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 BTG 406: METABOLIC ENGINEERING

 ASSIGNMENT

Mechanism of metabolic reaction

Metabolic pathways can be linear, e.g. glycolysis or can be cyclic, e.g. TCA. the rate of biosynthesis of cell components is also adjusted to immediate needs. However, the regulation of a metabolic pathway may occur at several levels namely;

1. The reaction rate of each enzymatic reaction
2. The action of regulatory enzymes

(1) The reaction rate of each enzymatic reaction or a function of the pH and the intracellular concentrations of its substrates products and cofactor which are pre-elements in the regulation of enzyme activity.

1. Substrate availability –A reduction in substrate concentration will decrease the activity of the enzyme (provided it is not saturated with substrate) and this could result in a decreased flux through the pathway. Similarly, an increase in (S) could stimulate the path-way. In general however, the constancy of the internal environment of the animal and the cell, as regards the substrates of metabolic pathways implies that such regulatory mechanisms are not common in higher animals.

A typical example of control by substrate availability is that by plasma concentration of fatty acids. The concentration of plasma fatty acids appears to play a fundamentally important role in the regulation of their oxidation by various tissues and in turn their oxidation can modify the rate of carbohydrate utilization by the animal.

1. Cofactor availability – Similar to control by substrate availability. However, substantial inhibition of enzyme activity could be achieved only if the concentration of the cofactor was reduced to very low levels. This may only be possible if the cofactor is specific for the particular pathway in question and is not required for other pathways.

A typical example is carmitine, a cofactor involved in fatty acid oxidation. Fatty acids are activated by an enzyme, fatty acyl-CoA synthetase to produce fatty acyl-CoA, a reaction that occurs in the cytoplasm. The β-oxidation of fatty acid occurs inside the mitochondrion. Therefore, the fatty acyl-CoA has to traverse the mitochondrial membranes. The inner mitochondrial membrane is not permeable to fatty acul-CoA; to overcome this barrier, fatty acyl-CoA is converted into fatty acyl-carmitone by the enzyme carmitine acyltransferase. Fatty acyl-carnitine is able to traverse the membrane and on getting into the mitochondrion, is converted back to fatty acyl-CoA and thus provides substrate for β-oxidation. Thus, variations in (carnitone) could regulate the rate of fatty acid oxidation without affecting other metabolic processes.

1. Product removal – If a pathway substrate is converted to the pathway product by a series of reactions, the removal of the product could control the rate of its formation from the substrate. Minor pathways or perhaps specific portions of metabolic pathways may be controlled by such a mechanism.

 A typical example is the conversion of pyruvate to lactate in muscle catalysed by lactate dehydrognase and the movement of lactate from the muscle to the blood. An increased blood flow through the muscle will increase the rate of lactate removal from the muscle which could therefore increase the rate of conversion of pyruvate to lactate.

Another possible, e.g. is the utilization of acetoacetic acid by extra-hepatic tissues.

(2) The second level of control of metabolic pathways is through the action of regulatory enzymes. There are 2 major types of regulatory enzymes:

(a) Allosteric enzymes: These are enzymes whose catalytic activity is modulated through the non-covalent binding of a specific metabolite at a site on the protein other than the catalytic site;

(b) Covalently modulated enzymes: These are enzymes that are interconverted between active and inactive forms by the action of other enzymes. They also respond to non-covalent allosteric modulators. The 2 types of regulatory enzymes respond to alterations in the metabolic state of a cell or tissue on a relatively short time scale – allosteric enzymes within seconds and covalently regulated enzymes within minutes.

(a) Allosteric enzymes.

Allosteric regulation acts to modulate enzymes situated at key steps in metabolic pathways. In metabolic pathways, the end product of the reaction sequence may inhibit an enzyme at or near the beginning of the sequence; such that the rate of the entire pathway is determined by the steady-state concentration of the end-product. Consider the reaction sequence:

 A $\frac{E\_{1}}{\rightarrow }$ B $\frac{E\_{2}}{\rightarrow }$ C $\frac{E\_{3}}{\rightarrow }$ D $\frac{E\_{4}}{\rightarrow }$ G

In this scheme, G represents an essential metabolite (lipid, protein, nucleotide). Here, G, the end-product inhibits the 1st step in the reaction sequence catalysed by E1. Therefore, when sufficient G is synthesized, it blocks further synthesis of itself. This phenomenon whereby product of a reaction sequence inhibits the activity of an enzyme early in the biosynthetic pathway is referred to as feedback inhibition or feedback regulation or end-product inhibition. The 1st enzyme in this sequence that is inhibited by the end product is called an Allosteric enzyme. The reaction catalysed by the allosteric enzyme is usually irreversible under intra-cellular conditions. It is often called the committing reaction or the rate-limiting step; once it occurs all the ensuing reactions of the sequence will take place. Typical examples include regulation of biosynthesis of amino acids in micro-organisms. In the synthesis of L-isoleucune from L-threonine.

(b) Covalently modulated enzymes.

This is a second group of regulatory enzymes that are inter-converted between active and inactive forms by other enzymes by covalent modification of specific amino acid residues on the enzyme surface. Covalent modification may either reinforce or counteract the effects of allosteric regulators and hence may either intensify or tend to nullify allosteric regulatory effects. Regulation by covalent modulation is well documented in animals.

 In mammalian systems, the 2 most common forms of covalent modification are Partial proteolysis and Phosphorylation and De-phosphorylation. Cells lack the ability to reunite the 2 portions of a protein produced by hydrolysis of a peptide bond, proteolysis constitutes an irreversible modification. In contrast, phosphorylation is a reversible. Phosphorylation takes place on seryl, threomyl, or tyrosyl residues and it is catalysed by a group of enzymes known as protein Kinases. PO4 action is versible, the hydrolytic removal of these phosphoryl groups is also possible and it is catalysed by enzymes called protein phosphatases.

The activities of protein Kinases and protein Phosphatases are themselves regulated; if not, their concerted action would be both thermodynamically and biologically unproductive.

A typical example of an enzyme regulated by covalent modification of its activity is glycogen phosphorylase of animal tissues which catalyses the breakdown of glycogen.

(Glucose)n + Pi (Glucose)n-1 + Glucose -1- P04 Glycogen Shortened glucogen molecule