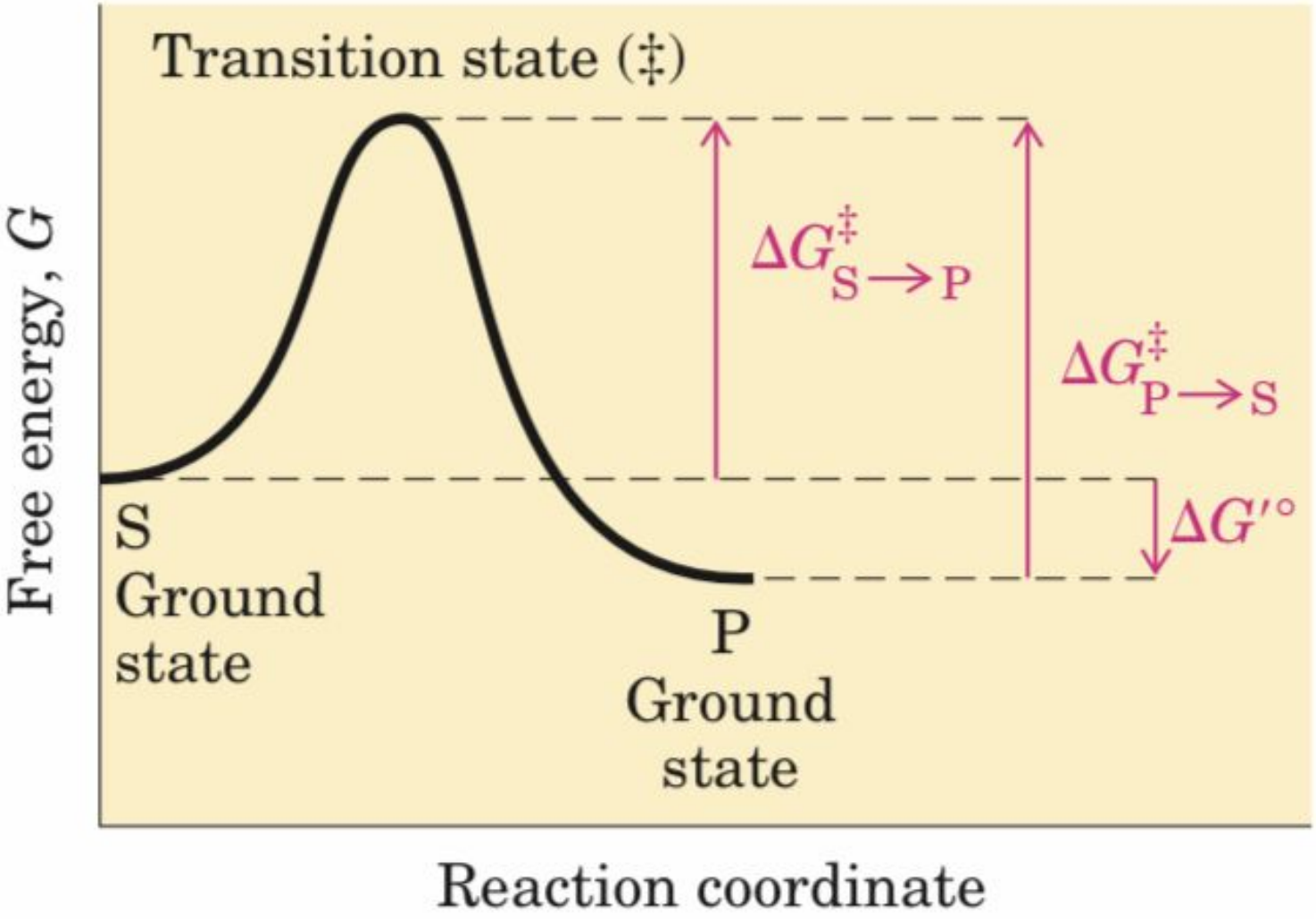
The function of a catalyst is to increase the rate of a reaction.

Catalysts do not affect reaction equilibria.

Any reaction, such as S $\rightleftharpoons$ P, can be described by a reaction coordinate diagram, a picture of the energy changes during the reaction.

In the coordinate diagram, the free energy of the system is plotted against the progress of the reaction (the reaction coordinate).

The starting point for either the forward or the reverse reaction is called the ground state.



The equilibrium between S and P reflects the difference in the free energies of their ground states.

In the example shown, the free energy of the ground state of P is lower than that of S.

ΔG for the reaction is negative and the equilibrium favors P.

Reaction coordinate diagram for a chemical reaction

The free energy of the system is plotted against the progress of the reaction S→P. (Lehninger)

KINETICS OF ENZYMATIC

- Reaction is thermodynamically possible, if the free energy of the ground state of product (P) is lower than that of substrate (S).

- A favorable equilibrium does not mean that the S→P conversion will occur at a detectable rate.

KINETICS OF ENZYMATIC CATALYSIS

- For conversion of S → P extra energy is needed (represented as energy barrier) that represents the energy required for alignment of reacting groups, formation of transient unstable charges, bond re- arrangements, and other transformations required for the reaction to proceed in either direction.

- The top of the energy barrier is called transition state, where certain events like bond breakage, bond formation etc decide the future course of the reaction.

ACTIVATION ENERGY (

- The difference between the energy levels of the ground state and transition state is called **activation energy (E_a)**.
- A higher activation energy corresponds to a slower reaction.
- Reaction rates can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier.
- **Catalysts (enzymes) enhance reaction rates by lowering activation energies.**

Enzymes (catalysts) do not affect reaction equilibria

Any enzyme that catalyzes the reaction S→P also catalyzes the reaction P→S.

The role of enzymes is to accelerate the interconversion of S and P.

The enzyme is not used up in the process, and the equilibrium point is unaffected.

However, the reaction reaches equilibrium much faster when the appropriate enzyme is present, because the rate of the reaction is increased.

Covalent interactions between Enzyme & Substrate

Catalytic functional groups on an enzyme (specific amino acid side chains, metal ions, and coenzymes) may form a transient covalent bond with a substrate and activate it for reaction, or a group may be transiently transferred from the substrate to the enzyme.

Covalent interactions between enzymes and substrates lower the activation energy (and thereby accelerate the reaction) by providing an alternative, lower-energy reaction path.

Non covalent interactions between Enzyme & Substrate

The interaction between substrate and enzyme in ES complex is mediated by hydrogen bonds and hydrophobic and ionic interactions .

The full complement of such interactions between substrate and enzyme is formed only when the substrate reaches the transition state.

Formation of each weak interaction in the ES complex is accompanied by release of a small amount of free energy called **binding energy**, G_B

Binding energy stabilizes the enzyme-substrate interaction and ΔG

It is a major source of free energy used by enzymes to lower the activation energies of reactions.

Catalytic Mechanisms employed by enzymes

Catalytic mechanisms employed by enzymes include

- Catalysis by Approximation
- General acid-base catalysis,
- Covalent catalysis, and
- Metal ion catalysis

Catalysis by Approximation

Many reactions have two distinct substrates.

In such cases, the reaction rate may be considerably enhanced by bringing the two substrates together along a single binding surface on an enzyme.

For example, carbonic anhydrase binds carbon dioxide and water in adjacent sites to facilitate their reaction.

General Acid- Base Catalysis

Many biochemical reactions involve the formation of unstable charged intermediates.

Charged intermediates can often be stabilized by the transfer of protons to form a species that breaks down more readily to products.

In the active site of an enzyme, a number of amino acid side chains can act as proton donors and acceptors .

Proton transfers are the most common biochemical reactions.

Covalent Catalysis

A number of amino acid side chains and the functional groups of some enzyme cofactors can serve as nucleophiles in the formation of covalent bonds with substrates.

These covalent complexes always undergo further reaction to regenerate the free enzyme.

Metal Ion Catalysis

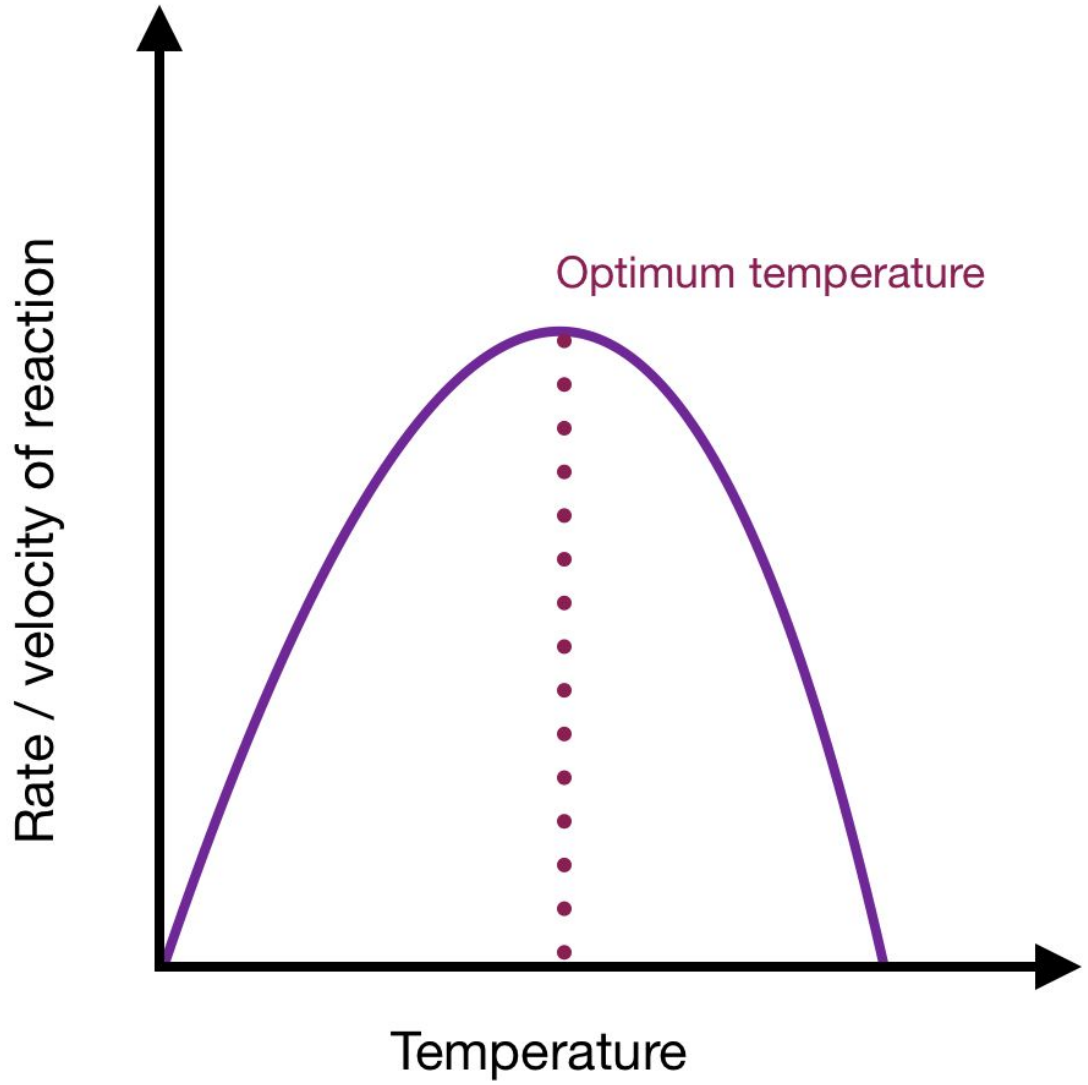
Ionic interactions between an enzyme-bound metal and a substrate stabilize charged reaction transition states.

Metals can also mediate oxidation-reduction reactions by reversible changes in the metal ion's oxidation state.

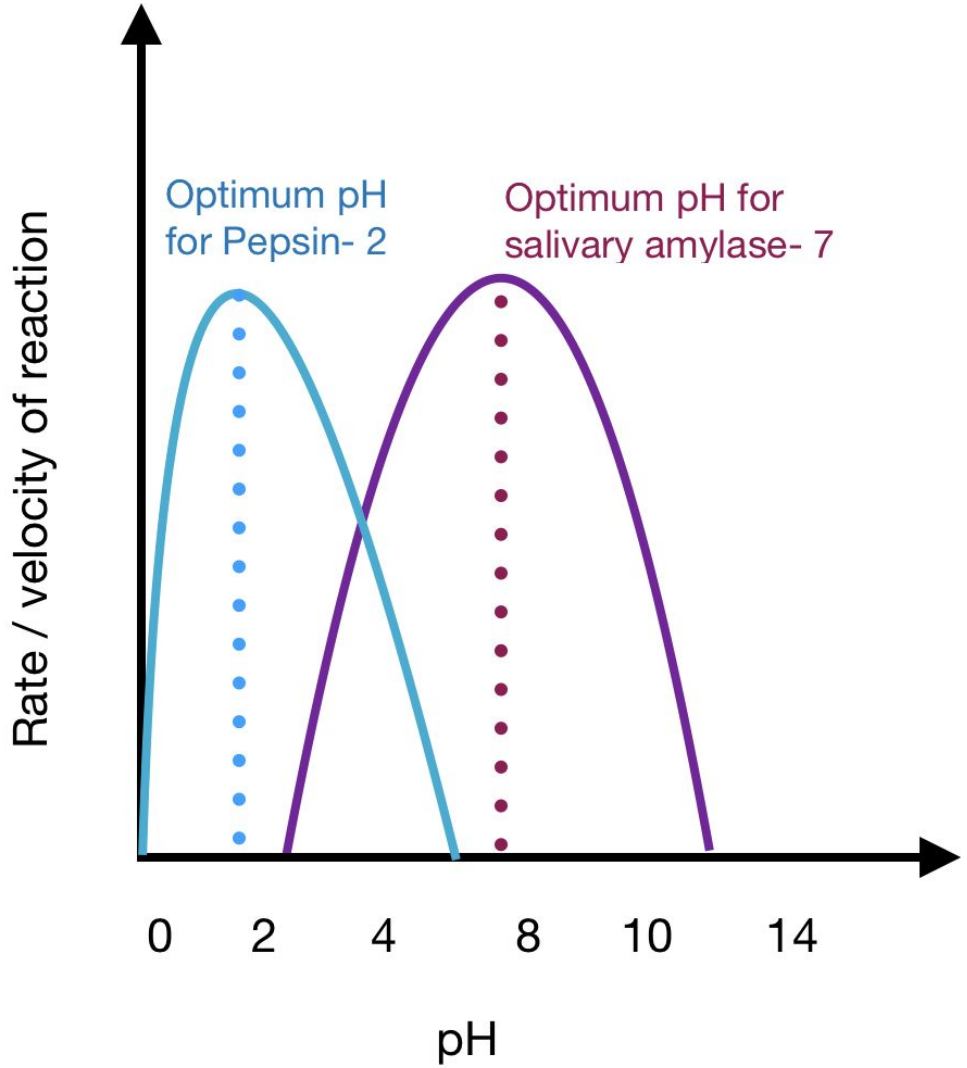
Nearly a third of all known enzymes require one or more metal ions for catalytic activity.

FACTORS AFFECTING ENZYME

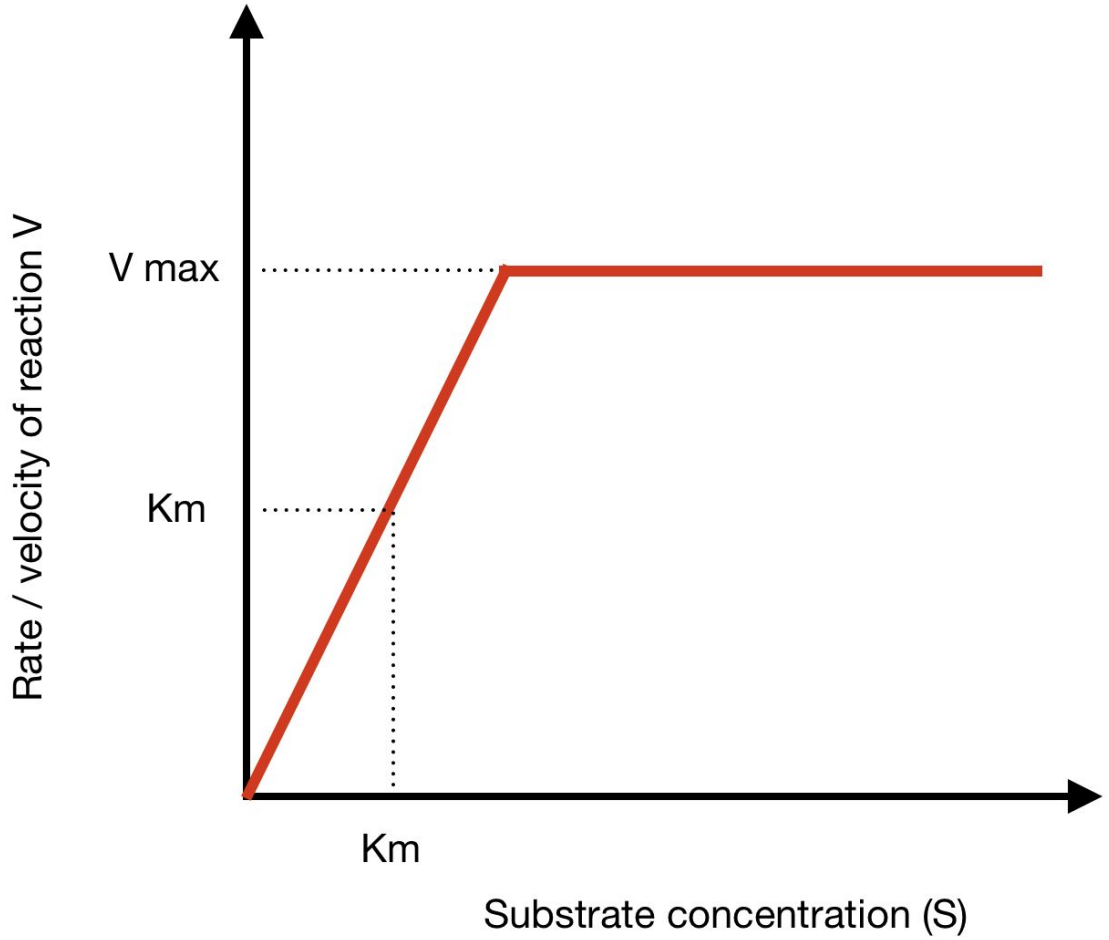
- Temperature
- pH
- Concentration of substrate molecule [S]
- Activators/ Inhibitors



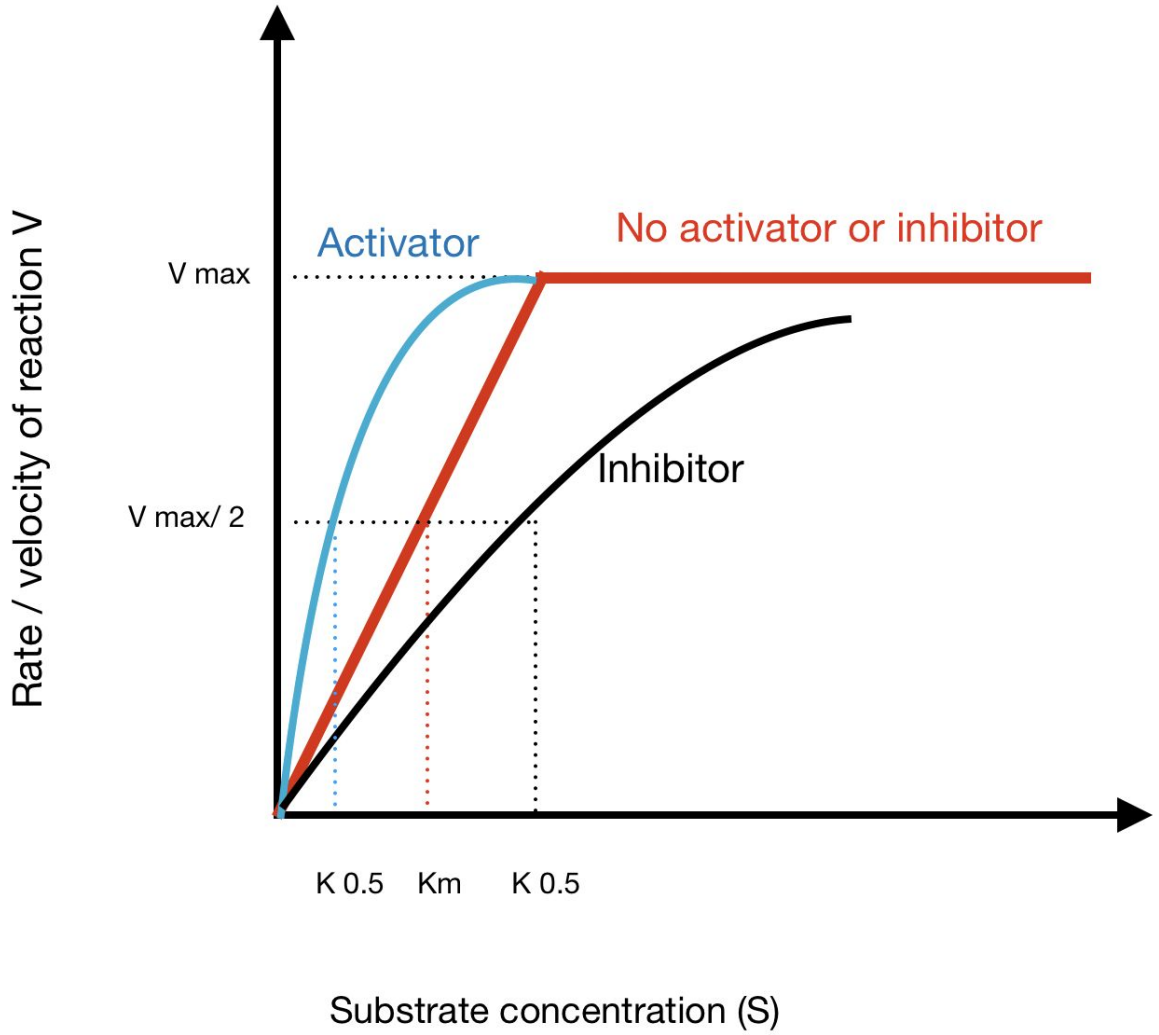
Effect of temperature on enzyme catalyzed reaction



Effect of pH on enzyme catalyzed reaction



Effect of substrate concentration on enzyme catalyzed reactions



Effect of activator and inhibitor on enzyme catalysed reaction

MICHAELIS- MENTEN EQUATION AND ITS SIGNIFICANCE:

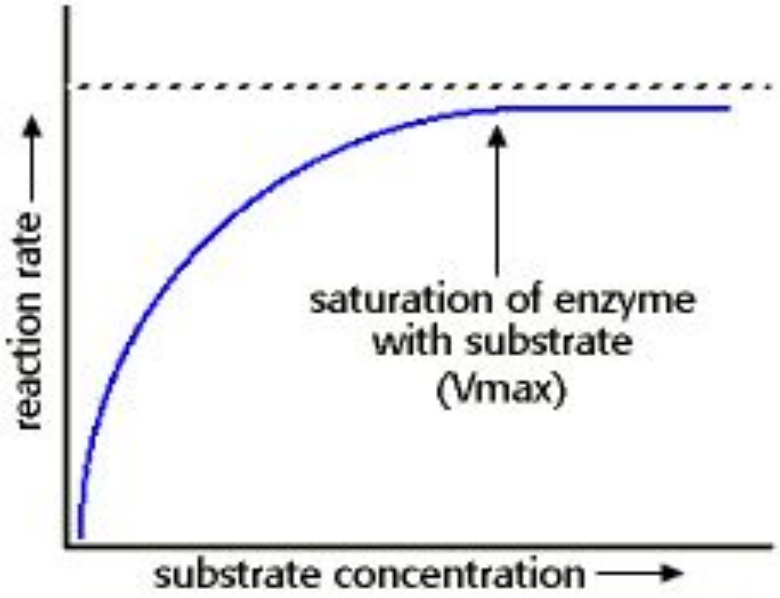
- Rate of reaction increase with the increase in substrate concentration.
- Finally, a point is reached when the addition of substrate has no effect on the rate of enzyme catalysed reaction.
- This is called maximum rate (Vmax) at which the enzyme is saturated with the substrate .
- This saturation effect is exhibited by nearly all enzymes.

MICHAELIS- MENTEN EQUATION

- If a graph is plotted between rate of reaction versus substrate concentration V/ [S], a rectangular hyperbolic curve is obtained.

- The mathematical expression of this curve was developed by **Leonor Michaelis and Maud Menten** (1912-1913).

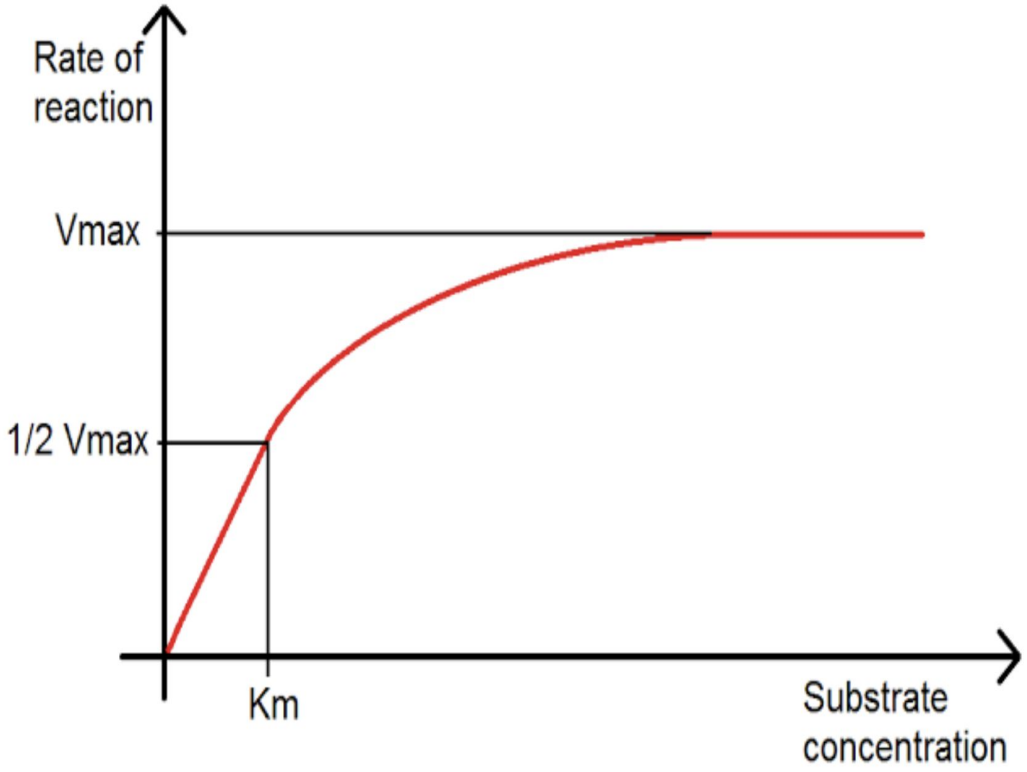
- The curve flattens at the maximum velocity (V_M), when all the active sites of the enzyme are filled with substrate.



MICHAELIS- MENTEN EQUATION

At half the maximum velocity, the substrate concentration in moles per litre (*M*) is equal to the **Michaelis- Menten constant Km**, which is a rough measure of the affinity of the substrate molecule for the enzyme.

- lower Km- higher binding affinity, higher rate of reaction.
- higher Km- lower binding affinity, lower rate of reaction.



$$Vo = \frac{V_{max}\,[S]}{Km + [S]}$$

V0 = Initial velocity (moles/times)

[S] = substrate concentration (molar)

V_{max}= maximum velocity

K_m = substrate concentration at half V_{max}

- The two variables in this equation are the reaction velocity V and the concentration of the substrate [S]
- Vmax and Km are the two constants
- By **Michaelis – Menten equation** we can calculate reaction rate of an enzyme at any given concentration of its substrate

LINEWEAVER BURK PLOT

- It is impossible to obtain a definitive value of Vmax and Km from a typical Michaelis-Menten plot.

Plotting the reciprocal of V/ [S] yields a "double-reciprocal" or Lineweaver-Burk plot (Hans Lineweaver and Dean Burk in 1934) . This provides a more precise way to determine $\mathbf{V_{max}}$ and $\mathbf{K_m}$.

Michaelis-Menten equation ⇒ $$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

invert

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

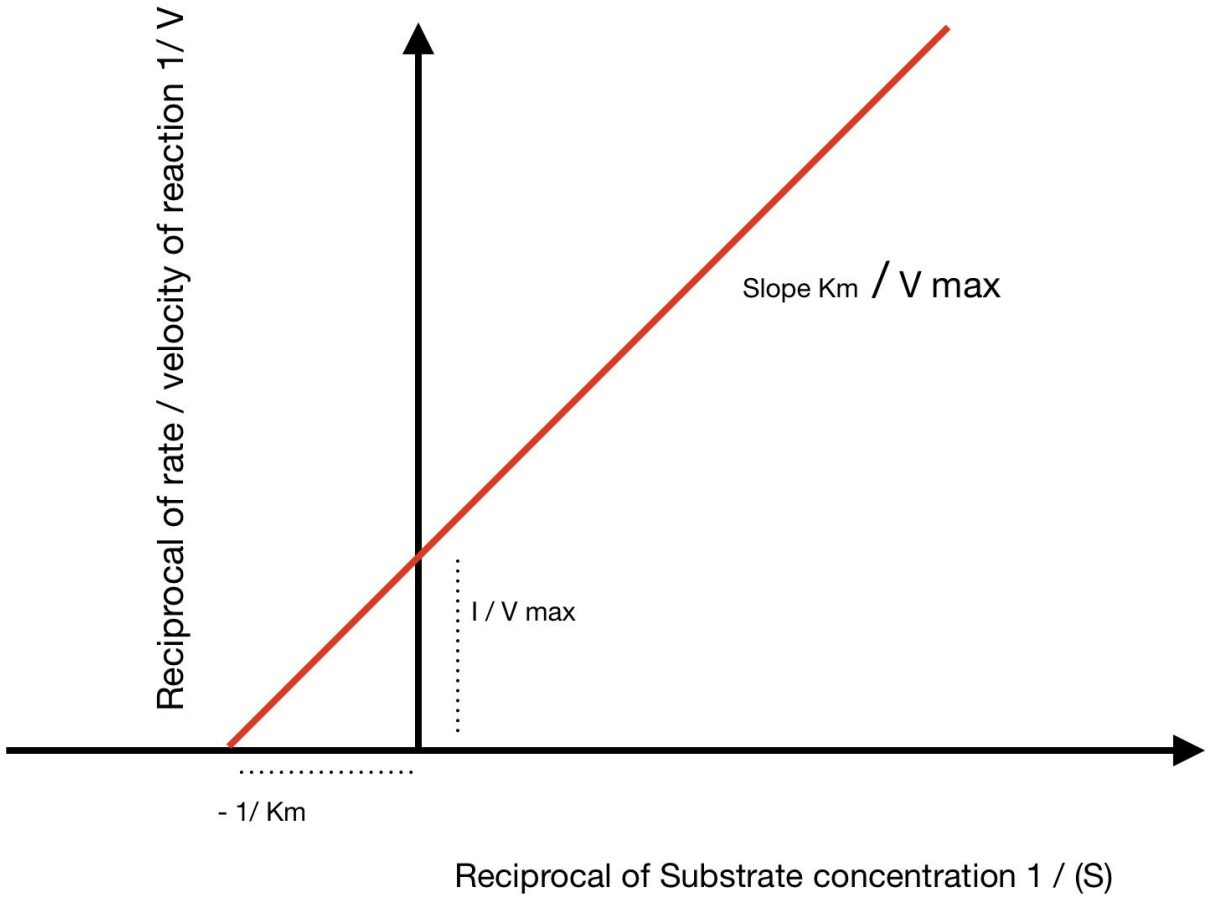
factor

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

and simplify

Double-Reciprocal or Lineweaver-Burk equation ⇒

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



A double-reciprocal plot of enzyme kinetics is generated by plotting $1/V_0$ versus 1/[S]. The slope is the K_M/V_{max}, the intercept on the vertical axis is $1/V_{max}$, and the intercept on the horizontal axis is $-1/K_M$.

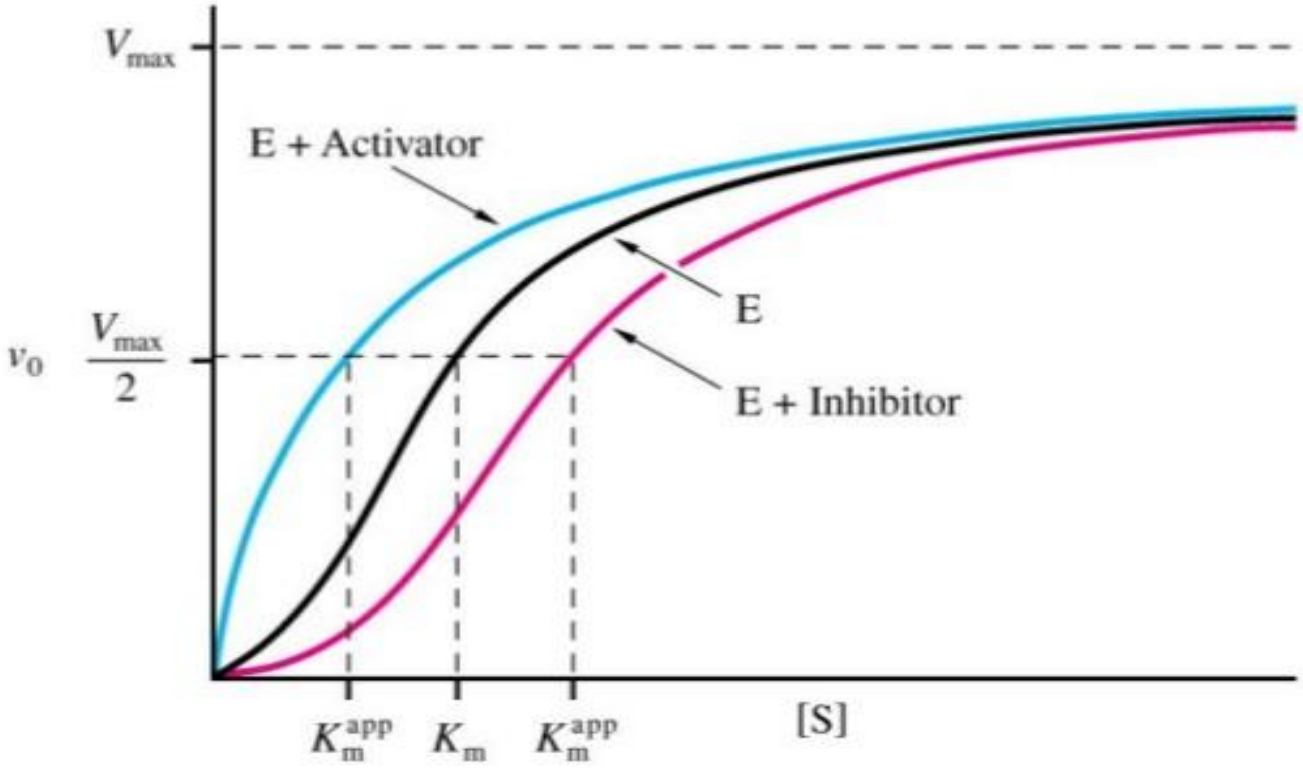
Kinetic properties of allosteric enzymes

- The allosteric enzymes often display sigmoidal saturation curve of the reaction velocity versus substrate concentration V/ [S] .

- Although value of [S] at half maximum velocity can be found out on sigmoid saturation curve, designation Km is not used because the enzyme does not follow the hyperbolic Michaelis- Menten relationship.

- Instead the symbol $[S]_{0.5}$ or $K_{0.5}$ is often used to represent the substrate concentration giving half maximum velocity of the reaction.

Vo vs [S] plots give sigmoidal curve for at least one substrate



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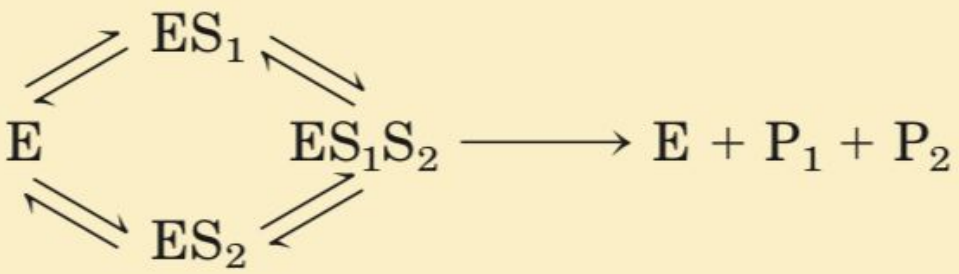
Reaction velocity (V) versus substrate concentration [S]

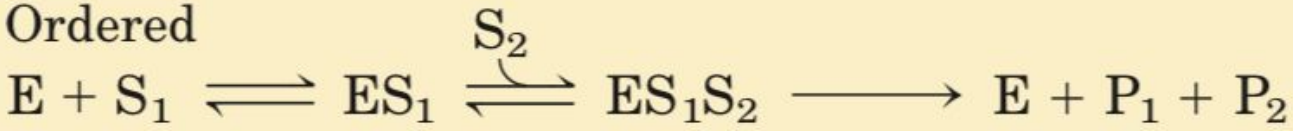
Enzyme-catalyzed bisubstrate reactions

(a) Enzyme reaction involving a ternary complex

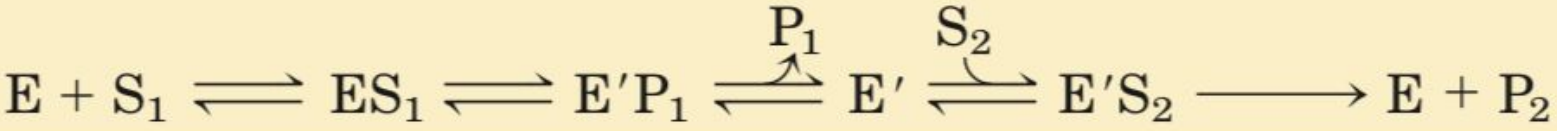
Random order



Ordered



(b) Enzyme reaction in which no ternary complex is formed



(a) The enzyme and both substrates come together to form a ternary complex.

(b) An enzyme-substrate complex forms, a product leaves the complex, the altered enzyme forms a second complex with another substrate molecule, and the second product leaves, regenerating the enzyme.

(Ref: Lehninger)

ENZYME INHIBITION

- **Reversible inhibition-**

Reversible inhibitors combine non-covalently with the enzyme and can therefore be readily removed by dialysis.

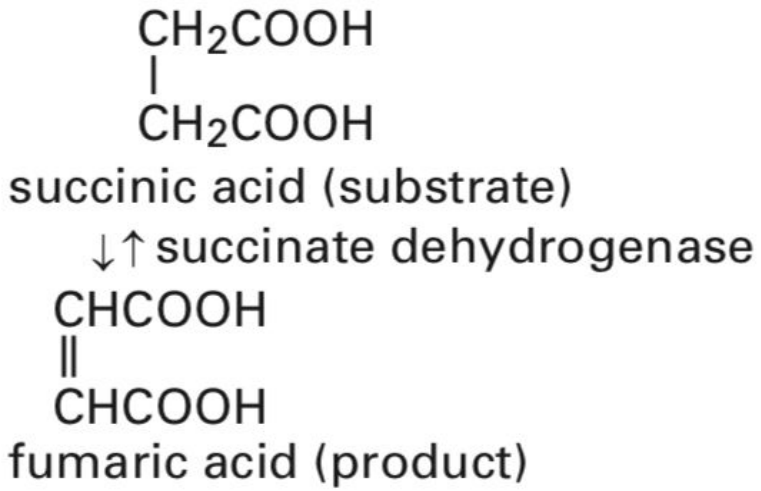
- Competitive reversible inhibition
- Non-competitive reversible inhibition
- Uncompetitive reversible inhibition
- End product inhibition

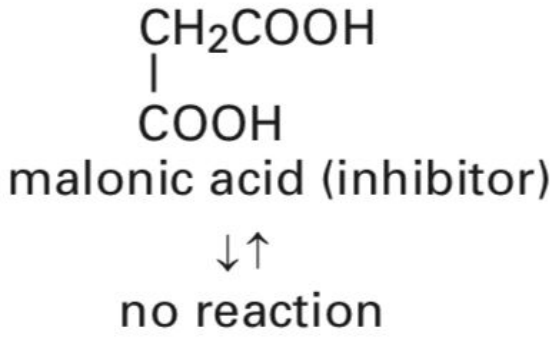
- **Irreversible inhibition**

Competitive reversible inhibition

Competitive reversible inhibitors combine at the same site as the substrate and must therefore be structurally related to the substrate.

$$E + S \rightleftharpoons ES \longrightarrow E + P$$
$$E + I \rightleftharpoons EI \longrightarrow \text{no reaction}$$





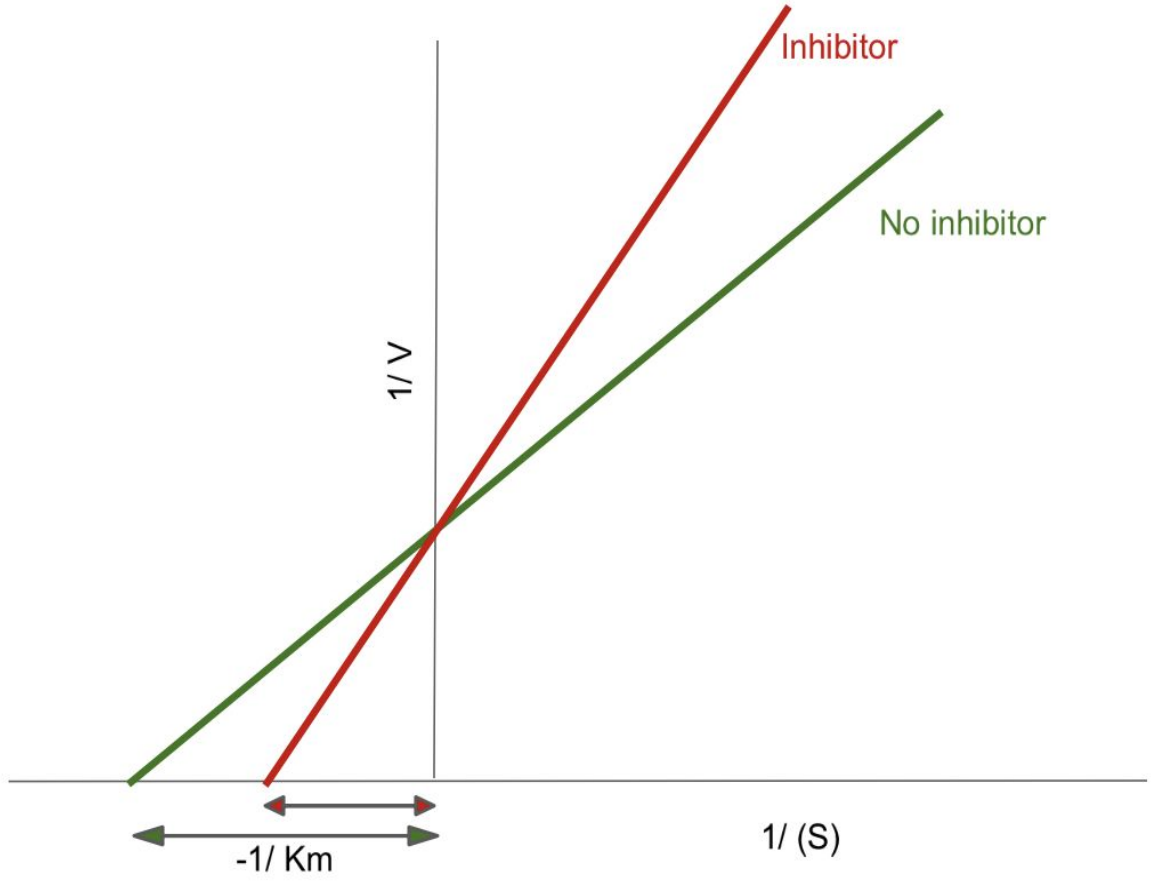
Competitive reversible inhibition

inhibition of succinate dehydrogenase by malonate

Competitive Inhibition

Since the binding of both substrate and inhibitor involves the same site, the effect of a competitive reversible inhibitor can be overcome by increasing the substrate concentration.

Vmax is unaffected but the concentration of substrate required to achieve it is increased (Km is increased).



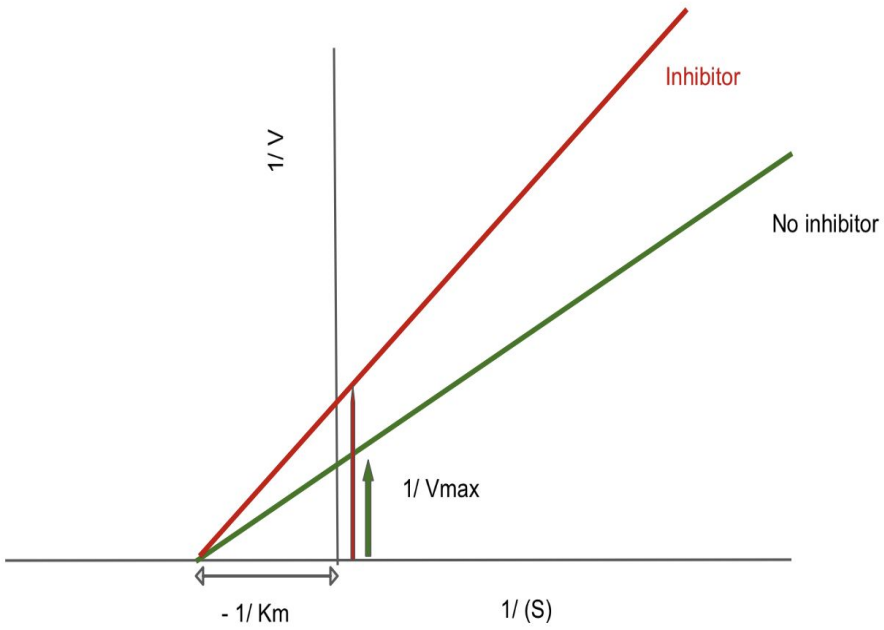
Competitive Inhibition - Km increased, Vmax unaffected

Non-competitive reversible inhibition

A non-competitive reversible inhibitor combines at a site distinct from that for the substrate.

Since this inhibition involves a site distinct from the catalytic site, the inhibition cannot be overcome by increasing the substrate concentration.

Vmax is reduced but Km is unaffected because the inhibitor does not affect the binding of substrate but it does reduce the amount of free ES that can proceed to the formation of product.



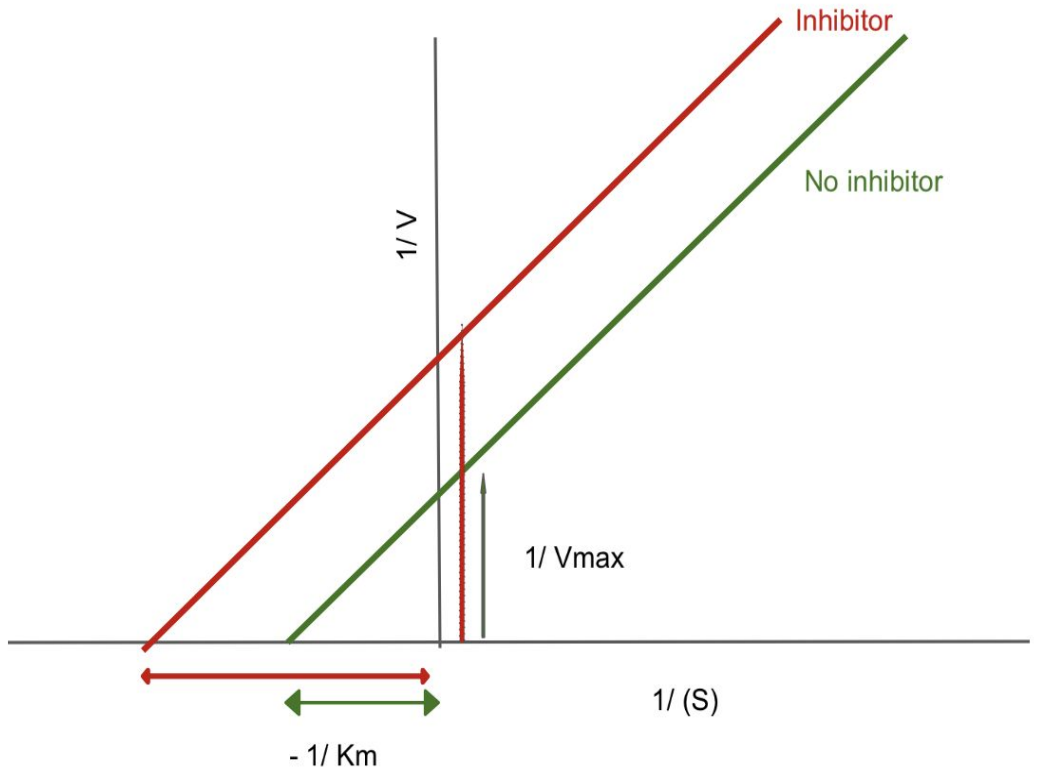
Non Competitive Inhibition - Km unaffected, Vmax reduced

Uncompetitive reversible inhibition

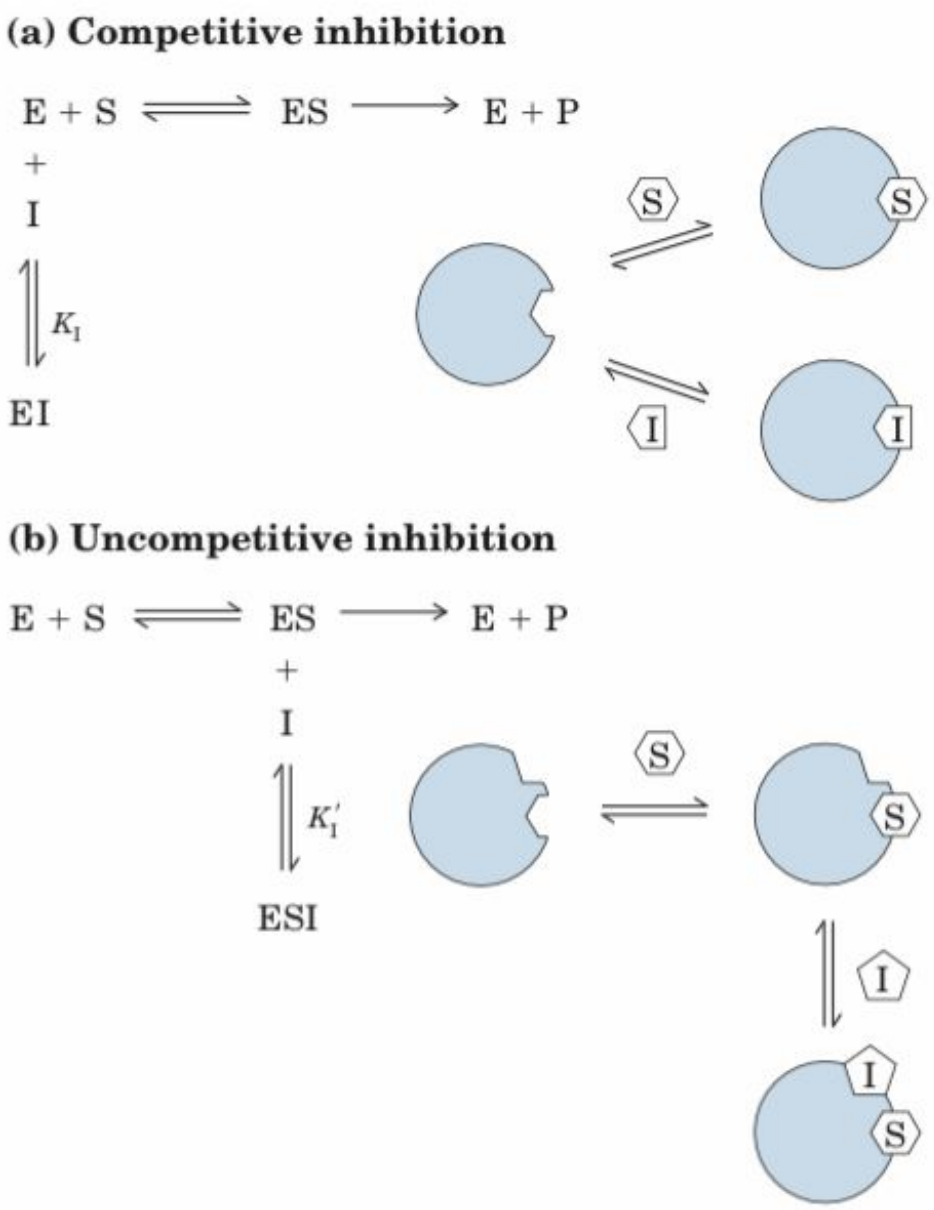
An uncompetitive reversible inhibitor can bind only to the ES complex and not to the free enzyme, so that inhibitor binding must be either at a site created by a conformational change induced by the binding of the substrate to the catalytic site or directly to the substrate molecule.

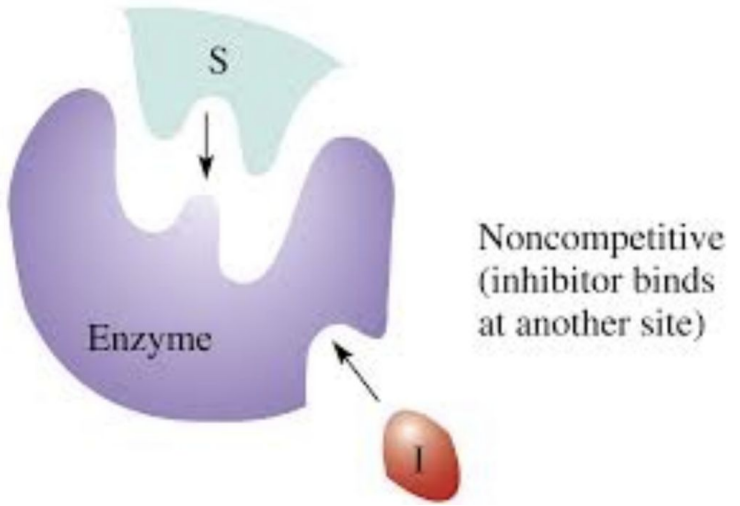
As with non-competitive inhibition, the effect cannot be overcome by increasing the substrate concentration.

Both Km and Vmax are reduced.



Uncompetitive Inhibition - Km reduced , Vmax reduced





Non Competitive Inhibition

Competitive & Uncompetitive Inhibition ref Lehninger, Stryer

End-product inhibition

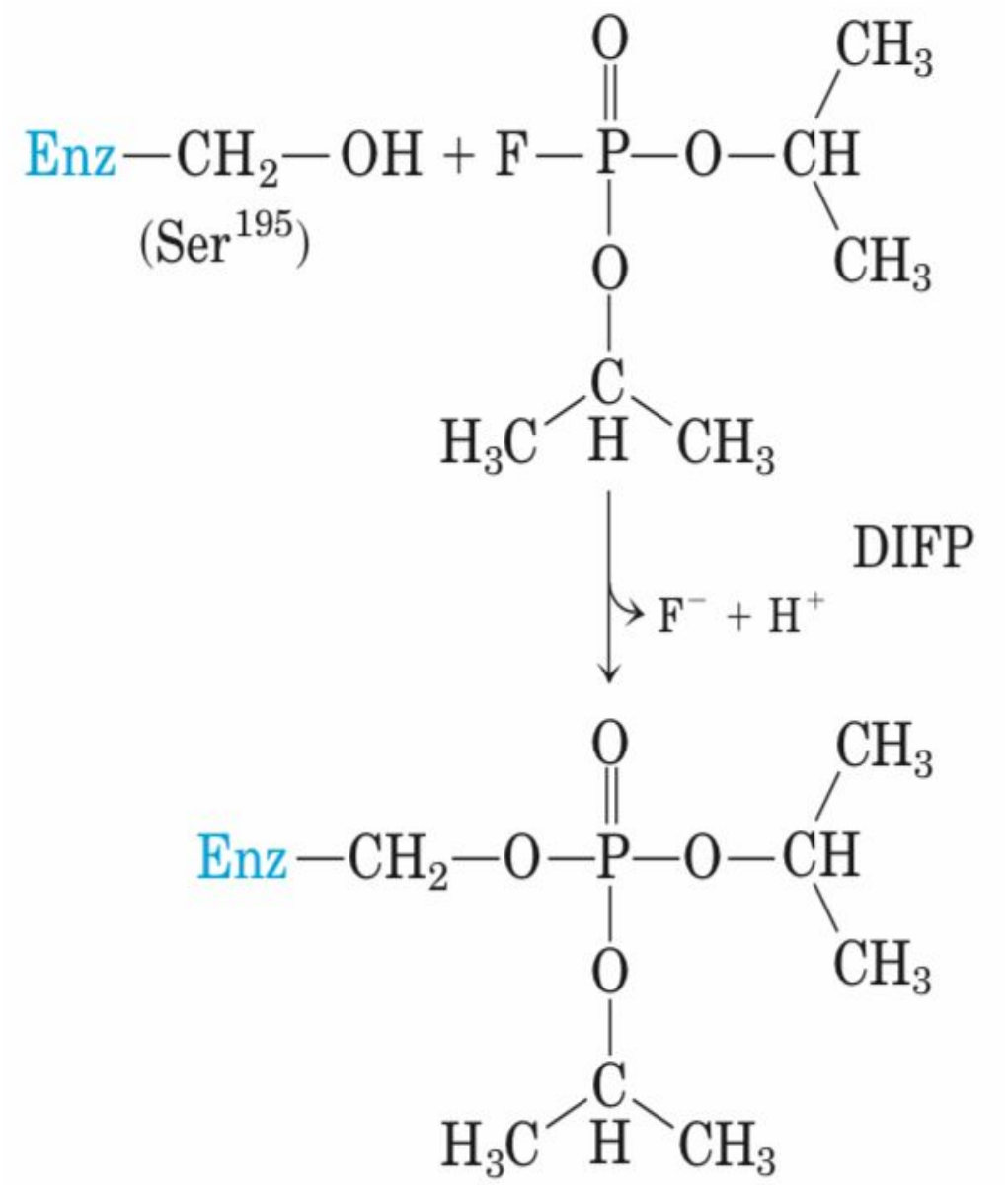
The first enzymes in an unbranched metabolic pathway are commonly regulated by end-product inhibition.

Here the final product of the pathway acts as an inhibitor of the first enzyme in the pathway thus switching off the whole pathway when the final product begins to accumulate.

Irreversible inhibition

Irreversible inhibitors, such as the organophosphorus and organomercury compounds, cyanide, carbon monoxide and hydrogen sulphide, react with a functional group, such as hydroxyl or sulfhydryl, or with a metal atom in the active site or a distinct allosteric site.

The organophosphorus compound, diisopropylphosphofluoridate (DIPF) , reacts with a serine group in the active site of esterases such as acetylcholinesterase and cause irreversible inhibition.



Irreversible inhibition

Reaction of chymotrypsin with DIFP irreversibly inhibits the enzyme.

This has led to the conclusion that **Ser^{195} is the key active-site Ser residue in chymotrypsin.**

Covalent inhibitors provide a means of mapping the enzyme's active site

Group-specific reagents (such as DIPF) react with specific side chains of amino acids.

DIPF modifies only 1 of the 28 serine residues in the proteolytic enzyme chymotrypsin and yet inhibits the enzyme, implying that this serine residue (Ser^{195}) is especially reactive and is indeed located at the active site.

DIPF also revealed a reactive serine residue in acetylcholinesterase, an enzyme important in the transmission of nerve impulses .

Thus, DIPF and similar compounds that bind and inactivate acetylcholinesterase are potent nerve gases.

Reference Books