INTRODUCTION TO ENZYMES

The use of enzymes in the diagnosis of disease is one of the important benefits derived from the intensive research in biochemistry since the 1940s. Enzymes have provided the basis for the field of clinical chemistry.

Given the dramatic growth of life science research over recent decades, interest in diagnostic enzymology has multiplied. New AI online tools aid researchers in the area of medical research in which the diagnostic potential of enzyme reactions can be found.

Prepared by Worthington Biochemical Corporation, this overview is a practical introduction to enzymology. As a result of close involvement over the years in the theoretical as well as the practical aspects of enzymology, Worthington's knowledge covers a broad spectrum of the subject. This information has been assembled here for the benefit of a new generation of researchers.

Enzymes and Life Processes

The living cell is the site of tremendous biochemical activity called metabolism. This is the process of chemical and physical change which goes on continually in the living organism. These changes include the build-up of new tissue, replacement of old tissue, conversion of food to energy, disposal of waste materials, reproduction, etc. – all the activities that we characterize as "life."

This building up and tearing down takes place in the face of an apparent paradox. The greatest majority of these biochemical reactions do not take place spontaneously. The phenomenon of catalysis makes possible biochemical reactions necessary for all life processes. Catalysis is defined as the acceleration of a chemical reaction by some substance which itself undergoes no permanent chemical change. The catalysts of biochemical reactions are enzymes and are responsible for bringing about almost all of the chemical reactions in living organisms. Without enzymes, these reactions take place at a rate far too slow for the pace of metabolism.

The oxidation of a fatty acid to carbon dioxide and water is not a gentle process in a test tube – extremes of pH, high temperatures and corrosive chemicals are required. Yet in the body, such a reaction takes place smoothly and rapidly within a narrow range of pH and temperature. In the laboratory, the average protein must be boiled for about 24 hours in a 20% HCl solution to achieve a complete breakdown. In the body, the breakdown takes place in four hours or less under conditions of mild physiological temperature and pH.

It is through attempts at understanding more about enzyme catalysts – what they are, what they do, and how they do it – that many advances in medicine and the life sciences have been brought about.

Early Enzyme Discoveries

The existence of enzymes has been known for well over a century. Some of the earliest studies were performed in 1835 by the Swedish chemist, Jon Jakob Berzelius who termed their chemical action *catalytic*. It was not until 1926, however, that the first enzyme was obtained in pure form, a feat accomplished by James B. Sumner of Cornell University. Sumner was able to isolate and crystallize the enzyme urease from the jack bean. His work was to earn him the 1947 Nobel Prize.

John H. Northrop and Wendell M. Stanley of the Rockefeller Institute for Medical Research shared the 1947 Nobel Prize with Sumner. They discovered a complex procedure for isolating and purifying pepsin. This precipitation technique devised by Northrop and Stanley has been used to crystallize several enzymes.

Chemical Nature of Enzymes

Most enzymes are proteins, although a few are catalytic RNA molecules. Catalytic RNA molecules are called ribozymes. Enzymes are high molecular weight compounds made up principally of chains of amino acids linked together by peptide bonds (**Figure 1**). Enzymes can be denatured and precipitated with salts, solvents and other reagents. They have molecular weights ranging from 10,000 to 2,000,000 Da.

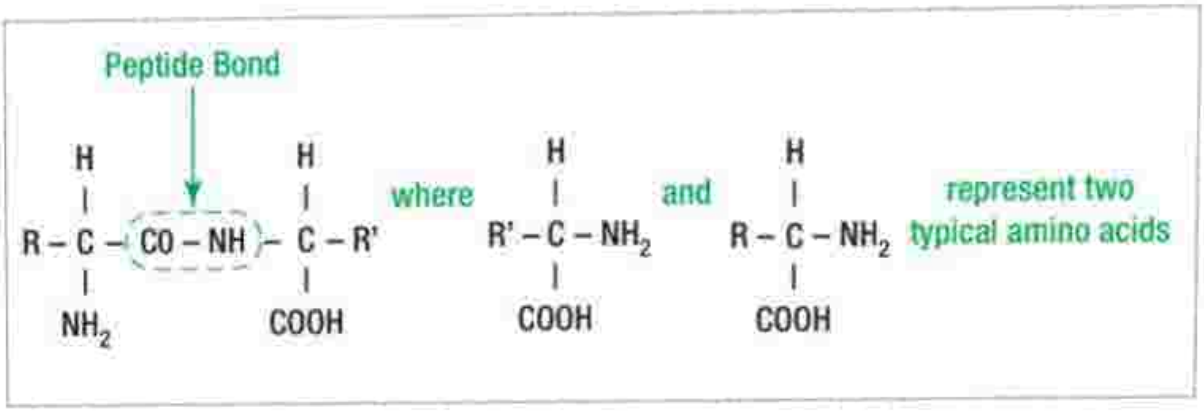

Figure 1: Typical protein structure – two amino acids joined by a peptide bond.

Many enzymes require the presence of other compounds – cofactors – before their catalytic activity can be exerted. This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor(s) (coenzyme, prosthetic group or metal-ion activator) **(Figure 2)**.

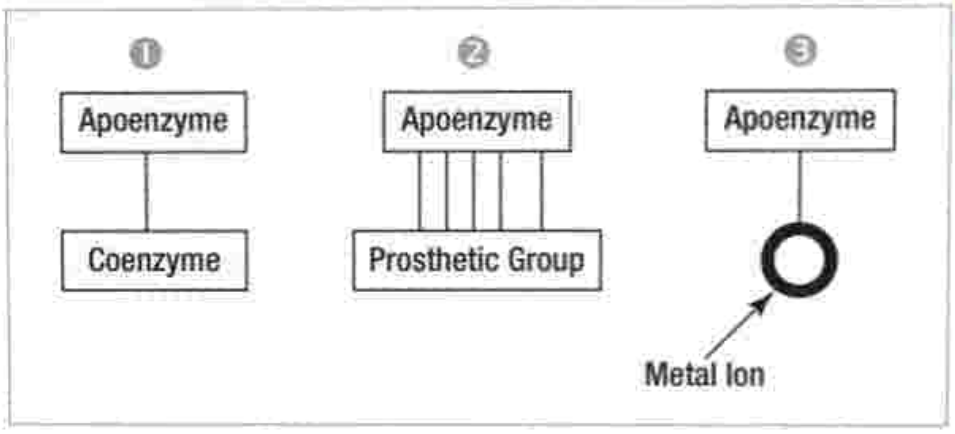

Figure 2: Holoenzymes plus various types of cofactors.

According to Holum, the cofactor may be:

1. **A coenzyme** – a non-protein organic substance which is dialyzable, thermostable and loosely attached to the protein part.
2. **A prosthetic group** – an organic substance which is dialyzable and thermostable which is firmly attached to the protein or apoenzyme portion.
3. **A metal-ion-activator** – these include K^+, Fe^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, and Mo^{3+}.

Specificity of Enzymes

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group.

In general, there are four distinct types of specificity:

1. **Absolute specificity** – the enzyme will catalyze only one reaction.
2. **Group specificity** – the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
3. **Linkage specificity** – the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
4. **Stereochemical specificity** – the enzyme will act on a particular steric or optical isomer.

Though enzymes exhibit great degrees of specificity, cofactors may serve many apoenzymes. For example, nicotinamide adenine dinucleotide (NAD) is a coenzyme for a great number of dehydrogenase reactions in which it acts as a hydrogen acceptor. Among them are the alcohol dehydrogenase, malate dehydrogenase and lactate dehydrogenase reactions.

Naming and Classification

Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase". The International Union of Biochemistry (I.U.B.) initiated standards of enzyme nomenclature which recommended that enzyme names indicate both the substrate acted upon and the type of reaction catalyzed. Under this system, the enzyme uricase is called urate: O_2 oxidoreductase, while the enzyme glutamic oxaloacetic transaminase (GOT) is called L-aspartate: 2-oxoglutarate aminotransferase.

The I.U.B. devised a system of classification and identification of enzymes in terms of the reactions they catalyse. This relies on a numerical system (the EC number) to classify enzymes in groups according to the types of reaction catalysed and systematic naming that describes the chemical reaction involved. This is now in widespread use, and the official list of enzymes classified can be found at ExplorEnz – The Enzyme Database (http://www.enzyme-database.org). The enzyme nomenclature is incorporated into many other resources, including the ExPASy-ENZYME, BRENDA and KEGG bioinformatics databases.

The EC number or Enzyme Commision number is a four-component identifier which classifies an enzyme according to class, subclass, sub-subclass and the final compontent being a serial number within that sub-subclass. The EC classes are currently listed as:

1. **Oxidoreductases** – To this class belong all enzymes catalysing oxidation reduction reactions. The substrate that is oxidized is regarded as hydrogen donor.
2. **Transferases** – Transferases are enzymes transferring a group, e.g. a methyl group or a glycosyl group, from one compound (generally regarded as donor) to another compound (generally regarded as acceptor).