CHAPTER 6 *Enzyme Chemistry*

LEARNING TARGETS:

At the end of the lesson, the students can:

- Define enzyme and identify its role in metabolic reactions,
- Classify and name enzymes.
- Describe the general nature of enzymes.
- identify and enumerate the factors that affect enzymatic activity.

What are ENZYMES?

Enzymes are the functional units of cells metabolism. Acting in organized sequences, they catalyze the hundreds of stepwise reactions by which nutrient molecules are degraded, chemistry energy is conserved and transformed, and cell macromolecules are made from simple precursors. Among many enzymes participating in metabolism, there is a special class called regulatory enzymes, which can sense various metabolic signals and change their catalytic rates accorded through their actions enzyme system are highly coordinated to yield a harmonious interplay between the many different metabolic activities necessary to sustain the living state.

Enzymes are defined as organic catalysts, produced by the living cell, that are not dependent on the intact cell for their activity. Each enzyme catalyzes only one or at most only a small number of reactions, Enzymes are thus reaction-specific are thus reaction-specific catalysts. Many enzymes maybe extracted from calls without outside the living cell.

There are two important characteristics of enzymes that should not be forgotten. The first is that the enzyme is not changed by entering into the reaction; it reverts to its original state the reaction is complete. The second is that the enzyme does not change the equilibrium constant of the reaction, it simply increases the rate at which the reaction takes place and achieved equilibrium.

ENZYME CLASSIFICATION AND NOMENCLATURE

Enzymes are usually named in terms of the reactions they catalyze. It is customary to add the suffix-ase to a major part of the name of the substrate acted upon. e.g. Urease acts on urea, arginase on arginine and tyrosinase on tyrosine. Trivial names introduced long ago persist and include such names as trypsin and pepsin. The international union of biochemistry (IUB) classified enzymes into 6 major classes:

CLASS 1
OkIDOREDUCTASES- these enzymes involved in the transfer of electrons; they catalyze reactions involving removal of electrons from an electron donor and transfer them to an appropriate electron accept usually these reactions ultimately lead to the formation of ATP.

CLASS 2
TRANSFERASES- these enzymes are involved in transferring functional groups between donors and acceptors. Amino, acyl, phosphate, transferred.

CLASS 3
HYDROLASES- this group of enzymes can be considered as a special class of the transferases which the donor is transferred to water acid anhydride, C-C, C-halide or C-N bonds are included reaction involves the addition or Introduction of the element of water across the bond which is to broken. Digestive enzymes of the GIT belong to these class enzymes.

CLASS 4
LYASES- they are enzymes that catalyze the removal of the group from a substrate by mechanism other than hydrolysis, leaving double bonds or they are enzymes which add or remove the element of water, ammonia or CO, to double bonds.

CLASS 5
ISOMERASES- this class includes all enzymes catalyzing the interconversion of optical geometric or positional isomers. These include cis-trans ketonol and aldose-ketose interconversions.

CLASS 6
LIGASES- since light means to bind, these enzymes are involved in synthetic reactions where two molecules are joined at the expense of an ATP "high-energy phosphate bond". The use of synthetase omit is reserved for this particular group of enzymes.

General Nature of Enzyme

a. All enzyme are proteins
All pure enzymes examined to date are proteins; moreover, their catalyst activity depends upon the integrity of their structure as proteins. If we disrupt the characteristic folding of the polypeptide chain (s) of a native enzyme protein by denaturing agents, the catalytic activity will also be lost.

b. Enzymes accelerate the rate of a chemical reaction but not consumed in the overall process. Enzymes are true catalysts. They greatly enhance the rate of specific chemical reactions that would otherwise occur only very slowly. They cannot change the equilibrium point of the reactions they promote nor are they used up or permanently changed by these reactions.

c. Enzymes exhibit a high degree of specificity of their substrate,
Enzymes are highly specific both in the reaction catalyzed and in their choice of reactants, which are called substrates. An enzyme usually catalyzes a single chemical reaction of a set of closely related reactions. The degree of specificity for the substrate is usually high and sometimes virtually absolute.

d. Enzymes catalysis involves the formation of an intermediate complex between the enzyme and its substrate or substrates.

The making and breaking of a chemical bond an enzyme is preceded by the formation of an enzyme- substrate (ES) complex. The substrate is bound to a specific region of the enzyme called the active site. Most enzymes are highly selective in their building of substrate. Indeed, the catalytic specificity of enzymes called the active specificity of the binding process. Furthermore, the control of enzymatic activity may also take place at this stage. The region of an enzyme that specifically interacts with the substrate is called the active site.

The active site of an enzyme is the region that binds the substrate (and the prosthetic group, if any) and contributes the residues that directly participate in the making and breaking of bonds. These residues are called the catalytic groups. The active site is therefore composed of the binding site and catalytic site.

Although enzymes differ widely in structure, specificity, and made of catalysis, a number of generalizations concerning their active sites can be stated:

1. The active site takes up a relatively small part of the total volume of an enzyme. Most of the amino acid residue in an enzyme is not in contact with the substrate.

2. The active site is a 3-dimensional entity. The active site of an enzyme is not a point, a line or even plane. It is an intricate 3-dimensional form made up of groups that come from different parts of a linear amino acid sequence.

3. Active sites are clefts or crevices. An all enzymes of known structure substrate molecules are bound to a cleft or crevice from which water is usually excluded unless it is reactant. The non-polar character of the cleft enhances the binding of the substrate.

4. The specificity of binding depends on the precisely defined arrangement of atoms in an active site. There are two types of specificity:

a. **Absolute specificity** - when a given enzyme acts only on one substrate e.g. urease only urea, carbonic anhydrase acts only on carbonic acid.
Absolute optical specificity - with the exception of epimerases which interconvert isomers, enzymes generally show absolute optical specificity for at least a portion of the substrate

molecule. Examples, maltase hydrolyzes & glucosides but not B-glucosides, mammalian enzymes act only one the L-isomer of amino acids.

b. Relative specificity - when a given enzyme acts on a particular kind of covalent bond in closely related substrates, e.g, proteolytic enzymes hydrolyze the peptide bonds of polypeptides, and lipase split the ester bonds of lipids.

(WITH DRAWING)

There are two theories or hypothesis that attempt to explain enzyme specificity.

a. **Lock and key mechanism -** this was forwarded by Emil Fisher which explains enzyme specificity on the basis of a rigid template. According to this concept, only the proper substrate can fit into a complementary enzyme surface, as the key fits into its lock. In other words, enzyme specificity depends on the shape of the active site of the enzyme and the inherent 3-dimensional shape of the substrate molecule.

b. Induce fit mechanism this was proposed by Koshland which based on the idea that the active Site is flexible; the protein enzyme may not have proper proximity of reactive groups in the active site until the substrate binds to it. Only the proper substrate can cause the precise alignment of catalytic groups needed for enzyme action. In this concept, there will be a conformation change on the enzyme as the substrate molecule approaches it.

C. Enzymes lower the activation energy required for a chemical reaction

A chemical reaction takes place because a certain fraction of the population of substrate molecules at any given Instant possesses enough energy to attain an activated condition, called the transitions to state, in which the probability is very high that a chemical bond will be made or broken to form the product. This transition state is at the top of the energy barrier separating the reactants and products. The activation energy of a reaction is the amount of energy in calories required to bring all the molecules in 1 mole of a substance at a given temperature to the transition state at are formed the free energy barrier.

Enzymes combine transiently with the reactants to produce a transition state having lower energy of activation than the transition sate of the catalyzed reaction. Thus, they accelerate chemical reactions by lowering the energy of activation. When the reaction products formed, the free catalyst is regenerated.

COFACTORS

A cofactor is a non-protein chemical compound that is bound (either tightly or loosely) to an enzyme and is required for catalysis.

Types of Cofactors
A. Coenzymes - The non-protein component, loosely bound to apoenzyme by a non-covalent bond.
Examples: vitamins or compound derived from vitamins.

B. Prosthetic groups - The non-protein component, tightly bound to the apoenzyme by covalent bonds is called a prosthetic group.

COENZYMES

Some enzymes depend for activity only on their structure as proteins, while others required is addition non-protein structure for activity. Same enzymes contain metallic or mineral ions known as cofactors. Many enzymes, however, require for their function the presence of certain organic, dialyzable, thermostable compounds. If such composed is rather firmly attached to the enzyme protein it is usually called a prosthetic group. The group or compound is called a coenzyme if its attachment to the protein is not very firm. The coenzymes are well-defined organic compounds and in many instances are structures related to the vitamins of the B-complex family.

The term **apoenzyme** is sometimes used for the protein portion of the system which becomes a holoenzyme when combined with the coenzyme. The function of the coenzyme in the enzymatic reaction is to assist in the cleavage of the substrate by acting as an acceptor for one of the cleavage produce.

Factors Influencing Enzyme Action

Since the enzyme is usually present biological systems in an amount too small to measure quantitively, their enzymatic activity is determined instead.
There are two ways in which enzyme activity is measured or determined:

1. The rate of disappearance of the substrate molecules
2. The rate of disappearance of the products in the system

A variety of factors influence the velocity of an enzyme-catalyzed reaction. Among them are the following:

A. Effect of pH or hydrogen lon concentration

Enzymes have an optimum pH. The optimum pH is that pH at which a certain enzyme causes a reaction to progress rapidly. On their side of the optimum, the rate of reaction is lower and at certain pH's an enzyme may be inactivated or even destroyed. Most enzymes have a pH activity curve which is bell shape there are few exceptions such as pepsin.

B. Effect of Temperature

All chemical reactions get accelerated with a rise in temperature, whether mediated by catalyst or not. There is an optimal temperature at which reaction is most rapid. The optimum temperature is usually reached at 40°C to 50°C for animal enzymes, whereas plant enzymes are higher usually 50°C to 60°C. Above this, the rate of reaction decreases because the enzyme is denatured at a faster than the increase in the reaction. Most enzymes are denatured above 60°C.

C. Effects of enzyme concentration

The velocity of an enzyme is directly proportional to the concentration of the enzyme, provided that the substrate is present in excess. This is particularly true at the beginning of the reaction, but it may not hold true as the reaction continues, especially as the substrate is used up.

D. Effect of substrate concentration

Let us examine the effect of varying concentration on the initial rate of an enzyme-catalyzed reaction when the enzyme concentration is held constant. At a very low concentration of substrate, the rate of reaction is very low, but it will increase with an increase in the substrate concentration. If we measuring the initial rate of the catalyzed reaction, we shall find that the rate increases by smaller and smaller amounts. Finally, a point will be reached beyond which there are only small increases in the reaction rate with increasing substrate concentration. No matter how high the substrate concentration is raised beyond this point, the reaction rate will approach but never quite reach a plateau. At this plateau, called the maximum velocity or rate (Vmax), the enzyme is "saturated" with its substrate and can function no faster.

E. Influence of time

As we have already noted, the velocity of the reaction is the amount of product produced per unit of time. The amount of product becomes constant while the time factor increases, i.e, The velocity the reaction product over the time decreases. This slowing may be attributable to several causes the amount of available substrate decreases; accumulation or product increases the reverse reaction: the product may be a deleterious effect on the reaction by denaturing the enzyme or changing the away from the optimum.

ENZYME INHIBITORS

Most enzymes can be poisoned or inhibited by certain chemical reagents. From the study of enzyme inhibitors valuable information has been obtained about the substrate

specificity or enzyme nature of the activity, Enzyme inhibitors also are very useful in elucidating metabolic pathways in cell Moreover, some drugs useful in medicine appear to function because they can inhibit certain oncelns.in malfunctioning cells. There are two major types of enzyme inhibitors:

a. Irreversible Inhibitors

They are those combine with or destroy a functional group on the enzyme molecule that is necessary for its catalytic activity. An example of an irreversible inhibitor is the compound dilsopropylfluorophosphate (DFP) which inhibit the enzyme acetylcholinesterase, important in the transmission on the nerve impulse.

b. Reversible inhibitors - there are three major types of reversible enzyme inhibition:

1. Competitive Inhibition
    a. Comparative inhibitor completes with the substrate for binding to the active site but, once bound, cannot be transformed by the enzyme.
    b. It can be reversed by simply increasing the substrate concentration
    c. Competitive inhibitors usually resemble the normal substrate in a three-dimensional structure. Because of this resemblance, the competitive inhibitor tricks the enzyme into binding it.
    d. The classical example is the competitive inhibitors of succinate dehydrogenase by the malonate

2. Uncompetitive inhibitors

a. The inhibitors do not combine with the free enzyme or affect its reaction with its normal substrate. However, it does not combine with the enzyme-substrate enzyme complex to give an inactive enzyme-substrate-inhibitor complex, which cannot undergo further to yield the normal product.
b. Uncompetitive inhibition is not reversed by increasing substrate concentration

3. Non-competitive inhibition

a. The inhibitor can combine with either the free enzyme of the enzyme-substrate complex, interfering with the action of both
b. The inhibit tor binds to a site on the enzyme other than the active site, often to deform the enzyme, so that it does not form the enzyme-substrate complex at its normal rate and, once formed, the ES complex does not at the normal rate to yield products.
c. This type of enzyme inhibition is not reversed by increasing the substrate concentrating

MULTIENZYME SYSTEMS

In the fact cell, enzyme usually works together in sequential chains in which the product of the first enzyme becomes the substrate of the next, and so on. Three levels of complexity of the molecular organization of the multienzyme system can be discerned:

1. In the simplest multienzyme system, the individual enzymes are in solution in the cytoplasm as independent molecular entities; they are not physically associated with each other at any time during their action. The small substrate molecules, which have high rates of diffusion, find their way from one enzyme molecule to the next very rapidly.

2. Other multienzyme systems are more rapidly organized so that the individual enzymes are physically associated and function together as enzyme complexes. This arrangement of enzyme molecules in a cluster in biologically advantageous in that limits the distance through which the substrate molecules most diffuse the course of the reaction sequence.

3. The most highly organized enzymes systems are those associated with a large supramolecular structure such as membranes or ribosomes. An important example is the chain of respiratory enzymes that is responsible for transferring electrons from substrate to oxygen; the individual enzyme molecule is attached to the inner membrane of the mitochondrion and indeed, from part of its structure.

ALLOSTERIC ENZYMES

In many multienzymes system, the end product of the reaction sequence may act as a specifies inhibitor of an enzyme at or near the beginning of the sequence, with the result that the rate of the entire sequence of reactions is determined by the steady-state concentration of the end product. This type of inhibition is called end-product inhibition, feedback inhibition or retroinhibition. The enzyme is this sequence which is inhibited by the end product is called the allosteric enzyme. The step catalyzed by this allosteric enzyme is often called committed reaction or rate limiting reaction which is us irreversible under intracellular conditions. In this case, the allosteric enzyme becomes the commit enzyme or rate-limiting enzyme. Once the committed reactions occur all the ensuing reactions of sequence takes place. Clearly, it is a good strategy for the cell to regulate a metabolic pathway at first step, to achieve maximum economy of metabolites.

The term "allosteric" denotes "another space" allosteric enzymes posses, in addition to the catalytic site, the "other space" to which the specific effectors or modulator is reversibility and non-covalently bound. In the other words, the catalytic activity of allosteric enzymes is regulated through the non-covalent binding of a specific metabolite (called effector) at a site on the protein enzyme other than the catalytic site (referred to an allosteric site)

In general, the allosteric site is as specific for binding the modulator and the catalytic site is for binding the substrate. Some modulators are inhibitory and therefore called negative

modulators. Other allosteric enzymes have stimulatory or positive modulators. When allosteric enzyme has only one specific site on the enzyme; it is said to be monovalent. Some allosteric enzymes respond to two or more specific modulators, each bound is a specific site on the enzyme; they are polyvalent. Moreover, a given allosteric enzyme may have both positive and negative modulators.

ZYMOGENS

Many proteolytic enzymes are produced by the cell in the form of inactive precursors called zymogens or proenzymes. The conversion of the new substance to active enzymes is affected by agents they are more or less specific. In many instances, die active enzymes that are found, can itself act as an activator of its own zymogen, an autocatalytic reaction. Activation involves limited hydrolysis which either allows the subsequent formation of the active site or removes an inhibitory peptide fragment. The zymogens of the digestive enzymes are an example of these proenzymes.

ISOENZYMES (ISOZYMES)

Many enzymes occur in more than one molecular in the same species in the same tissue, or even in the same cell. In such cases, different forms of the enzymes catalyze the same reaction, but since they have different forms of the enzymes catalyze the same reaction, but since they are different kinetic properties and distinctly different amino acid composition or sequence, they can be distinguished and separated by appropriate procedures. Such multiple forms or enzymes are called Isozymes. They are more specific in the diagnosis of certain diseases. One of the first enzymes found to have such multiple forms in lactate dehydrogenase.

DIAGNOSTIC VALUE OF SPECIFIC ENZYMES

This is determined by the clinical laboratory of the activity of the following enzymes can provide the physician with valuable confirmatory or suggestive diagnostic evidence.

1. AMYLASE - the plasma amylase level may be low in liver disease and increased in high intestinal obstruction, parotitis, acute pancreatitis, and diabetes.

2. TRANSAMINASES - two transaminases are of clinical interest: rum glutamic oxaloacetic transaminases (SGOT) and serum glutamic pyruvate transaminase (SGPT). Serum transaminase levels in normal subjects are low, but after extensive damage or tissue destruction, these enzymes are liberated in the blood. SGPT is a more reliable indicator of liver damage while SGOT is a more reliable indicator of myocardial (heart) damage.

3. LACTATE DEHYDROGENASE - in myocardial all infraction, the concentration of serum LDH rises 48 hours of the occurrence of the infarct and returns to the normal range within 5 to 6 days.

4. CREATINE PHOSPHOKINASE - the measurement of serum GPK activity is of value in the diagnostic of disorders affecting skeletal and cardiac muscle as well as in studies of families affected with pseudohypertrophic muscular dystrophy.

5. ACID PHOSPHATE - the level of enzymes capable of catalyzing the hydrolysis of various phosphate esters at acidic pH (acid phosphate activity) may be elevated in metastatic prostatic carcinoma.

6. ALKALINE PHOSPHATE - blood levels of this enzyme are elevated in rickets, obstructive jaundice, hyperparathyroidism, and osteoblastic sarcoma.