AL-MUSTAQBAL UNIVERSITY

College of Medical and Health Techniques

Medical Laboratories Techniques Departments

Biochemistry Lectures for 2nd Year Students

(2 Credit Hrs. Theory + 2 Credit Hrs. Practice / Week = 3 Credit Unit

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Lecture No. 1

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Course Organizers:

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Enzymes, Properties, Functions and Enzymes Classifications

Learning Objectives:

1. To study what are enzymes, their general properties.
2. What is meant by catalytic activity of enzymes?
3. Study the nomenclature and classification of enzyme as approved by International Union of Biochemistry (IUB).
4. To learn holoenzyme, apoenzyme, coenzyme and isoenzyme
5. To learn the mechanisms of enzyme catalyzed reactions and various factors affecting enzyme activity.
6. Study the role of metal ions in enzymes.
7. To know what is enzyme catalyzed reaction and how an enzyme functions by lowering the energy of activation.
8. Study lock-and-key theory and induced fit theory of mechanism of action of enzymes.
9. Enzyme specificity and learn different types of specificity.

Key Points:

1. Enzymes are protein or conjugate protein or nucleoproteins (RNA complexes with proteins) catalysts utilized by essentially all mammalian cells in specific biochemical reactions in different organs of the body and which may also be physically located in different organelles and structures within a cell.
2. Enzymes speed up these reactions by decreasing their activation energy which are thermodynamically possible.
3. In addition to certain narrow ranges of pH, temperature, and protein and salt concentration, most enzymes require additional organic molecules and / or inorganic ions for optimal enzyme function.
4. An understanding of enzyme kinetics allows for laboratory measurement of plasma enzyme levels as well as determination of possible enzyme inhibition.
5. Damaged or dying cells within organs can release enzymes into the circulation; these plasma enzyme levels can be used clinically to develop a differential diagnosis of a patient with respect to specific organ disease and dysfunction
6. Many enzymes have isozymes, i.e., polypeptide chains that differ in amino acid sequence but have similar enzymatic activity. Some enzymes are

composed of two or more different polypeptide chains giving rise to isozymes that differ in chain composition. In a number of diseases, specific isozymes become elevated in serum, facilitating diagnosis.

Enzymes are another important group of biomolecules synthesized by the living cells. They are ***catalysts of biological systems (hence are called as biocatalysts), colloidal, thermo labile and protein in nature.*** The striking characteristics of enzymes are their catalytic power and specificity. Actions of most enzymes are under strict regulation in a variety of ways. ***Substances on which enzymes act to convert them into products are called*** **substrates.**

Catalytic Activity of Enzymes:

Enzymes have immense catalytic power and accelerate reactions at least a million times, by ***reducing the energy of activation***. Before a chemical reaction can occur, the reacting molecules are required to gain a minimum amount of energy; this is called the **energy of activation**. It can be decreased by increasing the temperature of the reaction medium. But in human body which maintains a normal body temperature fairly constant, it is achieved by enzymes.

Enzymes and Free Energy Changes:

The ***free energy of activation*** is the energy absorbed by reactant molecules before they have a chance to convert to products. The free energy of activation is a barrier to chemical reactivity. When this barrier is large, the rate of a chemical reaction is very slow. The lower the barrier, the faster is the reaction rate. The barrier exists for almost all chemical reactions because, for bonds to be broken. Enzymes *lower* the free energy of activation, E_{act}. For chemical reaction to precede energy barrier must be overcome **(Fig.1)**

A + B ◄---------► AB ◄----------► C + D

Energy is needed to transform substrate into “transition state (AB)”. Transition state has the highest “Free energy”, “Gibbs Free Energy of Activation ($\Delta G^{\ddagger}$) ” of any component in the reaction pathway; “The free energy needed for a productive reaction is compared between un-catalyzed and enzyme-catalyzed reaction. Enzymes have developed as extremely efficient catalysts because their active sites have evolved to bind transition states very tightly. It is this tight binding, which stabilizes the transition state and lowers the free energy of activation.

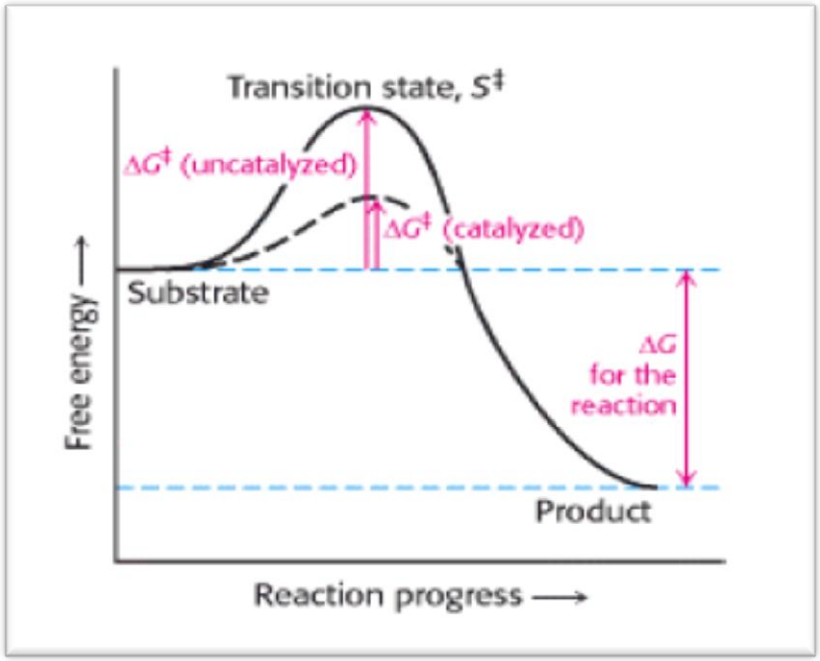

Fig. 1: Lowering of activation energy by enzymes

Protein Nature of Enzymes:

All physical and chemical properties of proteins with different techniques are applicable for enzymes because the enzymes are proteins in nature with some modification. All enzymes are proteins and may compose of non-protein compounds or cofactors. Therefore:

Holoenzyme = Apoenzyme + Cofactors (Metal ions or Coenzyme)

Active Enzyme = Large M. wt. + Small M. wt.

Some enzymes are active in the presence of apoprotein portion or apoenzyme only such as pancreatic ribonuclease. Others are composed coenzyme or metal ion (anion or cation) beside the apoprotein portion. The combination of cofactor plus the protein portion, the ***apoenzyme*****,** forms the complete catalytic entity and is known as the ***holoenzyme*** **as indicated above equation.** In general with the exception of *ribozymes* which are few RNA molecules with enzymatic activity, ***all the enzymes are protein in nature with large mol. wt.*** few enzymes are simple proteins while some are conjugated proteins. In such enzymes the ***non-protein part is called prosthetic group or coenzyme*** **and the protein part is called as *apoenzyme*.**

Enzymes are Biocatalysts:

A catalyst is a substance that increase or accelerate the rate of a particular chemical reaction without itself being consumed. At the end of a catalyzed reaction, the catalyst appears unchanged in form and quantity, whereas the main reaction materials have undergoes transformation into new product. The acceleration may occur in solution and the process is called homogeneous catalysis. Catalysis on an insoluble surface is termed heterogeneous catalysis.

Characteristics of Enzymes:

1. Almost all enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins.
2. They are heat labile.
3. They are water-soluble.

Enzyme Co-factors:

Co-factors: Organic cofactors are bound covalently or non-covalently to the apoenzyme include in metal ions or activated forms of water soluble vitamins. Covalently bound cofactors are sometimes referred to as ***prosthetic groups*.**

Coenzymes:

1. Enzymes may be simple proteins, or complex enzymes, containing a non-protein part, called the **prosthetic group.** The prosthetic group is called the **co-enzyme.** It is heat stable.
2. The protein part of the enzyme is then named the **apoenzyme.** It is heat labile, these two portions combined together are called the **holo-enzyme**.
3. Co-enzymes may be divided into **two groups**
    a. Those taking part in reactions catalyzed by **oxidoreductases** by donating or accepting hydrogen atoms or electrons.
    b. Those co-enzymes taking part in reactions transfer groups **other than hydrogen**.

Since the involvement of coenzyme in a given reaction on a substrate is so intimate that coenzyme is often called as ***co-substrate or second substrate*.** Coenzymes can be **classified according to the group whose transfer they facilitate**. Based on this concept we may classify coenzymes as follows:

1. **For transfer of groups other than hydrogen**

    CoA-SH, Thiamine pyrophosphate (TPP), Pyridoxal phosphate, Folate coenzymes, Biotin and Cobamide coenzyme.

2. For transfer of hydrogen

NAD^+, $NADP^+$; FMN, FAD ; Many coenzymes contain adenine, ribose and phosphate and are derivatives of adenosine monophosphate such as NAD^+ and FAD.

Water-soluble vitamin	Coenzymes	Typical reaction type	Consequences of deficiency
Thiamine, (B1)	Thiamine pyro-phosphate, TPP	Aldehyde transfer	Beriberi (weight loss, heart problems, neurological dysfunction)
Riboflavine, (B2)	FAD, FMN	Oxidation-reduction	Cheliosis and angular stomatitus (lesions of the mouth), dermatitis
Nicotinamide, (B3)	NAD^+ / NADH $NADP^+$/ NADPH	Oxidation-reduction	Pellagra (dermatitis, depression, diarrhea)
Pantothenic acid, (B5)	Acetyl-CoA	Acyl-group transfer	Hypertension
Pyridoxal, (B6)	Pyridoxal-5-phosphate	Group transfer to or from amino acids	Depression, confusion, Convulsions
Biotin	Biotin-lysine complexes (biocytin)	ATP-dependent carboxylation and carboxyl-group transfer	Rash about the eyebrows, muscle pain, fatigue (rare)
Folic acid	Tetrahydrofolate	Transfer of one-carbon components; thymine synthesis	Anemia, neural-tube defects in development, megaloblastic anemia
Cyano-cobalamin, (B12)	5-Deoxy-adenosyl cobalamin	Transfer of methyl groups; intra-molecular rearrangements	Anemia, pernicious anemia, methylmalonic acidosis

Metal Ions:

Inorganic cofactors that include mainly metal ions are listed below:

Mg^{2+}	Ca^{2+}
Fe^{2+}/Fe^{3+}	Mn^{2+}
Zn^{2+}	Co^{2+}
Cu^{+}/Cu^{2+}	Cl^{-}

Metal ions often used for one or more of the following:

1. Binding substrates in the proper orientation
2. Mediating oxidation-reduction reactions
3. Electrostatically stabilizing or shielding negative charges (electrostatic catalysis).

Types of metal ion binding include:

a. **Metalloenzymes** contain tightly bound metal ions: (usually Fe^{+2}, Fe^{+3}, Cu^{+2}, Zn^{+2}, or Mn^{+2})
b. **Metal-activated enzymes** contain loosely bound metal ions: (usually Na^{+}, K^{+}, Mg^{+2}, or Ca^{+2})
c. Some prosthetic groups are **metallo-organic** compounds, e.g. heme

Role of Metal Ions in Enzymes

The activity of many enzymes depends on the presence of certain metal ions such as K^{+}, Mg^{++}, Ca^{++}, Zn^{++}, Cu^{++}.

1. **Metal activated enzymes:** In certain enzymes the metals ***form a loose and easily dissociable complex.*** Such enzymes are called ***metal-activated enzymes***. The metal ions can be removed by dialysis or any other such method from the enzyme without causing any denaturation of apoenzyme.

2. **Metalloenzymes:** The second category of metal enzymes is called as ***metallo-enzymes*** . In this case ***metal ion is bound tightly to the enzyme and is not dissociated*** even after several extensive steps of purification.

Metals play variety of roles such as:

1. They help in either maintaining or producing (or both), active structural conformation of the enzyme,
2. Formation of enzyme-substrate complex,
3. Making structural changes in substrate molecule,
4. Accept or donate electrons.

Nomenclature and Enzyme Classification:

Enzymes are generally named after adding the suffix ***'ase'*** to the name of the substrate, sometimes the name also includes a designation of the type of reaction catalyzed. **Examples** (Ribonucleic acid (RNA) is hydrolyzed by an enzyme called *ribonuclease) and* (Lactic acid is oxidized to pyruvic acid by an enzyme called *lactate dehydrogenase, LD or LDH)*. **e.g.** enzymes acting on nucleic acids are known as *nucleases,* enzymes hydrolyzing dipeptides are called *dipeptidases***,** lactase acts on the substrate lactose, and the products glucose and galactose are formed. Enzymes that hydrolyze starch (amylose) are termed as amylases; those that dehydrogenate the substrates are called dehydrogenases. These are known as the **trivial names** of the enzymes. Few exceptions such as trypsin, pepsin, and chymotrypsin are still in use. Further, **few enzymes exist in their inactive forms** and are called as **proenzymes or zymogens,** e.g. *pepsin* has *pepsinogen* as its zymogen. The zymogens ***become active after undergoing some prior modification*** in its structure by certain agents. ***Many times the active form of enzyme acts on zymogen and catalyzes its conversion into active form and this process is called as autocatalysis.***

International Union of Biochemistry and Molecular Biology (IUBMB) in 1964, (modified in 1972 and 1978), suggested the IUBMB system of nomenclature of enzymes. In order to have a uniformity and unambiguity in identification of enzymes, ***International Union of Biochemistry (IUB)*** adopted a nomenclature system ***based on chemical reaction type and reaction mechanism.*** According to this system, enzymes are grouped in **six major classes.** Each enzyme is characterized by a code number (enzyme code number or EC No.) comprising four figures (digits) separated by points, As per this system, the name starts with the EC No followed by 4 digits.

1. **The first** digit represents the major class**.**
2. ***The second digit*** indicates the type of group involved in the reaction.
3. **The third** digit is the sub-sub class or subgroup which denotes the reaction more precisely indicating substrate on which the group acts.
4. **The fourth** digit gives the number of the particular enzyme or the serial number of the enzyme in the list.

Briefly, the four digits characterize class, sub-class, sub-sub-class and serial number of a particular enzyme.

The six classes of enzyme classification are:

1. **Oxidoreductase:** Enzymes involved in oxidations and reductions of their substrates, e.g. *alcohol dehydrogenase, lactate dehydrogenase, xanthine oxidase, glutathione reductase, glucose-6-phosphate dehydrogenase.*
2. **Transferases:** Enzymes that catalyze transfer of a particular group from one substrate to another, e.g. *aspartate and alanine transaminase (AST/ALT), hexokinase, phosphoglucomutase, hexose-1-phosphate uridyltransferase, ornithine carbamoyl transferase,* etc.
3. **Hydrolases:** Enzymes that bring about hydrolysis, e.g. *glucose-6-phosphatase, pepsin, trypsin, esterases, glycoside hydrolases,* etc.
4. **Lyases:** Enzymes that facilitate removal of small molecule from a large substrate, e.g. *fumarase, arginosuccinase, histidine decarboxylase*.
5. **Isomerases:** Enzymes involved in isomerisation of substrate, e.g. *UDP-glucose, epimerase, retinal isomerase, racemases, triosephosphate isomerase*.
6. **Ligases:** Enzymes involved in joining together two substrates, e.g. *alanyl-t. RNA synthetase, glutamine synthetase, DNA ligases.*

Example: Alcohol Dehydrogenase: EC 1.1.1.1

$$R\text{-}CH_2\text{-}OH + NAD^+ \leftrightarrow R\text{-}CO\text{-}H + NADH + H^+$$

Structure of the alcohol dehydrogenase enzyme (E.C.1.1.1.1.) complexes with nicotinamide adenine dinulceotide (NAD^+) and zinc.

Six Classes of Enzymes – Enzyme Classification

- **EC 1. Oxidoreductases**
- **EC 2. Transferases**
- **EC 3. Hydrolases**
- **EC 4. Lyases**
- **EC 5. Isomerases**
- **EC 6. Ligases**

The **major six classes of enzyme classification were indicated below:**

Class 1: ***Oxidoreductases:*** Transfer of hydrogen or addition of oxygen; e.g. Lactate dehydrogenase (NAD); Glucose-6-phosphate dehydrogenase (NADP); Succinate dehydrogenase (FAD); dioxygenases.

Class 2: ***Transferases:*** Transfer of groups other than hydrogen. Example, Aminotransferase. (Subclass: Kinase, transfer of phosphoryl group from ATP; e.g. Hexokinase).

Class 3: ***Hydrolases:*** Cleave bond and add water; e.g. Acetylcholine esterase; Trypsin.

Class 4: ***Lyases:*** Cleave without adding water, e.g. Aldolase; HMG-CoA lyase; ATP Citrate lyase. (Subclass: Hydratase;add water to a double bond).

Class 5: ***Isomerases:*** Intramolecular transfers. They include racemases and epimerases. Example, Triose phosphate isomerase.

Class 6: ***Ligases:*** ATP dependent condensation of two molecules, e.g. Acetyl-CoA carboxylase; Glutamine synthetase; PRPP synthetase.