4. Control of enzyme synthesis

Most of the enzymes, particularly the rate limiting ones, are present in very low concentration. Nevertheless, the amount of the enzyme directly controls the velocity of the reaction, catalysed by that enzyme. ***Many rate limiting enzymes have short half-lives***. This helps in the efficient regulation of the enzyme levels.

There are two types of enzymes—(a) ***Constitutive enzymes*** (house-keeping enzymes)—the levels of which are not controlled and remain fairly constant. (b) ***Adaptive enzymes***—their concentrations increase or decrease as per body needs and are well-regulated. The synthesis of enzymes (proteins) is regulated by the genes (***Refer Chapter 26***).

Induction and repression : The term induction is used to represent increased synthesis of enzyme while repression indicates its decreased synthesis. Induction or repression which ultimately determines the enzyme concentration at the gene level through the mediation of hormones or other substances.

Examples of enzyme induction : The hormone insulin induces the synthesis of ***glycogen synthetase***, glucokinase, phosphofructokinase and pyruvate kinase. All these enzymes are involved in the utilization of glucose. The hormone cortisol induces the synthesis of many enzymes e.g. pyruvate carboxylase, tryptophan oxygenase and tyrosine aminotransferase.

Examples of repression : In many instances, substrate can repress the synthesis of enzyme. Pyruvate carboxylase is a key enzyme in the synthesis of glucose from non-carbohydrate sources like pyruvate and amino acids. If there is sufficient glucose available, there is no necessity for its synthesis. This is achieved through repression of ***pyruvate carboxylase by glucose***.

5. Enzyme degradation

Enzymes are not immortal, since it will create a series of problems. There is a lot of variability in the half-lives of individual enzymes. For some, it is in days while for others in hours or in minutes, e.g. LDH_4 — 5 to 6 days; LDH_1 — 8 to 12 hours; amylase — 3 to 5 hours.

In general, the key and regulatory enzymes are most rapidly degraded. If not needed, they immediately disappear and, as and when required, they are quickly sysnthesized. Though not always true, an enzyme with long half-life is usually sluggish in its catalytic activity.

6. Isoenzymes

Multiple forms of the same enzyme will also help in the regulation of enzyme activity, Many of the isoenzymes are tissue-specific. Although isoenzymes of a given enzyme catalyse the same reaction, they differ in K_m, V_{max} or both. e.g. isoenzymes of LDH and CPK.

UNITS OF ENZYME ACTIVITY

Enzymes are never expressed in terms of their concentration (as mg or µg etc.), but are expressed only as activities. Various methods have been introduced for the estimation of enzyme activities (particularly for the plasma enzymes). In fact, the activities have been expressed in many ways, like King-Armstrong units, Somogyi units, Reitman-Frankel units, spectrophotometric units etc.

Katal

In order to maintain uniformity in the expression of enzyme activities (as units) worldover, the Enzyme Commission of IUB has suggested radical changes. A new unit – namely katal (abbreviated as kat) – was introduced. ***One kat denotes the conversion of one mole substrate per second*** (mol/sec). Activity may also be expressed as millikatals (mkat), microkatals (µkat) and so on.

International Units (IU)

Some workers prefer to use standard units or ***SI units*** (System International). One SI unit or International Unit (IU) is defined as the amount of enzyme activity that catalyses the ***conversion of one micromol of substrate per minute***. SI units and katal are interconvertible.

1 IU = 60 μkatal
(or)
1 nkatal = 1.67 IU

Laboratory use of enzyme units

In the clinical laboratories, however, the units — namely katal or SI units — are yet to find a place. Many investigators still use the old units like King-Armstrong units, Somogyi units etc. while expressing the enzyme activities. It is therefore, essential that the units of enzyme activity, along with the normal values, be invariably stated while expressing the enzymes for comparison.

NON-PROTEIN ENZYMES

Ribozymes

Ribozymes are a group of ***ribonucleic acids*** that function as biological ***catalysts***, and they are regarded as non-protein enzymes.

Altman and his coworkers, in 1983, found that ***ribonuclease P*** – an enzyme till then known to cleave precursors of tRNAs to give tRNAs – was functional ***due to RNA*** component present in the enzyme and not the protein part of the enzyme.

The RNA part isolated from ribonuclease P exhibited a true enzyme activity and also obeyed Michaelis-Menten kinetics. Later studies have proved that RNA, in fact, can function as an enzyme and bring about the catalysis.

RNA molecules are known to adapt a tertiary structure just as in the case of proteins (i.e. enzymes). The specific conformation of RNA may be responsible for its function as biocatalyst. It is believed that ribozymes (RNAs) were functioning as catalysts before the occurrence of protein enzymes during evolution.

APPLICATIONS OF ENZYMES

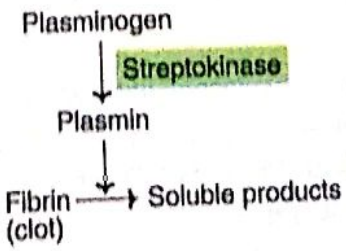
Certain enzymes are useful as therapeutic agents, analytical reagents, in genetic manipulations and for industrial applications (***Table 6.7***).

Table 6.7 A selected list of applications of enzymes

Enzyme	Application
Therapeutic applications	
Streptokinas/urokinase	To remove blood clots
Asparaginase	In cancer therapy
Papain	Anti-inflammatory
α_1-Antitrypsin	To treat emphysema (breathing difficulty due to distension of lungs)
Analytical application reagents (for estimation)	
Glucose oxidase and peroxidase	Glucose
Urease	Urea
Cholesterol oxidase	Cholesterol
Uricase	Uric acid
Lipase	Triacylglycerols
Luciferase	To detect bacterial contamination of foods
Alkaline phosphatase/ horse radish peroxidase	In the analytical technique ELISA
Applications in genetic engineering	
Restriction endonucleases	Gene transfer, DNA finger printing
Taq DNA polymerase	Polymerase chain reaction
Industrial applications	
Rennin	Cheese preparation
Glucose isomerase	Production of high fructose syrup
α-Amylase	In food industry to convert starch to glucose
Proteases	Washing powder

Enzymes as therapeutic agents

1. **Streptokinase** prepared from streptococcus is useful for clearing the blood clots. Streptokinase activates plasma plasminogen to plasmin which, in turn, attacks fibrin to convert into soluble products.



4. The enzyme **asparaginase** is used in the treatment of leukemias. Tumor cells are dependent on asparagine of the host's plasma for their multiplication. By administering asparaginase, the host's plasma levels of asparagine are drastically reduced. This leads to depression in the viability of tumor cells.

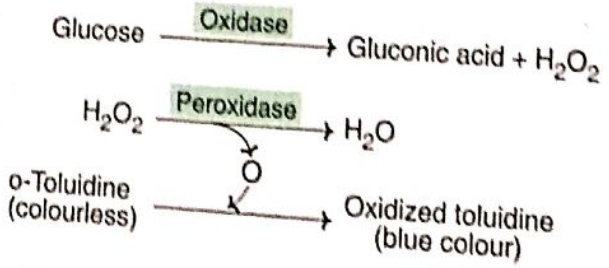
Enzymes as analytical reagents

Some enzymes are useful in the clinical laboratory for the measurement of substrates, drugs, and even the activities of other enzymes. The biochemical compounds (e.g. glucose, urea, uric acid, cholesterol) can be more accurately and specifically estimated by enzymatic procedures compared to the conventional chemical methods. A good example is the estimation of plasma glucose by glucose oxidase and peroxidase method.

Immobilized enzymes

Enzymes can be used as catalytic agents in industrial and medical applications. Some of these enzymes are immobilized by binding them to a solid, insoluble matrix which will not affect the enzyme stability or its catalytic activity. Beaded gels and cyanogen bromide activated sepharose are commonly used for immobilization of enzymes. The bound enzymes can be preserved for long periods without loss of activity.

Glucose oxidase and peroxidase, immobilized and coated on a strip of paper, are used in the clinical laboratory for the detection of glucose in urine.



The intensity of the blue colour depends on the concentration of glucose. Hence, the strip method is useful for semi-quantitative estimation of glucose in urine.

DIAGNOSTIC IMPORTANCE OF ENZYMES

Estimation of enzyme activities in biological fluids (particularly plasma/serum) is of great clinical importance. Enzymes in the circulation are divided into two groups – plasma functional and plasma non functional.

1. Plasma specific or plasma functional enzymes

Certain enzymes are normally present in the plasma and they have specific functions to perform. Generally, these enzyme activities are higher in plasma than in the tissues. They are mostly synthesized in the liver and enter the circulation e.g. lipoprotein lipase, plasmin, thrombin, choline esterase, ceruloplasmin etc.

Impairment in liver function or genetic disorders often leads to a fall in the activities of plasma functional enzymes e.g. deficiency of ceruloplasmin in Wilson's disease.

2. Non-plasma specific or plasma non- functional enzymes

These enzymes are either totally absent or present at a low concentration in plasma compared to their levels found in the tissues. The digestive enzymes of the gastrointestinal tract (e.g. amylase, pepsin, trypsin, lipase etc.) present in the plasma are known as ***secretory enzymes.*** All the other plasma enzymes associated with metabolism of the cell are collectively referred to as ***constitutive enzymes*** (e.g. lactate dehydrogenase, transaminases, acid and alkaline phosphatases, creatine phosphokinase).

Estimation of the activities of non-plasma specific enzymes is very important for the diagnosis and prognosis of several diseases.

The normal serum level of an enzyme indicates the balance between its synthesis and release in the routine cell turnover. The raised enzyme levels could be due to cellular damage, increased rate of cell turnover, proliferation of cells, increased synthesis of enzymes etc. Serum