Biomolecule - Part 3

Objectives

After going through this lesson, the learners will be able to understand the following:

- Metabolic basis for living
- The living state
- Chemical Reactions
- Enzymes
- Rate of reaction
- Nature of Enzyme Action
- Factors Affecting Enzyme Activity
- Classification and Nomenclature of Enzymes

Content Outline

- Introduction
- Metabolic Basis for Living
- The Living State
- Chemical Reactions
- Enzymes
- Rate of Reaction
- Nature of Enzyme Action
- Factors Affecting Enzyme Activity
- Classification and Nomenclature of Enzymes
- Co-factors
- Summary

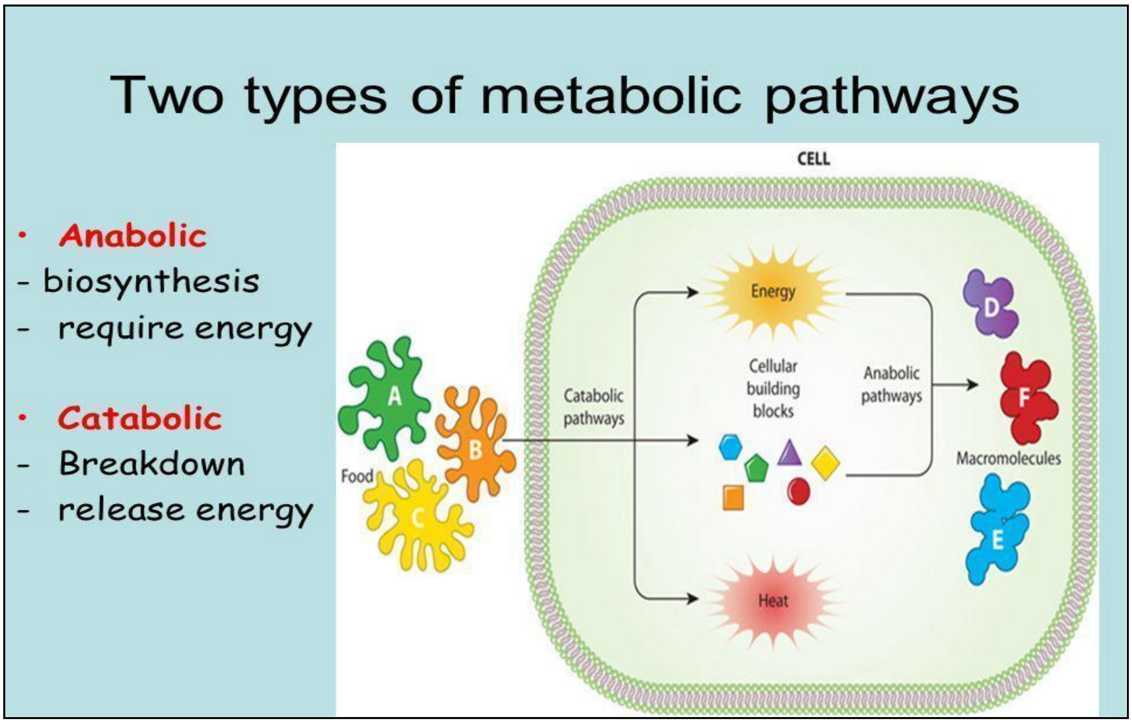
Introduction

What we have learnt till now is that living organisms, be it a simple bacterial cell, a protozoan, a plant or an animal, contain thousands of organic compounds. These compounds or biomolecules are present in certain concentrations (expressed as moles/cell or moles/litre etc.). One of the greatest discoveries ever made was the observation that all these biomolecules have a **turn over**. This means that they are constantly being changed into some other form and also made from some other biomolecules. This breaking and making is through chemical reactions constantly occurring in living organisms. Together all these chemical reactions are called **metabolism**. Each of the metabolic reactions results in the transformation of biomolecules. A few examples for such metabolic transformations are removal of CO_2 from amino acids making an amino acid into an amine, removal of amino group in a nucleotide base; hydrolysis of a glycosidic bond in a disaccharide, etc. We can list tens and thousands of such examples. Majority of these metabolic reactions do not occur in isolation but are always linked to some other reactions. In other words, metabolites are converted into each other in a series of linked reactions called metabolic pathways. These metabolic pathways are similar to the automobile traffic in a city. These pathways are either linear or circular. These pathways cross each other, i.e., there are traffic junctions. Flow of metabolites through metabolic pathway has a definite rate and direction like automobile traffic.

This metabolite flow is called the dynamic state of body constituents. What is most important is that this interlinked metabolic traffic is very smooth and without a single reported mishap for healthy conditions. Another feature of these metabolic reactions is that every chemical reaction is a **catalysed reaction**. There is no uncatalysed metabolic conversion in living systems.Even CO_2 dissolving in water, a physical process, is a catalysed reaction in living systems. The catalysts which hasten the rate of a given metabolic conversation are also proteins. These proteins with catalytic power are named **enzymes.**

Metabolic basis for living

Metabolic pathways can lead to a more complex structure from a simpler structure (for example, acetic acid becomes cholesterol) or lead to a simpler structure from a complex structure (for example, glucose becomes lactic acid in our skeletal muscle). The former cases are called biosynthetic pathways or **anabolic** pathways. The latter constitute degradation and hence are called **catabolic** pathways. Anabolic pathways, as expected, consume energy. Assembly of a protein from amino acids requires energy input. On the other hand, catabolic pathways lead to the release of energy.



For example, when glucose is degraded to lactic acid in our skeletal muscle, energy is liberated. This metabolic pathway from glucose to lactic acid which occurs in 10 metabolic steps is called glycolysis. Living organisms have learnt to trap this energy liberated during degradation and store it in the form of chemical bonds. As and when needed, this bond energy is utilized for biosynthetic, osmotic and mechanical work that we perform. The most important form of energy currency in living systems is the bond energy in a chemical called **adenosine triphosphate (ATP)**.

How do living organisms derive their energy? What strategies have they evolved? How do they store this energy and in what form? How do they convert this energy into work? You will study and understand all this under a sub-discipline called 'Bioenergetics' later in your higher classes.

The living state

You must understand that the tens and thousands of chemical compounds in a living organism, otherwise called metabolites, or biomolecules, are present at concentrations characteristic of each of them. For example, the blood concentration of glucose in a normal healthy individual is 4.5-5.0 mM, while that of hormones would be nanograms/mL. The most important fact of biological systems is that all living organisms exist in a steady-state characterised by concentrations of each of these biomolecules. These biomolecules are in a metabolic flux. Any chemical or physical process moves spontaneously to equilibrium. The steady state is a non-equilibrium state. One should remember from physics that systems at equilibrium cannot perform work. As living organisms work continuously, they cannot afford to reach equilibrium.

Hence, the living state is a non-equilibrium steady-state to be able to perform work; the living process is a constant effort to prevent falling into equilibrium. This is achieved by energy input. Metabolism provides a mechanism for the production of energy. Hence, the living state and metabolism are synonymous. Without metabolism there cannot be a living state.

Chemical Reactions

Chemical reaction is a process that changes, transforms Chemical compounds into other forms. The chemical compounds undergo two types of changes. A physical change simply refers to a change in shape without breaking of bonds. This is a physical process. Another physical process is a change in state of matter: when ice melts into water, or when water becomes a vapour. These are also physical processes. However, when bonds are broken and new bonds are formed during transformation, this will be called a chemical reaction. For example:

$$Ba(OH)_2 + H_2SO_4 \rightarrow BaSO_4 + 2H_2O$$

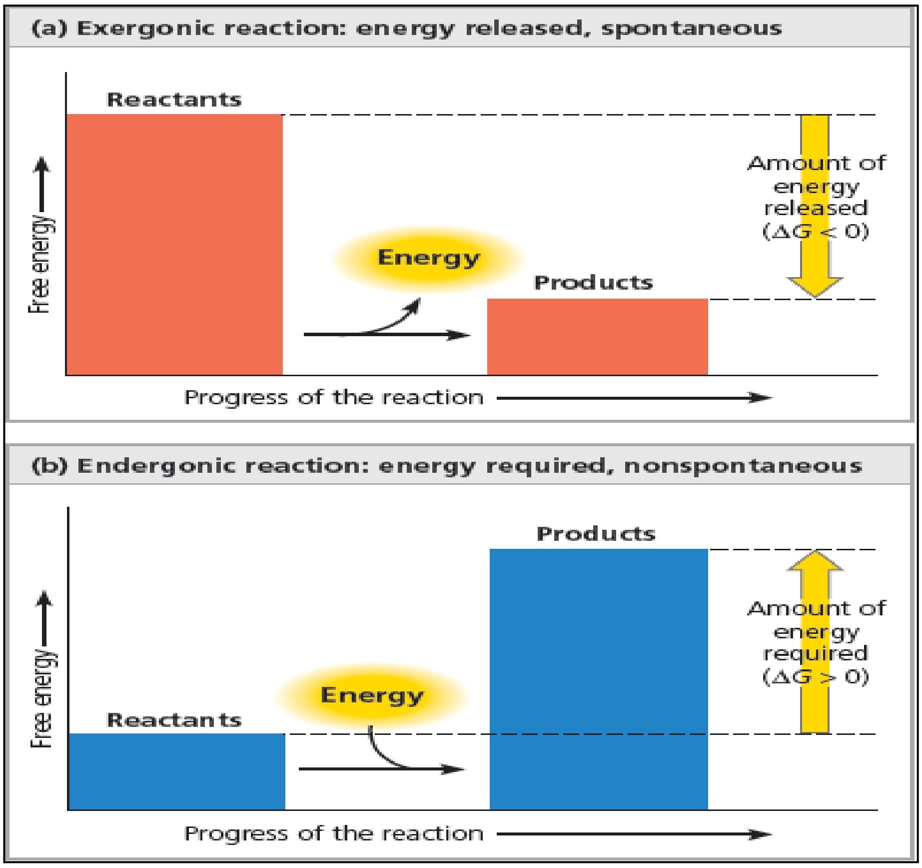
It is an inorganic chemical reaction. Similarly, hydrolysis of starch into glucose is an organic chemical reaction. A common example of chemical reaction in our body is the production of CO_2. The CO_2 produced is carried by blood to the lungs for exhalation. But this CO_2 is not completely dissolved in blood; actually it reacts with water to form highly soluble compound carbonic acid.

$$CO_2 + H_2O \rightarrow H_2CO_3$$

And this reaction is reversed in lungs, so as to release CO_2.

Energy In Reactions

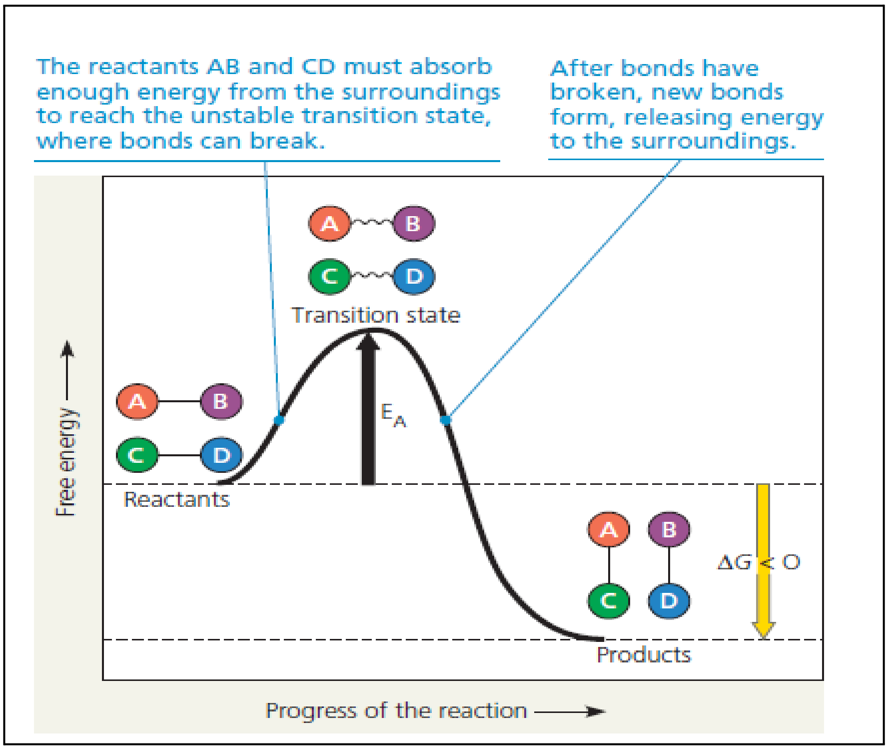
As in a chemical reaction there is transformation of bonds, so the breakage and formation of bonds involves changes in energy, some chemical reactions release energy and some chemical reactions absorb energy. Compounds undergo reaction are reactants and their resultant forms are the products. The reactions which usually release energy often occur quickly whereas those that absorb energy do not occur without the source of energy. Even the reactions which release energy do not occur so quickly. It's known as the Gibb's free energy or free energy which tells whether the reaction is spontaneous or not. This free energy represented by G is the energy that performs work even if the temperature and pressure is consistent throughout the system as in case of a living cell. Change in free energy represented by ΔG. This tells whether the reaction is energetically favorable or requires an input of energy. Following is the diagrammatic representation of the free energy changes in a reaction.



Even there is a need for some energy to start a reaction. This energy is known as the activation energy represented by E_A. After absorbing energy the reactants are in an unstable form that is the transition state. Like in graph shown below a reaction, reactants are

$$AB + CD \quad \rightarrow \quad AC + BD$$

Reactants **Products**



The EA acts as the barrier which tells whether the reaction proceeds further or not. For some reactions EA is low to reach transition state early but for some reaction EA is high and to reach transition state is not easily attained. There comes an enzyme which lowers the activation energy to reach the transition state.

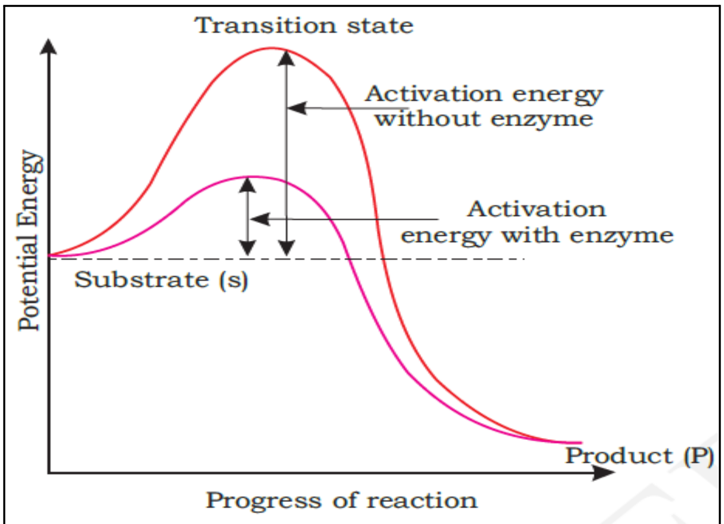


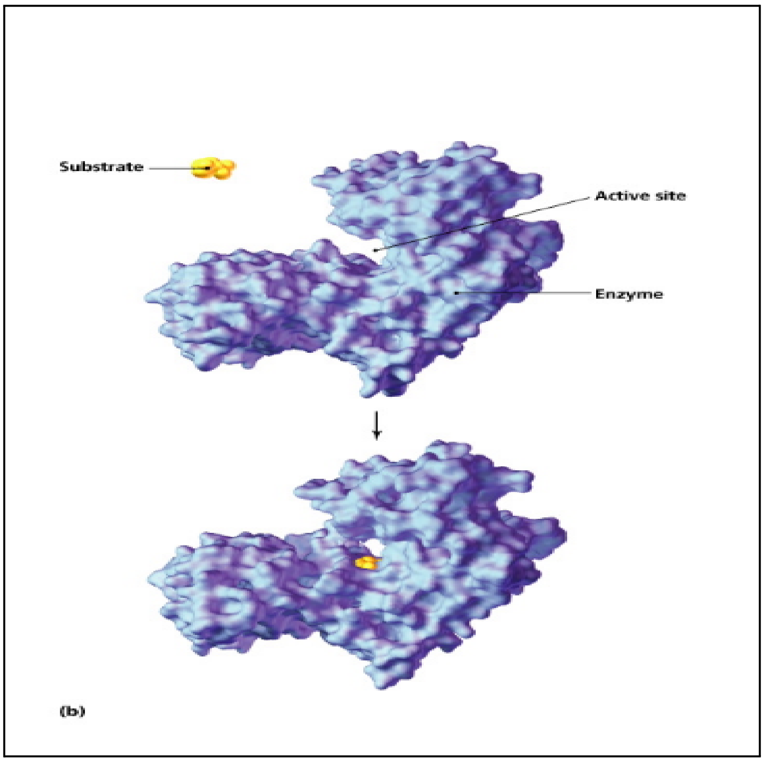
Figure 9.6 Concept of activation energy

The y-axis represents the potential energy content. The x-axis represents the progression of the structural transformation or states through the 'transition state'. You would notice two things: the energy level difference between S and P. If 'P' is at a lower level than 'S', the reaction is an exothermic reaction. One need not supply energy (by heating) in order to form the product. However, whether it is an exothermic or spontaneous reaction or an endothermic or energy-requiring reaction, the ‘S’ has to go through a much higher energy state or transition state.

The difference in average energy content of ‘S’ from that of this transition state is called ‘activation energy’. Enzymes eventually bring down this energy barrier making the transition of ‘S’ to ‘P’ more easy. So let’s define what is an enzyme?

Enzymes

Almost all enzymes are proteins. There are some nucleic acids that behave like enzymes. These are called ribozymes. One can depict an enzyme by a line diagram. An enzyme like any protein has a primary structure, i.e., amino acid sequence of the protein. An enzyme like any protein has the secondary and the tertiary structure. When you look at a tertiary structure you will notice that the backbone of the protein chain folds upon itself, the chain criss-crosses



itself and hence, many crevices or pockets are made. One such pocket is the ‘active site’. An active site of an enzyme is a crevice or pocket into which the substrate fits.

Thus enzymes, through their active site, catalyze reactions at a high rate. Enzyme catalysts differ from inorganic catalysts in many ways, but one major difference needs mention.

Inorganic catalysts work efficiently at high temperatures and high pressures, while enzymes get damaged at high temperatures (say above 40°C). However, enzymes isolated from organisms who normally live under extremely high temperatures (e.g., hot vents and sulphur springs), are stable and retain their catalytic power even at high temperatures (upto 80°-90°C). Thermal stability is thus an important quality of such enzymes isolated from thermophilic organisms.

Rate of reaction

Rate of a physical or chemical process refers to the amount of product formed per unit time. It can be expressed as:

$$\text{rate} = \frac{\delta P}{\delta t}$$

Rate can also be called velocity if the direction is specified. Rates of physical and chemical processes are influenced by temperature among other factors. A general rule of thumb is that rate doubles or decreases by half for every 10°C change in either direction. Catalysed reactions proceed at rates vastly higher than that of uncatalysed ones. When enzyme catalysed reactions are observed, the rate would be vastly higher than the same but uncatalysed reaction. For example,

$$\underset{\text{carbon dioxide}}{CO_2} + \underset{\text{water}}{H_2O} \xrightleftharpoons{\text{Carbonic anhydrase}} \underset{\text{carbonic acid}}{H_2CO_3}$$

In the absence of any enzyme this reaction is very slow, with about 200 molecules of H_2CO_3 being formed in an hour. However, by using the enzyme present within the cytoplasm called carbonic anhydrase, the reaction speeds dramatically with about 600,000 molecules being formed every second. The enzyme has accelerated the reaction rate by about 10 million times. The power of enzymes is incredible indeed!

There are thousands of types of enzymes each catalysing a unique chemical or metabolic reaction. A multistep chemical reaction, when each of the steps is catalysed by the same enzyme complex or different enzymes, is called a metabolic pathway. For example,

$$\text{Glucose} \rightarrow 2 \text{ Pyruvic acid}$$

$$C_6H_{12}O_6 + O_2 \rightarrow 2C_3H_4O_3 + 2H_2O$$

is actually a metabolic pathway in which glucose becomes pyruvic acid through ten different enzyme catalysed metabolic reactions. These reactions are part of respiration. At this stage you should know that this very metabolic pathway with one or two additional reactions gives rise to a variety of metabolic end products. In our skeletal muscle, under anaerobic conditions, lactic acid is formed.

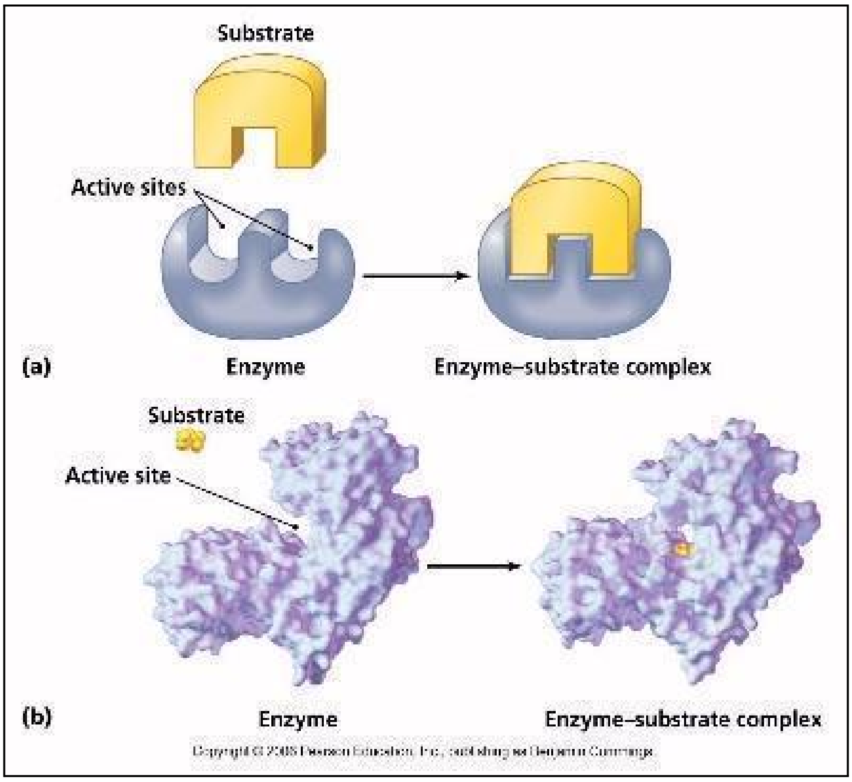
Under normal aerobic conditions, pyruvic acid is formed. In yeast, during fermentation, the same pathway leads to the production of ethanol (alcohol). Hence, in different conditions different products are possible.

How do Enzymes bring about such High Rates of Chemical Conversions?

To understand this we should study enzymes a little more. We have already understood the idea of an 'active site'. The chemical or metabolic conversion refers to a reaction. The chemical which is converted into a product called a 'substrate'. Hence enzymes, i.e., proteins with three dimensional structures including an 'active site', convert a substrate (S) into a product (P). symbolically, this can be depicted as:

$$S \rightarrow P$$

It is now understood that the substrate 'S' has to bind the enzyme at its 'active site' within a given cleft or pocket. The substrate has to diffuse towards the 'active site'. There is thus, an obligatory formation of an 'ES' complex. E stands for enzyme. This complex formation is a transient phenomenon.



During the state where the substrate is bound to the enzyme active site, a new structure of the substrate called transition state structure is formed. Very soon, after the expected bond breaking/making is completed, the product is released from the active site. In other words, the structure of the substrate gets transformed into the structure of product(s).

Nature of Enzyme Action

Each enzyme (E) has a substrate (S) binding site in its molecule so that a highly reactive enzyme-substrate complex (ES) is produced. This highly reactive enzyme-substrate complex (ES) is produced. This complex is short-lived and dissociates into its product(s) P and the unchanged enzyme with an intermediate formation of the enzyme-product complex (EP).
The formation of the ES complex is essential for catalysis.

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

The catalytic cycle of an enzyme action can be described in the following steps:

- First, the substrate binds to the active site of the enzyme, fitting into the active site.
- The binding of the substrate induces the enzyme to alter its shape, fitting more tightly around the substrate.

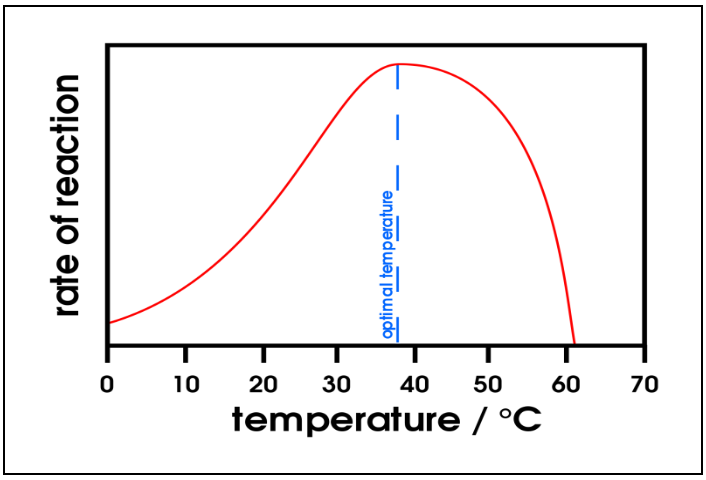
- The active site of the enzyme, now in close proximity to the substrate, breaks the chemical bonds of the substrate and the new enzyme- product complex is formed.
- The enzyme releases the products of the reaction and the free enzyme is ready to bind to another molecule of the substrate and run through the catalytic cycle once again.

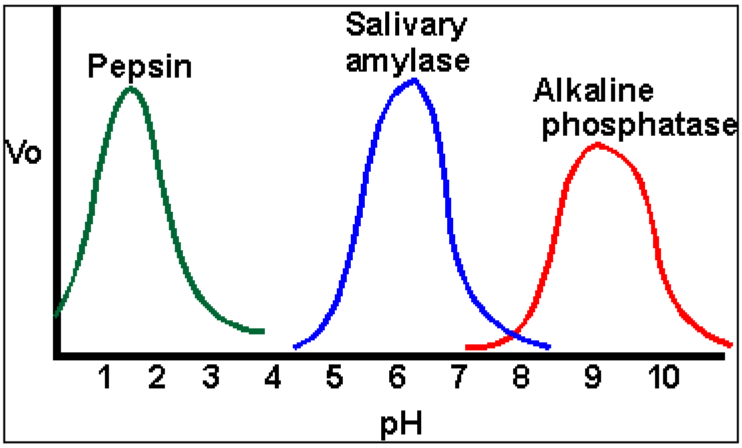
Factors Affecting Enzyme Activity

The activity of an enzyme can be affected by a change in the conditions which can alter the tertiary structure of the protein. These include temperature, pH, and change in substrate concentration or binding of specific chemicals that regulate its activity.

- **Temperature and pH**

Enzymes generally function in a narrow range of temperature and pH. Each enzyme shows its highest activity at a particular temperature and pH called the optimum temperature and optimum pH. Activity declines both below and above the optimum value.





Low temperature preserves the enzyme in a temporarily inactive state whereas high temperature destroys enzymatic activity because proteins are denatured by heat. Like in human beings optimal temperature for enzyme activity is 37ºC whereas bacteria say thermophilic bacteria tolerates 70ºC.

- **Concentration of Substrate**

With the increase in substrate concentration, the velocity of the enzymatic reaction rises at first. The reaction ultimately reaches a maximum velocity (Vmax) which is not exceeded by any further rise in concentration of the substrate. This is because the enzyme molecules are fewer than the substrate molecules and after saturation of these molecules; there are no free enzyme molecules to bind with the additional substrate molecules.The activity of an enzyme is also sensitive to the presence of specific chemicals that bind to the enzyme. When the binding of the chemical shuts off enzyme activity, the process is called **inhibition** and the chemical is called an **inhibitor**.

When the inhibitor closely resembles the substrate in its molecular structure and inhibits the activity of the enzyme, it is known as a competitive **inhibitor**. Due to its close structural similarity with the substrate, the inhibitor competes with the substrate for the substrate binding site of the enzyme. Consequently, the substrate cannot bind and as a result, the enzyme action declines, e.g., inhibition of succinic dehydrogenase by malonate which closely resembles the substrate succinate in structure. Such competitive inhibitors are often used in the control of bacterial pathogens.

Classification and Nomenclature of Enzymes

Thousands of enzymes have been discovered, isolated and studied. Most of these enzymes have been classified into different groups based on the type of reactions they catalyse. Enzymes are divided into 6 classes each with 4-13 subclasses and named accordingly by a four-digit number.

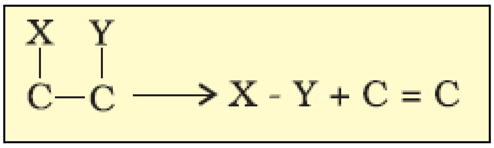
Oxidoreductases/dehydrogenases: Enzymes which catalyse oxidoreduction between two substrates S and S' e.g., S reduced + S' oxidised → S oxidised + S' reduced.

Transferases: Enzymes catalysing a transfer of a group, G (other than hydrogen) between a pair of substrate S and S' e.g.,

S – G + S' →S + S' – G

Hydrolases: Enzymes catalysing hydrolysis of ester, ether, peptide, glycosidic, C-C, C-halide or P-N bonds.

Lyases: Enzymes that catalyse removal of groups from substrates by mechanisms other than hydrolysis leaving double bonds.



Isomerases: Includes all enzymes catalysing inter-conversion of optical, geometric or positional isomers.

Ligases: Enzymes catalysing the linking together of 2 compounds, e.g., enzymes which catalyse joining of C-O, C-S, C-N, P-O etc. bonds.

Co-factors

Enzymes are composed of one or several polypeptide chains. However, there are a number of cases in which non-protein constituents called cofactors are bound to the enzyme to make the enzyme catalytically active. In these instances, the protein portion of the enzymes is called the apoenzyme. Three kinds of cofactors may be identified: prosthetic groups, coenzymes and metal ions.

Prosthetic groups are organic compounds and are distinguished from other cofactors in that they are tightly bound to the apoenzyme. For example, in peroxidase and catalase, which catalyze the breakdown of hydrogen peroxide to water and oxygen, haem is the prosthetic group and it is a part of the active site of the enzyme.

Coenzymes are also organic compounds but their association with the apoenzyme is only transient, usually occurring during the course of catalysis. Furthermore, coenzymes serve as cofactors in a number of different enzyme catalyzed reactions. The essential chemical components of many coenzymes are vitamins, e.g.,coenzyme nicotinamide adenine dinucleotide (NAD) and NADP contain the vitamin niacin.

A number of enzymes require metal ions for their activity which form coordination bonds with side chains at the active site and at the same time form one or more coordination bonds with the substrate, e.g., zinc is a cofactor for the proteolytic enzyme carboxypeptidase. Catalytic activity is lost when the cofactor is removed from the enzyme which testifies that they play a crucial role in the catalytic activity of the enzyme.

Key points

A chemical reaction involves changes in chemical bonds that join atoms in compounds.

Summary

To conclude a chemical reaction involves the transformation of bonds. So as to form products, reactions undergo catalysis, with the help of enzymes. Enzymes hasten the chemical reactions. Spontaneity of the reaction depends upon the activation energy. It's the enzymes which lowers the activation energy. Overall rate of reaction depends upon the activity of an enzyme.