16.1 Glycolysis Is an Energy-Conversion Pathway in Most Organisms

The first metabolic pathway that we encounter is glycolysis, an ancient energy-conversion pathway employed by a host of organisms. **Glycolysis** is the sequence of reactions that metabolizes one molecule of glucose to two molecules of pyruvate with the concomitant net production of two molecules of ATP. This process is anaerobic, meaning that it does not require O_2, because it evolved before substantial amounts of oxygen accumulated in the atmosphere. Glycolysis is common to virtually all cells, both prokaryotic and eukaryotic. The complete glycolytic pathway is also known as the *Embden-Meyerhof-Parnas pathway*, named after three pioneers of research on glycolysis. As observed accidentally by the Buchners, pyruvate can be further processed anaerobically by fermentation to lactic acid, ethanol, or a variety of other final products depending on the organism. Under aerobic conditions, pyruvate can be completely oxidized to CO_2, generating much more ATP, as will be described in Chapters 17 and 18. **Figure 16.1** shows a few possible fates of pyruvate produced by glycolysis.

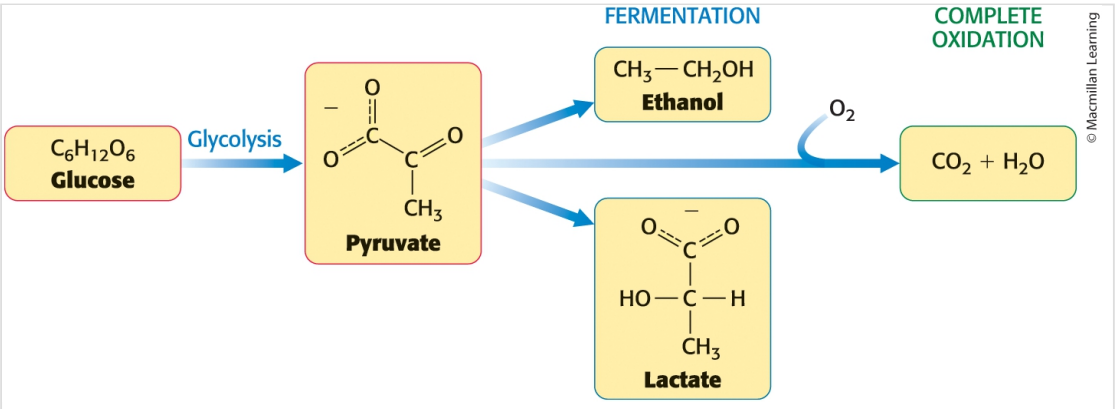


FIGURE 16.1 Glucose can be metabolized to different products depending on the organism and the presence or absence of oxygen.

Recall from Chapter 11 that glucose is a common but yet precious fuel for almost all organisms. In mammals, it is the only fuel that the brain uses under nonstarvation conditions and the only fuel that red blood cells can use at all. Because glucose is such a precious fuel, metabolic products, such as pyruvate and lactate, are salvaged to synthesize glucose in the process of **gluconeogenesis**, but the majority of glucose circulating in our blood each day originates directly from our diet.

Glucose is generated from dietary carbohydrates

In our diets, we typically consume a generous amount of glucose in the form of starch and a smaller amount as glycogen. These complex carbohydrates must be converted into simpler carbohydrates for absorption by the intestine and transport in the blood. Starch and glycogen are digested primarily by the pancreatic

and salivary enzyme α-*amylase*. Amylase cleaves the α-1,4 bonds of starch and glycogen, but not the α-1,6 bonds. The products are the di- and trisaccharides maltose and maltotriose. The material not digestible because of the α-1,6 bonds is called the *limit dextrin*.

α-Glucosidase (maltase) cleaves maltose and maltotriose, as well as any other α-1,4-linked oligosaccharides that may have escaped digestion by the amylase, into glucose molecules. α-Dextrinase further digests the limit dextrin. α-Glucosidase is located on the surface of the intestinal cells, as are sucrase and lactase. Sucrase degrades ingested sucrose to fructose and glucose, while lactase is responsible for degrading the milk sugar lactose into glucose and galactose. The monosaccharides are transported into the endothelial cells lining the intestine by the action of active transporters. Glucose then moves passively down its concentration gradient out of endothelial cells and into the bloodstream, and again passively into cells that will finally utilize it. How is this directional movement from one tissue to another accomplished?

A family of transporters enables glucose to enter and leave animal cells

Several glucose transporters (named GLUT1 to GLUT5) mediate the thermodynamically downhill movement of glucose across the plasma membranes of animal cells. Although all have a 12-transmembrane-helix structure similar to that of lactose permease (Section 13.3), the members of this family have distinctive roles, summarized in **Table 16.1**. GLUT1 and GLUT3, present in nearly all mammalian cells, are responsible for basal glucose uptake. Their K_M value for glucose is about 1 mM, significantly less than the normal blood serum-glucose range of 4–8 mM. Hence, GLUT1 and GLUT3

continually transport glucose into cells at an essentially constant rate.

TABLE 16.1 Family of glucose transporters

Name	Tissue location	K_M	Comments
GLUT 1	All mammalian tissues	1 mM	Basal glucose uptake
GLUT 2	Liver and pancreatic β cells	15–20 mM	In the pancreas, plays a role in the regulation of insulin In the liver, removes excess glucose from the blood
GLUT 3	All mammalian tissues	1 mM	Basal glucose uptake
GLUT 4	Muscle and fat cells	5 mM	Amount in muscle plasma membrane increases with endurance training and in response to insulin
GLUT 5	Small intestine	—	Primarily a fructose transporter

As described in Section 14.3, the number of GLUT4 transporters in the plasma membrane of muscle and fat cells increases rapidly in the presence of insulin, which signals the fed state. Thus, insulin promotes the uptake of glucose by muscle and fat. Endurance exercise training also increases the amount of this transporter

present in muscle membranes. GLUT2 is present in liver and pancreatic β cells but is distinctive in having a very high K_M value for glucose (15–20 mM). Because of this, glucose enters these tissues at a biologically significant rate only when there is much glucose in the blood. The pancreas can sense the glucose level and accordingly adjust the rate of insulin secretion. Lastly, while GLUT5 can also transport glucose, it functions primarily as a fructose transporter in the small intestine. Once finally in the cytoplasm of a glycolytically active cell, glucose and other monosaccharides are ready to enter the glycolytic pathway.

16.2 Glycolysis Can Be Divided into Two Parts

The 10 enzyme-catalyzed reactions that make up the glycolytic pathway can be thought of as comprising two stages (Figure 16.2). Stage 1 is the trapping and preparation phase. No ATP is generated in this stage. In stage 1, glucose is converted into fructose 1,6-bisphosphate in three steps: a phosphorylation, an isomerization, and a second phosphorylation reaction. The strategy of these initial steps in glycolysis is to trap the glucose in the cell and form a compound that can be readily cleaved into phosphorylated three-carbon units (Figure 16.3). Stage 1 is completed with the cleavage of the fructose 1,6-bisphosphate into two three-carbon fragments. These resulting three-carbon units are readily interconvertible. In stage 2, ATP is harvested when the three-carbon fragments are oxidized to pyruvate.

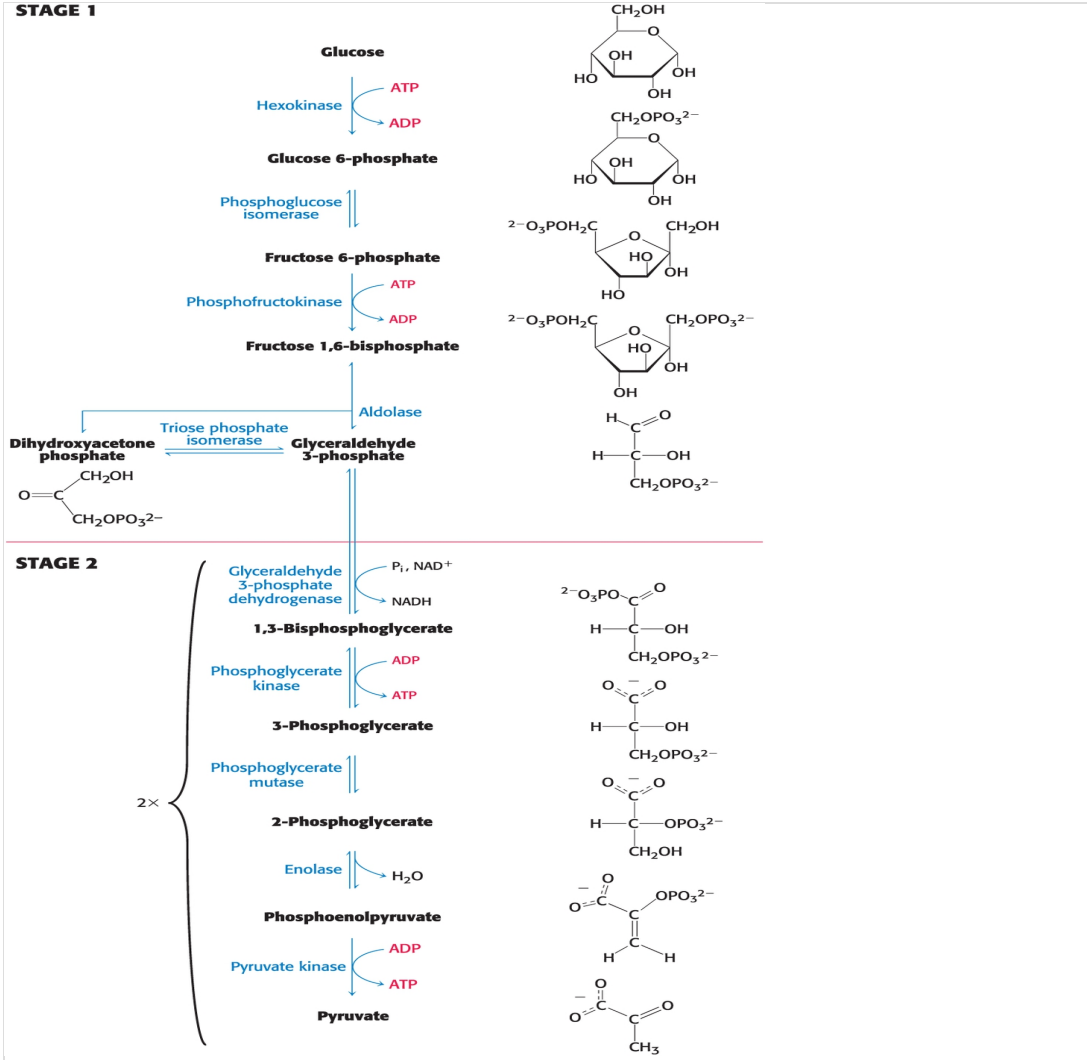


FIGURE 16.2 The glycolytic pathway can be divided into two stages. In Stage 1, glucose is trapped, destabilized, and cleaved into two interconvertible three-carbon molecules generated by cleavage of six-carbon fructose; and in Stage 2, ATP is generated.

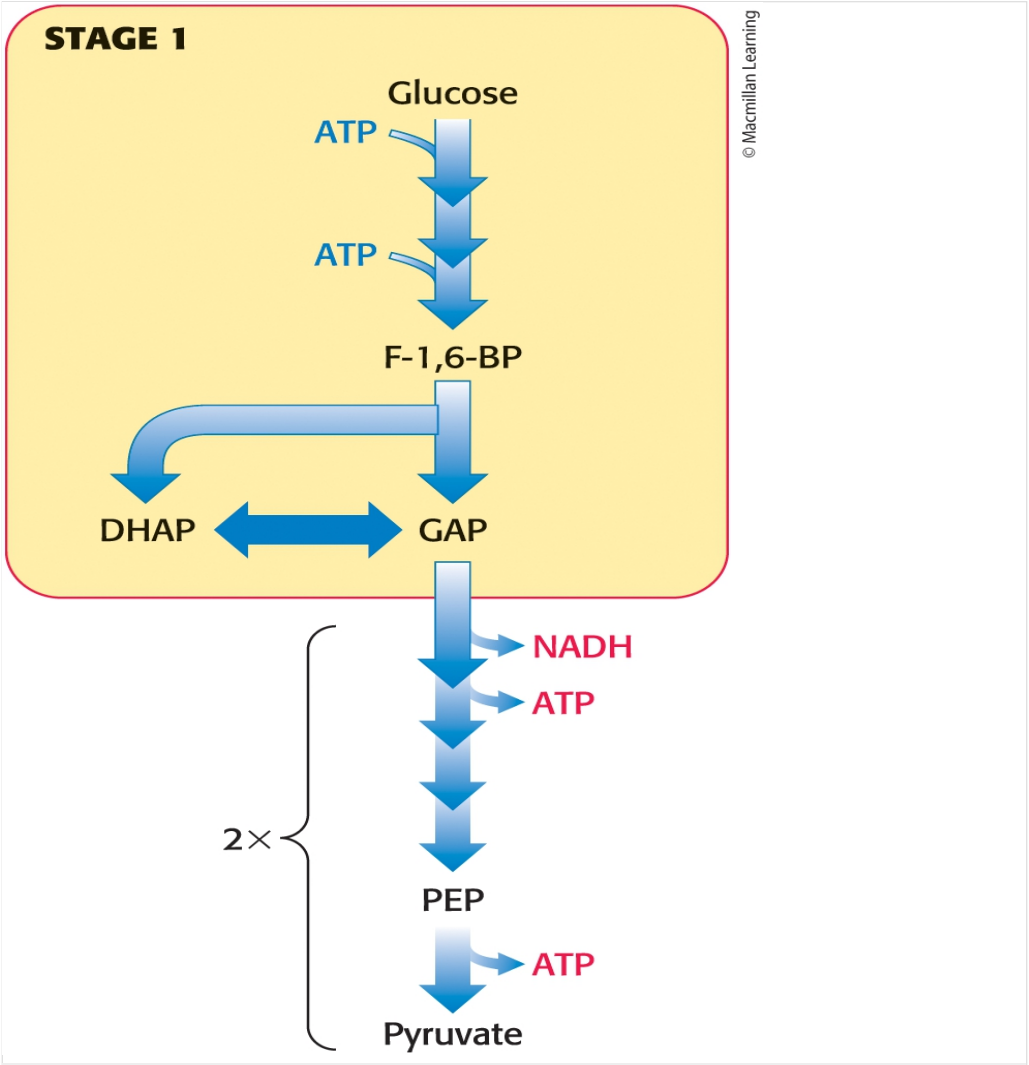
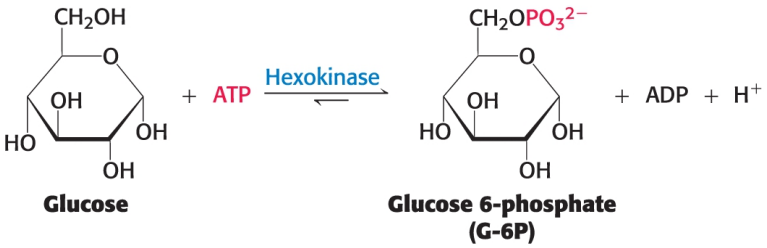


FIGURE 16.3 The first or "investment" stage of glycolysis uses the energy from two molecules of ATP. The products of this stage are two molecules of GAP for every molecule of glucose entering the pathway.

Stage 1 begins: Hexokinase traps glucose in the cell and begins glycolysis

Glucose entering a glycolytically active cell has one principal fate: it is phosphorylated by ATP to form glucose 6-phosphate. This step is notable for several reasons. Glucose 6-phosphate cannot pass through the membrane because of the negative charges on the phosphoryl groups, and it is not a substrate for glucose transporters. Also, the addition of the phosphoryl group facilitates the eventual metabolism of glucose into three-carbon molecules with high phosphoryl-transfer potential. The transfer of the phosphoryl group from ATP to the hydroxyl group on carbon 6 of glucose is catalyzed by hexokinase.



Phosphoryl transfer is a fundamental reaction in biochemistry. **Kinases** are enzymes that catalyze the transfer of a phosphoryl group from ATP to an acceptor, or to ADP from a phosphoryl donor. Hexokinase, then, catalyzes the transfer of a phosphoryl group from ATP to a variety of six-carbon sugars (hexoses), such as glucose and mannose. Hexokinase, like adenylate kinase (Section 6.1) and all other kinases, requires Mg^{2+} (or another divalent metal ion such as Mn^{2+}) for activity. The divalent metal ion forms a complex with ATP.

X-ray crystallographic studies of yeast hexo-kinase revealed that the binding of glucose induces a large conformational change in the enzyme. Hexokinase consists of two lobes, which move toward each other when glucose is bound (**Figure 16.4**). On glucose binding, one lobe rotates 12 degrees with respect to the other, resulting in movements of the polypeptide backbone of as much as 8 Å. The cleft between the lobes closes, and the bound glucose becomes surrounded by protein, except for the hydroxyl group of carbon 6, which will accept the phosphoryl group from ATP. The closing of the cleft in hexokinase is a striking example of the role of induced fit in enzyme action (Section 5.3).

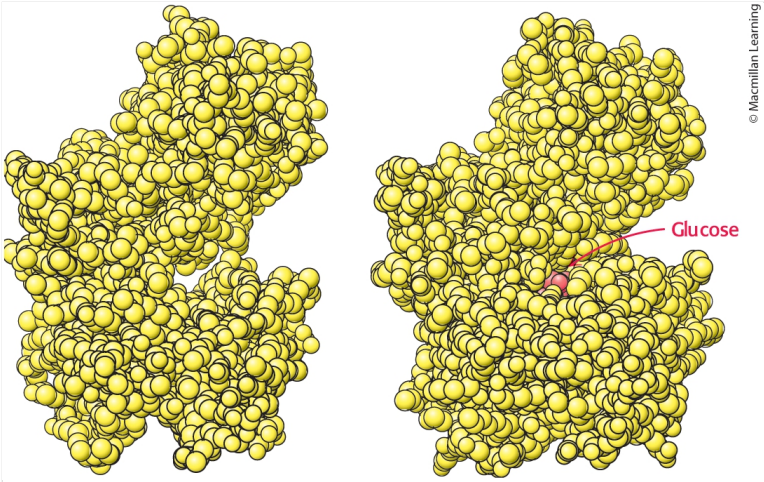


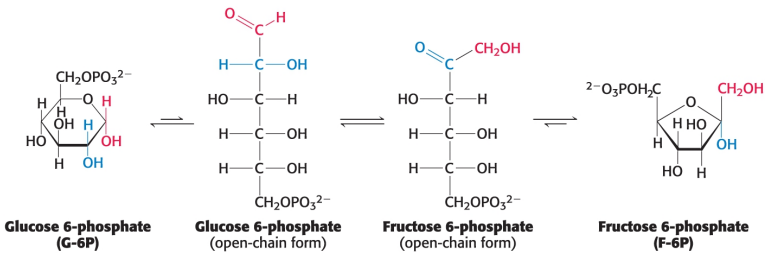
FIGURE 16.4 The induced fit of hexokinase is due to large structural changes upon binding the first of its two substrates. The two lobes of hexokinase are separated in the absence of glucose (left). The conformation of hexokinase changes markedly on binding glucose as the two lobes of the enzyme come together, creating the necessary environment for catalysis (right).

[Drawn from 2YHX.pdb and 1HKG.pdb.]

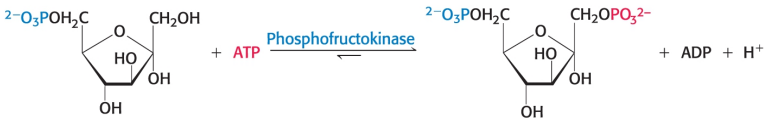
The glucose-induced structural changes are significant in two respects. First, the environment around the glucose becomes more nonpolar, which favors reaction between the hydrophilic hydroxyl group of glucose and the terminal phosphoryl group of ATP. Second, the conformational changes enable the kinase to discriminate against H_2O as a substrate. The closing of the cleft keeps water molecules away from the active site. If hexokinase were rigid, a molecule of H_2O occupying the binding site for the $-CH_2OH$ of glucose could attack the γ phosphoryl group of ATP, forming ADP and P_i. In other words, a rigid kinase would likely also be an ATPase.

Fructose 1,6-bisphosphate is generated from glucose 6-phosphate

A crucial step toward completion of the first phase of glycolysis — the formation of fructose 1,6-bisphosphate — is the isomerization of glucose 6-phosphate to fructose 6-phosphate. Recall that the open-chain form of glucose has an aldehyde group at carbon 1, whereas the open-chain form of fructose has a keto group at carbon 2. Thus, the isomerization of glucose 6-phosphate to fructose 6-phosphate is a conversion of an aldose into a ketose. The reaction catalyzed by phosphoglucose isomerase takes several steps because both glucose 6-phosphate and fructose 6-phosphate are present primarily in the cyclic forms. The enzyme must first open the six-membered ring of glucose 6-phosphate, catalyze the isomerization, and then promote the formation of the five-membered ring of fructose 6-phosphate.



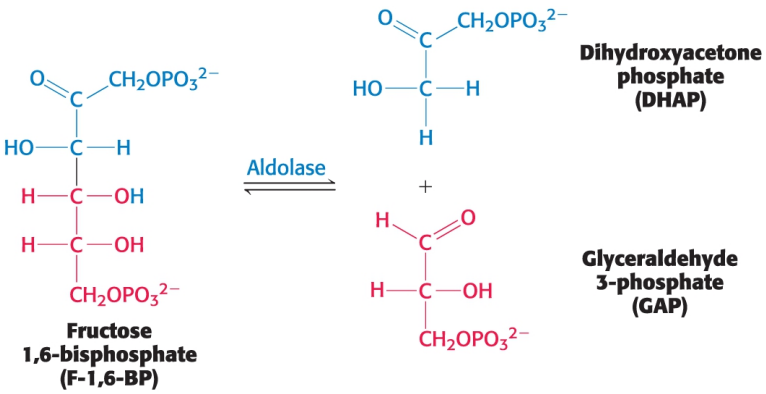
A second phosphorylation reaction follows the isomerization step. Fructose 6-phosphate is phosphorylated at the expense of ATP to fructose 1,6-bisphosphate (F-l,6-BP). The prefix *bis-* in bisphosphate means that two separate monophosphoryl groups are present, whereas the prefix *di-* in diphosphate (as in adenosine diphosphate) means that two phosphoryl groups are present and are connected by an anhydride linkage.



This reaction is catalyzed by phosphofructokinase (PFK), an allosteric enzyme that sets the pace of glycolysis. As we will learn, this enzyme plays a central role in the metabolism of many molecules in all parts of the body.

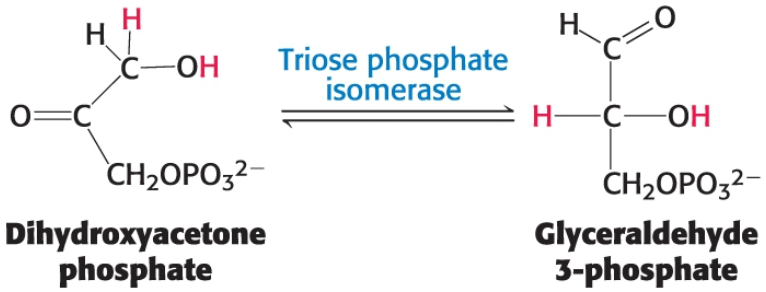
The six-carbon sugar is cleaved into two three-carbon fragments

The newly formed fructose 1,6-bisphosphate is cleaved into glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), completing stage 1 of glycolysis. The products of the remaining steps in glycolysis consist of three-carbon units rather than six-carbon units. This reaction, which is readily reversible, is catalyzed by aldolase. This enzyme derives its name from the nature of the reverse reaction, an aldol addition.



What is the biochemical rationale for the isomerization of glucose 6-phosphate to fructose 6-phosphate and its subsequent phosphorylation to form fructose 1,6-bisphosphate? First, phosphorylation of the fructose 6-phosphate to fructose 1,6-bisphosphate prevents the reformation of glucose 6-phosphate. Second, and perhaps more important, had the aldol cleavage taken place in the aldose glucose, a two-carbon and a four-carbon fragment would have resulted. Two different metabolic pathways — one to process the two-carbon fragment and one for the four-carbon fragment — would have been required to extract energy. Instead, the cleavage of fructose 1,6-bisphosphate yields two phosphorylated interconvertible three-carbon fragments that will be oxidized in the later steps of glycolysis to capture energy in the form of ATP.

Glyceraldehyde 3-phosphate is on the direct pathway of glycolysis, whereas dihydroxyacetone phosphate is not. Unless a means exists to convert dihydroxyacetone phosphate into glyceraldehyde 3-phosphate, a three-carbon fragment useful for generating ATP will be lost. These compounds are isomers that can be readily interconverted: dihydroxyacetone phosphate is a ketose, whereas glyceraldehyde 3-phosphate is an aldose. The isomerization of these three-carbon phosphorylated sugars is catalyzed by triose phosphate isomerase (TPI, sometimes abbreviated TIM).



This reaction is rapid and reversible. At equilibrium, 96% of the triose phosphate is dihydroxyacetone phosphate. However, the reaction proceeds readily from dihydroxyacetone phosphate to glyceraldehyde 3-phosphate because the subsequent reactions of glycolysis remove this product.

Mechanism: Triose phosphate isomerase salvages a three-carbon fragment

Much is known about the catalytic mechanism of triose phosphate isomerase (Figure 16.5). TPI catalyzes the transfer of a hydrogen atom from carbon 1 to carbon 2, an intramolecular oxidation–reduction. This isomerization of a ketose into an aldose proceeds through an enediol intermediate (Figure 16.6).

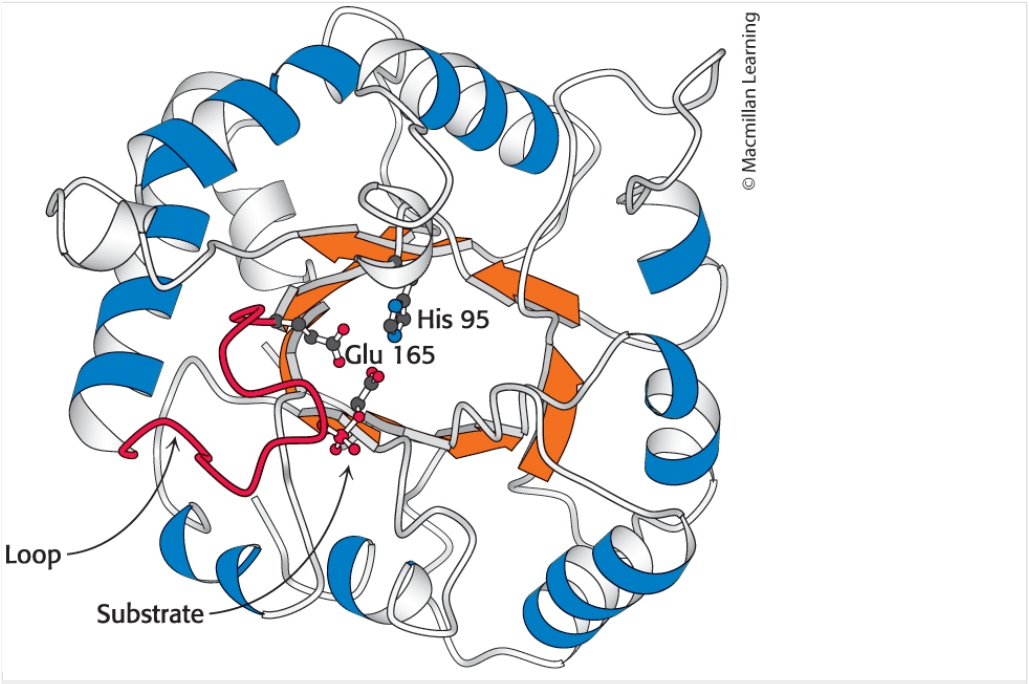


FIGURE 16.5 Triose phosphate isomerase is an example of a common "barrel" motif, with the active site buried in the center. This enzyme consists of a central core of eight parallel β strands (orange) surrounded by eight α helices (blue). This structural motif, called an αβ barrel or a TIM barrel because of this enzyme, is also found in the glycolytic enzymes aldolase, enolase, and pyruvate kinase. Notice that histidine 95 and glutamate 165, essential components of the active site of triose phosphate isomerase, are located in the barrel. A loop (red) closes off the active site on substrate binding.

[Drawn from 2YPI.pdb.]

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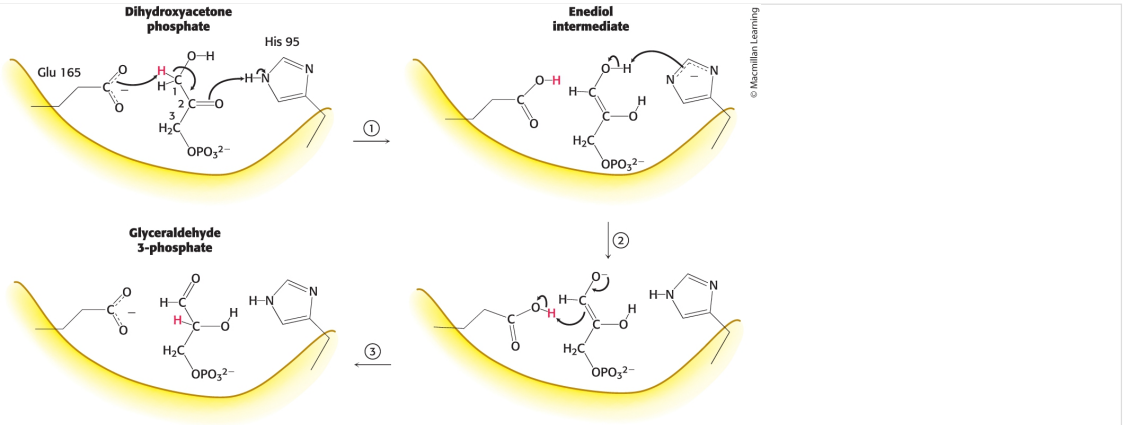
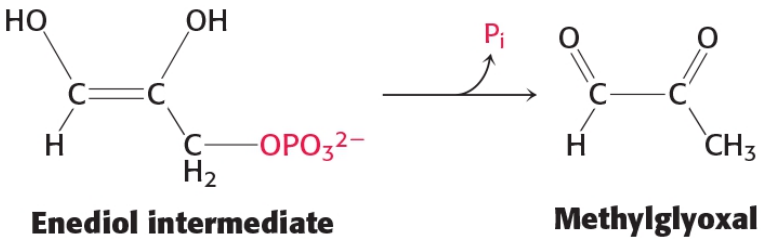


FIGURE 16.6 The catalytic mechanism of triose phosphate isomerase is an acid/base mechanism proceeding through an enediol intermediate. (1) Glutamate 165 acts as a general base by abstracting a proton (H^+) from carbon 1. Histidine 95, acting as a general acid, donates a proton to the oxygen atom bonded to carbon 2, forming the enediol intermediate. (2) Glutamic acid, now acting as a general acid, donates a proton to C-2, while histidine removes a proton from the OH group of C-1. (3) The product is formed, and glutamate and histidine are returned to their ionized and neutral forms, respectively.

X-ray crystallographic and other studies showed that glutamate 165 plays the role of a general acid–base catalyst: it abstracts a proton (H^+) from carbon 1 and then donates it to carbon 2. However, the carboxylate group of glutamate 165 by itself is not basic enough to pull a proton away from a carbon atom adjacent to a carbonyl group. Histidine 95 assists catalysis by donating a proton to stabilize the negative charge that develops on the C-2 carbonyl group.

Two features of this enzyme are noteworthy. First, TPI displays great catalytic prowess. It accelerates isomerization by a factor of 10^{10} compared with the rate obtained with a simple base catalyst such as acetate ion. Indeed, the k_{cat}/K_M ratio for the isomerization of glyceraldehyde 3-phosphate is $2 \times 10^8\ M^{-1}\ s^{-1}$, which is close to the diffusion-controlled limit. In other words, catalysis takes place every time that enzyme and substrate meet. The diffusion-controlled encounter of substrate and enzyme is thus the rate-limiting step in catalysis. TPI is an example of a kinetically perfect enzyme (Section 5.4). Second, TPI suppresses an undesired side reaction, the decomposition of the enediol intermediate into methylglyoxal and orthophosphate.



In solution, this physiologically useless reaction is 100 times as fast as isomerization. Moreover, methylglyoxal is a highly reactive compound that can modify the structure and function of a variety of biomolecules, including proteins and DNA. The reaction of methylglyoxal with a biomolecule is an example of deleterious reactions called advanced glycation end products, discussed earlier (AGEs, Section 11.1). Hence, TPI must prevent the enediol from leaving the enzyme. This labile intermediate is trapped in the active site by the movement of a loop of 10 residues (Figure 16.5). This loop serves as a lid on the active site, shutting it when the enediol is present and reopening it when isomerization is completed. We see here a striking example of one means of preventing an undesirable alternative reaction: the active site is kept closed until the desirable reaction takes place.

Thus, two molecules of glyceraldehyde 3-phosphate are formed from one molecule of fructose 1,6-bisphosphate by the sequential action of aldolase and triose phosphate isomerase. The economy of metabolism is evident in this reaction sequence. The isomerase funnels dihydroxyacetone phosphate into the main glycolytic pathway; a separate set of reactions is not needed.

Stage 2 begins: The oxidation of an aldehyde powers the formation of a compound with high phosphoryl-transfer potential

The preceding steps in glycolysis have transformed one molecule of glucose into two molecules of glyceraldehyde 3-phosphate, but no energy has yet been extracted. On the contrary, thus far, two molecules of ATP have been invested. We come now to the second stage of glycolysis — a series of steps that harvest some of the energy contained in glyceraldehyde 3-phosphate as ATP (**Figure 16.7**).

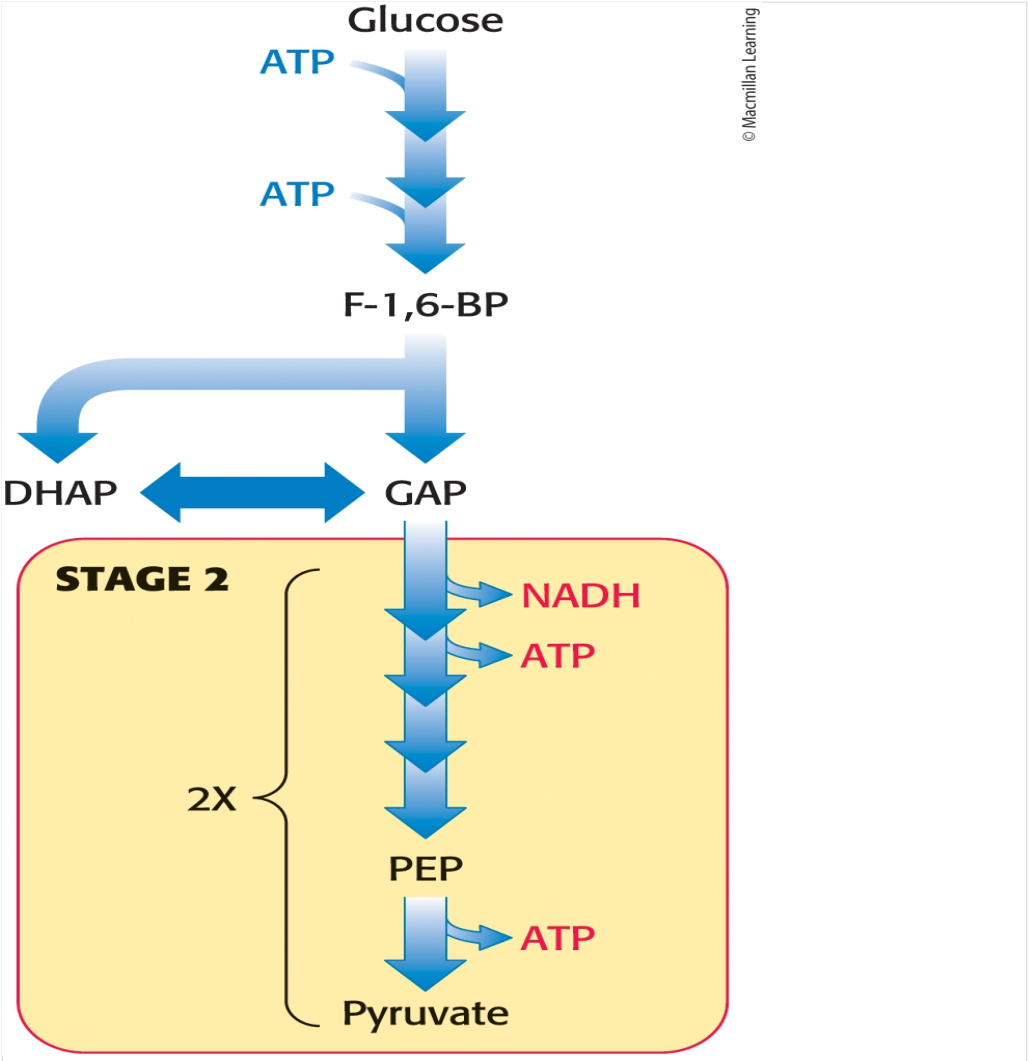
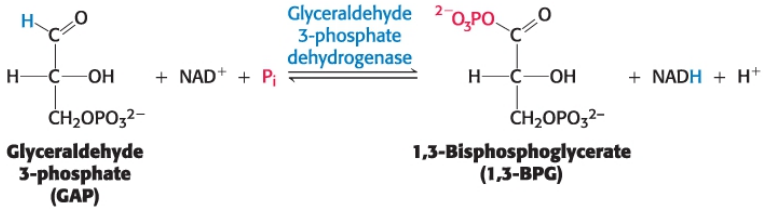


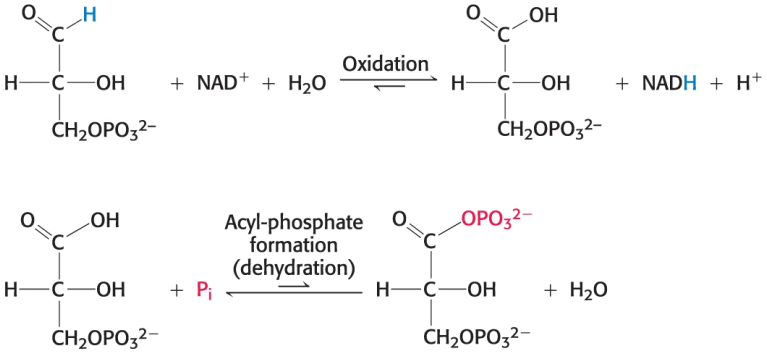
FIGURE 16.7 The second or "payoff" stage of glycolysis generates both ATP and NADH. Note that each reaction in the second stage occurs twice for each glucose molecule entering the pathway.

The initial reaction in this sequence is the conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate (1,3-BPG), a reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase (GAPDH).



1,3-Bisphosphoglycerate is an acyl phosphate, which is a mixed anhydride of phosphoric acid and a carboxylic acid. Such compounds have a high phosphoryl-transfer potential (Section 15.2); one of its phosphoryl groups is transferred to ADP in the next step in glycolysis.

The reaction catalyzed by GAPDH can be viewed as the sum of two processes: the oxidation of the aldehyde to a carboxylic acid by NAD^+, and the joining of the carboxylic acid and orthophosphate to form the acyl-phosphate product.



The first reaction is thermodynamically quite favorable, with a standard free-energy change, $\Delta G^{\circ\prime}$, of approximately $-50\ kJ\ mol^{-1}\ (-12\ kcal\ mol^{-1})$, whereas the second reaction is quite unfavorable, with a standard free-energy change of the same magnitude but the opposite sign. If these two reactions simply took place in succession, the second reaction would have a very large activation energy and thus not take place at a biologically significant rate. These two processes must be coupled so the favorable aldehyde oxidation can be used to drive the formation of the acyl phosphate.

How are these reactions coupled? The key is an intermediate, formed as a result of the aldehyde oxidation, that is connected to the enzyme by a thioester linkage (Section 15.4). The thioester intermediate, which is higher in free energy than the free carboxylic acid is, couples the favorable oxidation and the unfavorable phosphorylation reactions. This coupling preserves much of the free

energy released in the oxidation reaction. The thioester intermediate reacts with orthophosphate to form the high-energy compound 1,3-bisphosphoglycerate.

We see here the use of a covalent enzyme-bound intermediate as a mechanism of energy coupling. A free-energy profile of the GAPDH reaction, compared with a hypothetical process in which the reaction proceeds without this intermediate, reveals how this intermediate allows a favorable process to drive an unfavorable one (Figure 16.8).

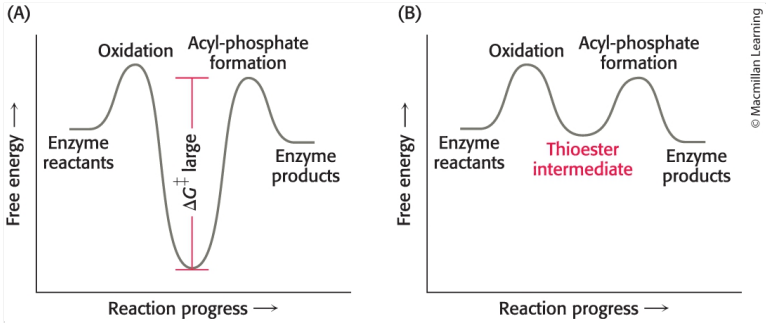


FIGURE 16.8 Free-energy profiles for glyceraldehyde oxidation followed by acyl-phosphate formation demonstrate the importance of the reaction coupling. (A) A hypothetical case with no coupling between the two processes. The second step would have a very large activation barrier, making the reaction very slow. (B) The actual case with the two reactions coupled through a thioester intermediate vastl accelerates the reaction and allows a favorable reaction to drive an unfavorable one.

Mechanism: Phosphorylation is coupled to the oxidation of glyceraldehyde 3-phosphate by a thioester intermediate

The active site of GAPDH includes a reactive cysteine residue, as well as NAD^+ and a crucial histidine (Figure 16.9).

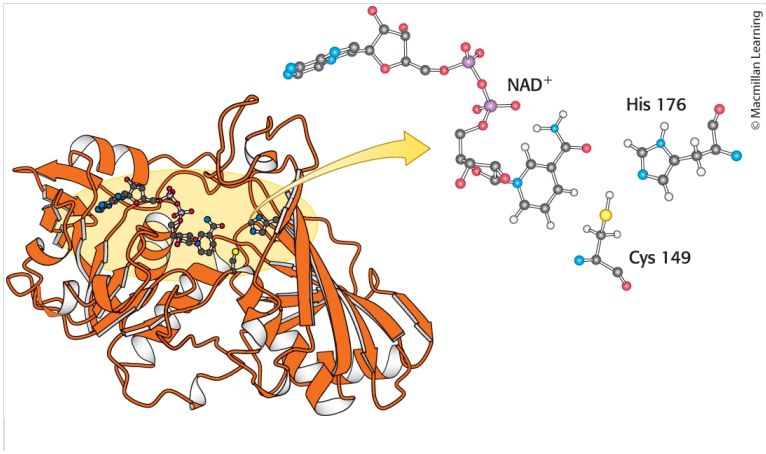


FIGURE 16.9 The active site of GAPDH includes cysteine and histidine residues adjacent to a bound NAD^+ molecule. The sulfur atom of cysteine will react with the substrate to form a transitory thioester intermediate.

[Drawn from 1GAD.pdb.]

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Let's consider in detail how these components cooperate in the reaction mechanism (Figure 16.10) in the following four steps:

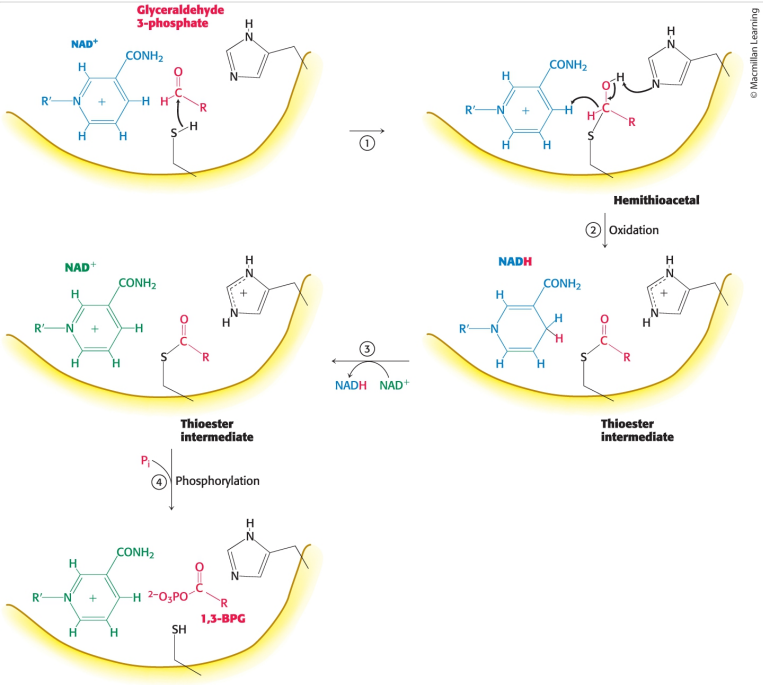


FIGURE 16.10 The catalytic mechanism of GAPDH is an example of covalent catalysis to form a key intermediate. The reaction proceeds through a thioester intermediate, which allows the oxidation of glyceraldehyde to be coupled to the phosphorylation of 3-phosphoglycerate. (1) Cysteine reacts with the aldehyde group of the substrate, forming a hemithioacetal. (2) An oxidation takes place with the transfer of a hydride ion to NAD^+, forming a thioester. This reaction is facilitated by the transfer of a proton to histidine. (3) The reduced NADH is exchanged for an NAD^+ molecule. (4) Orthophosphate attacks the thioester, forming the product 1,3-BPG.

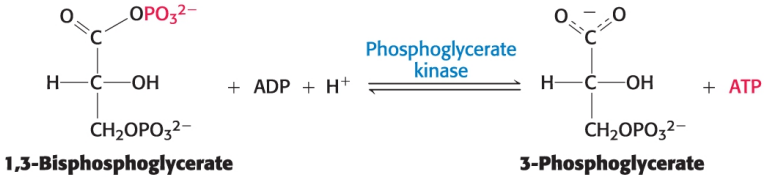
1. The aldehyde substrate reacts with the sulfhydryl group of cysteine 149 on the enzyme to form a hemithioacetal.

2. The hydride ion is transferred to a molecule of NAD^+ that is bound to the enzyme and is adjacent to the cysteine residue. This reaction is favored by the deprotonation of the hemithioacetal by histidine 176. The products are the reduced coenzyme NADH and a thioester intermediate. This thioester intermediate has a free energy close to that of the reactants (Figure 16.8).
3. The NADH formed from the aldehyde oxidation leaves the enzyme and is replaced by a second molecule of NAD^+. This step is important because the positive charge on NAD^+ polarizes the thioester intermediate to facilitate the attack by orthophosphate.
4. The orthophosphate attacks the thioester to form 1,3-BPG and free the cysteine residue.

This example illustrates the essence of energy transformations and of metabolism itself: energy released by carbon oxidation is converted into high phosphoryl-transfer potential.

ATP is formed by phosphoryl transfer from 1,3-bisphosphoglycerate

1,3-Bisphosphoglycerate is an energy-rich molecule with a greater phosphoryl-transfer potential than that of ATP (Section 15.2). Thus, 1,3-BPG can be used to power the synthesis of ATP from ADP. Phosphoglycerate kinase catalyzes the transfer of the phosphoryl group from the acyl phosphate of 1,3-bisphosphoglycerate to ADP; ATP and 3-phosphoglycerate are the products.



1,3-Bisphosphoglycerate **3-Phosphoglycerate**

The formation of ATP in this manner is referred to as **substrate-level phosphorylation** because the phosphate donor, 1,3-BPG, is a substrate with high phosphoryl-transfer potential. We will contrast this manner of ATP formation with the formation of ATP from ionic gradients in Chapters 18 and 19.

Thus, the outcomes of the reactions catalyzed by glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase are:

1. Glyceraldehyde 3-phosphate, an aldehyde, is oxidized to 3-phosphoglycerate, a carboxylic acid.
2. NAD^+ is concomitantly reduced to NADH.
3. ATP is formed from P_i and ADP at the expense of carbon-oxidation energy.

In essence, the energy released during the oxidation of glyceraldehyde 3-phosphate to 3-phosphoglycerate is temporarily trapped as 1,3-bisphosphoglycerate. This energy powers the transfer of

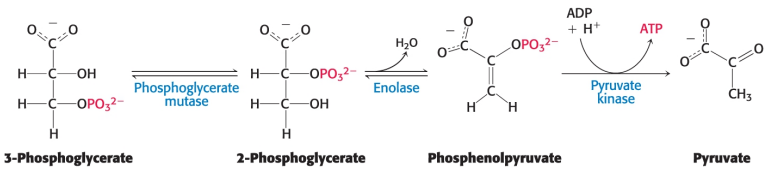
a phosphoryl group from 1,3-bisphosphoglycerate to ADP to yield ATP. Keep in mind that, because of the actions of aldolase and triose phosphate isomerase, two molecules of glyceraldehyde 3-phosphate were formed, and hence two molecules of ATP were generated. These ATP molecules make up for the two molecules of ATP consumed in the first stage of glycolysis.

Self-Check Question

Arsenate (AsO_4^{3-}) resembles P_i in structure and reactivity closely enough that GAPDH can incorporate arsenate instead of phosphate. The product, 1-arseno-3-phosphoglycerate, is highly unstable and rapidly hydrolyzed by the surrounding water molecules producing 3-phosphoglycerate and arsenate. Based on this information, what would be the effect of arsenate on ATP generation in a cell?

Additional ATP is generated with the formation of pyruvate

In the remaining steps of glycolysis, 3-phosphoglycerate is converted into pyruvate, and a second molecule of ATP is formed from ADP.



The first reaction is a rearrangement. The position of the phosphoryl group shifts in the conversion of 3-phosphoglycerate into 2-phosphoglycerate, a reaction catalyzed by phosphoglycerate mutase. In general, a mutase is an enzyme that catalyzes the intramolecular shift of a chemical group, such as a phosphoryl group. The phosphoglycerate mutase reaction has an interesting mechanism: the phosphoryl group is not simply moved from one carbon atom to another. This enzyme requires catalytic amounts of 2,3-bisphosphoglycerate (2,3-BPG) to maintain an active-site histidine residue in a phosphorylated form. This phosphoryl group is transferred to 3-phosphoglycerate to reform 2,3-bisphosphoglycerate.

Enz-His-phosphate + 3-phosphoglycerate $\rightleftharpoons$ Enz-His + 2,3-bisphosphoglycerate

The mutase then functions as a phosphatase: it converts 2,3-bisphosphoglycerate into 2-phosphoglycerate. The mutase retains the phosphoryl group to regenerate the modified histidine.

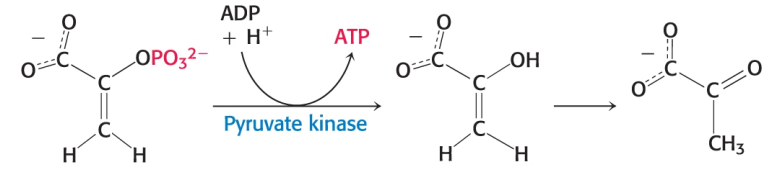
Enz-His + 2,3-bisphosphoglycerate $\rightleftharpoons$ Enz-His-phosphate + 2-phosphoglycerate

The sum of these reactions yields the mutase reaction:

3-Phosphoglycerate $\rightleftharpoons$ 2-phosphoglycerate

In the next reaction, the dehydration of 2-phosphoglycerate introduces a double bond, creating an enol. Enolase catalyzes this formation of the enol phosphate phosphoenolpyruvate (PEP). This dehydration markedly elevates the transfer potential of the phosphoryl group. An enol phosphate has a high phosphoryl-transfer potential, whereas the phosphate ester of an ordinary alcohol, such as 2-phosphoglycerate, has a low one. The $\Delta G^{\circ\prime}$ of the hydrolysis of a phosphate ester of an ordinary alcohol is $-13\ \text{kJ mol}^{-1}\ (-3\ \text{kcal mol}^{-1})$, while that of phosphoenolpyruvate is $-62\ \text{kJ mol}^{-1}\ (-15\ \text{kcal mol}^{-1})$.

Why does phosphoenolpyruvate have such a high phosphoryl-transfer potential? The phosphoryl group traps the molecule in its unstable enol form. When the phosphoryl group has been donated to ATP, the enol undergoes a conversion into the more stable ketone — namely, pyruvate.



Thus, the high phosphoryl-transfer potential of phosphoenolpyruvate arises primarily from the large driving force of the subsequent enol–ketone conversion. Hence, pyruvate is formed, and ATP is generated concomitantly. The virtually irreversible transfer of a phosphoryl group from phosphoenolpyruvate to ADP is catalyzed by pyruvate kinase.

What is the energy source for the formation of phosphoenolpyruvate? The answer to this question becomes clear when we compare the structures of 2-phosphoglycerate and pyruvate. The formation of pyruvate from 2-phosphoglycerate is, in essence, an internal oxidation–reduction reaction; carbon 3 takes electrons from carbon 2 in the conversion of 2-phosphoglycerate into pyruvate. Compared with 2-

phosphoglycerate, C-3 is more reduced in pyruvate, whereas C-2 is more oxidized. Once again, carbon oxidation powers the synthesis of a compound with high phosphoryl-transfer potential, phosphoenolpyruvate here and 1,3-bisphosphoglycerate earlier, which allows the synthesis of ATP.

Because the molecules of ATP used in forming fructose 1,6-bisphosphate have already been regenerated, the two molecules of ATP generated from phosphoenolpyruvate are "profit."

Two ATP molecules are formed in the conversion of glucose into pyruvate

The net reaction in the transformation of glucose into pyruvate is

$$\text{Glucose} + 2\ P_i + 2\ \text{ADP} + 2\ \text{NAD}^+ \rightarrow 2\ \text{pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ H^+ + 2\ H_2O$$

Thus, two molecules of ATP are generated in the conversion of glucose into two molecules of pyruvate. The reactions of glycolysis are summarized in Table 16.2.

TABLE 16.2 Reactions of glycolysis

Step	Reaction	Enzyme	Reaction type	$\Delta G^{\circ\prime}$ in kJ mol^{-1} (kcal mol
1	Glucose + ATP → glucose 6–phosphate + ADP + H^+	Hexokinase	Phosphoryl transfer	−16.7 (−4.0)
2	Glucose 6-phosphate ⇌ fructose 6-phosphate	Phosphoglucose isomerase	Isomerization	+1.7 (+0.4)
3	Fructose 6–phosphate + ATP → fructose 1,6–bisphosphate + ADP + H^+	Phosphofructokinase	Phosphoryl transfer	−14.2 (−3.4)
4	Fructose 1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	Aldolase	Aldol cleavage	+23.8 (+5.7)
5	Dihydroxyacetone phosphate ⇌ glyceraldehyde 3-phosphate	Triose phosphate isomerase	Isomerization	+7.5 (+1.8)

TABLE 16.2 Reactions of glycolysis

Step	Reaction	Enzyme	Reaction type	$\Delta G^{\circ\prime}$ in kJ mol^{-1} (kcal mol^{-1})
6	Glyceraldehyde 3-phosphate + P_i + NAD^+ ⇌ 1,3-bisphosphoglycerate + NADH + H^+	Glyceraldehyde 3-phosphate dehydrogenase	Phosphorylation coupled to oxidation	+6.3 (+1.5)
7	1,3-Bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	Phosphoryl transfer	−18.8 (−4.5)
8	3-Phosphoglycerate ⇌ 2-phosphoglycerate	Phosphoglycerate mutase	Phosphoryl shift	+4.6 (+1.1)
9	2-Phosphoglycerate ⇌ phosphoenolpyruvate + H_2O	Enolase	Dehydration	+1.7 (+0.4)
10	Phosphoenolpyruvate + ADP + H^+ → pyruvate + ATP	Pyruvate kinase	Phosphoryl transfer	−31.4 (−7.5)

Note: ΔG, the actual free-energy change, has been calculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiological conditions. Glycolysis can proceed only if the ΔG values of all reactions are negative. The small positive ΔG values of three of the above reactions indicate that the concentrations of metabolites in vivo in cells undergoing glycolysis are not precisely known.

The energy released in the anaerobic conversion of glucose into two molecules of pyruvate is about $-90\ \text{kJ mol}^{-1}$ ($-22\ \text{kcal mol}^{-1}$). We shall see in Chapters 17 and 18 that much more energy can be released from glucose in the presence of oxygen.

Self-Check Question

The conversion of one molecule of fructose 1,6-bisphosphate into two molecules of pyruvate results in the net synthesis of how many molecules each of NADH and ATP?

NAD^+ is regenerated from the metabolism of pyruvate

The conversion of glucose into two molecules of pyruvate has resulted in the net synthesis of ATP. However, an energy-converting pathway that stops at pyruvate will not proceed for long, because redox balance has not been maintained. As we have seen, the activity of glyceraldehyde 3-phosphate dehydrogenase, in addition to generating a compound with high phosphoryl-transfer potential, reduces NAD^+ to NADH. In the cell, there are limited amounts of NAD^+, which is derived from the vitamin niacin (B_3), a dietary requirement for human beings. Consequently, NAD^+ must be regenerated for glycolysis to proceed. Thus, the final process in the pathway is the regeneration of NAD^+ through the metabolism of pyruvate.

The sequence of reactions from glucose to pyruvate is similar in most organisms and most types of cells. In contrast, the fate of pyruvate is variable. Three reactions of pyruvate are of primary importance: conversion into ethanol, lactate, or carbon dioxide (Figure 16.11). The first two reactions are fermentations that take place in the absence of oxygen. A **fermentation** is an ATP-generating process in which organic compounds act both as the donor and as the acceptor of electrons; thus, they are redox neutral pathways for the production of ATP from an organic fuel molecule. In contrast, in the presence of oxygen — the most common situation in multicellular organisms and in many unicellular ones — pyruvate is oxidized to carbon dioxide and water through the citric acid cycle and the electron-transport chain, with oxygen serving as the final electron acceptor. We now take a closer look at these three examples of possible fates of pyruvate.

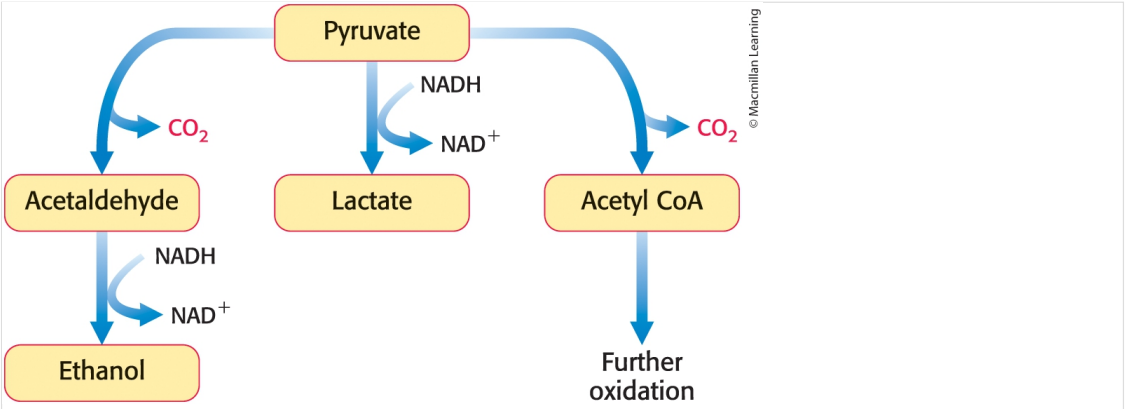
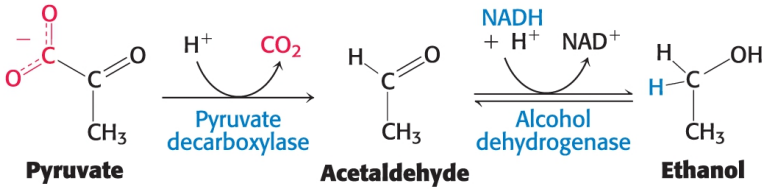


FIGURE 16.11 Pyruvate has many possible fates. Shown are just three possible fates, but many others exist. Ethanol and lactate can be formed by reactions that include NADH. Alternatively, a two-carbon unit from pyruvate can be coupled to coenzyme A (Chapter 17) to form acetyl CoA.

Ethanol fermentation

Ethanol is formed from pyruvate in yeast and several other microorganisms. The conversion of glucose into ethanol in anerobic conditions is called **ethanol fermentation**. The first step is the decarboxylation of pyruvate. This reaction is catalyzed by pyruvate decarboxylase, which requires the coenzyme

thiamine pyrophosphate that is derived from the vitamin thiamine (B_1). The second step is the reduction of acetaldehyde to ethanol by NADH, in a reaction catalyzed by alcohol dehydrogenase. This reaction regenerates NAD^+.



The active site of alcohol dehydrogenase contains a zinc ion that is coordinated to the sulfur atoms of two cysteine residues and a nitrogen atom of histidine (Figure 16.12). This zinc ion polarizes the carbonyl group of the substrate to favor the transfer of a hydride from NADH.

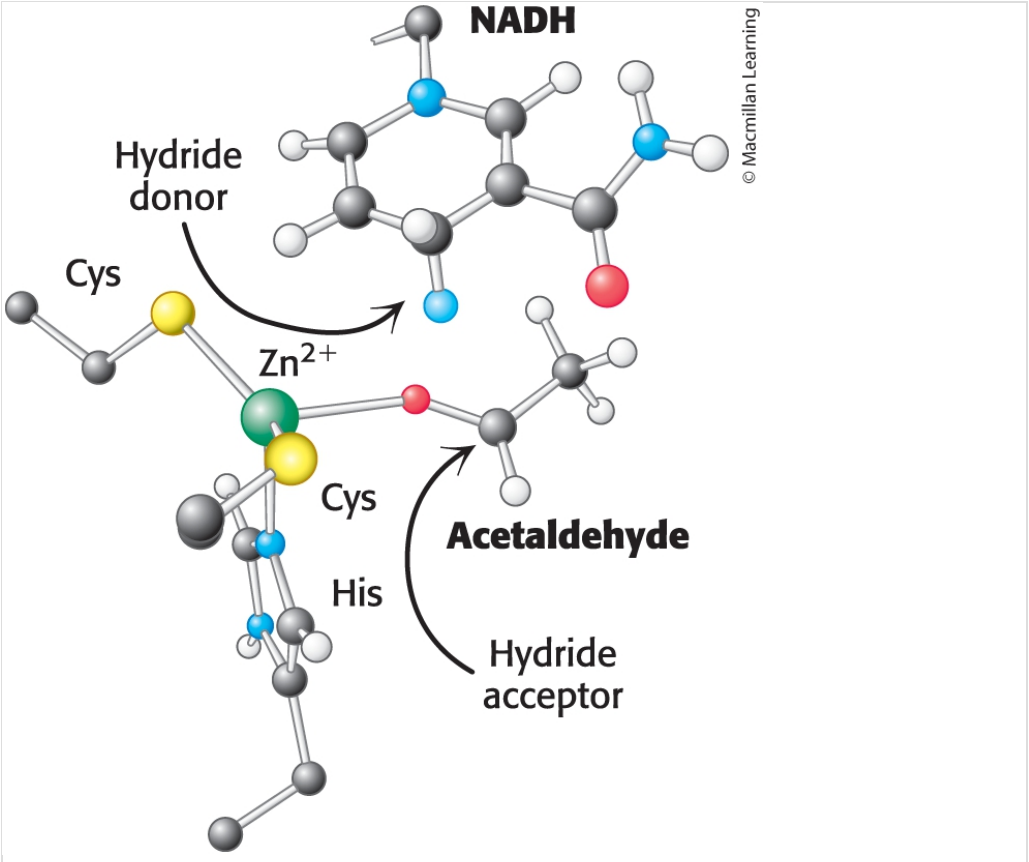


FIGURE 16.12 The active site of alcohol dehydrogenase contains a critical zinc ion bound to two cysteine residues and one histidine residue. The zinc ion binds the acetaldehyde substrate through its oxygen atom, polarizing the substrate so that it more easily accepts a hydride from NADH. Only the nicotinamide ring of NADH is shown.

The net result of this anaerobic process is

$$\text{Glucose} + 2\ P_i + 2\ \text{ADP} + 2\ H^+ \rightarrow 2\ \text{ethanol} + 2\ CO_2 + 2\ \text{ATP} + 2\ H_2O$$

Note that NAD^+ and NADH do not appear in this equation, even though they are crucial for the overall process. NADH generated by the oxidation of glyceraldehyde 3-phosphate is consumed in the reduction of acetaldehyde to ethanol. Thus, there is no net oxidation–reduction in the conversion of glucose into ethanol (Figure 16.13). The ethanol formed during fermentation provides a key ingredient for brewing and winemaking.

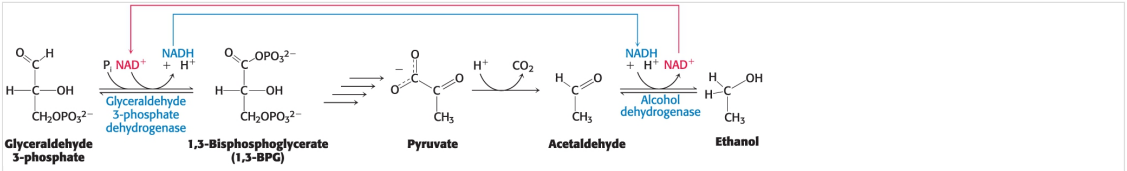
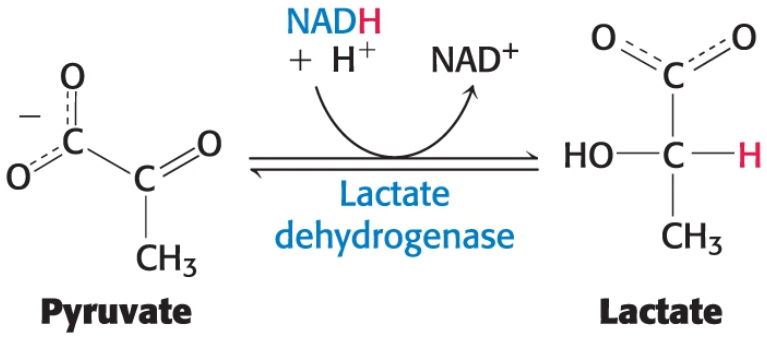


FIGURE 16.13 The NADH produced in glycolysis must be reoxidized to NAD^+ for the glycolytic pathway to continue. NADH is constantly generated by the glyceraldehyde 3-phosphate dehydrogenase reaction. In ethanol fermentation, alcohol dehydrogenase oxidizes NADH and generates ethanol.

Lactic acid fermentation

Lactate is formed from pyruvate under anaerobic conditions in a variety of microorganisms and in a variety of animal tissues. The conversion of glucose to lactate in anaerobic conditions is called **lactic acid fermentation**. After the Buchners' original discovery of ethanol fermentation by yeast, studies of muscle extracts later showed that many of the reactions of lactic acid fermentation were the same as those of ethanol fermentation. This second exciting discovery revealed an underlying unity in biochemistry. Lactic acid fermentation occurs regularly in the retina of the eye and in erythrocytes (red blood cells), even in the presence of oxygen, as well as in tissues like the outer layers of the skin that are perpetually limited in oxygen availability. Additionally, certain types of skeletal muscles in most animals can also function anaerobically for short periods. For example, lactic acid fermentation occurs when a specific type of muscle fiber, called fast-twitch or type IIb fibers, performs short bursts of intense exercise because the ATP needs rise faster than the ability of the body to provide oxygen to the muscle. The muscle functions anaerobically until fatigue sets in, which is caused, in part, by lactate buildup. Indeed, the pH of resting type IIb muscle fibers, which is about 7.0, may fall to as low as 6.3 during the bout of exercise. The drop in pH inhibits phosphofructokinase, as we will discuss shortly. A

lactate/H^+ symporter allows the exit of lactate from the muscle cell. The reduction of pyruvate by NADH to form lactate is catalyzed by lactate dehydrogenase.



The overall reaction in the conversion of glucose into lactate is

$$\text{Glucose} + 2\ P_i + 2\ \text{ADP} \rightarrow 2\ \text{lactate} + 2\ \text{ATP} + 2\ H_2O$$

As in ethanol fermentation, there is no net oxidation–reduction. The NADH formed in the oxidation of glyceraldehyde 3-phosphate is consumed in the reduction of pyruvate. The regeneration of NAD^+ in the reduction of pyruvate to lactate or ethanol sustains the continued process of glycolysis under anaerobic conditions (**Figure 16.14**).

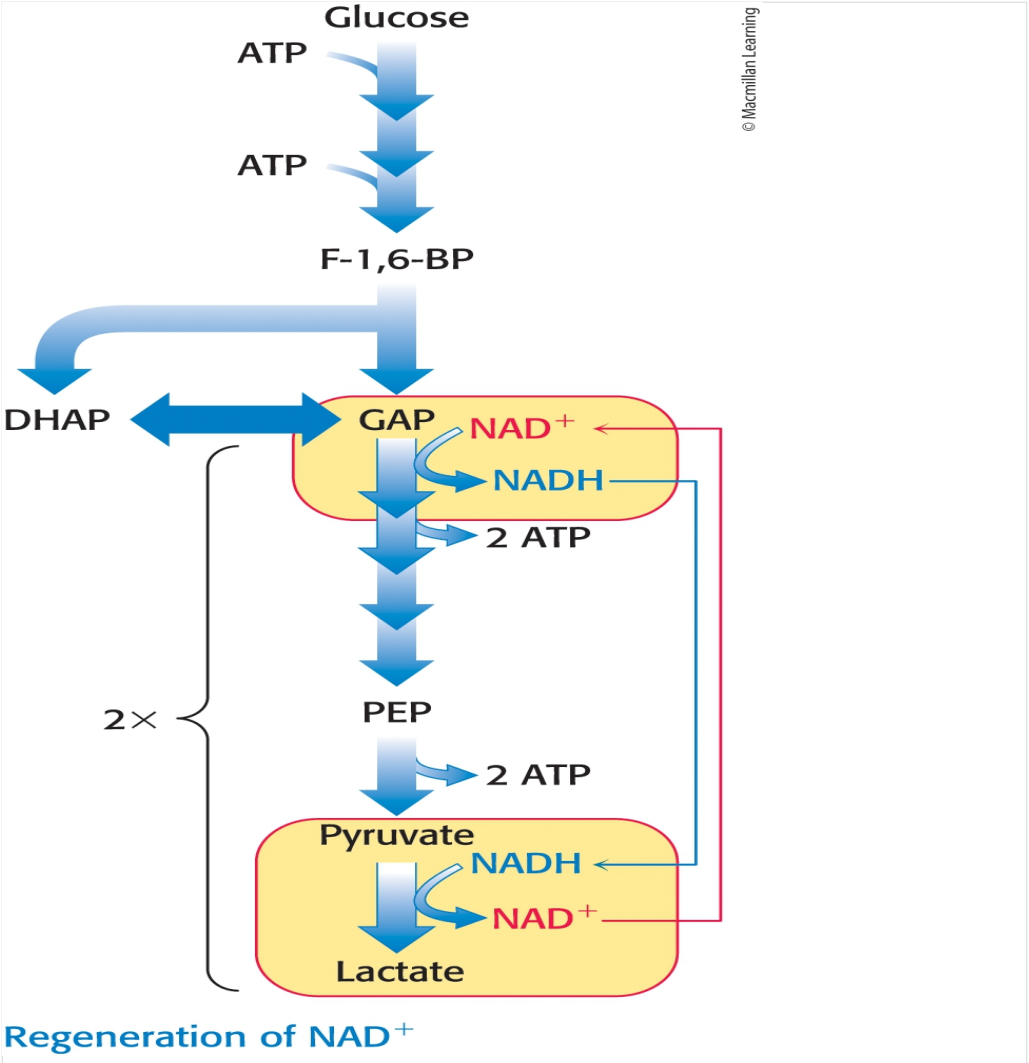


FIGURE 16.14 NAD^+ is regenerated by lactate dehydrogenase during lactic acid fermentation. Lactate dehydrogenase oxidizes NADH while generating lactic acid.

Oxidation by the citric acid cycle and the electron transport chain

Carbon dioxide and water are formed, and a great deal of energy extracted, when pyruvate is metabolized under aerobic conditions by means of the citric acid cycle and the electron-transport chain. In contrast, only a fraction of the energy of glucose is released in its anaerobic conversion into ethanol or lactate. The entry point to this oxidative pathway is acetyl coenzyme A (acetyl CoA), which is formed inside mitochondria by the oxidative decarboxylation of pyruvate.

$$\text{Pyruvate} + \text{NAD}^+ + \text{CoA} \rightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

This reaction, which is catalyzed by the pyruvate dehydrogenase complex, will be considered in detail in Chapter 17. The NAD^+ required for this reaction and for the oxidation of glyceraldehyde 3-phosphate is regenerated when NADH ultimately transfers its electrons to O_2 through the electron-transport chain in mitochondria.

Fermentations provide usable energy in the absence of oxygen

Fermentations yield only a fraction of the energy available from the complete combustion of glucose. Why is a relatively inefficient metabolic pathway so extensively used? The fundamental reason is that oxygen is not required. The ability to survive without oxygen affords a host of living accommodations such as soils, deep water, and skin pores. Some organisms, called **obligate anaerobes**, cannot survive in the presence of O_2, a highly reactive compound. The bacterium *Clostridium perfringens*, the cause of gangrene, is an example of an obligate anaerobe. Other pathogenic obligate anaerobes are listed in **Table 16.3**. Some organisms, such as yeast, are **facultative anaerobes** that metabolize glucose aerobically when oxygen is present and perform fermentation when oxygen is absent.

TABLE 16.3 Examples of pathogenic obligate anaerobes

Bacterium	Result of infection
Clostridium tetani	Tetanus (lockjaw)
Clostridium botulinum	Botulism (an especially severe type of food poisoning)
Clostridium perfringens	Gas gangrene (gas is produced as an end point of the fermentation, distorting and destroying the tissue)
Bartonella hensela	Cat-scratch fever (flu-like symptoms)
Bacteroides fragilis	Abdominal, pelvic, pulmonary, and blood infections

Many food products, including sour cream, yogurt, various cheeses, beer, wine, and sauerkraut, result from fermentation. Yogurt is produced by the fermentation of lactose in milk to lactate by a mixed culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus*. Sour cream begins with pasteurized light cream, which is fermented to lactate by *Streptococcus lactis*. The lactate is further fermented to ketones and aldehydes by *Leuconostoc citrovorum*. The second fermentation adds to the taste and aroma of sour cream. Yeast, *Saccharomyces cerevisiae*, ferments carbohydrates to ethanol and carbon dioxide, providing some of the ingredients for an array of alcohol beverages. Although we have considered only lactic acid and ethanol fermentation, microorganisms are capable of generating a wide array of molecules as end points of fermentation (**Table 16.4**).

TABLE 16.4 Starting and ending points of various fermentations

Glucose	→	Lactate
Lactate	→	Acetate
Glucose	→	Ethanol
Ethanol	→	Acetate
Purines	→	Formate
Ethylene glycol	→	Acetate
Threonine	→	Propionate
Leucine	→	2-Alkylacetate
Phenylalanine	→	Propionate

Note: The products of some fermentations are the substrates for others.

Self-Check Question

The conversion of one molecule of glucose into two molecules of lactate results in the net synthesis of how many ATP and how many NADH molecules?

Fructose is converted into glycolytic intermediates by fructokinase

Although glucose is the most widely used monosaccharide, others also are important fuels. Let us consider how fructose is funneled into the glycolytic pathway (Figure 16.15). There is no separate catabolic pathway for metabolizing fructose, and so the strategy is to convert this sugar into a metabolite of glucose.

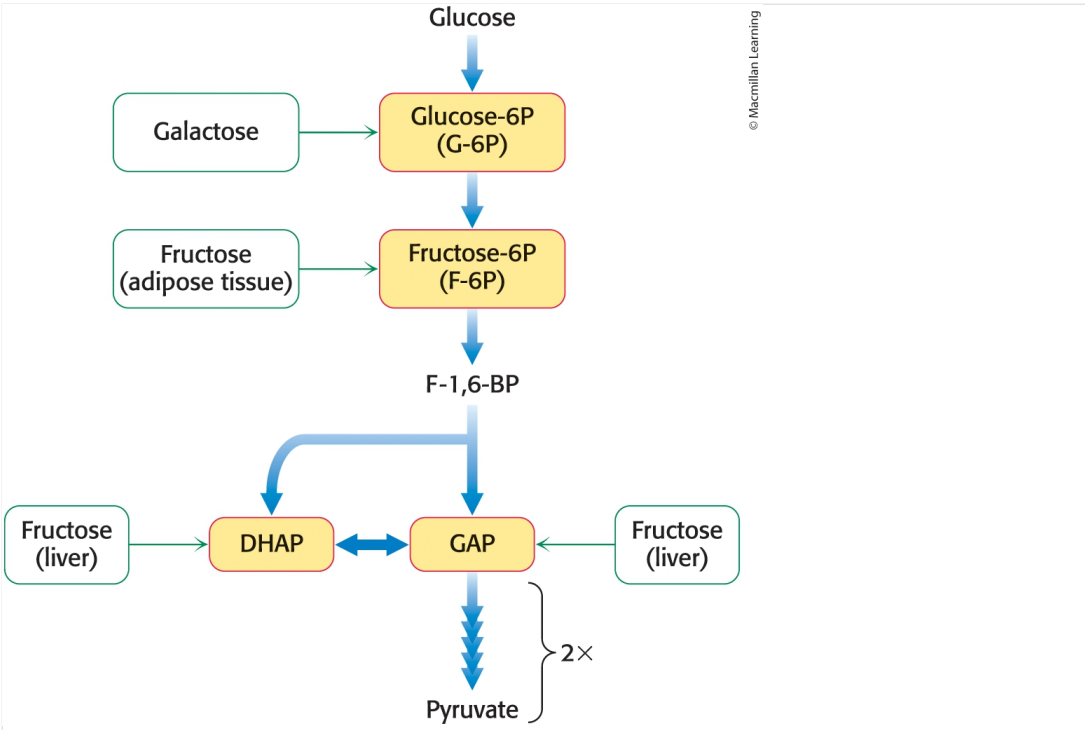


FIGURE 16.15 Additional sugars such as fructose and galactose can enter glycolysis at a variety of points.

The main site of fructose metabolism is the liver, using the fructose 1-phosphate pathway (Figure 16.16). The first step is the phosphorylation of fructose to fructose 1-phosphate by fructokinase. Fructose 1-phosphate is then split into glyceraldehyde and dihydroxyacetone phosphate, an intermediate in glycolysis. This aldol cleavage is catalyzed by a specific fructose 1-phosphate aldolase. Glyceraldehyde is then phosphorylated to glyceraldehyde 3-phosphate, a glycolytic intermediate, by triose kinase. In other tissues, such as adipose tissue, fructose can be phosphorylated to fructose 6-phosphate by hexokinase.

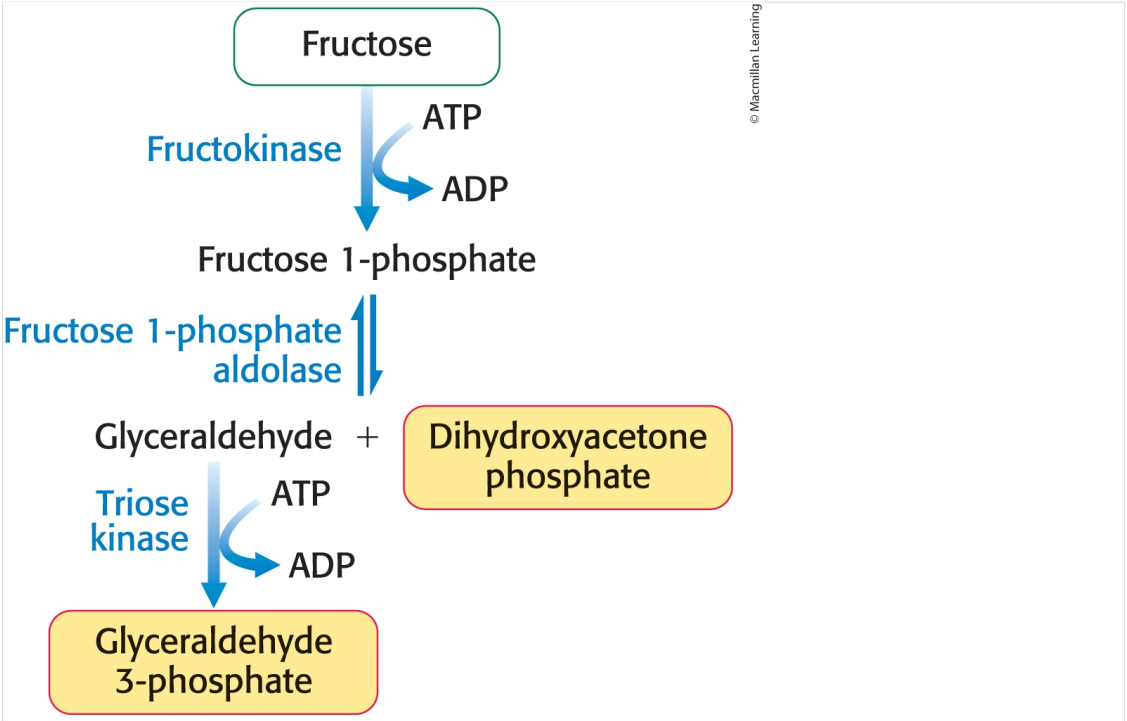


FIGURE 16.16 In the liver, fructose enters the glycolytic pathway through the fructose 1-phosphate pathway.

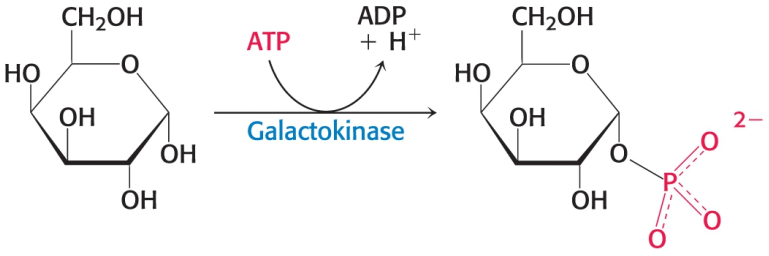
Fructose consumption, previously associated with pathological conditions, is likely inconsequential relative to caloric intake. Fructose, a commonly used sweetener, is a component of sucrose and high fructose corn syrup (which contains approximately 55% fructose and 45% glucose). Epidemiological as well as clinical studies previously linked excessive fructose consumption to fatty liver, insulin insensitivity, and obesity, while other studies found negligible or even beneficial effects of fructose consumption, relative to calorically equivalent quantities of other sugars like glucose and sucrose. Recent meta-analyses, large studies which methodically evaluate the results of many other experiments collectively, now indicate that the widely held belief in the negative effects of fructose is no longer supported by the current literature. Interestingly, the hypothesis that overconsumption of fructose, specifically, contributed to poor health outcomes has a biochemical underpinning.

Note that, as shown in Figure 16.16, the actions of fructokinase and triose kinase bypass the most important regulatory step in glycolysis, the phosphofructokinase-catalyzed reaction. Thus, the fructose-derived glyceraldehyde 3-phosphate and dihydroxyacetone phosphate are produced in an unregulated fashion. However, it is likely that additional regulatory enzymes prevent any further unregulated metabolism of the downstream product pyruvate. Although the type of sugar may be unimportant, the overconsumption of sugars and fats is still strongly associated with fatty liver, insulin insensitivity, and obesity, conditions that may eventually result in type 2 diabetes (Chapter 24). Excess pyruvate produced in glycolysis, from any source, is metabolized to acetyl CoA. As we will see in Chapter 22, this excess

acetyl CoA is converted to fatty acids, which can be transported to adipose tissue and result in obesity. The liver also begins to accumulate fatty acids, resulting in fatty liver. We will return to the topic of obesity and caloric homeostasis in Chapter 24.

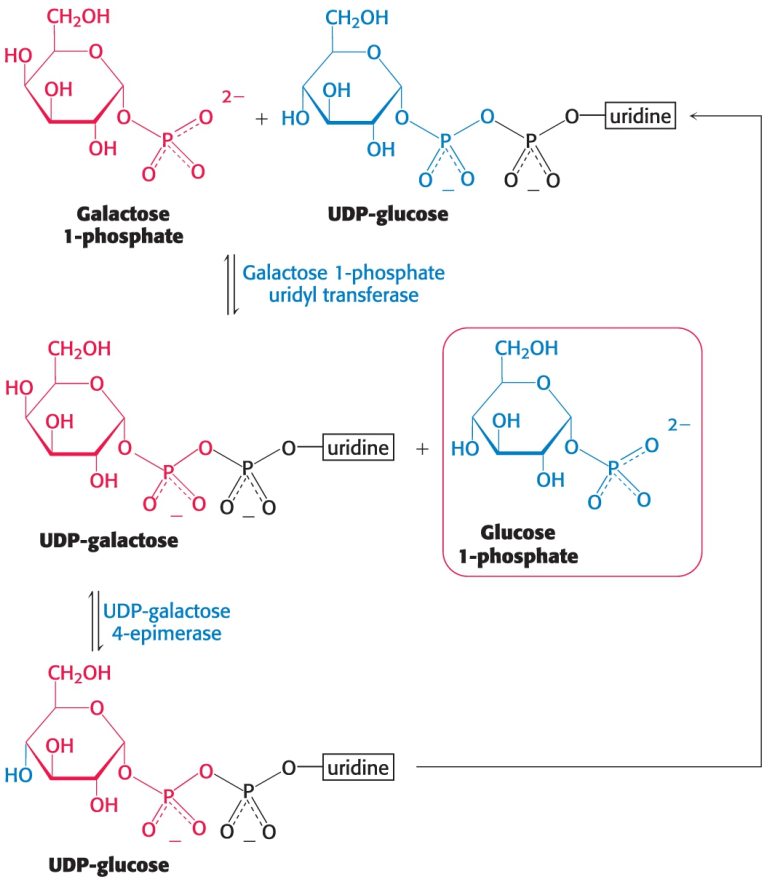
Galactose is converted into glucose 6-phosphate

Like fructose, galactose is an abundant sugar common in dairy products that must be converted into metabolites of glucose (Figure 16.15). Galactose is converted into glucose 6-phosphate in four steps. The first reaction in the galactose–glucose interconversion pathway is the phosphorylation of galactose to galactose 1-phosphate by galactokinase.



Galactose **Galactose 1-phosphate**

Galactose 1-phosphate then acquires a uridyl group from uridine diphosphate glucose (UDP-glucose), an activated intermediate in the synthesis of carbohydrates (Section 21.4).



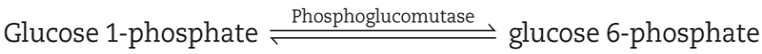
The products of this reaction, which is catalyzed by galactose 1-phosphate uridyl transferase, are UDP-galactose and glucose 1-phosphate. The galactose moiety of UDP-galactose is then epimerized to glucose. The configuration of the hydroxyl group at carbon 4 is inverted by UDP-galactose 4-epimerase.

The sum of the reactions catalyzed by galactokinase, the transferase, and the epimerase is

$$\text{Galactose} + \text{ATP} \rightarrow \text{glucose 1-phosphate} + \text{ADP} + \text{H}^+$$

Note that UDP-glucose is not consumed in the conversion of galactose into glucose, because it is regenerated from UDP-galactose by the epimerase. This reaction is reversible, and the product of the reverse direction also is important. The conversion of UDP-glucose into UDP-galactose is essential for the synthesis of galactosyl residues in complex polysaccharides and glycoproteins if the amount of galactose in the diet is inadequate to meet these needs.

Finally, glucose 1-phosphate, formed from galactose, is isomerized to glucose 6-phosphate by phosphoglucomutase.



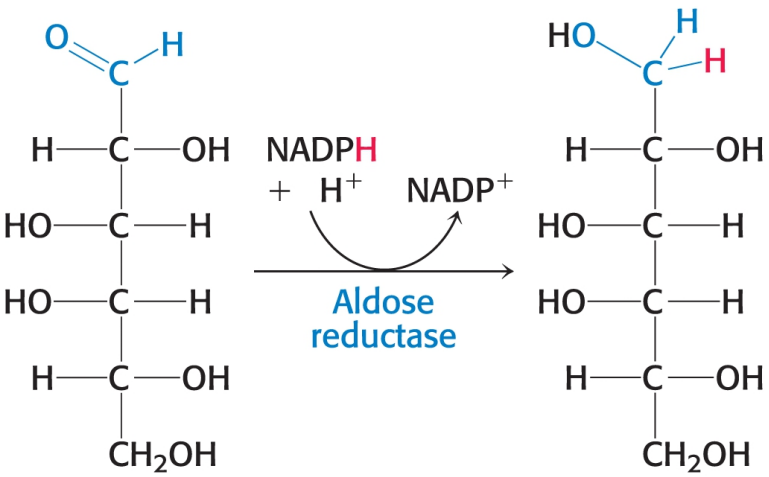
We shall return to this reaction when we consider the synthesis and degradation of glycogen, which proceeds through glucose 1-phosphate, in Chapter 21.

Galactose can be highly toxic with a defective metabolic pathway

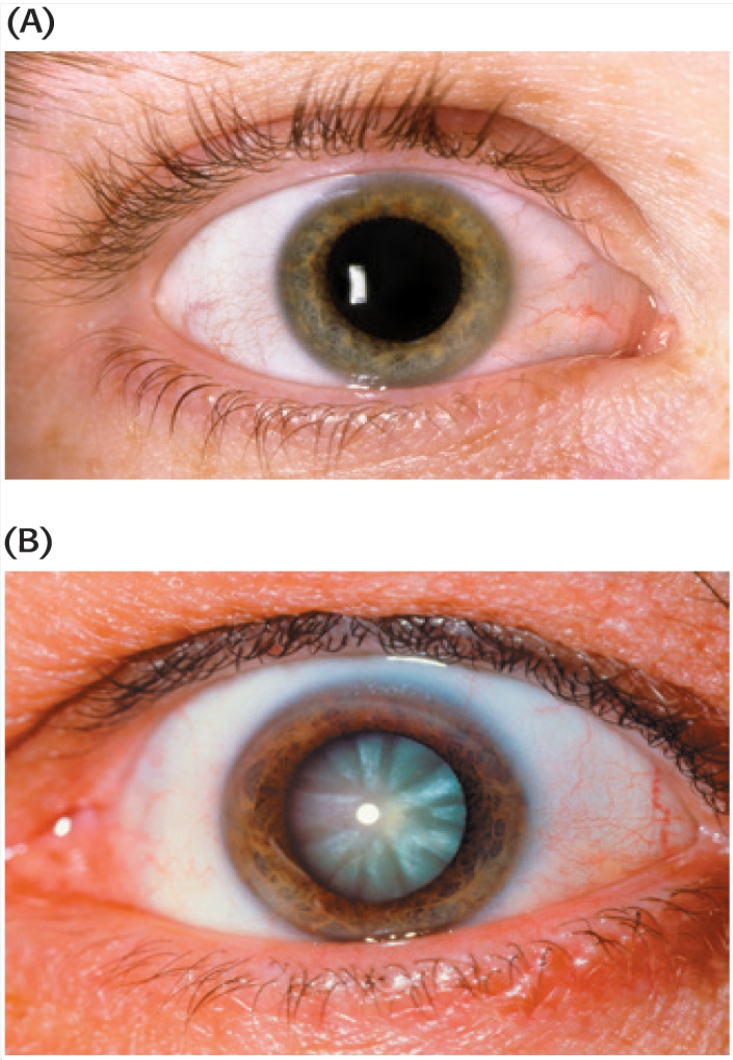
Rare disorders that interfere with the metabolism of galactose are collectively referred to as *galactosemias.* The most common form, called classic galactosemia, is an inherited deficiency in galactose 1-phosphate uridyl transferase activity. Afflicted infants fail to thrive; they vomit or have diarrhea after consuming milk, and enlargement of the liver and jaundice are common, sometimes progressing to cirrhosis. Cataracts will form, and lethargy and delayed neurological development also are common. The blood-galactose level is markedly elevated, and galactose is found in the urine.

The most common treatment is to remove galactose (and lactose) from the diet. An enigma of galactosemia is that, although elimination of galactose from the diet prevents liver disease and cataract development, the majority of patients still suffer from central nervous system malfunction, most commonly a delayed acquisition of language skills. Female patients also display ovarian failure.

Cataract formation is better understood. A cataract is the clouding of the normally clear lens of the eye due to pathological protein aggregation (**Figure 16.17**). If the transferase is not active in the lens of the eye, the presence of aldose reductase causes the accumulating galactose to be reduced to galactitol.



Galactose **Galactitol**



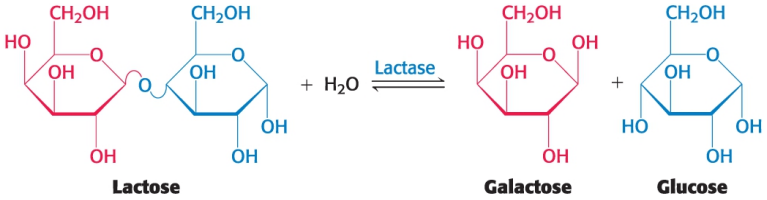
A: Tim Mainiero/Shutterstock; B: SPL/Science Source

FIGURE 16.17 Cataracts are evident as the clouding of the lens. (A) A healthy eye. (B) An eye with a cataract.

Galactitol is poorly metabolized and accumulates in the lens. Water will diffuse into the lens to maintain osmotic balance, triggering the formation of cataracts. In fact, there is a high incidence of cataract formation with age in populations that consume substantial amounts of milk into adulthood.

Many adults worldwide are intolerant of milk because they are deficient in lactase

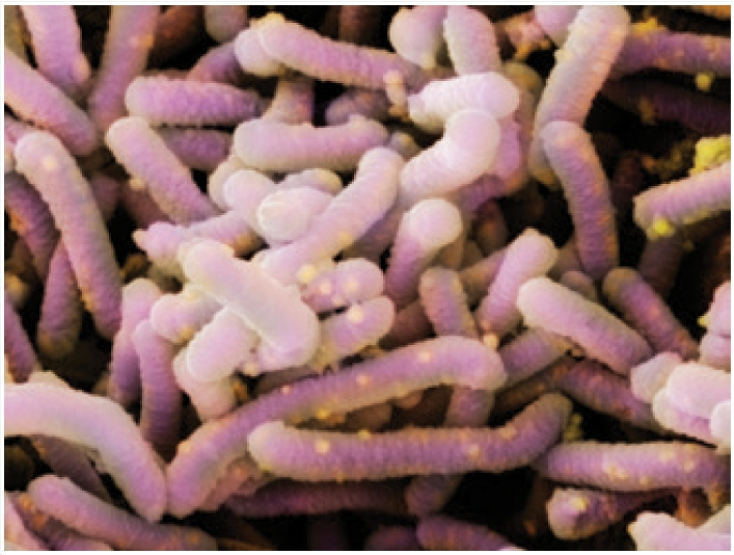
Many adults are unable to metabolize the milk sugar lactose and experience gastrointestinal disturbances if they drink milk. Lactose intolerance is most commonly caused by a deficiency of the enzyme lactase, which cleaves lactose into glucose and galactose.



"Deficiency" is not quite the appropriate term, because a decrease in lactase is normal in the course of development in all mammals. As children are weaned and milk becomes less prominent in their diets, lactase activity normally declines to about 5% to 10% of the level at birth. This decrease is not as pronounced with some groups of people, and people from these groups can continue to ingest milk without gastrointestinal difficulties.

How did this tolerance evolve? With the development of dairy farming, an adult with active lactase would have a selective advantage in being able to consume calories from the readily available milk. Indeed, estimates suggest that people with the mutation would have produced almost 20% more fertile offspring. Some form of lactose tolerance evolved independently at least four different times in different human populations in the last 10,000 years, indicating that the evolutionary selective pressure on lactase persistence must have been substantial, attesting to the biochemical value of being able to use milk as an energy source into adulthood.

What happens to the lactose in the intestine of a lactase-deficient person? The lactose is a good energy source for microorganisms in the colon (**Figure 16.18**), which ferment it to lactic acid while generating methane (CH_4) and hydrogen gas (H_2). The gas produced creates the uncomfortable feeling of gut distension and the annoying problem of flatulence. The lactate produced by these microorganisms is osmotically active and draws water into the intestine, as does any undigested lactose, resulting in diarrhea. If severe enough, the gas and diarrhea hinder the absorption of other nutrients such as fats and proteins. The simplest treatment is to avoid the consumption of products containing much lactose. Alternatively, the enzyme lactase can be ingested with milk products.



SPL/Science Source

FIGURE 16.18 *Lactobacillus* is one example of an industrially useful anaerobic bacterium. A scanning electron micrograph of an anaerobic bacterial species from the genus *Lactobacillus* is shown. As suggested by its name, this genus ferments glucose into lactic acid. *Lactobacillus* is widely used in the food industry and is an important component of the normal human bacterial flora of the urogenital tract, where it prevents the growth of harmful organisms by creating an acid environment.

Self-Check Question

Which condition is more detrimental to human health: lactose intolerance or classic galactosemia? Also, if a person were galactosemic, would it be better to be lactose tolerant or intolerant?

16.3 The Glycolytic Pathway Is Tightly Controlled

The glycolytic pathway has a dual role: it degrades glucose to generate ATP, and it provides building blocks for biosynthetic reactions. The rate of conversion of glucose into pyruvate is regulated to meet these two major cellular needs. In metabolic pathways, enzymes catalyzing essentially irreversible reactions are potential sites of control. In glycolysis, the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase are virtually irreversible, and each of them serves as a control site. These enzymes become more active or less so in response to the reversible binding of allosteric effectors or to covalent modification. In addition, the amounts of these important enzymes are varied by the regulation of transcription to meet changing metabolic needs. The time required for allosteric regulation, control by phosphorylation, and transcriptional change is measured typically in milliseconds, seconds, and hours, respectively. We will consider the control of glycolysis in two different tissues — skeletal muscle and liver.

Glycolysis in muscle is regulated to meet the need for ATP

Glycolysis in skeletal muscle provides ATP primarily to power contraction. Consequently, the primary regulation of muscle glycolysis is the ratio of ATP to AMP. Let's examine how each of the key regulatory enzymes responds to changes in the amounts of ATP and AMP present in the cell.

Phosphofructokinase

Phosphofructokinase is the most important control site in the mammalian glycolytic pathway (Figure 16.19). High levels of ATP allosterically inhibit the enzyme (a 340-kDa tetramer). ATP binds to a specific regulatory site that is distinct from the catalytic site.

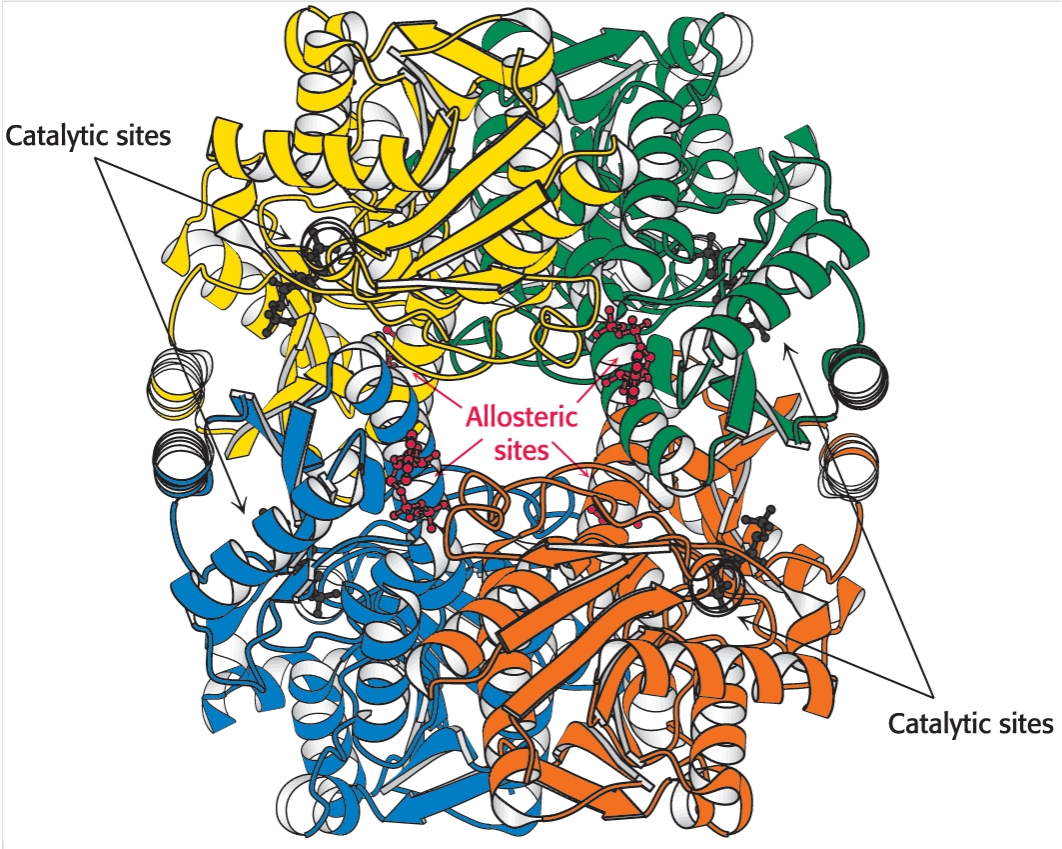


FIGURE 16.19 Phosphofructokinase from *E. coli* is comprised of four identical subunits with separate catalytic and allosteric sites. Each subunit of the human liver enzyme consists of two domains that are similar to the *E. coli* enzyme.

[Drawn from 1PFK.pdb.]

The binding of ATP lowers the enzyme's affinity for fructose 6-phosphate. Thus, a high concentration of ATP converts the hyperbolic binding curve of fructose 6-phosphate into a sigmoidal one (Figure 16.20). AMP reverses the inhibitory action of ATP, and so the activity of the enzyme increases when the ATP/AMP ratio is lowered. A decrease in pH also inhibits phosphofructokinase activity by augmenting the inhibitory effect of ATP. The pH might fall when fast-twitch muscle is functioning anaerobically, producing excessive quantities of lactic acid. The inhibitory effect protects the muscle from damage that would result from the accumulation of too much acid.

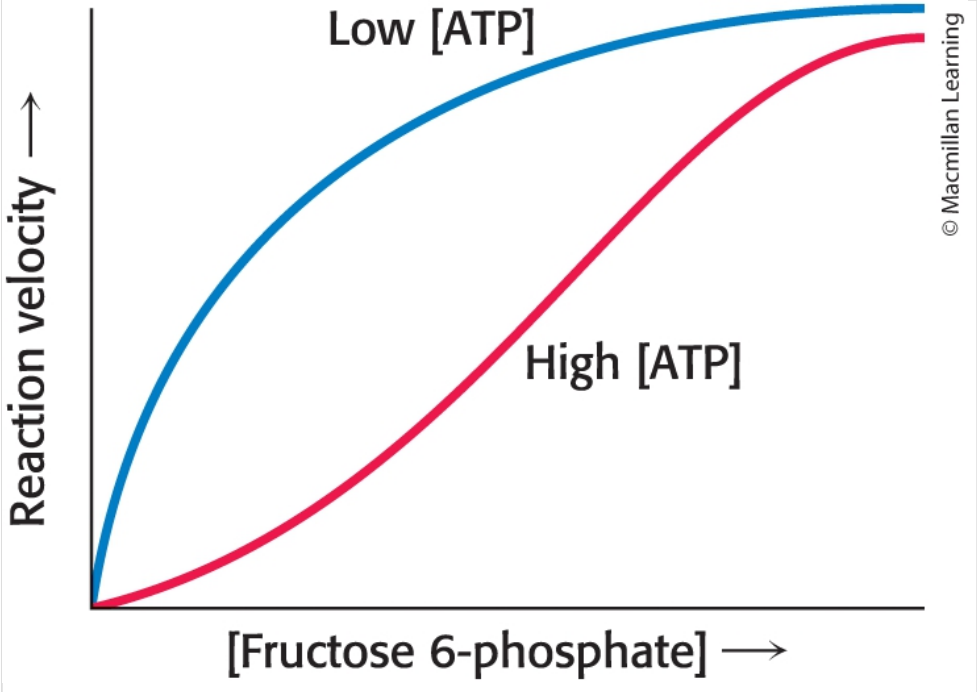


FIGURE 16.20 A high level of ATP allosterically inhibits phosphofructokinase by decreasing its affinity for fructose 6-phosphate.

Why is AMP and not ADP the positive regulator of phosphofructokinase? When ATP is being utilized rapidly, the enzyme adenylate kinase can

form ATP from ADP by the following reaction:

$$\mathrm{ADP} + \mathrm{ADP} \rightleftharpoons \mathrm{ATP} + \mathrm{AMP}$$

Thus, some ATP is salvaged from ADP, and AMP becomes the signal for the low-energy state.

Moreover, the use of AMP as an allosteric effector provides an especially sensitive regulation. We can understand why by considering the following. First, the total adenylate pool ([ATP], [ADP], [AMP]) in a cell is constant over the short term. Second, the concentration of ATP is greater than that of ADP, and the concentration of ADP is, in turn, greater than that of AMP. Consequently, small percentage changes in [ATP] result in larger percentage changes in the concentrations of the other adenylate nucleotides. This magnification of small changes in [ATP] to larger changes in [AMP] leads to tighter regulation by increasing the range of sensitivity of phosphofructokinase.

Hexokinase

Phosphofructokinase is the most prominent regulatory enzyme in glycolysis, but it is not the only one. Hexokinase, the enzyme catalyzing the first step of glycolysis, is inhibited by its product, glucose 6-phosphate. High concentrations of this molecule signal that the cell no longer requires glucose for energy or for the synthesis of glycogen, a storage form of glucose (Chapter 21), and the glucose will be left in the blood. A rise in glucose 6-phosphate concentration is a means by which phosphofructokinase communicates with hexokinase. When phosphofructokinase is inactive, the concentration of fructose 6-phosphate rises. In turn, the level of glucose 6-phosphate rises because it is in equilibrium with fructose 6-phosphate. Hence, the inhibition of phosphofructokinase leads to the inhibition of hexokinase.

Why is phosphofructokinase rather than hexokinase the pacemaker of glycolysis? The reason becomes evident on noting that glucose 6-phosphate is not solely a glycolytic intermediate. In muscle, glucose 6-phosphate can also be converted into glycogen. The first irreversible reaction unique to the glycolytic pathway, the **committed step**, is the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate. Thus, it is highly appropriate for phosphofructokinase to be the primary control site in glycolysis. In general, the enzyme catalyzing the committed step in a metabolic sequence is the most important control element in the pathway.

Pyruvate kinase

Pyruvate kinase, the enzyme catalyzing the third irreversible step in glycolysis, controls the outflow from this pathway. This final step yields ATP and pyruvate, a central metabolic intermediate that can be oxidized further or used as a building block. ATP allosterically inhibits pyruvate kinase to slow glycolysis when the energy charge is high. When the pace of glycolysis increases, fructose 1,6-bisphosphate — the product of the preceding irreversible step in glycolysis — activates the kinase to enable it to keep pace with the oncoming high flux of intermediates. Such a process is called **feedforward stimulation** or **feedforward activation**. A summary of the regulation of glycolysis in resting and active muscle is shown in **Figure 16.21**.

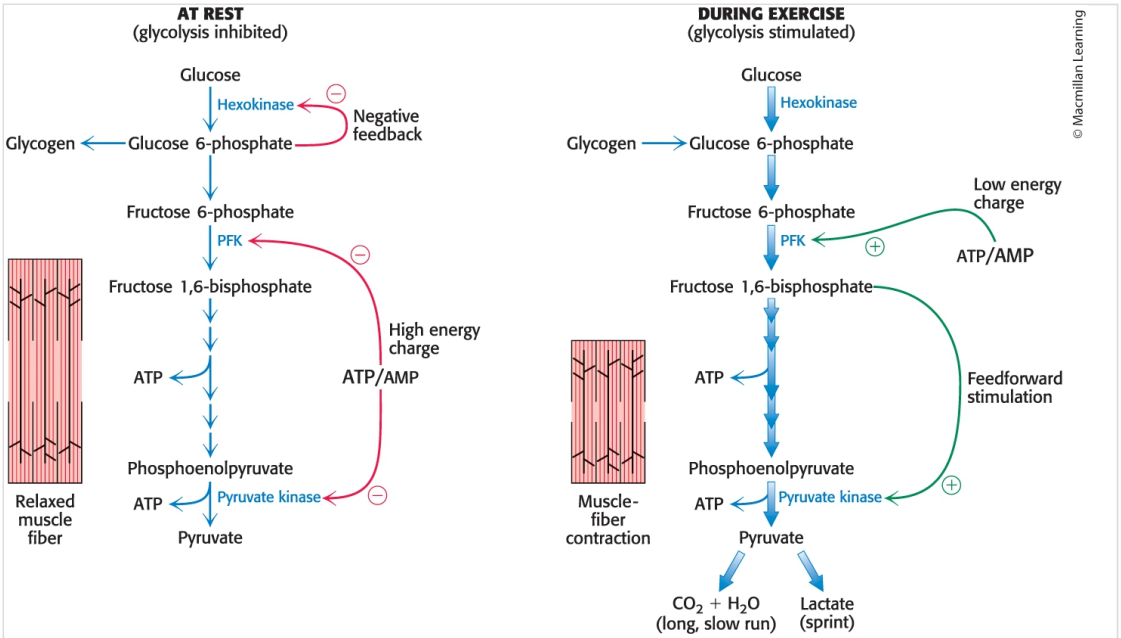


FIGURE 16.21 Regulation of glycolysis in muscle. At rest (left), glycolysis is not very active (thin arrows). The high concentration of ATP inhibits phosphofructokinase (PFK), pyruvate kinase, and hexokinase. Glucose 6-phosphate is converted into glycogen (Chapter 21). During exercise (right), the decrease in the ATP/AMP ratio resulting from muscle contraction activates phosphofructokinase and hence glycolysis. The flux down the pathway is increased, as represented by the thick arrows.

The regulation of glycolysis in the liver illustrates the biochemical versatility of the liver

The liver has more diverse biochemical functions than does muscle. Significantly, the liver maintains blood-glucose concentration: it stores glucose as glycogen when glucose is plentiful, and it releases glucose when supplies are low. It also uses glucose to generate reducing power

for biosynthesis (Section 20.3) as well as to synthesize a host of biochemicals. So, although the liver has many of the regulatory features of muscle glycolysis, the regulation of glycolysis in the liver is more complex.

Phosphofructokinase

Liver phosphofructokinase can be regulated by ATP as in muscle, but such regulation is not as important since the liver does not experience the sudden ATP needs that a contracting muscle does. Likewise, low pH is not an important metabolic signal for the liver enzyme, because lactate is not normally produced in the liver. Indeed, as we will see, lactate is converted into glucose in the liver.

Glycolysis in the liver furnishes carbon skeletons for biosyntheses, and so a signal indicating whether building blocks are abundant or scarce should also regulate phosphofructokinase. In the liver, phosphofructokinase is inhibited by citrate, an early intermediate in the citric acid cycle (Chapter 17). A high level of citrate in the cytoplasm means that biosynthetic precursors are abundant, and so there is no need to degrade additional glucose for this purpose. Citrate inhibits phosphofructokinase by enhancing the inhibitory effect of ATP.

The key means by which glycolysis in the liver responds to changes in blood glucose is through the signal molecule fructose 2,6-bisphosphate (F-2,6-BP), a potent activator of phosphofructokinase. In the liver, the concentration of fructose 6-phosphate rises when blood-glucose concentration is high, and the abundance of fructose 6-phosphate accelerates the synthesis of F-2,6-BP (**Figure 16.22**). Hence, an abundance of fructose 6-phosphate leads to a higher concentration of F-2,6-BP.

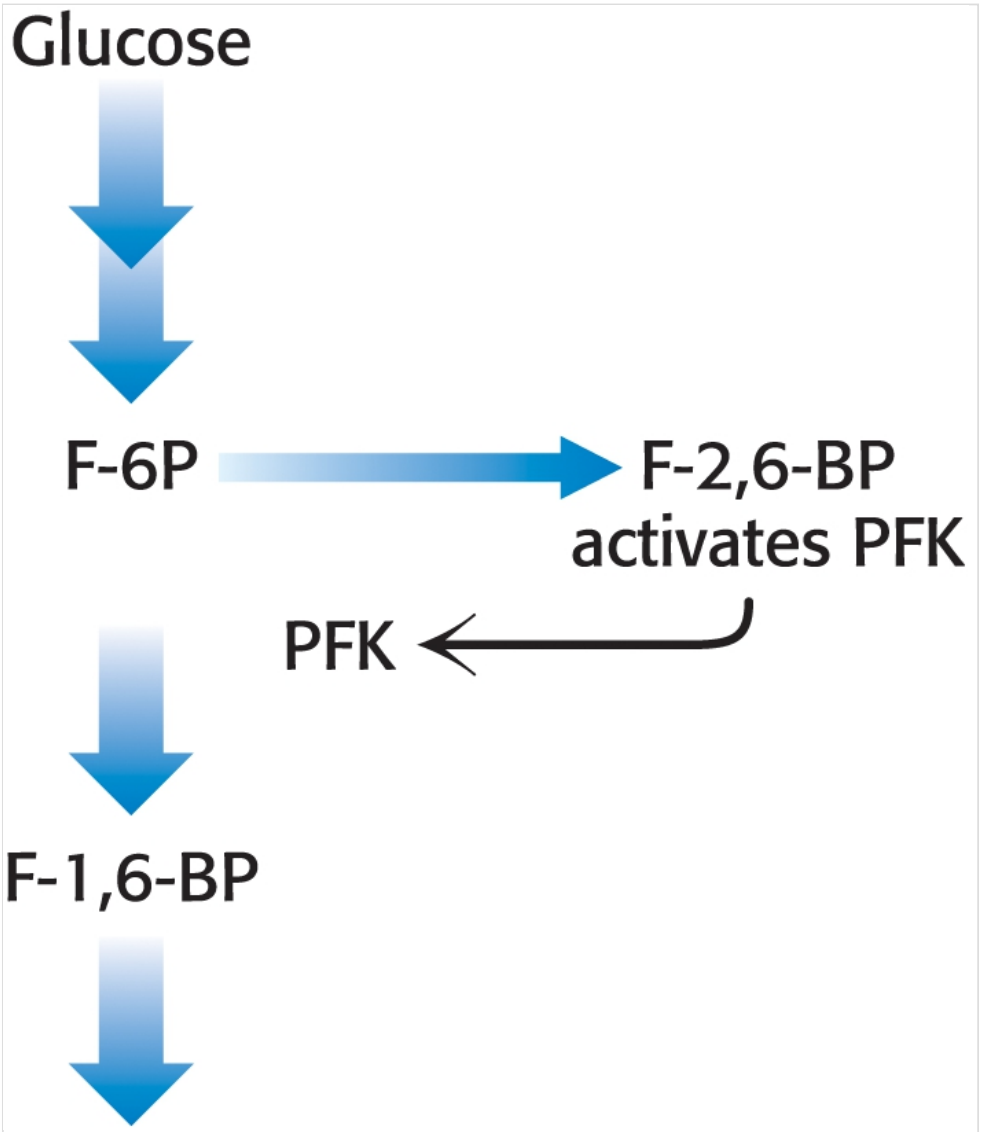
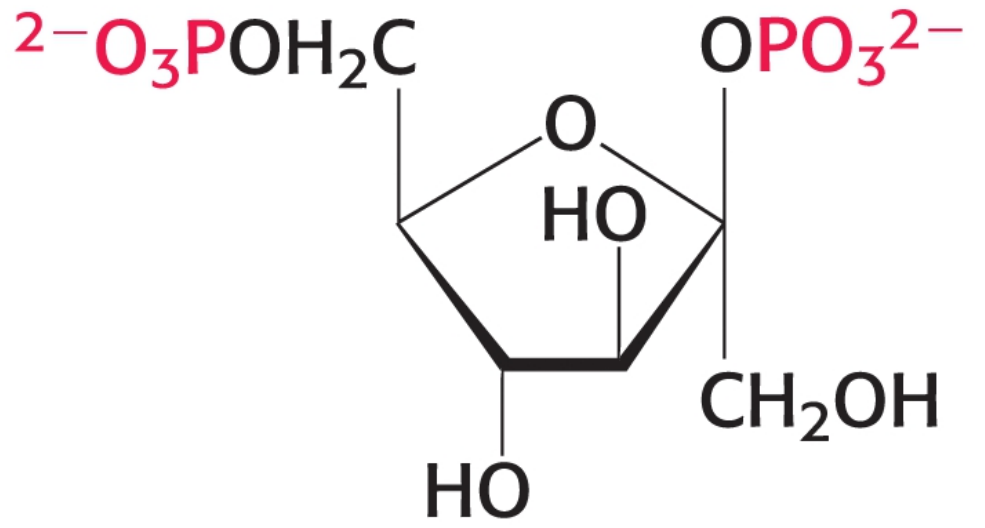


FIGURE 16.22 Fructose 2,6-bisphosphate allosterically activates phosphofructokinase. In high concentrations, fructose 6-phosphate (F-6P) activates the enzyme phosphofructokinase (PFK) through an intermediary, fructose 2,6-bisphosphate (F-2,6-BP).

The binding of fructose 2,6-bisphosphate increases the affinity of phosphofructokinase for fructose 6-phosphate and diminishes the

inhibitory effect of ATP (Figure 16.23). Glycolysis is thus accelerated when glucose is abundant. We will further explore the additional hormonal control over the synthesis and degradation of this important regulatory molecule after we have considered gluconeogenesis.



Fructose 2,6-bisphosphate (F-2,6-BP)

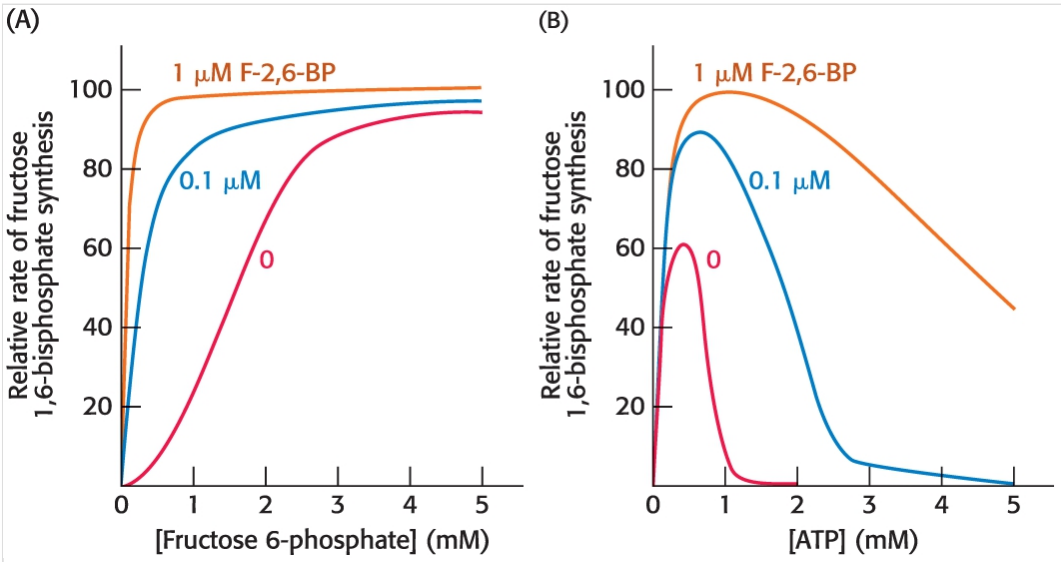


FIGURE 16.23 Activation of phosphofructokinase by fructose 2,6-bisphosphate changes the properties of the enzyme and counteracts inhibition by ATP. (A) The sigmoidal dependence of velocity on substrate concentration becomes hyperbolic in the presence of 1 μM fructose 2,6-bisphosphate. (B) ATP, acting as a substrate, initially stimulates the reaction. As the concentration of ATP increases, it acts as an allosteric inhibitor. The inhibitory effect of ATP is reversed by fructose 2,6-bisphosphate.

[Data from E. Van Schaftingen, M. F. Jett, L. Hue, and H. G. Hers, *Proc. Natl. Acad. Sci. U.S.A.* 78:3483–3486, 1981.]

Hexokinase and glucokinase

The hexokinase reaction in the liver is regulated as in the muscle. However, the liver, in keeping with its role as monitor of blood-glucose levels, possesses another specialized isozyme of hexokinase, called *glucokinase*, which is not inhibited by glucose 6-phosphate. The role of glucokinase is to provide glucose 6-phosphate for the synthesis of glycogen and for the formation of fatty acids (Chapter 22).

Remarkably, glucokinase displays the sigmoidal kinetics characteristic of an allosteric enzyme even though it functions as a monomer.

Glucokinase phosphorylates glucose only when glucose is abundant because the affinity of glucokinase for glucose is about 50-fold lower than that of hexokinase. Moreover, when glucose concentration is low, glucokinase is inhibited by the liver-specific glucokinase regulatory protein (GKRP), which sequesters the kinase in the nucleus until the glucose concentration increases. The low affinity of glucokinase for glucose gives the brain and muscles first call on glucose when its supply is limited, and it ensures that glucose will not be wasted when it is abundant.

 Glucokinase is also present in the β cells of the pancreas, which secrete the hormone insulin in response to the increased formation of glucose 6-phosphate by glucokinase when blood-glucose levels are elevated. Insulin signals the need to remove glucose from the blood for storage as glycogen or conversion into fat. Drugs that activate liver glucokinase or disrupt its interaction with GKRP are being evaluated as a treatment for type 2 diabetes, in which the sensitivity to natural levels of insulin has decreased.

Pyruvate kinase

Several isozymic forms of pyruvate kinase (a tetramer of 57-kDa subunits) encoded by different genes are present in mammals: The L type predominates in the liver, and the M type in muscle and the brain. The L and M forms of pyruvate kinase have many properties in common. Indeed, the liver enzyme behaves much like the muscle enzyme with regard to allosteric regulation, except that the liver enzyme is also inhibited by alanine (synthesized in one step from pyruvate), a signal that building blocks are available. Moreover, the isozymic forms differ in their susceptibility to covalent modification. The catalytic properties of the L form — but not of the M form — are also controlled by reversible phosphorylation (**Figure 16.24**). When the blood-glucose level is low, the glucagon-triggered cyclic AMP cascade

(Section 14.2) leads to the phosphorylation of pyruvate kinase, which diminishes its activity. This hormone-triggered phosphorylation prevents the liver from consuming glucose when it is more urgently needed by the brain and muscle. We see here a clear-cut example of how isoenzymes contribute to the metabolic diversity of different organs.

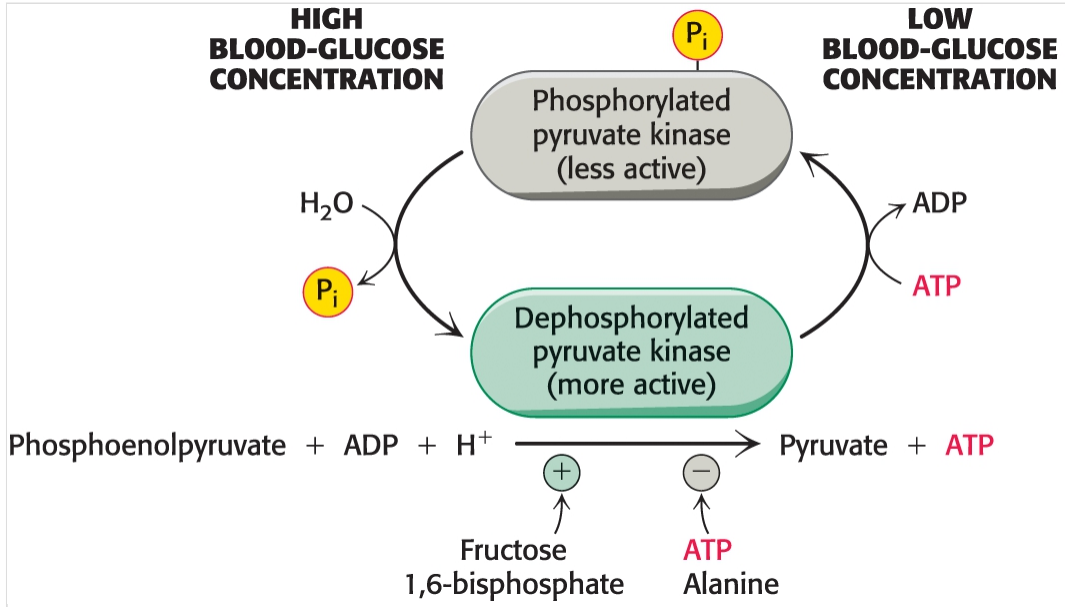


FIGURE 16.24 Pyruvate kinase activity is controlled by phosphorylation and dephosphorylation. Pyruvate kinase is automatically regulated by allosteric effectors but controlled by extracellular signals through reversible covalent modification. Fructose 1,6-bisphosphate allosterically stimulates the enzyme, while ATP and alanine are allosteric inhibitors. Glucagon, secreted in response to low blood glucose, promotes phosphorylation and inhibition of the enzyme. When blood glucose concentration is adequate, the enzyme is dephosphorylated and activated.

The enzymes of glycolysis are physically associated with one another

Evidence has accumulated that the enzymes of glycolysis in eukaryotes are organized into complexes. For example, in yeast, the glycolytic enzymes are associated with the mitochondria, while in mammalian erythrocytes, the enzymes are found bound to the inner surface of the cell membrane. Indeed, some level of organization appears to occur in all cell types. This arrangement increases enzyme efficiency by facilitating movement of substrates and products between enzymes — a process called **substrate channeling** — and prevents the release of any toxic intermediates. We will see that the organization of metabolic pathways into large complexes is a common occurrence inside the cell.

Aerobic glycolysis is a property of tumor cells and other rapidly growing cells

Tumors have been known for decades to display enhanced rates of glucose uptake and glycolysis. Indeed, rapidly growing tumor cells will metabolize glucose to lactate even in the presence of oxygen, a process called **aerobic glycolysis** or the *Warburg effect*, after Otto Warburg, the biochemist who first noted this characteristic of cancer cells in the 1920s. In fact, tumors with a high glucose uptake are particularly aggressive, and the cancer is likely to have a poor prognosis. A nonmetabolizable glucose analog, 2-^{18}F-2-D-deoxyglucose, detectable by a combination of positron emission tomography (PET) and computer-aided tomography (CAT), easily visualizes tumors and allows monitoring of treatment effectiveness (**Figure 16.25**).

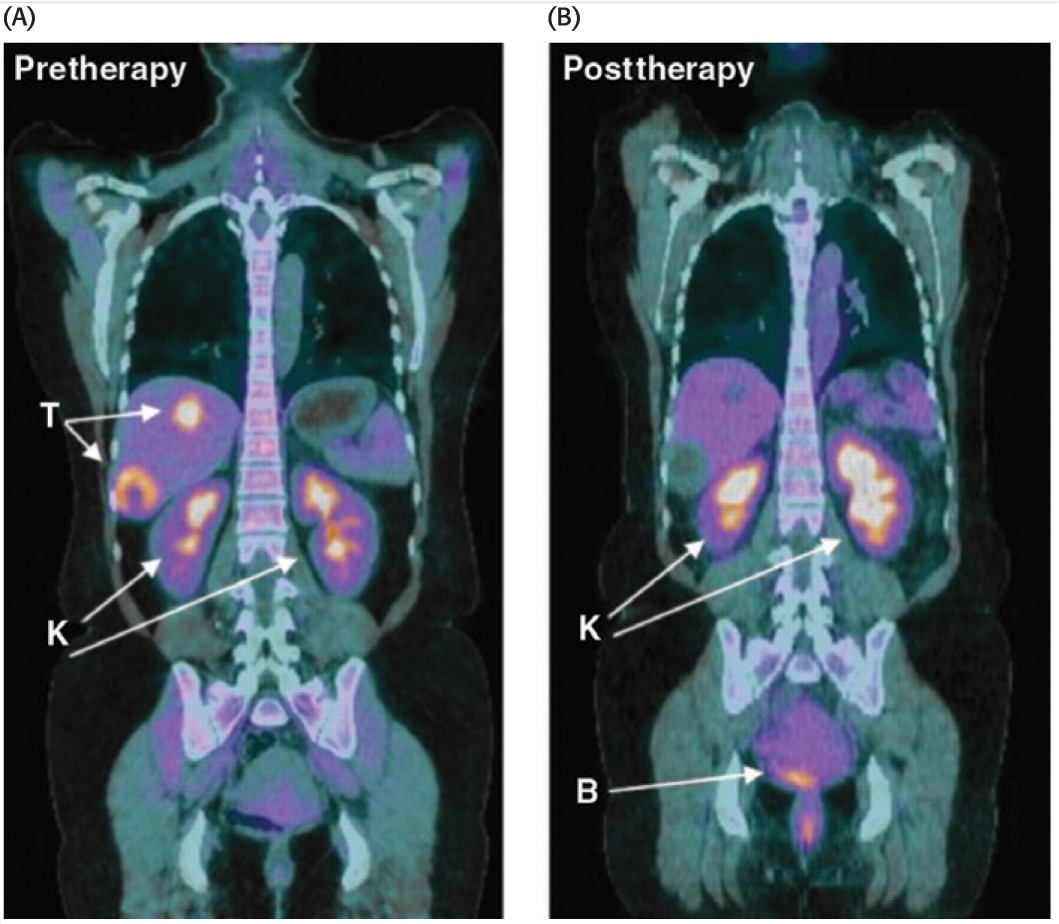


FIGURE 16.25 Tumors can be visualized with 2-^{18}F-2-D-deoxyglucose (FDG) and positron emission tomography. (A) A nonmetabolizable glucose analog infused into a patient and detected by a combination of positron emission and computer-aided tomography reveals the presence of a malignant tumor (T). (B) After 4 weeks of treatment with a tyrosine kinase inhibitc (Section 14.5), the tumor shows no uptake of FDG, indicating decreased metabolism. Excess FD which is excreted in the urine, also visualizes the kidneys (K) and bladder (B).

What selective advantage does aerobic glycolysis offer the tumor over the energetically more efficient oxidative phosphorylation? Researchers are actively pursuing the answer to this question, but we can speculate on the benefits. First, aerobic glycolysis generates lactic acid that is then

secreted. Acidification of the tumor environment has been shown to facilitate tumor invasion. Moreover, lactate impairs the activation of $CD8^+$ T and NK immune system cells that normally attack the tumor.

However, even leukemias perform aerobic glycolysis, and leukemia is not an invasive cancer. Second, and perhaps more importantly, the increased uptake of glucose and formation of glucose 6-phosphate provides substrates for another metabolic pathway — the pentose phosphate pathway (Section 20.3) — that generates biosynthetic reducing power, NADPH. Finally, cancer cells grow more rapidly than the blood vessels that nourish them; thus, as solid tumors grow, the oxygen concentration in their environment falls. In other words, they begin to experience a deficiency of oxygen, called *hypoxia*. The use of aerobic glycolysis reduces the dependence of cell growth on oxygen.

What biochemical alterations facilitate the switch to aerobic glycolysis? Again, the answers are not complete, but changes in gene expression of isozymic forms of two glycolytic enzymes may be crucial. Tumor cells express an isozyme of hexokinase that binds to mitochondria. There, the enzyme has ready access to any ATP generated by oxidative phosphorylation and is not susceptible to feedback inhibition by its product, glucose 6-phosphate. More importantly, an embryonic isozyme of pyruvate kinase, pyruvate kinase M, is also expressed. Remarkably, this isozyme has a lower catalytic rate than normal pyruvate kinase and creates a bottleneck, allowing the use of glycolytic intermediates for biosynthetic processes required for cell proliferation. The need for biosynthetic precursors is greater than the need for ATP, suggesting that even glycolysis at a reduced rate produces sufficient ATP to allow cell proliferation. Although originally observed in cancer cells, the Warburg effect is also seen in noncancerous, rapidly dividing cells.

Cancer and endurance training affect glycolysis in a similar fashion

The hypoxia that some tumors experience with rapid growth activates a transcription factor, hypoxia-inducible transcription factor (HIF-1). HIF-1 increases the expression of most glycolytic enzymes and the glucose transporters GLUT1 and GLUT3 (Table 16.5). These adaptations by the cancer cells enable a tumor to survive until blood vessels can grow. HIF-1 also increases the expression of signal molecules, such as vascular endothelial growth factor (VEGF), that facilitate the growth of blood vessels that will provide nutrients to the cells (Figure 16.26). Without new blood vessels, a tumor would cease to grow and either die or remain harmlessly small.

TABLE 16.5 Proteins in glucose metabolism encoded by genes regulated by hypoxia-inducible factor

GLUT1
GLUT3
Hexokinase
Phosphofructokinase
Aldolase
Glyceraldehyde 3-phosphate dehydrogenase
Phosphoglycerate kinase

Enolase
Pyruvate kinase

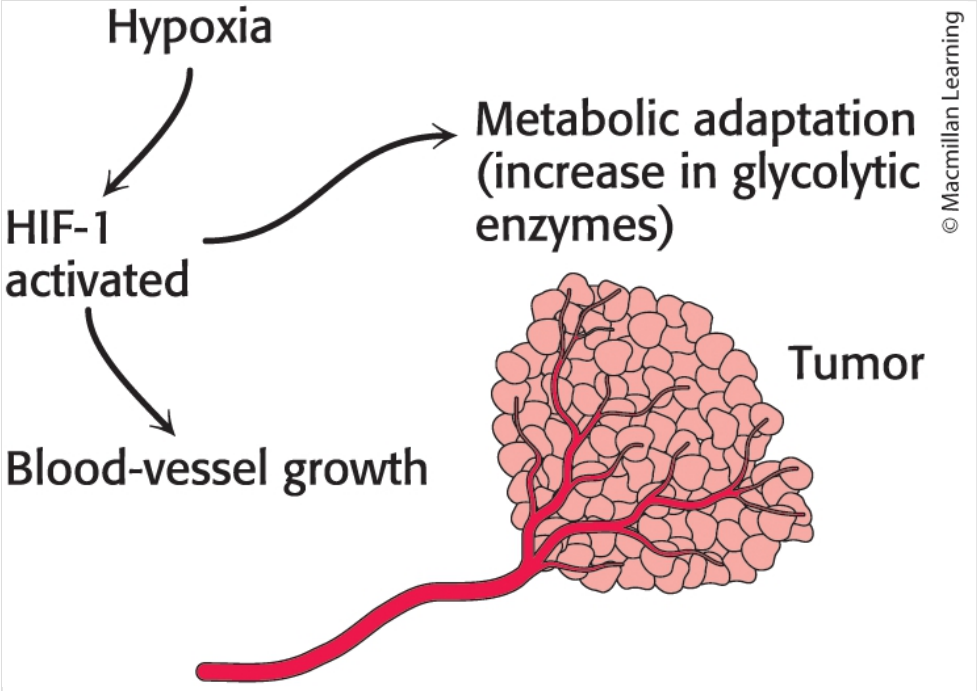


FIGURE 16.26 Hypoxia alters gene expression in tumors. The hypoxic conditions inside a tumor mass lead to the activation of the hypoxia-inducible transcription factor (HIF-1), which induces metabolic adaptation (an increase in glycolytic enzymes) and activates angiogenic factors that stimulate the growth of new blood vessels.

[Information from C. V. Dang and G. L. Semenza, *Trends Biochem. Sci.* 24:68–72, 1999.]

Efforts are underway to develop drugs that inhibit the growth of blood vessels in tumors. Indeed, bevacizumab, a monoclonal antibody that binds to VEGF and prevents activation of angiogenesis, has been approved for treatment of glioblastomas, which are fast-growing cancers of the central nervous system derived from glial cells.

Interestingly, anaerobic exercise training — forcing muscles to rely on lactic acid fermentation for ATP production — also activates HIF-1, producing the same effects as those seen in the tumor — enhanced ability to generate ATP anaerobically and a stimulation of blood-vessel growth. These biochemical effects account for the improved athletic performance that results from training and demonstrate how behavior can affect biochemistry. Other signals from sustained muscle contraction trigger muscle mitochondrial biogenesis, allowing for more efficient aerobic energy generation and forestalling the need to resort to lactic acid fermentation for ATP synthesis.

Self-Check Question

Muscle phosphofructokinase activity increases as a function of ATP concentration, but only up to a point, and then it falls rapidly. Explain these results and how they relate to the role of phosphofructokinase in glycolysis.