Introduction

Recall from your pervious classes that enzymes are biological catalyst which increase the rate of chemical reaction in living cells without being used in the process. Nearly every chemical process that takes place in living things is facilitated by an enzyme. The sum of all the chemical reactions that a cell or larger living thing carries out is its metabolism. Many activities in living things are controlled by metabolic pathways in which a series of interrelated steps are involved each one of them facilitated by an enzyme.

3.1 Enzyme Structure

Enzymes are proteins, and their function is determined by their complex structure. The reaction takes place in a small part of the enzyme called the active site, while the rest of the protein acts as "framework". The amino acids around the active site attach to the substrate molecule and hold it in position while the reaction takes place. This makes the enzyme specific for one reaction only, as other molecules would not fit into the active site.

3.2 Mode of Enzyme Action

Following two hypotheses explains mode of enzyme action

3.2.1 Lock and Key Hypothesis

Fischer in 1890 suggested that each enzyme had a particular shape into which the substrate fit exactly. This was known as the lock and key hypothesis. According to this hypothesis the substrate is imagined like a lock while the enzyme is imagined like a key. As one specific key can open only a specific lock, similarly a specific enzyme can break up only one specific substrate. The active site is regarded as rigid structure that does not modify or change during the reaction process. However later studies did not support this hypothesis in all type of reactions and therefore the hypothesis was modified into Induced fit hypothesis.

3.2.2 Induced-Fit Hypothesis

The attraction of the substrate and enzyme form an enzyme-substrate complex. It was originally referred to as the Lock and Key Enzyme Theory. The current theory suggests that the enzyme molecules are in an inactive form. To become active they must undergo a slight change in structure to more specifically accommodate the substrate(s). It is said to be "induced to fit" the substrate.

Think of way your hand changes shape slightly when you shake a person's hand. There are three ways of thinking about enzyme catalysis. They all describe the same process, though in different ways, and you should know about each of them.

A. Reaction Mechanism

In any chemical reaction, a substrate (S) is converted into a product (P):

S~P

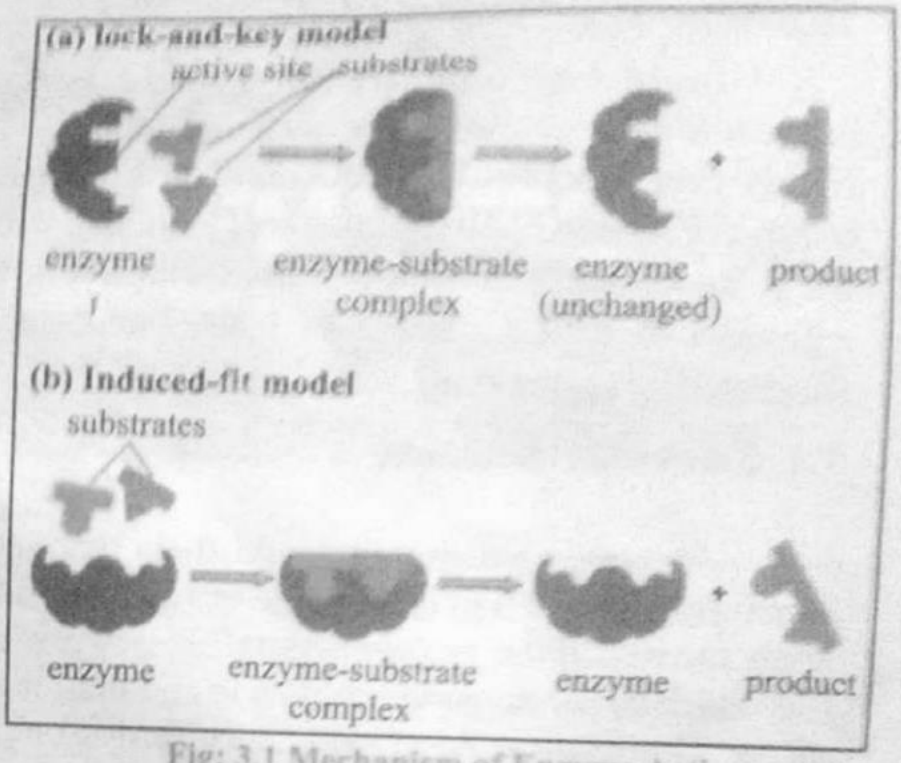


Fig: 3.1 Mechanism of Enzyme Action

There may be more than one substrate and more than one product, but that doesn't matter here. In an enzyme-catalyzed reaction, the substrate first binds to the active site of the enzyme to form an enzyme-substrate (ES) complex, then the substrate is converted into product while attached to the enzyme, and finally the product is released. This mechanism can be shown as in the Fig 3.2. The enzyme is then free to start again.

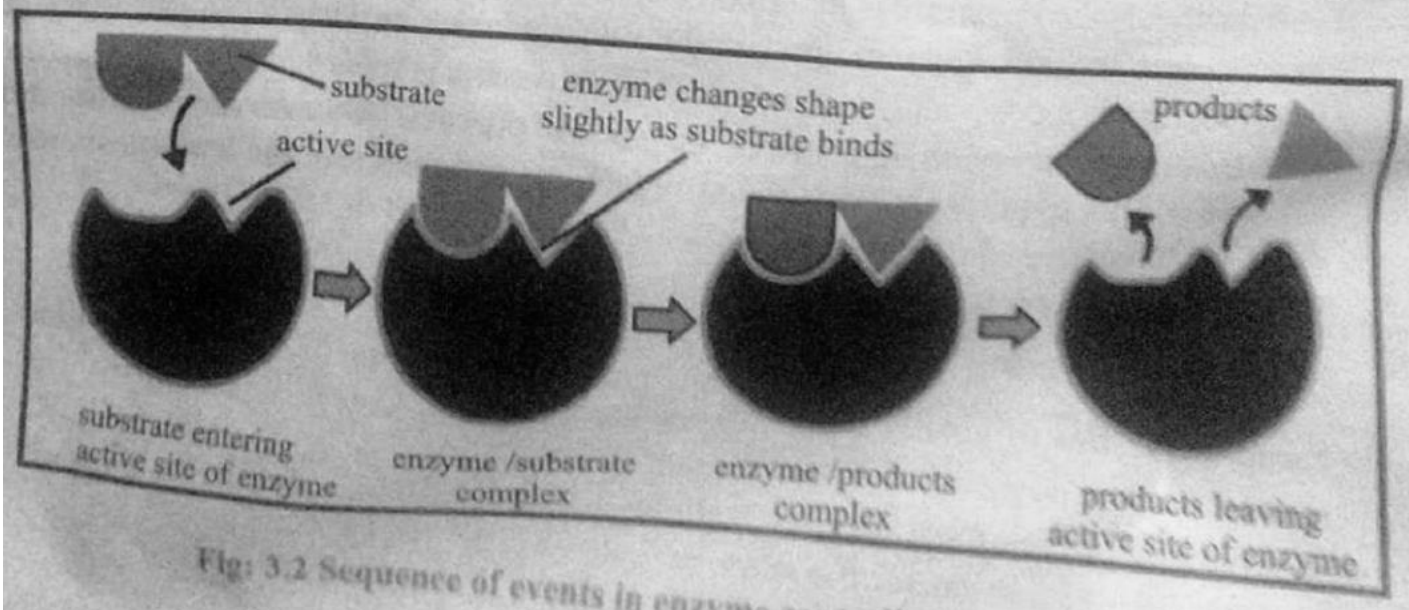


Fig: 3.2 Sequence of events in enzyme controlled reaction

B. Molecule Geometry

The substrate molecule fits into the active site of the enzyme molecule like a key fitting into a lock. Once there, the enzyme changes shape slightly, distorting the molecule in the active site, and making it more likely to change into the product. For example if a bond in the substrate is to be broken, that bond might be stretched by the enzyme, making it more likely to break. Alternatively the enzyme can make the local conditions inside the active site quite different from those outside (such as pH, water concentration, charge), so that the reaction is more likely to happen.

C. Energy Changes

The way enzymes work can also be shown by considering the energy changes that take place during a chemical reaction. We shall consider a reaction where the product has a lower energy than the substrate, so the substrate naturally turns into product (in other words the equilibrium lies in the direction of the product). Before it can change into product, the substrate must overcome an "energy barrier" called the activation energy (E_a). The larger the activation energy, the slower the reaction will be because only a few substrate molecules will by chance have sufficient energy to overcome the activation energy barrier. Enzymes dramatically reduce the activation energy of a reaction, so that most molecules can easily go over the activation energy barrier and quickly turn into product. For example, for the following reaction

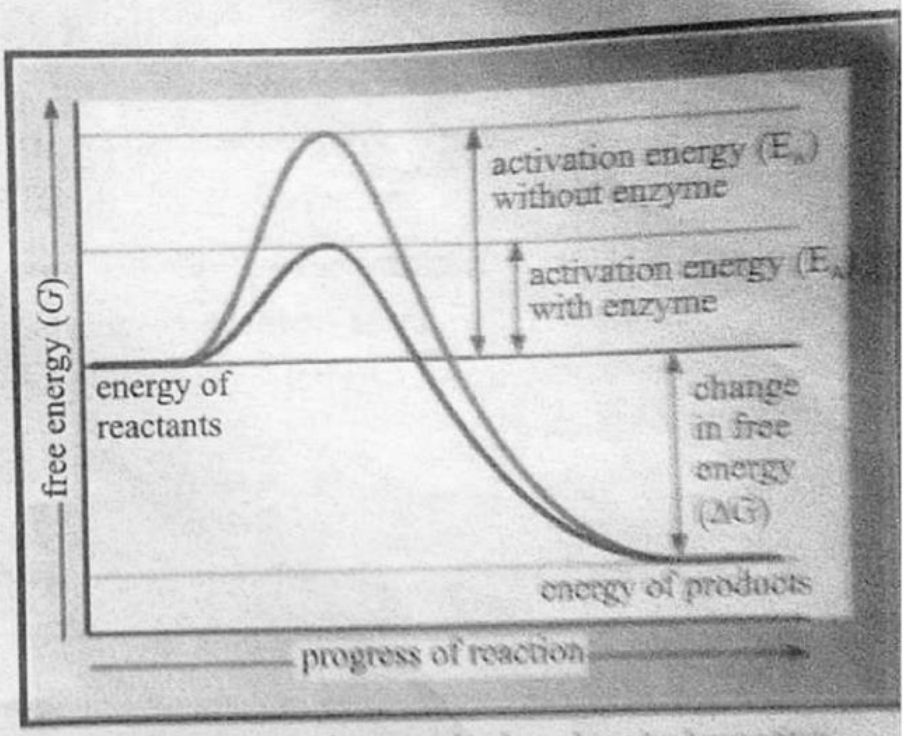


Fig: 3.3 Energy changes during chemical reaction.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

The activation energy is 86 kJ mol^{-1} with no catalyst and just 1 kJ mol^{-1} in the presence of the enzyme catalase.

The activation energy is actually the energy required to form the transition state, so enzymes lower the activation energy by stabilising the transition state, and they do this by changing the conditions within the active site of the enzyme.

3.3 Cofactors

Cofactors - are atoms, groups of atoms and molecules that join with enzymes altering their shape and making them functional. One can think of these cofactors as an" on-off" switch for activating an enzyme. If the cofactor is a non-protein like a metallic ion (i.e. zinc, copper, or iron) it is referred to as a prosthetic group. Some cofactors are small organic molecules called coenzymes. Like enzymes they are not permanently altered in the reactions.

Many of these coenzymes are derived from vitamins and minerals that are essential for life. The absence of these cofactors can lead to vitamin and mineral deficiency diseases e.g lack of Vitamin B produces beriberi . Examples of coenzymes are NAD^+, FAD^+, NADP.

3.4 Enzyme nomenclature

Many enzymes but not all end in the suffix "ase". (exceptions: pepsin, trypsin). They are named for the substrate they act on or the action they perform.

The following are the six major enzyme categories.

1. Oxidoreductases

These enzymes catalyze various types of oxidation-reduction reactions. Subclasses of this group contain oxidases, oxygenases and peroxidases.

2. Transferases

These enzymes catalyze reactions that involve the transfer of groups from one molecule to another. Examples of such groups include amino, carboxyl, methyl and carbonyl. Transcarboxylases and transmethylases are examples of transferases.

3. Hydrolases

These enzymes catalyze the reactions in which the cleavage of bonds is accomplished by the addition of water. The hydro lases include the esterases, phosphatases and peptidases

Table 3.1 Enzyme Nomenclature by Substrate

Substrate	Enzyme
Lipid	Lipase
Urea	Urease
Maltose	. Maltase
Ribonucleic Acid (RNA)	RNAase
A TP	ATPase
Dextrose	Dextrase
Protein	Proteinase

4. Lyases

Lyases catalyze reactions in which groups (e.g. H_2O, CO_2 and NH_3) are removed to form a double bond or added to a double bond .Decarboxylases, deaminases and synthases are examples of Lyases.

5. Isomerases

This is a heterogeneous group of enzymes which catalyze several types of intermolecular rearrangements. Epimerases and mutases are the examples.

6. Ligases

Ligases catalyze bond formation between two substrate molecules. The energy for these reactions is always supplied by ATP hydrolysis.

3.5 Factors that Affect the Rate of Enzyme Reactions

Rate of enzyme reactions depend on the following factors.

A. Temperature

Enzymes works best at an optimum temperature. Enzymes present in mammals works best at about 40°C. Animals present in different environments are adopted to range of temperature. for example, enzymes of the arctic snow flea work at -10°C whereas in thermophilic bacteria enzymes work at a temperature of 90°C.Upto the optimum temperature the rate increases geometrically with temperature (i.e. it's a curve, not a straight line). The rate increases because the enzyme and substrate molecules both have more kinetic energy so collide more often and also because more molecules have sufficient energy to overcome the (greatly reduced) activation energy. The increase in rate with temperature can be quantified as a Q IO, which is the relative increase for a 10°C rise in temperature.

The rate is not zero at O°C, so enzymes still work in the refrigerator (and food still goes off), but they work slowly. Enzymes can even work in ice, though the rate is extremely slow due to the very slow diffusion of enzyme and substrate molecules through the ice lattice.

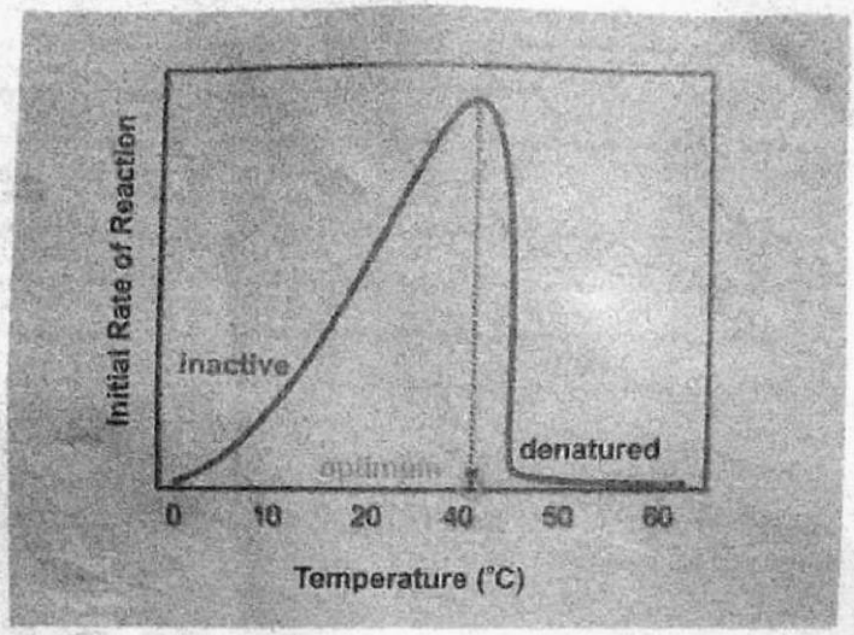


Fig: 3.4 Influence of temperature on the rate of enzyme-catalyzed reactions.

B. pH

Enzymes have an optimum pH at which they work fastest. For most enzymes this is about pH 7-8 (physiological pH of most cells), but a few enzymes can work at extreme pH, such as protease enzymes in animal stomachs, which have an optimum pH 1. The pH affects the charge of the amino acids at the active site more specifically, changes in pH ionizes amino acids forming an enzyme so the properties of the active site change and the substrate can no longer bind.

For your information

Enyzme	pH Optimum
Lipase (Pancreas)	8.0
Lipase (Stomach)	4.0-5.0
Lipase (Castor oil)	4.7
Pepsin	1.5-1.6
Trypsin	7.8-8.7
Urease	7.0
Invertase	4.5
Maltase	6.1-6.8
Amylase (Pancreas)	6.7-7.0
Amylase (malt)	4.6-5.2
Catalase	7.0

C. Enzyme concentration

As the enzyme concentration increases the rate of the reaction increases linearly, because there are more enzyme molecules available to catalyze the

reaction. At very high enzyme concentration the substrate concentration may become rate-limiting, so the rate stops increasing. Normally enzymes are present in cells in rather low concentrations.

D. Substrate concentration

The rate of an enzyme-catalyzed reaction shows a curved dependence on substrate concentration. As the substrate concentration increases, the rate increases because more substrate molecules can collide with enzyme molecules, so more reactions will take place. At higher concentrations the enzyme molecules become saturated with substrate, so there are few free enzyme molecules, so adding more substrate doesn't make much difference.

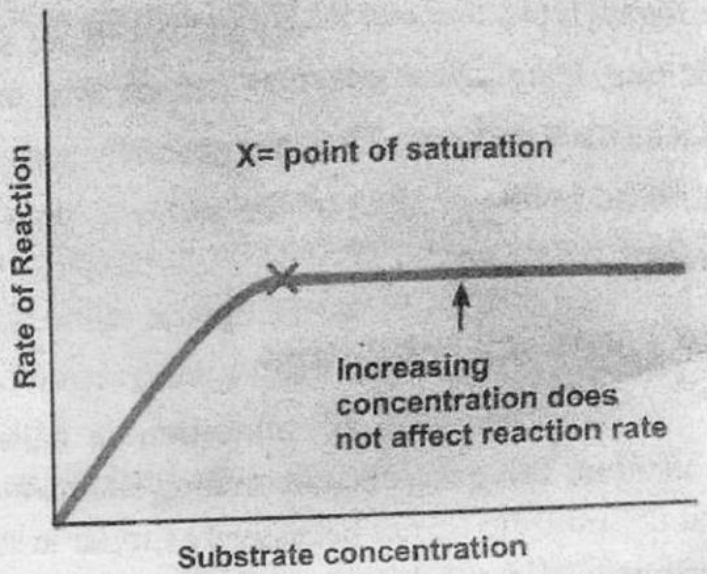


Fig: 3.5 Effect of substrate concentration on rate of reaction.

E. Inhibitors

Inhibitors inhibit the activity of enzymes, reducing the rate of their reactions. They are found naturally, but are also used artificially as drugs, pesticides and research tools. There are two kinds of inhibitors.

(a) **A competitive inhibitor molecule** has a similar structure to the normal substrate molecule, and it can fit into the active site of the enzyme. It therefore competes with the substrate for the active site, so the reaction is slower e.g the sulphonamide to an antibacterial drugs which act as competitive inhibitors.

(b) **A non-competitive inhibitor molecule** is quite different in structure from the substrate molecule and does not fit into the active site. It binds to another part of the enzyme molecule, changing the shape of the whole enzyme, including the active site, so that it can no longer bind substrate molecules. Inhibitors that bind weakly and can be removed out easily are sometimes called reversible inhibitors, while those that bind tightly and cannot be removed out are called irreversible inhibitors. Poisons like cyanide, heavy metal ions and some insecticides are all non-competitive inhibitors.

The activity of some enzymes is controlled by certain molecules binding to a specific regulatory (or allosteric) site on the enzyme, distinct from the active site. Different molecules can inhibit or activate the enzyme, allowing sophisticated control of the rate. Only a few enzymes can do this, and they are often at the start of a long biochemical pathway. They are generally activated by the substrate of the pathway and inhibited by the product of the pathway, thus only turning the pathway on when it is needed.

3.6 Feedback Inhibition

Another kind of inhibition is called feedback inhibition. In feedback inhibition, there is a second binding site on the enzyme where the inhibitor binds, so that the inhibitor is not necessarily similar in structure to the substrate. The absence or presence of the inhibitor at this second binding site activates or deactivates the enzyme, by changing the conformation of the enzyme so that the active site is made available or unavailable to the substrate. The inhibitor is usually the product of a reaction formed during the metabolic pathway.

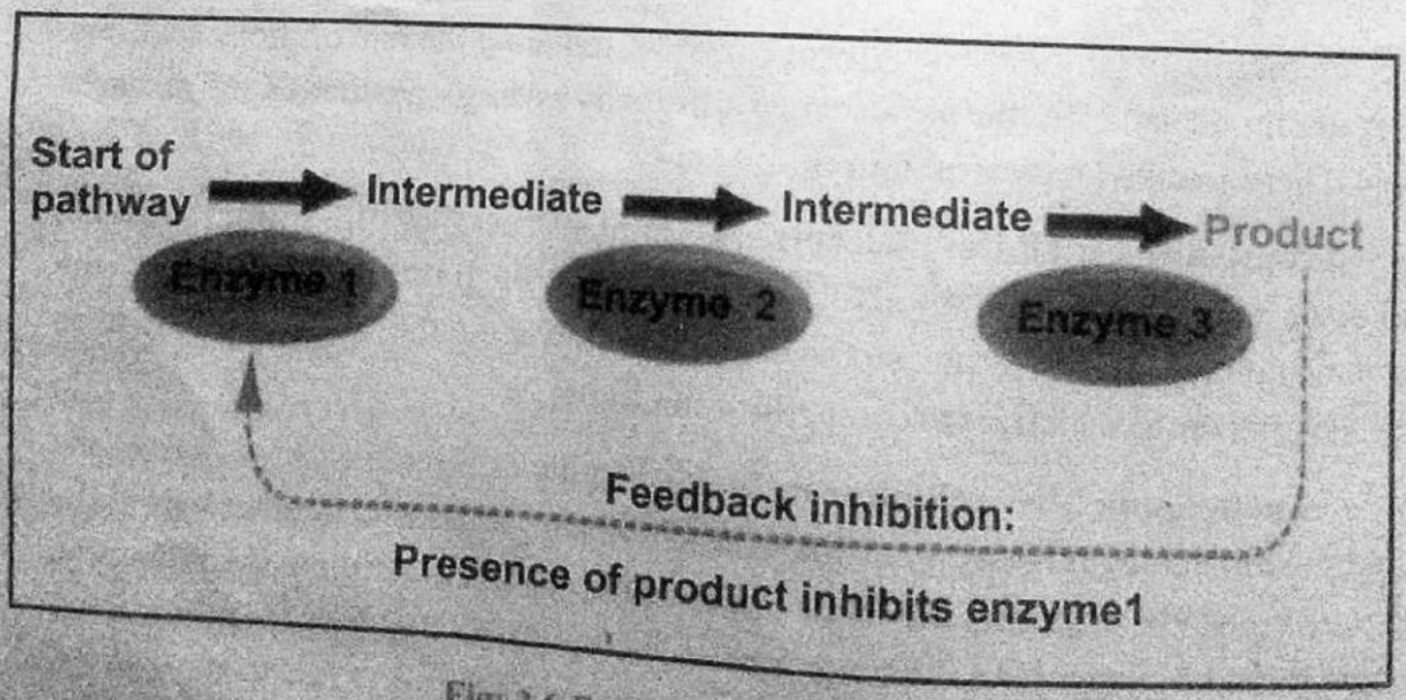


Fig: 3.6 Feedback inhibition.

KEY POINTS

Enzymes are organic chemical substances produced by the living organisms to speed up a particular reaction, but during this process these remain unchanged.

Enzymes are very specific in their action acting on a specific substrate.

When an enzymes acts on a specific substrate, enzymes substrates complex is formed.

When enzyme's shapes are disrupted it loses its characteristics biological activity.

The non protein part or prosthetic group of an enzyme is called cofactor.

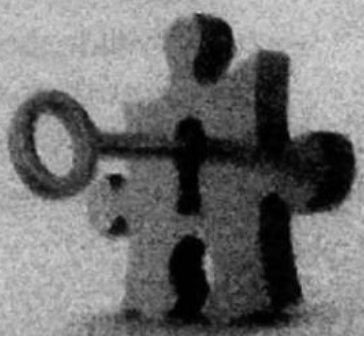
According to lock and key model of enzyme the active site of an enzyme is a rigid structure.

Modification to the lock and key model suggests that since enzymes are rather flexible structures, the active site is continually reshaped by interactions with the substrate as the substrate interacts with the enzyme.

An inhibitor is a chemical substance which can block the active site of an enzyme temporarily or permanently by stopping the activity of the enzyme.

The factors that affect the rate of enzyme action are: enzymes concentration, substrate concentration, temperature, pH of the medium.

A cellular control mechanism in which an enzyme that catalyzes the production of a particular substance in the cell is inhibited when that substance has accumulated to a certain level, thereby balancing the amount provided with the amount needed.



EXERCISE ?

A. Choose the correct answers in the following questions.

1. Which one enzyme catalyzes the oxidation-reduction reaction?
   a. Oxygenases
   b. Transmethylases
   c. Lyases
   d. Peptidases

2. Enzyme catalyzing rearrangement of atomic groupings without altering molecular weight or number of atoms is:
   a. ligase
   b. isomerase
   c. oxidoreductase
   d. hydrolase

3. Enzymes are polymers of:
   a. hexose sugar
   b. amino acids
   c. fatty acids
   d. inorganic molecules

4. Which one forms the raw material for coenzymes?
   a. Vitamins
   b. Carbohydrates
   c. Proteins
   d. Metals

5. What will happen to reaction if enzyme is added?
   a. Rate of reaction decreases
   b. Rate of reaction increase
   c. No effect on the rate of reaction
   d. Reaction is reversed

6. What is induced fit hypothesis?
   a. When enzyme change shape due to absence of substrate
   b. When enzyme do not change shape due to absence of substrate
   c. When enzyme change shape due to presence of substrate
   d. When enzyme do not change shape due to presence of substrate

7. Which enzyme digest' egg albumin into peptides and amino acids best in alkaline conditions?
   a. Catalase
   b. Lipase
   c. Pepsin
   d. Trypsin

8. Sometimes enzyme and substrate are held together by the kind of bonds called:
   a. Ionic
   b. hydrogen
   c. hydrophobic
   d. covalent

9. Which one of the following refers to non competitive inhibitors:
   a. Bind to the active site.
   b. Similar to the normal substrate with which energy interact
   c. Destroy the globular conformation of enzyme
   d. Bind to the binding site other than active site

EXERCISE

10. Which one of the following factors does not affect the rate of enzyme action?
    a. Enzymes concentration
    b. Substrate concentration
    c. Water concentration
    d. Temperature

11. The optimum pH value for pepsin to work is :
    a. 6.8
    b. 4.5
    c. 5.5
    d. 1.5

B. Write short answers to the following questions.

1. What is a cofactor? Give examples.
2. What are metal activators? Give three examples.
3. Differentiate the key difference between the Lock and Key Model and Induced Fit Hypothesis/model?
4. How pH of a cell affects the enzyme activity?

C. Write detailed answers to the following questions.

1. Describe the characteristics of enzymes.
2. Explain the process of enzyme inhibition. Make a list of some common enzymes inhibitors.
3. Write briefly the mode of action of an enzyme.
4. How do the enzyme and substrate concentrations affect the rate of enzyme action?

Projects

- Search and make a list of enzymes which are used for diagnostic purposes in your local diagnostic laboratory.
- List down some common venoms which can act as enzyme inhibitors.
- Enzymes are three-dimensional, create a unique three-dimensional enzyme model using low cost no cost material. Identify your model in terms of the name of enzyme and substrate, active site, enzyme substrate complex, products.