11

Cells and Metabolism: Putting it all Together

11.1 General aspects of metabolism

Much of what has been discussed so far has concentrated on the how and why of chemical reactions and their energetics. We have seen that some biochemical reactions will not proceed spontaneously in the desired direction, but they can be made to do so indirectly by means of coupled reactions, appropriate concentration ratios, and removal of substrates. Often the energy source to drive such pathways is ATP, the major readily available source of metabolic energy in the cell. Primary generation of ATP occurs by oxidative phosphorylation, which involves a series of redox reactions coupled in an electron transport chain (ETC) to generate a proton gradient. The energy available as a result of the proton gradient, the protonmotive force, drives the synthesis of ATP. The primary energy source driving oxidative phosphorylation in cells is glucose, which originates in photosynthetic cells by photosynthesis, another process involving ETCs and redox reactions.

Having dealt with the principles of chemical thermodynamics and with the major energy-producing processes in living organisms, we are now in a position for an overall look at the way these are integrated in individual cells as the processes of metabolism. The word metabolism derives from the Greek for 'change'. As mentioned in an earlier chapter, metabolism refers to the totality of chemical changes that convert the raw materials (foods and various nutrients) into energy plus the many chemical compounds required to nourish the cell.

Some of the metabolic pathway 'maps' found in biochemistry texts are mind boggling in their complexity; they may show hundreds of reactions and contain many interlocking pathways. I shall not deal with metabolism in such detail, as this is not a biochemistry text. My major objectives in this section are to provide an overview of metabolism within the framework that has already been set, and to show that essentially all organisms have the same basic set of metabolic pathways, underlining their common origins and the continuity of life on Earth from the earliest times to the present.

Metabolism in a cell consists of hundreds of reactions organized into metabolic pathways. Each pathway runs in a series of discrete steps, most of which are catalysed by a specific enzyme. By means of these pathways, chemical substrates are converted into various end products via a series of intermediate

chemical types. The term intermediary metabolism is often used to reflect this. Each pathway needs to be regulated, so that the overall balance of chemical metabolites is maintained. The 'correct' or 'desirable' balance of metabolites for each pathway will vary depending on the demands of the cell. When ATP is required, pathways that generate ATP will be activated until the demand is met. Feedback mechanisms that slow down the rate of ATP production come into operation. These feedback mechanisms often involve the allosteric inhibition of a key enzyme or enzymes early in the pathway by a metabolite further along the pathway. This makes sense, as a high concentration of a compound in the pathway close to the end product means that the end product concentration is likely to be high and the pathway can probably be closed down safely.

In Chapter 1 I discussed the concept of producers (autotrophs, or 'self-feeders') and consumers (heterotrophs, or 'feeders on others') in the biological world. This is a simplified classification and we are now in a position to extend it. If we arrange organisms in metabolic pathway terms to include the origins of their carbon-based chemicals, energy sources, and requirements for oxygen, a broader, more useful classification system emerges.

Note the electron donor requirement for all types of organism. Life on Earth depends on electron transfer as a major means of energy transduction. We have discussed redox reactions as a source of energy in the form of electron flow and typical examples of electron donors in such systems are included in Table 11.1.

Phototrophs are photosynthetic organisms, gaining their energy from light. Chemotrophs use organic compounds such as glucose (chemoheterotrophs) or certain simple inorganic compounds such as those listed in Table 11.1 (chemoautotrophs).

Table 11.1. Classification according to carbon, oxygen, and energy requirements.

Metabolic classification	Carbon source*	Energy source	Electron donor
Aerobic photoautotrophs Green plants, algae, cyanobacteria, protists	CO_2	Light/aerobic respiration	H_2O
Anaerobic photoautotrophs Photosynthetic bacteria	CO_2	Light/glycolysis	H_2S, S, and other inorganic compounds
Photoheterotrophs Non-sulphur purple bacteria	Organic compounds	Light	Organic compounds
Chemoautotrophs Nitrifying bacteria; sulphur, hydrogen, and iron bacteria	CO_2	Redox reactions	Inorganic compounds [H_2, H_2S, Fe^{2+}, NH_4^+ Mn^{2+}]
Aerobic heterotrophs All animals; protists, fungi; many microbes	Organic compounds	Aerobic respiration	Organic compounds, e.g. glucose
Anaerobic heterotrophs Fermenting bacteria	Organic compounds	Glycolysis	Organic compounds, e.g. glucose

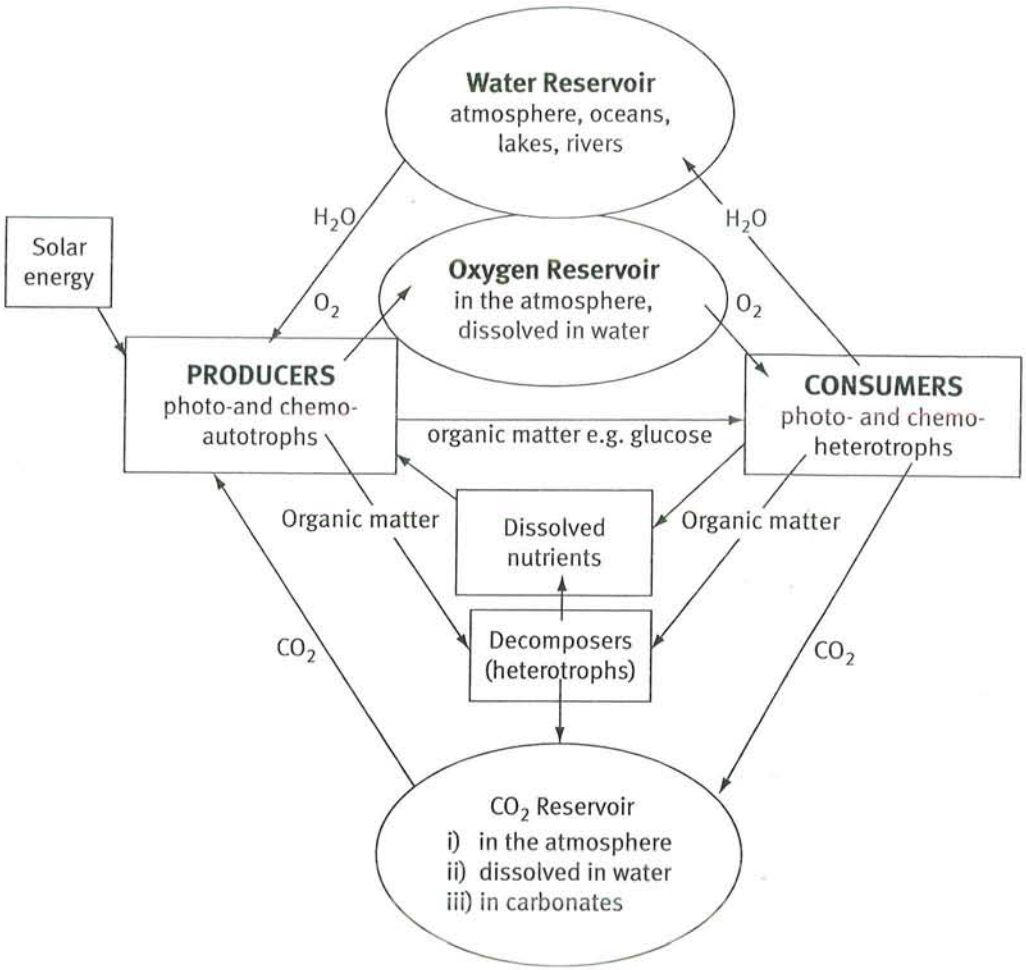


Figure 11.1. Summary of biogeochemical cycles.

Before looking more deeply into metabolic pathways, let's first look very broadly at the flow of energy in Earth's biosphere through some important biogeochemical cycles. This is important to have in mind as a lead-in to metabolic pathways because it reminds us of the interrelated nature of organisms and the biosphere (Figure 11.1).

The scheme in Figure 11.1 outlines the important carbon, oxygen, and water cycles that illustrate the purely chemical interrelationships between living organisms and their environment. Other important cycles are the nitrogen cycle, which refers to the movement of nitrogen through the food chain, the sulphur cycle, and the phosphorus cycle. All these cycles trace the flow of the respective essential nutrients and ingredients in the biosphere. For a healthy biosphere, the cycles should be kept in a state such that the chemical ingredients are present in sufficient amounts and in an accessible form, in other words in 'dynamic balance' or 'steady-state' conditions, terms we have met previously. Taking an idealized example, when a new generation of organisms comes into being, an old generation dies and begins to decompose. The essential nutrients in the decaying old generation enter the cycles and so replace those that have been removed by the new generation.

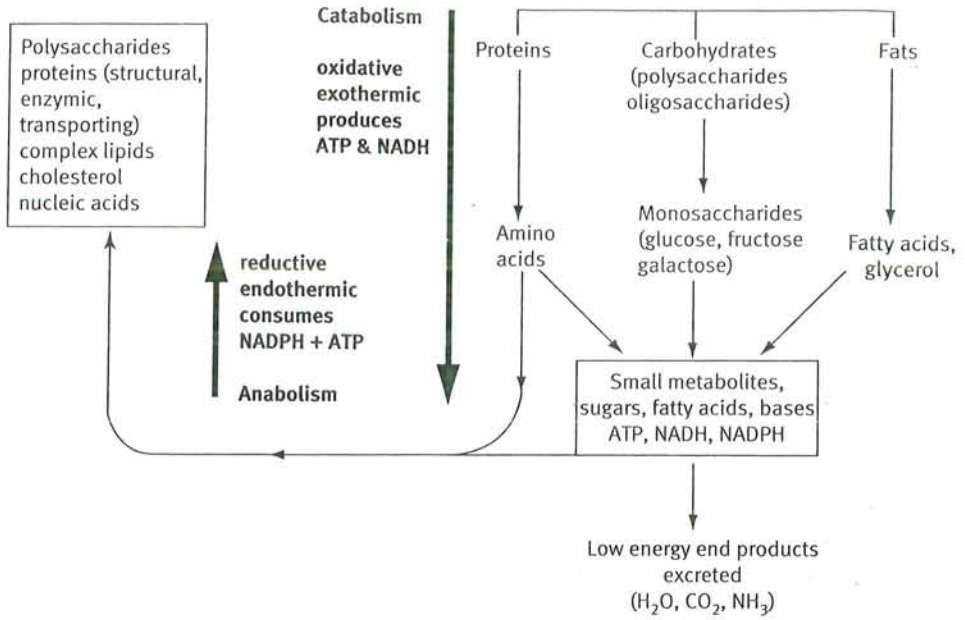


Figure 11.2. General features of anabolism and catabolism for a heterotrophic, aerobic cell.

What the balance may be will vary with the region in question. Knowledge of the state of the cycles can assist biologists and earth scientists to understand what is occurring in the broadest sense and allow planned intervention in cases where environmental deficiencies (or excesses) in nutrients, etc. may occur. If dissolved nutrients from over-irrigation accumulate, combined with a rising water-table, the resulting salination of groundwater can be devastating to crops and a serious problem for human consumers. This has occurred in parts of the Murrumbidgee irrigation area in eastern Australia, and even the billions of dollars which need to be spent might not cure the problems of the region. Too high a level of phosphate from fertilizers in a water system may result in a 'bloom' of toxic algae. Coral polyps on the Great Barrier Reef are dying from pollutants in runoff water from cattle-grazing and sugar-cane operations in Queensland. Recent legislation provides for fines of up to A$30,000 for non-compliance with the pesticide and fertilizer use laws. We are all familiar with one or more of these instances of imbalance.

In a similar fashion, within the autotrophs and heterotrophs embedded in the above cycles, there are metabolic pathways and cycles whose 'balance' and 'health' must be maintained. They are kept in control by various feedback mechanisms, but in addition they must have the correct ingredients at the input end. For animals such as ourselves, this basically means access to sufficient food, air, and nutrients; for plants, sufficient water, carbon dioxide, sunlight, and mineral nutrients; each other form of life has its own specific needs for survival. By looking more deeply into the metabolic pathways, we can draw further diagrams to show interrelationships, but firstly let's look at the two quite different purposes of metabolism. For convenience of study, metabolism may be divided into catabolism, which involves mainly oxidative, degradative pathways, and anabolism, which largely involves reductive, biosynthetic pathways (Figure 11.2).

Typically, catabolic pathways are involved in generating energy—they are exothermic—for use by the cell by transforming the chemical energy in foodstuffs and are usually oxidative in their chemistry. In contrast, anabolic pathways usually require the consumption of energy—they are endothermic—to build complex molecules required by the cell and are reductive in their chemistry. The synthesis of complex molecules is usually endothermic overall and is often achieved by the roundabout method of coupled reactions, as mentioned previously. Anabolic processes typically utilize NADPH as the coenzyme. Catabolic processes produce NADH, which is used as a source of chemical reducing power for the cell and much of this is ultimately transferred to the ETC, where it is used via oxidative phosphorylation to form ATP. The enzymes that utilize NADH vs NADPH can discriminate between the two coenzymes on the basis of the extra phosphate group in the latter. Catabolic processes typically produce ATP as their end product and, as we have seen, ATP is used to drive endothermic reactions.

The above descriptions deal with metabolism in very general terms. Starting with catabolism, let us be specific.

In the discussion that follows, the emphasis is on the overall significance rather than the individual steps to gain an appreciation of the pathways and cycles, and the ways in which they are interdependent. It is a problem that most of us have learned things, especially science, within a largely reductionist framework. When it is necessary to step outside the framework and take a more global view, we often have difficulty. Detail may obscure the broader patterns. To reduce this I shall dispense with chemical formulae as much as possible. Details are provided in appendices, as noted.

In a typical aerobic heterotrophic cell, most energy is derived from the catabolism of carbohydrates, proteins, and fats contained in food (Figure 11.3).

Stage 1 The metabolic pathways of these food materials begin separately, as they are broken down into their components, for example by the early stages of digestion.

Stage 2 In glycolysis, the components are further degraded, and converge to a common product, the two-carbon acetyl groups of acetyl-coenzyme A (acetyl-CoA).

Stage 3 Acetyl-CoA enters a metabolic pathway called the citric acid cycle (also known as the Krebs cycle or the tricarboxylic acid (TCA) cycle). The citric acid cycle plays a central and vital role in aerobic metabolism, feeding into the ETC, where oxidative phosphorylation generates large amounts of ATP. Glucose is the principal source of chemical energy for many life forms, from early anaerobes to today's mammals. For this reason I will concentrate on glucose pathways from now on and go into no further detail about the ways in which lipids and proteins (see Figure 11.3) are converted to pyruvate, to acetyl-CoA, or to compounds that can feed into the citric acid cycle.

11.2 Glycolysis

The metabolic pathway from glucose to pyruvate (pyruvic acid) is called glycolysis. Glycolysis itself is anaerobic, in keeping with its early evolutionary origins as a major pathway in anaerobic bacteria. It takes place in the cytoplasm

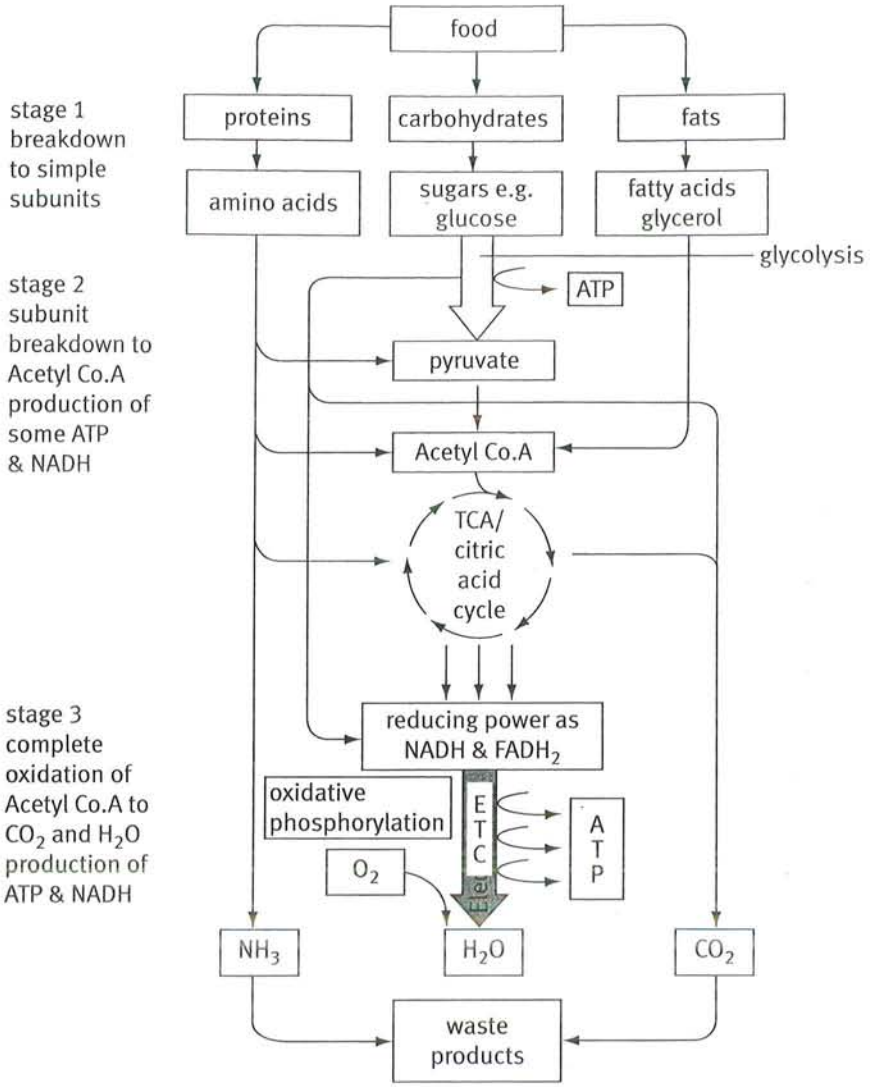


Figure 11.3. Catabolism. The three stages by which foods are broken down to generate ATP. ETP = electron transport chain/respiratory chain. (Illustration adapted from Pocock, G. and Richards, C.D. *Human Physiology*, p. 29. © 1999 Oxford University Press.)

of both prokaryotes and eukaryotes. It is believed to be the most ancient metabolic pathway, occurring in essentially all cells, having evolved before oxygen appeared in significant amounts in the atmosphere (Garrett and Grisham 1999 p. 609; Schopf 1999). Glycolysis has a dual role overall: to generate ATP and to produce intermediates that are used in a number of biosynthetic pathways, for example acetyl-CoA is the precursor for fatty acid biosynthesis. In glycolysis, two ATP are consumed and four ATP are generated, a net yield of two ATP per glucose molecule. Two NADH molecules are also formed and under aerobic conditions these yield further energy, as ATP, via the citric acid cycle, ETC, and oxidative phosphorylation (Figure 11.4).

The first phase of glycolysis actually consumes two ATP. It can be regarded as a preparatory phase that sets up the chemistry for the second, energy-producing phase.

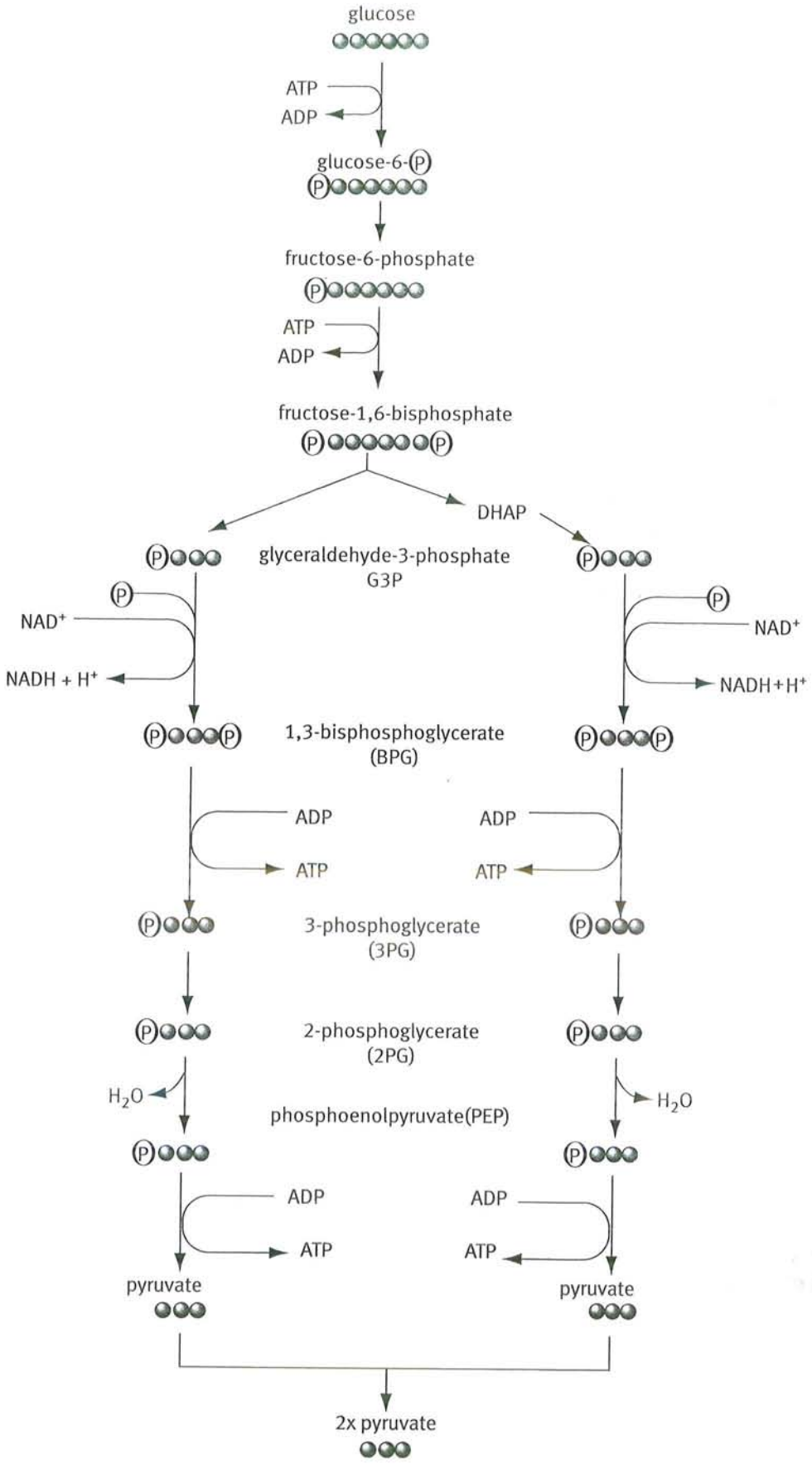


Figure 11.4. Glycolysis, showing the conversion of glucose to pyruvate. DHAP = dihydroxyacetone phosphate, which is immediately converted to G3P. ● represents one carbon atom.

11.3 The reactions of glycolysis

1. Phosphorylation

$$\text{glucose} + \text{ATP} \xrightarrow{\textit{hexokinase}} \text{glucose-6-Ⓟ} + \text{ADP}$$

($\Delta G^{0\prime} = -16.7\ \text{kJ mol}^{-1}$; $\Delta G = -33.9\ \text{kJ mol}^{-1}$ under cellular conditions).

Phosphorylation of glucose keeps it in the cell as this ionized compound cannot diffuse through non-polar membranes. The large negative ΔG makes it a site for regulation of glycolysis.

2. Isomerization

$$\text{glucose-6-Ⓟ} \xrightarrow[\textit{isomerase}]{\textit{phosphogluco-}} \text{fructose-6-Ⓟ}$$

($\Delta G^{0\prime} = +16.7\ \text{kJ mol}^{-1}$; $\Delta G = -2.9\ \text{kJ mol}^{-1}$ under cellular conditions).

The isomerization to fructose operates close to equilibrium in the cell (low ΔG). It produces the fructose molecule, which can undergo ready C_3-C_4 bond cleavage.

3. Phosphorylation

$$\text{fructose-6-Ⓟ} + \text{ATP} \xrightarrow[\textit{kinase}]{\textit{phosphofructo-}} \text{fructose-1,6-Ⓟ}_2 + \text{ADP}$$

($\Delta G^{0\prime} = -14\ \text{kJ mol}^{-1}$; $\Delta G = -19\ \text{kJ mol}^{-1}$ under cellular conditions).

Fructose-1,6-bisphosphate is ideally set up to be cleaved into two three-carbon fragments that are already phosphorylated. Phosphofructokinase is inhibited allosterically by two compounds: ATP and even more strongly by its product, fructose-1,6-bisphosphate. The reaction is therefore an important regulation point in glycolysis.

4. Cleavage

$$\text{fructose-1,6-Ⓟ}_2 \xrightarrow{\textit{aldolase}} \text{glyceraldehyde-3-Ⓟ} + \text{dihydroxyacetone-Ⓟ}$$

($\Delta G^{0\prime} = -24\ \text{kJ mol}^{-1}$; $\Delta G = -0.23\ \text{kJ mol}^{-1}$ under cellular conditions).

This important reaction leads, after reaction 5, to two identical three-carbon, phosphorylated compounds, that is two glyceraldehyde-3-phosphate molecules. The favourable chemistry for C3−C4 bond cleavage is demonstrated by two reactions:

1) Free fructose is cleaved chemically in alkaline solution at room temperature to glyceraldehyde and dihydroxyacetone, just as in reaction 4.
2) Under similar alkaline conditions, a mixture of glyceraldehyde and dihydroxyacetone forms substantial amounts of fructose, plus some C_3 and C_4 epimers. This reversible reaction is well known to organic chemists as an example of aldol condensation, after which the enzyme aldolase is named. Note the small value of ΔG, showing that the reaction is close to equilibrium *in vivo*.

5. Isomerization

$$\text{dihydroxyacetone-Ⓟ} \xrightarrow[\text{isomerase}]{\textit{triose phosphate}} \text{glyceraldehyde-3-Ⓟ}$$

($\Delta G^{0\prime} = +7.6\ \text{kJ mol}^{-1}$; $\Delta G = -2.4\ \text{kJ mol}^{-1}$ under cellular conditions).

From this point on, we have two molecules of glyceraldehyde-3-phosphate, etc. from each molecule of glucose, as illustrated in Figure 11.4. I emphasize this by adding (×2) after each heading. The chemistry has converged to one type of compound, simplifying the rest of glycolysis.

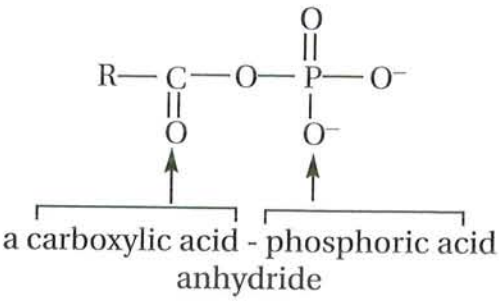
6. Oxidation (×2)

$$\text{glyceraldehyde-3-Ⓟ} + \text{NAD}^+ + \text{P}_\text{i} \xrightarrow[\text{dehydrogenase}]{\textit{glyceraldehyde-3-P}} \text{1,3-bisphosphoglycerate} + \text{NADH} + \text{H}^+$$

($\Delta G^{0\prime} = +6.3\ \text{kJ mol}^{-1}$; $\Delta G = -1.3\ \text{kJ mol}^{-1}$ under cellular conditions).

This can be considered as a two-stage reaction, involving an electron transfer oxidation and a phosphorylation that does not involve ATP. (The reaction involves a thioester intermediate in the active site of glyceraldehyde-3-phosphate dehydrogenase. See Box 11.1 for more on the importance of thioesters in metabolism.)

Despite the slightly positive ΔG, this reaction proceeds because of the overall favourable ΔG for glycolysis, and there is an advantage in saving energy for the next step in the form of the phosphate group on position 1 of 1,3-bisphosphoglycerate (BPG). This is in the form of an acid anhydride (specifically, an acyl phosphate) between phosphoric acid and the carboxyl group.



As such, it has a high phosphate group-transfer potential, capable of synthesis of ATP in step 7.

(In comparison, the familiar terminal phosphate in ATP is a phosphoric acid anhydride, also having high phosphate group-transfer potential.)

7. Phosphate transfer (×2)

$$\text{1,3-bisphosphoglycerate} + \text{ADP} \xrightarrow[\text{kinase}]{\textit{phosphoglycerate}} \text{3-phosphoglycerate} + \text{ATP}$$

($\Delta G^{0\prime} = -19\ \text{kJ mol}^{-1}$; $\Delta G = +0.1\ \text{kJ mol}^{-1}$ under cellular conditions).

Box 11.1 Thioesters and coenzyme A

As we have seen in many examples so far, phosphate in the form of phosphorylated compounds is ubiquitous in metabolism, especially in energy metabolism. Phosphate was scarce in the prebiotic world and it has been proposed that other 'high-energy' compounds must have been present to 'drive' ancient metabolic pathways before phosphorylated compounds became established. There is considerable evidence for this in the literature on the origins of life. One strongly supported candidate for such a role is the thioester. Chemically, thioesters are formed by the condensation of a carboxylic acid and a thiol:

$$\underset{}{\text{R-COOH} + \text{HSR}'} \longrightarrow \underset{\text{a thioester}}{\text{RCOSR}'} + \text{H}_2\text{O} \qquad (11.B1)$$

Thioesters have a $\Delta G^{0\prime}$ of hydrolysis much higher than ordinary esters, RCOOR.

In modern metabolic pathways, thioesters play a central role in the biological oxidation of carbonyl groups (−C=O) to carboxyl groups (−COOH). Biochemically, these reactions are of two types:

$$\underset{\text{aldehyde}}{\text{RCHO} + \text{HSR}'} \longrightarrow \text{RCOSR}' + 2e^- + 2H^+ \qquad (11.B2)$$

$$\underset{\substack{\| \ \| \\ \text{O O}}}{\text{RC–COH}} + \text{HSR}' \longrightarrow \text{RCOSR}' + 2e^- + 2H^+ + CO_2 \qquad (11.B3)$$

α-keto acid

These reactions bring about the direct formation of a high-energy thioester bond. They are 'driven' by the energy released by the electron transfers shown in eqns (11.B2) and (11.B3). (In vivo, the electron acceptors for reactions (11.B2) and (11.B3) are NAD^+ or $NADP^+$.)

Thioesters RCOSR′ have a high group-transfer potential for acyl groups, RC=O. Thus

$$\text{RCOSR}' + \text{P}_\text{i} \longrightarrow \text{RCO-P} + \text{R}'\text{SH} \qquad (11.B4)$$

$$\text{RCO-P} + \text{ADP} \longrightarrow \text{RCOOH} + \text{ATP} \qquad (11.B5)$$

$$\text{RCOSR}' + \text{P}_\text{i} + \text{ADP} \longrightarrow \text{RCOOH} + \text{RSH} + \text{ATP} \qquad (11.B6)$$

Overall reaction (11.B6) is the sum of (11.B4) and (11.B5). It results in the formation of a carboxylic acid, and via a coupling mechanism mediated by an appropriate enzyme, in the formation of ATP. This is an example of a substrate level phosphorylation, mentioned previously. As such it could have been a source of ATP in ancient organisms, before the advent of oxidative phosphorylation. This type of ATP formation occurs without the complex electron carriers involved

Box 11.1 (continued)

in the ETC, and without the need for a proton gradient and the protonmotive force. Most importantly, thioesters are central to energy metabolism in modern organisms.

1. Thioesters in glycolysis: an example of the reaction type

$$RCHO + HSR' \longrightarrow RCOSR' + 2e^- + 2H^+ \qquad (11.B2)$$

Reaction 6 of glycolysis (see above) is the oxidation of an aldehyde:

$$\text{glyceraldehyde-3-P} + NAD^+ + P_i \xrightarrow[\text{dehydrogenase}]{\text{glyceraldehyde-3-P}} \text{1,3-bisphosphoglycerate} + NADH + H^+ \qquad (11.B7)$$

This reaction, the exothermic oxidation of an aldehyde, is used to drive the synthesis of a high-energy compound, the acyl phosphate 1,3-BPG. The last step in the mechanism shows that an acyl thioester intermediate forms, which is attacked by the phosphate group to produce 1,3-BPG (Figure 11.B1) (Voet *et al.* 2002 p. 395).

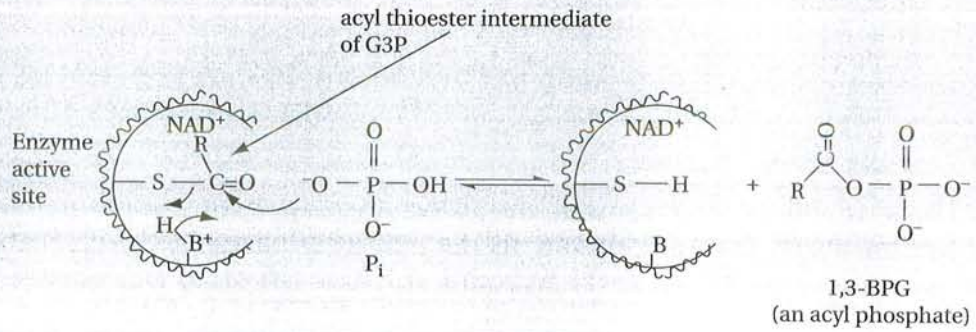


Figure 11.B1. The final step in the proposed mechanism for the oxidation of G3P to 1,3-bisphosphoglycerate (1,3-BPG), catalysed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH). R = [(P)OCH_2CHOH].

Acyl phosphates have a large negative free energy for acyl group transfer. The next step in glycolysis (step 7 above) uses this to generate ATP from ADP:

$$\text{1,3-BPG} + ADP \underset{\text{kinase}}{\overset{\text{phosphoglycerate}}{\rightleftharpoons}} \text{3-phosphoglycerate (3PG)} + ATP$$

The energy of aldehyde oxidation (of G3P) is conserved in two ways by steps 6 and 7 of glycolysis: the production of reducing power in the form of NADH, plus the formation of 1 mole of ATP. This is very efficient use of energy, which in glycolysis up to this point had actually consumed ATP. From step 7 onwards, glycolysis goes into its net energy-producing stage, as mentioned above in the discussion on glycolysis. The net formation of two ATP by glycolysis comes about by substrate level phosphorylation, which does not involve oxygen.

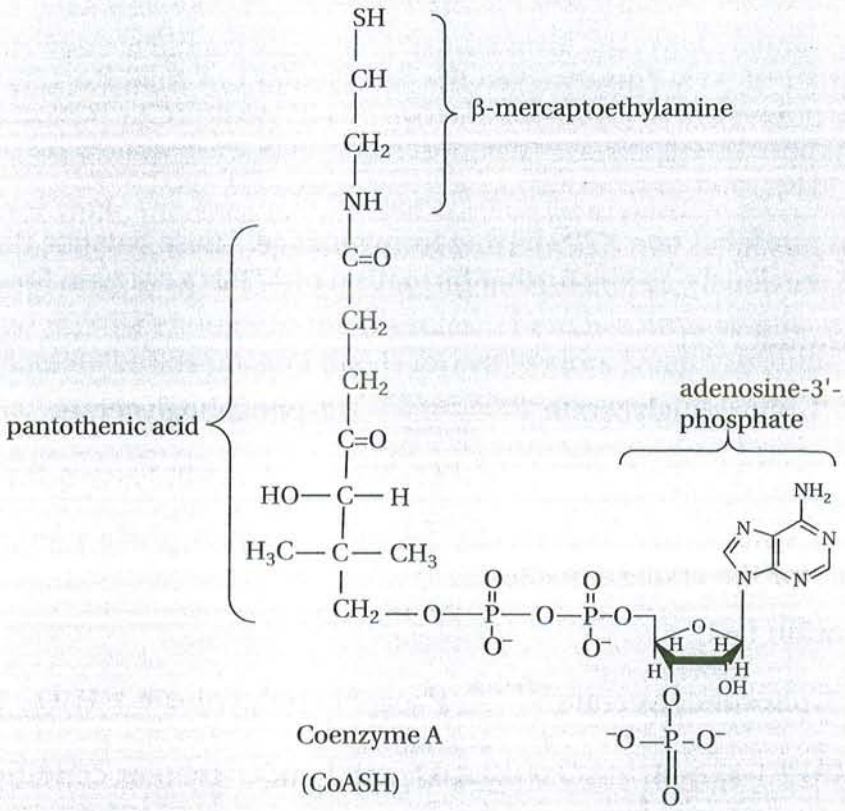
Box 11.1 (continued)

Glycolysis, as we have seen, can be used by anaeroboic organisms as their sole energy source. This is in keeping with glycolysis being an ancient metabolic pathway and the fact that a thioester is involved lends credence to the argument that thioesters may have been important in the emergence of early life.

2. Coenzyme-A: examples of the reaction type

$$\underset{\underset{\text{O}}{\|}}{\text{RC–}\underset{\underset{\text{O}}{\|}}{\text{C}}\text{OH}} + \text{HSR} \longrightarrow \text{RCOSR}' + 2e^- + 2H^+ + CO_2 \qquad (11.B3)$$

CoA is involved in two key steps involving the TCA cycle. In its uncombined form the abbreviation is CoA-SH because of the terminal thiol (SH) group. It has a complex molecular structure:



CoA-SH acts as a carrier of acetyl and other acyl groups. Acetyl CoA, or CoA-S-$COCH_3$, is a thioester of acetic acid having a $\Delta G^{0\prime}$ of hydrolysis of -31.5 kJ mol^{-1}, almost the same as that of ATP (-30.5 kJ mol^{-1}). Thus, formation of a thioester conserves energy that can be used in other reactions to drive endothermic processes. The linking of glycolysis to the TCA cycle allows access of pyruvate to oxidative phosphorylation. Instead of the formation of a mere two ATP per glucose molecule by glycolysis alone, a total of 32–38 ATP may be achieved. Significantly, the access of pyruvate to the TCA cycle involves not only thioesters. The conversion of pyruvate to acetyl CoA is carried out by a

Box 11.1 (continued)

multienzyme complex, pyruvate dehydrogenase. The complex contains three enzymes and carries out five sequential reactions. Overall:

$$\text{pyruvate} + \text{CoA-SH} + \text{NAD}^+ \longrightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \qquad (11.7)$$

Also involved are the coenzymes thiamine pyrophosphate (see Chapter 8), lipoamide, and FAD, making five coenzymes in all. This is an impressive array, reflective of the importance of this vital linkage between the two ancient metabolic pathways that played such a role in the development of modern metabolism.

The third and final example is the involvement of thioester chemistry in the TCA cycle, which takes place at step 6:

$$\text{succinyl-CoA} + \text{GDP} + \text{P}_\text{i} \longrightarrow \text{succinate} + \text{GTP} + \text{CoASH} \qquad (11.12)$$

$$\Delta G^{0\prime} = +4.4 \text{ kJ mol}^{-1}$$

As mentioned above, the formation of GTP is the energetic equivalent of formation of ATP. Thus, we see the involvement of thioesters at three crucial points, one in glycolysis and two involving the TCA cycle, all at the very centre of metabolism in the most highly evolved organisms on Earth.

This step produces two ATP starting from glucose. These balance the two ATP consumed previously, so any further formation of ATP is a net gain for glycolysis.

8. Isomerization (×2)

$$\text{3-phosphoglycerate} \xrightarrow[\text{mutase}]{\text{phosphoglycerate}} \text{2-phosphoglycerate}$$

($\Delta G^{0\prime} = +4.4$ kJ mol^{-1}; $\Delta G = +0.8$ kJ mol^{-1} under cellular conditions).

This step is necessary to correctly position the phosphate group on C_2 in preparation for the enolase reaction.

9. Dehydration (×2)

$$\text{2-phosphoglycerate} \xrightarrow{\text{enolase}} \text{Phosphoenolpyruvate} + \text{H}_2\text{O}$$

($\Delta G^{0\prime} = +2.1$ kJ mol^{-1}; $\Delta G = +1.1$ kJ mol^{-1} under cellular conditions).

By rearrangement of the bonding in the molecule, this reaction produces a phosphate with high group-transfer potential, another so-called 'high-energy' phosphate, to be used in step 10 to produce two further molecules of ATP.

10. Phosphate transfer (×2)

$$\text{Phosphoenolpyruvate} + \text{ADP} \xrightarrow{\text{pyruvate kinase}} \text{pyruvate} + \text{ATP}$$

($\Delta G^{0\prime} = -31.7$ kJ mol^{-1}; $\Delta G = -23$ kJ mol^{-1} under cellular conditions).

Pyruvate kinase is an allosteric enzyme made up of four subunits. It is inhibited by ATP, to slow down production of the latter when it is in high concentration in the cell.

(The above *in vivo* thermodynamic data are from Minakami and Yoshikawa (1965). They refer to glycolysis in red blood cells. Data referring to heart muscle (Newsholme and Start 1973, reported in Voet *et al.* 2002 p. 407) give somewhat different values, but qualitatively show the same trends.)

The net reaction of glycolysis from glucose to pyruvate is:

$$\text{glucose} + 2\text{ADP} + 2\text{P}_\text{i} + 2\text{NAD}^+ \longrightarrow 2\,\text{pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+ \quad (11.1)$$

The ATP formed during glycolysis is another example of the substrate-level phosphorylation that was mentioned in Chapter 10. The Gibbs energy to drive substrate-level phosphorylation comes from the group-transfer potential of certain phosphate groups. In glycolysis, these powerful phosphate donors to ADP are 1,3-BPG and phosphoenolpyruvate (PEP).

Note again the formation of two molecules of the three-carbon molecule pyruvate from each six-carbon molecule of glucose. Pyruvate may have one of three main fates (Figure 11.5).

In aerobic metabolism, it loses carbon dioxide and the remaining two-carbon fragment becomes linked to CoA to form acetyl-CoA. This enters the citric acid cycle. Two anaerobic fates are possible for pyruvate. It may be reduced to lactate (lactic acid). Some bacterial species such as *Lactobacillus*, responsible for the taste of sour milk, produce lactate and survive by anaerobic glycolysis. Animal examples include mammalian red blood cells, which rely on anaerobic glycolysis for energy, and muscle tissue during vigorous exercise, when oxygen cannot be supplied rapidly enough to the muscles. There is a limit to this; muscle pain occurs as lactate builds up, and one is forced to stop. Deep breathing eventually

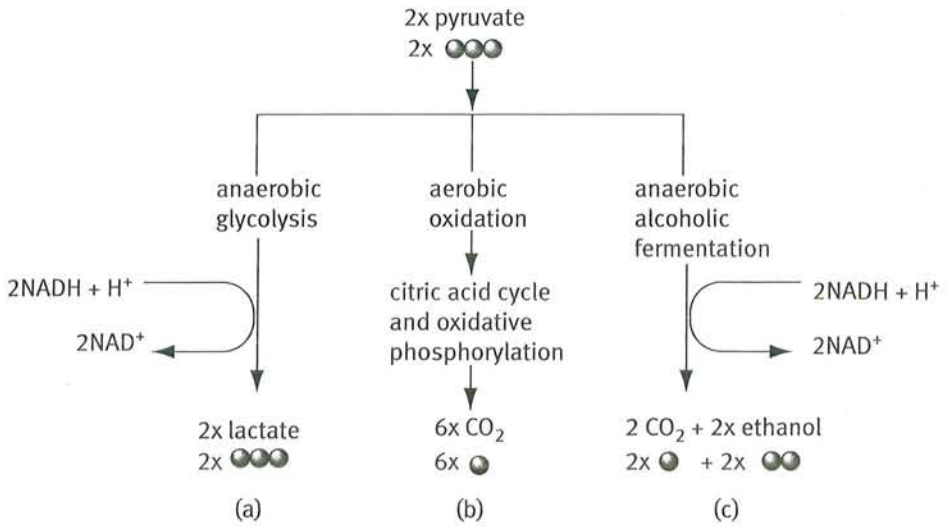


Figure 11.5. A summary of three fates of pyruvate derived from glucose. (a) Anaerobic formation of lactate, e.g. in exercising muscle tissue. (b) Formation of acetyl Co.A, entry to the citric acid cycle, oxidative phosphorylation to ATP, CO_2, and water. (c) Fermentation to form ethanol and CO_2. ● represents one carbon atom.

allows aerobic metabolism to take over, the lactate is oxidized to pyruvate then converted to acetyl-CoA.

The other anaerobic fate of pyruvate is its conversion to acetaldehyde then ethanol in those organisms capable of alcoholic fermentation, such as yeasts. This is utilized by the brewing industry to produce a variety of alcoholic beverages, notably beer and wine. Of overall biological importance is the ability of anaerobic glycolysis to produce enough ATP to sustain a variety of living organisms. It is possible to make an estimate of the efficiency of anaerobic glycolysis in the utilization of glucose. For fermentation to lactate:

$$\text{glucose} \longrightarrow 2\text{lactate} + 2H^+ \quad \Delta G^{0\prime} = -196 \text{ kJ mol}^{-1} \tag{11.2}$$

For fermentation to alcohol:

$$\text{glucose} \longrightarrow 2CO_2 + 2CH_3CH_2OH \quad \Delta G^{0\prime} = -235 \text{ kJ mol}^{-1} \tag{11.3}$$

Each of these reactions is coupled to the formation of 2ATP, which requires $2 \times \Delta G^{0\prime}$ for the synthesis of ATP, or $2 \times 30.5 = 61$ kJ mol^{-1}. The 61 kJ mol^{-1} is the energy return to the cell for the release of 196 or 235 kJ mol^{-1}. The rest is dissipated as heat. Thus, the efficiencies of lactate and alcoholic fermentation are (61/196) = 31% and (61/235) = 26%, respectively, under biochemical standard state conditions. Under cellular conditions, the efficiency has been calculated to be greater than 50% (data from Voet *et al.* 2002 p. 406). A comparison with oxidative phosphorylation, which yields up to 38 ATP per glucose (see below) reveals that anaerobic glycolysis is a much less efficient user of the free energy available from a mole of glucose. When oxygen became available to life courtesy of photosynthesis, it was soon 'chosen' by natural selection as the ultimate electron acceptor by a vast number of species.

On the other hand, it is fortunate that we mammals still have access to anaerobic glycolysis, as it can produce ATP up to 100 times faster than oxidative phosphorylation, a useful property in hard-working muscle tissue. The lactate is aerobically processed as soon as we are forced to slow down.

Close regulation of metabolic pathways is necessary to allow them to adapt to changing cellular conditions. Such control is often exercised near the start and end of a pathway, and sometimes at key intermediate steps. Regulation of glycolysis occurs at three points.

1) Conversion of glucose to glucose-6-phosphate (Figure 11.4) is catalyzed by hexokinase ($\Delta G^{0\prime} = -16.7$ kJ mol^{-1}, $\Delta G = -33.9$ kJ mol^{-1}). This reaction is inhibited by its product, glucose-6-phosphate. Kinases are enzymes that transfer the terminal phosphate group of ATP to nucleophilic acceptors. Hexokinase can use several hexoses such as glucose, fructose, and mannose as substrates. There are specific glucokinases in some tissues, for example mammalian liver and pancreas.
2) Conversion of fructose-6-phosphate to fructose-1,6-bisphosphate is catalyzed by phosphofructokinase ($\Delta G^{0\prime} = -14$ kJ mol^{-1}, $\Delta G = -19$ kJ mol^{-1}).

This enzyme is inhibited by ATP.

3) The last step in glycolysis, formation of pyruvate and ATP from PEP, is catalyzed by pyruvate kinase ($\Delta G^{0\prime} = -31.7$ kJ mol^{-1}, $\Delta G = -23$ kJ mol^{-1}). This reaction is also inhibited by ATP.

All three reactions have large negative $\Delta G^{0\prime}$ values, making them potentially useful as control points. Somewhat different values have been determined (Voet *et al.* 2002 p. 407) but the overall features are the same. Regulation at these points makes perfect logic, as high levels of all three inhibitors indicate that plenty of ATP is available; slowing down of its synthesis makes cellular sense.

11.4 The pentose phosphate pathway

Although ATP is the major energy source at any given instant, cells must have another essential 'currency' available. This is reducing power. As well as ATP, cells require a constant supply of NADPH for anabolic endothermic reactions such as the reductive biosynthesis of fatty acids and cholesterol. Metabolically, NADH and NADPH are not interchangeable. NADH uses the free energy of metabolite oxidation to synthesize ATP via oxidative phosphorylation, while NADPH uses the same energy source for reductive biosynthetic purposes. Both NADH and NADPH act as coenzymes for a variety of dehydrogenase enzymes. Each type of dehydrogenase is specific for either NADH or NADPH. They can discriminate between the two structures. Cells maintain their NAD^+/NADH ratio at about 1000/1. This favours oxidative, catabolic processes, as $NAD^+ \rightarrow NADH + H^+$ is oxidative. The $NADP^+$/NADPH ratio is maintained at about 0.01/1 and this favours the reductive processes typical of biosynthesis.

NADPH is formed from glucose-6-phosphate by the pentose phosphate pathway, an alternative to glycolysis. Tissues such as liver, adipose tissue, and the adrenal cortex are involved in lipid biosynthesis and are rich in pentose phosphate enzymes. They are located in the cytosol, the site of fatty acid biosynthesis. The pathway is versatile, producing three-, four-, five-, six-, and seven-carbon sugars. In muscle tissue, which is mainly involved in energy production, the pentose phosphate enzymes are essentially absent.

The overall reaction for the pathway is:

$$3\text{Glc6P} + 6\text{NADP}^+ + 3\text{H}_2\text{O} \rightleftharpoons 6\text{NADPH} + 6\text{H}^+ + 2\text{F6P} + 3\text{CO}_2 + \text{G3P} \qquad (11.4)$$

glucose-6-phosphate fructose-6-P glyceraldehyde-3-P

Although, as indicated, a number of sugars are formed during the cycle, I won't go into further detail. The important point is that reducing power in the form of 6NADPH is generated. One important sugar formed is ribose-5-phosphate (R5P), which can be directed to the synthesis of nucleotides and subsequently of DNA/RNA.

What regulates the level of activity of the pentose phosphate pathway? It is controlled by the activity of the first enzyme in the pathway, glucose-6-phosphate dehydrogenase. The activity of glucose-6-phoshate dehydrogenase is in turn regulated by the level of $NADP^+$:

$$\text{Glc6P} \xrightarrow{\text{Glc6P dehydrogenase}} \text{6-phosphogluconolactone} \qquad (11.5)$$
$$\text{NADP}^+ \qquad \text{NADPH} + \text{H}^+$$

$$\Delta G_{\text{reaction}} = -17.6 \text{ kJ mol}^{-1}$$

This reaction is well set up for a regulatory role. It has a large negative ΔG, so is essentially irreversible. As the cell consumes NADPH, the level of $NADP^+$ will increase, activate the enzyme, and stimulate the pentose phosphate pathway.

11.5 The citric acid or tricarboxylic acid cycle

The TCA cycle is much more than just an addition to the end of glycolysis. It is a central metabolic pathway for the conversion of several types of foodstuff to metabolic energy (see Figure 11.3). It also supplies the reactants for a number of biosynthetic pathways. The starting point for the study of the cycle is the formation of acetyl-CoA from pyruvate or from β-oxidation of fatty acids. The citric acid cycle, via a series of eight reactions, oxidizes acetyl-CoA to produce energy as guanosine triphosphate (GTP), the reducing power of NADH and $FADH_2$, plus CO_2. GTP is a purine nucleoside triphosphate, similar to ATP in structure and properties such as large negative ΔG of hydrolysis. Like ATP, it can be used to drive endothermic reactions via energy coupling, so in energy terms GTP = ATP. Oxidative phosphorylation and electron transport are tightly linked to the cycle (Chapter 10). The NAD/NADH redox system acts as a shuttle that carries electrons released from foods to the ETC, ultimately transferring them to oxygen and forming water. ATP is formed by oxidative phosphorylation, as described in Chapter 10.

Expressing this generally:

$$AH_2 + NAD^+ \longrightarrow A + NADH + H^+ \qquad (11.6)$$

where AH_2 represents a typical substrate, for example $-CH_2-$ in a foodstuff, which is oxidized to A, and NAD^+ is reduced to $NADH+H^+$. The $NADH+H^+$ is reoxidized to NAD^+ when it transfers its reducing power to the ETC in mitochondria. This step is the reverse of eqn (11.2), and the electron acceptor A is oxygen. It recycles the precious NAD^+. The citric acid cycle components are located in the mitochondrial matrix of eukaryotes, except for succinate dehydrogenase, which is embedded in the inner mitochondrial membrane (Figure 10.2). The TCA cycle is found in the cytosol of prokaryotes. In eukaryotes, all the substrates must be formed in the mitochondria or be capable of being transported there. The

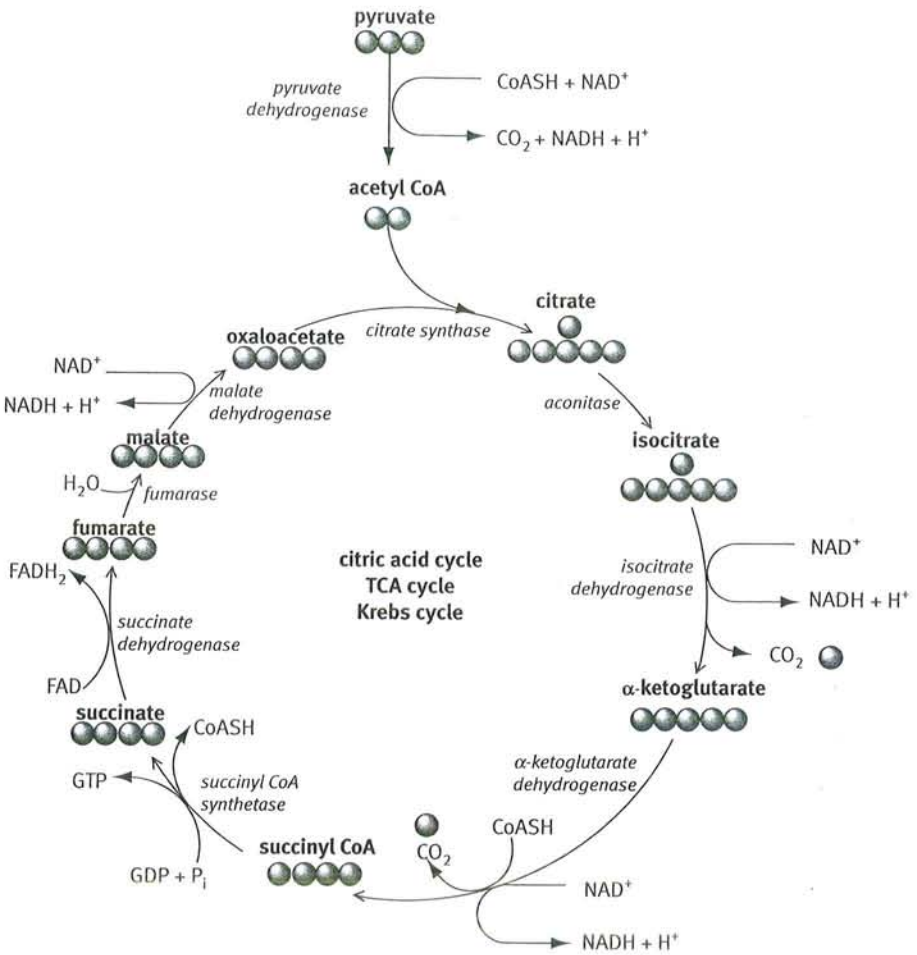


Figure 11.6. The citric acid or TCA cycle. One turn of the cycle oxidizes the equivalent of one acetyl group to two CO_2, while generating three ($NADH+H^+$), one $FADH_2$, and one GTP, and regenerating one oxaloacetate to continue the cycle. Two turns of the cycle are required to oxidize 1 mole of glucose, via input of two pyruvate from glycolysis. ● represents one carbon atom.

products are either consumed there or are capable of transport out of the mitochondrion to the cytosol.

In the following section, the $\Delta G^{0\prime}$ values are from Garrett and Grisham (2010 p. 572). The ΔG values shown are those pertaining to cellular conditions (Newsholme and Leech 1983).

1) Pyruvate from glycolysis in the cytoplasm enters the mitochondrion via a specific transporter (Figure 11.6). It undergoes activation to form a thioester with CoA (CoASH):

$$\text{pyruvate} + \text{CoASH} + \text{NAD}^+ \longrightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \qquad (11.7)$$

$$\Delta G^{0\prime} = -33.4\,\text{kJ mol}^{-1}$$

This reaction is catalyzed by the pyruvate synthase complex, which in mammals is made up of five enzymes (see Box 11.1).

2) Catalyzed by citrate synthase, acetyl-CoA (two carbons) is linked to oxaloacetate (four carbons) provided by the cycle to form citrate (six carbons).

$$\text{oxaloacetate} + \text{acetyl-CoA} + H_2O \longrightarrow \text{citrate} + \text{CoASH} \qquad (11.8)$$

$$\Delta G^{0\prime} = -31.5 \text{ kJ mol}^{-1}$$

$$\Delta G = -31.5 \text{ kJ mol}^{-1}$$

3) Catalyzed by aconitase, citrate is isomerized to isocitrate (still having six carbons), a reaction that involves moving an OH group from one carbon atom to a neighbouring one. Aconitase requires Fe^{2+} for activity.

$$\text{citrate} \longrightarrow \text{isocitrate} \quad \Delta G^{0\prime} = +6.7 \text{ kJ mol}^{-1} \qquad (11.9)$$

$$\Delta G = +0.8 \text{ kJ mol}^{-1}$$

4) Catalyzed by isocitrate dehydrogenase, isocitrate is converted to α-ketoglutarate. This is an oxidative decarboxylation.

$$\text{isocitrate} + NAD^+ \longrightarrow \alpha\text{-ketoglutarate} + NADH + H^+ + CO_2 \qquad (11.10)$$

$$\Delta G^{0\prime} = -8.4 \text{ kJ mol}^{-1}$$

$$\Delta G = -17.5 \text{ kJ mol}^{-1}$$

This step is notable as it is the first in the cycle to achieve an oxidation of one net carbon to CO_2 with concurrent reduction of NAD^+ to $NADH + H^+$. The whole objective of the large expenditure on metabolites and energy in the citric acid cycle is for it to produce much more energy than it consumes. The production of reducing power in the form of $NADH{+}H^+$ largely achieves this objective. As we have seen, the channelling of electrons from species such as $NADH/H^+$ and $FADH_2$ into the ETC generates ATP.

5) Catalyzed by α-ketoglutarate dehydrogenase complex, another oxidative decarboxylation step produces more $NADH/H^+$.

$$\alpha\text{-ketoglutarate} + NAD^+ + \text{CoASH} \rightleftharpoons \text{succinyl-CoA} + NADH + H^+ + CO_2 \qquad (11.11)$$

$$\Delta G^{0\prime} = -30 \text{ kJ mol}^{-1}$$

$$\Delta G = -44 \text{ kJ mol}^{-1}$$

In accord with our previous observations on metabolic control, this highly exothermic reaction is another point of regulation of the cycle. The reaction is complex, similar to that forming acetyl-CoA from pyruvate. It takes place in several steps and there is a requirement for TPP, FAD, lipoic acid, and Mg^{2+}. The CO_2 is removed, making the cycle irreversible in vivo. To this point, two molecules of CO_2 have been formed, leaving a four-carbon acid that needs to be converted back to oxaloacetate to continue the next cycle. The carbon atoms in the two CO_2 molecules released do not come from the acetyl of the acetyl-CoA added in the first step, as shown by specific carbon-labelling experiments.

6) Catalyzed by succinyl-CoA synthetase, succinate is formed and the large negative ΔG of hydrolysis of the succinyl-CoA is coupled to the synthesis of GTP, making more energy available through this 'high-energy' compound.

$$\text{succinyl-CoA} + \text{GDP} + \text{P}_\text{i} \longrightarrow \text{succinate} + \text{GTP} + \text{CoASH} \qquad (11.12)$$

$$\Delta G^{0\prime} = -3.3 \text{ kJ mol}^{-1}$$

$$\Delta G = \sim 0 \text{ kJ mol}^{-1}$$

7) Catalyzed by succinate dehydrogenase, succinate is oxidized to fumarate, with the generation of $FADH_2$, which as we have seen feeds into the mitochondrial ETC (Figure 10.2).

$$\text{succinate} + \text{FAD} \longrightarrow \text{fumarate} + \text{FADH}_2 \qquad (11.13)$$

$$\Delta G^{0\prime} = +0.4 \text{ kJ mol}^{-1}$$

$$\Delta G = \sim 0 \text{ kJ mol}^{-1}$$

8) Catalyzed by fumarase, water is added to fumarate to form malate.

$$\text{fumarate} + \text{H}_2\text{O} \longrightarrow \text{malate} \qquad (11.14)$$

$$\Delta G^{0\prime} = -3.8 \text{ kJ mol}^{-1}$$

$$\Delta G = \sim 0 \text{ kJ mol}^{-1}$$

9) Catalyzed by malate dehydrogenase, malate is oxidized to oxaloacetate, completing the cycle with the formation of further reducing power as $NADH/H^+$.

$$\text{malate} + \text{NAD}^+ \longrightarrow \text{oxaloacetate} + \text{NADH} + \text{H}^+ \qquad (11.15)$$

$$\Delta G^{0\prime} = +29.7 \text{ kJ mol}^{-1}$$

$$\Delta G = \sim 0 \text{ kJ mol}^{-1}$$

This final step is endothermic, but overall the citric acid cycle from pyruvate is exothermic:

$$\text{pyruvate} + 4\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{P}_\text{i} + 2\text{H}_2\text{O} \longrightarrow 2\text{CO}_2 + 4\text{NADH} + 4\text{H}^+ + \text{FADH}_2 + \text{GTP} \qquad (11.16)$$

For eqn (11.16) $\Delta G^{0\prime} = -77.7 \text{ kJ mol}^{-1}$

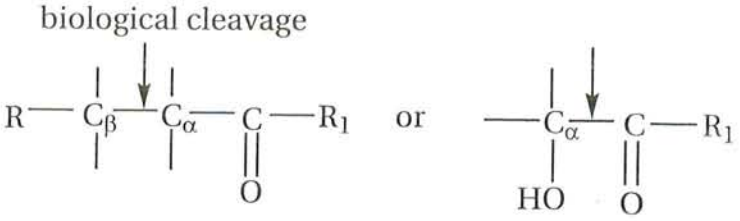
Starting from acetyl-CoA:

$$3\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{P}_\text{i} + \text{acetyl-CoA} + 2\text{H}_2\text{O} \longrightarrow 2\text{CO}_2 + 3\text{NADH} + \text{H}^+ + \text{FADH}_2 + \text{GTP} + \text{CoASH}$$

$$\Delta G^{0\prime} = -40 \text{ kJ mol}^{-1}$$

From either starting point, the cycle will run *in vivo*.

Why go through such a complex process to oxidize acetyl groups $CH_3-\overset{\overset{O}{\|}}{C}-$ to $2CO_2$, which is what the citric acid cycle really does? Direct oxidation of pyruvate would require cleavage of C–C bonds. In biochemical systems, the chemical mechanisms used cleaves C–C bonds (i) between carbon atoms α- and β- to a carbonyl group or (ii) α- to an α-hydroxyketone.



(i) (ii)

Acetate has no β-carbon and chemically hydroxylation of acetate is unfavourable.

The end product of the evolutionary 'search' for a means to oxidize acetate is hardly a compromise, considering the versatility of the resulting citric acid cycle.

The overall yield of ATP from the oxidation of glucose via glycolysis, the citric acid cycle, and oxidative phosphorylation is as follows:

glycolysis: glucose to pyruvate	2 net ATP
citric acid cycle	2 net ATP (as GTP)
oxidative phosphorylation	28–34 net ATP
total per glucose molecule	32–38 net ATP

The above numbers include some consensus values used in the calculation of ATP yield in oxidative phosphorylation (Campbell and Farrell 2006 p. 563; Garrett and Grisham 2010 p. 623). Phosphorylation and the redox reactions are not directly coupled, so the NADH/ATP and $FADH_2$/ATP ratios are not whole numbers. The modern consensus values are 2.5 and 1.5 ATP produced from one NADH and one $FADH_2$, respectively. Varying metabolic conditions can alter the estimate of ATP. Other estimates propose a yield of 36–38 net ATP (Campbell *et al.* 2008 p. 176; Voet *et al.* 2002 p. 484).

The citric acid cycle also has a role in producing a range of precursors for various biosynthetic (anabolic) pathways, and is integrated with these. Examples include:

- from acetyl-CoA: steroids and fatty acids
- from α-ketoglutarate: some amino acids, purines
- from oxaloacetate and fumarate: some amino acids, pyrimidines (purines and pyrimidines are components of DNA and RNA)
- from succinyl-CoA: porphyrins (found in the structures of haemoglobin and chlorophylls).

Thus, the citric acid cycle is involved in both catabolic and anabolic metabolism, and is said to be amphibolic for this reason. It is important to remember that in living cells many of the metabolic pathways are often operating

simultaneously. Every stage is under control to maintain optimal concentrations of substrates for the numerous reactions. It is just for the convenience of understanding that we discuss them separately.

11.6 Regulation of the citric acid cycle

The TCA cycle is situated between glycolysis and the ETC where oxidative phosphorylation occurs, that is, it is located at the heart of aerobic respiration in the cell and must be regulated carefully. If the cycle were to run uncontrolled, there would be an oversupply of ATP and NADH. If it were to run too slowly, there would soon be an undersupply of these components, and the energetic requirements of the cell could not be met. Control occurs at three points in the cycle, plus at the pyruvate to acetyl-CoA step. Why is control exerted at these points in particular?

Some of the reactions in the cycle operate close to equilibrium, that is $\Delta G \sim 0$. Thus, small changes in concentration of reactants or products could push these reactions either forwards or backwards. On the other hand, reactions that have a large negative ΔG under cellular conditions, if inhibited, can come to a complete stop but won't go into reverse. It is usually at these highly exothermic steps that metabolic control is manifested.

1) Pyruvate to acetyl-CoA: the pyruvate decarboxylase complex is inhibited by ATP and NADH.
2) Oxaloacetate to citrate: citrate synthase is inhibited by ATP, NADH, succinyl-CoA, and citrate. It is an allosteric enzyme. As the first reaction of the cycle it is also a logical candidate for regulation. This is commonly the case in metabolic pathways.
3) Isocitrate to α-ketoglutarate: isocitrate dehydrogenase is inhibited by ATP and NADH, and stimulated by ADP and NAD^+. ADP is an allosteric activator. There are many instances in which ATP and NADH inhibit enzymes of a pathway, while ADP and NAD^+ activate the same enzymes.
4) α-Ketoglutarate to succinyl-CoA: the 2-ketoglutarate dehydrogenase complex is inhibited by ATP, NADH, and succinyl-CoA. ADP and NAD^+ are activators.

Use of the inhibitors and activators above make perfect sense. When the ratios of ATP/ADP and NADH/NAD^+ are high, the cycle needs to be slowed, and vice versa. Some of the reactions are inhibited by their own products, which also makes perfect regulatory sense.

I should mention that in plants, plus some bacteria and algae, an extra pathway related to the citric acid cycle exists: the glyoxylate pathway. This pathway allows plants to use acetyl-CoA for the synthesis of carbohydrates, indeed for all the carbon-based compounds needed. This does not occur in animals. Animals can convert carbohydrates to fats, but not fats to carbohydrates. Although animals can produce acetyl-CoA from the catabolism of fatty acids, they do not possess the enzymes of the glyoxylate pathway to convert it to carbohydrates. While the citric acid cycle can provide some intermediates for biosynthesis, it cannot do so for carbohydrates. The citric acid cycle does not lead to a net synthesis of intermediates, as two CO_2 molecules are given off for

each two-carbon acetyl-CoA fragment that enters it. Thus, it would be impossible to build up large amounts of storage molecules such as carbohydrates. The availability of the extra enzymes of the glyoxylate pathway allows plants, etc. to overcome this problem by utilizing acetyl-CoA directly.

The macromolecular carbohydrates are called polysaccharides and, like all polymers, consist of monomers, in this case simple sugars—the monosaccharides—joined by covalent linkages. Examples are starch in plants, and glycogen in the muscle and liver of animals. Both these polysaccharides have a branched structure and consist of glucose units, but they are joined in slightly different ways. Enzymes exist in cells and are made available as appropriate to break down the efficient energy storage molecules starch and glycogen to glucose. The paths of synthesis and breakdown of polysaccharides are regulated by feedback controls similar in principle to those described for glycolysis and the citric acid cycle. Glucose can also be obtained directly in the diet or from other simple sugars by enzyme-catalyzed reactions. Whatever its source, the glucose may enter the glycolytic pathway and undergo metabolism as described above.

It is by such means that living cells self-regulate at key control points in their metabolic pathways and maintain the flow of energy that is essential for their survival.

Finally, here is a reminder of the different locations in the cell of the components of glycolysis, the TCA cycle, the ETC, and the ATP synthase (Figure 11.7).

Glucose comes from outside the cell (from the blood in mammals) by active transport via a specific protein channel. Oxygen diffuses into the cell from the blood capillaries and CO_2 diffuses out to the blood. The enzymes of glycolysis are in the cytosol, but pyruvate from glycolysis (and other pathways) is fed into the mitochondria, where the citric acid cycle enzymes and the ETC are located.

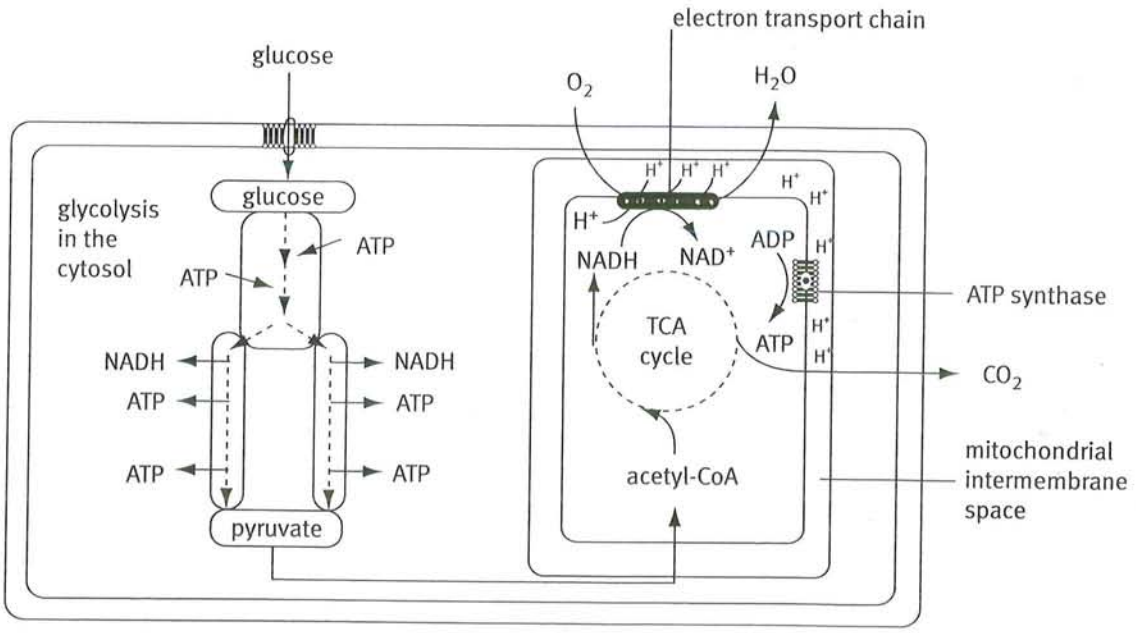


Figure 11.7. Location of the glycolytic and citric acid cycles, electron transport chain, and ATP synthase components within an aerobic, eukaryotic cell.

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