BTY 202 ENZYMOLOGY

UNIT- 1

ENZYME CLASSIFICATION

The human body is composed of different types of cells, tissues and other complex organs. For efficient functioning, our body releases some chemicals to accelerate biological processes such as respiration, digestion, excretion and a few other metabolic activities to sustain a healthy life. Hence, enzymes are pivotal in all living entities which govern all the biological processes.

"Enzymes can be defined as biological polymers that catalyze biochemical reactions."

The majority of enzymes are proteins with catalytic capabilities crucial to perform different processes. Metabolic processes and other chemical reactions in the cell are carried out by a set of enzymes that are necessary to sustain life.

The initial stage of metabolic process depends upon the enzymes, which react with a molecule and is called the substrate. Enzymes convert the substrates into other distinct molecules, which are known as products.

The regulation of enzymes has been a key element in clinical diagnosis because of their role in maintaining life processes. The macromolecular components of all enzymes consist of protein, except in the class of RNA catalysts called ribozymes. The word ribozyme is derived from the ribonucleic acid enzyme. Many ribozymes are molecules of ribonucleic acid, which catalyze reactions in one of their own bonds or among other RNAs.

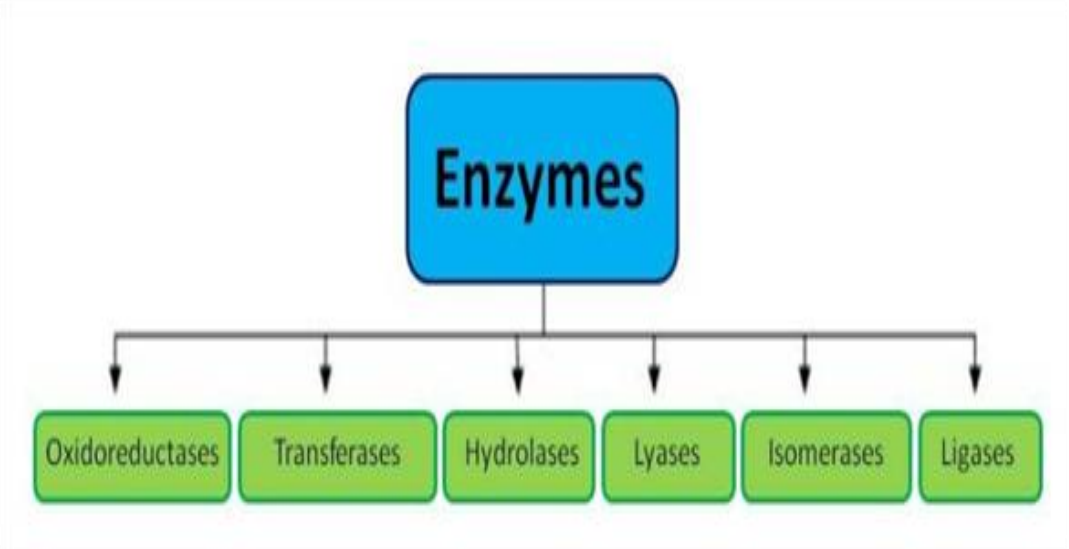
Enzymes are found in all tissues and fluids of the body. Catalysis of all reactions taking place in metabolic pathways is carried out by intracellular enzymes. The enzymes in the plasma membrane govern the catalysis in the cells as a response to cellular signals and enzymes in the circulatory system regulate the clotting of blood. Most of the critical life processes are established on the functions of enzymes.

Enzyme Structure

Enzymes are a linear chain of amino acids, which give rise to a three-dimensional structure. The sequence of amino acids specifies the structure, which in turn identifies the catalytic activity of the enzyme. Upon heating, the enzyme's structure denatures, resulting in a loss of enzyme activity, which typically is associated with temperature.

Compared to its substrates, enzymes are typically large with varying sizes, ranging from 62 amino acid residues to an average of 2500 residues found in fatty acid synthase. Only a small section of the structure is involved in catalysis and is situated next to the binding sites. The catalytic site and binding site together constitute the enzyme's active site. A small number of ribozymes exist which serve as an RNA-based biological catalyst. It reacts in complex with proteins.

Enzymes Classification



Earlier, enzymes were assigned names based on the one who discovered them. With further research, classification became more comprehensive.

According to the International Union of Biochemists (I U B), enzymes are divided into six functional classes and are classified based on the type of reaction in which they are used to catalyze. The six kinds of enzymes are hydrolases, oxidoreductases, lyases, transferases, ligases and isomerases.

Listed below is the classification of enzymes discussed in detail:

Types	Biochemical Property
Oxidoreductases	The enzyme Oxidoreductase catalyzes the oxidation reaction where the electrons tend to travel from one form of a molecule to the other.
Transferases	The Transferases enzymes help in the transportation of the functional group among acceptors and donor molecules.
Hydrolases	Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction by adding water to cleave the bond and hydrolyze it.
Lyases	Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds.
Isomerases	The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.
Ligases	The Ligases enzymes are known to charge the catalysis of a ligation process.

Oxidoreductases

These catalyze oxidation and reduction reactions, e.g. pyruvate dehydrogenase, catalysing the oxidation of pyruvate to acetyl coenzyme A.

Transferases

These catalyze transferring of the chemical group from one to another compound. An example is a transaminase, which transfers an amino group from one molecule to another.

Hydrolases

They catalyze the hydrolysis of a bond. For example, the enzyme pepsin hydrolyzes peptide bonds in proteins.

Lyases

These catalyze the breakage of bonds without catalysis, e.g. aldolase (an enzyme in glycolysis) catalyzes the splitting of fructose-1, 6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

Isomerases

They catalyze the formation of an isomer of a compound. Example: phosphoglucomutase catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate (phosphate group is transferred from one to another position in the same compound) in glycogenolysis (glycogen is converted to glucose for energy to be released quickly).

Ligases

Ligases catalyze the association of two molecules. For example, DNA ligase catalyzes the joining of two fragments of DNA by forming a phosphodiester bond.

Factors affecting enzyme action

Enzymes are biological catalysts that accelerate the rate of chemical reactions in the biological system of living beings. Like catalysts, enzymes are also affected by a number of factors that regulate enzyme action. These factors are related to the chemical nature of enzymes as enzymes are proteins, and proteins are affected by most of these factors.

1. Temperature

Enzymes are thermolabile or heat-sensitive because they are proteinaceous in nature.

Temperature affects the speed of the reaction by changing the activity of the enzyme involved.

Like in most proteins, the rate of an enzyme action increases with the rise in temperature.

The rate increases by two to three-fold with every 10°C rise in temperature.

However, as the temperature becomes high, the activity of an enzyme decreases. Temperature above 60°C causes destruction and coagulation of enzymes.

This temperature is detrimental to enzymatic reactions as the structure of the enzymes changes irreversibly.

Some enzymes found in dry tissues, however, can endure higher temperatures like 100°C to 120°C.

While studying the effect of increasing temperature, it can be observed that the initial velocity of the reaction steadily increases with temperature.

However, once a particular temperature is crossed, the enzymatic activity begins to cease with less and less product being formed.

That particular temperature or temperature range is termed as an optimal temperature of an enzyme. This temperature not easy to determine precisely because it is a somewhat vague concept, and will depend on the length of time over which the measurements are made.

Despite that, the approximate values obtained often show a distinct correlation with the body temperatures of the organisms from which the enzyme came.

Thus, enzymes found in mammals have optimal temperature in the range of 35°C to 45°C, but enzymes in bacteria living in hot springs may have an optimal temperature of 80°C.

At low temperatures, the catalytic activity of the enzyme predominates, although some thermal denaturation takes place during this period.

As the temperature reaches 0°C, inactivation of the enzyme might be observed, which is a reversible type of change and the enzyme regains its catalyzing power upon increasing the temperature to the optimum.

The effect of temperature and heat is also observed during the storage of enzymes as the best preservation of enzyme preparations is by refrigeration or quick freezing.

2. pH (using buffer solutions)

pH is another important parameter that affects the activity of the enzyme by changing its shape and structure.

Like temperature, pH, or the H+ ion concentration of the medium where the enzyme is present bring about significant changes in the activity of such enzymes.

The change in pH causes ionization of amino acid atoms and molecules while also changing the degree of dissociation of the substrate.

Besides, a change in pH might also bring changes in the charges present on the enzyme, which affects the formation of the enzyme-substrate complex.

Thus, enzymes have a particular value of pH or concentration of H+ ion at which the enzyme acts best.

The activity of the enzyme, however, decreases with any change (increase or decrease) in the said pH value.

This specific pH at which the activity of an enzyme is maximum is termed as the optimal pH for that particular enzyme.

This pH might be specific t each enzyme and is determined by various factors like the composition and structure of the enzyme.

Other factors that determine the optimum pH for an enzyme include the nature of the buffer system, the presence of other colloids, activators, or inhibitors, the age of the cell tissue, and the nature of the substrate.

Change in the pH of the buffer solution used brings about changes in the activity of an enzyme, as it affects the structure and shape of the enzyme.

The use of different buffer solutions with lower or higher pH values might affect the ionization state of the acidic (carboxyl) or basic (amine) groups.

With the change in the ionized state of amino acid, the ionic bonds maintaining the three-dimensional structure of the enzymes are also affected.

This might leads to a reduction in enzyme activity and even inactivation.

pH is also found to change the structure and shape of substrates which prevents the binding of substrate to the active site of the enzyme.

These changes might be reversible for a narrow range of pH, but if the pH change is significant, enzymes and substrate might be denatured, causing permanent loss of activity.

3. Enzyme concentration

The effect of enzyme concentration on the activity of an enzyme can only be observed when the substrate is present in excess, causing the reaction to be independent of the substrate concentration.

In that case, any change in the number of products formed over a particular period of time will be dependent on the enzyme concentration.

Thus, to observe the effect of enzyme concentration, zero-order reactions are to be studied.

In order to determine the concentration of enzyme in a system, the amount of substrate catalyzed is to be determined. This, in turn, depends on other factors like temperature and pH.

However, to determine the relationship between the concentration of enzyme and rate of enzyme action, the substrate must be present in excess, resulting in a zero-order reaction.

Under such conditions, enzyme action increases linearly with time, causing double the amount of products to be formed as the process is run for double time.

The speed of the reaction or the activity of the enzyme increases with the increase in enzyme concentration as long as there as enough substrate molecules to bind to the active sites of the enzyme.

Once all the active sites are filled, the enzyme activity doesn't increase.

4. Substrate concentration

The effect of the increase in substrate concentration on the enzymatic action is to be determined at a constant concentration of the enzyme.

When the concentration of the enzyme is constant, the rate of a chemical reaction or the activity of the enzyme increases with the increase in substrate concentration up to a point where it is maximum.

After this point, the increase in substrate doesn't change the activity of the enzyme or the rate of the reaction.

It is because, with an increase in substrate concentration, the number of substrate molecules binding to the active site of the enzyme increases.

But once all the active sites are filled, increasing substrate concentration doesn't affect the activity of the enzyme.

Thus, the enzyme action is the highest when all enzyme molecules are present in the form of the enzyme-substrate complex.

5. Inhibitor concentration

Inhibitors are compounds that convert the enzymes into inactive substances and thus adversely affect the rate of enzymatically-catalyzed reaction is called an enzyme inhibitor, and the process involved is termed enzyme inhibition.

These molecules might affect the activity of the enzyme by either binding to the active site or some other regions of the enzyme.

This prevents the binding of substrate to the active site, affecting the rate of formation of the enzyme-substrate complex.

The concentration of such inhibitors is indirectly proportional to the rate of enzyme action.

With the increase in the concentration of inhibitors, the rate of formation of enzyme-substrate complex decreases which, in turn, decreases the concentration of products formed.

Depending on the nature of inhibition (competitive or non-competitive), the effect of inhibitors might be reduced by changing the concentration of the substrate.

In competitive inhibition, the rate of enzyme action can be increased by increasing the substrate concentration in the medium.

In non-competitive inhibition, however, the rate cannot be increased even with the increase in substrate concentration.

Lock and Key Model- Mode of Action of Enzymes

Enzymes are biological catalysts. These are commonly proteins but also include RNA (ribozymes) molecules that catalyze chemical reactions by lowering the activation energy of a reaction. These are known to speed up the rate of a reaction millions of times faster than the reaction without enzymes. Nearly all biological reactions require enzymes to transform substrate into products. The substrate is the reactant molecule upon which enzymes act during a chemical reaction, and products are the substances formed as a result of a chemical reaction. A single reactant molecule can decompose to give multiple products.

Similarly, two reactants can enter into a reaction to yield products. These are reusable even after the completion of the reaction. Chemical properties such as charge and pH are vital in enzymatic reactions.

Binding between enzymes and reactant molecules takes place in such a way that chemical bond-breaking and bond-forming processes occur more readily. Meanwhile, no change in Δthe *G* value of a reaction takes place, thereby not altering the energy-releasing or energy-absorbing process of the reaction. However, it lowers the energy of the transition state, the topmost unstable state where the activated complex is formed from reactants that later give products.

Enzyme's Active site and Substrate Specificity

Enzymes are relatively larger than the substrates, whose only a small fraction is involved in catalysis by reducing chemical activation energy, also known as the catalytic site, and the other portion for binding with the substrate and orienting them also known as the binding site. The catalytic site and binding site altogether form the active site of an enzyme. Usually, there are two active sites in an enzyme.

The active site of enzymes is a cleft portion, composed of a small number of a unique combination of amino acid residues, usually three to four in number, which make up only ~10-20% of the volume of an enzyme.

The remaining amino acids are used to maintain tertiary structure by proper scaffold folding through non-covalent interactions.

Non-covalent interaction between enzyme and substrate in correct orientation favors their reaction. These interactions include hydrogen bonds, hydrophobic bonds, ionic interactions, and Van der Waal's interactions.

However, transient covalent bonds between enzymes and substrates are also formed during the time of reaction.

Side chains of amino acids play an important role in highly specific three-dimensional conformation at the level of the active site. These are large or small, hydrophilic or hydrophobic, acidic or basic.

The specific shape, size, and chemical behavior of enzymes are determined by the nature of amino acids and their 3D space in the active site.

Specificity is a distinctive feature of enzymes where they have a unique ability to choose an exact substrate from a group of similar chemical molecules. Their specificity towards their substrate varies to a different extent. These are of different types, namely: Bond specificity, Group specificity. Substrate specificity, Stereospecificity, Geometrical specificity, and Co-factor specificity

Substrate specificity is also k/a absolute specificity for the enzyme's specificity towards one substrate and one reaction. For e.g., Lactase acts on the B-1-4 glycosidic linkage of lactose to yield galactose and glucose. The restrictive nature of enzymes towards the choice of substrate can be attributed to the enzymatic activity

of two oxidoreductase enzymes. Alcohol dehydrogenase uses its substrate alcohol while lactic acid dehydrogenase act on lactic acid. Although these two enzymes function with the mechanism of oxidation and reduction reaction, their substrates can't be used interchangeably. This is because the different structure of each substrate prevents their fitting into the active site of the alternative enzyme.

In most cases, cofactors, the non-protein molecules, are required to ensure an efficient enzyme-facilitated chemical reaction. These function to bind with enzymes via either ionic interaction or covalent interactions. Metal ions (such as minerals) and co-enzymes (vitamin derivatives) are cofactors.

Lock and Key Model

A German scientist, Emil Fischer postulated the lock and key model in 1894 to explain the enzyme's mode of action. Fischer's theory hypothesized that enzymes exhibit a high degree of specificity towards the substrate. This model assumes that the active site of the enzyme and the substrate fit perfectly into one another such that each possesses specific predetermined complementary geometric shapes and sizes. Thus, the shape of the enzyme and substrate do not influence each other. This specificity is analog to the lock and key model, where the lock is the enzyme, and the key is the substrate. In certain circumstances, if a second substrate similar in shape and size to the primary substrate is made to bind to the enzyme, this second substrate also fits in the active site too.

Unit – 2

Enzymes are biological catalysts; they speed up chemical reactions but they are not changed by the reaction. Enzymes are *reusable;* because they are not consumed by the reaction one enzyme can carry out hundreds, even thousands, of reactions. To understand how enzymes function requires first understanding the relevant vocabulary.

Enzyme — A molecule that facilitates a chemical reaction, but is not part of the chemical reaction. Usually made of proteins (but can be RNA-based).

- **Substrate(s)** — The reactant(s) of the chemical reaction facilitated by the enzyme. The substrate(s) must bind to the enzyme to initiate the reaction.
- **Active Site** — The location on the enzyme where the substrates bind and where the chemical reaction occurs. Usually the active site has a very fixed, unique shape that perfectly matches the substrate(s).
- **Inhibitor** — A chemical molecule that can bind to an enzyme and slow or prevent the chemical reaction.

Types of Enzyme Inhibition

The two main types of enzyme inhibition are **reversible inhibition** and **irreversible inhibition**. The difference is determined by the strength of the interaction between the enzyme and the inhibitor. In addition, reversible inhibition can be further subdivided into **competitive**, **non-competitive**, and **uncompetitive** inhibition based on *where* the inhibitor binds to the enzyme.

Reversible and Irreversible Enzyme Inhibition

All enzyme inhibitors act either reversibly (weak, non-covalent interactions that are not permanent) or irreversibly (covalently alters the shape of the enzyme and/or active site).

- **Irreversible Inhibition** — An inhibitor chemically modifies the enzyme, usually by forming covalent bonds with the R-groups of certain amino acids. The interaction permanently inactivates the enzyme. The most commonly altered amino acids are located at the active site, but irreversible inhibition can occur anywhere on the enzyme.
    - *Example:* The figure shows how the irreversible inhibitor **diisopropylfluorophosphate (DFP)** chemically alters a critical serine R-group (��2−��) at the active site of the enzyme **acetylcholine esterase**, an enzyme necessary for proper neural functioning. Because

it permanently inactivates this important neural enzyme, DFP is classified as a potent neurotoxin, and a dose of less than 100 mg can be lethal.

- **Reversible Inhibition** — An inhibitor temporarily binds to the enzyme and either blocks access to or alters the shape of the active site. However, the effect can be reversed once the inhibitor is not longer bound to the enzyme. When the inhibitor leaves, the enzyme returns to normal functioning.
    - *Example:* As mentioned, the antibiotic **penicillin** inhibits bacterial cell wall formation. It binds to the enzyme **transpeptidase**, which has the important job of joining together peptidoglycan molecules to form the protective bacterial cell wall. Penicillin binds to transpeptidase at its active site and prevents the peptidoglycan molecules from binding. However, the binding of penicillin is *not permanent,* it will eventually leave the active site of the enzyme so its normal functioning can resume

Competitive, Non-competitive, and Uncompetitive Inhibition

Reversible inhibitors can be further classified as competitive, noncompetitive, or uncompetitive inhibitors based solely on *where* on the enzyme they temporarily bond.

Competitive Inhibitors

Competitive inhibitors compete with the substrate at the active site and therefore increase Km (the Michaelis-Menten constant). However, Vmax is unchanged because, with enough substrate concentration, the reaction can still complete. The graph plot of enzyme activity against substrate concentration would be shifted to the right due to the increase of the Km, whilst the Lineweaver-Burke plot would be steeper when compared with no inhibitor.

Non-Competitive Inhibitors

Non-competitive inhibitors bind to another location on the enzyme and as such decrease VMAX. However, KM is unchanged. This is demonstrated by a lower maximum on a graph plotting enzyme activity against substrate concentration and a higher y-intercept on a Lineweaver-Burke plot when compared with no inhibitor.

Allosteric Inhibition

Allosteric enzymes display a sigmoidal curve in contrast to the hyperbolic curve displayed by Michaelis-Menten Enzymes. This is because most allosteric enzymes contain multiple sub-units which can affect each other when the substrate binds to the enzyme. Inhibition can affect either K0.5, which is the substrate concentration for half-saturation, Vmax or both. This results in a shift of the curve to the right, and in the case of reducing Vmax, shifts the curve down.

Allosteric enzymes have two states: a low-affinity state dubbed the "T" state and the high-affinity "R" state. Inhibitors work by preferentially binding to the T state of an allosteric enzyme, causing the enzyme to maintain this low-affinity state.

This is extremely useful to limit the amount of an enzyme's product, as the product can then go on to inhibit the same type of enzyme to ensure the amount of product is not excessive. This is known as feedback inhibition. For example, ATP allosterically inhibits pyruvate kinase to prevent increased formation of pyruvate, so less ATP is eventually formed. Additionally, phosphofructokinase is allosterically inhibited by citrate, an intermediate of the TCA cycle. This means that glyco lysis will be limited when there is high ATP generation from the TCA cycle.

Enzyme kinetics

Enzyme kinetics involves the measurement of the rate at which chemical reactions that are catalyzed by enzymes occur. Knowledge about the kinetics of an enzyme can reveal useful information about its catalytic mechanism, role in metabolism, factors that impact its activity, and mechanisms of inhibition.

This article will cover the basic principles of enzyme kinetics, including the reaction equation, rate of reaction and maximal velocity (V_{max}) and Michaelis Constant (K_m).

Rate of reaction

Enzymes are thought to form a complex with the substrates to catalyze the reaction. This process can be illustrated with the simplified equation, where e is the enzyme, S is the substrate, and P is the product:

$$E + S \Leftrightarrow ES \Rightarrow P + E$$

The first step of the equation, which is reversible, has the reaction rate constant of k_{+1} to produce the enzyme substrate complex and k_{-1} for the reverse reaction. The reaction rate constant for the second step of the equation, which is not reversible, is k_{+2}.

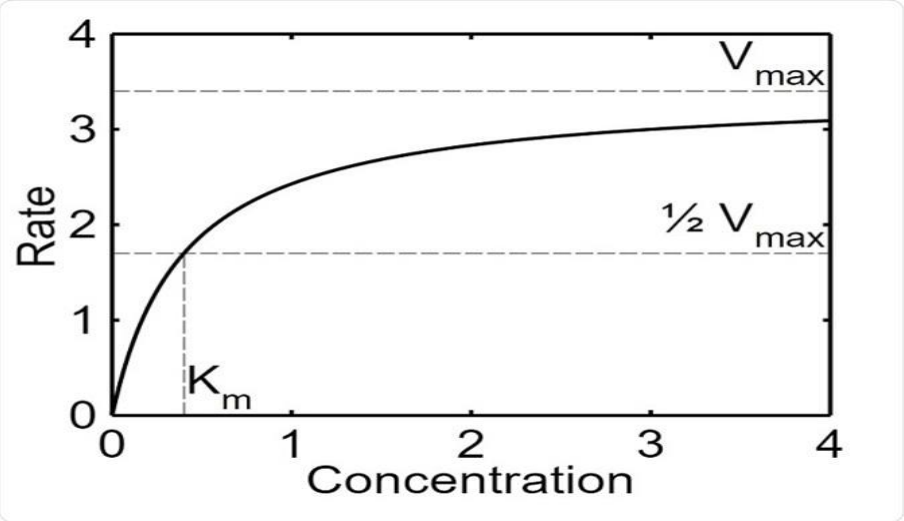
The rate of reaction (v), which is the rate at which the product is formed, is defined by the following equation: $v = d[P]/dt = k_{+2}[ES]$

The square brackets in the above equation represent the molar concentration of the substance specified within, so [P] refers to the molar concentration of the product and [ES] the molar concentration of the enzyme substrate.

V_{max} and K_m

Leonor Michaelis and Maud Menten introduced a mathematical illustration to describe the action of enzymes with two constants, V_{max} and K_m.

The maximal velocity (V_{max}) refers to the point at which the increase the concentration of the substrate does not increase the rate of a reaction catalyzed by an enzyme. This occurs because the substrate molecules saturate the active sites of the enzyme and are not able to form more complexes with the enzyme. This value is given as a rate (mmol/s), which is the maximum velocity of the reaction when the enzyme is saturated.



The Michaelis constant (K_m) is the concentration of the substrate when half of the active binding sites of an enzyme are occupied by the substrate. The constant helps to depict the affinity of the enzyme for their substrate. This value is given as the concentration of the substrate (mM) at half of V_{max}. An enzyme with a high K_m has a low affinity for the substrate, and a high concentration of the substrate is needed in order for the enzyme to become saturated. Conversely, an enzyme with a high K_m has a high affinity for the substrate and the enzyme may become saturated even with a low amount of substrate.

Applications of enzyme kinetics

There are many practical uses of enzyme kinetics. For example, the kinetic constants can help explain how enzymes work and assist in the prediction of the behavior of enzymes in living organisms.

V_{max} and K_m both play a key role in understanding the metabolism of the human body. Knowledge of the enzyme kinetic constants allows us to gain a better understanding of the enzymes and processes that take place in human metabolism. This knowledge could then be used for medical purposes to improve patient health outcomes.

UNIT – 3

Enzyme catalysis

Enzyme catalysts or enzymes as a catalyst are biocatalysts that can be utilised in the transformation of organic compounds. A natural enzyme is generally a biological macromolecule that is produced by living organisms. Basically, these are complex nitrogenous proteins that help to catalyse the biochemical reactions in living organisms. More significantly, all biochemical reactions occurring in living organisms depend on catalysts.

There are also enzymes that have been more or less isolated and are employed in biocatalysis or enzyme catalysis. With the help of modern biotechnology, non-natural enzymes are also produced in labs today. With such developments, the modified enzymes are widely used to catalyze novel small molecule transformations that may be difficult to achieve using classical synthetic organic chemistry. When natural or modified enzymes are used to perform certain organic synthesis, it is known as chemoenzymatic synthesis. The reactions that are performed using the enzyme are classified as chemoenzymatic reactions.

All in all, enzyme catalysts usually increase the rate of chemical reactions, which further result in the conversion of substrate into a product. There are mainly two types of enzyme catalysts – activation enzymes and inhibitory enzymes. We will learn more about them in the following paragraphs.

Characteristics of an Enzyme Catalyst

In comparison to inorganic catalysts which include metals, acids and bases, enzymes are very particular when it comes to reactions. A certain type of enzyme can react with only one particular compound or its substrate. As far as the mechanism is concerned, when enzymes act as catalysts, they tend to weaken the substrate bonds, thereby lowering the overall activation energy. Reactions take place and the product is formed. A single enzyme molecule can be used repeatedly to transform several substrate molecules. Lets us also quickly go through some of the important characteristics of an enzyme catalyst below.

1. Specificity of Enzymes

Enzymes are highly specific in nature. This specific nature is of the following types:

(a) Group-specific: These enzymes will act on molecules having a specific functional group like amines, acids,

etc. Enzymes are not only structural specific but also specific to the chemical groups surrounding them.

Example: Pepsin hydrolyses a peptide bond in which the amino group (NH_2 group) is contributed by an aromatic amino acid such as phenylalanine, tryptophan.

(b) Linkage-specific: In this type, the activity of the enzyme depends upon the linkage of enzyme molecules with functional groups.

(c) Bond-specific: Enzymes are specific to the substrate having a similar bond and similar structure.

Example: α-amylase enzyme can hydrolyse α-1-4 glycosidic bond in glycogen and starch. Here, enzyme is specific to the α-1-4 glycosidic bond and not to the substrate.

(d) Substrate-specific: Enzyme specific for one substrate and one reaction.

Example: Maltase acts only on maltose.

(e) Optical-specific: Enzymes are specific to the optical configuration of the substrate.

Example: L-amino acid oxidase acts only on L-amino acids.

(f) Geometrical-specific: Enzymes can act on a different substrate having the same molecular geometry.

Example: Alcohol dehydrogenase can oxidize both ethanol and methanol to give corresponding aldehydes.

2. Optimum Temperature

High-temperature causes the deactivation of enzymes. So, most enzymes function effectively at an optimum temperature of 25 – 35°C.

3. Enzyme Activators

Certain substances increase enzyme activity to a very high or enormous rate. This activation exists as a molecule that is bound to an allosteric site of enzymes which “increase” the activation centre on the enzyme.

Example:

1. Hexokinase (I) acts as an activator to extract the glucose in the glycolysis pathway.

2. Glucokinase is an enzyme activator that combines with enzymes released by pancreatic cells used in the treatment of diabetes.

4. Enzyme Inhibitors

A certain molecule binds the active site of an enzyme and decreases its activity, which are known as enzyme inhibitors. These may be drugs, pathogens, pesticides. A drug acts as an enzyme inhibitor and attacks the active site.

Example: Hydrolysis of cane sugar

Sugarcane is sucrose ($C_{12}H_{22}O_{11}$), which basically is a dextro rotator with a specific angle of rotation +62.5. In the process, enzyme invertase sucrose undergoes hydrolysis to give α – D(+) glucose and β – D(-) fructose as products. The solutes contain more leave rotatory fructose whose angle of the specific rotator is -92.2. Hence, this mixture is known as invert sugar and the process is the inversion of cane sugar.

This conversion of Dextro rotators sucrose to fructose is done by enzyme and block the attack of substrate, which are known as competitive inhibitors.

5. Optimum pH

pH is a very important characteristic feature of biochemical reactions. Higher pH reactions generally deactivate the enzyme activity, and lower pH media encourages the growth of microbes. So, optimum pH conditions are required for enzyme catalysis. Generally, pH from 7.2 to 7.4 is required for the enzymatic reactions.

6. Activation Centre

Enzymes are biological catalysis with a greater number of activation centres and large surface area. They are more efficient than inorganic catalysis due to more activation centre enzymes are enzyme catalysis.

Example 1: Hydrolysis of starch.

Starch is a complex polysaccharide, which is present in potato, rice and other grains. It contains α – D(+) glucose with $C_1 – C_4$ glycosidic linkage, diastase enzyme hydrolysis the complex polysaccharide starch into simple monosaccharide glucose.

Example 2: Ethyl alcohol production

Ethanol is obtained from glucose by the enzymatic action, glucose is converted into ethyl alcohol and CO_2 by the enzyme catalysis of zymase enzyme produced from yeast.

Example 2: Hydrolysis of urea

Urea is an excretory chemical produced in the metabolic reactions of a living organism. It undergoes hydrolysis to give ammonia and CO_2. Due to the offensive smell of NH_3 public toilets are often with bad smell. This hydrolysis is catalysed by the enzymatic action of the urease enzyme.

Enzyme Catalysis

When there is an increase in the rate of a chemical process by a biological molecule typically an enzyme, the process is known as enzyme catalysis. Such processes involve chemical reactions and the catalysis generally occurs at a localized site known as the active site.

Mechanism of Enzyme Catalysis

The mechanisms of enzyme catalysis usually vary in different processes. However, they are quite similar in principle to other types of chemical catalysis. There is a reduction of energy barrier(s), therefore, separating the reactants from the products. With reduced activation energy the fraction of reactant molecules that can overcome this barrier increases and the product is formed.

An important factor that we need to consider is that enzymes can catalyze reactions in both directions but cannot take a reaction forward nor change the equilibrium position. The enzyme is not changed or consumed during the reaction and it can perform many catalyses repeatedly.

Chymotrypsin mechanism

Chymotrypsin is a digestive enzyme belonging to a super family of enzymes called serine proteases. It uses an active serine residue to perform hydrolysis on the C-terminus of the aromatic amino acids of other proteins. Chymotrypsin is a protease enzyme that cleaves on the C-terminal phenylalanine (F), tryptophan (W), and tyrosine (Y) on peptide chains. It shows specificity for aromatic amino acids because of its hydrophobic pocket.

Introduction

Chymotrypsin is one of the most studied enzymes due to its two phase kinetics: pre-steady-state and steady state. The study of these two kinetic states gives evidence of the "Ping-Pong" mechanism, the formation of covalent complexes leading to covalent hydrolysis reactions, and the rate of the catalyzed reactions. Synthesis of chymotrypsin occurs primarily in the pancreas. Instead of the active form, however, it is produced as an inactive zymogen called chymotrypsinogen to prevent its protease activity from digesting the pancreas. Upon secretion into the lumen of the small intestine, it is converted to its active form by another enzyme called trypsin. This dependence of a different enzyme for the activation of a protease is a common way for the body to prevent the digestion of organs and other harmful enzymatic side-effects.

Chymotrypsin operates through a general mechanism known as the ping-pong mechanism (Figure 7.2.17.2.1) whereby the enzyme reacts with a substrate to form an enzyme intermediate. This intermediate has different properties than the initial enzyme, so to regenerate the initial enzymatic activity, it must react with a secondary substrate. This process is illustrated below:

More specifically, chymotrypsin operates through a particular type of ping-pong mechanism called covalent hydrolysis. This means that the enzyme first forms a covalent bond with the target substrate, displacing the more stable moiety into solution. This enzyme-substrate complex is called the enzyme intermediate. The intermediate then reacts with water, which displaces the remaining part of the initial substrate and reforms the initial enzyme.

Chymotrypsin, like most enzymes, is specific in the types of substrates with which it reacts. As a protease, it cleaves polypeptides, and its inherent specificity allows it to act only on the carboxy-terminal of aromatic residues. It is a somewhat complicated mechanism, and is best explained in a series of steps.

Step 1: The target enters the active site of chymotrypsin, and it is held there by hydrophobic interactions between exposed non-polar groups of enzyme residues and the non-polar aromatic side-chain of the substrate. It is important to note the hydrogen bond between the Schiff nitrogen on histidine-57 and the oxygen side-chain of serine-195.

Step 2: Aided by the histidine-serine hydrogen bonding, the hydroxyl group on serine-195 performs a nucleophilic attack on the carbonyl carbon of an aromatic amino acid while simultaneously transferring the hydroxyl hydrogen to the histidine Schiff nitrogen. This attack pushes the pi carbonyl electrons onto the carbonyl oxygen, forming a short-lived intermediate consisting of a c-terminal carbon with four single

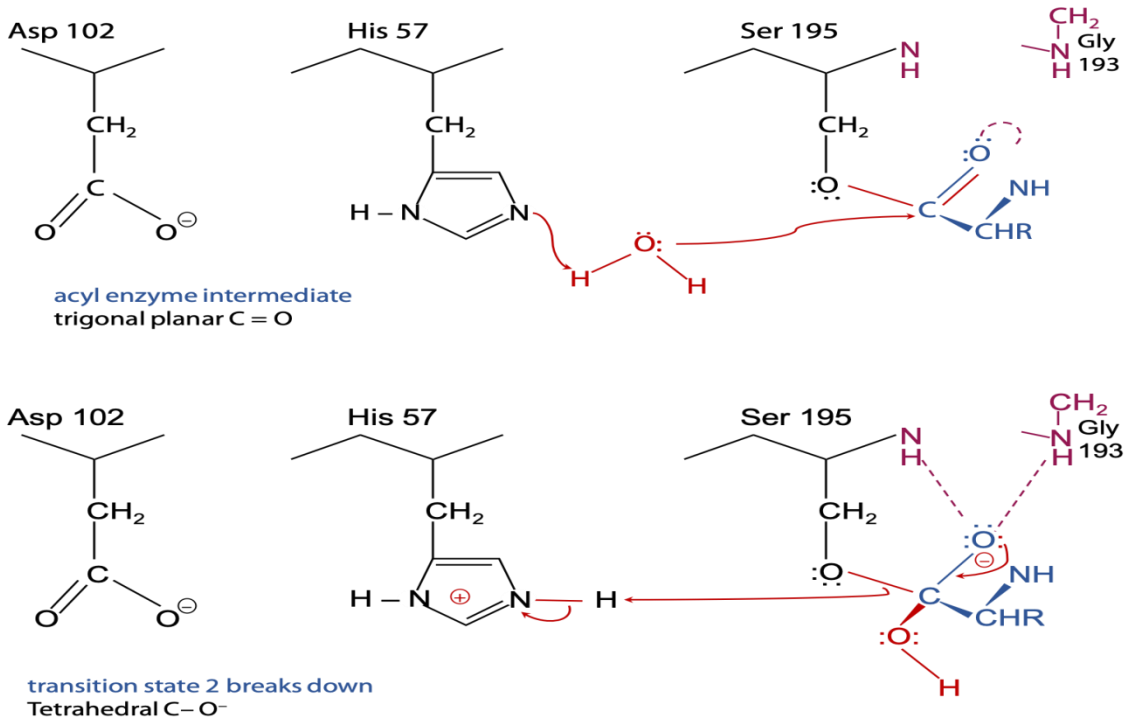
bonds: an oxygen anion, the beta-carbon of the aromatic amino acid, the n-terminus of the subsequent amino acid of the substrate protein, and the serine-195 side-chain oxygen.

Step 3: This intermediate is short-lived, as the oxyanion electrons reform the pi bond with the c-terminus of the aromatic amino acid. The bond between the carboxy-terminus of the aromatic amino acid and the n-terminus of the subsequent residue is cleaved, and its electrons are used to extract the hydrogen of the protonated Schiff nitrogen on histidine-57. The bonds between the carbonyl carbon and the serine-195 oxygen remain in an ester configuration. This is called the acyl-enzyme intermediate. The c-terminal side of the polypeptide is now free to dissociate from the active site of the enzyme.

Step 4: Water molecules are now able to enter and bind to active site through hydrogen bonding between the hydrogen atoms of water and the histidine-57 Schiff nitrogen.

Step 5: The water oxygen now makes a nucleophilic attack on the carbonyl carbon of the acyl-enzyme intermediate, pushing the carbonyl's pi electrons onto the carbonyl carbon as histidine-57 extracts one proton from water. This forms another quaternary carbon covalently bonded with serine, a hydroxyl, an oxyanion, and the aromatic amino acid. The proton on the recently protonated histidine-57 is now able to make a hydrogen bond with the serine oxygen.

Step 6: The oxyanion electrons reform the carbonyl pi bond, cleaving the bond between the carbonyl carbon and the serine hydroxyl. The electrons in this bond are used by the serine oxygen to deprotonate the histidine Schiff nitrogen and reform the original enzyme. The substrate no longer has affinity for the active site, and it soon dissociates from the complex.



Kinetics

Spectrophotometric analysis of chymotrypsin acting on nitrophenylacetate showed that nitrophenolate was produced at a rate independent of substrate concentration, proving that the only factor contributing to the rate of product formation is the concentration of enzyme; this is typical for enzyme-substrate kinetics. However, when the slope of the 0-order absorbance plot was traced back to the starting point (time = 0), it was found that the initial concentration of nitrophenolate was not 0. In fact, it showed a 1:1 stoichiometric ratio with the amount of chymotrypsin used in the assay. This can only be explained by the fact that hydrolysis by chymotrypsin is biphasic in nature, meaning that it proceeds in two distinct steps.

1. The first step, which describes the **initial burst** of nitrophenolate seen in Hartley and Kilby's absorbance plot, is the fastest. The attack of the nitrophenyl acetate substrate by chymotrypsin immediately cleaves the nitrophenolate moiety and leaves the acetate group attached to chymotrypsin, rendering the enzyme inactive.
2. The second step has been deduced to involve the hydrolysis of the acetate group from the inactivated chymotrypsin to regenerate the original enzyme.

Unit -4

Enzyme regulation

Allosteric enzymes are enzymes that have an additional binding site for effector molecules other than the active site. The binding brings about conformational changes, thereby changing its catalytic properties. The effector molecule can be an inhibitor or activator. All the biological systems are well regulated. There are various regulatory measures in our body, that control all the processes and respond to the various inside and outside environmental changes. Whether it is gene expression, cell division, hormone secretion, metabolism or enzyme activity, everything is regulated to ensure proper development and survival. Allostery is the process of enzyme regulation, where binding at one site influences the binding at subsequent sites.

Allosteric Enzyme Properties

- Enzymes are the **biological catalyst,** which increases the rate of the reaction
- Allosteric enzymes have an additional site, other than the active site or substrate binding site. The substrate-binding site is known as C-subunit and effector binding site is known as R-subunit or regulatory subunit

- There can be more than one allosteric sites present in an enzyme molecule
- They have an ability to respond to multiple conditions, that influence the biological reactions
- The binding molecule is called an effector, it can be inhibitor as well as activator
- The binding of the effector molecule changes the conformation of the enzyme
- Activator increases the activity of an enzyme, whereas inhibitor decreases the activity after binding
- The velocity vs substrate concentration graph of allosteric enzymes is **S-curve** as compared to the usual hyperbolic curve

Allosteric Regulation Mechanism

There are two types of allosteric regulation **on the basis of substrate and effector molecules**: **Homotropic Regulation:** Here, the substrate molecule acts as an effector also. It is mostly enzyme activation and also called cooperativity, e.g. binding of oxygen to haemoglobin. **Heterotropic Regulation:** When the substrate and effector are different. The effector may activate or inhibit the enzyme, e.g. binding of CO_2 to haemoglobin. **On the basis of action performed by the regulator**, allosteric regulation is of two types, inhibition and activation. **Allosteric Inhibition:** When an inhibitor binds to the enzyme, all the active sites of the protein complex of the enzyme undergo conformational changes so that the activity of the enzyme decreases. In other words, an allosteric inhibitor is a type of molecule which binds to the enzyme specifically at an allosteric site. **Allosteric Activation:** When an activator binds, it increases the function of active sites and results in increased binding of substrate molecules. There are two models proposed for the mechanism of regulation of allosteric enzymes:

1. **Simple Sequential Model-** It was given by **Koshland.** In this model, the binding of substrate induces a change in the conformation of the enzyme from T (tensed) to R (relaxed). The substrate binds according to the induced fit theory. A conformational change in one unit stimulates similar changes in other subunits. This explains the cooperative binding.The same way inhibitors and activators bind, the T form is favoured, when the inhibitor binds and R form is favoured, when the activator binds. The binding at one subunit affects the conformation of other subunits.The sequential model explains the negative cooperativity in enzymes, e.g. tyrosyl tRNA synthetase, where the binding of substrate inhibits the binding of another substrate.
2. **Concerted or Symmetry Model-**According to this model, there is a simultaneous change in all the subunits of an enzyme. All the subunits are either present in R form (active form) or T form (inactive form), having less affinity to a substrate.An inhibitor shifts the equilibrium of T ⇄ R, towards T, and

activator shifts the equilibrium towards R form and favours the binding. It explains the cooperative regulation of activators as well as inhibitors.

Allosteric Enzyme Examples

There are many allosteric enzymes that take part in the biochemical pathways so that the system is well controlled and modulated. **Aspartate Transcarbamoylase (ATCase)**

- ATCase catalyses the biosynthesis of pyrimidine
- Cytidine triphosphate (CTP) is the end product and also inhibits the reaction. It is known as feedback regulation
- ATP (adenosine triphosphate), a purine nucleotide activates the process, high concentration of ATP can overcome inhibition by CTP
- This ensures the synthesis of pyrimidine nucleotide when a high concentration of purine nucleotide is present

Glucokinase

- It plays an important role in glucose homeostasis. It converts glucose to glucose-6-phosphate and enhances glycogen synthesis in the liver. It also senses the concentration of glucose for the release of insulin from pancreatic beta cells
- The glucokinase has low affinity for glucose, so it acts when more concentration of glucose is present in the liver, which should be converted to glycogen
- The activity of glucokinase is regulated by glucokinase regulatory proteins

Acetyl-CoA Carboxylase

- Acetyl-CoA carboxylase regulates the process of lipogenesis
- This enzyme is activated by citrate and inhibited by a long chain acyl-CoA molecule such as palmitoyl-CoA, which is an example of negative feedback inhibition by product
- Acetyl-CoA carboxylase is also regulated by phosphorylation/ dephosphorylation controlled by hormones such as glucagon and epinephrine

Isolation and purification of enzymes

Purification of enzymes is often a complicated process, requiring a combination of techniques to achieve sufficiently high purity levels. 1) A high final degree of purity is one of the most important requirements for an efficient enzyme purification strategy. 2) High overall enzyme activity recovery. 3) Reproducibility. Recall that extraction procedures release the desired enzyme into the medium along with a variety of other cell components, such as other enzymes and proteins, nucleic acids, and polysaccharides, which, due to their polymeric structure, tend to raise the viscosity of the solution. Following homogenization of the original raw material, the first stage in purification is to separate any leftover cell debris (if any) using differential sedimentation or supernatant precipitation, or centrifugation or filtration for a faster and clearer extract.

Purification and separation of enzymes are usually based on solubility, size, polarity, and binding affinity. The production scale, timeline, and properties of the enzymes should all be deliberated when choosing the appropriate separation method.

Types of enzyme separation

Solubility based separation: The principle of the kind of separation is that enzyme solubility changes drastically when the pH, ionic strength, or dielectric constant changes.

Mass based method: Enzymes are relatively large molecules, separation based on the size or mass of molecules favors purification of enzymes, particularly the ones with high molecular weight. Dialysis is a frequently used method, where semipermeable membranes are used to eliminate salts, small organic molecules, and peptides.

Polarity based separation: Like other proteins, enzymes can be alienated on the basis of polarity, more specially, their net charge, charge density, and hydrophobic interactions. In ionexchange chromatography, a column of beads comprising negatively or positively charged functional groups are used to separate enzymes. The cationic enzymes can be alienated on anionic columns, and anionic enzymes on cationic column.

Affinity or ligand based purification: Affinity chromatography is additional powerful and commonly pertinent means of purifying enzymes. This method takes benefit of the high affinity of many enzymes for particular chemical groups. In general, affinity chromatography can be successfully used to isolate a protein that recognizes a definite group by covalently attaching this group or a derived of it to a column, adding a mixture of proteins to this column, which is then washed with buffer to eliminate unbound proteins and eluting the required protein by adding a high concentration of a soluble form of the affinity group or altering the circumstances to diminution binding affinity.

Affinity adsorbents based on immobilized triazine dyes offer significant advantages circumventing many of the difficulties associated with biological ligands. The main drawback of dyes is their moderate selectivity for proteins. Rational attempts to tackle this problem are realized through the biomimetic dye model according to which new dyes, the biomimetic dyes, are designed to mimic natural ligands. Biomimetic dyes are estimated to exhibit augmented affinity and purifying capability for the targeted proteins. Biocomputing offers a predominant method to biomimetic ligand design. The successful corruption of contemporary computational techniques in molecular design needs the knowledge of the three-dimensional structure of the target protein, or at least, the amino acid sequence of the target protein and the three-dimensional structure of an extremely homologous protein. From such data one can then design, on a graphics workstation, the model of the protein and also a number of appropriate synthetic ligands which mimic natural biological ligands of the protein. There are numerous examples of enzyme purifications (trypsin, urokinase, kallikrein, alkaline phosphatase, malate dehydrogenase, formate dehydrogenase, oxaloacetate decarboxylase and lactate dehydrogenase) where synthetic biomimetic dyes have been used effectively as affinity chromatography tools.