The metabolic reactions which provide the energy that a cell needs in order to carry out its physiological activities and to grow and divide are vitally important. Humans and other animals obtain their energy by breakdown of organic molecules which they ingest as food. Carbohydrates (in particular glucose), lipids and amino acids can all be utilized as energy sources.

In this chapter and the next we will examine how the free energy contained in the chemical bonds of a glucose molecule is released and utilized by the cell. We will discover that the process is a multistep metabolic pathway involving a variety of enzymes and a series of intermediate compounds that are formed during the gradual breakdown of glucose. It is important that we study the individual steps in the pathway, but equally important that we do not lose sight of the overall purpose of the pathway as a whole. We will therefore begin with an overview of the process, so that throughout the next two chapters we will be aware of the broader context for the individual reactions being studied.
Chapter 8

8.1 An overview of energy generation

The complete breakdown of a molecule of glucose yields six molecules of carbon dioxide and six of water:

$$\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$$

Oxygen is used up during the reaction, so in chemical terms the process is an oxidation.

Glucose oxidation is a highly exergonic reaction, yielding 2870 kJ of energy for every mole of glucose that is broken down. In biochemical terms, this is a substantial amount of energy; a typical endergonic enzyme-catalyzed reaction requires only about 10 kJ of energy to convert a mole of substrate into a mole of product. The cell therefore breaks glucose down gradually, releasing smaller units of energy at different stages of the process. These packets of energy are stored in activated carrier molecules.

Box 8.1 Units of energy

In biochemistry, quantities of energy are expressed as kilojoules per mole, written as kJ mol$^{-1}$. Kilojoules and moles are standard SI units that are defined as follows:

- A kilojoule is 1000 joules, a joule being the work done by a force of one newton when its point of application moves through a distance of one meter in the direction of the force.

The complete oxidation of glucose yields 2870 kJ mol$^{-1}$ of energy. In other words, the $\Delta G$ for this reaction is $-2870$ kJ mol$^{-1}$, the negative value indicating that this is an exergonic reaction (see Section 7.2.1).

8.1.1 Activated carrier molecules store energy for use in biochemical reactions

In Section 7.2.1 we learnt that the energy needed to drive an endergonic biochemical reaction is often obtained by hydrolysis of a molecule of ATP. ATP is an example of an activated carrier molecule, which is a molecule that acts as a temporary store of the free energy released by breakdown of glucose and other organic compounds.

ATP is the most important biological energy carrier, with a typical human cell containing approximately $10^8$ molecules of ATP, which in some cells is completely used up (and replaced by new ATP molecules) once every few minutes. Hydrolysis of ATP releases 30.84 kJ mol$^{-1}$ of energy and results in ADP and inorganic phosphate (Fig. 8.1).

\[ \text{ATP} \rightarrow \text{ADP} + \text{P}_i + 30.84 \text{ kJ mol}^{-1} \]

In other words, the activation energy is 30.84 kJ mol$^{-1}$ and $\Delta G$ is $-30.84$ kJ mol$^{-1}$.

Figure 8.1 Hydrolysis of ATP. Abbreviations: ATP, adenosine 5‘-triphosphate; ADP, adenosine 5‘-diphosphate; P$_i$, inorganic phosphate.
The phosphate–phosphate linkage that is broken during ATP hydrolysis is sometimes called a ‘high-energy’ bond, but this is a confusing description and not a correct interpretation of the source of the energy released when ATP is hydrolyzed. The energy that is released does not come directly from the splitting of a phosphate–phosphate bond, and is certainly not the bond energy for that linkage. Instead, the free energy arises, as in all chemical reactions, because of the $\Delta G$ between the reactants and products. In this case, the $\Delta G$ between the reactants (ATP and water) and the products (ADP and inorganic phosphate) is relatively large because of differences between the resonance (distribution of electrons) and solvation (interaction with water) properties of ATP and ADP. Because of these resonance and solvation differences, ADP is, in thermodynamics terms, a more ordered system than ATP, and so has a lower free energy content. The conversion of ATP to ADP therefore releases energy.

ATP may be the most important activated carrier molecule in living cells, but it is by no means the only compound of this type. A second type of nucleotide, GTP, also acts as an energy carrier, particularly during the reactions that result in synthesis of proteins.

Some enzyme cofactors are also activated carrier molecules. These include NAD$^+$ and NADP$^+$, each of which can carry energy in the form of a pair of electrons and a proton (H$^+$ ion), converting the molecules into their reduced forms referred to as NADH and NADPH. The chemical equations for reduction of NAD$^+$ and NADP$^+$ are therefore:

$$\text{NAD}^+ + \text{H}^+ + 2\text{e}^- \rightarrow \text{NADH}$$
$$\text{NADP}^+ + \text{H}^+ + 2\text{e}^- \rightarrow \text{NADPH}$$

Reversal of these reactions releases the stored energy. NADH acts as an energy carrier between different components of the energy-generating pathway, as we will see below, whereas NADPH is mainly used in anabolic reactions leading to synthesis of large organic molecules from smaller ones.

FAD and FMN act in a similar way, but reacting with two protons rather than one:

$$\text{FAD} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{FADH}_2$$
$$\text{FMN} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{FMNH}_2$$

Both FAD and FMN, like NAD$^+$, are involved in the energy-generating pathway.

### 8.1.2 Biochemical energy generation is a two-stage process

The series of reactions that release the energy contained in a glucose molecule in incremental steps, transferring it to ATP molecules, can be described as a two-stage process (Fig. 8.2). The first stage is called glycolysis. Each six-carbon glucose molecule is broken down to two molecules of the three-carbon sugar called pyruvate. Glycolysis does not require oxygen and so can occur in all cells of all organisms. However, it releases less than 7% of the total free energy content of glucose. This released energy is used to synthesize two molecules of ATP. In addition, two molecules of NADH are made for every molecule of glucose that is metabolized.

The second stage of the process requires oxygen, and therefore only occurs under aerobic conditions in cells capable of carrying out respiration. This stage comprises two linked pathways. First, the tricarboxylic acid (TCA) cycle completes the breakdown of the pyruvate molecules resulting from glycolysis. Before entering the TCA cycle, pyruvate is converted into acetyl-CoA, generating another molecule of NADH. The TCA cycle then breaks down the acetyl-CoA, with each molecule of acetyl-CoA yielding one molecule of ATP, in addition to three of NADH and one of FADH$_2$. Acetyl-CoA is also obtained from the breakdown of storage fats, which means that the TCA cycle can utilize energy from this other energy store. The second pathway
is the electron transport chain, which uses the energy contained in the NADH and FADH\(_2\) molecules to synthesize another three molecules of ATP per NADH and two per FADH\(_2\).

In summary therefore, each molecule of glucose yields 38 molecules of ATP:

- Glycolysis results in eight of these – two molecules of ATP made directly during the glycolysis pathway and six more from the NADH molecules that glycolysis generates.
- Another six ATP molecules are obtained from the two NADH molecules produced when the two pyruvates resulting from glycolysis are converted into two acetyl-CoA molecules.
- Two further ATP molecules are made during the TCA cycle, one from each of the two acetyl-CoA molecules.
- The final 22 ATPs are generated from the NADH and FADH\(_2\) molecules that also result from the TCA cycle.

The 38 ATP molecules corresponds to 38 \times 30.84 = 1173 \text{kJ mol}^{-1} of energy. This is only 41% of the total energy contained in glucose. What happens to the remainder? It is lost as heat, which in warm-blooded creatures such as humans helps to maintain our body temperature.

8.2 Glycolysis

As we have seen, the first stage of the process that releases energy from glucose is called glycolysis. In the remainder of this chapter we will consider this process from four angles. First, we will look in detail at the steps in the glycolytic pathway, in particular highlighting those that result in transfer of energy to an ATP molecule. Secondly, we will study the role of glycolysis in anaerobic organisms – those that cannot respire and therefore depend on glycolysis as their principal energy source. Thirdly, we will ask how sugars other than glucose enter the pathway, and finally we will examine how glycolysis is regulated so that the amount of glucose that is consumed is appropriate for the energy needs of the cell.

8.2.1 The glycolytic pathway

The glycolysis pathway is shown in outline in Figure 8.3. We will run through the individual steps in the pathway and then look in more detail at the key features.
Glycolysis converts one molecule of glucose into two of pyruvate

The individual steps in glycolysis are as follows.

**Step 1.** To begin the pathway, glucose is phosphorylated by ATP to form glucose 6-phosphate and ADP. The reaction is catalyzed by the enzyme *hexokinase*.
**Step 2.** Glucose 6-phosphate is converted to fructose 6-phosphate by phosphoglucoisomerase.

Glucose 6-phosphate is an aldose sugar whereas fructose 6-phosphate is a ketose. The conversion of one to the other is therefore an isomerization reaction, which is more easily visualized by looking at its effect on the linear versions of the two compounds.

**Step 3.** Fructose 6-phosphate is phosphorylated by ATP to form fructose 1,6-bisphosphate and ADP. The enzyme catalyzing this step is phosphofructokinase.

**Step 4.** Aldolase splits fructose 1,6-bisphosphate, which is a six-carbon sugar, into two three-carbon compounds. These compounds are glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.
**Step 5.** Dihydroxyacetone phosphate cannot itself be used in the remainder of the glycolytic pathway. It is therefore converted into glyceraldehyde 3-phosphate by an isomerization reaction catalyzed by triose phosphate isomerase.

![Dihydroxyacetone phosphate to glyceraldehyde 3-phosphate conversion](image)

**Step 6.** Glyceraldehyde 3-phosphate is converted to 1,3-bisphosphoglycerate. The reaction is catalyzed by glyceraldehyde 3-phosphate dehydrogenase and uses inorganic phosphate (P\(_i\)) and NAD\(^+\). It generates one molecule of NADH and hence is the first step in the pathway at which some of the energy content of the original glucose molecule becomes stored in an activated carrier.

![Glyceraldehyde 3-phosphate dehydrogenase](image)

**Step 7.** Phosphoglycerate kinase catalyzes the transfer of a phosphate group from 1,3-bisphosphoglycerate to ADP, generating ATP and 3-phosphoglycerate.

![Phosphoglycerate kinase](image)

**Step 8.** 3-Phosphoglycerate is converted to 2-phosphoglycerate by phosphoglycerate mutase. This reaction moves the phosphate group present in 3-phosphoglycerate to a different carbon atom within the same molecule.

![Phosphoglycerate mutase](image)
**Step 9.** Enolase catalyzes the removal of water from 2-phosphoglycerate, giving phosphoenolpyruvate.

![2-phosphoglycerate to phosphoenolpyruvate](image)

**Step 10.** In the final reaction of the pathway, pyruvate kinase catalyzes the transfer of the phosphate group from phosphoenolpyruvate to ADP to form ATP and pyruvate.

![Phosphoenolpyruvate to Pyruvate](image)

**Glycolysis uses up ATP in order to make more ATP**

The glycolysis pathway can be divided into two phases, the first phase comprising steps 1–5 and culminating in synthesis of glyceraldehyde 3-phosphate, and the second phase made up of steps 6–10, when glyceraldehyde 3-phosphate is metabolized into pyruvate. The first phase does not generate ATP. In fact, the reverse is true, because two molecules of ATP are needed to convert one molecule of glucose (which has no phosphate groups) into two molecules of glyceraldehyde 3-phosphate (both of which have a single phosphate). It is only in phase 2 of glycolysis that ATP molecules are produced, two for every molecule of glyceraldehyde 3-phosphate, and hence four for each starting molecule of glucose (Fig. 8.4). The pathway as a whole therefore achieves a net gain of two ATPs per glucose molecule. This net gain can be increased to eight in respiring organisms where glycolysis is linked to the electron transport chain, because now the energy contained in the NADH molecule generated in step 6 can be used to synthesize three further ATPs. Again, we double this number to get the number of ATPs obtained from a single starting glucose molecule.

The two ATPs that are used in the first phase of the pathway are recovered in the final step, when phosphoenolpyruvate is converted to pyruvate. Why use up two ATPs during the first phase of glycolysis when they are simply recovered again at the end of the pathway? There are two reasons. First, the initial phosphorylation ensures that glucose continues to flow into the cell. Remember that in Section 5.2.2 we learnt how the GLUT1 uniporter transports glucose across the plasma membrane. Glucose transport is an example of facilitated diffusion, so to be transported into the cell the internal glucose concentration must be lower than that outside of the cell. Conversion of glucose to glucose 6-phosphate, which is not a substrate for the GLUT1 uniporter, immediately or soon after transport ensures that the internal glucose concentration remains low (Fig. 8.5). In effect, the phosphorylation traps the energy source within the cell so it is not lost if the external glucose concentration drops.

The second reason for the initial phosphorylations is that these favor the reactions occurring during steps 6 and 7. These two steps result in conversion of glyceraldehyde 3-phosphate to 3-phosphoglycerate, generating one molecule of ATP and one of NADH. These steps therefore make up the critical part of the pathway because
this is when the net energy gain is achieved. Glyceraldehyde 3-phosphate, as its names implies, is an aldehyde, and 3-phosphoglycerate is a type of carboxylic acid. The conversion from one to the other is an oxidation reaction. The first of the two enzymes involved in this conversion, glyceraldehyde 3-phosphate dehydrogenase, uses inorganic phosphate as the source of the oxygen to carry out the oxidation, generating 1,3-bisphosphoglycerate (see step 6, above). The displaced hydrogen is used to reduce NAD\(^+\) to NADH, capturing a portion of the energy released by the oxidation reaction. The 1,3-bisphosphoglycerate molecule is immediately passed to the second enzyme, phosphoglycerate kinase, which transfers the phosphate group to ADP, converting the latter to ATP (step 7, above). These reactions would be possible with glyceraldehyde as the substrate instead of glyceraldehyde 3-phosphate, but with glyceraldehyde the energy barrier that would have to be surmounted to bring the oxidation to completion is greater. The increased energy input needed to oxidize glyceraldehyde would reduce the overall energy balance of these two steps to the extent that insufficient energy would be left over to generate either the ATP or NADH molecules. The two phosphorylations in the first phase of glycolysis, by reducing the energy barrier, therefore make it possible for the energy released during the oxidation to drive the production of ATP and NADH.

### 8.2.2 Glycolysis in the absence of oxygen

Glycolysis does not require the presence of molecular oxygen and so can take place under anaerobic conditions. As glycolysis results in a net yield of ATP molecules, a cell operating under anaerobic conditions is able to generate energy, even though it is unable to utilize the additional energy contained in the NADH molecules generated in step 6. This is a disadvantage, but the main problem that an anaerobic cell faces is that if these NADH molecules are not re-oxidized then its supply of NAD\(^+\) may become low. As NAD\(^+\) is a substrate for step 6 of glycolysis, a shortage of this compound would, amongst other things, cause glycolysis to stall at this point, before the pathway has reached the steps where the net gain in ATP is achieved. As we will see, different species have evolved different strategies for converting the NADH back to NAD\(^+\).

**In exercising muscles, pyruvate is converted to lactate**

In animals, oxygen can become limiting in muscles after a period of prolonged exercise. The TCA cycle and electron transport chain are then unable to work rapidly enough to regenerate all the NAD\(^+\) needed to maintain glycolysis at its maximal rate. To alleviate this problem, some of the pyruvate that now accumulates in the muscle cells is converted to lactate by the enzyme lactate dehydrogenase.

![Figure 8.5 Phosphorylation traps glucose within the cell. Immediately after transport into the cell, glucose is converted into glucose 6-phosphate. The latter cannot pass back through the GLUT1 uniporter and so remains inside the cell even if the external glucose concentration drops.](image)

This conversion of pyruvate to lactate is a reduction and hence can be coupled to the oxidation of NADH to NAD\(^+\), ensuring that the cell’s supply of the latter remains sufficient for glycolysis to continue.

What happens to the lactate that is now being produced? Lactate cannot be metabolized into any other useful compounds and so the only way to get rid of it is to convert it back to pyruvate. This reverse reaction can also be catalyzed by lactate dehydrogenase, but of course would consume NAD\(^+\) molecules in the muscle. Instead, the lactate is transported from the anaerobic muscle environment, via the bloodstream, to the liver, whose cells are unaffected by exercise and will still be...
operating in an aerobic environment. The lactate is then oxidized back to pyruvate by lactate dehydrogenase.

The pyruvate in the liver could now enter the TCA cycle, but usually this does not occur. This is because the liver is able to generate sufficient energy for its own needs without this pyruvate boost. Instead, a process called **gluconeogenesis** converts the pyruvate back to glucose, which is then passed into the bloodstream for use by other tissues. If the period of exercise that initiated this process is prolonged and severe, then its maintenance may be dependent on the muscle cells accessing this new supply of glucose. The combination of glycolysis and lactate production in muscle cells linked to regeneration of pyruvate and glucose in the liver is called the **Cori cycle** (Fig. 8.6). The cycle, and the exercise it is supporting, cannot continue indefinitely because there is a net loss of ATP. This is because gluconeogenesis consumes six ATP molecules for every pyruvate that is converted back to glucose, and only two of these ATPs are recovered when the glucose is converted back to pyruvate via glycolysis.

---

**Box 8.2 Biochemical synthesis of ATP**

ATP is the most important activated carrier molecule and reactions that result in its synthesis are critical for maintaining the energy supply available to the cell. There are two ways in which ATP can be generated, by **substrate-level phosphorylation** and by **oxidative phosphorylation**.

In substrate-level phosphorylation, the phosphate used to generate ATP from ADP comes from a sugar–phosphate intermediate, which is one of the substrates of the reaction. We can denote this intermediate as $R$-OPO$_3^{2-}$, where $R$ is the sugar component of the compound:

$\text{ADP} + \text{R}-\text{OPO}_3^{2-} \rightarrow \text{R-OH} + \text{ATP}$

The energy released when the phosphate group is detached from the intermediate is conserved and used to drive the transfer of this group to ADP. **Steps 7 and 10** of glycolysis are both substrate-level phosphorylations.

In oxidative phosphorylation, an ATP synthase enzyme synthesizes ATP from ADP and inorganic phosphate:

$\text{ADP} + P_i \rightarrow \text{ATP}$

The energy needed to drive this reaction is obtained by **oxidation** of NADH or FADH$_2$. We will study the process in detail in **Section 8.2.3**.

Respiring cells obtain most of their ATP by oxidative phosphorylation. Substrate-level phosphorylation is more important in tissues that are suffering from oxygen starvation and in organisms that live in natural environments that lack oxygen.

---

**Figure 8.6 The Cori cycle.**

Lactate synthesized in exercising muscle is transported to the liver, where it is converted to pyruvate by lactate dehydrogenase, and then to glucose by the gluconeogenesis pathway. During periods of extreme exercise, the glucose can be sent back to the muscle in order to maintain glycolysis in the muscle cells.
Box 8.3  Aerobes and anaerobes

Microorganisms can be classified according to their requirement for oxygen:

- **An obligate aerobe** must have oxygen in order to grow. These organisms obtain the bulk of their ATP from oxidative phosphorylation, hence their requirement for oxygen. Most fungi and algae are obligate aerobes, as are many bacteria.

- **A facultative anaerobe** is able to use oxygen to make ATP, but can also grow in the absence of oxygen. The yeast *Saccharomyces cerevisiae* is a typical facultative anaerobe. If no oxygen is available then yeasts can use fermentation to regenerate NAD⁺, enabling them to continue to obtain ATP by substrate-level phosphorylation during glycolysis.

- **An obligate anaerobe** never uses oxygen. Indeed oxygen is lethal to many organisms of this type, because they are unable to detoxify compounds such as superoxide (O₂⁻) and hydrogen peroxide (H₂O₂), which then accumulate in their cells causing oxidative damage to enzymes and membranes. Some obligate anaerobes convert pyruvate from glycolysis into lactate or some other compound whose synthesis enables the NAD⁺ used in glycolysis to be regenerated. Others regenerate NAD⁺ and possibly synthesize ATP via modified versions of the electron transport chain, in which a compound other than oxygen is used as the final electron acceptor. For example, *Desulfobacter* uses sulfate as its final electron acceptor, and is therefore a type of sulfate-reducing bacterium.

Yeast converts pyruvate to alcohol and carbon dioxide

Various microorganisms, including the yeast *Saccharomyces cerevisiae*, can live in natural environments that lack oxygen. These species are therefore called **facultative anaerobes**, to distinguish them from **obligate aerobes**, which are organisms that must have oxygen in order to grow. When oxygen is available, yeast carries out the full energy-generation pathway including the TCA cycle and electron transport chain. But when the oxygen supply falls below a certain level, the TCA cycle and electron transport chain are temporarily switched off, and the yeast cell relies only on glycolysis for provision of ATP.

Under anaerobic conditions, yeast regenerates the NADH resulting from glycolysis by a two-step process called **alcoholic fermentation** (Fig. 8.7).

**Step 1.** Pyruvate is converted to acetaldehyde by **pyruvate decarboxylase**.

![Pyruvate decarboxylase](image)

**Step 2.** Acetaldehyde is converted to ethanol by **alcohol dehydrogenase**.

![Alcohol dehydrogenase](image)

The products of alcoholic fermentation are therefore NAD⁺, ethanol and carbon dioxide.

For yeast cells, the purpose of alcoholic fermentation is to regenerate NAD⁺ for use in glycolysis. For humans, the commercial importance of the pathway is synthesis of the ethanol byproduct, the use of yeast to produce this compound representing the earliest example of prehistoric biotechnology. Alcoholic beverages are made by allowing yeast to carry out alcoholic fermentation of sugar contained in natural products such as grapes, to produce wine, and barley, to produce beer. The archaeological record
suggests that a type of rice wine was being made in China about 9000 years ago, and at about the same time beer was being brewed in Mesopotamia. The carbon dioxide produced during alcoholic fermentation is exploited during bread-making; addition of yeast to the flour generates carbon dioxide that causes the resulting dough to rise and produces nice bouncy bread. Certain chemicals such as baking soda (sodium bicarbonate) that release carbon dioxide during baking can be used instead of yeast. Agents such as yeast and baking soda which cause bread dough to rise are called leavening agents and the resulting bread is called leavened bread. The ancient Egyptians were making bread leavened with yeast 2500 years ago, and there is evidence that this type of bread-making was being practiced in Greece some 1000 years earlier than that. Today, the production of alcoholic beverages and bread are important industries worldwide.

8.2.3 Glycolysis starting with sugars other than glucose

Glucose is one of three sugars, fructose and galactose being the others, that can be absorbed into the bloodstream during digestion. Having considered glucose, we must now look at how these other sugars enter the glycolytic pathway.

Fructose has two routes into glycolysis, which are used in different tissues

Fructose is common in the human diet, being present in many fruits and most root vegetables, and is also the major sugar in honey. Sucrose is a disaccharide of fructose and glucose and so, after digestion, is another major dietary source of fructose.

In most tissues, the presence of fructose rather than glucose does not cause any difficulty because hexokinase, which catalyzes the conversion of glucose to glucose 6-phosphate, can also use fructose as a substrate (Fig. 8.8). The resulting fructose 6-phosphate then enters step 3 of glycolysis.

A difficulty arises in liver cells, because these make use of an alternative means of phosphorylating glucose, using the enzyme glucokinase instead of hexokinase. Glucokinase does not recognize fructose as a substrate, so in these cells fructose must enter glycolysis via a different route. This is achieved by the fructose 1-phosphate pathway (Fig. 8.9). This pathway has three steps:

Step 1. Fructokinase phosphorylates fructose, converting it into fructose 1-phosphate.

\[
\text{fructose} + \text{ATP} \xrightarrow{\text{fructokinase}} \text{fructose 1-phosphate} + \text{ADP} + \text{H}^+ 
\]

Step 2. Fructose 1-phosphate aldolase splits fructose 1-phosphate into glyceraldehyde and dihydroxyacetone phosphate.

\[
\text{fructose 1-phosphate} \xrightarrow{\text{fructose 1-phosphate aldolase}} \text{glyceraldehyde + dihydroxyacetone phosphate}
\]

Figure 8.8 The conversion of fructose to fructose 6-phosphate catalyzed by hexokinase.

Figure 8.9 The fructose 1-phosphate pathway.
The dihydroxyacetone enters the glycolytic pathway at step 5 and is converted into glyceraldehyde 6-phosphate by triose phosphate isomerase.

**Step 3. Triose kinase** phosphorylates glyceraldehyde to give glyceraldehyde 3-phosphate.

\[
\text{glyceraldehyde} \quad \xrightarrow{\text{triose kinase}} \quad \text{glyceraldehyde 3-phosphate}
\]

The fructose 1-phosphate pathway therefore yields two molecules of glyceraldehyde 3-phosphate, just like the first phase of the standard glycolysis pathway.

**Galactose is converted to glucose before use in glycolysis**

Galactose is less common than glucose and fructose in fruits and vegetables, though it is present in sugar beet. In the human diet, its main source is milk and dairy products, which contain lactose, a disaccharide of galactose and glucose. Babies are able to split lactose into its component sugars using the enzyme lactase and the resulting glucose and galactose are then absorbed into the bloodstream. Humans who display lactase persistence, in which lactase is still active in adulthood, are also able to metabolize lactose in this way.

The molecular structures of galactose and glucose differ only in the arrangement of the –H and –OH groups around carbon number 4 (Fig. 8.10). Conversion of one to the other therefore requires an isomerization reaction, specifically the type of isomerization called epimerization, in which chemical groups are rearranged around a chiral carbon. The **galactose–glucose interconversion pathway** (Fig. 8.11) carries out this epimerization in four steps.
**Step 1.** Galactokinase phosphorylates galactose to galactose 1-phosphate.

\[
\begin{align*}
\text{galactose} & \quad \text{ATP} \quad \text{ADP} + \text{H}^+ \\
\text{galactokinase} & \quad \text{galactose 1-phosphate}
\end{align*}
\]

**Step 2.** Galactose 1-phosphate uridylyl transferase transfers a uridine group from UDP–glucose to galactose 1-phosphate. This gives one molecule of UDP–galactose and one of glucose 1-phosphate.

\[
\begin{align*}
\text{galactose 1-phosphate} & \quad \text{UDP-glucose} \\
\text{UDP-galactose} & \quad \text{glucose 1-phosphate}
\end{align*}
\]

**Step 3.** UDP–galactose 4-epimerase converts UDP–galactose to UDP–glucose. This step therefore regenerates the UDP–glucose molecule used in step 2.

\[
\begin{align*}
\text{UDP-galactose} & \quad \text{UDP-glucose}
\end{align*}
\]
**Step 4. Phosphoglucomutase** repositions the phosphate group on the glucose 1-phosphate molecule formed in step 2.

```
\[
\begin{align*}
\text{glucose 1-phosphate} & \quad \xrightarrow{\text{phosphoglucomutase}} \quad \text{glucose 6-phosphate} \\
\end{align*}
\]
```

This reaction produces glucose 6-phosphate, which enters at step 2 of the standard glycolytic pathway.

### 8.2.4 Regulation of glycolysis

The final aspect of glycolysis that we must consider is how the pathway is regulated. Glycolysis has two main roles: it degrades glucose to generate ATP and it produces intermediates that act as precursors for biosynthetic pathways, such as those involved in the synthesis of fatty acids. Glycolysis must therefore be regulated to ensure that these two roles are fulfilled.

**The conversion of fructose 6-phosphate to fructose 1,6-bisphosphate is the main control point in glycolysis**

The main control point in the glycolytic pathway is step 3, when fructose 6-phosphate is phosphorylated by ATP to form fructose 1,6-bisphosphate and ADP. In eukaryotes, the enzyme that catalyzes this step, phosphofructokinase, is inhibited by three of the later products of glycolysis (ATP, citrate, and hydrogen ions) enabling the pathway as a whole to be regulated in response to different physiological conditions (Fig. 8.12).

The most straightforward of the inhibitory effects on phosphofructokinase is that brought about by ATP. Clearly, if the cell is lacking ATP then it needs to increase the rate at which glycolysis is taking place and, conversely, when ATP is abundant the pathway should be slowed down. This is achieved by ATP attaching to the surface

---

**Figure 8.12 Phosphofructokinase is the main control point in glycolysis.**

The first five steps of glycolysis are shown, with the step catalyzed by phosphofructokinase highlighted.
of the phosphofructokinase enzyme. This attachment is away from the active site of the enzyme, where ATP also binds in order to participate in the phosphorylation catalyzed by the enzyme (Fig. 8.13). ATP therefore acts as an allosteric regulator of phosphofructokinase. AMP competes with ATP for attachment to the allosteric site, and reverses the inhibitory effect of ATP. The rate of the phosphofructokinase reaction, and hence the flow of metabolites through the subsequent steps of the glycolytic pathway, is therefore regulated in response to the relative amounts of AMP and ATP in the cell. When ATP is plentiful the rate goes down so that the ATP pool does not become over-abundant, and when ATP is scarce the rate increases so that the cell’s ATP supplies are replenished.

Citrate affects phosphofructokinase activity by promoting the binding of ATP to the allosteric site on phosphofructokinase. Increased levels of citrate therefore result in decreased phosphofructokinase activity, so glycolysis slows down. This is logical because citrate is one of the intermediates of the TCA cycle, and its accumulation in the cell would indicate that the energy-generating pathway as a whole is over-active. There is, however, some doubt about the role of citrate inhibition in living cells. Its effect on phosphofructokinase has been studied in the test tube, but in the cell it is possible that any excess of citrate resulting from the TCA cycle is immediately used as a source of acetyl-CoA for fatty acid synthesis. If this is the case then citrate is unlikely to accumulate sufficiently to have a significant effect on phosphofructokinase activity.

Hydrogen ions also inhibit phosphofructokinase, again by increasing the allosteric effect of ATP. This means that at low pH values, phosphofructokinase activity is reduced and glycolysis slows down. Why should pH be an important influence on the rate of glycolysis? The answer lies with the accumulation of lactate that occurs in active muscle tissue. Excess amounts of lactate can damage muscle tissue and also

**Box 8.4 Why is phosphofructokinase regulated by AMP and not ADP?**

The product of phosphofructokinase activity is ADP, not AMP, and so it might appear logical that ADP would be the positive regulator of this enzyme. AMP plays this role because the level of ADP is not always an accurate indication of the energy requirements of the cell. This is because ADP can be directly converted to ATP by adenylate kinase.

![Figure 8.13 ATP binding at the active and regulatory sites of phosphofructokinase. Phosphofructokinase is a tetramer of four identical subunits, one of which is shown in this drawing.](image)

This conversion, which occurs when ATP is used up rapidly, decreases the amount of ADP in the cell under conditions when ATP is required. ADP could not therefore act as the positive regulator of phosphofructokinase, because its concentration drops when ATP is needed. On the other hand, the AMP produced by adenylate kinase supplements the very small amount of this compound that is usually present in the cell. The resulting large increase in AMP concentration enables AMP to act as the positive regulator of phosphofructokinase. It out-competes ATP for occupancy of the allosteric site, so the enzyme is stimulated to increase the flow of metabolites through the glycolytic pathway and hence increase the synthesis of ATP.
bring about acidosis, when the blood pH falls to dangerously low levels. Inhibition of phosphofructokinase by hydrogen ions means that, at low pH values, glycolysis is slowed down so less lactate is produced and its dangerous effects are ameliorated. This is one of the reasons why excessive exercise cannot be continued indefinitely. Although muscle cells can switch to anaerobic respiration when their oxygen supply becomes limiting, and some of the lactate that is then produced can be transported to the liver and converted back to pyruvate and glucose, at some point the rate of accumulation of lactate will defeat the body’s attempts to adapt, and energy production will begin to decline because of the hydrogen ion effect.

**Substrate availability also regulates phosphofructokinase activity**

So far we have examined how the activity of phosphofructokinase can be inhibited by the products of glycolysis, so that the flow of metabolites through the pathway is increased or decreased depending on how much ATP, citrate or lactate is being produced. Phosphofructokinase is also regulated by the amount of substrate that is present. This stimulatory effect is mediated by fructose 2,6-bisphosphate, a phosphorylated sugar with a structure slightly different to the fructose 1,6-bisphosphate that is produced by phosphofructokinase activity (Figure 8.14).

Fructose 2,6-bisphosphate is synthesized from fructose 6-phosphate by an enzyme called **phosphofructokinase 2**. This is a different enzyme to the phosphofructokinase involved in glycolysis, but it catalyzes a similar reaction.

\[
\begin{align*}
\text{fructose 6-phosphate} + \text{ATP} & \overset{\text{phosphofructokinase 2}}{\rightarrow} \text{fructose 2,6-bisphosphate} \\
+ \text{ADP} + \text{H}^+ & \\
\end{align*}
\]

The only difference between the activities of the two types of phosphofructokinase is the number of the carbon to which the phosphate group is attached. Phosphofructokinase 2 attaches this phosphate to carbon number 2, whereas phosphofructokinase uses carbon 1.

The reverse reaction, converting fructose 2,6-bisphosphate back to fructose 6-phosphate is catalyzed by **fructose bisphosphatase 2**.

\[
\begin{align*}
\text{fructose 2,6-bisphosphate} & \overset{\text{fructose bisphosphatase 2}}{\rightarrow} \text{fructose 6-phosphate} \\
& + \text{P}_i \\
\end{align*}
\]

Although phosphofructokinase 2 and fructose bisphosphatase 2 are different enzyme activities, both are catalyzed by the same protein. The activity of this protein is regulated by fructose 6-phosphate in two separate ways (Figure 8.15):

- Fructose 6-phosphate *stimulates* the phosphofructokinase 2 activity, and hence promotes its own conversion into fructose 2,6-bisphosphate.
- Fructose 6-phosphate *inhibits* the fructose bisphosphatase 2 activity, and hence reduces its synthesis from fructose 2,6-bisphosphate.

The net result of these two complementary regulatory activities is that fructose 6-phosphate exerts self-control over its concentration in the cell. When the amount of fructose 6-phosphate increases, the excess is converted into fructose 2,6-bisphosphate rather than proceeding down the glycolytic pathway with the possible over-production of ATP. If the level of fructose 6-phosphate falls, then more can be obtained from the fructose 2,6-bisphosphate pool, so the rate of glycolysis is maintained.
Fructose 6-phosphate is the product of glucose, in steps 1 and 2 of glycolysis, so the regulation exerted by fructose 6-phosphate and fructose 2,6-bisphosphate is responsive to the amount of glucose available to the cell. Glucose also has its own more direct effect on this regulatory network. When the amount of glucose in the bloodstream falls, the hormone called glucagon is released by the pancreas. Glucagon triggers a series of reactions that result in modification of the phosphofructokinase 2/fructose bisphosphatase 2 protein. The modified protein has an increased fructose bisphosphatase 2 activity and reduced phosphofructokinase 2 activity (Figure 8.16). This means that more fructose 2,6-bisphosphate is converted to fructose 6-phosphate, maintaining the rate of glycolysis even though the availability of glucose has become low (see Box 8.5).

We have examined the phosphofructokinase 2/fructose bisphosphatase 2 control system in some detail, not just because it is a key aspect of glycolysis regulation, but also because this system illustrates the exquisite complexity and fitness-for-purpose that regulatory networks can display in living organisms. The direct and indirect influences of glucose and fructose 6-phosphate on this bifunctional enzyme, which is not itself an integral part of the glycolysis pathway, enables the rate of glycolysis to be precisely set so that the most efficient use is made of the amount of substrates that are available to the cell.
Control of fructose 6-phosphate levels by glucagon

The process by which glucagon controls the fructose 6-phosphate content of the cell provides a typical example of a signal transduction pathway (Section 5.2.2). Glucagon is the extracellular signaling compound, and the signal from cell membrane to target enzyme is transduced via a second messenger system involving cAMP.

The first stage of any signal transduction pathway is the attachment of the extracellular signaling compound to the outside of the cell. Glucagon binds to the glucagon receptor, which is a transmembrane protein with seven α-helices forming a barrel-shaped structure that spans the cell membrane.

Because of their structure, this class of receptor is called seven-transmembrane-helix or 7TM proteins.

Binding of glucagon to the outer surface of the receptor induces a conformational change in the positioning of the loops on the internal side of the protein. This in turn activates a G protein that is associated with the receptor. A G protein is a small protein that binds either a molecule of GDP or GTP. When GDP is bound the G protein is inactive. The change in conformation of the glucagon receptor causes the GDP to be replaced by GTP, converting the G protein to its active form. Because it works via a G protein the glucagon receptor is called a G-protein coupled receptor.

Once activated, the G protein interacts with adenylate cyclase which, like the receptor protein, is attached to the cell membrane with its active site on the internal side. The interaction with the G protein changes the conformation of adenylate cyclase, changing it to its active form, which now converts ATP to cAMP (see Figure 5.29). The increased cellular level of cAMP in turn activates protein kinase A, which adds a phosphate group to one of the serines in the phosphofructokinase 2/fructose bisphosphatase 2 enzyme. This phosphorylation is the modification that increases the fructose bisphosphatase 2 activity and reduces the phosphofructokinase 2 activity.

When the blood glucose level increases, glucagon is no longer released by the pancreas and the signal transduction pathway is switched off. A phosphatase enzyme now removes the phosphate from phosphofructokinase 2/fructose bisphosphatase 2, so this enzyme reverts back to its alternative state, with reduced fructose bisphosphatase 2 activity and increased phosphofructokinase 2 activity.
Hexokinase and pyruvate kinase are also control points in glycolysis

Although phosphofructokinase is the main control point in glycolysis, two other enzymes have important roles in regulating the pathway. These are hexokinase and pyruvate kinase, which catalyze the first and last steps in the pathway, respectively.

Hexokinase is inhibited by its product, glucose 6-phosphate. Step 2 of glycolysis, in which glucose 6-phosphate is converted to fructose 6-phosphate by phosphoglucoisomerase, is a reversible reaction. This means that there is a balance between the amounts of glucose 6-phosphate and fructose 6-phosphate in the cell, the relative amounts kept in equilibrium by the reversible nature of their interconversion. So when phosphofructokinase, the enzyme for step 3 of the pathway, is inhibited, and fructose 6-phosphate accumulates, glucose 6-phosphate also accumulates. The latter then inhibits hexokinase, the enzyme responsible for its synthesis, so that additional glucose does not enter the pathway until it is needed (Figure 8.17).

Why is phosphofructokinase the main control point for glycolysis and not hexokinase, the first enzyme in the pathway? The answer is because glucose 6-phosphate is not only used as a substrate for glycolysis. Some glucose 6-phosphate is used to synthesize glycogen, and some is also used by the pentose phosphate pathway, which generates the NADPH needed for synthesis of fatty acids and other biomolecules. If hexokinase was the main control point for glycolysis, then the availability of glucose 6-phosphate would be subject to a regulatory process that did not take account of the requirements of these other metabolic pathways (Figure 8.18). Phosphofructokinase is therefore the first commitment step in glycolysis, and hence is the main control step.

Pyruvate kinase, catalyzing the last step in glycolysis, can be looked on as regulating the junction between this pathway and the TCA cycle, into which pyruvate enters prior to its complete breakdown into carbon dioxide and water. Pyruvate kinase is activated by fructose 1,6-bisphosphate and inhibited by ATP, exactly as we might anticipate from what we have learnt so far about the respective effects of substrates and products on the control of glycolysis. Pyruvate kinase is also inhibited by glucagon, and so is a second site at which this hormone achieves a slow-down of glycolysis when blood glucose levels are low. Finally, pyruvate kinase is inhibited by the amino acid alanine. Amino acids are one of several types of biomolecule that are made from intermediates synthesized during the TCA cycle. A relatively high amount of alanine indicates that the cell has a plentiful supply of these biomolecules, reducing the need for pyruvate to be fed into the TCA cycle.
Further reading


Self-assessment questions

Multiple choice questions

Only one answer is correct for each question. Answers can be found on the website: www.scionpublishing.com/biochemistry.

1. How much energy is produced by the complete oxidation of 1 mole of glucose?
   (a) 287 cal
   (b) 287 kJ
   (c) 2870 cal
   (d) 2870 kJ

2. What are the activated carrier molecules synthesized during glycolysis?
   (a) ATP and NADH
   (b) ATP and FADH₂
   (c) ATP, NADH and FADH₂
   (d) ATP and NADPH

3. One NADH molecule can generate how many ATPs when entered into the electron transport chain?
   (a) 1
   (b) 2
   (c) 3
   (d) 4

4. Glycolysis results in a net gain of how many ATP molecules?
   (a) 2
   (b) 4
   (c) 6
   (d) 8

5. Which enzyme catalyzes the first step in the glycolysis pathway?
   (a) Aldolase
   (b) Enolase
   (c) Hexokinase
   (d) Phosphoglucoisomerase

6. Which compound is split to give one molecule of glyceraldehyde 3-phosphate and one of dihydroxyacetone phosphate?
   (a) Fructose 1,6-bisphosphate
   (b) Fructose 2,6-bisphosphate
   (c) Fructose 6-phosphate
   (d) Glucose 6-phosphate

7. Which compound is converted into pyruvate by the enzyme pyruvate kinase?
   (a) Acetyl CoA
   (b) 2-Phosphoglycerate
   (c) 3-Phosphoglycerate
   (d) Phosphoenolpyruvate

8. What is the production of ATP by phosphoglycerate kinase called?
   (a) Activation
   (b) Kinasing
   (c) Oxidative phosphorylation
   (d) Substrate-level phosphorylation
9. In exercising muscle cells, what is excess pyruvate converted into?
   (a) Acetyl CoA
   (b) Alcohol and carbon dioxide
   (c) Lactate
   (d) Phosphoenolpyruvate

10. Which one of the following statements is correct with regard to the Cori cycle?
    (a) Acetyl CoA is used as a substrate
    (b) Lactate from muscles is transported to the liver where it is converted to glucose
    (c) It is responsible for alcohol production by yeast
    (d) It results in a net gain of ATP molecules

11. What is Saccharomyces cerevisiae an example of?
    (a) Facultative anaerobe
    (b) Obligate aerobe
    (c) Obligate anaerobe
    (d) None of the above

12. What are the two enzymes involved in alcoholic fermentation?
    (a) Lactate dehydrogenase and alcohol dehydrogenase
    (b) Lactate dehydrogenase and pyruvate decarboxylase
    (c) Pyruvate decarboxylase and alcohol dehydrogenase
    (d) Pyruvate decarboxylase and triose kinase

13. To be used in glycolysis, fructose is first converted to which of the following?
    (a) Fructose 1,6-bisphosphate
    (b) Fructose 1-phosphate
    (c) Fructose 6-phosphate
    (d) Glucose

14. Which of the following is UDP-glucose involved in?
    (a) Entry of fructose into glycolysis
    (b) Galactose-glucose interconversion pathway
    (c) Regulation of glycolysis
    (d) The Cori cycle

15. The main control point in glycolysis is the step that results in synthesis of what?
    (a) Fructose 1,6-bisphosphate
    (b) Fructose 6-phosphate
    (c) Glucose 6-phosphate
    (d) Pyruvate

16. Which one of the following is not an inhibitor of phosphofructokinase?
    (a) ADP
    (b) ATP
    (c) Citrate
    (d) Hydrogen ions

17. Which compound regulates phosphofructokinase activity in response to substrate availability?
    (a) Fructose 1,6-bisphosphate
    (b) Fructose 2,6-bisphosphate
    (c) Glucose 6-phosphate
    (d) Glucose 1,6-bisphosphate

18. The glucagon receptor protein is an example of what?
    (a) G-protein coupled receptor
    (b) Integral membrane protein
    (c) Seven-transmembrane-helix protein
    (d) All of the above

19. Hexokinase is inhibited by which one of these compounds?
    (a) ADP
    (b) Glucose
    (c) Glucose 1-phosphate
    (d) Glucose 6-phosphate

20. Regulation of pyruvate kinase involves which one of the following?
    (a) Activation by ATP and inhibition by fructose 1,6-bisphosphate
    (b) Activation by fructose 1,6-bisphosphate and inhibition by ATP
    (c) Activation by both ATP and fructose 1,6-bisphosphate
    (d) Inhibition by both ATP and fructose 1,6-bisphosphate
Short answer questions

These questions do not require additional reading.

1. Giving examples, describe the biochemical role of activated carrier molecules.

2. Explain in detail how a single molecule of glucose can yield 38 molecules of ATP.

3. Draw an outline of the glycolytic pathway, showing the substrates, products and enzymes for each step.

4. Give a detailed description of those steps of glycolysis that either consume or synthesize ATP. Based on your description, explain why glycolysis results in a net gain of two ATPs per glucose molecule.

5. What is the role of the GLUT1 uniporter in glycolysis?

6. Describe the special features of glycolysis in (a) exercising muscle, and (b) yeast cells growing under anaerobic conditions.

7. Outline how fructose and galactose are entered into the glycolytic pathway.

8. Describe why conversion of fructose 6-phosphate to fructose 1,6-bisphosphate is the main control point in glycolysis.


10. Outline the signal transduction pathway that enables glucagon to influence the intracellular level of fructose 6-phosphate.

Self-study questions

These questions will require calculation, additional reading and/or internet research.

1. Although glycolysis, the TCA cycle and electron transport chain can yield 38 molecules of ATP per molecule of glucose, it has been estimated that most cells can only generate 30–32 ATPs per glucose. What might be the reason(s) for this discrepancy?

2. Identify which carbon atom(s) in pyruvate correspond to carbons 1 and 4 of the glucose molecule that entered the glycolysis pathway. What assumption must be made in order to answer this question?

3. Arsenate ions ($\text{AsO}_4^{3-}$) are able to replace phosphate in many biochemical reactions, including the one catalyzed by glyceraldehyde 3-phosphate dehydrogenase. The resulting compound is unstable and immediately breaks down to give 3-phosphoglycerate. Describe the impact that arsenate will have on energy generation during glycolysis.

4. Pyruvate kinase deficiency (PKD) is estimated to affect 51 per million Caucasians. Individuals with this disorder present a range of symptoms, with anemia usually the most prevalent. Without treatment, patients can experience severe and possibly lethal complications, but if the anemia is managed, most individuals enjoy relatively good health. Those patients with a more mild form of PKD may not have any symptoms at all. Bearing in mind the essential role that pyruvate kinase plays in glycolysis, explain why PKD is not lethal in all patients, and why there are severe and mild forms of the disorder.

5. From the information provided about the Cori cycle (Section 8.2.2 and Fig. 8.6), predict what would happen if glycolysis and gluconeogenesis operated simultaneously in the same cell.