22 Generation of ATP from Glucose: Glycolysis

Glucose *is the universal fuel for human cells. Every cell type in the human is able to generate adenosine triphosphate (ATP) from glycolysis, the pathway in which glucose is oxidized and cleaved to form pyruvate. The importance of glycolysis in our fuel economy is related to the availability of glucose in the blood, as well as the ability of glycolysis to generate ATP in both the presence and absence of* O_2*. Glucose is the major sugar in our diet and the sugar that circulates in the blood to ensure that all cells have a continuous fuel supply. The brain uses glucose almost exclusively as a fuel.*

Glycolysis *begins with the phosphorylation of glucose to glucose 6-phosphate (**glucose-6-P**) by **hexokinase** (HK). In subsequent steps of the pathway, one glucose-6-P molecule is oxidized to two **pyruvate** molecules with generation of two molecules of **NADH** (Fig. 22.1). A net generation of two molecules of ATP occurs through direct transfer of **high-energy phosphate** from intermediates of the pathway to ADP (**substrate level phosphorylation**).*

*Glycolysis occurs in the **cytosol** and generates cytosolic NADH. Because NADH cannot cross the inner mitochondrial membrane, its reducing equivalents are transferred to the electron transport chain by either the **malate-aspartate shuttle** or the **glycerol 3-phosphate shuttle** (see Fig. 22.1). Pyruvate is then oxidized completely to* CO_2 *by pyruvate dehydrogenase and the TCA cycle. Complete **aerobic oxidation** of glucose to* CO_2 *can generate approximately **30 to 32 moles of ATP per mole of glucose**.*

*When cells have a limited supply of oxygen (e.g., kidney medulla), or few or no mitrochondria (e.g., the red cell), or greatly increased demands for ATP (e.g., skeletal muscle during high-intensity exercise), they rely on **anaerobic glycolysis** for generation of ATP. In anaerobic glycolysis, **lactate dehydrogenase** oxidizes the NADH generated from glycolysis by reducing pyruvate to **lactate** (Fig. 22.2). Because* O_2 *is not required to reoxidize the NADH, the pathway is referred to as anaerobic. The energy yield from anaerobic glycolysis (2 moles of ATP per mole of glucose) is much lower than the yield from aerobic oxidation. The lactate (lactic acid) is released into the blood. Under pathologic conditions that cause **hypoxia,** tissues may generate enough lactic acid to cause **lactic acidemia**.*

*In each cell, glycolysis is regulated to ensure that **ATP homeostasis** is maintained, without using more glucose than necessary. In most cell types, **hexokinase (HK)**, the first enzyme of glycolysis, is inhibited by glucose 6-phosphate (see Fig. 22.1). Thus, glucose is not taken up and phosphorylated by a cell unless glucose-6-P enters a metabolic pathway, such as glycolysis or glycogen synthesis. The control of glucose-6-P entry into glycolysis occurs at phosphofructokinase-1(**PFK-1**), the rate-limiting enzyme of the pathway. PFK-1 is **allosterically inhibited** by **ATP** and **allosterically activated** by **AMP**. AMP increases in the cytosol as ATP is hydrolyzed by energy-requiring reactions.*

For glucose 6-phosphate and other sugar phosphoesters, the phosphate group will be denoted with "P," as in glucose-6-P.

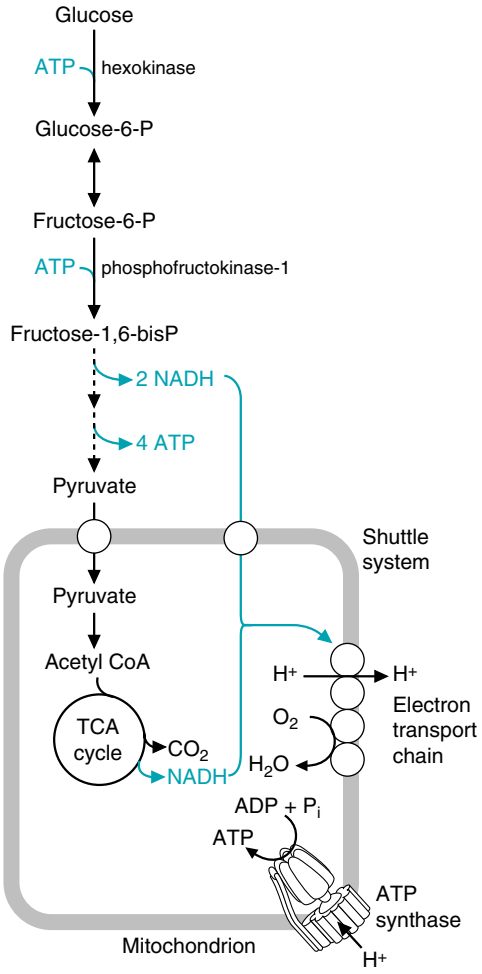


Fig. 22.1. Overview of glycolysis and the TCA cycle.

Glycolysis has functions in addition to ATP production. For example, in liver and adipose tissue, this pathway generates pyruvate as a precursor for ***fatty acid biosynthesis****. Glycolysis also provides precursors for the synthesis of compounds such as amino acids and 5-carbon sugar phosphates.*

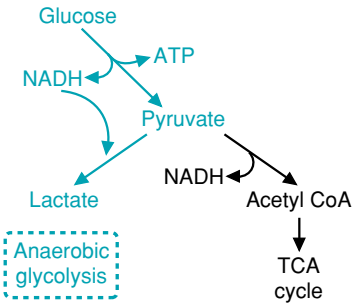


Fig. 22.2. Anaerobic glycolysis (shown in blue). The conversion of glucose to lactate generates 2 ATP from substrate-level phosphorylation. Because there is no net generation of NADH, there is no need for O_2, and, thus, the pathway is anaerobic.

THE WAITING ROOM

Lopa Fusor is a 68-year-old woman who is admitted to the hospital emergency room with very low blood pressure (80/40 mm Hg) caused by an acute hemorrhage from a previously diagnosed ulcer of the stomach. Lopa's bleeding stomach ulcer has reduced her effective blood volume severely enough to compromise her ability to perfuse (deliver blood to) her tissues. She is, therefore, a "low perfuser." She is also known to have chronic obstructive pulmonary disease (COPD) as a result of 42 years of smoking two packs of cigarettes per day. Her respiratory rate is rapid and labored, her skin is cold and clammy, and her lips are slightly blue (cyanotic). She appears anxious and moderately confused.

As appropriate emergency measures are taken to stabilize her and elevate her blood pressure, blood is sent for immediate blood typing and cross-matching, so that blood transfusions can be started. A battery of laboratory tests are ordered, including venous hemoglobin, hematocrit, and lactate levels, and arterial blood pH, partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2), bicarbonate, and oxygen saturation. Results show that the hemorrhaging and COPD have resulted in hypoxemia, with decreased oxygen delivery to her tissues, and both a respiratory and metabolic acidosis.

Otto Shape, a 26-year-old medical student, had gained weight during his first sedentary year in medical school. During his second year, he began watching his diet, jogging for an hour 4 times each week, and playing tennis twice a week. He has decided to compete in a 5-km race. To prepare for the race, he begins training with wind sprints, bouts of alternately running and walking.

Ivan Applebod is a 56-year-old morbidly obese accountant (see Chapters 1–3). He decided to see his dentist because he felt excruciating pain in his teeth when he ate ice cream. He really likes sweets and keeps hard candy in his pocket. The dentist noted from Mr. Applebod's history that he had numerous cavities as a child in his baby teeth. At this visit, the dentist found cavities in two of Mr.Applebod's teeth.

The hematocrit (the percentage of the volume of blood occupied by packed red blood cells) and hemoglobin content (g hemoglobin in 100 mL blood) are measured to determine whether the oxygen-carrying capacity of the blood is adequate. They can be decreased by conditions that interfere with erythropoiesis (synthesis of red blood cells in bone marrow), such as iron deficiency. They also can be decreased during chronic bleeding, but not during immediate acute hemorrhage, if interstitial fluid replaces the lost blood volume and dilutes out the red blood cells. The pCO_2 and pO_2 are the partial pressures of CO_2 and O_2 in the blood. The pO_2 and oxygen saturation determine whether adequate oxygen is available for tissues. Measurement of the pCO_2 and bicarbonate can distinguish between a metabolic and a respiratory acidosis (see Chapter 4).

I. GLYCOLYSIS

Glycolysis is one of the principle pathways for generating ATP in cells and is present in all cell types. The central role of glycolysis in fuel metabolism is related to its ability to generate ATP with, and without, oxygen. The oxidation of glucose to pyruvate generates ATP from substrate-level phosphorylation (the transfer of phosphate from high-energy intermediates of the pathway to ADP) and NADH. Subsequently, the pyruvate may be oxidized to CO_2 in the TCA cycle and ATP generated from electron transfer to oxygen in oxidative phosphorylation. However, if the pyruvate and NADH from glycolysis are converted to lactate (anaerobic glycolysis), ATP can be generated in the absence of oxygen, via substrate-level phosphorylation.

Glucose is readily available from our diet, internal glycogen stores, and the blood. Carbohydrate provides 50% or more of the calories in most diets, and glucose is the major carbohydrate. Other dietary sugars, such as fructose and galactose, are oxidized by conversion to intermediates of glycolysis. Glucose is stored in cells as glycogen, which can provide an internal source of fuel for glycolysis in emergency situations (e.g., decreased supply of fuels and oxygen during ischemia, a low blood flow). Insulin and other hormones maintain blood glucose at a constant level (glucose homeostasis), thereby ensuring that glucose is always available to cells that depend on glycolysis for generation of ATP.

In addition to serving as an anaerobic and aerobic source of ATP, glycolysis is an anabolic pathway that provides biosynthetic precursors. For example, in liver and adipose tissue, this pathway generates pyruvate as a precursor for fatty acid biosynthesis. Glycolysis also provides precursors for the synthesis of compounds such as amino acids and ribose-5-phosphate, the precursor of nucleotides. The integration of glycolysis with other anabolic pathways is discussed in Chapter 36.

After a high-carbohydrate meal, glucose is the major fuel for almost all tissues. Exceptions include intestinal mucosal cells, which transport glucose from the gut into the blood, and cells in the proximal convoluted tubule of the kidney, which return glucose from the renal filtrate to the blood. During fasting, the brain continues to oxidize glucose because it has a limited capacity for the oxidation of fatty acids or other fuels. Cells also continue to use glucose for the portion of their ATP generation that must be met by anaerobic glycolysis, due to either a limited oxygen supply or a limited capacity for oxidative phosphorylation (e.g., the red blood cell).

A. The Reactions of Glycolysis

The glycolytic pathway, which cleaves 1 mole of glucose to 2 moles of the 3-carbon compound pyruvate, consists of a preparative phase and an ATP-generating phase. In the initial preparative phase of glycolysis, glucose is phosphorylated

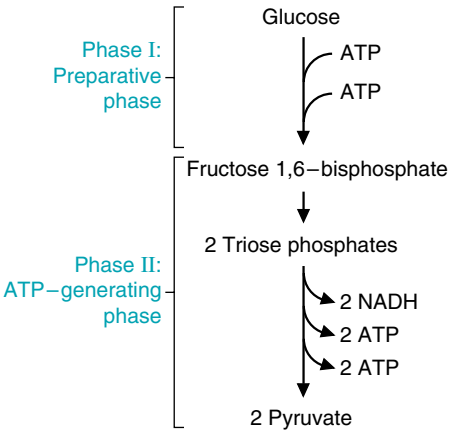


Fig. 22.3. Phases of the glycolytic pathway.

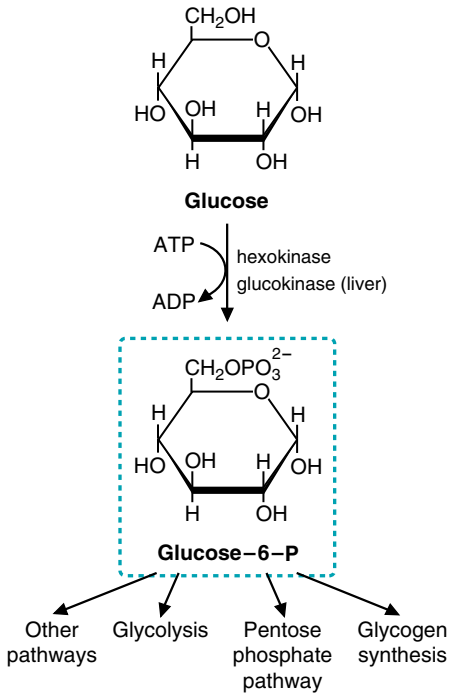


Fig. 22.4. Glucose 6-phosphate metabolism.

Hexokinases, other kinases, and many other enzymes that catalyze reactions involving the hydrolysis of ATP require Mg^{2+}. The Mg^{2+} forms a complex with the phosphate groups of ATP. Kinases also require K^{+}.

twice by ATP and cleaved into two triose phosphates (Fig. 22.3). The ATP expenditure in the beginning of the preparative phase is sometimes called "priming the pump," because this initial utilization of 2 moles of ATP/ mole of glucose results in the production of 4 moles of ATP/mole of glucose in the ATP-generating phase.

In the ATP-generating phase, glyceraldehyde 3-phosphate (a triose phosphate) is oxidized by NAD^+ and phosphorylated using inorganic phosphate. The high-energy phosphate bond generated in this step is transferred to ADP to form ATP. The remaining phosphate is also rearranged to form another high-energy phosphate bond that is transferred to ADP. Because there were 2 moles of triose phosphate formed, the yield from the ATP-generating phase is 4 ATP and 2 NADH. The result is a net yield of 2 moles of ATP, 2 moles of NADH, and 2 moles of pyruvate per mole of glucose.

1. CONVERSION OF GLUCOSE TO GLUCOSE 6-PHOSPHATE

Glucose metabolism begins with transfer of a phosphate from ATP to glucose to form glucose-6-P (Fig. 22.4). Phosphorylation of glucose commits it to metabolism within the cell because glucose-6-P cannot be transported back across the plasma membrane. The phosphorylation reaction is irreversible under physiologic conditions because the reaction has a high negative $\Delta G^{0\prime}$. Phosphorylation does not, however, commit glucose to glycolysis.

Glucose-6-P is a branchpoint in carbohydrate metabolism. It is a precursor for almost every pathway that uses glucose, including glycolysis, the pentose phosphate pathway, and glycogen synthesis. From the opposite point of view, it also can be generated from other pathways of carbohydrate metabolism, such as glycogenolysis (breakdown of glycogen), the pentose phosphate pathway, and gluconeogenesis (the synthesis of glucose from non-carbohydrate sources).

Hexokinases, the enzymes that catalyze the phosphorylation of glucose, are a family of tissue-specific isoenzymes that differ in their kinetic properties. The isoenzyme found in liver and β cells of the pancreas has a much higher K_m than other hexokinases and is called glucokinase. In many cells, some of the hexokinase is bound to porins in the outer mitochondrial membrane (voltage-dependent anion channels; see Chapter 21), which gives these enzymes first access to newly synthesized ATP as it exits the mitochondria.

2. CONVERSION OF GLUCOSE-6-P TO THE TRIOSE PHOSPHATES

In the remainder of the preparative phase of glycolysis, glucose-6-P is isomerized to fructose 6-phosphate (fructose-6-P), again phosphorylated, and subsequently cleaved into two 3-carbon fragments (Fig 22.5). The isomerization, which positions a keto group next to carbon 3, is essential for the subsequent cleavage of the bond between carbons 3 and 4.

The next step of glycolysis, phosphorylation of fructose-6-P to fructose 1,6-bisphosphate (fructose-1,6-bisP) by phosphofructokinase-1 (PFK-1), is generally considered the first committed step of the pathway. This phosphorylation requires ATP and is thermodynamically and kinetically irreversible. Therefore, PFK-1 irrevocably commits glucose to the glycolytic pathway. PFK-1 is a regulated enzyme in cells, and its regulation controls the entry of glucose into glycolysis. Like hexokinase, it exists as tissue-specific isoenzymes whose regulatory properties match variations in the role of glycolysis in different tissues.

Fructose-1,6-bisP is cleaved into two phosphorylated 3-carbon compounds (triose phosphates) by aldolase (see Fig. 22.5). Dihydroxyacetone phosphate (DHAP) is isomerized to glyceraldehyde 3-phosphate (glyceraldehyde-3-P), which is a triose phosphate. Thus, for every mole of glucose entering glycolysis, 2 moles of glyceraldehyde-3-P continue through the pathway.

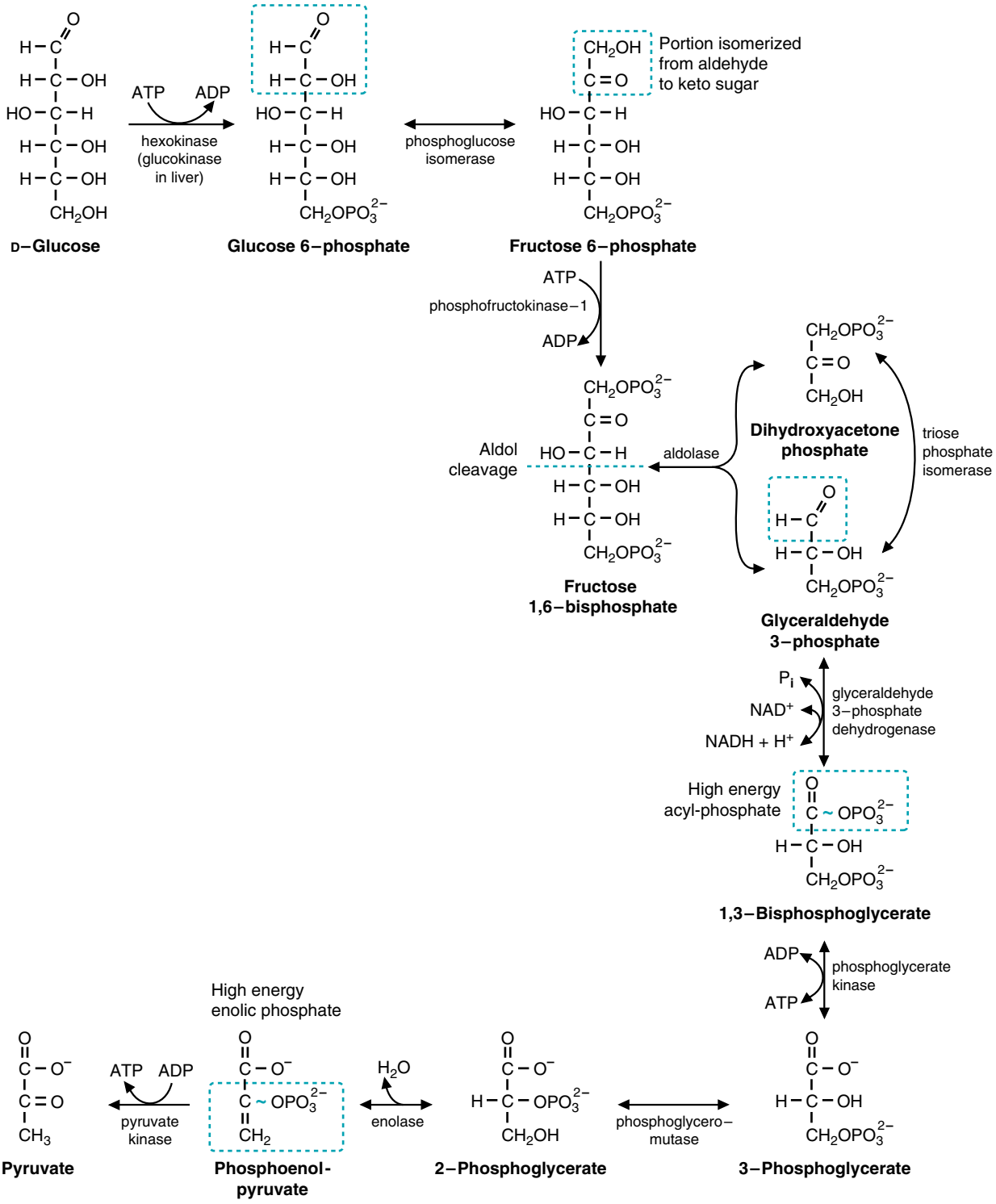


Fig. 22.5. Reactions of glycolysis. High-energy phosphates are shown in blue.

Aldolase is named for the mechanism of the forward reaction, which is an aldol cleavage, and the mechanism of the reverse reaction, which is an aldol condensation. The enzyme exists as tissue-specific isoenzymes, which all catalyze the cleavage of fructose 1,6-bisphosphate but differ in their specificities for fructose 1-P. The enzyme uses a lysine residue at the active site to form a covalent bond with the substrate during the course of the reaction. Inability to form this covalent linkage inactivates the enzyme.

3. OXIDATION AND SUBSTRATE LEVEL PHOSPHORYLATION

In the next part of the glycolytic pathway, glyceraldehyde-3-P is oxidized and phosphorylated so that subsequent intermediates of glycolysis can donate phosphate to ADP to generate ATP. The first reaction in this sequence, catalyzed by glyceraldehyde-3-P dehydrogenase, is really the key to the pathway (see Fig. 22.5). This enzyme oxidizes the aldehyde group of glyceraldehyde-3-P to an enzyme-bound carboxyl group and transfers the electrons to NAD^+ to form NADH. The oxidation step is dependent on a cysteine residue at the active site of the enzyme, which forms a high-energy thioester bond during the course of the reaction. The high-energy intermediate immediately accepts an inorganic phosphate to form the high-energy acyl phosphate bond in 1,3-bisphosphoglycerate, releasing the product from the cysteine residue on the enzyme. This high-energy phosphate bond is the start of substrate-level phosphorylation (the formation of a high-energy phosphate bond where none previously existed, without the utilization of oxygen).

In the next reaction, the phosphate in this bond is transferred to ADP to form ATP by 3-phosphoglycerate kinase. The energy of the acyl phosphate bond is high enough (~13 kcal/mole) so that transfer to ADP is an energetically favorable process. 3-phosphoglycerate is also a product of this reaction.

To transfer the remaining low-energy phosphoester on 3-phosphoglycerate to ADP, it must be converted into a high-energy bond. This conversion is accomplished by moving the phosphate to the second carbon (forming 2-phosphoglycerate) and then removing water to form phosphoenolpyruvate (PEP). The enolphosphate bond is a high-energy bond (its hydrolysis releases approximately 14 kcal/mole of energy), so the transfer of phosphate to ADP by pyruvate kinase is energetically favorable (see Fig. 22.5). This final reaction converts PEP to pyruvate.

Kinases transfer a phosphate from ATP to another compound. Hexokinase transfers a phosphate to glucose or another hexose to form a hexose phosphate. 3-Phosphoglycerate kinase is named for the reaction that is the reverse of glycolysis, transfer of phosphate from ATP to 3-phosphoglycerate to form 1,3-bisphosphoglycerate. Pyruvate kinase is also named for the reverse reaction (phosphorylation of pyruvate by ATP), although this direction does not occur under physiologic conditions.

4. SUMMARY OF THE GLYCOLYTIC PATHWAY

The overall net reaction in the glycolytic pathway is:

$$\text{Glucose} + 2NAD^+ + 2P_i + 2ADP \rightarrow 2\text{Pyruvate} + 2NADH + 4H^+ + 2ATP + 2H_2O$$

The pathway occurs with an overall negative $\Delta G^{0'}$ of approximately –22 kcal. Therefore, it cannot be reversed without the expenditure of energy.

B. Oxidative Fates of Pyruvate and NADH

The NADH produced from glycolysis must be continuously reoxidized back to NAD^+ to provide an electron acceptor for the glyceraldehyde-3-P dehydrogenase reaction and prevent product inhibition. Without oxidation of this NADH, glycolysis cannot continue. There are two alternate routes for oxidation of cytosolic NADH (Fig. 22.6). One route is aerobic, involving shuttles that transfer reducing equivalents across the mitochondrial membrane and ultimately to the electron transport chain and oxygen (see Fig. 22.6A). The other route is anaerobic (without the use of oxygen). In anaerobic glycolysis, NADH is reoxidized in the cytosol by lactate dehydrogenase, which reduces pyruvate to lactate (see Fig. 22.6B).

The fate of pyruvate depends on the route used for NADH oxidation. If NADH is reoxidized in a shuttle system, pyruvate can be used for other pathways, one of which is oxidation to acetyl-CoA and entry into the TCA cycle for complete oxidation. Alternatively, in anaerobic glycolysis, pyruvate is reduced to lactate and diverted away from other potential pathways. Thus, the use of the shuttle systems allows for more ATP to be generated than by anaerobic glycolysis by both oxidizing the cytoplasmically derived NADH in the electron transport chain and by allowing pyruvate to be oxidized completely to CO_2.

The reason that shuttles are required for the oxidation of cytosolic NADH by the electron transport chain is that the inner mitochondrial membrane is impermeable

The confusion experienced by **Lopa Fusor** in the emergency room is caused by an inadequate delivery of oxygen to the brain. Neurons have very high ATP requirements, and most of this ATP is provided by aerobic oxidation of glucose to pyruvate in glycolysis, and pyruvate oxidation to CO_2 in the TCA cycle. The brain has little or no capacity to oxidize fatty acids, and, therefore, its glucose consumption is high (approximately 125–150 g/day in the adult). Its oxygen demands are also high. If cerebral oxygen supply were completely interrupted, the brain would last only 10 seconds. The only reason consciousness lasts longer during anoxia or asphyxia is that there is still some oxygen in the lungs and in circulating blood. A decrease of blood flow to approximately ½ of the normal rate results in a loss of consciousness.

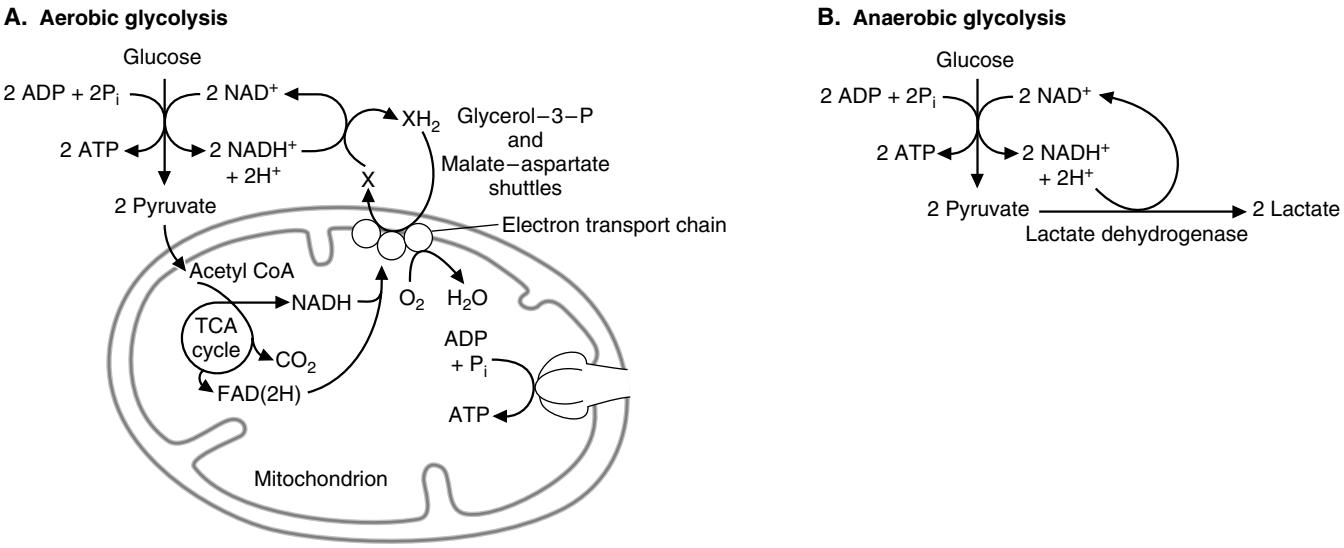


Fig. 22.6. Alternate fates of pyruvate. **A.** The pyruvate produced by glycolysis enters mitochondria and is oxidized to CO_2 and H_2O. The reducing equivalents in NADH enter mitochondria via a shuttle system. **B.** Pyruvate is reduced to lactate in the cytosol, thereby using the reducing equivalents in NADH.

to NADH, and no transport protein exists that can directly translocate NADH across this membrane. Consequently, NADH is reoxidized to NAD^+ in the cytosol by a reaction that transfers the electrons to DHAP in the glycerol 3-phosphate (glycerol-3-P) shuttle and oxaloacetate in the malate–aspartate shuttle. The NAD^+ that is formed in the cytosol returns to glycolysis while glycerol-3-P or malate carry the reducing equivalents that are ultimately transferred across the inner mitochondrial membrane. Thus, these shuttles transfer electrons and not NADH per se.

1. GLYCEROL 3–PHOSPHATE SHUTTLE

The glycerol 3–phosphate shuttle is the major shuttle in most tissues. In this shuttle, cytosolic NAD^+ is regenerated by cytoplasmic glycerol 3-phosphate dehydrogenase, which transfers electrons from NADH to DHAP to form glycerol 3-phosphate (Fig. 22.7). Glycerol 3-phosphate then diffuses through the outer mitochondrial membrane to the inner mitochondrial membrane, where the electrons are donated to a membrane-bound flavin adenive dinucleofide (FAD)-containing glycerophosphate dehydrogenase. This enzyme, like succinate dehydrogenase, ultimately donates electrons to CoQ, resulting in an energy yield of approximately 1.5 ATP from oxidative phosphorylation. Dihydroxyacetone phosphate returns to the cytosol to continue the shuttle. The sum of the reactions in this shuttle system is simply:

$$NADH_{cytosol} + H^+ + FAD_{mitochondria} \rightarrow NAD^+_{cytosol} + FAD(2H)_{mitochondria}$$

2. MALATE–ASPARTATE SHUTTLE

Many tissues contain both the glycerol-3-P shuttle and the malate–aspartate shuttle. In the malate–aspartate shuttle (Fig. 22.8), cytosolic NAD^+ is regenerated by cytosolic malate dehydrogenase, which transfers electrons from NADH to cytosolic oxaloacetate to form malate. Malate is transported across the inner mitochondrial membrane by a specific translocase, which exchanges malate for α-ketoglutarate. In the matrix, malate is oxidized back to oxaloacetate by mitochondrial malate dehydrogenase, and NADH is generated. This NADH can donate electrons to the electron transport chain with generation of approximately 2.5 moles of ATP per mole of NADH. The newly formed oxaloacetate cannot pass back through the inner mitochondrial membrane under physiologic conditions, so aspartate is used to

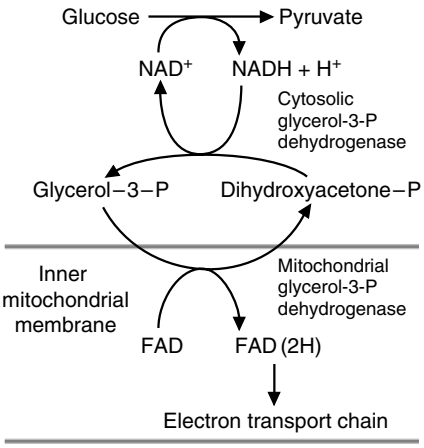


Fig. 22.7. Glycerol 3-phosphate shuttle. Because NAD^+ and NADH cannot cross the mitochondrial membrane, shuttles transfer the reducing equivalents into mitochondria. Dihydroxyacetone phosphate (DHAP) is reduced to glycerol-3-P by cytosolic glycerol 3-P dehydrogenase, using cytosolic NADH produced in glycolysis. Glycerol-3-P then reacts in the inner mitochondrial membrane with mitochondrial glycerol-3-P dehydrogenase, which transfers the electrons to FAD and regenerates DHAP, which returns to the cytosol. The electron transport chain transfers the electrons to O_2, which generates approximately 1.5 ATP for each FAD(2H) that is oxidized.

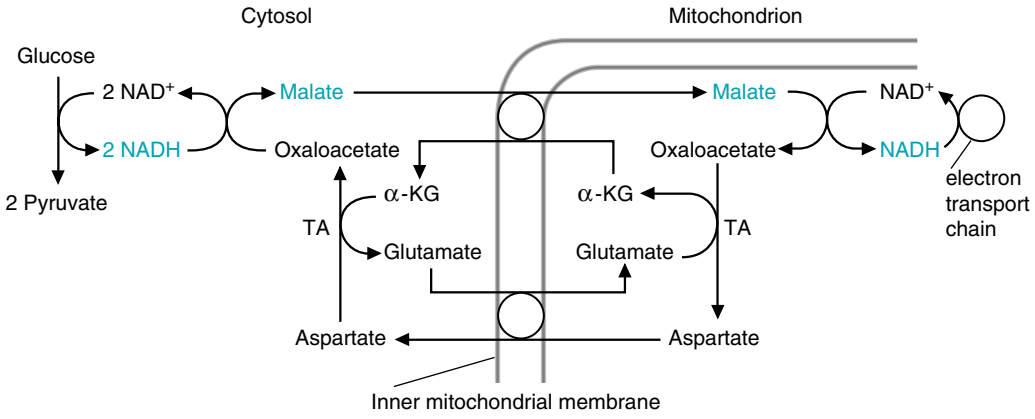


Fig. 22.8. Malate–aspartate shuttle. NADH produced by glycolysis reduces oxaloacetate (OAA) to malate, which crosses the mitochondrial membrane and is reoxidized to OAA. The mitochondrial NADH donates electrons to the electron transport chain, with 2.5 ATPs generated for each NADH. To complete the shuttle, oxaloacetate must return to the cytosol, although it cannot be directly transported on a translocase. Instead, it is transaminated to aspartate, which is then transported out to the cytosol, where it is transaminated back to oxaloacetate. The translocators exchange compounds in such a way that the shuttle is completely balanced. TA = transamination reaction. α-KG = α-ketoglutarate.

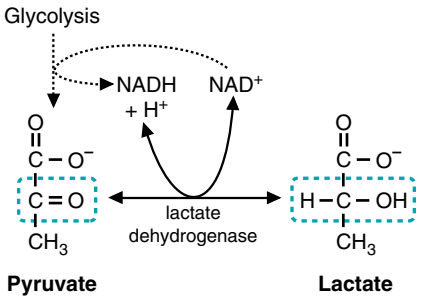


Fig. 22.9. Lactate dehydrogenase reaction. Pyruvate, which may be produced by glycolysis, is reduced to lactate. The reaction, which occurs in the cytosol, requires NADH and is catalyzed by lactate dehydrogenase. This reaction is readily reversible.

What are the energy-generating steps as pyruvate is completely oxidized to carbon dioxide to generate 12.5 molecules of ATP per pyruvate?

return the oxaloacetate carbon skeleton to the cytosol. In the matrix, transamination reactions transfer an amino group to oxaloacetate to form aspartate, which is transported out to the cytosol (using an aspartate/glutamate exchange translocase) and converted back to oxaloacetate through another transamination reaction. The sum of all the reactions of this shuttle system is simply:

$$NADH_{cytosol} + NAD^+_{matrix} \rightarrow NAD^+_{cytosol} + NADH_{matrix}.$$

C. Anaerobic Glycolysis

When the oxidative capacity of a cell is limited (e.g., the red blood cell, which has no mitochondria), the pyruvate and NADH produced from glycolysis cannot be oxidized aerobically. The NADH is therefore oxidized to NAD^+ in the cytosol by reduction of pyruvate to lactate. This reaction is catalyzed by lactate dehydrogenase (LDH) (Fig. 22.9). The net reaction for anaerobic glycolysis is:

$$Glucose + 2\ ADP + 2\ P_i \rightarrow 2\ Lactate + 2\ ATP + 2\ H_2O + 2\ H^+$$

1. ENERGY YIELD OF AEROBIC VERSUS ANAEROBIC GLYCOLYSIS

In both aerobic and anaerobic glycolysis, each mole of glucose generates 2 moles of ATP, 2 of NADH and 2 of pyruvate. The energy yield from anaerobic glycolysis (glucose to 2 lactate) is only 2 moles of ATP per mole of glucose, as the NADH is recycled to NAD^+ by reducing pyruvate to lactate. Neither the NADH nor pyruvate produced is thus used for further energy generation. However, when oxygen is available, and cytosolic NADH can be oxidized via a shuttle system, pyruvate can also enter the mitochondria and be completely oxidized to CO_2 via PDH and the TCA cycle. The oxidation of pyruvate via this route generates roughly 12.5 moles of ATP per mole of pyruvate. If the cytosolic NADH is oxidized by the glycerol 3-P shuttle, approximately 1.5 moles of ATP are produced per NADH. If, instead, the NADH is oxidized by the malate–aspartate shuttle, approximately 2.5 moles are produced. Thus, the two NADH molecules produced during glycolysis can lead to 3 to 5 molecules of ATP being produced, depending on which shuttle system is used to transfer the reducing equivalents. Because each pyruvate produced can give rise to 12.5 molecules of ATP, altogether 30 to 32 molecules of ATP can be produced from one mole of glucose oxidized to carbon dioxide.

In response to the hypoxemia caused by **Lopa Fusor's** COPD, she has increased hypoxia-inducible factor-1 (HIF-1) in her tissues. HIF-1 is a gene transcription factor found in tissues throughout the body (including brain, heart, kidney, lung, liver, pancreas, skeletal muscle, and white blood cells) that plays a homeostatic role in coordinating tissue responses to hypoxia. Each tissue will respond with a subset of the following changes. HIF-1 increases transcription of the genes for many of the glycolytic enzymes, including PFK-1, enolase, phosphoglycerate kinase, and lactate dehydrogenase. HIF-1 also increases synthesis of a number of proteins that enhance oxygen delivery to tissues, including erythropoietin, which increases the generation of red blood cells in bone marrow; vascular endothelial growth factor, which regulates angiogenesis (formation of blood vessels); and inducible nitric oxide synthase, which synthesizes nitric oxide, a vasodilator. As a consequence, Mrs. Fusor was able to maintain hematocrit and hemoglobin levels that were on the high side of the normal range, and her tissues had an increased capacity for anaerobic glycolysis.

To produce the same amount of ATP per unit time from anaerobic glycolysis as from the complete aerobic oxidation of glucose to CO_2, anaerobic glycolysis must occur approximately 15 times faster, and use approximately 15 times more glucose. Cells achieve this high rate of glycolysis by expressing high levels of glycolytic enzymes. In certain skeletal muscles and in most cells during hypoxic crises, high rates of glycolysis are associated with rapid degradation of internal glycogen stores to supply the required glucose-6-P.

2. ACID PRODUCTION IN ANAEROBIC GLYCOLYSIS

Anaerobic glycolysis results in acid production in the form of H^+. Glycolysis forms pyruvic acid, which is reduced to lactic acid. At an intracellular pH of 7.35, lactic acid dissociates to form the carboxylate anion, lactate, and H^+ (the pKa for lactic acid is 3.85). Lactate and the H^+ are both transported out of the cell into interstitial fluid by a transporter on the plasma membrane and eventually diffuse into the blood. If the amount of lactate generated exceeds the buffering capacity of the blood, the pH drops below the normal range, resulting in lacticacidosis (see Chapter 4).

3. TISSUES DEPENDENT ON ANAEROBIC GLYCOLYSIS

Many tissues, including red and white blood cells, the kidney medulla, the tissues of the eye, and skeletal muscles, rely on anaerobic glycolysis for at least a portion of their ATP requirements (Table 22.1). Tissues (or cells) that are heavily dependent on anaerobic glycolysis usually have a low ATP demand, high levels of glycolytic enzymes, and few capillaries, such that oxygen must diffuse over a greater distance to reach target cells. The lack of mitochondria, or the increased rate of glycolysis, is often related to some aspect of cell function. For example, the mature red blood cell has no mitochondria because oxidative metabolism might interfere with its function in transporting oxygen bound to hemoglobin. Some of the lactic acid generated by anaerobic glycolysis in skin is secreted in sweat, where it acts as an antibacterial agent. Many large tumors use anaerobic glycolysis for ATP production, and lack capillaries in their core.

In tissues with some mitochondria, both aerobic and anaerobic glycolysis occur simultaneously. The relative proportion of the two pathways depends on the mitochondrial oxidative capacity of the tissue and its oxygen supply and may vary between cell types within the same tissue because of cell distance from the capillaries. When a cell's energy demand exceeds the capacity of the rate of the electron transport chain and oxidative phosphorylation to produce ATP, glycolysis is activated, and the increased NADH/NAD^+ ratio will direct excess pyruvate into lactate. Because under these conditions pyruvate dehydrogenase, the TCA cycle, and the electron transport chain are operating as fast as they can, anaerobic glycolysis is meeting the need for additional ATP.

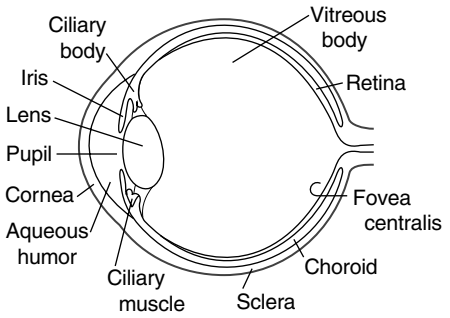
The dental caries in **Ivan Applebod's** mouth were caused principally by the low pH generated from lactic acid production by oral bacteria. Below a pH of 5.5, decalcification of tooth enamel and dentine occurs. Lactobacilli and *S. mutans* are major contributors to this process because almost all of their energy is derived from the conversion of glucose or fructose to lactic acid, and they are able to grow well at the low pH generated by this process. Mr. Applebod's dentist explained that bacteria in his dental plaque could convert all the sugar in his candy into acid in less than 20 minutes. The acid is buffered by bicarbonate and other buffers in saliva, but saliva production decreases in the evening. Thus, the acid could dissolve the hydroxyapatite in his tooth enamel during the night.

Table 22.1. Major Tissue Sites of Lactate Production in a Resting Man. An average 70-kg man consumes about 300 g of carbohydrate per day.

Daily Lactate Production (g/day)	
Total lactate production	115
Red blood cells	29
Skin	20
Brain	17
Skeletal muscle	16
Renal medulla	15
Intestinal muscosa	8
Other tissues	10

A: In the complete oxidation of pyruvate to carbon dioxide, four steps generate NADH (pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase). One step generates FAD(2H) (succinate dehydrogenase), and one substrate level phosphorylation (succinate thiokinase). Thus, because each NADH generates 2.5 ATPs, the overall contribution by NADH is 10 ATP molecules. The FAD(2H) generates an additional 1.5 ATP, and the substrate-level phosphorylation provides one more. Therefore, 10 + 1.5 + 1 = 12.5 molecules of ATP.

The tissues of the eye are also partially dependent on anaerobic glycolysis.



The eye contains cells that transmit or focus light, and these cells cannot, therefore, be filled with opaque structures such as mitochondria, or densely packed capillary beds. The corneal epithelium generates most of its ATP aerobically from its few mitochondria but still metabolizes some glucose anaerobically. Oxygen is supplied by diffusion from the air. The lens of the eye is composed of fibers that must remain birefringent to transmit and focus light, so mitochondria are nearly absent. The small amount of ATP required (principally for ion balance) can readily be generated from anaerobic glycolysis even though the energy yield is low. The lens is able to pick up glucose and release lactate into the vitreous body and aqueous humor. It does not need oxygen and has no use for capillaries.

Lactate dehydrogenase (LDH) is a tetramer composed of A subunits (also called M for skeletal muscle form) and B subunits (also called H for heart). Different tissues produce different amounts of the two subunits, which then combine randomly to form five different tetramers (M_4, M_3H_1, M_2H_2, M_1H_3, and H_4). These isoenzymes differ only slightly in their properties, with the kinetic properties of the M_4 form facilitating conversion of pyruvate to lactate in skeletal muscle and the H_4 form facilitating conversion of lactate to pyruvate in the heart.

4. FATE OF LACTATE

Lactate released from cells undergoing anaerobic glycolysis is taken up by other tissues (primarily the liver, heart, and skeletal muscle) and oxidized back to pyruvate. In the liver, the pyruvate is used to synthesize glucose (gluconeogenesis), which is returned to the blood. The cycling of lactate and glucose between peripheral tissues and liver is called the Cori cycle (Fig. 22.10).

In many other tissues, lactate is oxidized to pyruvate, which is then oxidized to CO_2 in the TCA cycle. Although the equilibrium of the lactate dehydrogenase reaction favors lactate production, flux occurs in the opposite direction if NADH is being rapidly oxidized in the electron transport chain (or being used for gluconeogenesis):

$$\text{Lactate} + NAD^+ \rightarrow \text{Pyruvate} + NADH + H^+$$

The heart, with its huge mitochondrial content and oxidative capacity, is able to use lactate released from other tissues as a fuel. During an exercise such as bicycle riding, lactate released into the blood from skeletal muscles in the leg might be used by resting skeletal muscles in the arm. In the brain, glial cells and astrocytes produce lactate, which is used by neurons or released into the blood.

II. OTHER FUNCTIONS OF GLYCOLYSIS

Glycolysis, in addition to providing ATP, generates precursors for biosynthetic pathways (Fig. 22.11). Intermediates of the pathway can be converted to ribose 5-phosphate, the sugar incorporated into nucleotides such as ATP. Other sugars, such as UDP-glucose, mannose, and sialic acid, are also formed from intermediates of glycolysis. Serine is synthesized from 3-phosphoglycerate, and alanine from pyruvate. The backbone of triacylglycerols, glycerol 3-phosphate, is derived from dihydroxyacetone phosphate in the glycolytic pathway.

The liver is the major site of biosynthetic reactions in the body. In addition to those pathways mentioned previously, the liver synthesizes fatty acids from the pyruvate generated by glycolysis. It also synthesizes glucose from lactate, glycerol 3-phosphate, and amino acids in the gluconeogenic pathway, which is principally a reversal of glycolysis. Consequently, in liver, many of the glycolytic enzymes exist as isoenzymes with properties suited for these functions.

The bisphosphoglycerate shunt is a "side reaction" of the glycolytic pathway in which 1,3-bis-phosphoglycerate is converted to 2,3-bis-phosphoglycerate (2,3-BPG). Red blood cells form 2,3-BPG to serve as an allosteric inhibitor of oxygen binding to heme (see Chapter 44). 2,3-BPG reenters the glycolytic pathway via dephosphorylation to 3-phosphoglycerate. 2,3-BPG also functions as a coenzyme in the conversion of 3-phosphoglycerate to 2-phosphoglycerate by the glycolytic

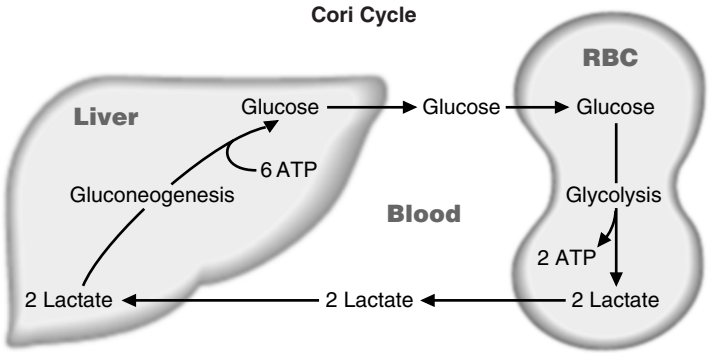


Fig. 22.10. Cori cycle. Glucose, produced in the liver by gluconeogenesis, is converted by glycolysis in muscle, red blood cells, and many other cells, to lactate. Lactate returns to the liver and is reconverted to glucose by gluconeogenesis.

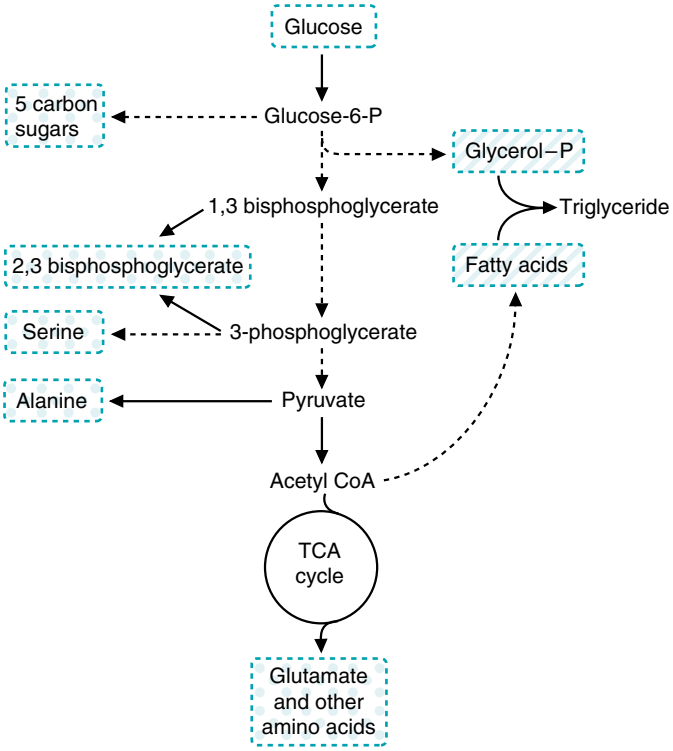


Fig. 22.11. Biosynthetic functions of glycolysis. Compounds formed from intermediates of glycolysis are shown in blue. These pathways are discussed in subsequent chapters of the book. Dotted lines indicate that more than one step is required for the conversion shown in the figure.

enzyme phosphoglyceromutase. Because 2,3-BPG is not depleted by its role in this catalytic process, most cells need only very small amounts.

III. REGULATION OF GLYCOLYSIS BY THE NEED FOR ATP

One of the major functions of glycolysis is the generation of ATP, and, therefore, the pathway is regulated to maintain ATP homeostasis in all cells. Phosphofructokinase-1 (PFK-1) and pyruvate dehydrogenase (PDH), which links glycolysis and the TCA cycle, are both major regulatory sites that respond to feedback indicators of the rate of ATP utilization (Fig. 22.12). The supply of glucose-6-P for glycolysis is tissue dependent and can be regulated at the steps of glucose transport into cells, glycogenolysis (the degradation of glycogen to form glucose), or the rate of glucose phosphorylation by hexokinase isoenzymes. Other regulatory mechanisms integrate the ATP-generating role of glycolysis with its anabolic roles.

All of the regulatory enzymes of glycolysis exist as tissue-specific isoenzymes, which alter the regulation of the pathway to match variations in conditions and needs in different tissues. For example, in the liver, an isoenzyme of pyruvate kinase introduces an additional regulatory site in glycolysis that contributes to the inhibition of glycolysis when the reverse pathway, gluconeogenesis, is activated.

A. Relationship between ATP, ADP, and AMP Concentrations

The AMP levels within the cytosol provide a better indicator of the rate of ATP utilization than the ATP concentration itself (Fig. 22.13). The concentration of AMP

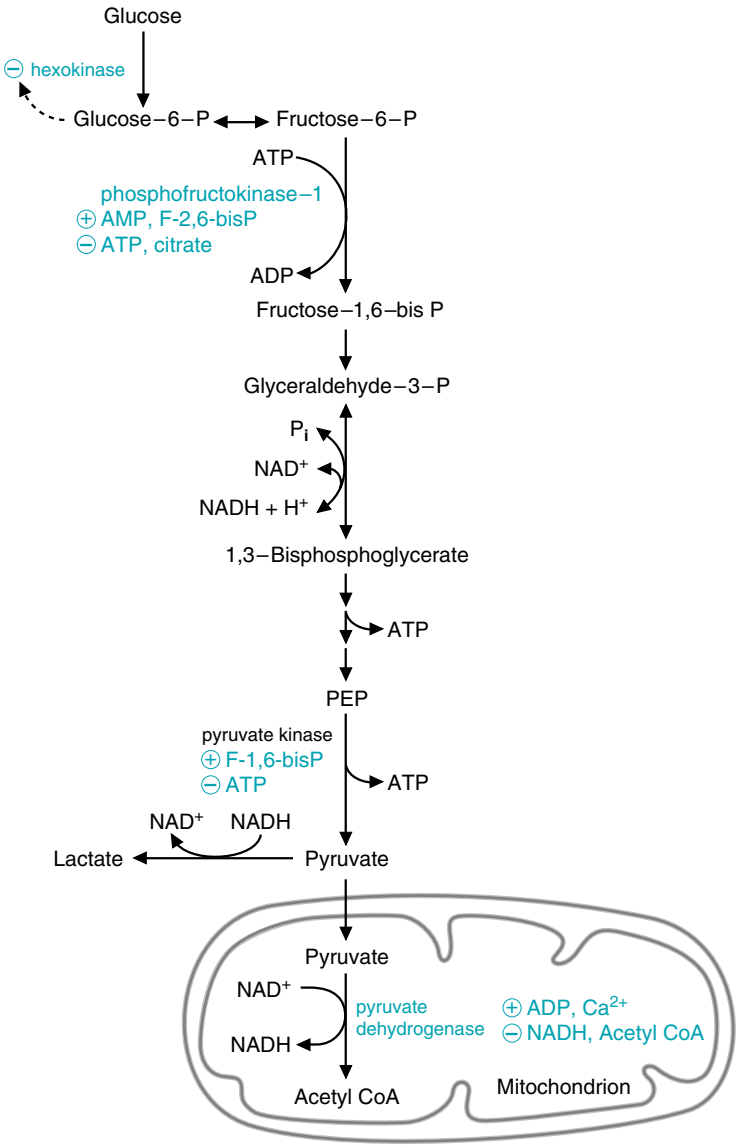


Fig. 22.12. Major sites of regulation in the glycolytic pathway. Hexokinase and phosphofructokinase-1 are the major regulatory enzymes in skeletal muscle. The activity of pyruvate dehydrogenase in the mitochondrion determines whether pyruvate is converted to lactate or to acetyl CoA. The regulation shown for pyruvate kinase only occurs for the liver (L) isoenzyme.

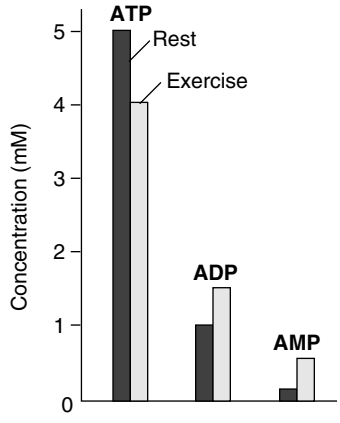


Fig. 22.13. Changes in ATP, ADP, and AMP concentrations in skeletal muscle during exercise. The concentration of ATP decreases by only approximately 20% during exercise, and the concentration of ADP rises. The concentration of AMP, produced by the adenylate kinase reaction, increases manyfold and serves as a sensitive indicator of decreasing ATP levels.

in the cytosol is determined by the equilibrium position of the adenylate kinase reaction.

$$2\ \text{ADP} \overset{\text{adenylate kinase}}{\leftrightarrow} \text{AMP} + \text{ATP}$$

The equilibrium is such that hydrolysis of ATP to ADP in energy-requiring reactions increases both the ADP and AMP contents of the cytosol. However, ATP is present in much higher quantities than AMP or ADP, so that a small decrease of ATP concentration in the cytosol causes a much larger percentage increase in the small AMP pool. In skeletal muscles, for instance, ATP levels are approximately 5 mM and decrease by no more than 20% during strenuous exercise (see Fig. 22.13). At the same time, ADP levels may increase by 50%, and AMP levels, which are in

the micromolar range, increase by 300%. AMP activates a number of metabolic pathways, including glycolysis, glycogenolysis, and fatty acid oxidation (particularly in muscle tissues), to ensure that ATP homeostasis is maintained.

B. Regulation of Hexokinases

Hexokinases exist as tissue-specific isoenzymes whose regulatory properties reflect the role of glycolysis in different tissues. In most tissues, hexokinase is a low-K_m enzyme with a high affinity for glucose (see Chapter 9). It is inhibited by physiologic concentrations of its product, glucose-6-P (see Fig. 22.12). If glucose-6-P does not enter glycolysis or another pathway, it accumulates and decreases the activity of hexokinase. In the liver, the isoenzyme glucokinase is a high-K_m enzyme that is not readily inhibited by glucose-6-P. Thus, glycolysis can continue in liver even when energy levels are high so that anabolic pathways, such as the synthesis of the major energy storage compounds, glycogen and fatty acids, can occur.

C. Regulation of PFK-1

Phosphofructokinase-1 (PFK-1) is the rate-limiting enzyme of glycolysis and controls the rate of glucose-6-P entry into glycolysis in most tissues. PFK-1 is an allosteric enzyme that has a total of six binding sites: two are for substrates (Mg-ATP and fructose-6-P) and four are allosteric regulatory sites (see Fig. 22.12). The allosteric regulatory sites occupy a physically different domain on the enzyme than the catalytic site. When an allosteric effector binds, it changes the conformation at the active site and may activate or inhibit the enzyme (see also Chapter 9). The allosteric sites for PFK-1 include an inhibitory site for MgATP, an inhibitory site for citrate and other anions, an allosteric activation site for AMP, and an allosteric activation site for fructose 2,6-bisphosphate (fructose-2,6-bisP) and other bisphosphates. Several different tissue-specific isoforms of PFK-1 are affected in different ways by the concentration of these substrates and allosteric effectors, but all contain these four allosteric sites.

1. ALLOSTERIC REGULATION OF PFK-1 BY AMP AND ATP

ATP binds to two different sites on the enzyme, the substrate binding site and an allosteric inhibitory site. Under physiologic conditions in the cell, the ATP concentration is usually high enough to saturate the substrate binding site and inhibit the enzyme by binding to the ATP allosteric site. This effect of ATP is opposed by AMP, which binds to a separate allosteric activator site (Figure 22.14). For most of the PFK-1 isoenzymes, the binding of AMP increases the affinity of the enzyme for fructose 6-P (e.g., shifts the kinetic curve to the left). Thus, increases in AMP concentration can greatly increase the rate of the enzyme (see Fig. 22.14), particularly when fructose-6-P concentrations are low.

2. REGULATION OF PFK-1 BY FRUCTOSE 2,6-BISPHOSPHATE

Fructose-2,6-bisP is also an allosteric activator of PFK-1 that opposes the ATP inhibition. Its effect on the rate of activity of PFK-1 is qualitatively similar to that of AMP, but it has a separate binding site. Fructose-2,6-bisP is NOT an intermediate

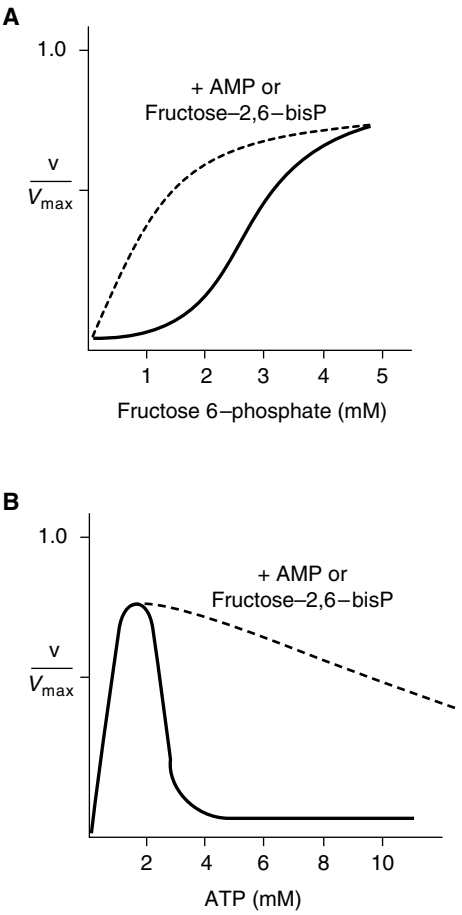


Fig. 22.14. Regulation of PFK-1 by AMP, ATP and fructose-2,6-bisP. **A.** AMP and fructose 2,6-bisphosphate activate PFK-1. **B.** ATP increases the rate of the reaction at low concentrations, but allosterically inhibits the enzyme at high concentrations.

Otto Shape has started high-intensity exercise that will increase the production of lactate in his exercising skeletal muscles. In skeletal muscles, the amount of aerobic versus anaerobic glycolysis that occurs varies with intensity of the exercise, with duration of the exercise, with the type of skeletal muscle fiber involved, and with the level of training. Human skeletal muscles are usually combinations of type I fibers (called fast glycolytic fibers, or white muscle fibers) and type IIb fibers (called slow oxidative fibers, or red muscle fibers). The designation of fast or slow refers to their rate of shortening, which is determined by the isoenzyme of myosin ATPase present. Compared with glycolytic fibers, oxidative fibers have a higher content of mitochondria and myoglobin, which gives them a red color. The gastrocnemius, a muscle in the leg used for running, has a high content of type IIb fibers. However, these fibers will still produce lactate during sprints when the ATP demand exceeds their oxidative capacity.

PFK-1 exists as a group of tissue-specific isoenzymes whose regulatory features match the role of glycolysis in different tissues. Three different types of PFK-1 isoenzyme subunits exist: M (muscle), L (liver), and C. The three subunits show variable expression in different tissues, with some tissues having more than one type. For example, mature human muscle expresses only the M subunit, the liver expresses principally the L subunit, and erythrocytes express both the M and the L subunits. The C subunit is present in highest levels in platelets, placenta, kidney, and fibroblasts but is relatively common to most tissues. Both the M and L subunits are sensitive to AMP and ATP regulation, but the C subunits are much less so. Active PFK-1 is a tetramer, composed of four subunits. Within muscle, the M4 form predominates but within tissues that express multiple isoenzymes of PFK-1 heterotetramers can form that have full activity.

Under ischemic conditions, AMP levels within the heart rapidly increase because of the lack of ATP production via oxidative phosphorylation. The increase in AMP levels activates an AMP-dependent protein kinase (protein kinase B), which phosphorylates the heart isoenzyme of PFK-2 to activate its kinase activity. This results in increased levels of fructose-2,6-bisP, which activates PFK-1 along with AMP such that the rate of glycolysis can increase to compensate for the lack of ATP production via aerobic means.

During **Cora Nari**'s myocardial infarction (see Chapter 20), her heart had a limited supply of oxygen and blood-borne fuels. The absence of oxygen for oxidative phosphorylation would decrease the levels of ATP and increase those of AMP, an activator of PFK-1 and the AMP-dependent protein kinase, resulting in a compensatory increase of anaerobic glycolysis and lactate production. However, obstruction of a vessel leading to her heart would decrease lactate removal, resulting in a decrease of intracellular pH. Under these conditions, at very low pH levels, glycolysis is inhibited and unable to compensate for the lack of oxidative phosphorylation.

of glycolysis but is synthesized by an enzyme that phosphorylates fructose 6-phosphate at the 2 position. The enzyme is therefore named phosphofructokinase-2 (PFK-2); it is a bifunctional enzyme with two separate domains, a kinase domain and a phosphatase domain. At the kinase domain, fructose-6-P is phosphorylated to fructose-2,6-bisP and at the phosphatase domain, fructose-2,6-bisP is hydrolyzed back to fructose-6-P. PFK-2 is regulated through changes in the ratio of activity of the two domains. For example, in skeletal muscles, high concentrations of fructose-6-P activate the kinase and inhibit the phosphatase, thereby increasing the concentration of fructose-2,6-bisP and activating glycolysis.

PFK-2 also can be regulated through phosphorylation by serine/threonine protein kinases. The liver isoenzyme contains a phosphorylation site near the amino terminal that decreases the activity of the kinase and increases the phosphatase activity. This site is phosphorylated by the cAMP-dependent protein kinase (protein kinase A) and is responsible for decreased levels of liver fructose-2,6-bisP during fasting conditions (as modulated by circulating glucagon levels, which is discussed in detail in Chapters 26 and 31). The cardiac isoenzyme contains a phosphorylation site near the carboxy terminal that can be phosphorylated in response to adrenergic activators of contraction (such as norepinephrine) and by increased AMP levels. Phosphorylation at this site increases the kinase activity and increases fructose-2, 6-bisP levels, thereby contributing to the activation of glycolysis.

3. ALLOSTERIC INHIBITION OF PFK-1 AT THE CITRATE SITE

The function of the citrate–anion allosteric site is to integrate glycolysis with other pathways. For example, the inhibition of PFK-1 by citrate may play a role in decreasing glycolytic flux in the heart during the oxidation of fatty acids.

D. Regulation of Pyruvate Kinase

Pyruvate kinase exists as tissue-specific isoenzymes. The form present in brain and muscle contains no allosteric sites, and pyruvate kinase does not contribute to the regulation of glycolysis in these tissues. However, the liver isoenzyme can be inhibited through phosphorylation by the cAMP-dependent protein kinase, and by a number of allosteric effectors that contribute to the inhibition of glycolysis during fasting conditions. These allosteric effectors include activation by fructose-1,6-bisP, which ties the rate of pyruvate kinase to that of PFK-1, and inhibition by ATP, which signifies high energy levels.

E. Pyruvate Dehydrogenase Regulation and Glycolysis

Pyruvate dehydrogenase is also regulated principally by the rate of ATP utilization (see Chapter 20) through rapid phosphorylation to an inactive form. Thus, in a normal respiring cell, with an adequate supply of O_2, glycolysis and the TCA cycle are activated together, and glucose can be completely oxidized to CO_2. However, when tissues do not have an adequate supply of O_2 to meet their ATP demands, the increased NADH/NAD^+ ratio inhibits pyruvate dehydrogenase, but AMP activates glycolysis. A proportion of the pyruvate will then be reduced to lactate to allow glycolysis to continue.

IV. LACTIC ACIDEMIA

Lactate production is a normal part of metabolism. In the absence of disease, elevated lactate levels in the blood are associated with anaerobic glycolysis during exercise. In lactic acidosis, lactic acid accumulates in blood to levels that significantly affect the pH (lactate levels greater than 5 mM and a decrease of blood pH below 7.2).

Lactic acidosis generally results from a greatly increased NADH/NAD^+ ratio in tissues (Fig.22.15). The increased NADH concentration prevents pyruvate oxidation in the TCA cycle and directs pyruvate to lactate. To compensate for the decreased ATP production from oxidative metabolism, PFK-1, and, therefore, the entire glycolytic pathway is activated. For example, consumption of high amounts of alcohol, which is rapidly oxidized in the liver and increases NADH levels, can result in a lactic acidosis. Hypoxia in any tissue increases lactate production as cells attempt to compensate for a lack of O_2 for oxidative phosphorylation.

A number of other problems that interfere either with the electron transport chain or pyruvate oxidation in the TCA cycle result in lactic acidemia (see Fig.22.15). For example, OXPHOS diseases (inherited deficiencies in subunits of complexes in the electron transport chain, such as MERFF) increase the NADH/NAD^+ ratio and

Q: Lactate and pyruvate are in equilibrium in the cell, and the ratio of lactate to pyruvate reflects the NADH/NAD^+ ratio. Both acids are released into blood, and the normal ratio of lactate to pyruvate in blood is approximately 25:1. This ratio can provide a useful clinical diagnostic tool. Because lactic acidemia can be the result of a number of problems, such as hypoxia, MERFF, thiamine deficiency, and pyruvate dehydrogenase deficiency, under which of these conditions would you expect the *lactate/pyruvate ratio* in blood to be much greater than normal?

Lopa Fusor had a decreased arterial pO_2 and elevated arterial pCO_2 caused by underperfusion of her lungs. The elevated CO_2 content resulted in an increase of H_2CO_3 and acidity of the blood (see Chapter 4). The decreased O_2 delivery to tissues resulted in increased lactate production from anaerobic glycolysis, and an elevation of serum lactate to 10 times normal levels. The reduction in her arterial pH to 7.18 (reference range, 7.35–7.45) resulted, therefore, from both a mild respiratory acidosis (elevated pCO_2) and a more profound metabolic acidosis (elevated serum lactate level).

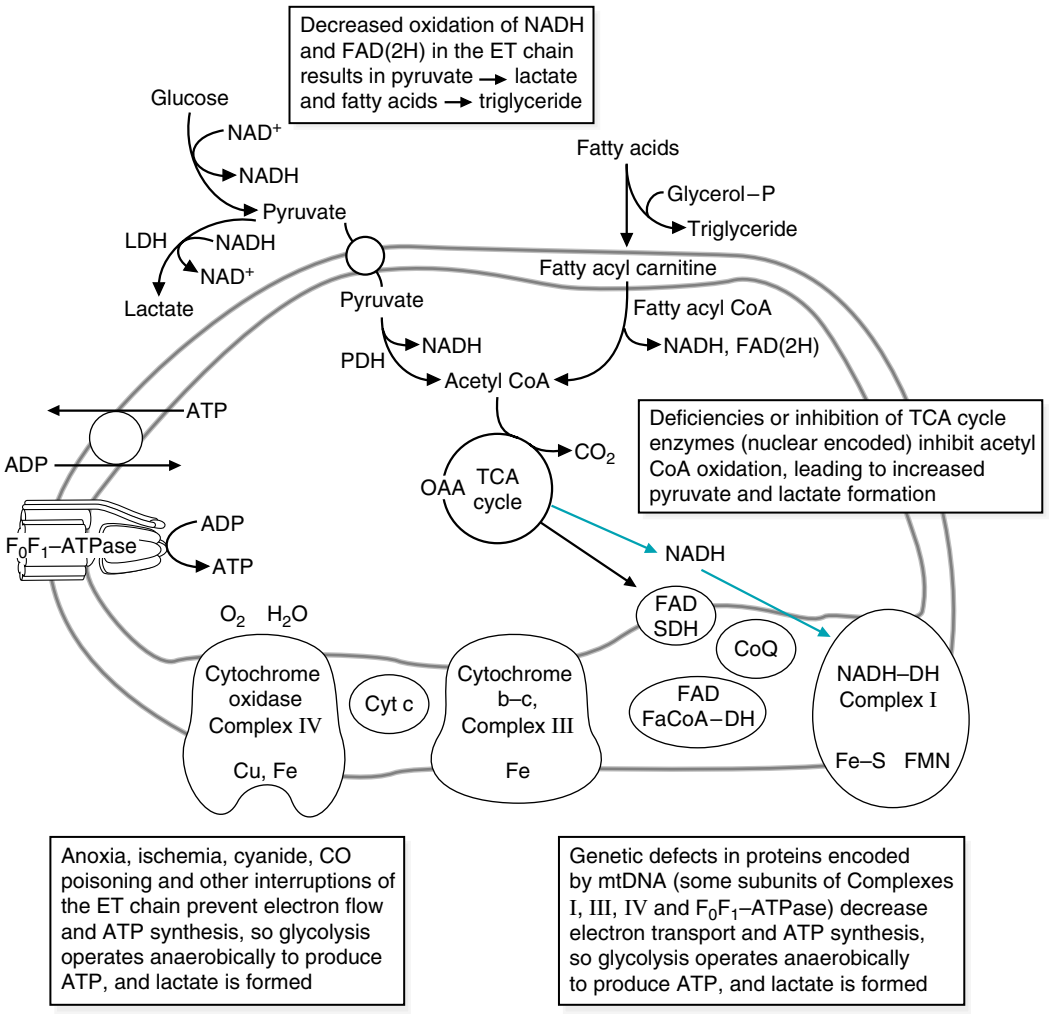


Fig. 22.15. Pathways leading to lactic acidemia.

A: Hypoxia and inherited deficiencies of subunits in the electron transport chain impair NADH oxidation, resulting in a higher NADH/NAD^+ ratio in the cell, and, therefore, a higher lactate/pyruvate ratio in blood. In contrast, conditions that cause lactic acidemia as a result of defects in the enzymes of pyruvate metabolism (thiamine deficiency or pyruvate dehydrogenase deficiency) would increase both pyruvate and lactate in the blood and have little effect on the ratio.

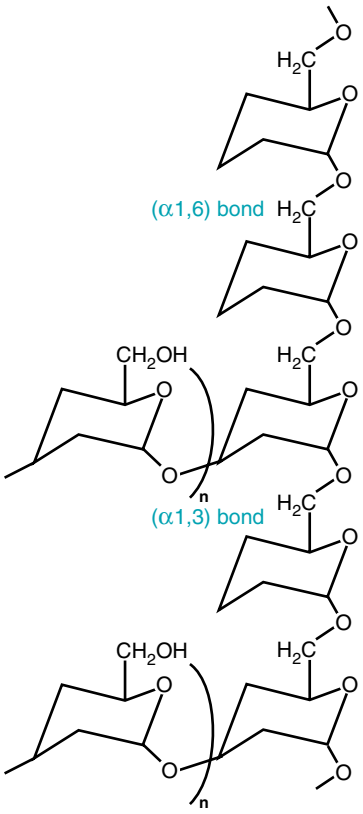


Fig. 22.16. General structure of dextran. Glucosyl residues are linked by α-1,3, α-1,6, and some α-1,4 bonds.

inhibit PDH (see Chapter 21). Impaired PDH activity from an inherited deficiency of E_1 (the decarboxylase subunit of the complex), or from severe thiamine deficiency, increases blood lactate levels (see Chapter 20). Pyruvate carboxylase deficiency also can result in lactic acidosis (see Chapter 20), because of an accumulation of pyruvate.

Lactic acidosis can also result from inhibition of lactate utilization in gluconeogenesis (e.g., hereditary fructose intolerance, which is due to a defective aldolase gene). If other pathways that use glucose-6-P are blocked, glucose-6-P can be shunted into glycolysis and lactate production (e.g., glucose 6-phosphatase deficiency).

CLINICAL COMMENTS

Lopa Fusor was admitted to he hospital with severe hypotension caused by an acute hemorrhage. Her plasma lactic acid level was elevated and her arterial pH was low. The underlying mechanism for Ms. Fusor's derangement in acid-base balance is a severe reduction in the amount of oxygen delivered to her tissues for cellular respiration (hypoxemia). Several concurrent processes contributed to this oxygen lack. The first was her severely reduced blood pressure caused by a brisk hemorrhage from a bleeding gastric ulcer. The blood loss led to hypoperfusion and, therefore, reduced delivery of oxygen to her tissues. The marked reduction in the number of red blood cells in her circulation caused by blood loss further compromised oxygen delivery. The preexisting chronic obstructive pulmonary disease (COPD) added to her hypoxemia by decreasing her ventilation, and, therefore, the transfer of oxygen to her blood (low pO_2). In addition, her COPD led to retention of carbon dioxide (high pCO_2), which caused a respiratory acidosis because the retained CO_2 interacted with water to form carbonic acid (H_2CO_3), which dissociates to H^+ and bicarbonate.

In skeletal muscles, lactate production occurs when the need for ATP exceeds the capacity of the mitochondria for oxidative phosphorylation. Thus, increased lactate production accompanies an increased rate of the TCA cycle. The extent to which skeletal muscles use aerobic versus anaerobic glycolysis to supply ATP varies with the intensity of exercise. At low-intensity exercise, the rate of ATP utilization is lower, and fibers can generate this ATP from oxidative phosphorylation, with the complete oxidation of glucose to CO_2. However, when **Otto Shape** sprints, a high-intensity exercise, the ATP demand exceeds the rate at which the electron transport chain and TCA cycle can generate ATP from oxidative phosphorylation. The increased AMP level signals the need for additional ATP and stimulates PFK-1. The NADH/NAD^+ ratio directs the increase in pyruvate production toward lactate. The fall in pH causes muscle fatigue and pain. As he trains, the amount of mitochondria and myoglobin will increase in his skeletal muscle fibers, and these fibers will rely less on anaerobic glycolysis.

Ivan Applebod had two sites of dental caries: one on a smooth surface and one in a fissure. The decreased pH resulting from lactic acid production by lactobacilli, which grow anaerobically within the fissure, is a major cause of fissure caries. *Streptococs mutans (S. mutans)* plays a major role in smooth surface caries because it secretes dextran, an insoluble polysaccharide, which forms the base for plaque. *S. mutans* contains dextran-sucrase, a glucosyltransferase that transfers glucosyl units from dietary sucrose (the glucose-fructose disaccharide in sugar and sweets) to form the α(1→6) and α(1→3) linkages between the glucosyl units in dextran (Fig. 22.16). Dextran-sucrase is specific for sucrose and does not catalyze the polymerization of free glucose, or glucose from other disaccharides or polysaccharides. Thus sucrose is responsible for the cariogenic potential of candy. The sticky water-insoluble dextran mediates the attachment of *S. mutans* and other

bacteria to the tooth surface. This also keeps the acids produced from these bacteria close to the enamel surface. Fructose from sucrose is converted to intermediates of glycolysis and is rapidly metabolized to lactic acid. Other bacteria present in the plaque produce different acids from anaerobic metabolism, such as acetic acid and formic acid. The decrease in pH that results initiates demineralization of the hydroxyapatite of the tooth enamel. **Ivan Applebod's** caries in his baby teeth could have been caused by sucking on bottles containing fruit juice. The sugar in fruit juice is also sucrose, and babies who fall asleep with a bottle of fruit juice in their mouth may develop caries. Rapid decay of these baby teeth can harm the development of their permanent teeth.

BIOCHEMICAL COMMENTS

How is the first high-energy bond created in the glycolytic pathway? This is the work of the glyceraldehyde 3-phosphate dehydrogenase reaction, which converts glyceraldehyde-3-P to 1,3 bisphosphglycerate. This reaction can be considered to be two separate half reactions, the first being the oxidation of glyceraldehyde-3-P to 3-phosphoglycerate, and the second the addition of inorganic phosphate to 3-phosphoglycerate to produce 1,3 bisphosphoglycerate. The $\Delta G^{0\prime}$ for the first reaction is approximately -12 kcal/mole; for the second reaction, it is approximately $+12$ kcal/mole. Thus, although the first half reaction is extremely favorable, the second half reaction is unfavorable and would not proceed under cellular conditions. So how does the enzyme help this reaction to proceed? This is accomplished through the enzyme forming a covalent bond with the substrate, using an essential cysteine residue at the active site to form a high-energy thioester linkage during the course of the reaction

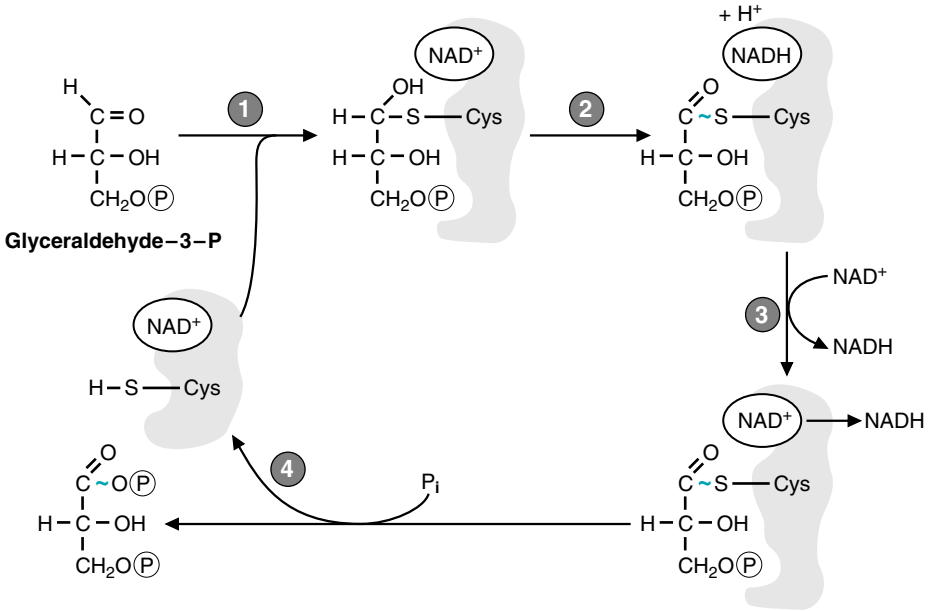


Fig. 22.17. Mechanism of the glyceraldehyde 3-phosphate dehydrogenase reaction. 1. The enzyme forms a covalent linkage with the substrate, using a cysteine group at the active site. The enzyme also contains bound NAD^+ close to the active site. 2. The substrate is oxidized, forming a high-energy thioester linkage (in blue), and NADH. 3. NADH has a low affinity for the enzyme and is replaced by a new molecule of NAD^+. 4. Inorganic phosphate attacks the thioester linkage, releasing the product 1,3 bisphosphoglycerate, and regenerating the active enzyme in a form ready to initiate another reaction.

(Fig. 22.17). Thus, the energy that would be released as heat in the oxidation of glyceraldehyde-3-P to 3-phosphoglycerate is conserved in the thioester linkage that is formed (such that the $\Delta G^{0\prime}$ of the formation of the thioester intermediate from glyceraldehyde-3-P is close to zero). Then, replacement of the sulfur with inorganic phosphate to form the final product, 1,3 bisphosphoglycerate, is relatively straightforward, as the $\Delta G^{0\prime}$ for that conversion is also close to zero, and the acylphosphate bond retains the energy from the oxidation of the aldehyde. This is one example of how covalent catalysis by an enzyme can result in the conservation of energy between different bond types.

Suggested References

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REVIEW QUESTIONS—CHAPTER 22

1. A major role of glycolysis is which of the following?

   (A) To synthesize glucose
   (B) To generate energy
   (C) To produce FAD(2H)
   (D) To synthesize glycogen
   (E) To use ATP to generate heat

2. Starting with glyceraldehyde 3-phosphate and synthesizing one molecule of pyruvate, the net yield of ATP and NADH would be which of the following?

   (A) 1 ATP, 1 NADH
   (B) 1 ATP, 2 NADH
   (C) 1 ATP, 4 NADH
   (D) 2 ATP, 1 NADH
   (E) 2 ATP, 2 NADH
   (F) 2 ATP, 4 NADH
   (G) 3 ATP, 1 NADH
   (H) 3 ATP, 2 NADH
   (I) 3 ATP, 4 NADH

3. When glycogen is degraded, glucose 1-phosphate is formed. Glucose 1-phosphate can then be isomerized to glucose 6-phosphate. Starting with glucose 1-phosphate, and ending with 2 molecules of pyruvate, what is the net yield of glycolysis, in terms of ATP and NADH formed?

   (A) 1 ATP, 1 NADH
   (B) 1 ATP, 2 NADH
   (C) 1 ATP, 3 NADH
   (D) 2 ATP, 1 NADH
   (E) 2 ATP, 2 NADH
   (F) 2 ATP, 3 NADH
   (G) 3 ATP, 1 NADH
   (H) 3 ATP, 2 NADH
   (I) 3 ATP, 3 NADH

4. Which of the following statements correctly describes an aspect of glycolysis?

   (A) ATP is formed by oxidative phosphorylation.
   (B) 2 ATP are used in the beginning of the pathway.
   (C) Pyruvate kinase is the rate-limiting enzyme.
   (D) One pyruvate and three CO_2 are formed from the oxidation of one glucose molecule.
   (E) The reactions take place in the matrix of the mitochondria.

5. How many moles of ATP are generated by the complete aerobic oxidation of 1 mole of glucose to 6 moles of CO_2?

   (A) 2–4
   (B) 10–12
   (C) 18–22
   (D) 30–32
   (E) 60–64