Enzymes (continued)

Factors influencing enzyme activity

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Factors influencing enzyme activity:

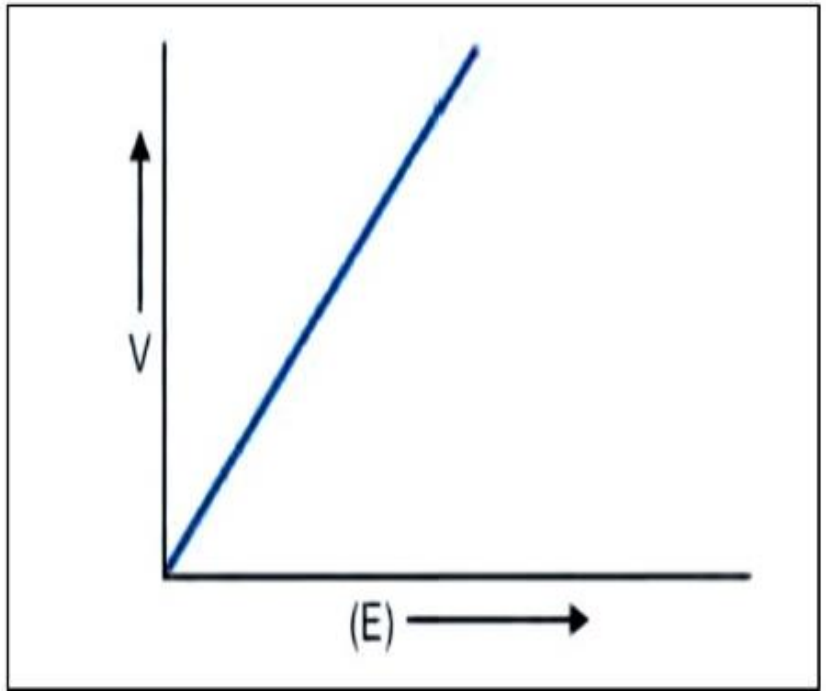
The various factors which affect enzyme activity are:

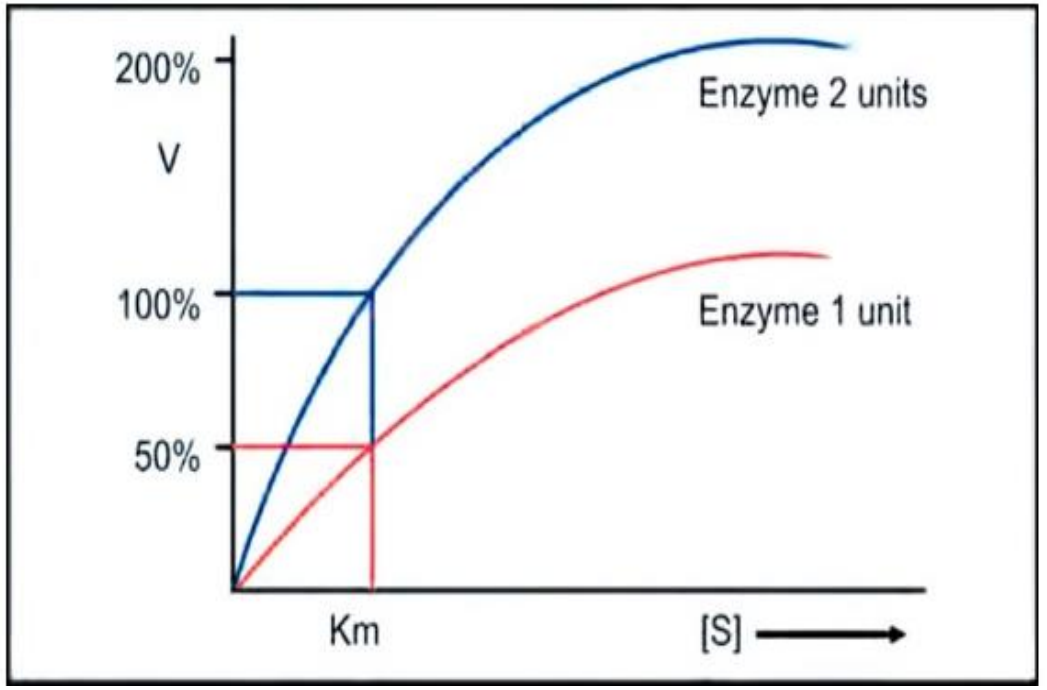
1. Enzyme concentration.
2. Substrate concentration.
3. Product concentration.
4. Temperature.
5. Hydrogen ion concentration (pH).
6. Allosteric regulation.
7. Covalent modification.
8. Presence of activators.
9. Presence of inhibitors.

Factors influencing enzyme activity:

1. Enzyme concentration:

The rate of a reaction or velocity (V) is directly proportional to the enzyme concentration, when sufficient substrate is present. Velocity of reaction is increased proportionately with the concentration of enzyme, provided substrate concentration is unlimited.





Factors influencing enzyme activity:

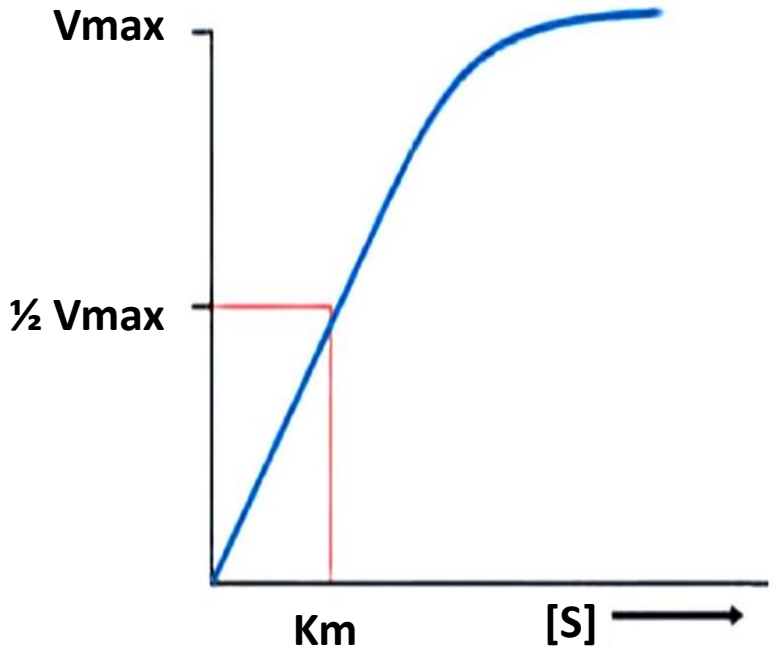
2. Substrate concentration:

The velocity of the enzymatic reaction is directly proportional to the substrate concentration. This is true up to a point when a further increase in the substrate concentration is not accompanied by increase in the reaction velocity. At this point, the enzyme concentration is the limiting factor.

The maximum velocity (Vmax) is the point when all enzyme molecules are in the form of E-S complex and the maximal rate of the reaction is reached. The substrate concentration at ½ Vmax is constant for each enzyme and is called ***Michaelis constant (Km)***.

Definition of Km:

i. Km value is substrate concentration at half-maximal velocity.
ii. It denotes that 50% of enzyme molecules are bound with substrate molecules at that particular substrate concentration.
iii. Km is independent of enzyme concentration.
iv. Km is the Signature of the Enzyme. It is the characteristic feature of a particular enzyme for a specific substrate.
v. Km denotes the affinity of enzyme for substrate. The lesser the numerical value of Km, the more affinity of enzyme for substrate.



Factors influencing enzyme activity:

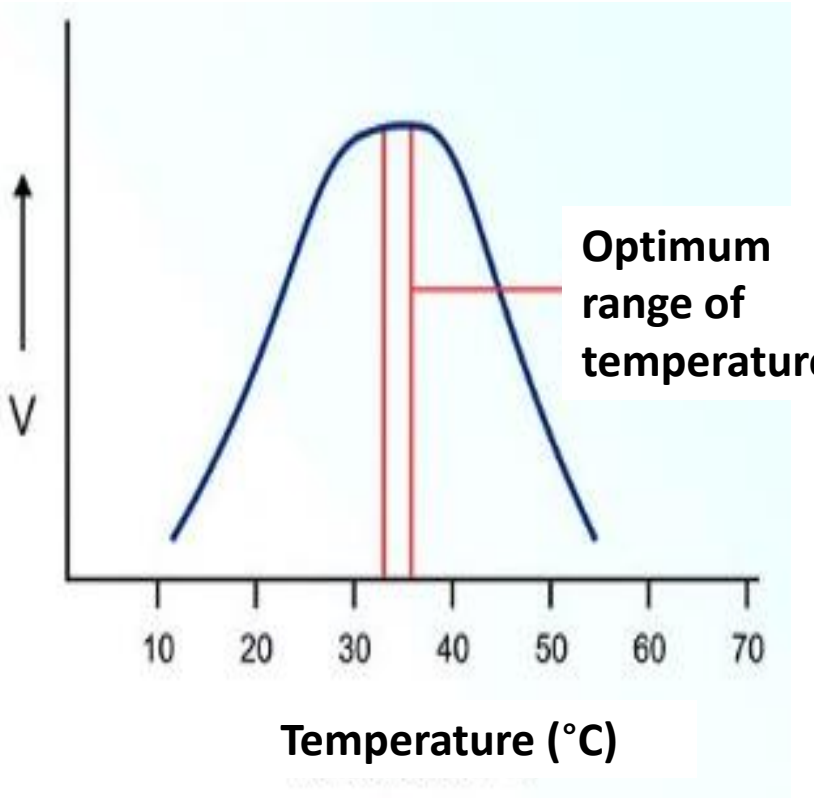
3. Concentration of products:

In a reversible reaction, S↔P, when equilibrium is reached, (as per the law of mass action) the reaction rate is slowed down. So when product concentration is increased, the reaction is slowed, stopped or even reversed.

Factors influencing enzyme activity:

4. Effect of temeparture:

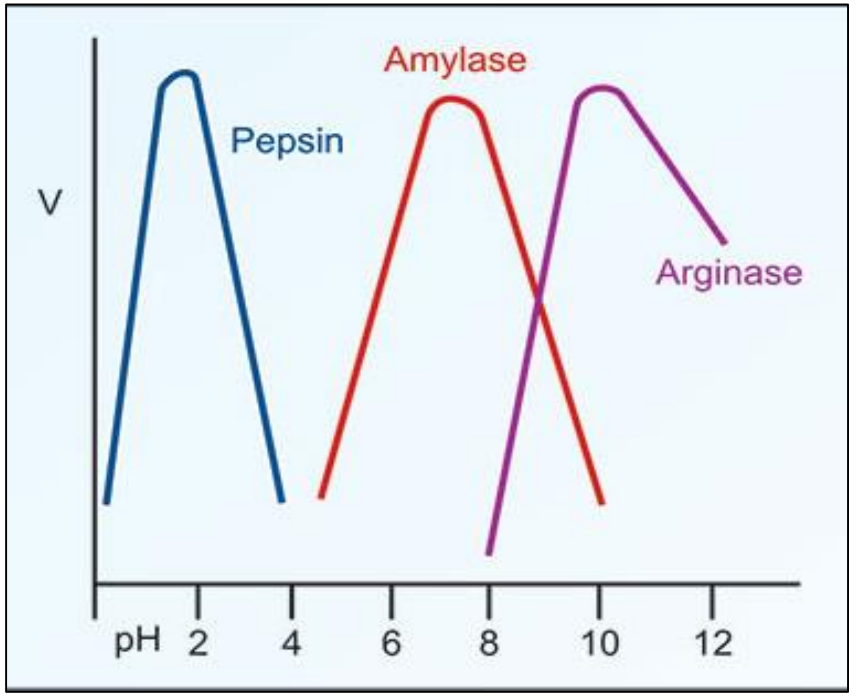
- Velocity of enzyme reaction increases when temperature of the medium is increased; reaches a maximum and then falls (Bell shaped curve).
- The temperature at which maximum amount of the substrate is converted to product per unit time is the optimum temperature.
- When temperature is > 50°C, heat denaturation and consequent loss of tertiary structure of protein occurs. So activity of the enzyme is decreased. Most human enzymes have the optimum temperature around 37°C.



Factors influencing enzyme activity:

5. Effect of pH:

- Each enzyme has an optimum pH, on both sides of which the velocity will be drastically reduced.
- The pH decides the charge on the amino acid residues at the active site. The net charge on the enzyme protein would influence substrate binding and catalytic activity.
- Usually enzymes have the optimum pH between 6 and 8. Some important exceptions are pepsin (pH 1-2); alkaline phosphatase (pH 9-10) and acid phosphatase (4-5).



Factors influencing enzyme activity:

6. Allosteric regulation:

Allosteric enzyme has one catalytic site where the substrate binds and another separate allosteric site where the modifier binds.

- The binding of the regulatory molecule can either enhance the activity of the enzyme **(allosteric activation),** or inhibit the activity of the enzyme **(allosteric inhibition).**
- The binding of substrate to one of the subunits of the enzyme may enhance
- substrate binding by other subunits **(Positive co-operativity).**
- If the binding of substrate to one of the subunits decreases the avidity of substrate binding by other sites **(negative co-operativity).**

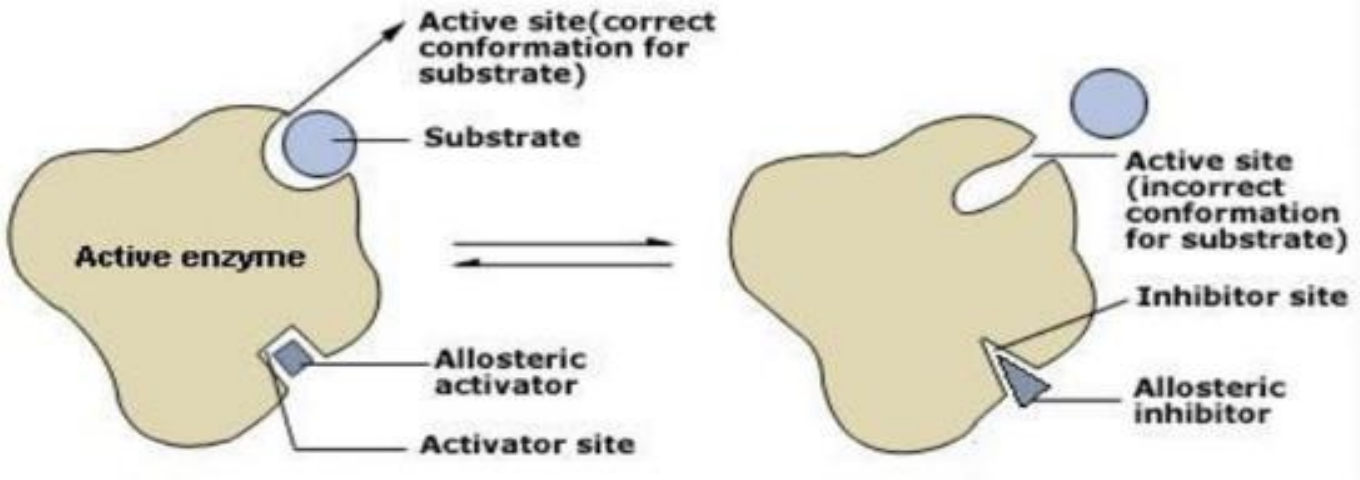
Factors influencing enzyme activity:

6. Allosteric regulation:

Body uses allosteric enzymes for regulating metabolic pathways. Such a regulatory enzyme in a particular pathway is called the key enzyme or rate limiting enzyme.

$$\text{Succinyl CoA + glycine} \xrightarrow{\textit{ALA synthase}} \text{delta-amino-levulinic acid (ALA)}$$

This is the first step in heme biosynthesis. The end product, heme will allosterically inhibit the ALA synthase. This enzyme is the key enzyme of heme synthesis.



Factors influencing enzyme activity:

7. Covalent Modification:

- The enzyme activity may be ↑ or ↓ by covalent modification. It means, either addition of a group to the enzyme protein by a covalent bond; or removal of a group by cleaving a covalent bond.
- **Zymogen activation by partial proteolysis** is an example of covalent activation. Addition or removal of a particular group brings about covalent modification of enzyme. This is a reversible reaction.
- Commonest type of covalent modification is the reversible protein **phosphorylation** (phosphate group may be attached to serine, threonine or tyrosine residues). **Phosphorylation activates glycogen phosphorylase, citrate lyase, phosphorylase kinase, HMGCoA reductase kinase. Phosphorylation is the basis of mechanism of action of insulin.**
- Dephosphorylation activates acetyl CoA carboxylase, glycogen synthase, pyruvate dehydrogenase, HMGCoA reductase, pyruvate kinase and PFK2.

Factors influencing enzyme activity:

8- Presence of Enzyme Activators:

A. **Presence of certain inorganic ions, some enzymes show higher activity** (Cl^- activate salivary amylase and Ca^{+2} activate lipases)
B. **Conversion of inactive pro-enzyme or zymogen to active enzyme:**
    i. By splitting a single peptide bond, and removal of a small polypeptide from trypsinogen, the active trypsin is formed. This results in unmasking of the active centre.
    ii. Similarly trypsin activates chymotrypsinogen
    iii. All gastrointestinal enzymes are synthesized as pro-enzymes, and only after secretion into the alimentary canal, they are activated. This prevents autolysis of cellular structural proteins.
    iv. Coagulation factors are seen in blood as zymogen form.

Factors influencing enzyme activity:

9- Enzyme Inhibitors: substances inhibit enzyme action; classified into:

A. Non Specific: inhibit wide variety of enzymes or almost all enzymes:

i. **Agents which denaturate or precipitate proteins.**
ii. **Salts of heavy metals** as mercuric chloride, oxidizing agents as K ferric cyanide and alkylating agents as iodoacetic acid. All of them are sulfyhydryl inhibitors.

B. Specific Inhibitors: They are inhibitors do their action on one enzyme only or a small group of related enzymes.

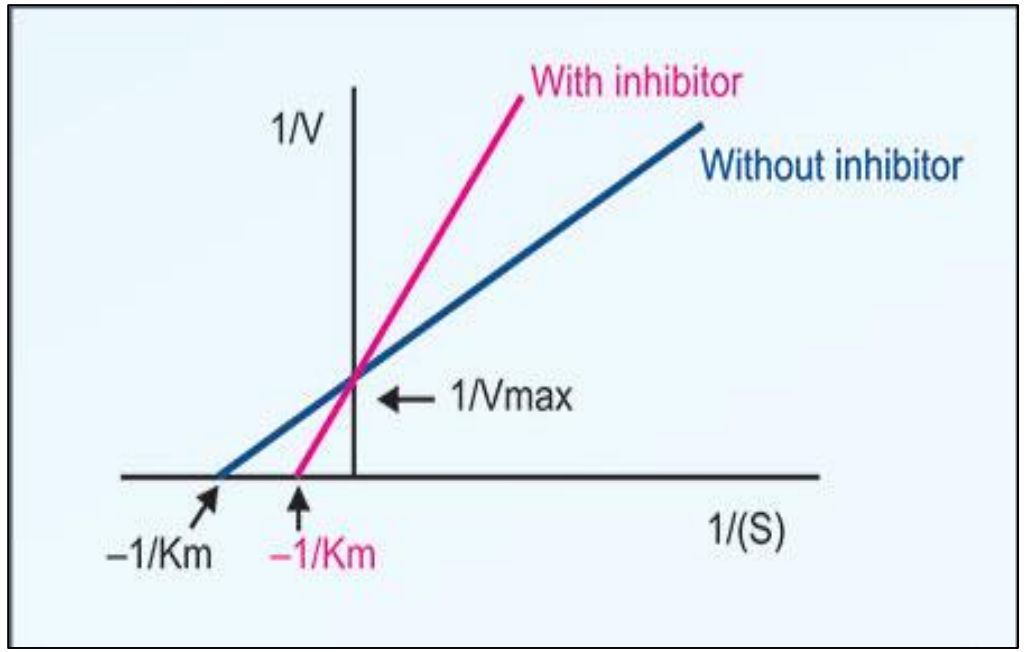
i. **Competitive Inhibitors (Reversible):**
ii. **Non-competitive Inhibitors (Irreversible)**
iii. **Uncompetitive Inhibitors:**
iv. **Feedback Inhibition**
v. **Suicide Inhibitors:**

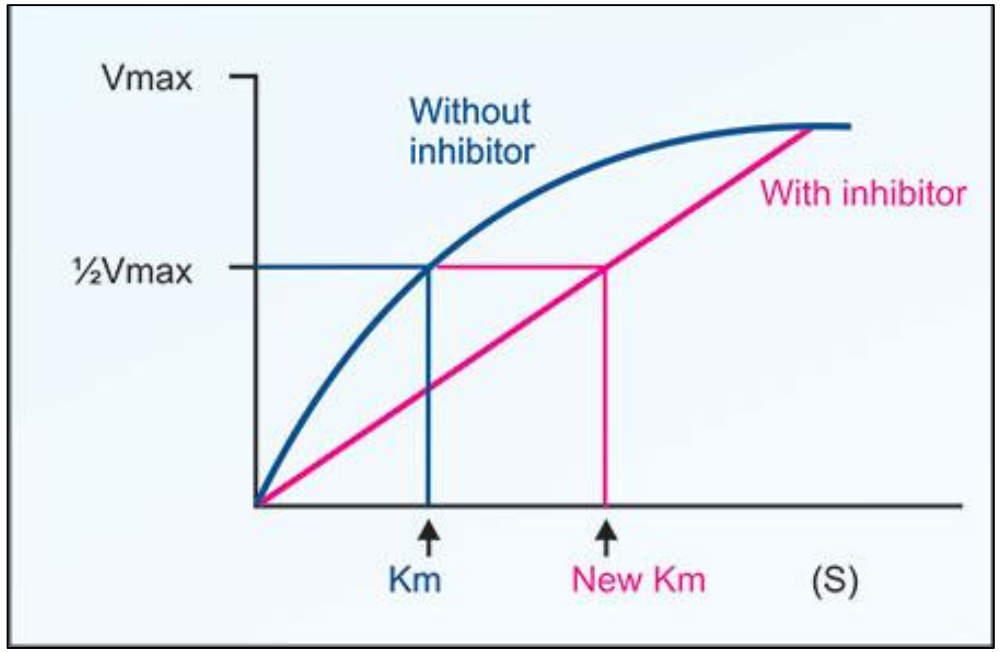
Factors influencing enzyme activity:

B. Specific Inhibitors:

i. Competitive Inhibitors (Reversible):

- Inhibitors that resemble the substrate in structure and compete with substrate for binding with active site of enzyme.
- Their inhibitory effect can be inhibited by increasing substrate concentration. e.g. Inhibition of succinic dehydrogenase by malonic acid. Malonic acid resembles succinic acid (substrate) in structure.





Clinical Significance of Competitive Inhibition:

Pharmacological action of drugs may be explained by competitive inhibition.

i. Sulphonamides: common antibacterial agents. Sulpha drugs, being structural analogues of PABA, will inhibit the folic acid synthesis in bacteria, and they die. The drug is nontoxic to human cells, because human beings cannot synthesize folic acid.

ii. Methotrexate: a structural analogue of folic acid, and so can competitively inhibit folate reductase enzyme, which is essential for DNA synthesis and cell division. So, methotrexate is anticancer drug.

iii. Dicumarol: It is structurally similar to vitamin K and can act as an anticoagulant by competitively inhibiting the vitamin K activity.

iv. Isonicotinic acid hydrazide (INH): a common antituberculous drug, structurally similar to pyridoxal and prolonged use of INH may cause pyridoxal deficiency and peripheral neuropathy