Lecture 18

APOENZYMES, COENZYMES AND COFACTORS, ISOZYMES

A complete, catalytically active enzyme together with its coenzyme and/or metal ions is called holoenzyme.

- The **protein part of an enzyme** is called **apoenzyme or apoprotein**.
- Enzymes require an additional non-protein component to carry out its catalytic functions.
- Generally these **non-protein components** are called as **cofactors.**
- The cofactors may be either one or more **inorganic ions such as Fe^{2}+, Mg^{2}+, Mn^{2}+ and Zn^{2}+** or a complex **organic molecules called coenzymes**.
- A coenzyme or metal ion that is covalently bound to the enzyme protein is called **prosthetic group.**
- Some enzymes require both coenzyme and one or more metal ions for their activity
- Coenzymes function as transient carriers of specific functional groups

Cofactors

- Metals are required as cofactors in approximately two thirds of all enzymes.
- **Metalloenzymes** contain a definite quantity of functional metal ion that is retained
  throughout whereas metal-activated enzymes bind metals less tightly but require added metals.
- The distinction between metalloenzymes and metal activated enzymes thus rests on the **affinity of a particular enzyme for its metal ion.**
- The mechanisms whereby metal ions perform their function appear to be **similar** both in metalloenzymes and metal activated enzymes.
- Metals participate through their **ability to act as Lewis acids and through chelate formation. Eg.** For metal functioning as a Lewis acid is the **zinc** in **carbonic anhydrase.**
- The metal can also **promote catalysis by binding substrate at the site of bond cleavage.** In **carboxypeptidase**, the carbonyl oxygen is chelated to the zinc.

The **iron-sulfur enzymes** are unique class of metalloenzymes in which the active centre consists of one or more clusters of **sulfur-bridged iron chelates**. These are of greater importance in plant systems

Isoenzymes

- **Enzymes which exist in multiple forms within a single species of organism or even in a single cell are called isoenzymes or isozymes.**

- Such multiple forms can be detected and separated by gel electrophoresis of cell extracts.

- Since they are **coded by different genes**, they **differ in amino acid composition and thus in their isoelectric pH values.**

- **Lactate dehydrogenase** is an example for the isoenzymes which occur as five different forms in the tissues of the human and other vertebrates.

- All the five isozymes catalyze the same reaction.

  Lactate + NAD+ ----------. Pyruvate + NADH + H+

- They have the molecular weight of about 134,000 and contain four polypeptides.

- The five isozymes consist of five different combinations of two different kinds of polypeptides **M and H.**

- Kinetic study of lactate dehydrogenase isozymes has revealed that although they catalyze the same reaction, they **differ significantly in their Km values for their substrates as well as Vmax values.**

- The two polypeptide chains in LDH are coded by **two different genes.**

- Skeletal muscle contains four identical M chains and designated as M4; whereas heart muscle contains four identical H chains and designated as H4.

- LDH of other tissues are a mixture of the five possible forms H4, H3M, H2M2, HM3 and M4.

- A **determination of the relative amounts of the five LDH isozymes** and the total concentration of LDH in a serum sample can provide valuable **diagnostic information about which tissues have been damaged and the extent of the damage.**