

ENZYMOLGY

Regulation of enzyme activity

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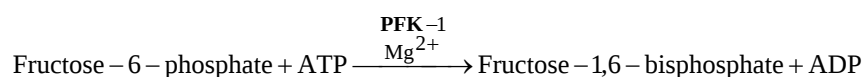
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Keywords

Metabolic pathway; Enzyme activity regulation; Metabolic regulation; Feedback inhibition; Covalent modification; Enzyme anchoring to membranes; Induction; Repression.

Introduction

In cells of an organism, the biochemical transformations taking place in a relationship of 'substrate-product-substrate' through enzyme catalyzed sequence of reactions are called 'metabolic pathways'. The sequence of reactions in glycolysis, tricarboxylic acid cycle, pathway of fatty acid catabolism and reactions of nucleotide biosynthesis, etc. are a few examples of 'metabolic pathway'. The flux (activities) through these pathways increase or decrease as per the requirement of the organism or cell. Most of the enzymes involved follow the kinetic pattern that has been described in detail in previous chapter. It is also considered that not all enzymatic reactions occur to the same extent in a cell. As some compounds are required in large amounts so their synthesis is needed to occur in higher rates and, at the same time, where the demand of a compound is less its synthesis would occur in small amounts. However, in any metabolic pathway there exist one or more enzymes which catalyze rate-limiting reactions and thereby control the overall flux (rate) of the metabolic pathway. These enzymes are called regulatory enzymes. These enzymes catalyze a non-equilibrium reaction and their activities are controlled by factors other than the substrate concentration. Allosteric enzymes are very good examples of regulatory enzymes. How an allosteric enzyme is involved in the regulation of metabolic flux through a metabolic pathway can be explained by discussing the example of enzyme phosphofructokinase-1 (PFK-1) involved in muscle glycolysis. PFK-1 catalyzes the following reaction in glycolytic pathway:



The control of glycolysis is the main regulatory property of PFK-1 which is exercised in following manner:

The activity of PFK-1 is inhibited by high concentration of ATP and this inhibition is overcome by AMP, Pi and fructose-6-phosphate (F-6-P). This enzyme is a tetrameric (4-subunits) enzyme and exists in two conformational states (designated as R and T) which are in equilibrium ($R \rightleftharpoons T$). As seen from the above reaction, ATP is a substrate but, it also acts as allosteric inhibitor. Each subunit has two binding sites for ATP, one as substrate-site and the other as inhibitor-site. The substrate-site in either conformation (R or T) binds ATP equally well but the inhibitor-site binds ATP only in the T conformation. F-6-P has preferential binding to enzyme in R conformation. Under metabolic conditions when ATP concentration is high (more than required by the muscle cell), it acts as a heterotropic allosteric inhibitor of PFK-1 and binds to T conformation. This binding shifts the equilibrium of $R \rightleftharpoons T$ in favour of T thereby resulting a decrease in affinity of PFK for F-6-P. If a graph is plotted between the velocity of reaction and F-6-P concentration each under the conditions of low (non-inhibitory) and high (inhibitory) concentrations of ATP and compared then it is found, as per Fig. 1, that a hyperbolic curve (at low ATP) is transformed into a sigmoidal curve (at high ATP) which is a characteristic of allosteric enzymes. Thus at high concentration of ATP (i.e. when its demand is low) the allosteric inhibition of enzyme by ATP lowers the flux through the pathway. On the other hand, at the inhibitory ATP concentration the ATP inhibition of enzyme is overcome by AMP which has a preferential binding for R conformation of enzyme and thus it apparently activates the enzyme. This can be seen from the Fig.2.

In muscular activity (i.e. muscle contraction) ATP is consumed for providing energy and broken down into ADP and Pi as per reaction given below:

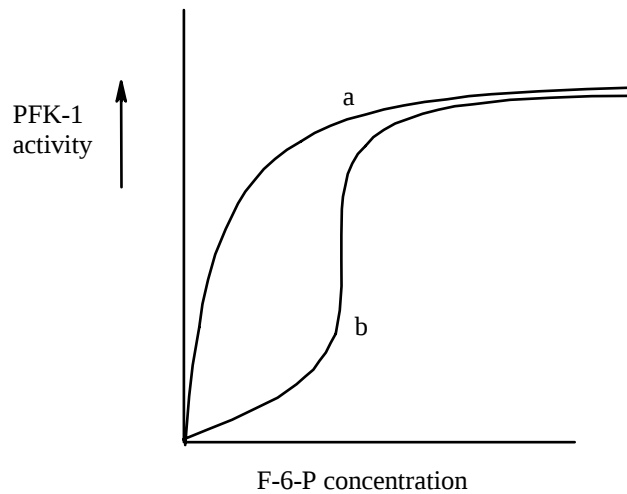


Fig. 1: Plot of PFK-1 activity as a function of F-6-P concentration at non-inhibitory (curve a) and in inhibitory (curve b) concentrations of ATP

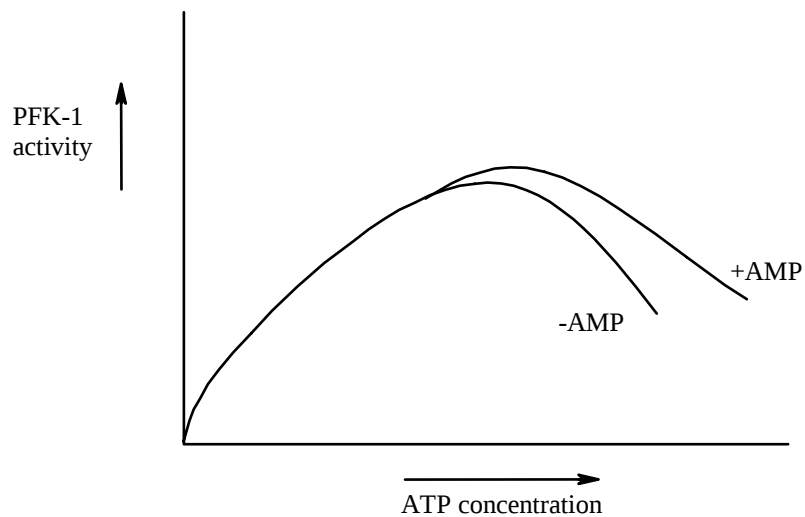
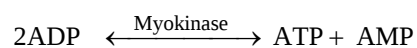


Fig. 2: Plot of PFK-1 activity against ATP concentration in absence and presence of AMP

The ADP thus formed is acted upon by enzyme myokinase to form ATP and AMP;



In addition, there are other mechanisms which make enzyme activity within a cell more efficient and well coordinated. These mechanisms, involved in regulation of enzyme activity and thereby the metabolism, are described below.

I. Regulation of activity by feedback inhibition

When in a metabolic pathway a substrate, S, is transformed into a product, P, through a series of enzymatic reactions (Fig.3) and if P accumulates in amounts that are not immediately needed by the cell then this product specifically inhibits the action of the first enzyme, E_1 , of the pathway. Thus, further transformation of S in that direction is stopped. This is called feedback inhibition or end-product inhibition. Two noteworthy points of this inhibition are following:

- (i) None of the intermediate products inhibits the enzyme E_1 .
- (ii) The other enzymes in the pathway except E_1 are not inhibited by P.

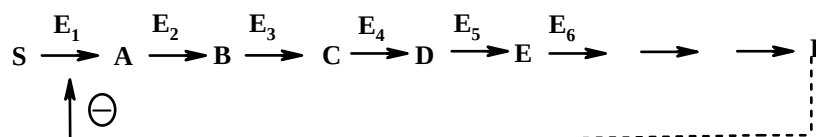


Fig. 3: A schematic presentation of pathway involving Feedback inhibition

So, only regulatory enzymes are subjected to feedback inhibition. These enzymes are allosteric in nature. The following are the well established examples showing feedback inhibition:

1. Inhibition of enzyme aspartate transcarbamoylase (ATCase) by nucleotide cytidine triphosphate (CTP).

ATCase catalyzes the synthesis of nucleoside triphosphate, CTP, from aspartic acid and carbamoyl phosphate through a sequence of reactions. The end product of the pathway, CTP, is responsible for inhibition of first enzyme when needed, as shown below:

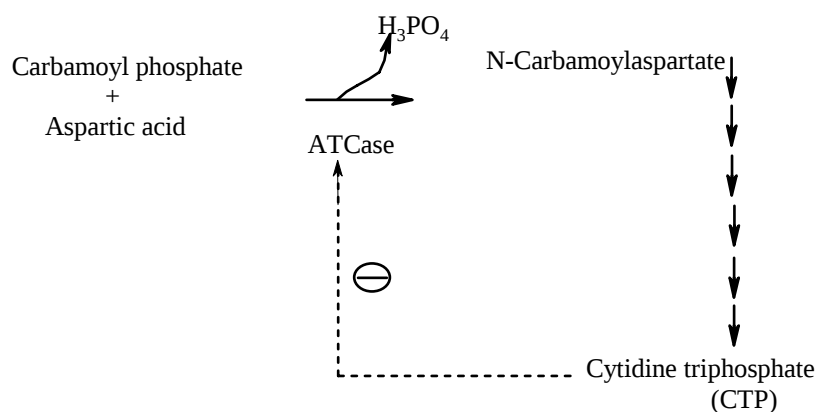


Fig. 4: Feedback inhibition by CTP of enzyme ATCase

2. Inhibition of enzyme L-threonine deaminase by amino acid isoleucine

Threonine deaminase initiates conversion of L-threonine to α -ketobutyric acid and subsequently through a series of reactions synthesis of isoleucine takes place. Isoleucine regulates its own synthesis by inhibiting the first enzyme of the pathway as shown below:

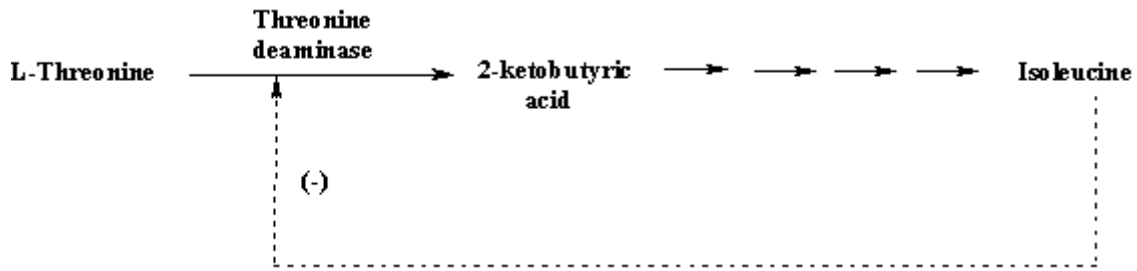


Fig. 5: Feedback inhibition of threonine deaminase by isoleucine

The inhibition of these activities by the end-product can also be shown *in vitro*.

This type of regulatory mechanism involves enzymes undergoing through changes in weak interactions and is an example of fine control of metabolism because these regulatory enzymes are responsive to changes taking place in cells on second-to-second basis.

Many metabolic pathways are characterized by a number of branch points. In the example shown in Fig. 6, the substrate, S, may be converted either to end product P₁ or P₂ or P₃ or P₄ or all. In such a pathway, the enzyme affected by feedback inhibition would be present at the branch points as shown. For example, if product P₂ is present in excess then it would inhibit its synthesis and thus requirement of the intermediate metabolite, A, would decrease. However, the production of A continues to some extent to meet the needs of pathway responsible for the synthesis of P₁, P₃ and P₄. If the production of excess P₂ inhibits not only the pathway unique for its production but does so at other sites then this would stop synthesis of other end-products, i.e. P₁, P₃ and P₄. There are however, mechanisms which overcome such situations. These are following:

Cumulative feedback inhibition: In this case the inhibition by one end product is only partial. Thus, the total inhibitory effect of more than one end product on a regulatory enzyme is strictly additive.

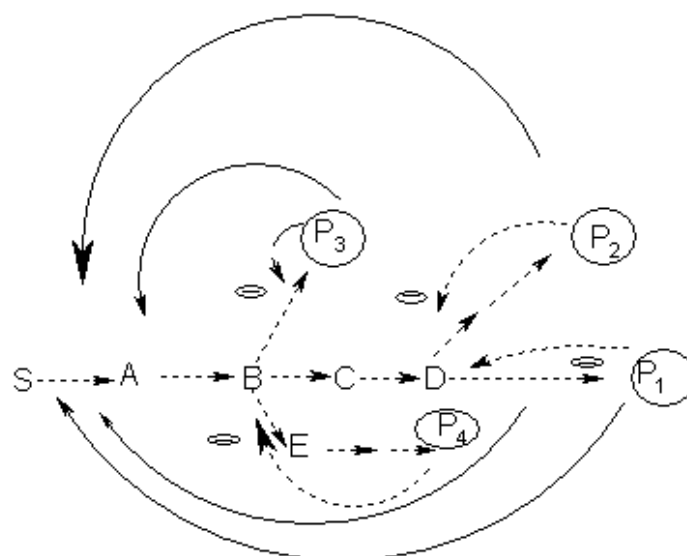


Fig. 6: Multiple sites of feedback inhibition by various end products in a branched metabolic pathway

Concerted feedback inhibition: In this case the total inhibition is observed when two or more end products in excess are simultaneously present.

One such example is of enzyme aspartokinase in microorganisms, e.g. in *Escherichia coli*. This enzyme initiates the synthesis of *threonine*, *methionine* and *lysine* in a branched pathway in a fashion depicted in Fig. 7. In *E. coli*, there are three isozymes of aspartokinase. Two forms of isoenzymes are subject to allosteric regulation; one by lysine and the other by threonine. The synthesis of third form is subject to repressive control by amino acid, methionine. In this, methionine acts in repressing the expression of corresponding specific gene. It is, therefore, called corepressor.

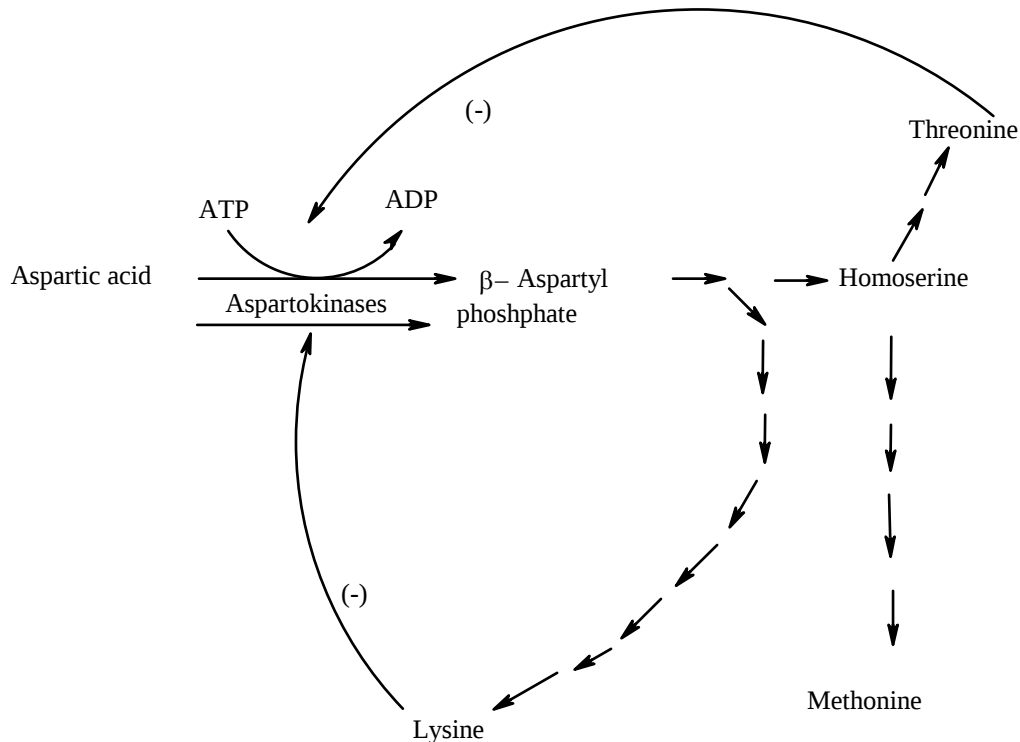


Fig. 7: Concerted feedback inhibition of aspartokinase by lysine, threonine and methionine in *Escherichia coli*.

II. Regulation of activity by covalent modification

(a) *Reversible covalent modification*

This is also one of the major ways of controlling the enzyme activity to exercise a regulatory control over metabolism. In this the enzyme protein gets activated or inhibited by undergoing through a covalent modification. These modifications are reversible and require two enzymes. Depending on the metabolic milieu of the cell one enzyme incorporates a covalently linked group and the other enzyme removes it from the enzyme protein whose activity is being controlled (Fig.8).

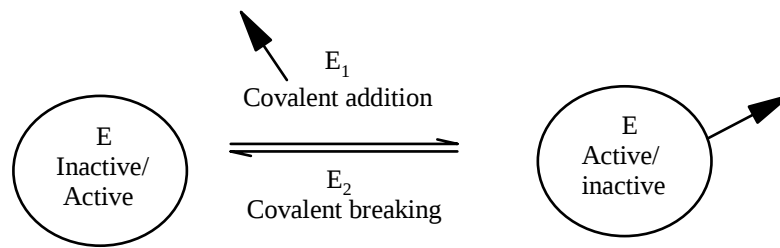
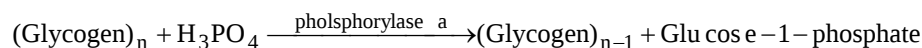


Fig. 8: A schematic presentation of covalent modifications of an enzyme protein

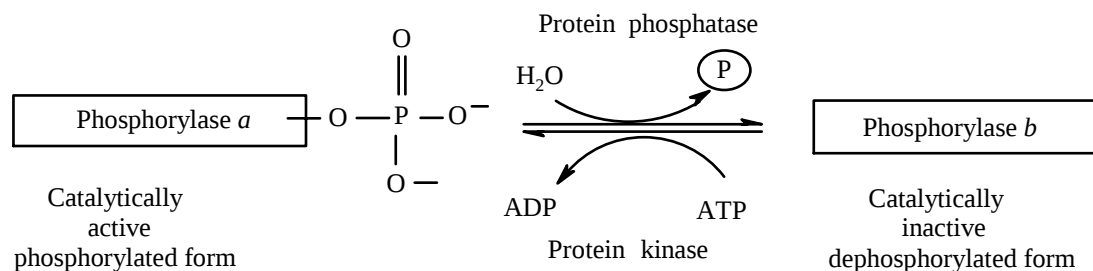
The following are some well established examples of covalent modification:

1. Glycogen phosphorylase: Activated by phosphorylation of enzyme protein.

The enzyme liberates glucose-1-phosphate from glycogen in muscle. A glucose residue at the non-reducing end of the chain is removed by breaking the glycosidic bond involving a phosphoric acid molecule. Thus, a molecule of glucose -1-phosphate is released that acts as a source of energy and glycogen chain becomes shorter by one glucose unit at each step as shown below:



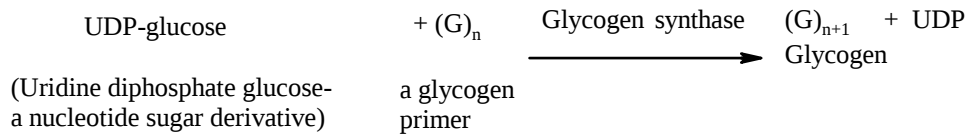
The enzyme phosphorylase in active form is phosphorylated and called phosphorylase **a**. Under the conditions where the breakdown of glycogen is not needed this active enzyme is converted into inactive form called phosphorylase **b**. These two forms of enzyme are interconvertible with the help of two enzymes; a protein phosphatase and a protein kinase as shown below:



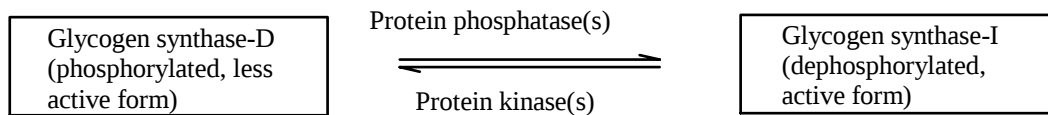
In this enzyme catalyzed interconversion of phosphorylase into *inactive* and *active* forms, respectively by a protein phosphatase and a protein kinase, the incorporation of phosphate group takes place at the -OH group of amino acid serine in regulatory protein.

2. Glycogen synthase: Activated by dephosphorylation

This enzyme is active in dephosphorylated form and turns less active when modified into phosphorylated form. The enzyme is involved in glycogen synthesis in muscle by following reaction:



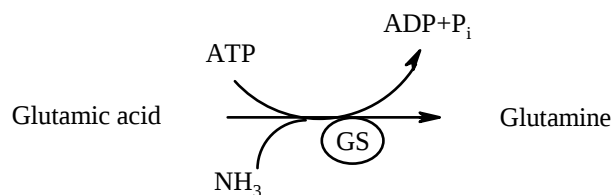
The 'active' and 'less active' forms of glycogen synthase are interconvertible by undergoing covalent modification in following fashion:



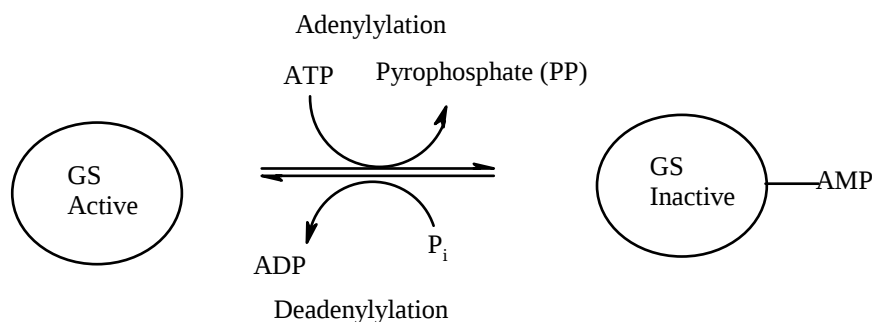
The phosphorylation and dephosphorylation of enzyme glycogen synthase is more complex than seen in case of phosphorylase. However, the regulation of enzymes by phosphorylation and dephosphorylation is very common and this regulation is under the influence of hormones.

3. Glutamine synthase (GS): Activated by deadenylation

This enzyme is involved in the synthesis of glutamine from glutamic acid:



This enzyme in *E. coli* has 12 subunits. The activity of the enzyme is regulated by *adenylation* of each subunit in which a tyrosine residue of enzyme reacts with ATP to form adenylyl derivative under the influence of enzyme *adenylyl transferase*.



The adenylyl and deadenylyl reactions are complex series of reactions and catalyzed by the same enzyme which is a complex made up of two proteins: *adenylyl transferase* and a *regulatory* protein. In general, such mechanisms are called nucleotidylation. When there is incorporation of AMP it is *adenylylation* and when UMP is involved then it is *uridylylation*. Regulation of activity of glutamine synthetase in animal cells is not known.

(b) Irreversible covalent modification

The type of mechanism is exemplified by conversion into active form of digestive enzymes e.g. trypsin and chymotrypsin. These enzymes are synthesized in pancreas in their inactive forms called trypsinogen and chymotrypsinogen, respectively. The general name for such catalytically inactive forms is zymogen or proenzyme. The zymogen form of the enzymes is slightly longer and this inactive form is broken down by the action of a protease to result the formation of active enzyme.

The zymogen form on synthesis in pancreas is secreted in pancreatic juice which then brings it to duodenum where first the N-terminal end of trypsinogen is removed by a protease (enteropeptidase) of duodenum. This results in formation of active trypsin which subsequently activates other zymogens to form respective active enzymes (Fig.9). The process is called zymogen activation.

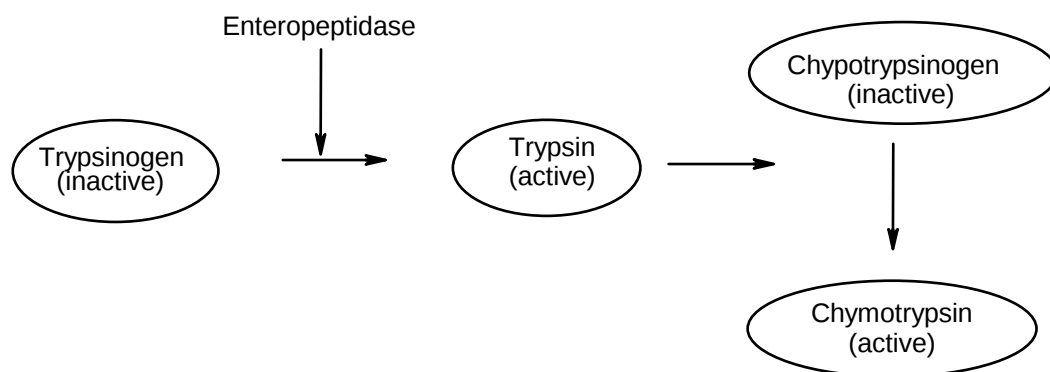


Fig. 9: Sequence of zymogen activation

It should be noted that the secretion of inactive zymogen forms of these enzymes is a protective mechanism to safeguard pancreas from enzymic active form otherwise this would pose a serious crisis for the organ. The zymogen activation can not be reversed.

III. Regulation of activity by anchoring of enzymes in membranes: Spatial relationship

There are many enzymes which are inserted into either in plasma membrane or in subcellular membranes such as mitochondria, chloroplast, ER, etc. These proteins are present either as transmembrane proteins or peripheral proteins. This spatial relationship with the lipid bilayer makes these enzymes more efficient in their function. For example, Na^+ , K^+ -ATPase of mammalian plasma membrane and H^+ -ATPase of plant/microbial cell plasma membrane. These enzymes efficiently couple the enzymic breakdown of ATP with building-up of ion-gradients across the membrane to facilitate the energization of transport processes. In addition, there are examples of regulation of enzyme activity by reversibly binding to membranes. For instance, the activity of enzyme CDP-Choline: 1,2-diacylglycerol phosphocholine transferase undergoes 'active-inactive' cycle on getting bound to endoplasmic reticulum and back to cytosolic form, respectively.

IV. Regulation of activity by enzyme synthesis and degradation

The earlier mechanisms described relate the regulation of activities of enzymes which are already present in the cell, but there are many enzymes that are generally not present all the time in a cell or organism but their need occurs at a particular stage of cell cycle or development.

(a) Regulation by synthesis

This type of mechanism is operative only under the circumstances when there is especial need encountered by the cell. The enzymes that perform the routine general functions are not regulated by this method.

This type of control in cells is exercised at the gene level. If the gene for that enzyme is activated then enzyme synthesis takes place and the process is called enzyme induction. On the contrary, if enzyme synthesis is inhibited it is called repression. This type of control mechanism is operative at the level of either transcription or translation, but mostly it is seen at transcription level. The most common example is the synthesis of β -galactosidase in *E. coli*. This bacterium rapidly multiplies in a medium containing glucose. If lactose is also incorporated in this medium then this disaccharide remains unutilized by the bacterium till glucose is available. During this period the cells of *E. coli* do not possess the activity of enzymes needed for lactose metabolism. However, if this organism is forced to grow under the conditions where lactose is the only available source of carbon then it is capable to synthesize the enzyme(s) needed for lactose metabolism. These enzymes include a protein called *lactose permease*, responsible for transporting lactose into bacterium cells, and enzyme β -galactosidase responsible for converting lactose into glucose and galactose. These monosaccharides are metabolized by *E. coli*. The observations indicated that *E. coli* genome carries the genes responsible to code for the needed proteins. It was also observed that the cells of *E. coli* cultivated in absence of lactose also possessed the above proteins and their mRNA molecules, though only in very small amounts. On transferring these cells into the medium containing lactose, there occurs permeation of small amount of lactose into the cells mediated by the available molecules of lactose permease. The lactose that could enter the cell triggers transcription of gene which facilitates generation of more permease molecules to cause rapid lactose uptake by the cells followed by production of large amount of β -galactosidase enzyme to ensure rapid lactose breakdown. Now if you remove lactose from the medium, both permease and β -galactosidase genes are suppressed and return to their original state.

(b) Regulation by degradation

The enzymes that appear in cells in response to a particular situation or requirement (as stated above) may not be needed by the cells at a later stage. If under these circumstances the biosynthesis of enzyme continues then it may pose a threat to the survival of the cell. In general, the enzyme molecules do not have persistent presence in cells but they have a lifetime. There are enzymes that last for many days and at the same time there are enzymes which have survival time of minutes or even less. Proteolytic degradation of cellular enzymes is the general mechanism determining their survival time. The enzymes which are present at key control points in metabolic pathways are rapidly degraded and, if required, they are equally rapidly synthesized. Similarly, there are proteins having longer survival time so they do not rapidly disappear and when required they do take sometime in synthesis.

The degradation of enzymes is also important for the removal of faulty proteins which otherwise would be harmful for the cell. Thus, cell can either activate or inhibit various metabolic pathways by controlling the amount of enzyme(s) at any time. Such genetic control on the level of enzymes has a response time which ranges from minutes to hours. For instance, in a rapidly growing microorganism it is in minutes and in higher organisms it is in hours.

V. Regulation of activity by other means: Specialized controls

The major control mechanism of enzyme activity have been described earlier, however, there are some specialized ways that are useful in regulating the enzyme activities. These are following:

- (a) Regulation by modulator proteins:** Activities of some enzymes are influenced by binding to some proteins, which are called modulator proteins. One important example is that of cAMP dependent protein kinase. This enzyme protein in its inactive form is a tetramer made up of two catalytic subunits (i.e. enzyme protein) and two regulatory subunits (modulator proteins). The enzyme is activated by its dissociation from modulator subunits mediated by cAMP. The active enzyme is a monomer. Reassociation with modulator protein inactivates the enzyme (Fig. 10).

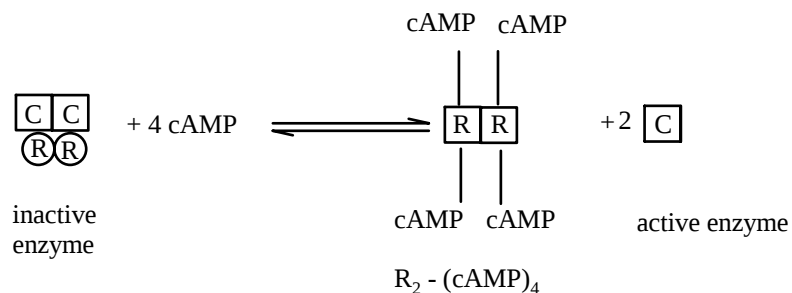


Fig. 10: cAMP –dependent modulation of protein kinase activity

- (b) Regulation by Isozymes:** There are many enzymes which exist in various tissues in multiple molecular forms. The most prominent example of isoenzymes is lactate dehydrogenase (LDH).

The various forms of LDH are expressed in different proportions in different tissues as per the metabolic need of the tissue. These forms differ in their kinetic properties such as affinity for the substrate and sensitivity to product inhibition. For example, in muscle the most predominant isozymic form is M_4 which has higher affinity for $NADH+H^+$. During glycolysis under anaerobic condition in muscle pyruvate and NADH are produced and it needs LDH to regenerate NAD^+ from NADH so that glycolysis can continue. It has been found that muscle M_4 form of LDH works efficiently in the direction of regenerating NAD^+ . Compared to this, the heart tissue, which works under aerobic condition, uses lactic acid as fuel to convert it to pyruvic acid so that pyruvate is oxidized via TCA cycle and energy is produced. The predominant LDH isozymic form in heart is H_4 and this is sensitive to inhibition by high pyruvate concentration. This type of regulatory mechanism stops the wastage of fuel. Thus isozymic forms regulate the metabolic flow depending on the need of the tissue.

One more example of the role of isozymic forms of enzymes in regulation of activity is that of aspartokinase in *E. coli*. As was discussed earlier under feedback inhibition that each isozyme is subjected to allosteric regulation by a separate corresponding amino acid (threonine or lysine). Though essentially it is a case of regulation by feedback inhibition but the involvement of isozymic forms of enzyme has a role in fine control of metabolism.

Thus, we have seen the various strategies adopted by a cell/tissue in regulation of enzyme activities aimed ultimately to enhance the economy and efficiency during metabolism.

Suggested Readings

1. Mathews, C.K. & Van Holde, H.E., Biochemistry, The Benjamin/ Cummings, 1990.
2. Nelson, D.L. & Cox, M.M, Lehninger Principles of Biochemistry, 4th ed., W.H. Freeman & Co., New York, 2005.
3. Voet, D. & Voet, J.G. , Biochemistry 3rd ed., John-Wiley & Sons, 2004.
4. Zubay, G.L., Biochemistry 4th ed., WCB, 1998.
5. Fell, D., Understanding the control of metabolism., Portland Press, 1997.
6. Campbell, M.K., Biochemistry 3rd ed., Harcourt Brace &co., 1999.