Enzymes

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Definition

Enzymes are organic catalysts that accelerate biochemical reaction. They may undergo physical change during a reaction but revert to their initial stage at the end of the reaction.

History behind enzyme discovery

Biological catalysis was first recognized and described in the late 1700s, in studies on the digestion of meat by secretions of the stomach, and research continued in the 1800s with examinations of the conversion of starch to sugar by saliva and various plant extracts. In the 1850s, Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by "ferments." He postulated that these ferments were inseparable from the structure of living yeast cells; this view, called vitalism, prevailed for decades. Then in 1897 Eduard Buchner discovered that yeast extracts could ferment sugar to alcohol, proving that fermentation was promoted by molecules that continued to function when removed from cells. **Frederick W. Kühne called these molecules enzymes.** As vitalistic notions of life were disproved, the isolation of new enzymes and the investigation of their properties advanced the science of biochemistry. The isolation and crystallization of urease by James Sumner in 1926 provided a breakthrough in early enzyme studies.

Cofactors and Prosthetic groups

Enzymes, like other proteins, have molecular weights ranging from about 12,000 to more than 1 million. Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional chemical component called a **cofactor—either one or more inorganic ions, such as** Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+}

Some Inorganic Elements That Serve as Cofactors for Enzymes

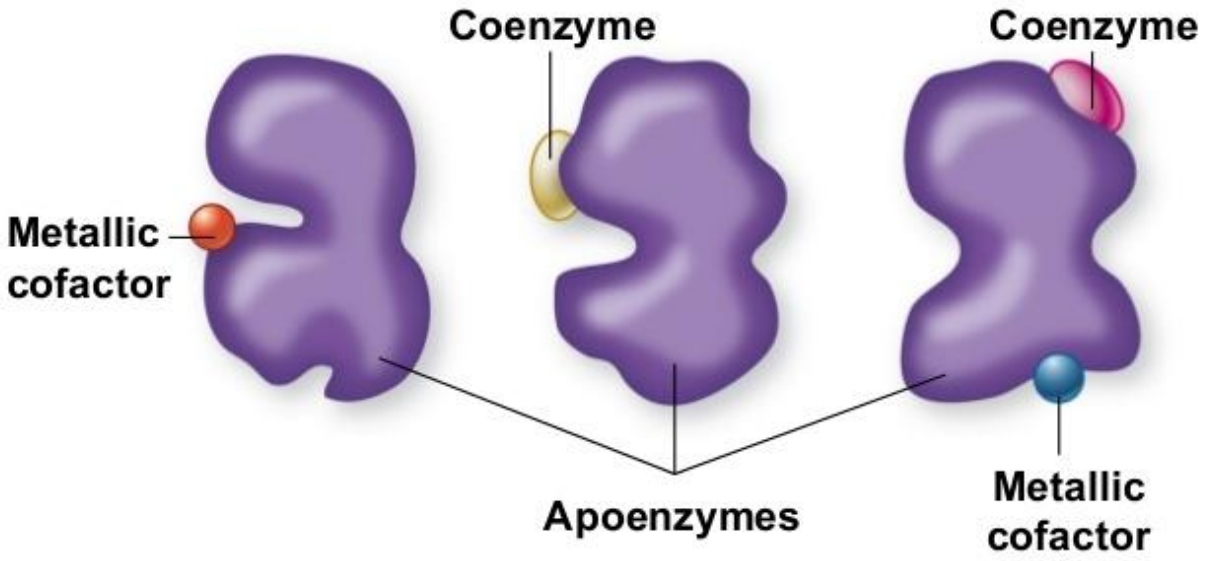
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

- or a complex organic or metalloorganic molecule called a **coenzyme**. Some enzymes require *both a coenzyme* and one or more metal ions for activity. A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a **prosthetic group.** A complete, catalytically active enzyme together with its bound coenzyme and or metal ions is called a **holoenzyme.** The protein part of such an enzyme is called the **apoenzyme or apoprotein.**

Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	*Examples of chemical groups transferred*	*Dietary precursor in mammals*
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Conjugated Enzyme Structure



International Classification of Enzymes

No.	*Class*	*Type of reaction catalyzed*
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

What exactly does an EC number designate?

- The first digit of the EC classification code denotes the general type of reaction catalyzed by the enzyme and ranges from one to six.
- The series of three numbers that follow this further define and narrow the details of the reaction type. The second and third numbers are the enzyme's sub-class and sub-sub-class, respectively, and describe the reaction with respect to the compound, group, bond or product involved in the reaction.
- The final number, or serial identifier, zeros in on specific metabolites and cofactors involved [3].
- **For example**, all type II restriction enzymes (**Type II site-specific deoxyribonuclease or Type II restriction enzyme)** (like those used in cloning) have the code EC 3.1.21.4, which breaks down as follows:
- **3**, denotes a hydrolase (see Table 1).
- **1**, indicates that it acts on ester bonds
- **21** tells us it is a endodeoxyribonuclease producing 5′-phosphomonoesters.
- **4** shows that it's a Type II site-specific deoxyribonuclease.

Examples

Oxidoreductase:

Pyruvic Acid + NADH ⟶ Lactic Acid + NAD^+

Transferase:

$C_6H_{12}O_6$ + ATP ⟶ G_6P +ADP

Lyase:

Malic Acid ⟶ Fumaric Acid + H_2O

Isomerase:

G_6P ⟶ F_6P

Ligase:

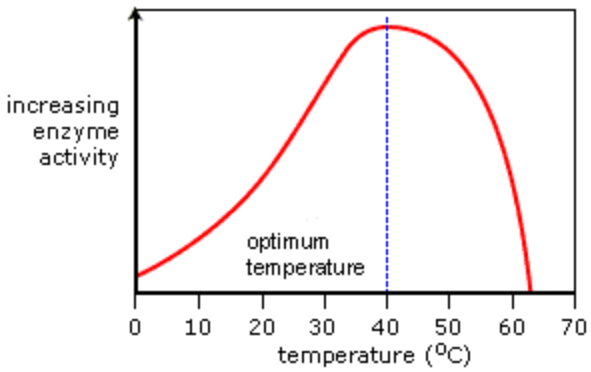
Succinic Acid ⟶ Succinyl Co-A

Properties of Enzyme

1. Specificity
    a. Relative Group Specificity e.g., Pepsin
    b. Absolute group Specificity e.g., Alcohol dehydrogenase
    c. Absolute Specificity e.g., Urease
    d. Stereo Specificity e.g., L-Arginase
2. Catalytic property
3. Reversibility e.g., 

4. Optimum temperature

Temperature Effects. Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. A **ten degree Centigrade** rise in temperature will increase the activity of most enzymes by 50 to 100%.



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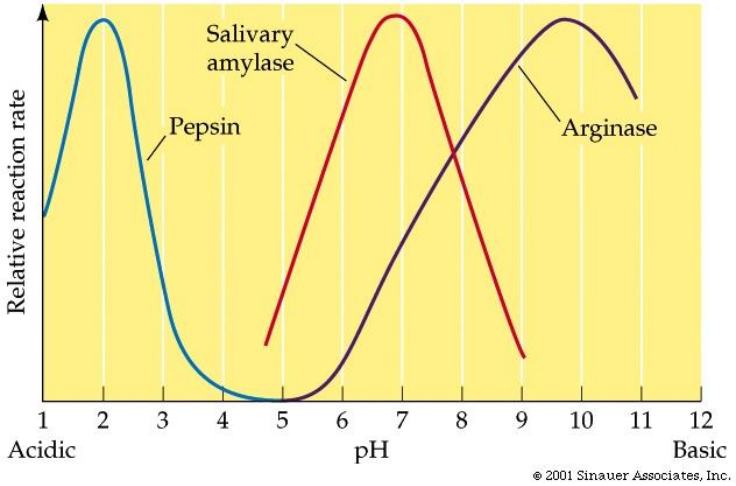
5.Optimum pH

Enzymes are affected by changes in **pH**. The most favorable **pH** value - the point where the **enzyme** is most active - is known as the optimum **pH**. This is graphically illustrated in Figure. Extremely high or low **pH** values generally result in complete loss of **activity** for most **enzymes**.



6. Nature

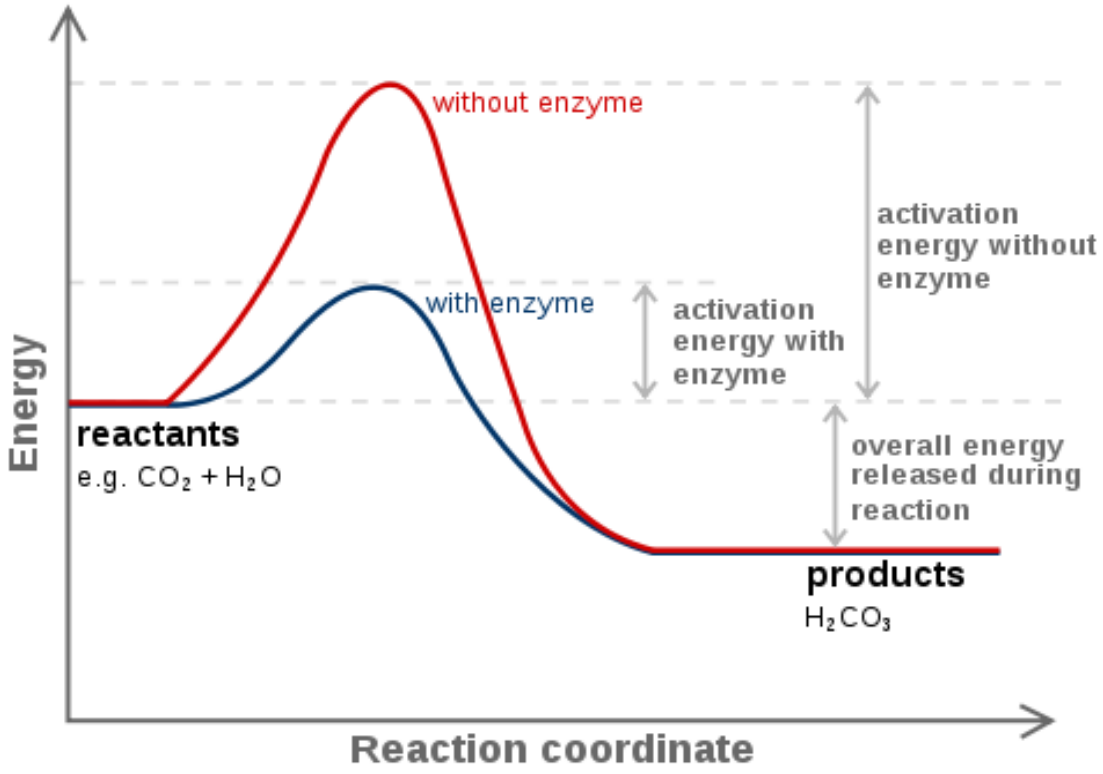
7. Solubility

Organic solvents commonly used are methanol, ethanol, and acetone.

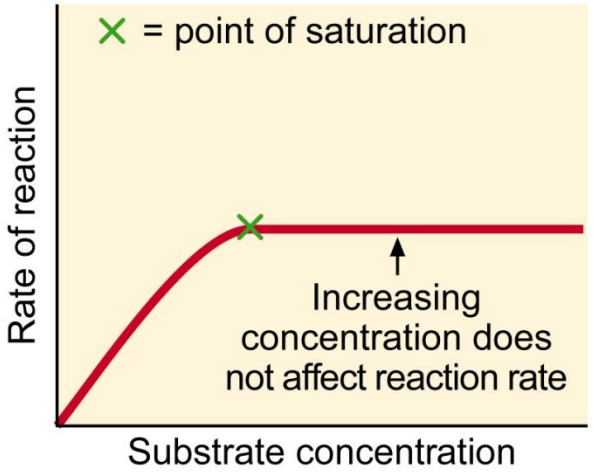
8. Colloidal property

Colloidal Nature. **Enzyme** molecules are of giant size. Their molecular weights range from 12,000 to over 1 million. Being **colloidal** in **nature**.

9. Activation of action of Enzyme



- $2KClO_3 + [MnO_2] = 2KCl + 3O_2\uparrow + [MnO_2]$



10.Substrate concentration

11. Product concentration

12. Presence of Inhibitor

- e.g., aspirin (acetylsalicylate) inhibits the enzyme that catalyzes the first step in the synthesis of prostaglandins

13. Presence of Inducer

- **enzyme inducers** include aminoglutethimide, barbiturates, carbamazepine, glutethimide, griseofulvin, phenytoin, primidone, rifabutin, rifampin, and troglitazone. Some drugs, such as ritonavir, may act as either an **enzyme inhibitor** or an **enzyme inducer**, depending on the situation.

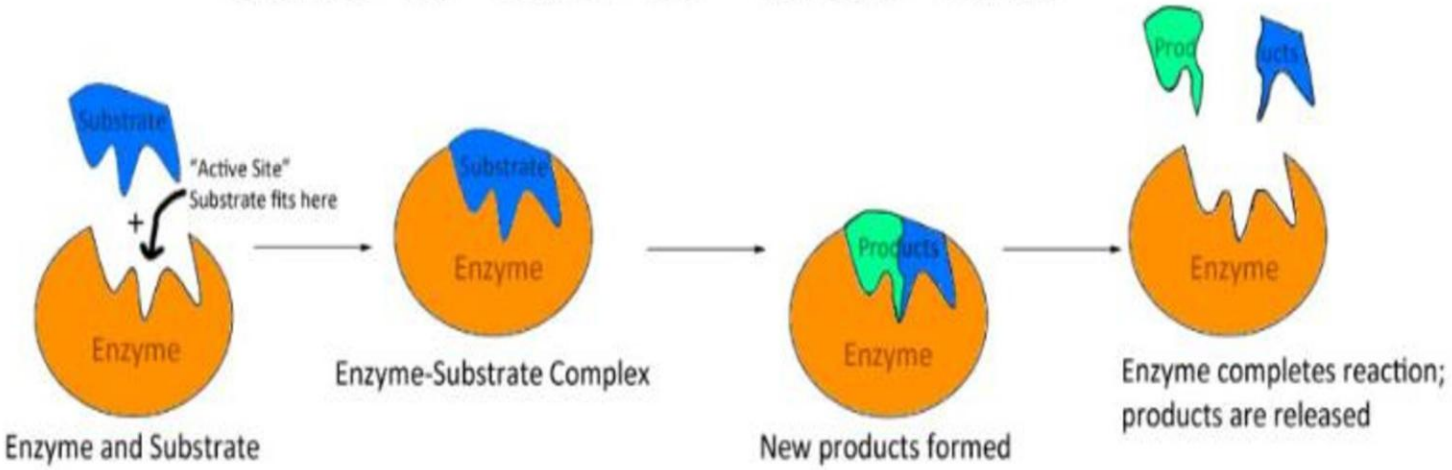
Mode of enzyme action

Interaction of substrate and enzymes

- Mechanism of enzyme action has been explained by two theories :---
- 1. **Lock and key theory** (rigid model of the catalytic site) and 2. **Induced fit theory**
- **1. Lock and key theory : Emil Fisher (1890)**
- According to this concept a structurally well defined catalytic site will accept only those substrate molecules which have a matching shape and will repel others that differ structurally

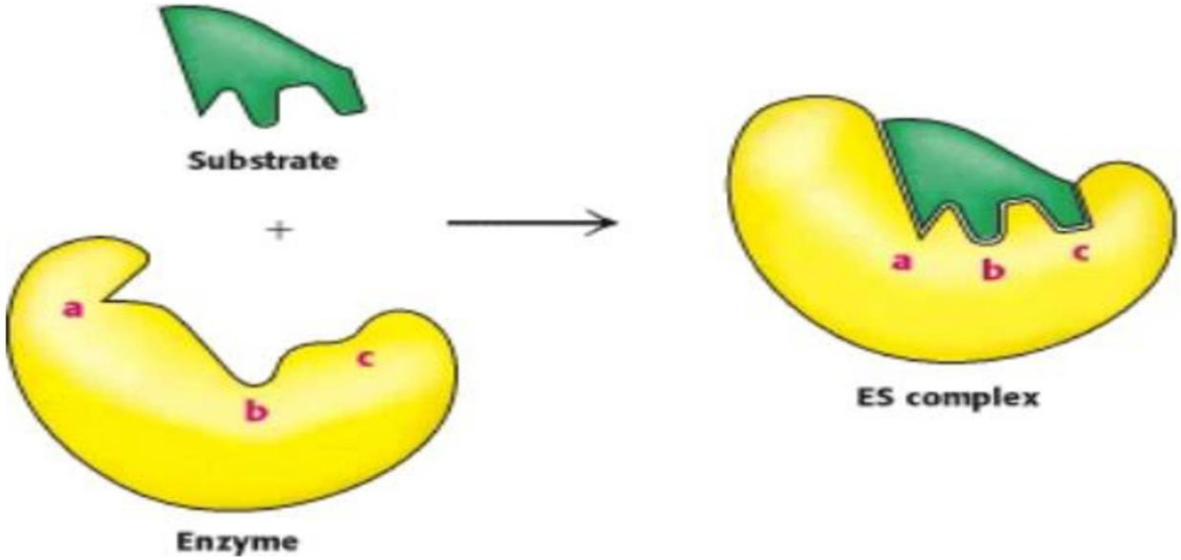
The Lock and Key Theory of Enzymes and Substrates

Substrate= "Key" Enzyme= "Lock" Active Site= "Key hole"



- **Induce fit theory** (flexible model of the catalytic site):--- **Daniel koshland** (1958)
- According to this model,the catalytic sites of some enzyme are not rigid. In these enzymes the shape of the catalytic site is modified by the binding of substrate.The catalytic site has a shape complementary to that of the substrate only after the substrate is bound.This process of dynamic recognition is called **induced fit.**

Induced fit model of enzyme action



Enzyme Kinetics

Enzyme Kinetics

- Enzyme Kinetics – Quantitative measurement of the rates of enzyme catalyzed reactions

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- The systematic study of factors that affect these rates

- Enzyme kinetics began in 1902 when Adrina Brown reported an investigation of the rate of hydrolysis of sucrose as catalyzed by the yeast enzyme inveratase.

- Brown demonstrated – when sucrose concentration is much higher than that of the enzyme, reaction rate becomes independent of sucrose concentration

- This idea was expanded into a general theory of enzyme action, particularly by Leonor Michaelis and Maud Menten in 1913. They postulated that the enzyme first combines reversibly with its substrate to form an enzyme-substrate complex in a relatively fast reversible step:

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES}$$

- The ES complex then breaks down in a slower second step to yield the free enzyme and the reaction product P:

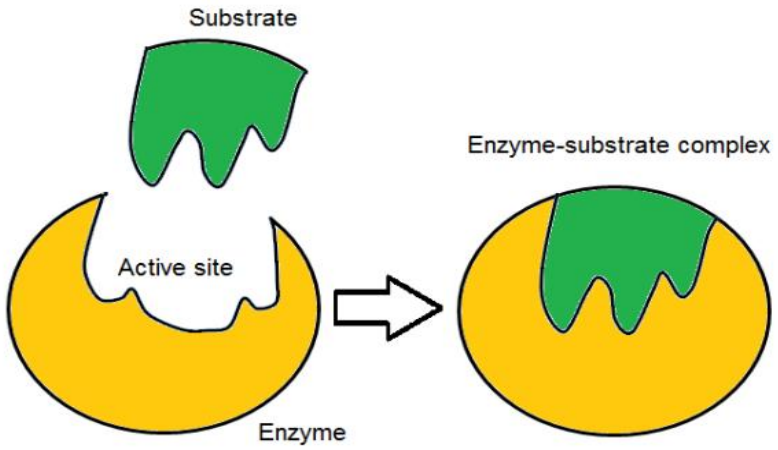
$$\mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

Enzyme Kinetics

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

- Here E, S, ES and P symbolize the enzyme, substrate, enzyme-substrate complex and products

- Because the slower second reaction must limit the rate of the overall reaction, the overall rate must be proportional to the concentration of the species that reacts in the second step, that is, ES.
- At any given instant in an enzyme-catalyzed reaction, the enzyme exists in two forms, the free or uncombined form E and the combined form ES.

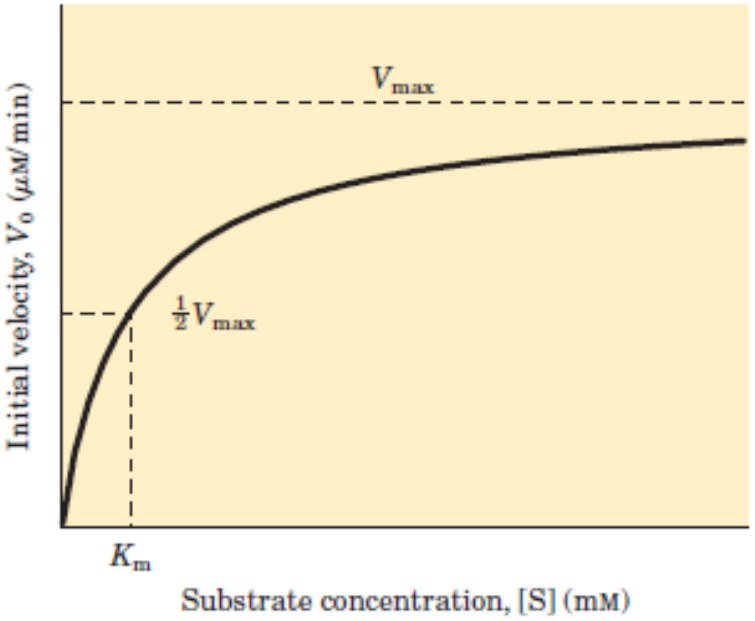


- At low [S], most of the enzyme is in the uncombined form E. Here, the rate is proportional to [S] because the equilibrium is pushed toward formation of more ES as [S] increases.

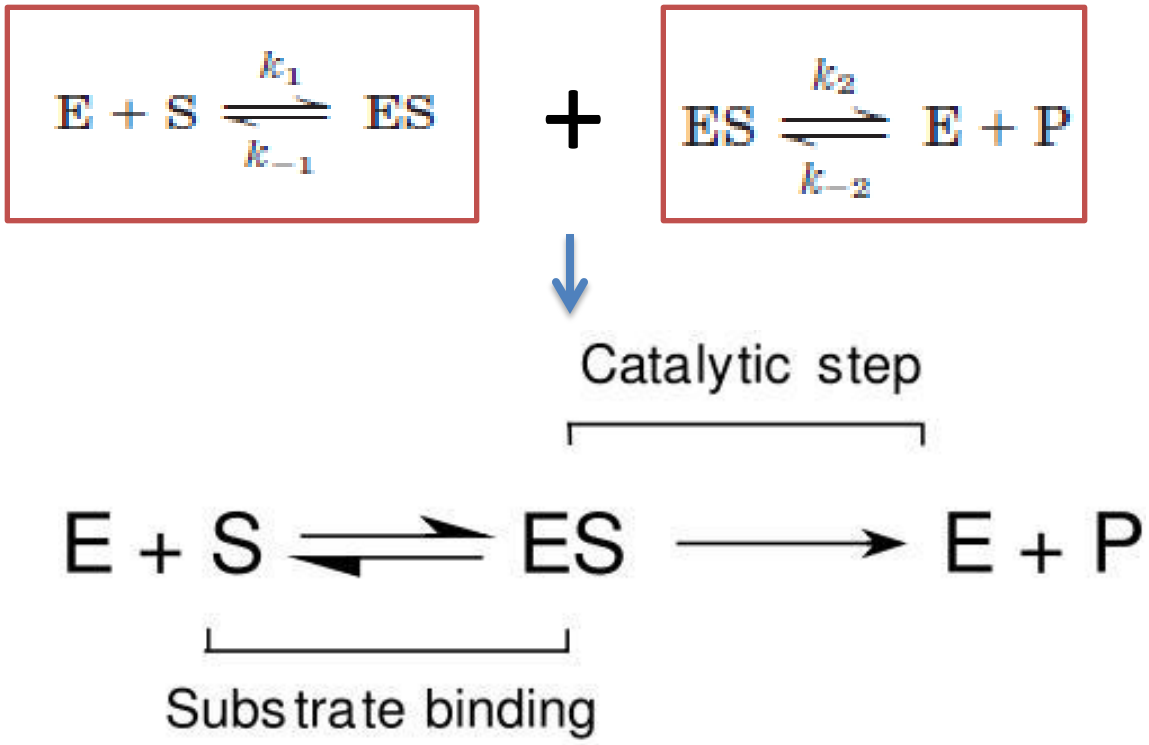
$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES}$$

- The maximum initial rate of the catalyzed reaction (*V max) is observed when virtually all the* enzyme is present as the ES complex and [E] is vanishingly small.
- Under these conditions, the enzyme is "saturated" with its substrate, so that further increases in [S] have no effect on rate.
- When the enzyme is first mixed with a large excess of substrate, there is an initial period, the **pre–steady state, during which the concentration of ES builds up.** This period is usually too short to be easily observed, lasting just microseconds.

The reaction quickly achieves a **steady state in which [ES]** (and the **concentrations** of any other intermediates) remains approximately constant over time. The concept of a steady state was introduced by G. E. Briggs and Haldane in 1925. The measured *V_0 generally reflects the steady state, even* though *V_0 is limited to the early part of the reaction*, and analysis of these initial rates is referred to as **steady-state kinetics.**



- Michaelis and Menten derived this equation starting from their basic hypothesis that the rate limiting step in enzymatic reactions is the breakdown of the ES complex to product and free enzyme.
- The derivation starts with the two basic steps of the formation and breakdown of ES



- V_0 is determined by the breakdown of ES to form product, which is determined by [ES]:

$$V_0 = k_2[\mathrm{ES}]$$

- *Step 1:* The 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:

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

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

Step 2: The initial rate of reaction reflects a steady state in which [ES] is constant—that is, the rate of formation of ES is equal to the rate of its breakdown. This is called the **steady-state assumption.**

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

Step 3: In a series of algebraic steps, we now solve the upper. First, the left side is multiplied out and the right side simplified to give as:

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

Adding the term k1[ES][S] to both sides of the equation and simplifying gives:

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

We then solve this equation for [ES]:

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

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

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

- The term (k2 k1)/k1 is defined as the **Michaelis constant, Km**. Substituting this Equation simplifies the expression to:

$$[\mathrm{ES}] = \frac{[\mathrm{E_t}][\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

- *Step 4:* We can now express V0 in terms of [ES]. Substituting the right side of upper equation for [ES] in equation $V_0 = k_2[\mathrm{ES}]$

$$V_0 = \frac{k_2[\mathrm{E_t}][\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

- This equation can be further simplified. Because the maximum velocity occurs when the enzyme is saturated (that is, with [ES] [Et]) *V max* can be defined as k2[Et].

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

Where,

V_0 is the initial velocity

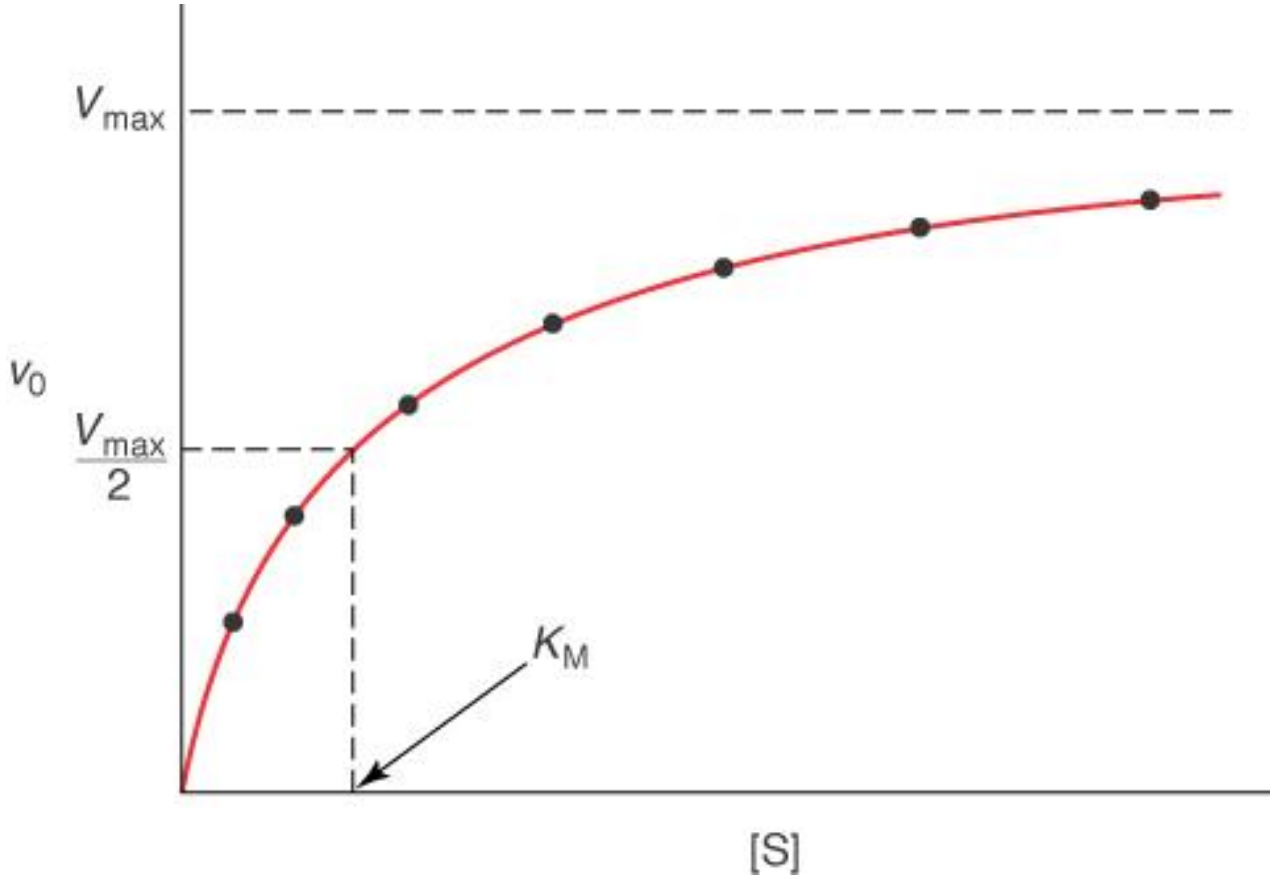
V_{max} is the maximum velocity

[S] is the substrate concentration

Km (Michaelis-Menten constant) is the substrate concentration at which the reaction velocity is the half of the maximum velocity.

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

Graphical determination of K_M



When [S] is equals to K_M

- An important numerical relationship emerges from the Michaelis-Menten equation in the special case **when V0 is exactly one-half *V max***. Then

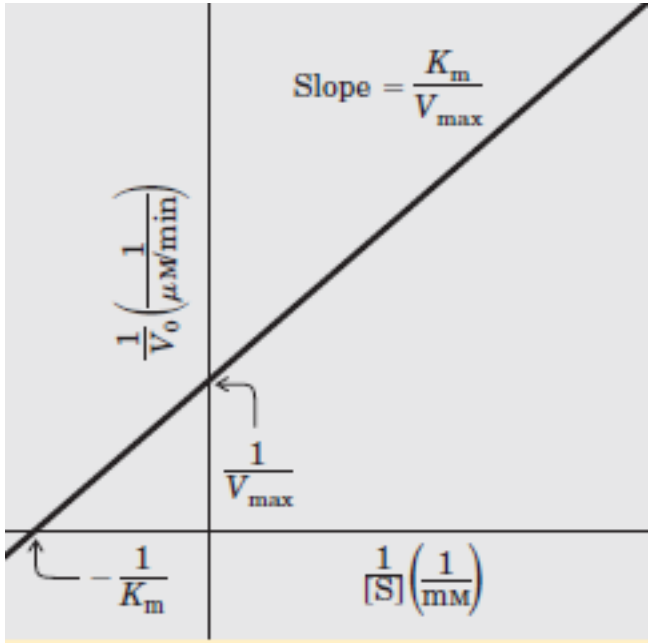
$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$$

On dividing by V_{max}, we obtain

$$\frac{1}{2} = \frac{[S]}{K_m + [S]}$$

Solving for K_m, we get $K_m + [S] = 2[S]$, or

$$K_m = [S], \text{ when } V_0 = \frac{1}{2}V_{max}$$



A double-reciprocal or Lineweaver-Burk plot.

- This is a very useful, practical definition of *Km: Km is* equivalent to the substrate concentration at which ***Vo is* one-half *V max.*** The Michaelis-Menten equation can be algebraically transformed into versions that are useful in the practical determination of *Km and Vmax*.

When [S] is much less than half-maximal velocity i.e., K_M

Addition of [S] to K_M will change its value very little [S] term can be removed from the denominator. Therefore, the equation is changed as follows:

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

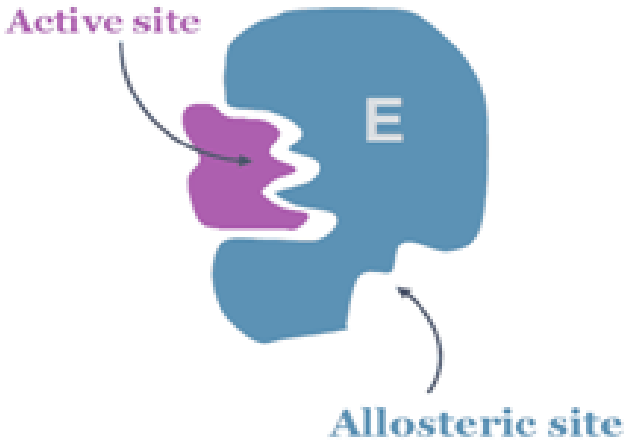
When [S] is much greater than K_M

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

Allosteric Enzyme

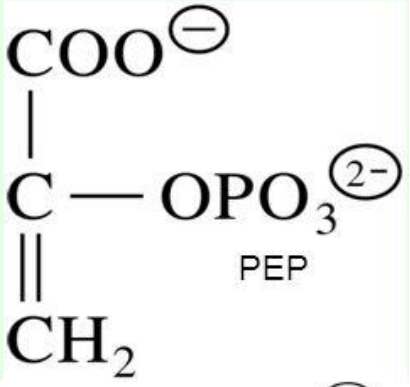
- The surface of enzyme other than the active site is called Allosteric site. Inhibition of enzyme activity by binding of an end product of reaction on the Allosteric site is called Allosteric inhibition. The enzyme inactivated by Allosteric inhibition is called Allosteric Enzyme.

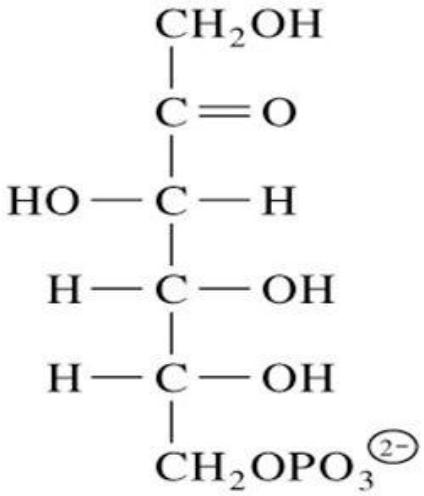
  - Allosteric means **"other site"**
  - These enzymes have **two receptor sites**
  - One site fits the substrate like other enzymes
  - The other site fits an inhibitor or activator molecule



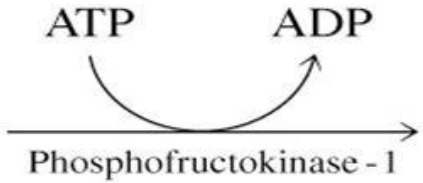
Example of allosteric enzyme - phosphofructokinase-1 (PFK-1)

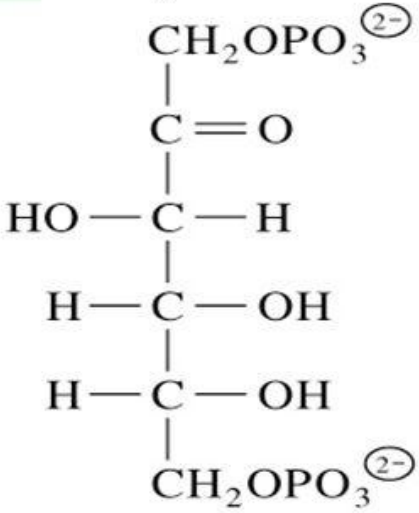
- PFK-1 catalyzes an early step in glycolysis
- Phosphoenol pyruvate (PEP), an intermediate near the end of the pathway is an allosteric inhibitor of PFK-1





Fructose 6-phosphate

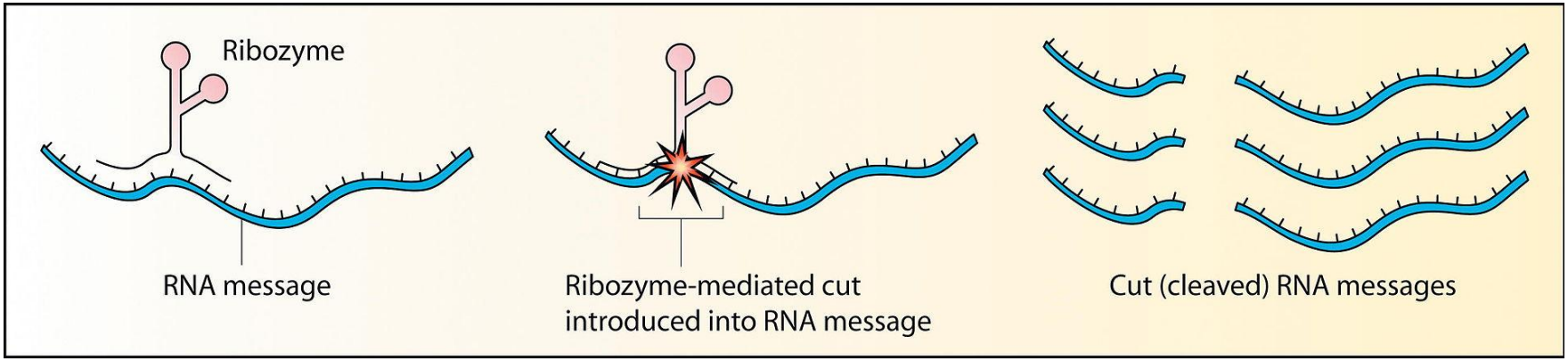




Fructose 1,6-*bis*phosphate

Ribozyme

Ribozymes (**ribo**nucleic acid en**zyme**s) are RNA molecules that are capable of catalyzing specific biochemical reactions, similar to the action of protein enzymes.



Inhibitors and modulators help control enzyme activity in cells

- **Irreversible Inhibitior:** Certain compounds interact with enzyme so tightly that their effect is irreversible. Eg., Di-isopropyl phospho fluoridate (DIPF) is an irreversible inhibitor of the enzyme chymotrypsin

- **Competitive Inhibitior :**In competitive inhibition, the inhibitor is a substance that directly competes with a substrate for binding to the enzyme's active site. E.g., Malonate is a competitive inhibitor of Succinate dehydroginase.

- **Mixed Inhibitior**: In mixed inhibition, the inhibitor binds to a site of the enzyme other than the active site and elicits a conformational change. As a result, the apparent V_{max} decreases and the apparent K_m may increase or decrease