ENZYMES

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Enzymes and Life Processes

- The living cell is the place of incredible number of biochemical reactions resulting in the overall essence of life.
- Enzymes are biological catalysts that speed up the Biochemical reactions.
- This word was first used by a German physiologist Wilhelm Kühne in 1878 while describing the ability of yeast to produce alcohol from sugars [Is derived from the Greek words en (meaning 'within') and zume (meaning 'yeast')].
- Enzymes accelerate the rates of reactions relative to non-enzyme catalysed reactions by factors of 10^6 to 10^{15}. In absence of enzymes, the rate of these reactions would be far too slow to keep pace with metabolism.

Early Enzyme Discoveries

- Some of the earliest studies on enzymes were performed in 1835 by the Swedish chemist Jon Jakob Berzelius who termed their chemical action catalytic [*an substance (organic, inorganic, organometallic, protein or RNA) which enhances the rate of a reaction without itself being changed and consumed during the overall reaction. A catalyst has NO effect on the equilibrium of a reaction, it only increases the rate of approach to equilibrium)*].
- 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 isolated and crystallized the enzyme urease from the jack bean.
- John H. Northrop and Wendell M. Stanley of the Rockefeller Institute for Medical Research shared the 1947 Nobel Prize with Sumner, who discovered a complex procedure for isolating pepsin.

Chemical Nature of Enzymes

Chemical Nature of Enzymes

- Until 1980s all enzymes were thought to be proteins having high molecular weight ranging from 10,000 to 2,000,000.
- These made up principally of chains of amino acids linked together by peptide bonds.
- These can be denatured and precipitated with salts, solvents and other reagents.

Chemical Nature of Enzymes

- However, in 1980s it was found that some ribonucleic acid (RNA) molecules had property to exert catalytic effects.
- These RNAs, having catalytic activities were termed as called ribozymes and have been shown to play an important role in gene expression.
- In the same decade, biochemists also developed the technology to generate antibodies possessing catalytic properties.
- These antibodies have been named as 'abzymes' and have significant potential both as novel industrial catalysts and in therapeutics.

Chemical Nature of Enzymes ……

- Amino acid-based enzymes are globular proteins that range in size from less than 100 to more than 2 000 amino acid residues.
- These amino acids can be arranged in form of one or more polypeptide chains that are folded and bent to form a specific three-dimensional structure, incorporating a small area known as the active site, where the substrate actually binds.
- The active site involves only a small number (less than 10) of the constituent amino acids.
- The shape and charge of the active site enable the enzyme to bind to a single type of substrate molecule, so that the enzyme is able to exhibit substantial specificity in its catalytic activity.

Chemical Nature of Enzymes

- The active site alone binds to the substrate and is responsible for the catalysis.
- The role of the rest of the protein molecule is to stabilize the active site and provide an suitable environment for interaction of the active site with the substrate molecule.
- The active site cannot be separated out from the rest of the protein without loss of catalytic activity.
- However, some laboratory-based directed (or forced) evolution studies have shown that it is sometimes possible to generate smaller enzymes that do retain activity.

Chemical Nature of Enzymes

- Although a large number of enzymes consist solely of protein, many also contain a non-protein component, known as a cofactor, that is necessary for the enzyme's catalytic activity.
- A cofactor may be another organic molecule, in which case it is called a coenzyme, or it may be an inorganic molecule, typically a metal ion such as iron, manganese, cobalt, copper or zinc.
- A coenzyme that binds tightly and permanently to the protein is generally referred to as the prosthetic group of the enzyme.
- When an enzyme requires a cofactor for its activity, the inactive protein component is generally referred to as an apoenzyme, and the apoenzyme plus the cofactor (i.e. the active enzyme) is called a holoenzyme .

MANY OF THE COFACTORS ARE DERIVATIVES OF VITAMINS OR DECORATED METAL CENTRES

S.No.	Vitamin	Coenzyme	Human Deficiency Disease
1.	B1 (Thiamine)	Thiamine pyrophosphate (TPP)	Beriberi
2.	B2 (Riboflavin)	Flavin coenzymes (e.g., FAD)	Deficiency in humans rare
3.	B3 (Pantothenate)	Coenzyme A (CoA)	Deficiency in humans rare
4.	B6 (Pyridoxine)	Pyridoxal phosphate (PLP)	Deficiency in humans rare
5.	B12 (Cobalamin)	Cobalamin coenzymes (e.g., 5 deoxyadenosyl cobalamin)	Pernicious anemia
6.	Biotin	Biocytin	Deficiency in humans rare
7.	Folic Acid	Tetrahydrofolate	Megaloblastic anemia
8.	Nicotinamide	Nicotinamide cofactors	Pellagra

Models of Enzyme Substrate binding

Lock and Key Hypothesis

- The hypothesis suggests that enzyme specificity results from the complementary nature of the substrate and its active site.
- This was first proposed by the German chemist Emil Fischer in 1894, and is known as Fischer's 'lock and key hypothesis'.
- This hypothesis assumes the substrate to be a key of the correct size and shape that fits the active site which resembles the keyhole of the lock (the enzyme).
- It is astounding that this theory was proposed at a time when it was not even established that enzymes were proteins.

INDUCED-FIT MODEL

- In 1958 Daniel Koshland extended Fischer's ideas and presented the 'induced-fit model' of substrate and enzyme binding,
- This model states the enzyme molecule changes its shape slightly to accommodate the binding of the substrate.
- The analogy that is commonly used is the 'hand-in-glove model', where the hand and glove are broadly complementary in shape, but the glove is moulded around the hand as it is inserted in order to provide a perfect match.

Specificity of Enzymes

Specificity of Enzymes

- The Enzymes show specificity in the reactions they catalyse.
- This makes them very useful as diagnostic and research tools.
- A few enzymes show absolute specificity and catalyse only one particular reaction.
- Other enzymes show specificity for a particular type of chemical bond or functional group.

Specificity of Enzymes

In general, there are four distinct types of specificity

- Absolute specificity - catalyse only one reaction.
- Group specificity - act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
- Linkage specificity - act on a particular type of chemical bond regardless of the rest of the molecular structure.
- Stereochemical specificity - act on a particular steric or optical isomer.

Specificity of Enzymes

- Though enzymes show great degrees of specificity, cofactors 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.
  - It binds alcohol dehydrogenase, malate dehydrogenase and lactate dehydrogenase reactions.

Naming and Classification

- Except for some of the originally studied enzymes such as pepsin and trypsin, most enzyme names end in "ase".
- The International Union of Biochemistry (I.U.B.) has set standards for enzyme nomenclature which recommend that enzyme names indicate both the substrate acted upon and the type of reaction catalysed.
- In this system, the enzyme uricase is named as urate: O2 oxidoreductase, while the enzyme glutamic oxaloacetic transaminase (GOT) is called L-aspartate: 2-oxoglutarate aminotransferase.

Enzyme Classification

Enzymes can be classified by the kind of chemical reaction catalysed.

- Addition or removal of water
  - Hydrolases - these include esterases, carbohydrases, nucleases, deaminases, amidases, and proteases
  - Hydrases such as fumarase, enolase, aconitase and carbonic anhydrase

Enzyme Classification

- Transfer of electrons
  - Oxidases
  - Dehydrogenases
- Transfer of a radical
  - Transglycosidases - of monosaccharides
  - Transphosphorylases and phosphomutases - of a phosphate group
  - Transaminases - of amino group
  - Transmethylases - of a methyl group
  - Transacetylases - of an acetyl group

Enzyme Classification

- Splitting or forming a C-C bond
  - Desmolases
- Changing geometry or structure of a molecule
  - Isomerases
- Joining two molecules through hydrolysis of pyrophosphate bond in ATP or other tri-phosphate
  - Ligases

Enzyme Classification: Main classes of enzymes in EC system

First EC digit	Enzyme class	Reaction type
1.	Oxidoreductases	Oxidation/reduction
2.	Transferases	Atom/group transfer (excluding other classes)
3.	Hydrolases	Hydrolysis
4.	Lyases	Group removal (excluding 3.)
5.	Isomerases	Isomerization
6.	Ligases	Joining of molecules linked to the breakage of a pyrophosphate bond

SUMMARY

- Enzymes are highly specific, powerful catalysts of biological systems that help to accelerate reactions taking place in organisms by factors of millions or more.
- Most catalysts in biology are proteins. The exception is the ribosome (the translation factory), ribozymes and abzymes
- Enzymes catalyze their reactions stereospecifically and are highly specific for their substrates.
- The region of the protein where the chemical reaction occurs is called the active site and there may be as few as 10 amino acids surrounding (enclosing) this site.

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