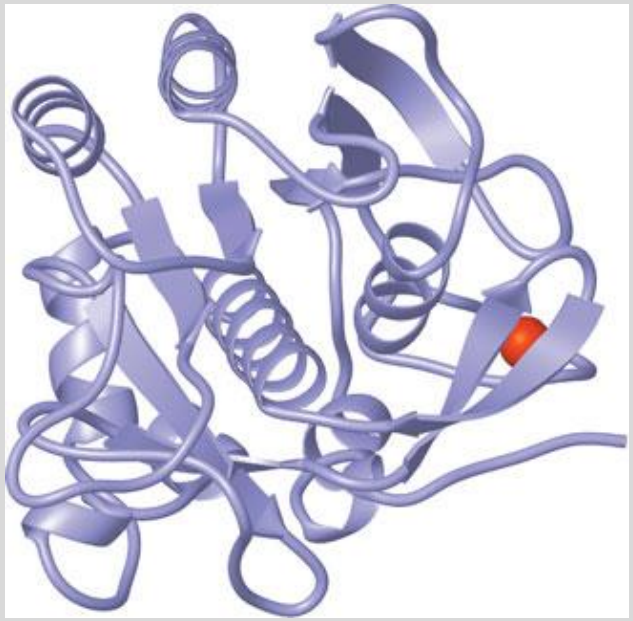
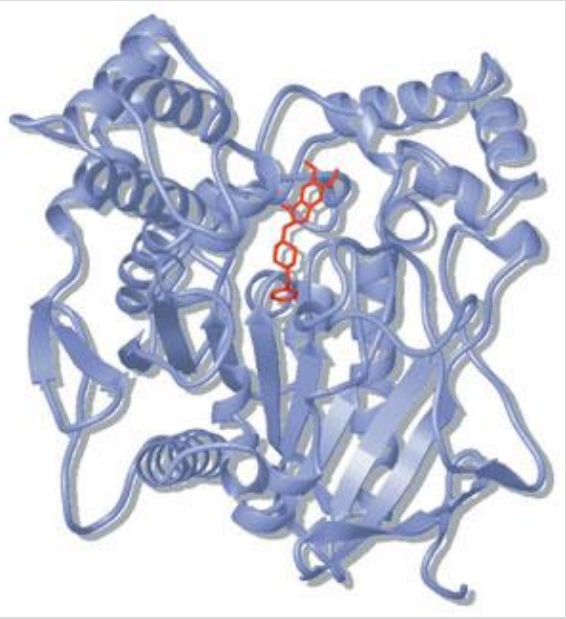
Al-Rasheed University College
Department of Medical Laboratory Technologies
Second class
Biochemistry

Lecture 1
Enzymes



By
Dr. Rusul H. Hamza



Enzymes are biological catalyst produced by living tissues. They are **proteins** (except a small group of RNA acting as ribozyme) that have the property of **accelerating specific chemical reactions** without being consumed in the process.

COFACTORS (COENZYME AND ACTIVATOR)

Some enzymes require an additional **nonprotein component** for its optimum activity. This additional component is called **cofactor**

These cofactors may be:

1. Organic compounds, called **coenzymes**

Examples of coenzyme:

FAD (Flavin Adenine Dinucleotide), NAD^+ (Nicotinamide Adenine Dinucleotide), Coenzyme A, TPP (Thiamine pyrophosphate).

COFACTORS (COENZYME AND ACTIVATOR)

2. Inorganic ions, called **activators**.

Examples of activators: copper, iron

Ferroxidase (ceruloplasmin) requires Copper

Catalase requires Iron as cofactor

- Enzymes without its cofactor is referred to as an **apoenzyme.**
- the complete catalytically active enzyme is called **holoenzyme.**

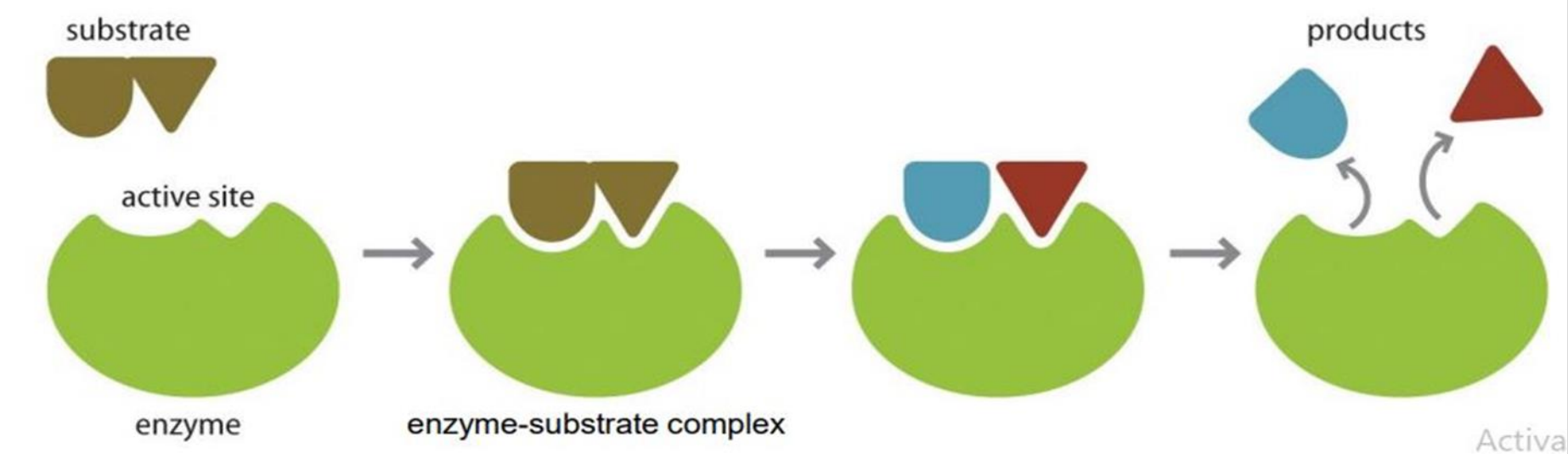
Apoenzyme + cofactor = holoenzyme

MECHANISM OF ENZYME ACTION

- **Substrate** is bound through multiple noncovalent interactions at the **active site** of the enzyme forming an **enzyme-substrate (ES)** complex which is subsequently converted to product and free enzyme.

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

The **active site** of an enzyme is the region that binds the substrate and which contains the specific amino acid residues.

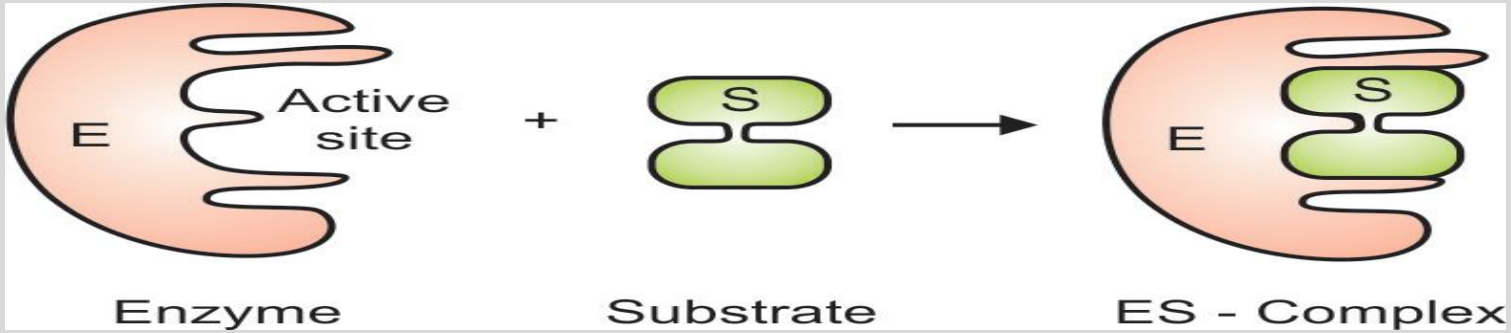


The models of Enzyme action (mechanism)

1. Lock and key model or rigid template model.
2. Induced fit model or hand-in-glove model.

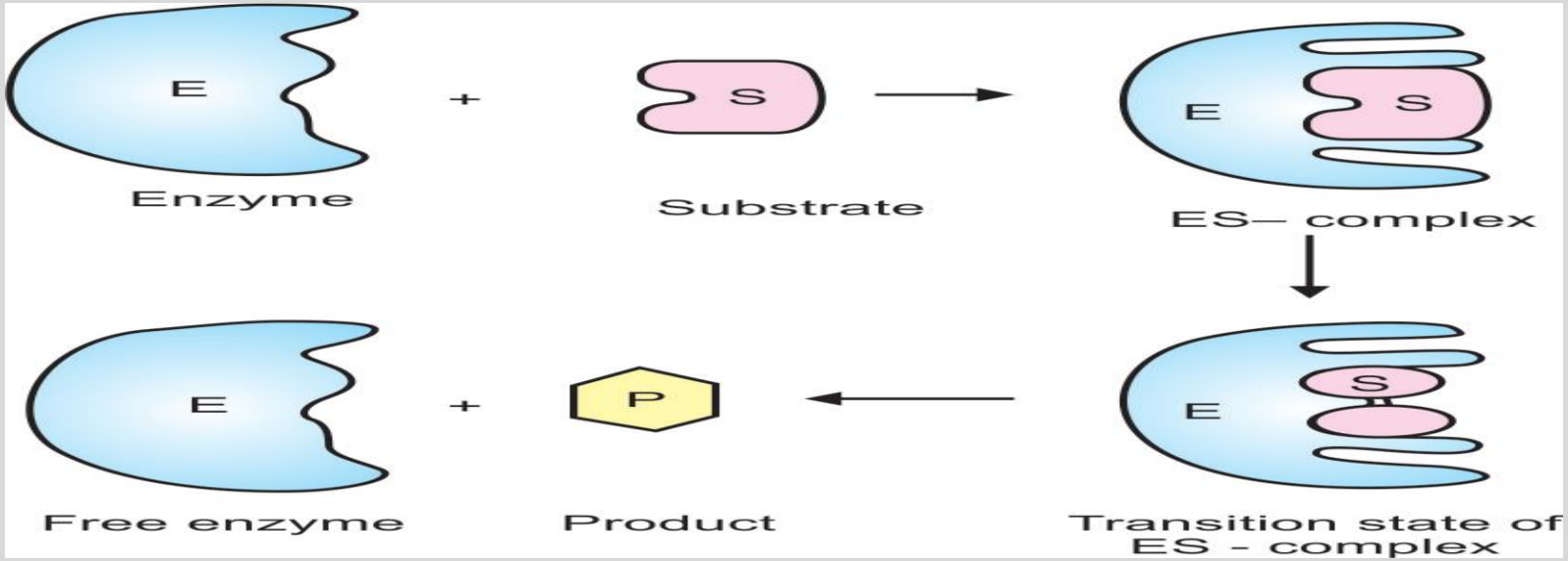
Lock and Key Model or Rigid Template Model

- This model is called lock and key model, because substrate fits into the active site in the same way that a key fits into a lock.
- The active site of the enzyme is complementary in shape to that of substrate



Induced Fit Model or Hand-in-glove Model

- **enzymes are flexible and shapes of the active site can be modified by the binding of the substrate**.
- the substrate induces a conformational change in the enzyme, in the same manner in which placing a hand (substrate) into a glove (enzyme) induces changes in the glove's shape (**hand-in glove model**).



Enzymes: nomenclature

Enzymes are commonly given names derived from the reaction that they catalyze and/or the compound or type of compound on which they act.

For example,

- lactate dehydrogenase speeds up the removal of hydrogen from Lactate.

As can be seen from these examples, the names of most enzymes end in "-**ase**."

ENZYME CLASSIFICATION

- The classification of enzyme by International Union of Biochemistry (IUB).

- each enzyme is characterized by a code number called :

  enzyme code number or **'EC' number,** consisting of four digits.

Example: lactate dehydrogenase (**EC 1.1.1.27**)

According to the IUB system, **enzymes are classified into six major classes** (according to the type of reaction they catalyze) as follows:

ENZYME CLASSIFICATION

EC-1 Oxidoreductases :

catalyze oxidation–reduction reactions.

Examples:-

Dehydrogenases

Reductases

Oxidases

EC-2 Transferases

catalyze the transfer of a group such as, amino, carboxyl from one molecule to another.

Examples:-

Amino transferase

Kinase

Transcarboxylase

EC-3 Hydrolases

catalyze the cleavage of C-O, C-N, C-C and some other bonds with the addition of water.

Examples:-

Acid phosphatase

All digestive enzymes like α-amylase, pepsin, trypsin, chymotrypsin, etc

EC-4 Lyases

catalyze the cleavage of C-O, C-C and C-N bonds giving rise to compound with double bonds or by the addition of group to a double bond.

Examples:-

Aldolase

Carbonic anhydrase

EC-5 Isomerases

catalyze intramolecular structural rearrangement in a molecule.

Examples:-

Triphosphate isomerase

Phosphohexose isomerase

EC-6 Ligases (Synthetases)

Ligases catalyze the joining of two molecules coupled with the hydrolysis of ATP.

Examples:-

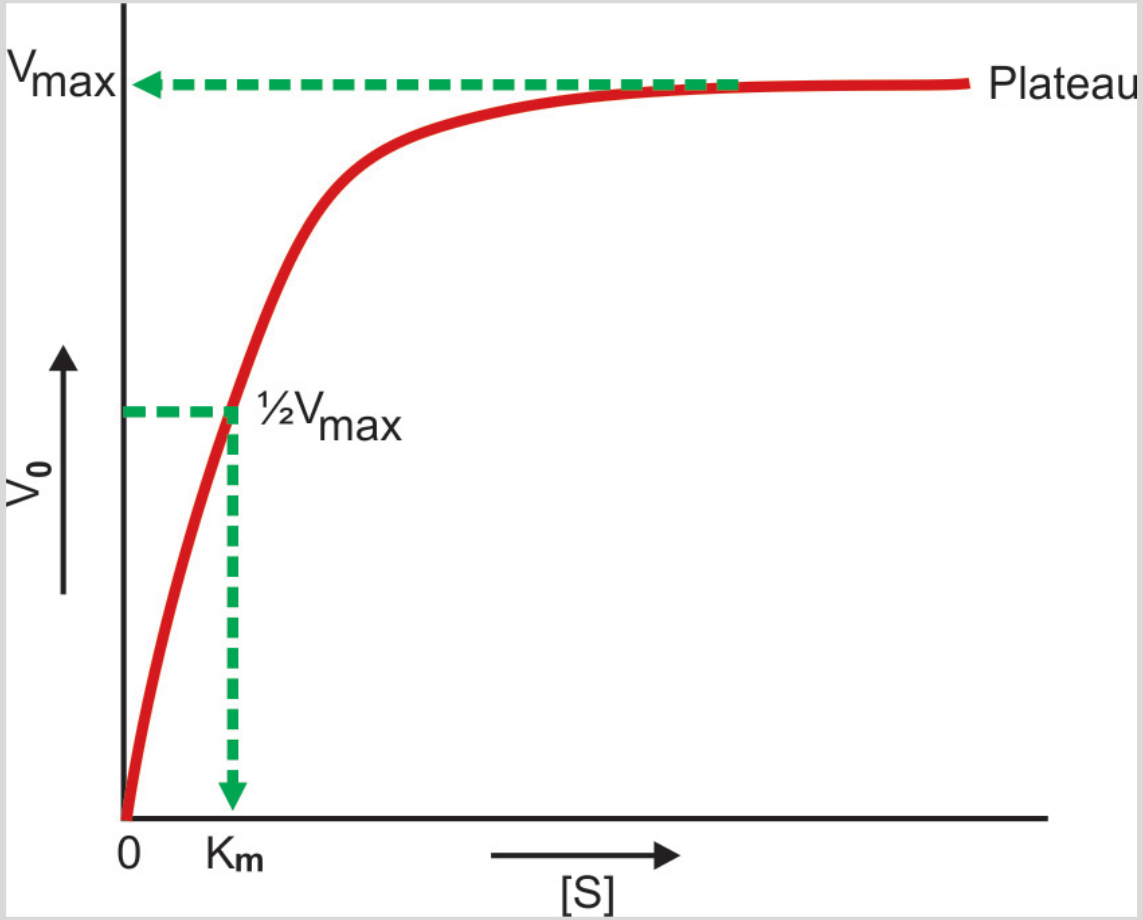
Glutamine synthetase

Pyruvate carboxylase

DNA ligases

Michaelis-Menten Equation

The **Michaelis-Menten equation** describes how reaction velocity varies with substrate concentration.



'**Michaelis-Menten equation**' :

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

V_0 = initial reaction velocity
Vmax = maximum velocity
Km = Michaelis-Menten constant
[S] = Substrate concentration

Km, the Michaelis constant is equal to the substrate concentration at which the reaction rate is half of its maximal value.

It is a measure of the **affinity of the enzyme for its substrate**:

- a high Km indicates weak binding
- a low Km indicates strong binding

Line weaver-Burk Plot

- A **more accurate** method of determining values for V_{max} and K_m
- This equation is obtained by taking the reciprocal of the Michaelis-Menten equation.

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

- slope = K_m/V_{max}.
- intersects the y-axis= $1/V_{max}$.
- intersects the x-axis = $-1/K_m$.

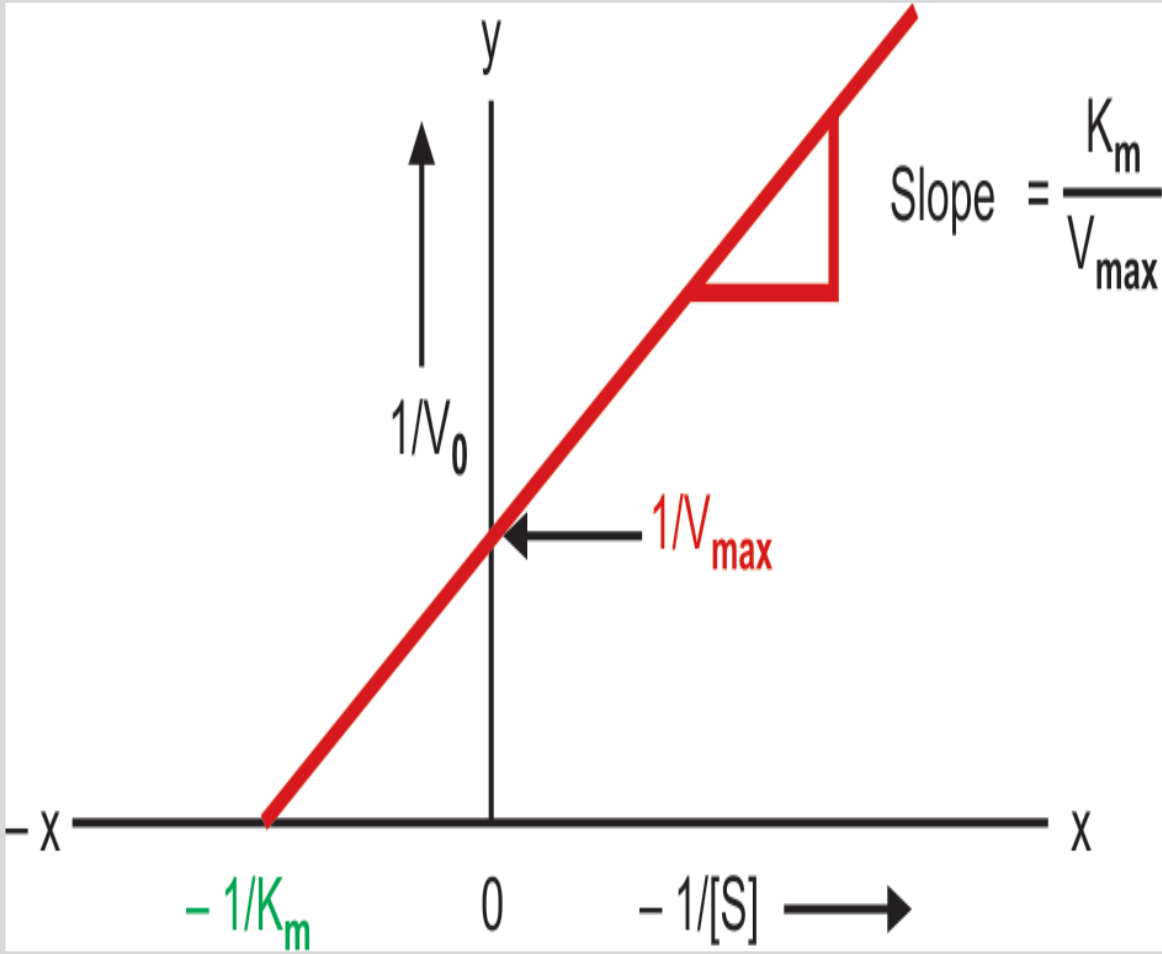


Figure : Lineweaver-Burk plot

Factors affecting enzyme activity

- Substrate concentration
- Enzyme concentration
- pH
- Temperature
- Product concentration
- Activators and coenzymes
- Time
- Physical agents
- Inhibitors

Effect of Substrate Concentration

the velocity of the reaction increases as the concentration of the substrate is increased.

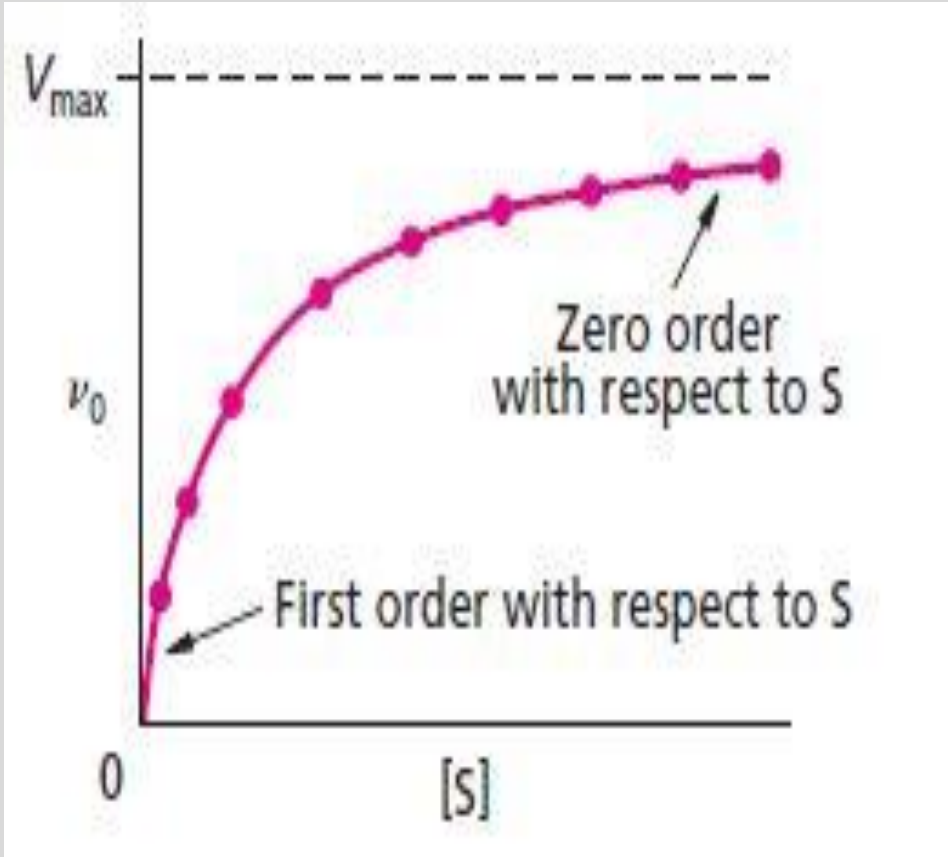
At first, this relationship is almost linear but later, the reaction curve becomes hyperbolic in shape

i. Low substrate concentrations, V_0 increases linearly with an increase in [S], a condition known as **First order kinetics**.

ii. Higher substrate concentrations, V_0 increases by smaller amounts in response to increase in [S].

iii. Finally, only small increase in V_0 with increasing [S], a condition known as **zero order kinetics** and a plateau is called **maximum velocity, V_{max}**

(all the free enzymes will have been converted into ES form so that any further increase in substrate concentration has no effect on the rate and the reaction achieves a steady state)



Effect of pH

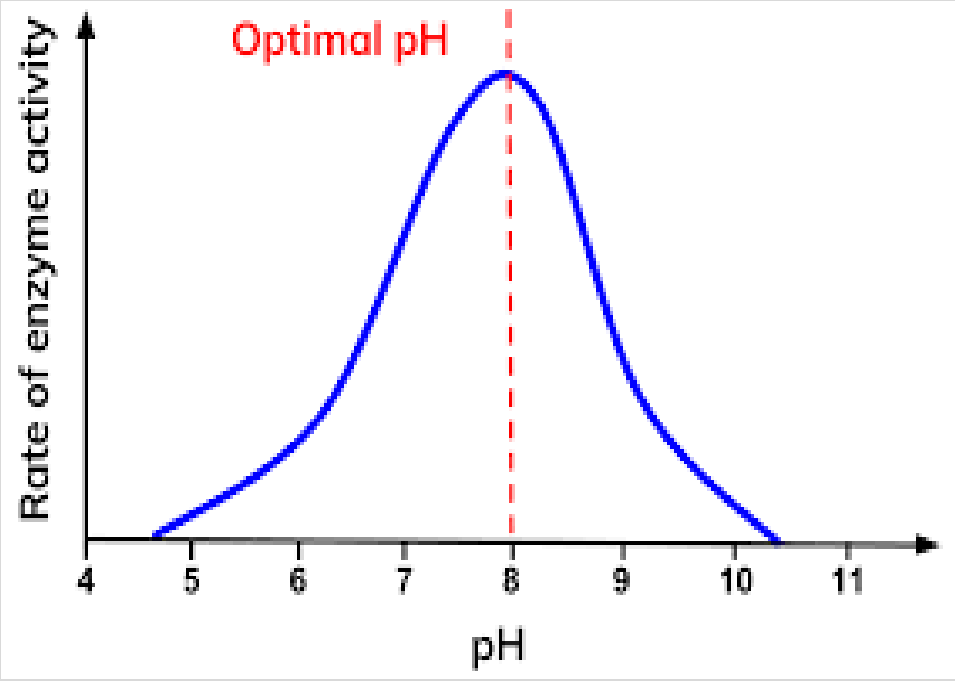
Each enzyme has an **optimum pH**, a pH at which the enzyme activity is maximum. Below or above this pH, enzyme activity is decreased.

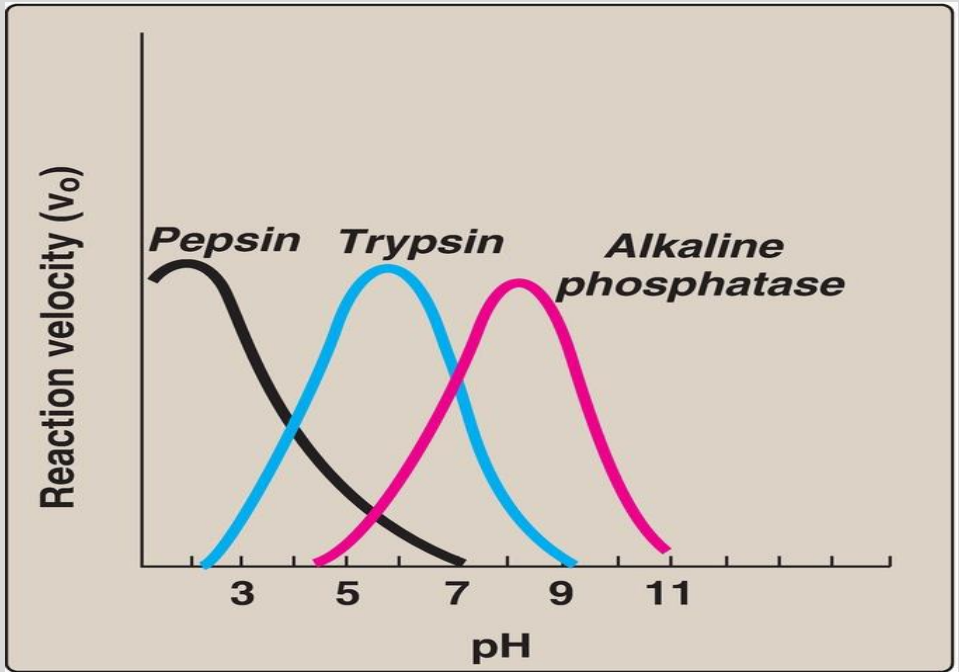
Changes in pH can alter the following:

Ionization state of the active site of the enzyme, the ionization state of the substrate, denatures the enzyme protein.

Pepsin = 1.2

Trypsin = 8.0





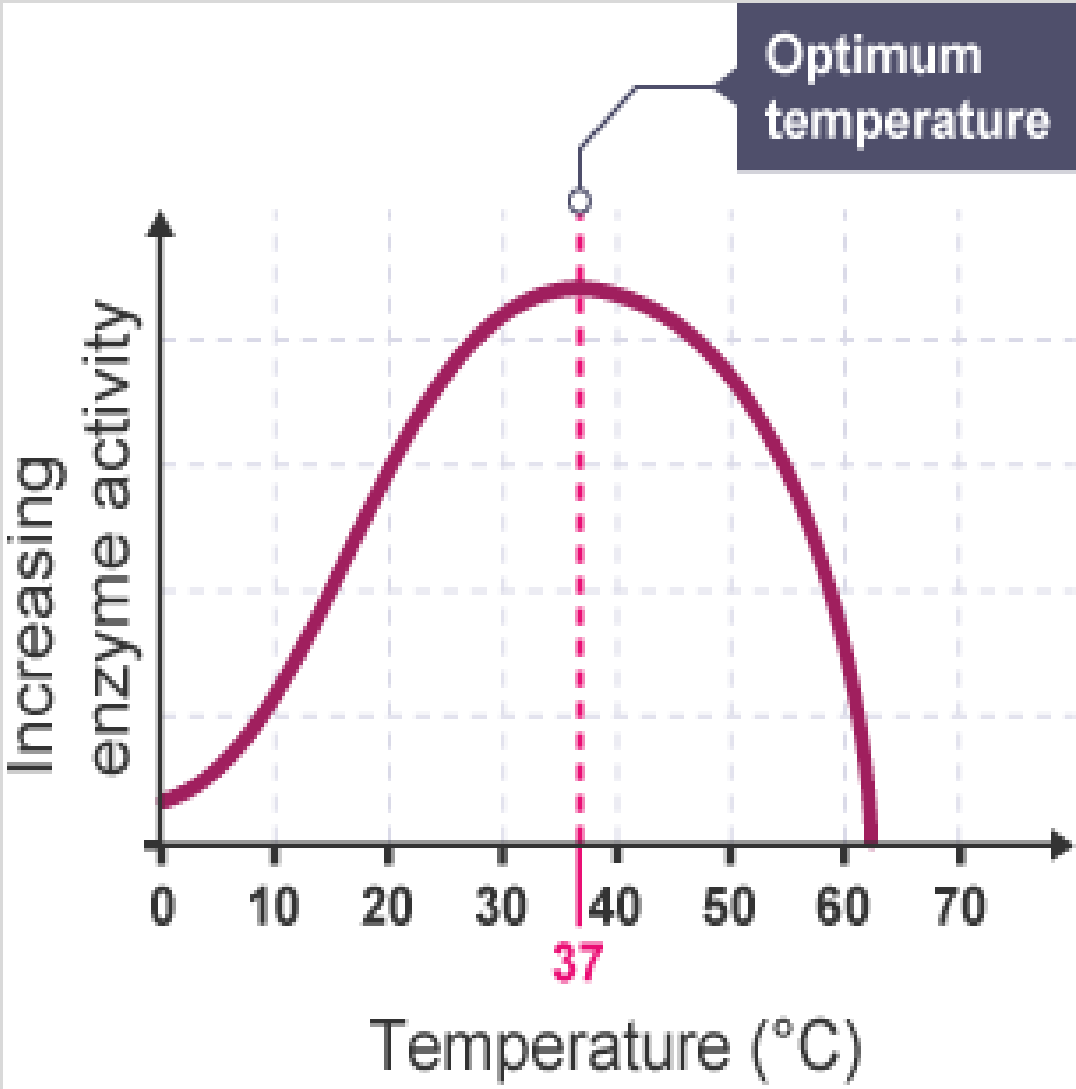
Effect of Temperature

- **increase** in reaction rate with **increasing** temperature only within a relatively low temperature range.
- Each enzyme shows the highest activity at a particular temperature called **optimum temperature**.
- Further elevation of the temperature

results in a decrease in reaction velocity due to denaturation of the enzyme protein.

✓The **optimum temperature** for most **human enzymes** is between **35°C and 40°C (37°C)**. Human enzymes start to denature at temperatures above 40°C,

✓Thermophilic bacteria found in hot springs have optimum temperatures of 70°C.



References

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I wish you a happiness success and good luck

Dr. Rusul H. Hamza

▼ Maud Menten (1879–1960).