UNIT THREE

ENZYMES

Learning objectives

Nature of enzymes of enzymes .	• Define enzymes and explain the properties • Explain how enzymes are named and then classify them according to their structure. • Conduct an experiment to show the specificity of an enzyme. • Appreciate the importance of enzymes in industries and local products.
Functions of enzymes energy .	• Explain how enzymes lower activation • Explain the mechanism of enzyme action. • Discuss the action of apo- and coenzymes. • Give examples of vitamins and minerals in food that act as cofactors.
Factors affecting the function of enzymes	• Explain factors that affect enzyme activity • Investigate the destruction of an enzyme by heat. • Show how temperature, pH, substrate concentration and enzyme concentration.

3.1 natures of enzymes

All living cells carryout different types of biochemical reaction which support life activates. These biochemical reactions are takes place rapidly due to the activity of enzymes. The ability of speedup or slowdown chemical reaction is so called catalyzing.

All enzymes are globular proteins (all globular proteins have a unique tertiary structure, which gives them a unique shape). The ability of catalyzing chemical reaction is depends on specific shape so called active site.

Active site is the part of an enzyme molecule that binds with its substrate so that the enzyme can catalyses the chemical reaction in living cells.

Substrate a substance upon which an enzyme acts in a biochemical reaction.

An enzyme is a globular protein with a uniquely shaped active site; it acts as a biological catalyst for a specific reaction, but remains unaltered by the reaction.

The intermediate formed, temporarily, when an enzyme binds to its substrate is called **enzyme–substrate complex.**

An enzyme is a globular protein with a uniquely shaped active site; it acts as a biological catalyst for a specific reaction, but remains unaltered (unchanged) by the reaction.

Catalyst a substance that speeds up a chemical reaction and remains unchanged at the end of the reaction.

Properties of enzymes

There are various types of enzymes used to catalyze thousands of chemical reactions in living organisms so all enzymes shares common characteristics. Which are?

- They are all proteins (but all proteins are not enzymes)
- They are biological catalysts: they speed up a reaction without being used up, so they can be used over and over again.
- They are specific: they catalyze one reaction only.
- A small amount of enzyme can bring about a change in a large amount of its substrate.
- Enzymes are affected by pH and temperature.

A. What are catalysts?

Catalysts are chemical substances that speed up chemical reaction without changing itself. There is no overall change to:

- The nature of the products.
- The catalyst itself.
- The energy change that takes place during the reaction.
- All chemical reactions that take place in living things are catalyzed by enzymes.

B. Why are enzymes specific?

The active site of enzymes is specific to substrate that catalyzed. Each chemical reaction has its own enzyme that speed up chemical reaction of living organism. It can be expressed by

I. Lock and Key model: It is assumed that the substrate and the active site have structural similarity as a key fits its padlock.

II. Induced fit model: Approaching of the substrate to an enzyme active site brings about a conformational change on the active site.

C. How are enzymes affected by pH and by temperature?

I. PH

The acidic (basic) nature of chemical affects enzyme to speed up chemical reaction. For example ***pepsin is effective in acidic environment***. It works at narrow range of PH.

II. Temperature

Temperature affects enzyme action in two ways: a higher temperature gives the enzyme molecules (and their substrate molecules) more kinetic energy; they move around faster and form more enzyme–substrate complexes a higher temperature affects the chemical bonds holding the tertiary structure of the enzyme in place (particularly those in the active site); as more and more of these bonds break, the shape of the active site changes and it can no longer bind with its substrate. Higher temperature than optimum temperature affects the active site which is so called denaturation.

Example if we boiled egg the egg is changed from semi solid to solid due to high temperature these shows as that protein can affected by temperature.

How do we name and classify enzymes?

Enzymes can be named based on different (various) types of criteria. Some of the criteria are:-

I. Names given by adding ‘ase’ to part of the name of the substrate. **For example,** *lipase* (lipid hydrolyzing enzyme), *sucrase* (sucrose hydrolysing enzyme).

II. Names given by the type of chemical reaction catalyzed. **For example**, *polymerase* (aids in polymerisation – joining similar units together), *dehydrogenase* (removal of hydrogen atoms or ions).

III. Depending on the identification for the first time. For example, *papayin* from papaya.
IV. Name given according to the protein catalyzes (the name of such enzymes are ends by in). For example, *pepsin, trypsin*, etc. These enzymes usually have alternative names that tell you rather more about them.

Because of the varied ways in which enzymes had been named, biologists at the **Enzyme Commission** decided to produce a systematic way of naming enzymes, based on the ways in which the enzymes act. Systematic name is name given by biologists study about enzymes to avoid ambiguity of naming enzyme through the world. Enzymes are generally classified on the basis of the type of reactions that they catalyze. According to these there are six groups of enzymes which are

Classification of enzymes

1. Oxidoreductases an enzyme that used for Transfer of hydrogen and oxygen atoms or electrons from one substrate to another. Example Dehydrogenases Oxidases
2. Transferases an enzyme that used for transfer of a specific group (a phosphate or methyl, etc.) from one substrate to another. Example Transaminase Kinases
3. Hydrolases an enzyam that catalyze Hydrolysis of a substrate Esterases. example Digestive enzymes
4. Isomerases an enzyme that catalyze Change of the molecular form of the substrate Example Phosphohexoisomerase Fumerase
5. Lyases (Non hydrolytic) removal of a group or addition of a group to a substrate Example Decarboxylases Aldolases
6. Ligase (Synthetases) is used to Joining of two molecules by the formation of new bondsCitric acid synthetase. Example DNA ligase

Each class of enzymes contains several different, but related, subclasses. Each subclass is further divided into sub-subclasses. Within the sub-subclasses, each enzyme has a number.

So, in the systematic naming of enzymes, an enzyme will have a name' such as EC 3.4.11.1. Each part of the description tells us something about the enzyme:

- EC stands for Enzyme Commission
- The first number shows to which of the six main classes the enzyme belongs
- The second figure indicates a subclass
- The third figure gives a sub-subclass
- The fourth figure is the serial number of the enzyme in its subsubclass.Enzyme EC 3.4.11.1 is:
- A hydrolase – all the enzymes in class 3 hydrolyse some kind of bond
- A peptidase – all the enzymes in subclass 4 of class 3 are peptidases and hydrolyse peptide bonds
- An amino-peptidase – all the enzymes in sub-subclass 11 of subclass 4 are amino-peptidases; they hydrolyse peptide bonds at the amino end of a polypeptide chain
- Leucyl-amino-peptidase – this particular amino-peptidase is number 1 of this sub-subclass

What do enzymes do for you?

Enzymes are very important to as in different ways because all chemical reaction that takes place in our body is catalyzed by enzyme. Life is impossible without enzyme. For example metabolism is unthinkable without enzyme. In addition enzyme is applied in our home for production of food and drink it is also important to produce in our home. Enzymes are also used in industry for production of various products some of the industrial products are:-

Sector	Application area	Benefits
Dairy	• Biochymosin to produce cheese • Lactase to produce lactose-free milk	• Supplies of natural rennet from calves livers are limited • Lactose-intolerant people suffer fewer cramps
Detergents	• Use of proteases, lipases and amylases in biological washing powders • Use of proteases and amylases in dishwasher detergents	• Many biological stains are removed efficiently at low temperatures (saving energy) • Remove food particles at lower temperatures and require fewer bleaching products to be added
Textiles	• Proteases to remove hair and lipases to degrease animal hides • Use of cellulase to 'bio-polish' cotton fabrics • Use of cellulase to 'bio-stone' denim	• Process is carried out much quicker than by traditional methods • Produces a smoother and glossier finish • The enzyme gives the 'stone-washed' effect much more easily
Food processing	• Pectinase to process fruit juice • Inverase to produce liquid-centre chocolates	• Clarifies fruit juice • Sucrose paste in the chocolate is made liquid by injection of the enzyme
Pulp and paper	• Amylases used in starch conversion • Use of xylanase enzymes in pre-bleaching the pulp • Use of esterases in control of 'stickies' (glues introduced during paper recycling)	• Reduces the quantity of starch in the paper and improves quality • Produces a whiter paper • Stickies would otherwise clog the machinery and reduce the quality of the paper
Medicine	• Glucose oxidase in clinistix strips, tests for glucose • Liver enzymes • Pulmozyme to treat cystic fibrosis	• Allows easy diagnosis of diabetes by testing urine • Testing for high levels of these in the blood confirms liver damage • Reduces viscosity (stickiness) of mucus
Pharma-ceutical	• Streptokinase to dissolve clots of heart-attack patients • Production of abacavir sulphate is controlled by enzymes	• Restores blood supply to area of heart muscle • Abacavir sulphate is an important anti-AIDS drug

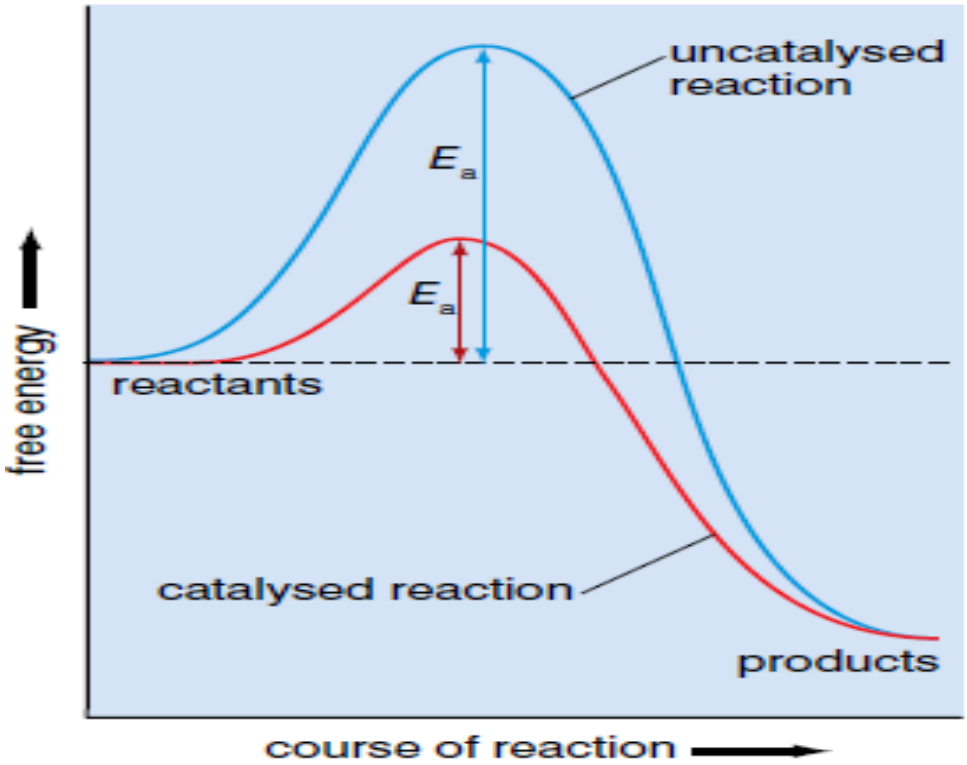
3.2 Functions of enzymes

At the end of this section the student should be able to:

- Explain how enzymes lower activation energy.
- Explain the mechanism of enzyme action.
- Define the action of apo- and coenzymes.
- Give examples of vitamins, minerals in food that act as co factor and define it properly.

As our previous definition:-enzymes are catalysts that increase the rate of chemical reaction by lowering activation energy without changing itself. Activation energy is the energy that must be provided to substances to result in chemical reaction (the energy required to form or break the bonds of reactant molecules). Catalysts speed up chemical reactions. In order for molecules to

react, they must have sufficient energy. This energy to start off the reaction is called **activation energy** (or E_a). Imagine a reaction in which substance A reacts with substance B to form substance AB. We can write an equation for this as: A + B → AB. The equation gives only the reactants (starting materials) and the products. It does not show how the energy changes as the reaction takes place. The reactant must 'climb an activation energy hill' before anything happens. Under normal conditions, very few molecules of A and B have sufficient kinetic energy to 'climb the activation energy hill', so the reaction proceeds slowly. A catalyst lowers the activation energy required for the reaction.



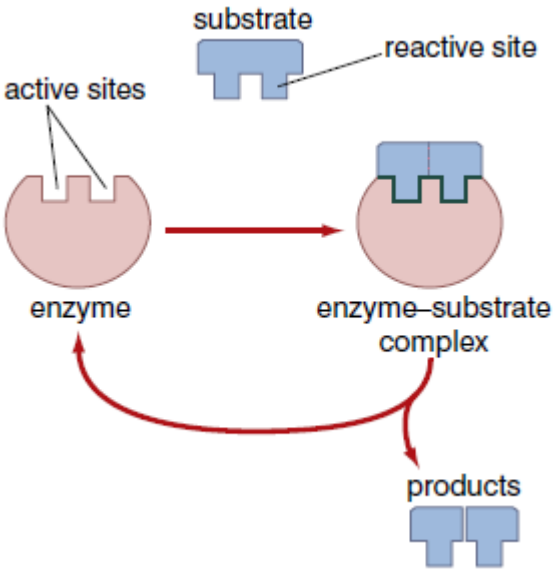
*This figure shows Activation energy for **uncatalysed** reaction and the same reaction **with** a **catalyst.***

There are two models of enzyme action which shows the way that enzymes catalyze chemical reactions by lowering the activation energy.

A. The lock-and-key model

This model proposes that the shapes of the substrate molecules are *complementary* to that of the active site, rather like the shape of a key is complementary to that of the lock it fits. According to this theory the enzyme and substrate shape do not influence each other because they are alredy in a predetermined perfectly complementary shape.

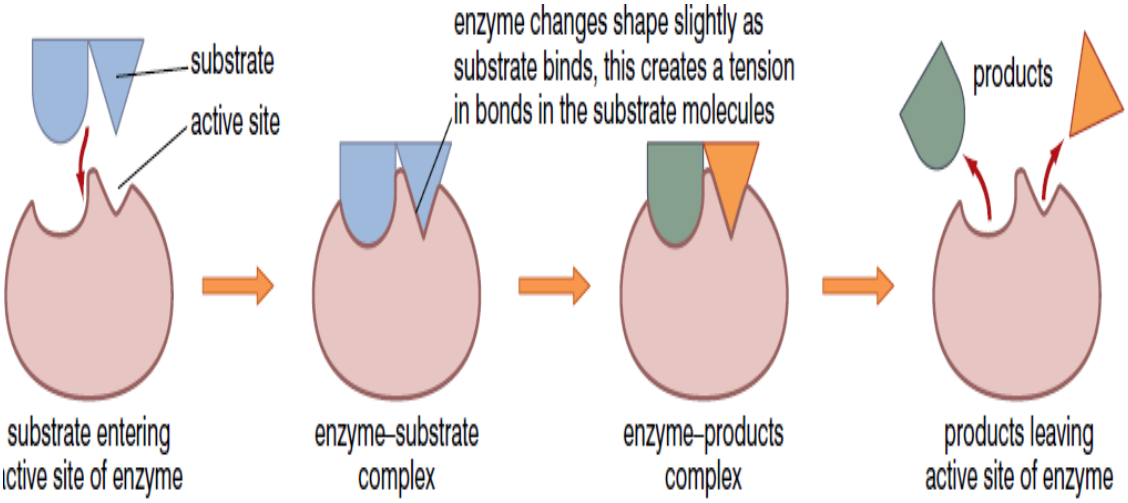
Example: - an egg sitting in an egg cup.

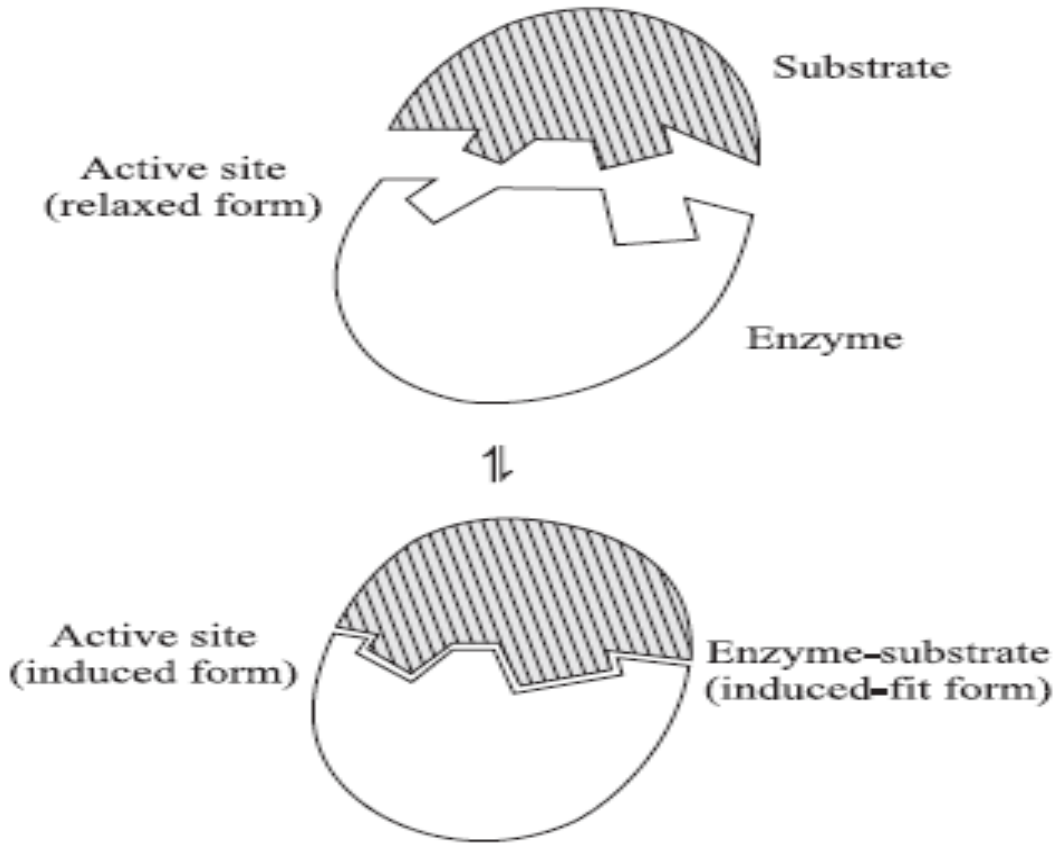


The diagram above shows the key and lock model

B. Induced fit model

The induced fit model is a model for enzyme- substrate interaction. It describes that the only the proper substrate is capable of inducing the proper alignment of active site that will enable the enzyme to perform its catalytic function. It is the modified form of key and lock model it is different from key and lock model because it is more flexible, so this model suggests that the active site and the substrate aren't naturally complementary in shape, but the binding of substrate molecules produces a conformational change (change in shape) in the active site. This allows the substrate and active site to bind fully. The conformational change also puts the substrate molecules under tension, so they enter a 'transition state' and are able to react because of the lowered activation energy. In the transition state, bonds in the reactants are put under strain and break more easily and rejoin with other bonds to form the products.





The above diagram shows induced fit model

Apoenzymes and Cofactors

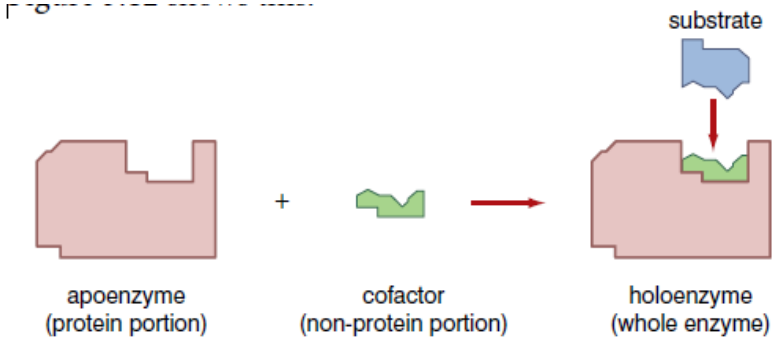
Why do some enzymes need cofactors?

Sometimes an active enzyme isn't just a single molecule, but is made from two molecules , neither of which has enzymic activity without the other. The two parts are the apoenzyme and the cofactor. Some enzymes are only protein which have ability of catalyzing chemical reaction example pepsin. The other two are apoenzyme and cofactor.

Apoenzyme – a protein that combines with a cofactor, to form an active enzyme (a protein part of an enzyme). It is non catalytic by itself without cofactor.

Cofactor – a small non-protein particle essential for the activity of some enzymes (A non protein part of enzyme). It is non catalytic by itself without Apoenzyme.

Coenzyme is a non polypeptide compound (an organic compound) that serve as cofactor and if its inorganic it contains ion such as Ca^{2+} , Zn^{2+}, CU^{2+} and vitamins (Coenzymes are substances that enhance the action of enzymes).



The diagram shows Apoenzyme, Cofactor and Holoenzyme

The following table indicates Common coenzymes and their functions

Coenzyme	**Vitamin**	**Enzyme**	**Function**
Nicotinamide adenine dinucleotide (NAD)	Niacin	Oxidoreductase in respiration	Oxidation or hydrogen transfer in respiration
Flavin adenine dinucleotide (FAD)	Riboflavin	Oxidoreductase in respiration	Oxidation or hydrogen transfer in respiration

Some group of enzymes can only function properly in the presence of certain mineral ions. These bind loosely with the enzyme to give it its catalytic activity.

The following table indicates Enzymes that require mineral ions as cofactors

Enzyme	**Mineral ion**	**Function**
Carbonic Anhydrase	Zinc ions (Zn^{++})	Causes CO_2 to react with water to form hydrogen carbonate
Alcohol Dehydrogenase	Zinc ions (Zn^{++})	Oxidises alcohol
Cytochrome Oxidase	Copper ions (Cu^{++} or Cu^{+})	Transfers electrons to oxygen during respiration

3.3 Factors affecting the functions of enzymes

There are various factors that affects the activity of enzymes the most common factors are (the activity of the enzyme are influenced by a number of external factors, including)

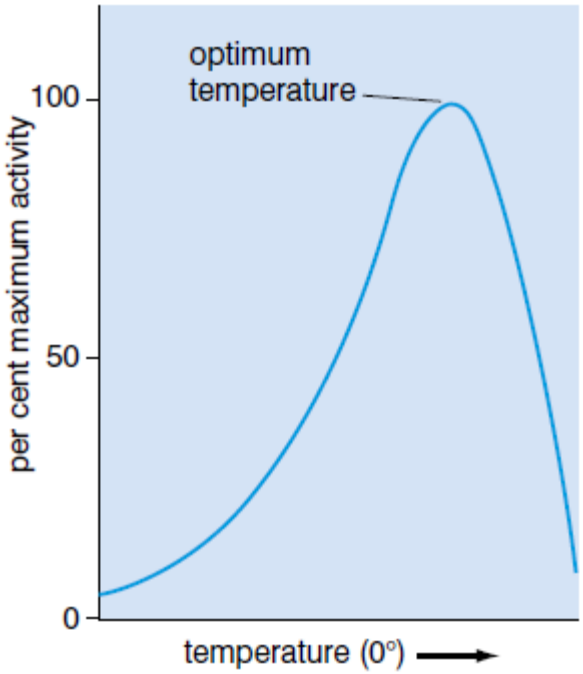
A. Temperature
B. pH
C. Substrate concentration
D. The presence of inhibitors
E. Enzyme concentration

A. Effect of Temperature

Since temperature (heat) increases the motion of molecules,(enzyme and substrate molecules also moves faster this increase the rate of chemical reaction). When the temperature is raised, particles are given more kineticenergy. This has two main effects

- 'Free' particles move around more quickly. This increases the probability that a substrate particle will collide with an enzyme molecule.
- Particles within a molecule vibrate more energetically. This puts strain on the bonds that hold the atoms in place. Bonds begin to break and, in the case of an enzyme, the shape of the molecule and the active site in particular, begin to change. The enzyme begins to lose its tertiary structure (remember it is a protein) and **denature.**
- **Denature** *the alteration of the tertiary structure of a protein; in living cells this is reversible.*

Therefore, the catalytic activity of enzyme works at normal temperature which is called optimum temperature. Enzymes require different optimum temperature for different organism.



The above graph shows the effect of temperature on the chemical reaction catalyzed.

B. The effect of PH on the activity of enzymes

Ph is the scale that used to measure the acidity or alkalinity of substances (The **pH scale** is a measure of the hydrogen ion concentration of a solution or other liquid system). The PH of the medium can have direct effect on the bonding responsible for the secondary and tertiary structure of enzymes. The pH scale ranges from 0 to 14. Solutions with a pH of less than 7 are acidic, those with a pH of more than 7 are alkaline and a solution with a pH of exactly 7 is neutral. Significant changes in pH can affect an enzyme molecule by:

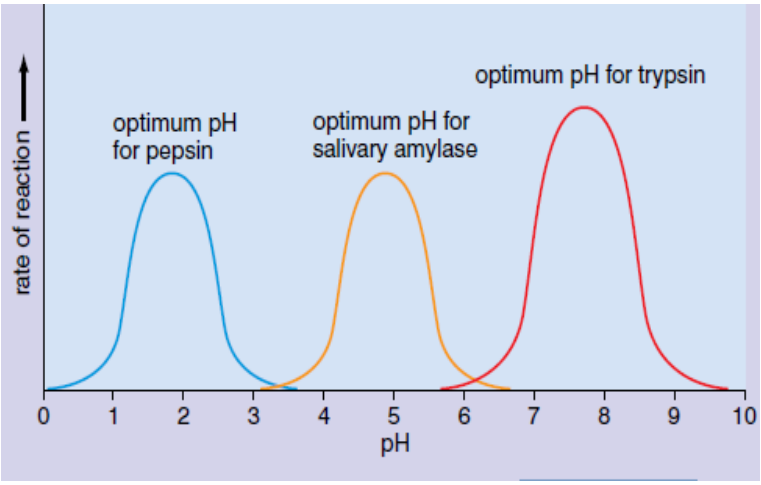
- Breaking ionic bonds that hold the tertiary structure in place; this leads to denaturation of the enzyme molecule

- ❖ Altering the charge on some of the amino acids that form the active site; this makes it more difficult for substrate molecules to bind

The following table lists some enzymes optimum catalyzing PH value

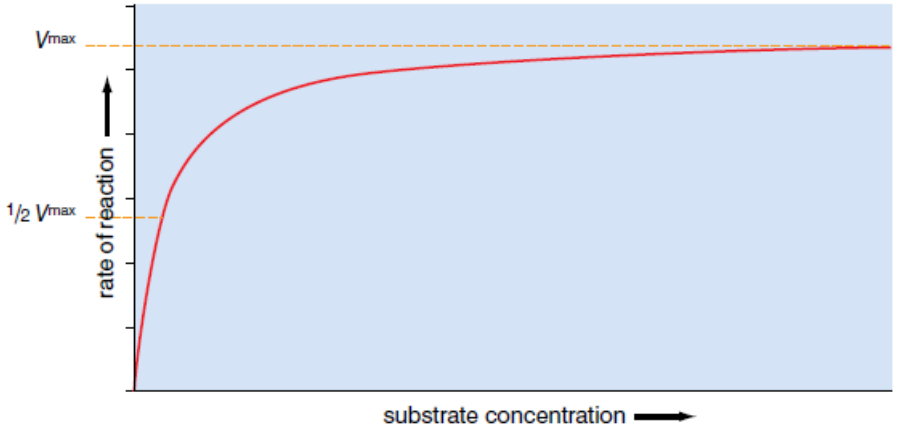
Enzyme	Optimum PH
Pepsin	2.00
Salivary Amylase	6.2-7.7
Pancreatic lipase	7.0
Catalase	7.6
Trypsin	7.5

The graph bellow indicates optimum PH of *some human digestive enzyme.*



C. The effect Substrate concentration on the activity of enzymes.

When considering the rate of an enzyme catalyzed reaction the proportion of enzyme and substrate should be considered. The activity of an enzyme depends on the number of substrate molecules per second that bind to form enzyme–substrate complexes. So the number of substrate molecules present must have an effect. A small number of substrate molecules mean few collisions and so only a few enzyme–substrate complexes form. Increasing the concentration of the substrate means more collisions and more enzyme–substrate complexes. So, the overall rate of reaction is increased. Eventually, because of the high substrate concentration, each enzyme molecule could be working at maximum turnover – that is, each active site is binding with substrate molecules all the time and there is no 'spare capacity' in the system. Increasing the substrate concentration beyond this point will have no effect on the activity of the enzyme because all the active sites are occupied all the time.



He above graph shows the effect of concentration on enzyme activity.

D. The presence of inhibitors

Inhibitors are substances that bind to enzymes and prevent them from forming enzyme–substrate complexes, as a result it causes, stop, or slow down, chemical reaction. There are two main types of inhibitors:

I. Irreversible inhibitors.

Irreversible inhibitors are bind tightly (strongly) and permanently to enzyme and affect their catalytic properties (Irreversible inhibitors bind strongly to enzymes, usually by a covalent bond, permanently altering the structure of the enzyme molecule and inactivating it). Example insecticides, pesticides, pain killer, heavy metal etc

II. Reversible inhibitor

Some substances inhibit enzymes action temporarily (bind to enzymes only weakly and the bond that holds them breaks easily releasing the inhibitor). This allows the enzyme to become active again. There are two main kinds of reversible inhibitors:

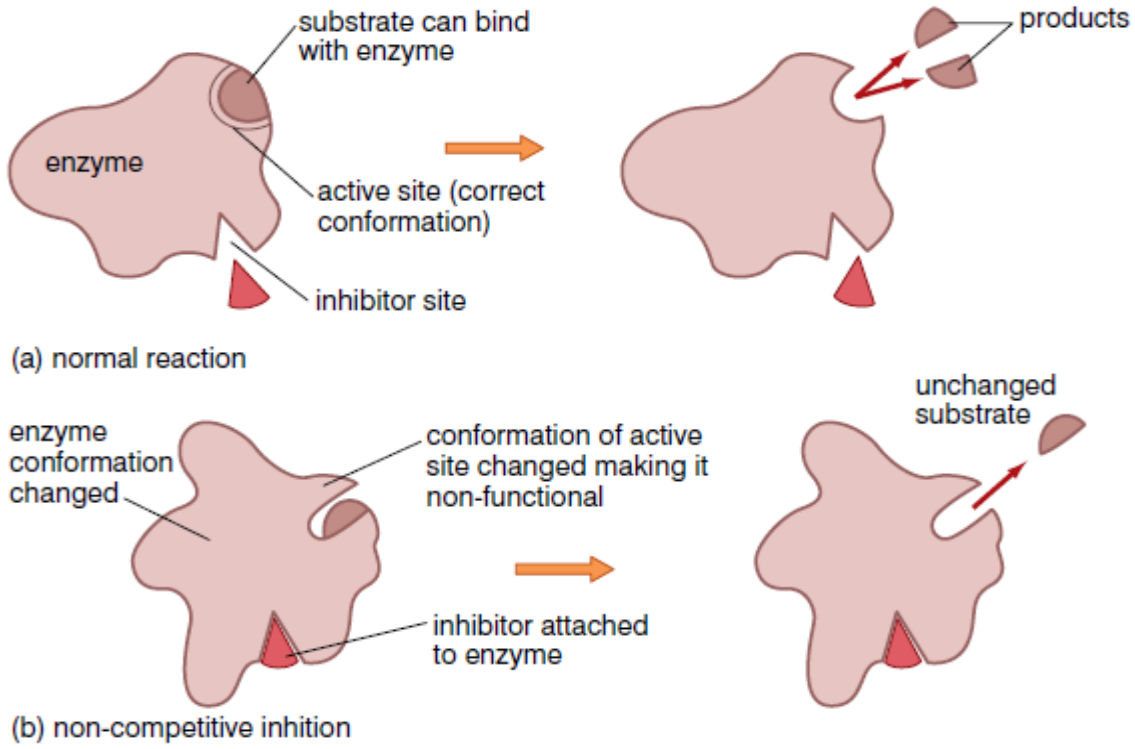
A. **Competitive reversible inhibitors**.

Competitive inhibitor competes with the normal substrate for binding to active site of enzyme because its similar to substrate structurally these allow binding or fitting to active site of enzyme. So, a competitive inhibitor blocks the active site so substrate molecules cannot bind. The overall effect on the rate of reaction depends on the relative concentrations of substrate and inhibitor molecules. Each molecule of competitive inhibitor can inhibit (temporarily) one enzyme molecule – but only if it can collide with the enzyme molecule and bind with the active site. ***Example*** Succinic dehydragenase which converts succinate to fumaric acid. It analogous to the structure of succinic acid . If its added to the reaction, the activity of succinic dehydragenase falls but the activity can be restored by increasing the concentration of succinic acid.

B. Non-competitive inhibitors

A molecule that alters the conformation of the active site by binding with the allosteric site of the enzyme; it prevents the substrate from binding and inhibits enzyme activity (do not compute with the substrate molecule for the active site). Such inhibitor has no structural similarity to the substrate but bind to other part of the enzyme which distorted the active site of the enzyme. Allosteric (regulatory) enzymes have distinct regulatory and catalytic site so, their activity can be enhanced (inhibited) by organic compounds that occur as intermediates in the sequence of reaction or an enzyme which have additional site for an effectors to bind.

The following figure shows the non competitive inhibitor



How do inhibitors control enzyme activity in living `cells?

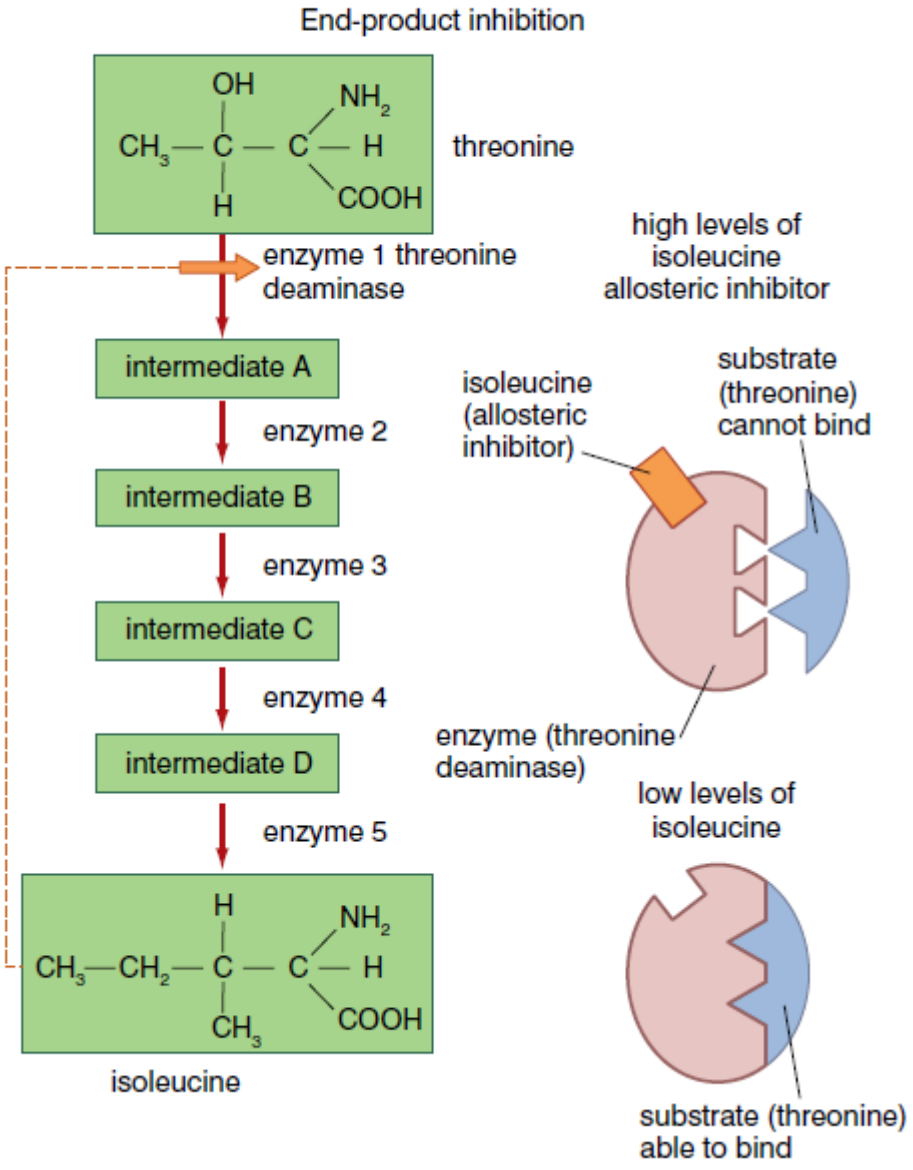
Many substances are produced in cells as a result of a metabolic pathway (a series of reactions), which can be represented as:

$$\text{Substance A} \xrightarrow{E1} \text{Substance B} \xrightarrow{E2} \text{Substance C} \xrightarrow{E3} \text{Substance D}$$

E1, E2 and E3 are enzymes catalyzing the reactions

All the reactions in this sequence are enzyme controlled. Therefore, inhibition of any of these enzymes will interrupt the process. However, the main function of this pathway is to produce substance D for use by the cell. If the requirement for substance D in the cell decreases, then the concentration of D will increase. This is at least inefficient (producing something that is not being used) and may be potentially harmful because high concentrations could be toxic. Such reaction sequences are often controlled by **end-product inhibition**.

- **end-product inhibition** ***when an end product inhibits the enzyme controlling the firststage of a reaction sequence*** **activator** ***a substance that removes an inhibitor.***



The above figure shows a metabolic pathway controlled by end product inhibition.

Review question of the unit

I. Give short answer for the following questions

1. What is enzyme and its nature?
2. List the main factors that affect the activity of enzyme and define it properly?
3. What is the difference between Coenzymes, Haloenzymes, Apoenzymes and cofactor?
4. Define the two models that represent enzyme action?
5. Define the following terms Inhibitor, irreversible inhibitor. reversible inhibitor?

Unit Four
Cell Biology

General objective of the unit
At the end of the lesson the student be able to:-

- Define the history of cell.
- Describe cell theory and investigate the size, structure and shape of cells.
- State the basic functions of cells.
- Appreciate that all life on Earth originates from cells.
- Compare the cell of plant and animal.

How did the modern cell theory develop?

The word cell is derived from a latin word called cellula which means room in latin.