UNIT 11 METABOLIC REGULATION

Structure

11.1 INTRODUCTION

In the previous units, you studied some metabolic pathways and their energetics. The energy released in the process of metabolism is utilised in carrying out the functions of the living cells. These cells exhibit a marked economy in the release and utilisation of metabolic energy and in the synthesis of complex organic molecules and their precursors. This is achieved by regulation of the rates of various metabolic pathways in such a way that only the necessary amount of energy is released or precursor synthesised. This flexibility in the rates of metabolic processes arises in several ways. In this unit, we will study some of them taking examples from the metabolic pathways described in the Units 9 and 10. It will, therefore, be necessary for you to recapitulate the preceding units.

Objectives

After studying this unit, you should be able to:

- describe some of the ways in which the living organisms regulate their metabolic rates and
- identify the regulatory relationship between some catabolic and anabolic pathways.

11.2 GENERAL FEATURES OF METABOLIC REGULATION

As mentioned above, for life to proceed in orderly fashion, the flow of metabolites participating in anabolic and catabolic pathways must be regulated. Regulation of metabolic processes is achieved in a number of ways. Since all cellular reactions are catalysed by specific enzymes, the rate of a metabolic pathway depends on the concentrations (also called levels) and catalytic efficiency of enzymes of that pathway. Not all the enzymes of any pathway are under regulatory control, nor it is necessary to do so from the cellular economy point of view. An overall regulation is achieved by modulation of the rates of only some reactions which occur at critical points along the pathway, e.g., the first step or a branching point where more than one pathways meet, etc.

Enzyme concentrations, or levels, depend on the rates of their synthesis and degradation. Some enzymes are always synthesised by the cell at a constant rate. They

are referred to as the **constitutive enzymes**. Their cellular levels are fairly constant. With several other enzymes, the rate of synthesis increases tremendously when the cells are exposed to their specific substrates or analogous compounds. Such enzymes are said to be **induced** and the substrate, or the analogous compound, is referred to as the **inducer**. Details of the latter process, regulation of the rate of enzyme synthesis, will be discussed in Unit 15.

The catalytic efficiency of some of the enzymes, not their levels, vary in response to their environments. You have studied the regulation of enzyme activity in Unit 6 of this course. Therefore, we will briefly recapitulate the previously explained mechanisms. In one of the ways, the end product of a biosynthetic pathway may frequently inhibit the first enzyme of that pathway. This is called **feedback regulation**. In a catabolic pathway, which serves the purpose of release of metabolic energy and its capture in the form of ATP, the latter may be considered as the end product. It is found that several enzymes of glycolysis are inhibited by ATP. Since the end product may have no structural similarity with the substrate of the first enzyme, this phenomenon has been referred to as **allosteric regulation**. The specific compound affecting the catalytic efficiency of such a regulatory enzyme is called an **allosteric effector**, which may be an activator or an inhibitor.

The interaction between an enzyme and its allosteric effector is noncovalent and freely reversible. Another mechanism of regulation is via a **covalent modification** of the enzyme. For example, the first enzyme required for mobilisation of glycogen, the reserve carbohydrate in animals, is glycogen phosphorylase (Unit 9). It is activated by phosphorylation of a specific serine residue in its molecule. The active form reverts to the inactive form when this phosphate group is removed.

In addition to the above, in higher multicellular organisms, the rates of metabolic pathways are also under **hormonal control**. Rate of hormone release and consequently their levels in the blood stream may vary in response to a variety of stimuli. The level of a hormone is sensed by the cells of its target tissue because their membranes contain specific proteins, called **receptors**. This initiates a sequence of events which bring about an alteration of the rates of metabolic processes. This regulation is generally achieved

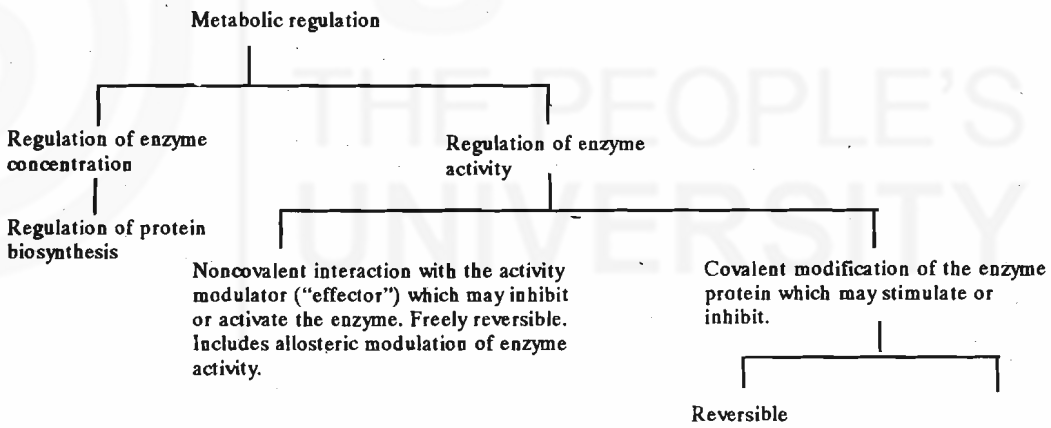


Fig. 11.1 : Summary of the different modes of metabolic regulation

by a combination of the two mechanisms mentioned above, namely by noncovalent interaction with effectors and by reversible covalent modification of the enzyme protein.

A summary of the various modes of metabolic regulation is given in Fig. 11.1.

Energy Charge

Energy charge of the cell is the extent to which the ATP-ADP-AMP system is "filled" with high energy phosphate groups.

As mentioned above, some enzymes of the catabolic pathways are inhibited by ATP. In general, these enzymes are activated by ADP and AMP. Thus, the relative concentrations of the constituents of the adenylate pool (ATP, ADP and AMP) play an important role in metabolic regulation. Their proportion also reflects the energy status of the cell, i.e., of the ready availability of metabolic energy. Daniel Atkinson defined an expression called **energy charge**.

$$\text{Energy charge} = \frac{[ATP] + 1/2[ADP]}{[ATP] + [ADP] + [AMP]}$$

The upper limit of the energy charge is unity, when the pool is made up of ATP only. The lower limit will be zero, when the whole of it exists as AMP. Thus the values for the energy charge can vary from 0 to 1. When the cells are alive, their energy charge lies between 0.85 and 0.95, i.e., ATP predominates. Further, a high energy charge is found to inhibit the ATP generating pathways and stimulate the ATP requiring pathways.

In the following sections, we will discuss some examples of metabolic regulation taken from the pathways described in Units 9 and 10. Before that try to solve the following SAQ.

SAQ 1

Concentration of adenine nucleotides in isolated perfused rat heart tissues under anaerobic conditions was found to be:

ATP = 3.4 mM, ADP= 1.2 mM and AMP = 0.2 mM

Calculate the energy charge from the above.

.....

.....

11.3 REGULATION OF GLYCOLYSIS

Regulation of metabolic paths generally takes place at such steps which are either particularly irreversible due to large free energy drop or where more than one

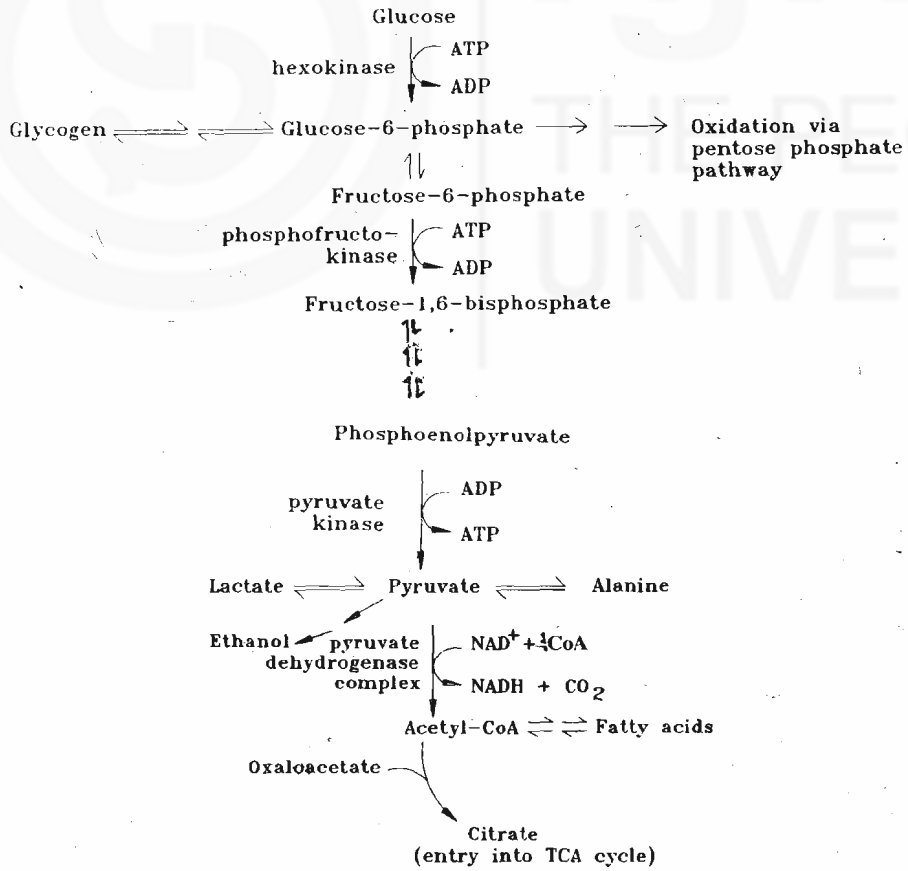


Fig. 11.2 : Conversion of glucose into pyruvate and acetyl-CoA showing practically irreversible steps and branching points in brown colour

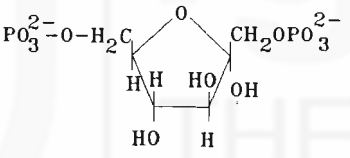
metabolic pathways meet. These meeting points are referred to as branching points. While discussing different metabolic pathways we indicated their important regulatory points. You would recall from Unit 9 that the glycolytic pathway contains three nonequilibrium reactions. These are the ones catalysed by hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase (PK). These steps along with the branching points of glycolytic pathway are shown in Fig. 11.2. Regulation of each of these steps contribute to the overall regulations of this pathway. We shall take up the regulation of PFK first. The justification for the 'out of turn' consideration would follow.

11.3.1 Phosphofructokinase

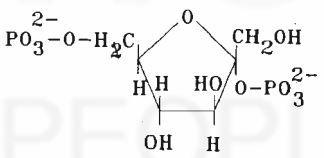
It is probably the most important control element in glycolysis. It is affected by a number of allosteric effectors, some of these activate while others inhibit it. It is inhibited by ATP, NADH and citrate and activated by ADP and AMP. You know that one outcome of glycolysis is the net conversion of ADP into ATP. In a way, ATP may be considered as an end product. Thus the end product inhibits one of the initial reactions of the pathway. Another function of glycolysis is to provide precursors like pyruvate and acetyl-CoA, which are also feed materials for TCA cycle. Citrate is probably an indicator molecule in this case. Its high concentration indicates an abundant supply of pyruvate and acetyl-CoA from alternative sources, e.g., from alanine and fatty acids respectively, Fig. 11.2.

It is noteworthy that AMP and ADP are activators of this enzyme, whereas ATP is an inhibitor. This suggests a strong effect of the energy charge on the activity of PFK. Its catalytic efficiency, and consequently the rate of glycolysis increases when the energy charge decreases.

Another activator of PFK and of glycolysis was discovered in 1980 by H.G. Hers and E. van Schaftingen. It was identified as fructose-2,6-bisphosphate (F-2,6-bis-P). Note the difference between structures of this effector and the product of the reaction catalysed by this enzyme, namely, fructose-1,6-bisphosphate.



Fructose-1,6-bisphosphate
(product)



Fructose-2,6-bisphosphate
(effector)

F-2,6-bis-P is effective at very small concentrations, in the range 0.1-1.0 μM. It enhances the affinity of the enzyme for its substrate, namely, fructose-6-phosphate, and decreases that for the inhibitor, i.e., ATP. Let us try to understand how is this done.

F-2,6-bis-P is formed from fructose-6-phosphate and ATP. The reaction is catalysed by **phosphofructokinase-2** (PFK-2). This activator can be hydrolysed back to the reactants in a reaction catalysed by the enzyme **fructose bisphosphatase-2** (FBPase-2). These two enzymes are different from the ones doing the similar job for fructose-1,6-bisphosphate. Fructose-6-phosphate reacts with ATP, the inhibitor for PFK and gives F-2,6-bis-P. Once formed, it activates its own formation by activating PFK-2. Simultaneous inhibition of FBPase-2 ensures a building up of the concentration of F-2,6-bis-P. This, in turn now activates PFK and makes the glycolysis process start. When the level of glucose in blood is low, a glucagon triggered cascade leads to activation of the FBPase-2 and inhibits PFK-2 whereby the level of F-2,6-bis-P decreases. This causes the deactivation of PFK and slowing down of glycolysis.

It is interesting to note that the activities of PFK-2 and FBPase-2 are exhibited by the same protein. F-2,6-bis-P activates PFK-2 and inhibits FBPase-2.

Inhibition of PFK leads to the accumulation of fructose-6-phosphate and also of glucose-6-phosphate, because the two are in equilibrium with each other. Since hexokinase is inhibited by its own product, namely glucose-6-phosphate, inhibition of PFK will also cause inhibition of hexokinase and, therefore, of the entry of glucose into glycolytic chain.

In most metabolic processes, the first "committed step" is the site of regulation. The word "committed" is used here with reference to a "commitment" to carry out some task. Once the reaction of the committed step has taken place, the process goes to completion. The reaction catalysed by PFK is the commited step for glycolysis and hence this step is the site of regulation for glycolysis. Let us see why can't the reaction catalysed by HK be a site of regulation in this. Have a look at Fig. 11.2 again. You can see that if the conversion of glucose to Gl-6-P is checked by inhibiting the activity of enzyme HK, Gl-6-P can be made available by the breakdown of glycogen and the glycolytic process can still continue. On the other hand if HK is activated and PFK is inhibited, then inspite of enough Gl-6-P the glycolytic process does not proceed. This Gl-6-P can be utilised for other metabolic pathways, e.g., oxidation via the pentose phosphate pathway and for the synthesis of glycogen (not discussed in this course).

SAQ 2

In most metabolic pathways, the modulation is exerted at the first step which is practically irreversible. In glycolysis, the most important control element is phosphofructokinase and not hexokinase although the later enzyme catalyses the first practically irreversible step with glucose as the substrate. What is the explanation for this apparent anomaly?

. . . . . .

. . . . . .

. . . . . .

. . . . . .

11.3.2 Pyruvate Kinase

This is the third control element in glycolysis. Its significance as a control element in mammals varies from one tissue to another. This is made possible by the occurrence of this enzyme in three different forms, **isozymes**. The L-type isozyme predominates in the liver, M-type in muscle and brain and A-type in the remaining tissues. The liver enzyme is inhibited by ATP and alanine. It may be noted that the product of this reaction, namely, pyruvate is readily interconvertible with alanine (Fig. 11.2). The inhibition by ATP may be considered as inhibition at higher energy charge and that by alanine as inhibition in the presence of excess pyruvate. Thus, the inhibitory properties of pyruvate kinase are similar to those of PFK described above.

Isozymes are the multiple forms of a given enzyme that occur within a single species of organism or even in a single cell.

The L-type pyruvate kinase is stimulated by fructose-1,6-bisphosphate, which is the product of the earlier irreversible step. The reaction catalysed by pyruvate kinase may be considered as the "exit" point from glycolysis. The exit point is stimulated, or "primed", to deal with the increasing flux represented by the higher levels of fructose-1,6-bisphosphate.

11.4 REGULATION OF PYRUVATE DEHYDROGENASE COMPLEX

You read in Unit 10 that pyruvate dehydrogenase complex (PDC) catalyses the following reaction leading to the production of acetyl-CoA which can enter TCA cycle.

$$\text{Pyruvate} + NAD^+ + CoA \rightarrow \text{Acetyl-}CoA + NADH + H^+ + CO_2$$

The enzyme complex is made up of three constituent enzymes, namely, pyruvate decarboxylase (E_1), dihydrolipoyl transacetylase (E_2) and dihydrolipoyl dehydrogenase (E_3).

The substrate, pyruvate, arises from glycoysis and also from alanine. The product of this reaction, acetyl-CoA, also arises from more than one sources, Fig. 11.2. Thus, this reaction connects two branching points in metabolism. Further, the above reaction is practically irreversible. It is not surprising, therefore, that pyruvate dehydrogenase complex is rigorously controlled. This is brought about in the following three ways:

i) Product Inhibition

The product of the reaction, acetyl-CoA, inhibits the activity of the enzyme and helps in regulating it. Let us see how does this happen. You would recall from Sec. 10.3.5, Unit 10 that pyruvate, the reactant for this reaction can be converted to oxaloacetate by the enzyme pyruvate carboxylase. This enzyme is activated by acetyl-CoA.

Under the conditions of biosynthetic requirements α-ketoglutarate is removed from the TCA cycle whereby the regeneration of oxaloacetate, essential for smooth running of the cycle is hampered. This leads to slowing down of citrate synthesis which means an accumulation of acetyl-CoA due to reduced consumption. The increased concentration of acetyl-CoA inhibits pyruvate dehydrogenase complex and activates pyruvate carboxylase. As a consequence more and more of oxaloacetate is formed which is required for smooth running of TCA cycle and biosynthetic work. Once the biosynthetic work is over, α-ketoglutarate is not removed any more. The regeneration of oxaloacetate is resumed and it starts consuming more of acetyl-CoA leading to a lower concentration of acetyl-CoA. This in turn lifts the inhibitory effect of acetyl-CoA on pyruvate dehydrogenase complex and the enzyme starts working faster. Concomitantly the activity of pyruvate carboxylase is lowered in accordance to the lower requirements of oxaloacetate from pyruvate.

Another product of the reaction, namely, NADH also inhibits the activity of pyruvate dehydrogenase complex. When the concentration of NADH is high it binds to E_2 of PDC and inhibits the activity leading to slowing down of production of acetyl-CoA. This, in turn slows down TCA cycle and also the generation of NADH. When reduced coenzyme is required, NAD^+ activates the enzyme to produce more of a etyl-CoA and thereby more of NADH.

ii) Regulation by Energy Charge

Energy charge also works in a way similar to NADH regulation. Under conditions of high energy charge, GTP, which is equivalent to ATP in energy, interacts with E_1 and inhibits it. This decreases the production of ATP. Therefore, the energy charge is lowered. When ATP is required, the AMP activates E_1 and accelerates the cycle.

iii) Regulation by Reversible Covalent Modification

In addition to the above effect of metabolites another mechanism operates to regulate the activity of PDC. It involves covalent modification and is referred to as phosphorylation-dephosphorylation mechanism. PDC contains a few molecules of protein kinase and protein phosphorylase. When ATP is high, protein kinase phosphorylates a specific semi residue of E_1 to give pyruvate dehydrogenase phosphate. This renders the enzyme inactive leading to lower production of acetyl-CoA and consequently of ATP. When ADP is high and pyruvate is available, protein phosphatase hydrolyses the pyruvate dehydrogenase phosphate to reactivate the enzymes.

This reaction is stimulated by Ca^{2+} ions and insulin. Later in this unit, we will come across another example of the activation/inactivation of an enzyme, or a complex, by reversible phosphorylation. This behaviour is observed with many regulatory enzymes.

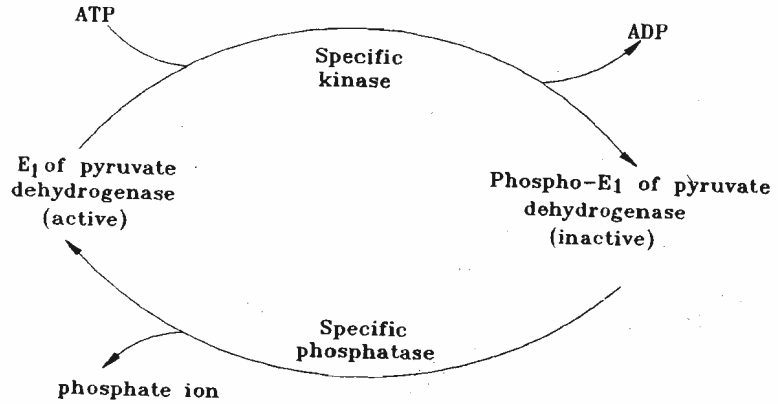


Fig. 11.3 : Regulation of pyruvate dehydrogenase complex by reversible covalent modification of a constituent enzyme

As we can see from the above, the regulation of PDC is, in a way, regulation of TCA cycle from outside. The TCA cycle is also regulated from within the cycle. Before reading about that try to answer the following SAQ.

SAQ 3

Fill in the blank spaces with appropriate words.

i) Pyruvate kinase is inhibited by _____ and _____ in liver.

ii) The regulatory enzyme catalysing the conversion of coenzyme-A to acetyl-CoA is

_____.

iii) NADH inhibits the activity of pyruvate dehydrogenase complex by way of _____.

11.5 REGULATION OF TRICARBOXYLIC ACID CYCLE

You know that the TCA cycle has a central role in metabolism in the sense that the entering metabolite, namely, acetyl-CoA, arises from different pathways (Fig. 11.2). Further, the cycle provides precursors for several biomolecules, like porphyrins and some amino acids. Besides this the cycle is regulated to provide efficient energy production in the cell. Energy production should increase or decrease on the demands of the cell. Thus, the rate of turnover of the cycle must be regulated so as to coordinate its various functions. This is achieved by three control points/elements, Fig. 11.4.

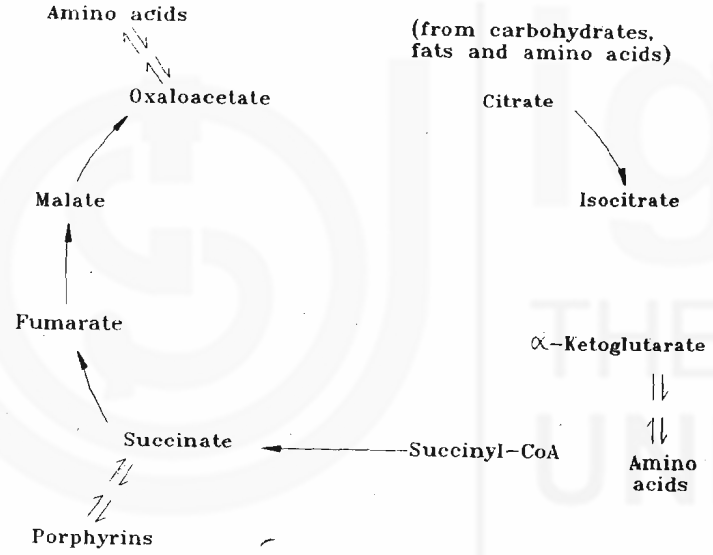


Fig. 11.4 : Tricarboxylic acid cycle showing the control points. The steps shown with brown colour arrows are regulated by energy charge and NADH/NAD$^+$ ratios.

i) **Citrate synthase**, which catalyses the condensation of acetyl-CoA with oxaloacetate, i.e., the entry of acetyl-CoA into the cycle, is inhibited by higher energy charge and NADH. The inhibition by energy charge is not direct. Recall that PDC is also inactivated at higher energy charge whereby the amount of acetyl-CoA formed is reduced. It, in turn slows down the formation of citrate. NADH, regulates citrate synthase negatively. A low NADH/NAD$^+$ ratio favours (as expected) the formation of citrate and hence a better rate of TCA. In the presence of ATP (allosteric effector), the K_m (Michaelis constant, Unit 6, Sec. 6.5) for acetyl-CoA increases. Consequently, the rate of citrate formation decreases.

ii) **Isocitrate dehydrogenase**, which catalyses the conversion of isocitrate of α-ketoglutarate, is negatively regulated by NADH and positively regulated by the second order of NAD$^+$ concentration. This makes the NADH/NAD$^+$ ratio very important for the regulation of its activity. Besides this, ATP/ADP ratio is also significant for this regulation. ATP acts as inhibitor and ADP is a positive modifier of its activity.

iii) **α-Ketoglutarate dehydrogenase complex**, which catalyses the third control point in TCA cycle, is structurally similar to pyruvate dehydrogenase complex and is regulated in a similar manner as described for the later enzyme complex. It is inhibited by succinyl-CoA and NADH (product inhibition) and also by high concentrations of ATP. High concentration of ATP leads to the deactivation of the enzyme and thus accumulation of α-ketoglutarate. α-Ketoglutarate can exit from the cycle by getting converted to glutamate which is needed directly for protein synthesis and also as precursor to many amino acids. When NADH is low the enzyme gets activated leading to increased generation of ATP and decrease in the biosynthetic activity which requires ATP. The net effect of these control elements is to slow down the TCA cycle when ATP/ADP or $NADH/NAD^+$ ratios are high.

SAQ 4

What is the importance of the regulation of the TCA cycle?

. . . . . .

. . . . . .

. . . . . .

. . . . . .

. . . . . .

11.6 REGULATION OF OXIDATIVE PHOSPHORYLATION

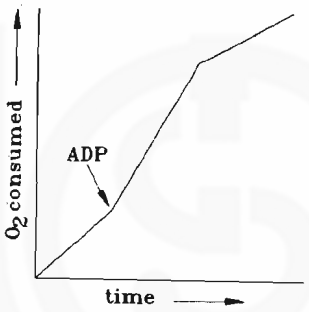


Fig. 11.5 : Schematic diagram showing the rate of oxygen consumption with time. Under normal conditions most of the adenylate pool has ATP with very little ADP and the rate of O_2 consumption is small. Due to sudden energy requirements and consequent conversion of ATP into ADP, the rate may increase upto tenfold. Similar response is produced on the addition of ADP to isolated mitochondria.

You studied in the previous units that the reduced coenzymes, NADH and $FADH_2$, formed on the oxidation of substrates like carbohydrates, fatty acids, etc. are reoxidised on the inner mitochondrial membrane by transferring their electrons to molecular oxygen via the electron transport chain. The electron transport is tightly coupled to the synthesis of ATP from ADP and phosphate ion. Together, they are referred to as oxidative phosphorylation, which you have studied in Unit 8. You would recall that in the electron transport chain protons are pumped from the matrix to the space between the inner and the outer membranes of mitochondria, thereby setting up a proton gradient. As the protons flow back down the gradient into the matrix via H^+-ATPase, also called ATP synthase, the free energy stored in the proton gradient is utilised to drive the endergonic synthesis of ATP from ADP and phosphate ion. Thus, oxidative phosphorylation requires the presence of reduced coenzymes, molecular oxygen, ADP and phosphate ions. The rate of this process is very sensitive to ADP concentration as can be seen in experiments using isolated mitochondria.

The rate of oxygen consumption, and therefore of oxidative phosphorylation, is very small in the resting state called the "**idling rate**". The rate increases by a factor of about ten when saturated concentration of ADP is added to the system. This has been shown schematically in Fig. 11.5. When all the added ADP has been converted into ATP, the rate drops again to the idling rate. This phenomenon is called the **acceptor control** or **respiratory control** of oxidative phosphorylation.

The experimental observation depicted in Fig. 11.5 is frequently used to check the integrity of a mitochondrial preparation, because old and damaged mitochondria do not respond to the addition of ADP.

In Unit 8, you studied that certain compounds called uncouplers bring about an uncoupling of electron transport from ATP synthesis. The uncouplers are presumably attached to the inner mitochondrial membrane and provide an alternative and more facile route for the protons to flow back into the matrix instead of going through the ATP synthase. In their presence, the reduced coenzymes are reoxidised in the usual manner and the resulting free energy is dissipated as heat. A similar principle operates in some physiological systems where it may be necessary to produce heat for maintaining the body temperature, e.g., in hibernating animals, some newborn animals (including humans) and cold-adapted mammals. Their brown adipose tissue is rich in mitochondria in which the inner membrane contains a large amount of a protein called **thermogenin**, also called the uncoupler protein. Thermogenin provides an alternative route for the back flow of protons into the matrix, thus dissipating the free energy,

released during electron transport in the form of heat.

SAQ 5

State whether the following are true or false. Give a brief explanation for your answer.

From the known effects of an uncoupler on the process of oxidative phosphorylation one may infer that its administration might cause,

i) fever in the case of some living beings.

.....

ii) general fatigue and muscle weakness due to deficiency of ATP synthesis.

.....

iii) decreased catabolism of carbohydrate, liquid and protein.

.....

iv) decreased need for oxygen by the body.

.....

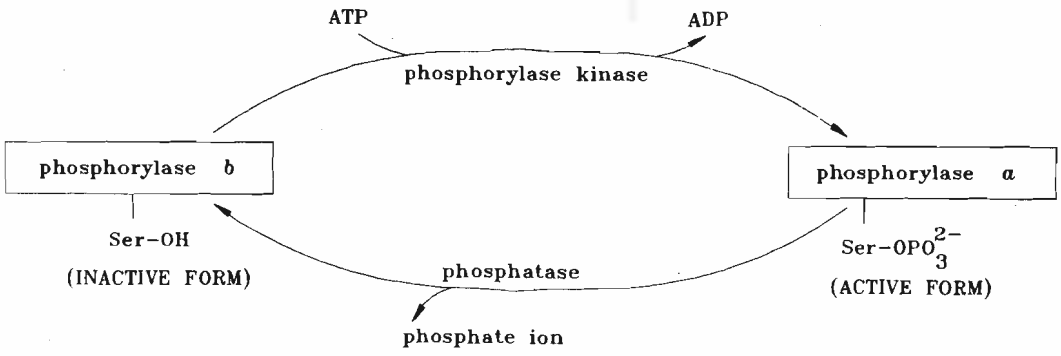
11.7 REGULATION OF GLYCOGEN METABOLISM

As mentioned in Unit 9, glycogen is a reserve polysaccharide made up of glucose units. The latter are mobilised one at a time by means of the following reaction.

$$\underset{\text{glycogen}}{G_n} + \text{Phosphate ion} \xrightarrow[\text{phosphorylase}]{\text{glycogen}} \underset{\text{glucose-1-phosphate}}{\text{Gl-1-P}} + G_{n-1}$$

The product, Gl-1-P, gets isomerised to glucose-6-phosphate which then undergoes glycolysis and other reactions. We would briefly discuss the enzyme catalysing this reaction, i.e., **glycogen phosphorylase** or simply **phosphorylase**. This enzyme exists in two interconvertible forms, namely, an active form called phosphorylase *a* and an inactive form called phosphorylase *b*.

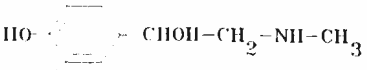
In phosphorylase *a*, some serine residues of the enzyme protein are phosphorylated. The phosphate groups are absent in phosphorylase *b*. Reaction of phosphorylase *b* with ATP, catalysed by **phosphorylase kinase**, gives rise to phosphorylase *a*. The latter is dephosphorylated in the presence of specific phosphatase and is, thereby, reconverted into phosphorylase *b*. This can be shown as follows:



This modulation of enzyme activity by reversible covalent modification, specifically by phosphorylation-dephosphorylation, of the enzyme protein is similar to the regulation of pyruvate dehydrogenase complex described above.

The activities of the two enzymes involved in the interconversion of phosphorylase *a* and phosphorylase *b*, i.e., of phosphorylase kinase and phosphatase, must also be stringently controlled. Otherwise the interconversion reactions provide a route for a wasteful hydrolysis of ATP into ADP and phosphate ion. Such possible wasteful cycles

are called **futile cycles**. It is found that the interconversion of the two forms of phosphorylase is in fact the terminal step in a cascade of reactions. The details of which will not be discussed. These reactions take place when a hormonal signal is detected in the blood stream. The specific hormone involved here is epinephrine, also called adrenalin.



Epinephrine

Epinephrine is synthesised in adrenal medula and may be referred to as an "emergency hormone". Its major function is to enhance the rate of release of metabolic energy. For this purpose, the reserve carbohydrate, glycogen, is mobilised to increase the supply of monosaccharides.

11.8 REGULATION AND COORDINATION OF METABOLIC PATHWAYS: AN OVERVIEW

In the preceding section, our discussion has been mostly limited to the various ways in which some individual enzymes are regulated and how it affects the concerned metabolic pathways. These pathways, however, do not proceed in isolation. They are coordinated with other metabolic pathways with a constant exchange of precursor molecules and energy. A complete description of all the pathways is not necessary at this stage. However, even an introductory description of metabolic regulation will be incomplete, and to some extent distorted, if a description of their interrelationship is not included specially with respect to their regulatory aspects. These relationships form the subject matter of this section.

In the complete oxidation of glucose, we saw three major branching or junction points where exchange of precursors with other metabolic pathways is most noticeable. These are at glucose-6-phosphate, pyruvate and acetyl-CoA stages. Metabolic fates of these species and the relationships of various pathways are summarised in Fig. 11.6.

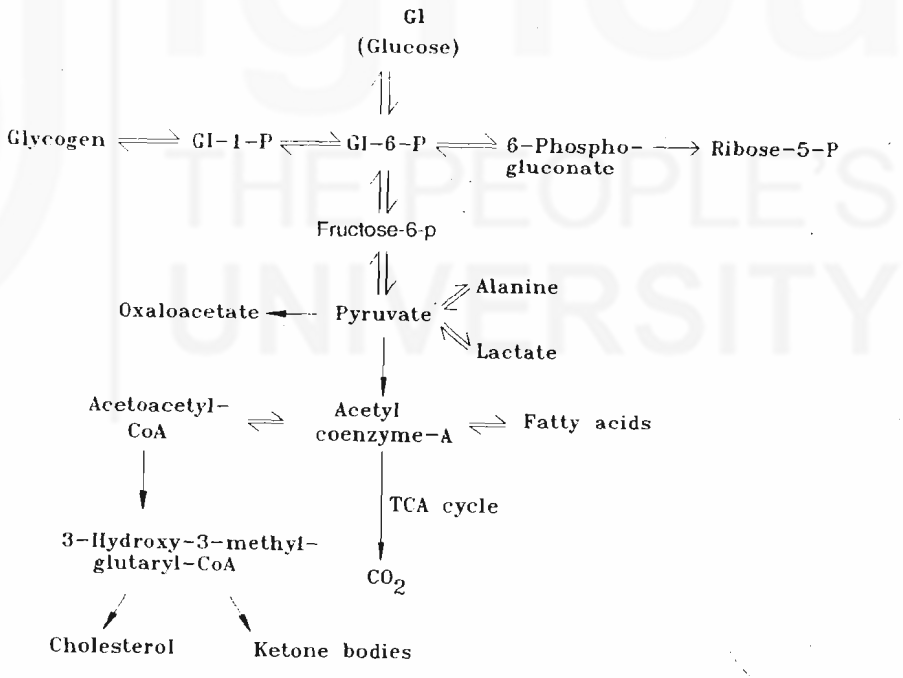


Fig. 11.6 : Branching points and interrelationships of some metabolic pathways. The arrows do not necessarily suggest a single step conversion.

Glucose entering the cell is converted into glucose-6-phosphate (Gl-6-P). The fate of Gl-6-P depends on the metabolic/physiological state of the cell. When there is an abundant supply of ATP and precursors like pyruvate and citrate, the phosphofructokinase step is inhibited. As explained earlier, this prevents the entry of Gl-6-P into the glycolytic pathway. Under these conditions, it is converted into glucose-1-phosphate and glycogen which is the reserve form of carbohydrates. If there

is a pronounced requirement for ATP and precursors for building other biomolecules, the inhibition of phosphofructokinase is reversed and Gl-6-P enters glycolysis. In such an eventuality, even the reserve carbohydrate may also be mobilised to yield more of Gl-6-P.

Alternatively, Gl-6-P can also be degraded via the pentose phosphate pathway (not discussed in this course) which provides NADPH and ribose-5-phosphate. The former functions as the reducing agent in the biosynthetic pathways, e.g., in the biosynthesis of fatty acids and the latter is the source of 5-carbon sugars for the synthesis of nucleotides and nucleic acids. The relative proportions of NADPH and ribose-5-phosphate produced are regulated according to the cellular requirements by slight alterations in the detailed reaction scheme.

Glycolytic breakdown of Gl-6-P leads to the formation of two molecules each of pyruvate and NADH, generating a small amount of ATP. In strenuous exercise, the need of the actively contracting muscles for ATP cannot be met from TCA cycle and electron transport chain for want of adequate supply of oxygen. Under such conditions, glycolysis can be made to run anaerobically for some time by regenerating NAD^+ from NADH by reducing pyruvate to lactate. Lactate is transported to liver, where it is reconverted into pyruvate which is metabolised further according to the needs. We can appreciate how our body transfers a part of the metabolic responsibility from muscles to the liver at the time of necessity.

Interconversion of pyruvate and alanine is one of the links between carbohydrate and amino acid metabolism. Pyruvate from both the sources is converted into acetyl-CoA. This step is inhibited by acetyl-CoA, NADH and GTP which signal the abundance of energy (NADH and GTP) and the precursors. Another "fate" of pyruvate is its carboxylation to form oxaloacetate, catalysed by pyruvate carboxylase (Unit 9). The latter may be converted into fructose-6-phosphate and finally to glucose by gluconeogenesis. This reaction also helps to maintain the concentrations of TCA cycle metabolites at the desired level. This cycle is the link between the anaerobic glycolysis and aerobic electron transport chain. This cycle is also central to the complete oxidation of carbohydrates, fatty acids and several amino acids.

Acetyl-CoA is formed from pyruvate and from fatty acids. Other sources of this metabolite are certain amino acids, called **ketogenic** amino acids. Its entry into the TCA cycle is regulated by inhibition of citrate synthase by ATP, which signals an abundant supply of readily available source of energy. Consequently, further metabolic breakdown and production of ATP is slowed down considerably. Instead, the available acetyl-CoA is utilised for the synthesis of fatty acids, which is another reserve form of metabolic energy. The first step in the biosynthesis of fatty acids, namely the ATP-dependent conversion of acetyl-CoA into malonyl-CoA (Unit 10), is activated by citrate. Third fate of acetyl-CoA is its conversion into β-hydroxy-3-methylglutaryl-CoA (HMG-CoA) which is the precursor in the biosynthesis of cholesterol and all the steroid hormones. Also, HMG-CoA is the source of ketone bodies like acetone, β-ketobutyrate and β-hydroxybutyrate. The formation of ketone bodies becomes significant under starvation or prolonged fasting conditions when the body has depleted its carbohydrate reserves and switches over to its fat deposits for the energy requirements.

In some plants and microorganisms, the acetyl moieties of two molecules of acetyl-CoA combine to give rise to one molecule of succinate (a 4-carbon acid) via glyoxylate cycle (not discussed in this course). Succinate is then converted into oxaloacetate via the reactions of TCA cycle and then to glucose by gluconeogenesis. Consequently, these organisms can convert fats into carbohydrates. Human beings and other mammals lack isocitrate lyase which is a key enzyme of the glyoxylate cycle. Therefore, they cannot convert fats into carbohydrates. The reverse transformation is possible; Fig. 11.6.

It has been mentioned above that alanine can be converted into pyruvate. Some other amino acids can be converted into TCA cycle intermediates like α-ketoglutarate, succinyl-CoA, fumarate or oxaloacetate. Since all these intermediates can be converted into glucose by gluconeogenesis via oxaloacetate, such amino acids are referred to as **glucogenic** amino acids. They constitute another important source of blood glucose under conditions of prolonged starvation. Other amino acids give rise to acetoacetyl-CoA and acetyl-CoA and are called **ketogenic** amino acids, because their

degradation produces ketone bodies as explained above. Some amino acids are both ketogenic as well as glucogenic.

SAQ 6

Under prolonged starvation conditions, can human beings maintain the desired blood glucose level by

i) degradation of body fats?

ii) degradation of amino acids and proteins?

Explain your answer in brief.

. . . . . .

. . . . . .

. . . . . .

. . . . . .

11.9 SUMMARY

Let us briefly summarise what all has been discussed about regulation of metabolism in this unit. The requirements of the living cells for energy and precursor molecules vary with conditions. Therefore, some processes have to be stimulated and others inhibited in response to the changing environments of the cell. This is achieved by modulation of the concentrations or the catalytic efficiency of the concerned enzymes. In many biosynthetic pathways, the end product inhibits the first enzyme of that pathway thus preventing unnecessary accumulation of that product or its intermediates (feedback inhibition). Modulation of an enzyme activity by noncovalent interaction with such specific substances is referred to as allosteric modulation. The catalytic efficiency of some enzymes is also regulated by reversible covalent modification of the enzyme protein. In higher multicellular organisms, the hormonal regulation of metabolism is generally a consequence of a combination of allosteric modulation and reversible covalent modification.

Rate of glycolysis is modulated mainly by regulating the activity of phosphofructokinase (PFK), hexokinase (HK) and pyruvate kinase (PK).

Conversion of pyruvate into acetyl-CoA by pyruvate dehydrogenase complex is inhibited by acetyl-CoA, NADH and GTP. Further, this enzyme complex is inactivated by phosphorylation of one of the constituent enzymes.

Tricarboxylic acid cycle occupies a central position in the final oxidation of acetyl moiety of acetyl-CoA formed from the metabolic breakdown of carbohydrates, fats and some amino acids. The regulatory enzymes of this cycle are citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase complex. Together, their effect is to slow down the cycle at higher values of ATP/ADP or NADH/NAD^+ ratios.

When necessary, glycogen is mobilised to one unit of glucose at a time, with the help of the enzyme glycogen phosphorylase. The activity of this enzyme is regulated by reversible covalent modification as a consequence of the appearance of a hormone, namely, epinephrine.

In the living cells, the various metabolic pathways do not proceed in isolation. They are interdependent and coordinated with other pathways with constant exchange of precursors, energy and "information" in the form of relative concentrations of various key molecules like ATP/ADP, NADH/NAD^+, NADPH/$NADP^+$ ratios. The levels of available precursors, e.g., pentose sugars required for nucleic acid biosynthesis, also stimulate some and inhibit other pathways. Under the conditions of prolonged starvation, the animal body (including human) mobilises its fat deposits and even amino acids to satisfy the energy requirements and to maintain a satisfactory blood glucose level.

11.10 TERMINAL QUESTIONS

1) During a strenuous muscular exercise, the energy charge of the cells decreased suddenly from its normal value of 0.89 to 0.70 and then gradually returned to the normal.
   i) Which changes in the glycolytic pathway might have possibly brought about the recovery?
   ii) What changes in the activity of pyruvate dehydrogenase complex and the enzymes of TCA cycle might also have contributed to the recovery?
   iii) What is the effect of energy charge on the rate of oxidative phosphorylation?
2) The level of ketone bodies is very small under normal conditions, but it increases steeply under starvation conditions. Explain.
3) From your knowledge of metabolic processes, explain whether humans can
   i) convert glucose into fatty acids, and
   ii) convert fatty acids into glucose?
4) Write in short about the coordination in regulation of different metabolic pathways.

11.11 ANSWERS

Self Assessment Questions

1) Energy charge = 0.83
2) The product of the reaction catalysed by hexokinase, namely, glucose-6-phosphate, may be diverted to glycogen synthesis and to pentose phosphate pathway. Other sugars enter glycolysis in between the reaction catalysed by hexokinase and phosphofructokinase. The latter enzyme catalyses a reaction which is the first committed step unique to glycolysis of all sugars.
3) i) ATP, alanine
   ii) pyruvate dehydrogenase complex
   iii) product inhibition
4) TCA cycle has a central role in metabolism and it is regulated to provide efficient and required energy production and precursors for the biosynthetic pathways.
5) i) True, since electron transport but not ATP synthesis can continue in presence of uncoupler and the energy produced is dissipated as heat.
   ii) True, because an uncoupler decreases ATP synthesis.
   iii) False, because a decrease in the ability to synthesise ATP would necessitate increased ATP production through substrate level phosphorylation.
   iv) False. When electron transport is uncoupled from ATP synthesis, its rate increases increasing the need for oxygen.
6) i) No, Human beings do not have isocitrate lyase enzyme which is required for fatty acid conversion to glucose.
   ii) Yes, degradation of glucogenic amino acids can help to maintain the desired blood glucose level.

Terminal Questions

1) i) At lower energy charge, the proportion of ADP and AMP must have increased in the adenylate pool. This must have activated phosphofructokinase and, thereby, stimulated glycolysis. This will lead to the conversion of ADP into ATP.

   ii) Pyruvate dehydrogenase complex is stimulated at lower energy charge. Citrate synthase and isocitrate dehydrogenase are both inhibited by ATP. This inhibition must have been partly reversed at lower energy charge. The overall

effect will be to enhance the rate of oxidation of pyruvate formed during glycolysis.

iii) Oxidative phosphorylation is known to be stimulated by ADP. Consequently, this process will proceed at a faster rate at lower energy charge.

2) Under normal conditions, the acetate moiety of acetyl-CoA is oxidised via TCA cycle and oxidative phosphorylation. An excess of acetyl-CoA is converted into fatty acids. Under prolonged starvation conditions, the supply of nutrient carbohydrates has been stopped and the reserve carbohydrate, glycogen, has been depleted. For its energy requirements, the body mobilises the fat deposits which are rapidly degraded to acetyl-CoA. Since TCA cycle depends on carbohydrates for anaplerosis and maintenance of the levels of its intermediates, it is slowed down under starvation conditions and cannot deal with all the acetyl-CoA formed from fatty acid breakdown. Consequently, acetyl-CoA is diverted to the formation of ketone bodies.

3) i) Yes. Human beings have all the enzymes required for the conversion of glucose into pyruvate and acetyl-CoA and those for the biosynthesis of fatty acids from acetyl-CoA.

ii) No. Acetyl-CoA formed from fatty acids can be converted into glucose with the help of glyoxylate cycle enzymes only. Human beings do not have isocitrate lyase which is a key enzyme of this cycle. Therefore, they cannot convert fatty acids into glucose.

4) See Sec. 11.8 for the answer.