1.12.6 Regulatory enzymes

Regulatory enzymes exhibit increased or decreased catalytic activity in response to specific signals. Activities of regulatory enzymes can be modulated through reversible non-covalent binding by regulatory compounds or by reversible covalent modification.

Regulation by non-covalent modification : Allosteric enzymes

Allosteric enzymes (Greek words: *allo* meaning other and *steric* meaning place or site) are a class of regulatory enzymes which increases or decreases catalytic activities in response to certain signals. Allosteric enzymes function through *reversible*, noncovalent binding of regulatory compounds called *modulators* or *effectors*. The modulators for allosteric enzymes may be inhibitory or stimulatory. Often the modulator is the substrate itself; regulatory enzymes for which substrate and the modulator are identical are called **homotropic**. When the modulator is a molecule other than the substrate, the enzyme is said to be **heterotropic**.

Most allosteric enzymes are oligomeric (consisting of multiple subunits). Allosteric enzymes may have two functionally different binding sites. One of the site, the active site, binds the substrate and catalyzes the reaction. The other type of site, known as *allosteric* or *regulatory site* binds with molecule which is called a modulator. Modulator molecules are of two types: positive modulator (called *activators*) that enhance enzyme activity and negative modulator (or *inhibitors*) which inhibit enzyme activity. Binding of modulator causes conformational changes in the allosteric enzymes which influence their catalytic activity. Enzymes with several modulators generally have different specific binding sites for each.

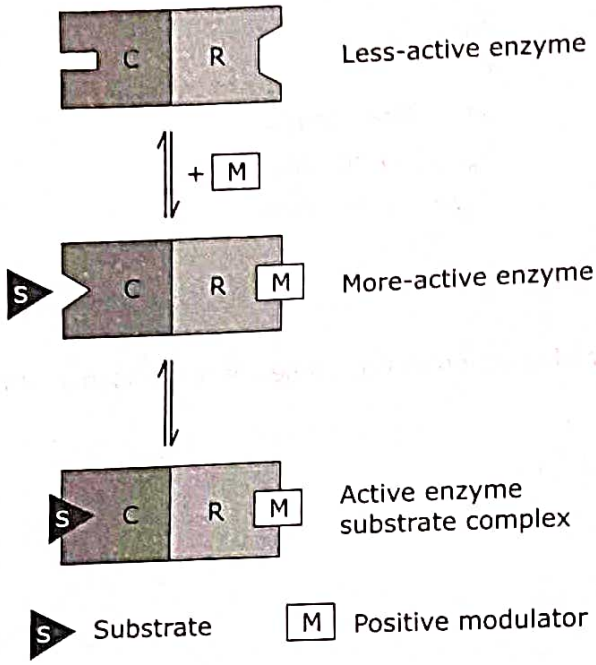
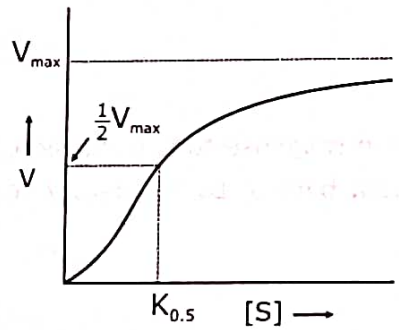
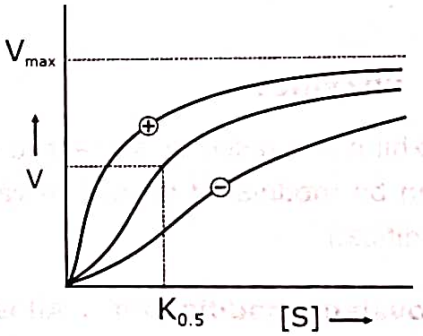


Figure 1.96
Allosteric enzymes are a class of regulatory enzymes which increases or decreases catalytic activities in response to modulators.

Allosteric enzymes don't obey Michaelis-Menten kinetics. Allosteric enzymes show relationships between V_0 and [S] that differ from Michaelis-Menten kinetics. They do exhibit saturation with the substrate when (S) is sufficiently high, produce a sigmoid saturation rather than the hyperbolic curve typical of non-regulatory enzymes. Sigmoid kinetic behavior generally reflects cooperative interactions between protein subunits. The principles are particularly well illustrated by a nonenzyme; O_2 binding to hemoglobin. Sigmoid kinetic behavior is explained by the *concerted* and *sequential models* for subunit interaction.



The sigmoid curve of a homotropic allosteric enzyme



The effects of a positive modulator (+) and a negative modulator (–) on heterotropic allosteric enzyme. The central curve shows the substrate-activity relationship without a modulator

Regulation by reversible covalent modification

Activity of some regulatory enzymes is modulated by *reversible covalent modification*. Covalent modification includes phosphorylation, adenylylation, methylation, uridylylation and ADP-ribosylation. Phosphorylation is the most common type of covalent modification. An important example of regulation by phosphorylation occurs in *glycogen phosphorylase* of muscle and liver, which catalyzes the glycogenolysis. Glycogen phosphorylase occurs in two forms: Glycogen phosphorylase *a* (active form) and glycogen phosphorylase *b* (less active form). In glycogen phosphorylase *a*, specific *Ser* residues, one on each subunit, are phosphorylated. Glycogen phosphorylase *a* is converted to the less active glycogen phosphorylase *b* by the enzymatic loss of these phosphoryl group. Glycogen phosphorylase *b* can be reconverted to glycogen phosphorylase *a* by the action of phosphorylase kinase.

Table 1.28 Some enzymes whose activity can be controlled by reversible covalent modification

Enzyme	*Modification*
Glycogen phosphorylase	Phosphorylation
Glycogen synthase	Phosphorylation
Acetyl-CoA carboxylase	Phosphorylation
Glutamine synthetase (*E. coli*)	Adenylylation
Nitrogenase	ADP-ribosylation

Feedback inhibition and feedback repression

In *feedback inhibition* (or end product inhibition), the end product of a biosynthetic pathway inhibits the *activity* of the first enzyme that is unique to the pathway, thus controlling production of the end product. The first enzyme in the pathway is an allosteric enzyme. Its allosteric site will bind to the end product of the pathway which alters its active site so that it cannot mediate the enzymatic reaction. It is a negative regulation. It prevents enzyme from acting. Enzymes can also be subject to positive regulation, in which the enzyme's activity is stimulated by regulatory molecule rather than being suppressed.

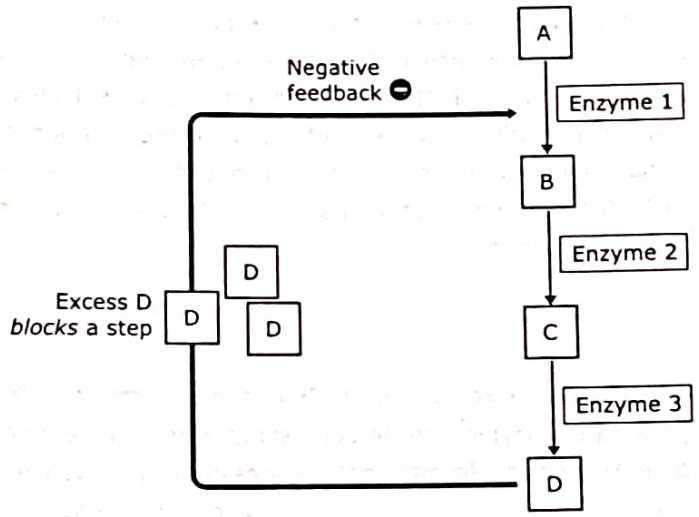


Figure 1.97 The pathway is regulated by the process of feedback inhibition. Compound D, the end product of the pathway, is the effector molecule that binds to the allosteric site of enzyme 1, the first enzyme in the pathway. When compound D is bound to the enzyme the catalytic (active) site of enzyme 1 is altered so that it is unable to react with its substrates and the synthesis of B is inhibited.

If a metabolic pathway branches, leading to the synthesis of two products, each end product can control its own synthesis without affecting the other.

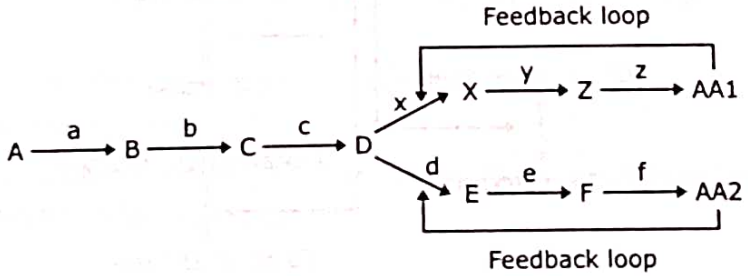


Figure 1.98 Generalized scheme for regulation of a branched metabolic pathway by the process of feedback inhibition.

The feedback inhibition is different from feedback repression. An inhibitory feedback system in which the end product produced in a metabolic pathway acts as a co-repressor and represses the *synthesis* of an enzyme that is required at an earlier stage of the pathway is called *feedback repression*.

1.12.7 Isozymes

Multiple forms of an enzyme that catalyzes the same reaction but differ from each other in their amino acid sequence composition, substrate affinity, V_{max}, and /or regulatory properties are described as *isozymes* (isoenzymes). A common example is lactate dehydrogenase (LDH) which catalyzes the reversible conversion of pyruvate to lactate. There are five LDH isoenzymes. Each is a homo– or heterotetramer of one or two different types of subunits called H and M. Two different subunits combine randomly with each other forming five isozymes.

HHHH (H_4)	LDH1	In the heart and RBCs
HHHM (H_3M)	LDH2	In the reticuloendothelial system
HHMM (H_2M_2)	LDH3	In the lungs
HMMM (HM_3)	LDH4	In the kidneys, placenta
MMMM (M_4)	LDH5	In the liver and striated muscle

Similarly, there are four important mammalian **hexokinase** isozymes that vary in subcellular locations and kinetics with respect to different substrates and conditions. They are designated hexokinases I, II, III and IV or hexokinases A, B, C and D. Hexokinases catalyze the phosphorylation of hexose (such as glucose). While hexokinases I, II and III are capable of phosphorylating several hexoses, hexokinase IV (also referred to as *glucokinase*) acts only on glucose as hexose. Glucokinase is mainly expressed in liver and pancreatic β-cells and is characterized by a low affinity to its substrate glucose. Hexokinases I, II and III are referred to as 'low-K_m' isozymes because of a high affinity for glucose even at low concentrations (below 1 mM) and all three are strongly inhibited by their product, glucose-6-phosphate. Glucokinase can only phosphorylate glucose if the concentration of this substrate is high enough (its K_m for glucose is 100 times higher than that of hexokinases I, II and III) and is not allosterically inhibited by its product, glucose-6-phosphate.

1.12.8 Zymogen

An inactive precursor of an enzyme is called *zymogen*. Zymogen is cleaved to form the active enzyme. Many proteolytic enzymes like chymotrypsin, trypsin are initially synthesized as inactive precursor chymotrypsinogen and trypsinogen. Specific cleavage causes conformational changes in the inactive precursor that expose the enzyme active site. In the duodenum, the pancreatic zymogens, *trypsinogen, chymotrypsinogen, proelastase* and *procarboxypeptidase* are converted into active enzymes by enteropeptidase and trypsin, as shown in figure 1.99.

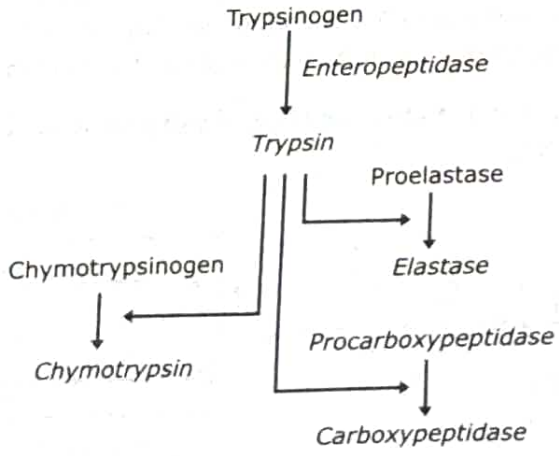


Figure 1.99 Activation of pancreatic zymogens.

Chymotrypsinogen, a single polypeptide chain of 245 amino acid residues, is converted to α-chymotrypsin, which has three polypeptide chains linked by two of the five disulfide bonds present in the primary structure of chymotrypsinogen.

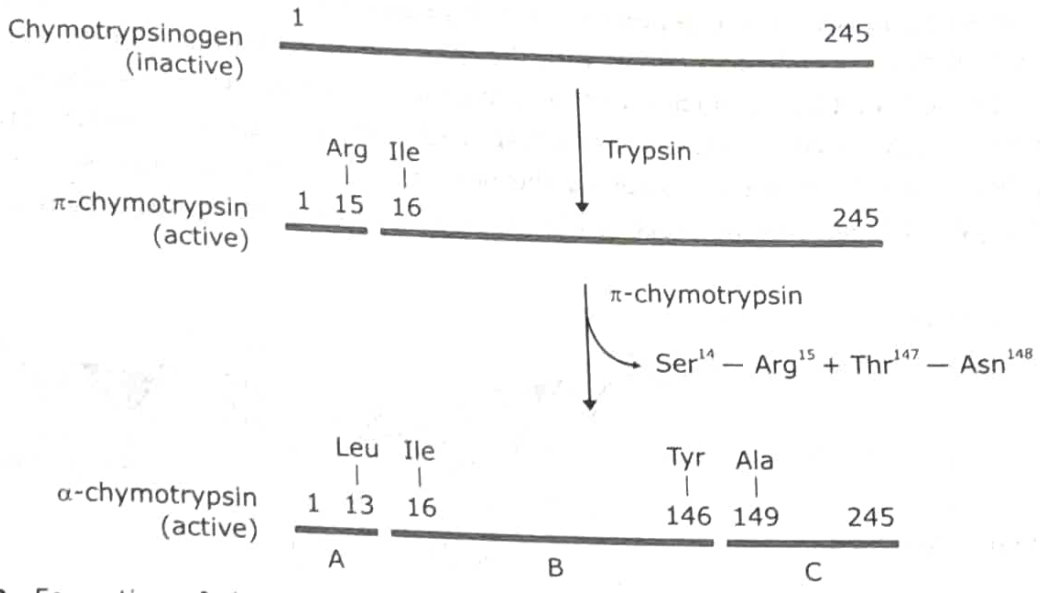


Figure 1.100 Formation of chymotrypsin (active) from chymotrypsinogen (inactive). The numbering of amino acid residues represents their positions in the primary sequence of the zymogen, chymotrypsinogen. The three polypeptide chains (A, B and C) of chymotrypsin are linked by disulfide bonds.

Defination of Enzyme's Unit

1) **International Unit:** International unit of an enzyme is that amount of the catalysis can form 1 um product per minute. (IU).

2) **Unit activity:** Unit activity can be defined as the amount of enzyme which will convert 1 um of substrate into product in one minute.

3) **Specific activity:** It is defined as total enzyme unit present per milligram of protein.
It is usually the index of purity of enzyme, increase in purification of enzyme and becoming maximum and constant when the enzyme is in the pure state.

$$\text{Specific activity} = \frac{\text{enzyme unit}}{\text{mg of protein}}$$

$$= \frac{\text{Activity of enzyme present}}{\text{mg of protein}}$$

4) **T.O.N =** It is defined as the number of substrate molecules transformed per minute by a single enzyme molecule when the enzyme is the rate limiting factor.

5) **Katal:** One katal is the amount of enzyme activity that transforms one mole of substrate per second. Katal is the enzyme activity unit in SI system.

$$1\ IU = \frac{1}{60}\ \mu Katal = 16.67\ n.Katal$$

Enzymes & Activation Energy