chapter 14

THE CITRIC ACID CYCLE

[Tom Merton/OJO Images/Getty Images, Inc.]

▸▸ WHERE does exhaled CO_2 come from?

Inflating a toy balloon is one way to capture exhaled breath, which contains CO_2. It is tempting to believe that in air-breathing animals, the oxygen that is inhaled is transformed into carbon dioxide that is exhaled. In fact, the two types of molecules never directly interact inside the body. In this chapter we will see that exhaled CO_2, a waste product of cellular metabolism, is generated mostly by operation of the citric acid cycle. This metabolic pathway converts the carbons of metabolic fuels into CO_2, saving their energy for ATP synthesis.

THIS CHAPTER IN CONTEXT

Part 1 Foundations

Part 2 Molecular Structure and Function

Part 3 Metabolism

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Part 4 Genetic Information

Do You Remember?

- **Enzymes accelerate chemical reactions using acid–base catalysis, covalent catalysis, and metal ion catalysis (Section 6-2).**
- **Coenzymes such as NAD^+ and ubiquinone collect electrons from compounds that become oxidized (Section 12-2).**
- **Metabolic pathways in cells are connected and are regulated (Section 12-2).**
- **Many vitamins, substances that humans cannot synthesize, are components of coenzymes (Section 12-2).**
- **Pyruvate can be converted to lactate, acetyl-CoA, or oxaloacetate (Section 13-1).**

The **citric acid cycle** is a pathway that occupies a central place in the metabolism of most cells. It converts two-carbon groups, in the form of acetyl-CoA, into CO_2 and therefore represents the final stage in the oxidation of metabolic fuels—not just carbohydrates but also fatty acids and amino acids (Fig. 14-1). As the carbons become fully oxidized to CO_2, their energy is conserved and subsequently used to produce ATP. The eight reactions of the citric acid cycle take place in the cytosol of prokaryotes and in the mitochondria of eukaryotes.

Unlike a linear pathway such as glycolysis (see Fig. 13-2) or gluconeogenesis (see Fig. 13-10), the citric acid cycle always returns to its starting position, essentially behaving as a multistep catalyst. However, it is still possible to follow the chemical transformations that occur at each step.

An examination of the citric acid cycle also illustrates an important feature of metabolic pathways in general, namely, that a pathway is less like an element of plumbing and more like a web or network. In other words, the pathway does not function like a simple pipeline, where one substance enters one end and another emerges from the other end. Instead, the pathway's intermediates can participate in many reactions, serving as both precursors and products of a large variety of biological molecules.

Although the carbon atoms that enter the citric acid cycle may be derived from amino acids, fatty acids, or carbohydrates, we will use pyruvate, the end product of glycolysis, as the starting point for our study of the citric acid cycle. We will examine the eight reactions of the citric acid cycle and discuss how this sequence of reactions might have evolved. Finally, we will consider the citric acid cycle as a multifunctional pathway with links to other metabolic processes.

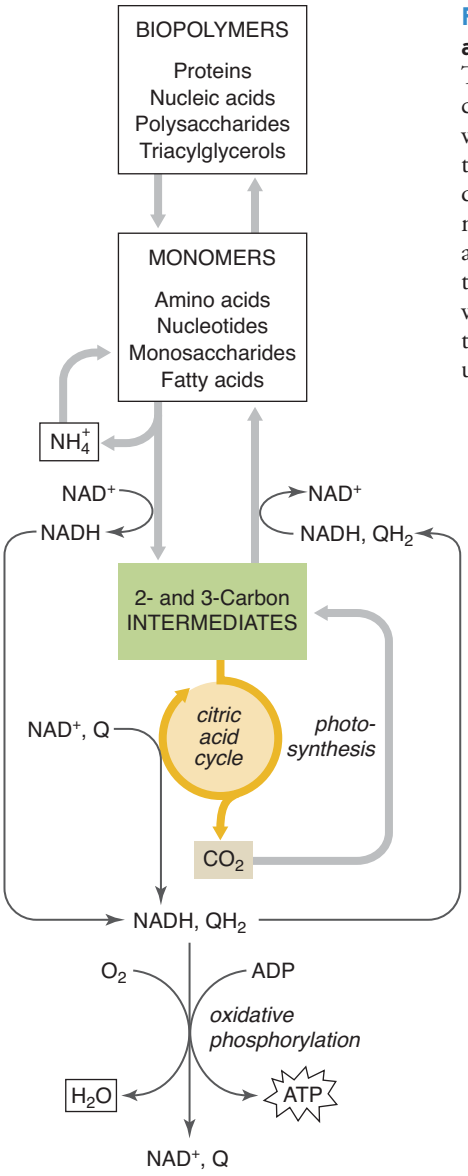


Figure 14-1 The citric acid cycle in context. The citric acid cycle is a central metabolic pathway whose starting material is two-carbon acetyl units derived from amino acids, monosaccharides, and fatty acids. These are oxidized to the waste product CO_2, with the reduction of the cofactors NAD^+ and ubiquinone (Q).

14-1 The Pyruvate Dehydrogenase Reaction

The end product of glycolysis is the three-carbon compound pyruvate. In aerobic organisms, these carbons are ultimately oxidized to 3 CO_2 (although the oxygen atoms come not from molecular oxygen but from water and phosphate). The first molecule of CO_2 is released when pyruvate is decarboxylated to an acetyl unit. The second and third CO_2 molecules are products of the citric acid cycle.

KEY CONCEPT

- The pyruvate dehydrogenase complex includes three types of enzymes that collectively remove a carboxylate group from pyruvate and produce acetyl-CoA and NADH.

The pyruvate dehydrogenase complex contains multiple copies of three different enzymes

The decarboxylation of pyruvate is catalyzed by the pyruvate dehydrogenase complex. In eukaryotes, this enzyme complex, and the enzymes of the citric acid cycle itself, are located inside the mitochondrion (an organelle surrounded by a double membrane and whose interior is called the **mitochondrial matrix**). Accordingly, pyruvate produced by glycolysis in the cytosol must first be transported into the mitochondria.

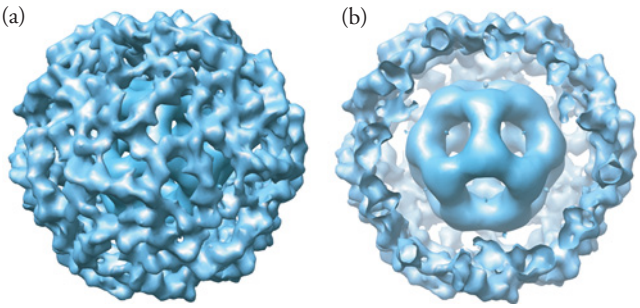


Figure 14-2 Model of the pyruvate dehydrogenase complex from *B. stearothermophilus*. These images are based on cryoelectron microscope studies of the pyruvate dehydrogenase complex. (a) Surface view. (b) Cutaway view showing the core of 60 E2 subunits. In this model the outer shell contains only E3; in the native pyruvate dehydrogenase complex, the outer shell contains both E1 and E3, which occupy similar positions. The space between the two layers of protein is about 75–90 Å. [Courtesy Jacqueline L. S. Milne and Sriram Subramaniam, National Cancer Institute, National Institutes of Health.]

For convenience, the three kinds of enzymes that make up the pyruvate dehydrogenase complex are called E1, E2, and E3. Together *they catalyze the oxidative decarboxylation of pyruvate and the transfer of the acetyl unit to coenzyme A*:

$$\text{pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH}$$

The structure of coenzyme A, a nucleotide derivative containing the vitamin pantothenate, is shown in Figure 3-3a.

In *E. coli*, the pyruvate dehydrogenase complex contains 60 protein subunits (24 E1, 24 E2, and 12 E3) and has a mass of about 4600 kD. In mammals and some other bacteria, the enzyme complex is even larger, with 42–48 E1, 60 E2, and 6–12 E3 plus additional proteins that hold the complex together and regulate its enzymatic activity. The pyruvate dehydrogenase complex from *Bacillus stearothermophilus* consists of a core of 60 E2 subunits that form a dodecahedron (a 12-sided polyhedron) surrounded by an outer protein shell (Fig. 14-2).

Pyruvate dehydrogenase converts pyruvate to acetyl-CoA

The operation of the pyruvate dehydrogenase complex requires several coenzymes, whose functional roles in the five-step reaction are described below.

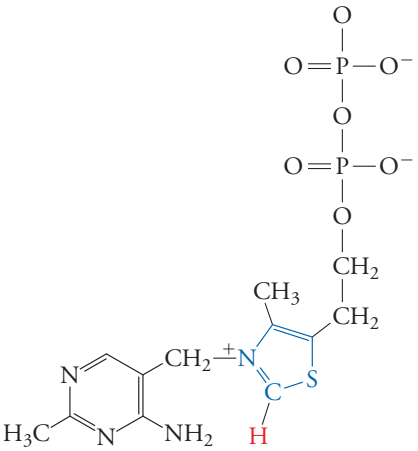
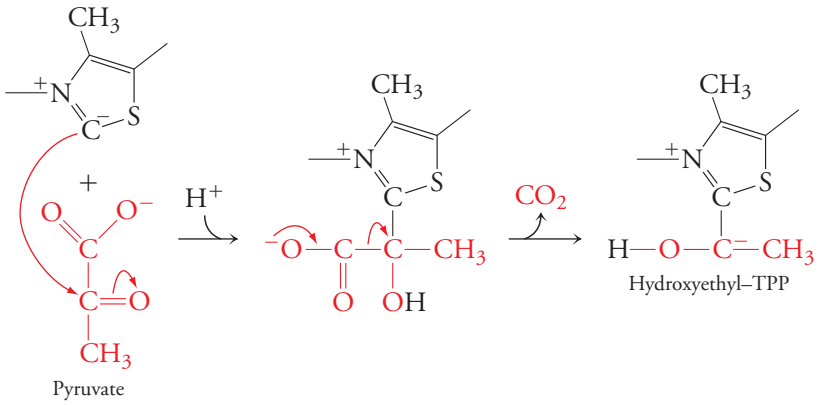
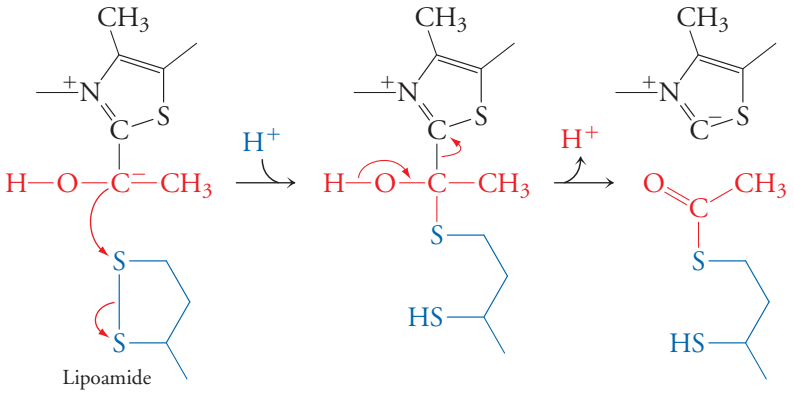


Figure 14-3 Thiamine pyrophosphate (TPP). This cofactor is the phosphorylated form of thiamine, also known as vitamin B_1 (see Section 12-2). The central thiazolium ring (blue) is the active portion. An acidic proton (red) dissociates, and the resulting carbanion is stabilized by the nearby positively charged nitrogen. TPP is a cofactor for several different decarboxylases.

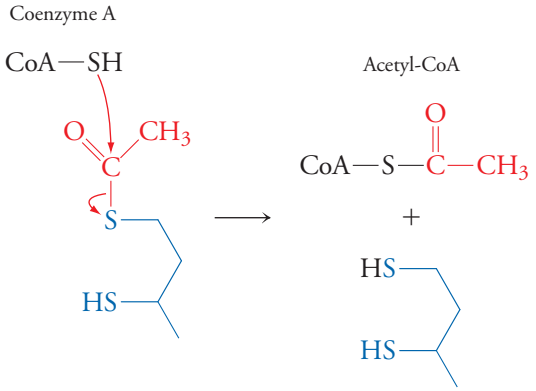
1. In the first step, which is catalyzed by E1 (also called pyruvate dehydrogenase), pyruvate is decarboxylated. This reaction requires the cofactor thiamine pyrophosphate (TPP; Fig. 14-3). TPP attacks the carbonyl carbon of pyruvate, and the departure of CO_2 leaves a hydroxyethyl group attached to TPP. This carbanion is stabilized by the positively charged thiazolium ring group of TPP:



2. The hydroxyethyl group is then transferred to E2 of the pyruvate dehydrogenase complex. The hydroxyethyl acceptor is a lipoamide prosthetic group (Fig. 14-4). The transfer reaction regenerates the TPP cofactor of E1 and oxidizes the hydroxyethyl group to an acetyl group:

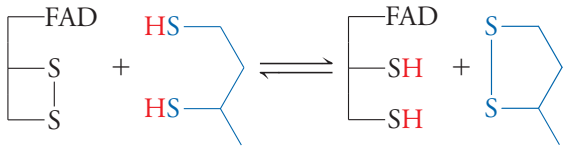


3. Next, E2 transfers the acetyl group to coenzyme A, producing acetyl-CoA and leaving a reduced lipoamide group.

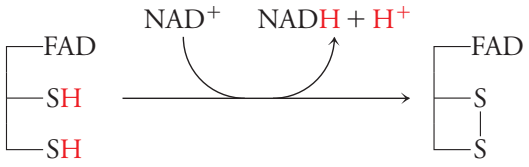


Recall that acetyl-CoA is a thioester, a form of energy currency (see Section 12-3). Some of the free energy released in the oxidation of the hydroxyethyl group to an acetyl group is conserved in the formation of acetyl-CoA.

4. The final two steps of the reaction restore the pyruvate dehydrogenase complex to its original state. E3 reoxidizes the lipoamide group of E2 by transferring electrons to a Cys–Cys disulfide group in the enzyme.



5. Finally, NAD^+ reoxidizes the reduced Cys sulfhydryl groups. This electron-transfer reaction is facilitated by an FAD prosthetic group (the structure of FAD, a nucleotide derivative, is shown in Fig. 3-3c).



During the five-step reaction (summarized in **Fig. 14-5**), the long lipoamide group of E2 acts as a swinging arm that visits the active sites of E1, E2, and E3 within the multienzyme complex. The arm picks up an acetyl group from an E1 subunit and transfers it to coenzyme A in an E2 active site. The arm then swings to an E3 active site, where it is reoxidized. Some other multienzyme complexes also include swinging arms, often attached to hinged protein domains to maximize their mobility.

A **multienzyme complex** such as the pyruvate dehydrogenase complex can carry out a multistep reaction sequence efficiently because *the product of one reaction can quickly become the substrate for the next reaction without diffusing away or reacting*

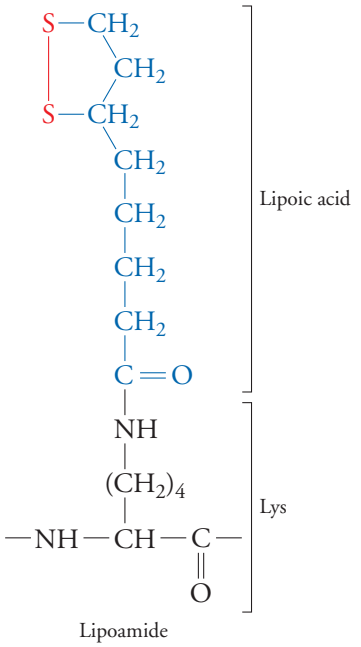
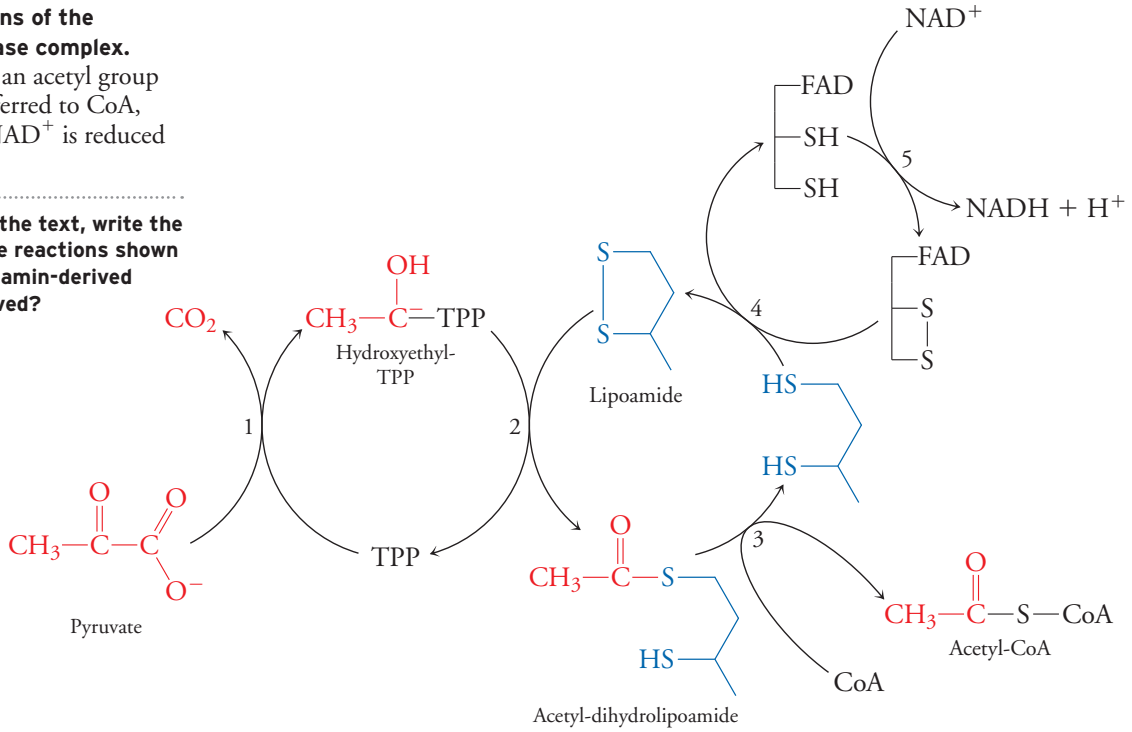


Figure 14-4 Lipoamide. This prosthetic group consists of lipoic acid (a vitamin) linked via an amide bond to the ε-amino group of a protein Lys residue. The active portion of the 14-Å-long lipoamide is the disulfide bond (red), which can be reversibly reduced.

Figure 14-5 Reactions of the pyruvate dehydrogenase complex. In these five reactions, an acetyl group from pyruvate is transferred to CoA, CO_2 is released, and NAD^+ is reduced to NADH.

? Without looking at the text, write the net equation for the reactions shown here. How many vitamin-derived cofactors are involved?



with another substance. There is also evidence that the individual enzymes of glycolysis and the citric acid cycle associate loosely with each other so that the close proximity of their active sites can increase flux through their respective pathways.

Flux through the pyruvate dehydrogenase complex is regulated by product inhibition: Both NADH and acetyl-CoA act as inhibitors. The activity of the complex is also regulated by hormone-controlled phosphorylation and dephosphorylation, which suits its function as the gatekeeper for the entry of a metabolic fuel into the citric acid cycle.

CONCEPT REVIEW

- Describe the functional importance of the coenzymes that participate in the reactions carried out by the pyruvate dehydrogenase complex.
- What is the advantage of a multienzyme complex?

14-2 The Eight Reactions of the Citric Acid Cycle

KEY CONCEPTS

- The citric acid cycle is a set of eight reactions in which an acetyl group is condensed with oxaloacetate, two CO_2 are lost, and oxaloacetate is regenerated.
- Each round of the citric acid cycle generates three NADH, one QH_2, and one GTP or ATP.
- Flux through the citric acid cycle is regulated primarily by feedback inhibition at three steps.
- The citric acid cycle likely evolved by the combination of oxidative and reductive pathways.

See Guided Exploration. Citric acid cycle overview.

An acetyl-CoA molecule derived from pyruvate is a product of carbohydrate catabolism as well as a product of amino acid catabolism, since the carbon skeletons of many amino acids are broken down to pyruvate. Acetyl-CoA is also a direct product of the degradation of certain amino acids and of fatty acids. In some tissues, the bulk of acetyl-CoA is derived from the catabolism of fatty acids rather than carbohydrates or amino acids.

Whatever its source, acetyl-CoA enters the citric acid cycle for further oxidation. This process is highly exergonic, and free energy is conserved at several steps in the form of a nucleotide triphosphate (GTP) and reduced cofactors. *For each acetyl group that enters the citric acid cycle, two molecules of fully oxidized CO_2 are produced, representing a loss of four pairs of electrons.* These electrons are transferred to 3 NAD^+ and 1 ubiquinone (Q) to produce 3 NADH and 1 QH_2. The net equation for the citric acid cycle is therefore

$$\text{acetyl-CoA} + \text{GDP} + P_i + 3\,NAD^+ + Q \rightarrow 2\,CO_2 + \text{CoA} + \text{GTP} + 3\,\text{NADH} + QH_2$$

In this section we examine the sequence of eight enzyme-catalyzed reactions of the citric acid cycle, focusing on a few interesting reactions. The entire pathway is summarized in **Figure 14-6**.

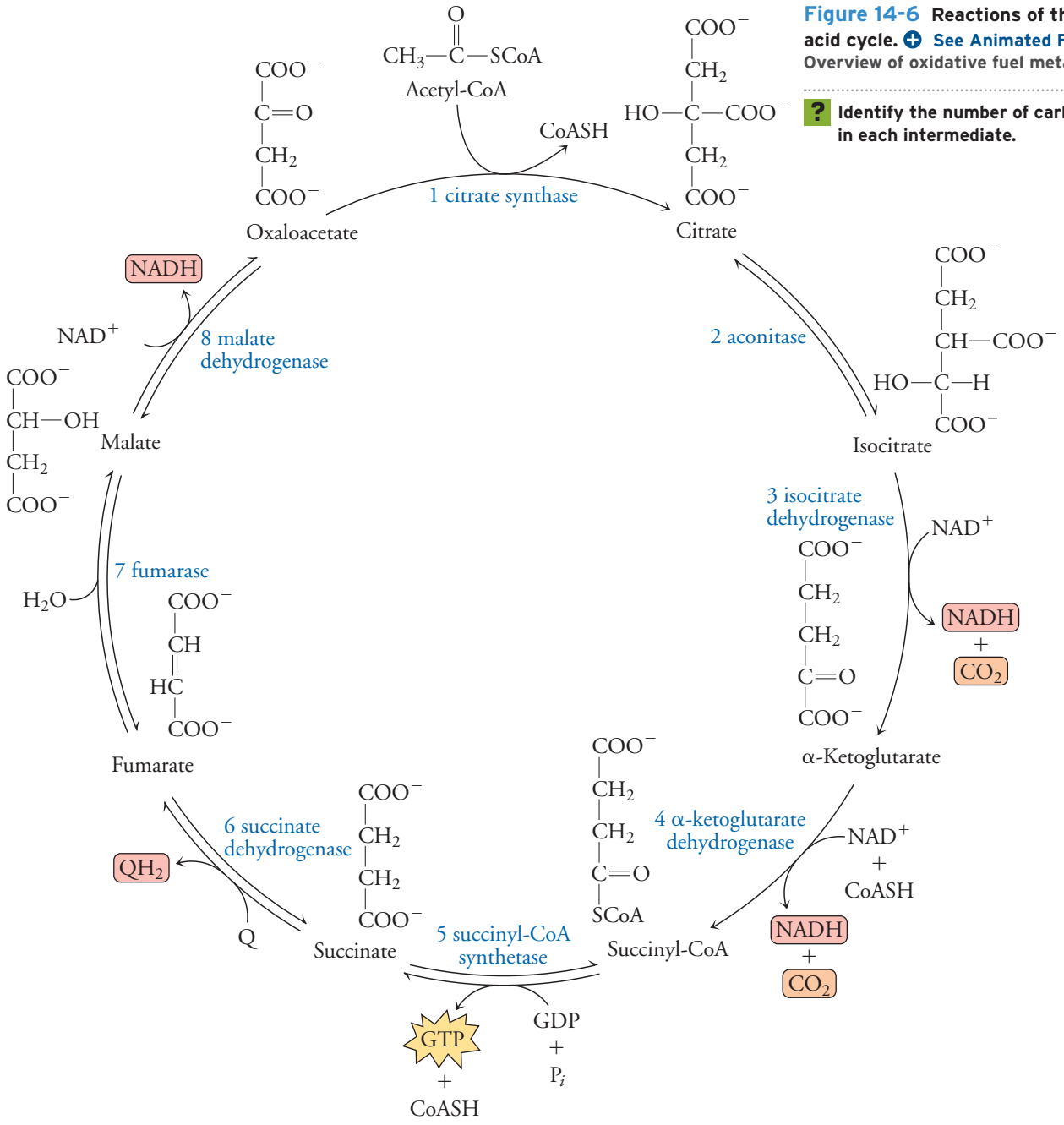
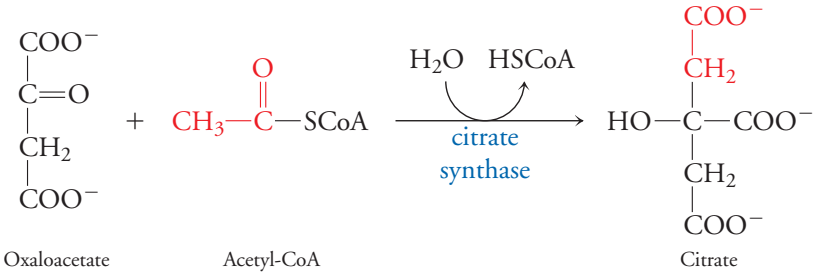


Figure 14-6 **Reactions of the citric acid cycle.** ⊕ **See Animated Figure.** Overview of oxidative fuel metabolism.

? **Identify the number of carbon atoms in each intermediate.**

1. Citrate synthase adds an acetyl group to oxaloacetate

In the first reaction of the citric acid cycle, the acetyl group of acetyl-CoA condenses with the four-carbon compound oxaloacetate to produce the six-carbon compound citrate:



Citrate synthase is a dimer that undergoes a large conformational change upon substrate binding (Fig. 14-7).

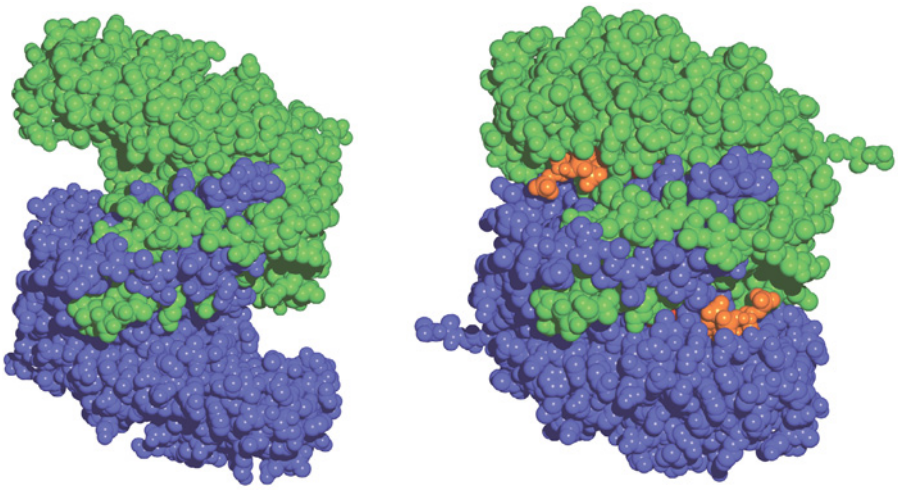


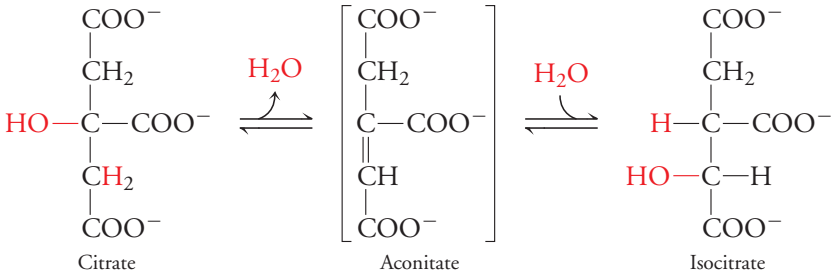
Figure 14-7 Conformational changes in citrate synthase. (a) The enzyme in the absence of substrates. The two subunits of the dimeric enzyme are colored blue and green. (b) When oxaloacetate (red, mostly buried) binds, each subunit undergoes a conformational change that creates a binding site for acetyl-CoA (an acetyl-CoA analog is shown here in orange). This conformational change explains why oxaloacetate must bind to the enzyme before acetyl-CoA can bind. [Structure of chicken citrate synthase alone (pdb 5CSC) determined by D.-I. Liao, M. Karpusas, and S. J. Remington; structure of citrate synthase with oxaloacetate and carboxymethyl-CoA (pdb 5CTS) determined by M. Karpusas, B. Branchaud, and S. J. Remington.] **See Interactive Exercise. Conformational changes in citrate synthase.**

Citrate synthase is one of the few enzymes that can synthesize a carbon–carbon bond without using a metal ion cofactor. Its mechanism is shown in **Figure 14-8**. The first reaction intermediate may be stabilized by the formation of low-barrier hydrogen bonds, which are stronger than ordinary hydrogen bonds (see Section 6-3). The coenzyme A released during the final step can be reused by the pyruvate dehydrogenase complex or used later in the citric acid cycle to synthesize the intermediate succinyl-CoA.

The reaction catalyzed by citrate synthase is highly exergonic ($\Delta G^{\circ\prime} = -31.5$ kJ · mol^{-1}, equivalent to the free energy of breaking the thioester bond of acetyl-CoA). We will see later why the efficient operation of the citric acid cycle requires that this step have a large free energy change.

2. Aconitase isomerizes citrate to isocitrate

The second enzyme of the citric acid cycle catalyzes the reversible isomerization of citrate to isocitrate:



The enzyme is named after the reaction intermediate.

Citrate is a symmetrical molecule, yet only one of its two carboxymethyl arms (—CH_2—COO^-) undergoes dehydration and rehydration during the aconitase reaction. This stereochemical specificity long puzzled biochemists, including Hans Krebs, who first described the citric acid cycle (also known as the Krebs cycle).

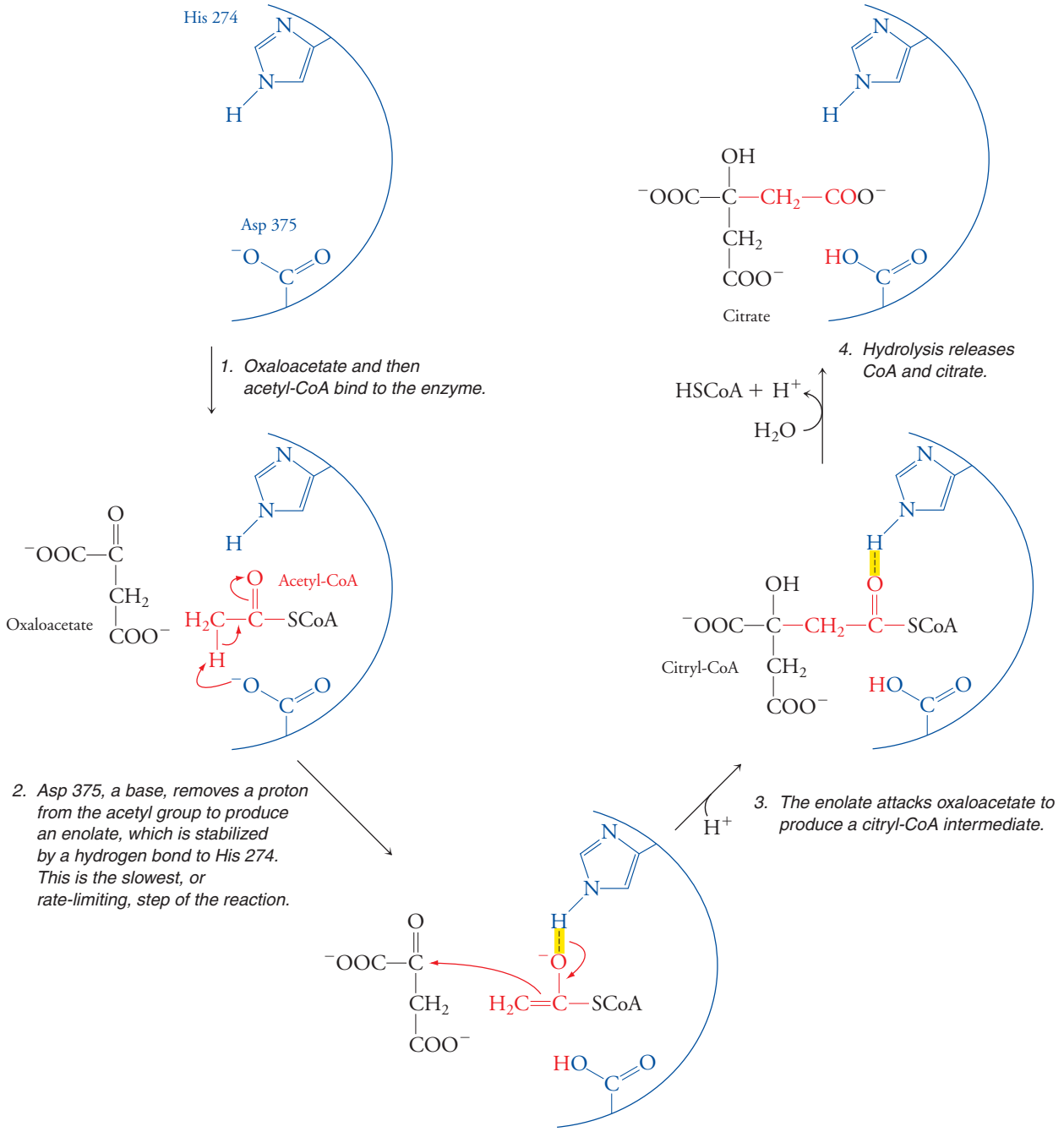


Figure 14-8 **The citrate synthase reaction.**

Eventually, Alexander Ogston pointed out that although citrate is symmetrical, its two carboxymethyl groups are no longer identical when it is bound to an asymmetrical enzyme (Fig. 14-9). In fact, a three-point attachment is not even necessary for an enzyme to distinguish two groups in a molecule such as citrate, which are related by mirror symmetry. You can prove this yourself with a simple organic chemistry model kit. By now you should appreciate that biological systems, including enzyme, are inherently chiral (also see Section 4-1).

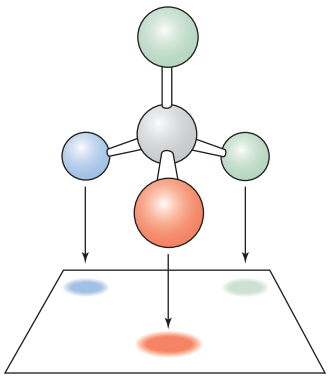
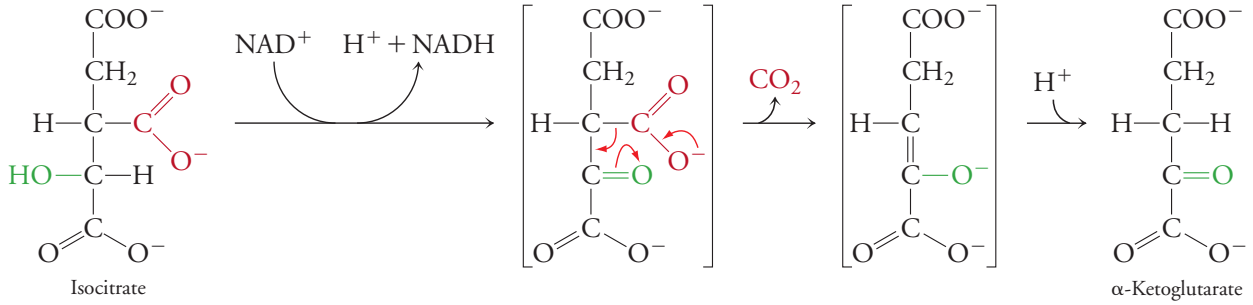


Figure 14-9 **Stereochemistry of citrate synthase.** The three-point attachment of citrate to the enzyme allows only one carboxymethyl group (shown in green) to react.

3. Isocitrate dehydrogenase releases the first CO_2

The third reaction of the citric acid cycle is the oxidative decarboxylation of isocitrate to α-ketoglutarate. The substrate is first oxidized in a reaction accompanied by the reduction of NAD^+ to NADH. Then the carboxylate group β to the ketone function (that is, two carbon atoms away from the ketone) is eliminated as CO_2.

An Mn^{2+} ion in the active site helps stabilize the negative charges of the reaction intermediate.

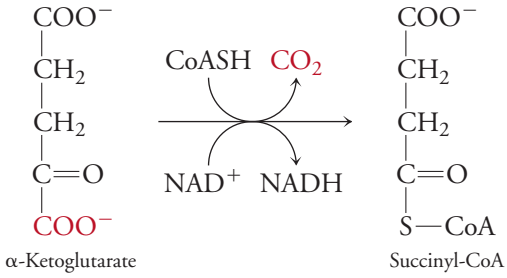


▶▶ WHERE does exhaled CO_2 come from?

The CO_2 molecules generated by isocitrate dehydrogenase—along with the CO_2 generated in the following reaction and the CO_2 produced by the decarboxylation of pyruvate—diffuse out of the cell and are carried in the bloodstream to the lungs, where they are breathed out. Note that these CO_2 molecules are produced through oxidation–reduction reactions: The carbons are oxidized, while NAD^+ is reduced. As described at the start of the chapter, O_2 is not directly involved in this process.

4. α-Ketoglutarate dehydrogenase releases the second CO_2

α-Ketoglutarate dehydrogenase, like isocitrate dehydrogenase, catalyzes an oxidative decarboxylation reaction. It also transfers the remaining four-carbon fragment to CoA:



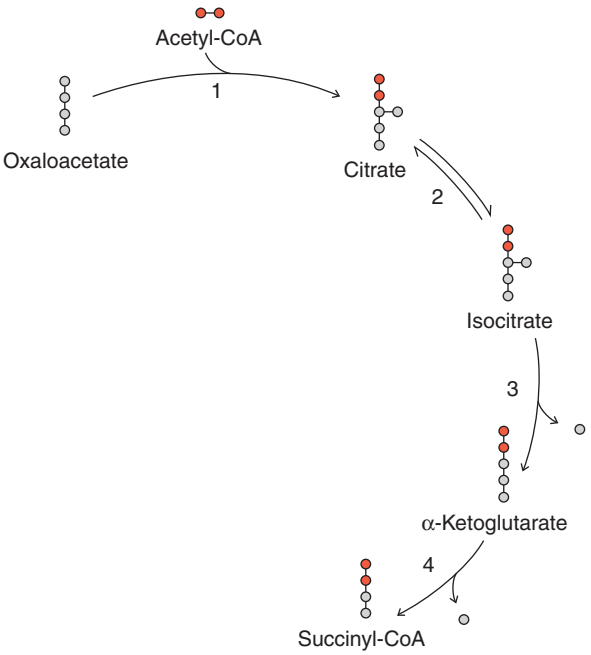


Figure 14-10 Fates of carbon atoms in the citric acid cycle. The two carbon atoms that are lost as CO_2 in the reactions catalyzed by isocitrate dehydrogenase (step 3) and α-ketoglutarate dehydrogenase (step 4) are not the same carbons that entered the cycle as acetyl-CoA (red). The acetyl carbons become part of oxaloacetate and are lost in subsequent rounds of the cycle.

The free energy of oxidizing α-ketoglutarate is conserved in the formation of the thioester succinyl-CoA. α-Ketoglutarate dehydrogenase is a multienzyme complex that resembles the pyruvate dehydrogenase complex in both structure and enzymatic mechanism. In fact, the same E3 enzyme is a member of both complexes.

The isocitrate dehydrogenase and α-ketoglutarate dehydrogenase reactions both release CO_2. These two carbons are not the ones that entered the citric acid cycle as acetyl-CoA; those acetyl carbons are released in subsequent rounds of the cycle (Fig. 14-10). *However, the net result of each round of the citric acid cycle is the loss of two carbons as CO_2 for each acetyl-CoA that enters the cycle.*

5. Succinyl-CoA synthetase catalyzes substrate-level phosphorylation

The thioester succinyl-CoA releases a large amount of free energy when it is cleaved ($\Delta G^{\circ\prime} = -32.6$ kJ · mol^{-1}). This is enough free energy to drive the synthesis of a nucleoside triphosphate from a nucleoside diphosphate and P_i ($\Delta G^{\circ\prime} = 30.5$ kJ · mol^{-1}). The change in free energy for the net reaction is near zero, so the reaction is reversible. In fact, the enzyme is named for the reverse reaction. Succinyl-CoA synthetase in the mammalian citric acid cycle generates GTP,

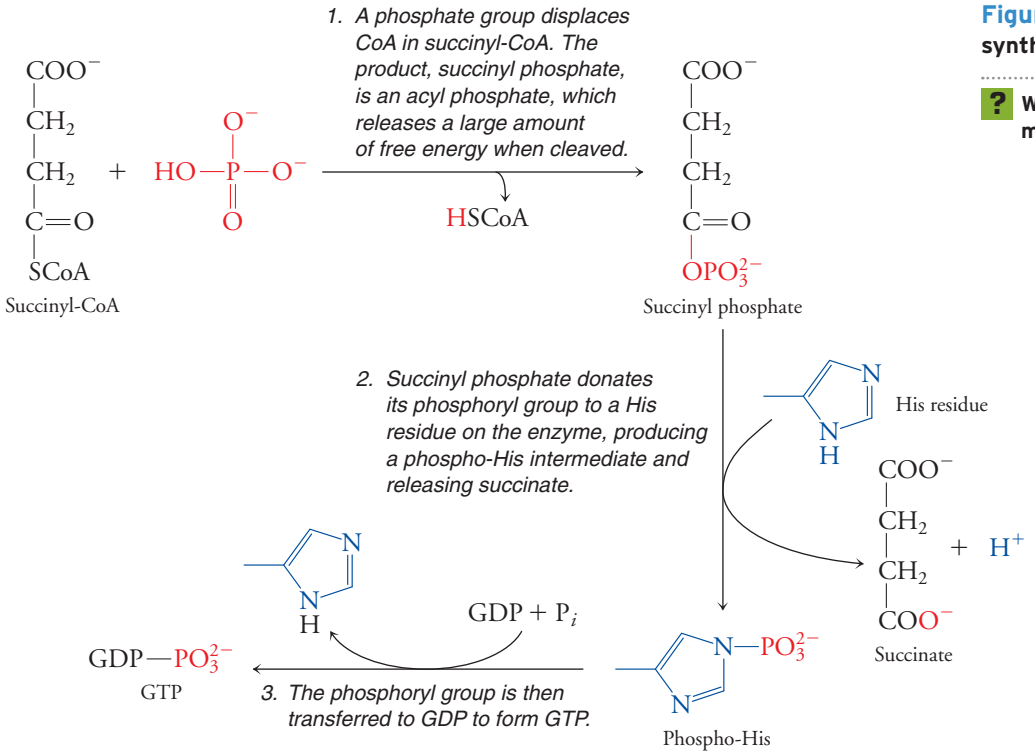


Figure 14-11 **The succinyl-CoA synthetase reaction.**

? **What is the fate of the free CoA molecule?**

whereas the plant and bacterial enzymes generate ATP (recall that GTP is energetically equivalent to ATP). An exergonic reaction coupled to the transfer of a phosphoryl group to a nucleoside diphosphate is termed **substrate-level phosphorylation** to distinguish it from oxidative phosphorylation (Section 15-4) and photophosphorylation (Section 16-2), which are more indirect ways of synthesizing ATP.

How does succinyl-CoA synthetase couple thioester cleavage to the synthesis of a nucleoside triphosphate? The reaction is a series of phosphoryl-group transfers that involve an active-site His residue **(Fig. 14-11)**. The phospho-His reaction intermediate must move a large distance to shuttle the phosphoryl group between the succinyl group and the nucleoside diphosphate **(Fig. 14-12)**.

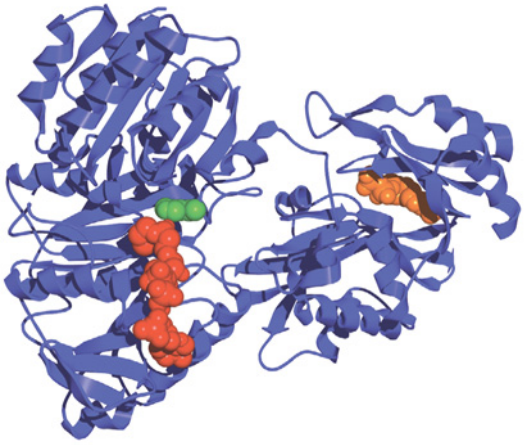
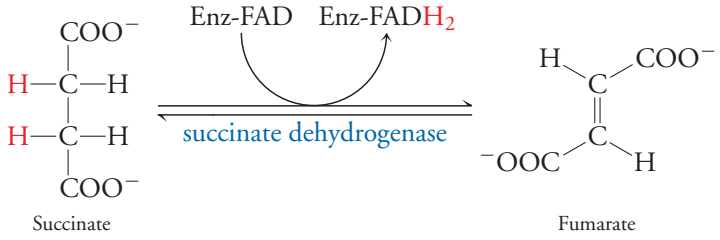


Figure 14-12 **Substrate binding in succinyl-CoA synthetase.** Succinyl-CoA (represented by coenzyme A, red) binds to the enzyme, and its succinyl group is phosphorylated. The succinyl phosphate then transfers its phosphoryl group to the His 246 side chain (green). A protein loop containing the phospho-His side chain must undergo a large movement because the nucleoside diphosphate awaiting phosphorylation (ADP, orange) binds to a site about 35 Å away. [Structure of *E. coli* succinyl-CoA synthetase (pdb 1CQI) determined by M. A. Joyce, M. E. Fraser, M. N. G. James, W. A. Bridger, and W. T. Wolodko.]

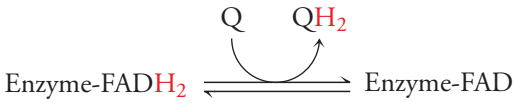
6. Succinate dehydrogenase generates ubiquinol

The final three reactions of the citric acid cycle convert succinate back to the cycle's starting substrate, oxaloacetate. Succinate dehydrogenase catalyzes the reversible dehydrogenation of succinate to fumarate. This oxidation–reduction reaction requires an FAD prosthetic group, which is reduced to $FADH_2$ during the reaction:



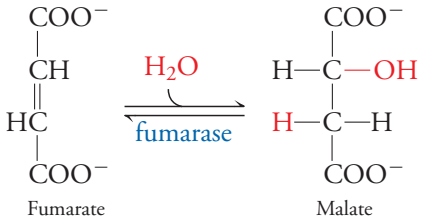
To regenerate the enzyme, the $FADH_2$ group must be reoxidized. Since succinate dehydrogenase is embedded in the inner mitochondrial membrane (it is the only one of the eight citric acid cycle enzymes that is not soluble in the mitochondrial

matrix), it can be reoxidized by the lipid-soluble electron carrier ubiquinone (see Section 12-2) rather than by the soluble cofactor NAD^+. Ubiquinone (abbreviated Q) acquires two electrons to become ubiquinol (QH_2).



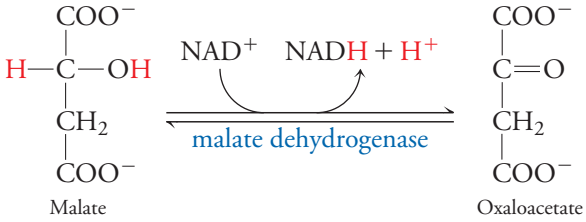
7. Fumarase catalyzes a hydration reaction

In the seventh reaction, fumarase (also known as fumarate hydratase) catalyzes the reversible hydration of a double bond to convert fumarate to malate:



8. Malate dehydrogenase regenerates oxaloacetate

The citric acid cycle concludes with the regeneration of oxaloacetate from malate in an NAD^+-dependent oxidation reaction:



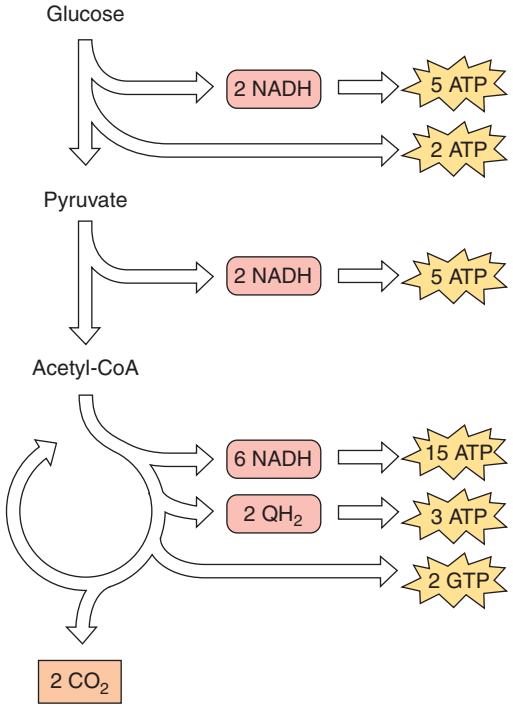
The standard free energy change for this reaction is $+29.7\ kJ \cdot mol^{-1}$, indicating that the reaction has a low probability of occurring as written. However, the product oxaloacetate is a substrate for the next reaction (Reaction 1 of the citric acid cycle). The highly exergonic—and therefore highly favorable—citrate synthase reaction helps pull the malate dehydrogenase reaction forward. This is the reason for the apparent waste of free energy released by cleaving the thioester bond of acetyl-CoA in the first reaction of the citric acid cycle.

The citric acid cycle is an energy-generating catalytic cycle

Because the eighth reaction of the citric acid cycle returns the system to its original state, *the entire pathway acts in a catalytic fashion to dispose of carbon atoms derived from amino acids, carbohydrates, and fatty acids.* Albert Szent-Györgyi discovered the catalytic nature of the pathway by observing that small additions of organic compounds such as succinate, fumarate, and malate stimulated O_2 uptake in a tissue preparation. Because the O_2 consumption was much greater than would be required for the direct oxidation of the added substances, he inferred that the compounds acted catalytically.

We now know that oxygen is consumed during oxidative phosphorylation, the process that reoxidizes the reduced cofactors (NADH and QH_2) that are produced by the citric acid cycle. Although the citric acid cycle generates one molecule of GTP (or ATP), considerably more ATP is generated when the reduced cofactors are reoxidized by O_2. Each NADH yields approximately 2.5 ATP, and each QH_2 yields approximately 1.5 ATP (we will see in Section 15-4 why these values are not whole numbers). Every acetyl unit that enters the citric acid cycle can therefore generate a

total of 10 ATP equivalents. The energy yield of a molecule of glucose, which generates two acetyl units, can be calculated:



A muscle operating anaerobically produces only 2 ATP per glucose, but under aerobic conditions when the citric acid cycle is fully functional, each glucose molecule generates about 32 ATP equivalents. This general phenomenon is called the **Pasteur effect,** after Louis Pasteur, who first observed that the rate of glucose consumption by yeast cells decreased dramatically when the cells were shifted from anaerobic to aerobic growth conditions.

The citric acid cycle is regulated at three steps

Flux through the citric acid cycle is regulated primarily at the cycle's three metabolically irreversible steps: those catalyzed by citrate synthase (Reaction 1), isocitrate dehydrogenase (Reaction 3), and α-ketoglutarate dehydrogenase (Reaction 4). The major regulators are shown in **Figure 14-13**.

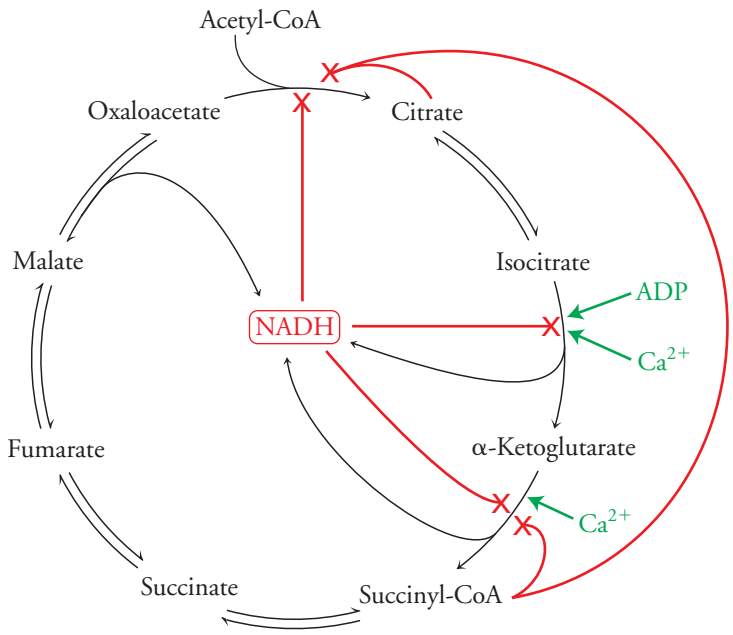


Figure 14-13 Regulation of the citric acid cycle. Inhibition is represented by red symbols, activation by green symbols.

Neither acetyl-CoA nor oxaloacetate is present at concentrations high enough to saturate citrate synthase, so flux through the first step of the citric acid cycle depends largely on the substrate concentrations. The product of the reaction, citrate, inhibits citrate synthase (citrate also inhibits phosphofructokinase, thereby decreasing the supply of acetyl-CoA produced by glycolysis). Succinyl-CoA, the product of Reaction 4, inhibits the enzyme that produces it. It also acts as a feedback inhibitor by competing with acetyl-CoA in Reaction 1.

The activity of isocitrate dehydrogenase is inhibited by its reaction product, NADH. NADH also inhibits α-ketoglutarate dehydrogenase and citrate synthase. Both dehydrogenases are activated by Ca^{2+} ions, which generally signify the need to generate cellular free energy. ADP, also representing the need for more ATP, activates isocitrate dehydrogenase. For reasons that are not entirely clear, many cancer cells develop mutations in isocitrate dehydrogenase (Box 14-A).

The citric acid cycle probably evolved as a synthetic pathway

A circular pathway such as the citric acid cycle must have evolved from a linear set of preexisting biochemical reactions. Clues to its origins can be found by examining

BOX 14-A CLINICAL CONNECTION

Mutations in Citric Acid Cycle Enzymes

Possibly because the citric acid cycle is a central metabolic pathway, severe defects in any of its components are expected to be incompatible with life. However, researchers have documented mutations in the genes for several of the cycle's enzymes, including α-ketoglutarate dehydrogenase, succinyl-CoA synthetase, and succinate dehydrogenase. These defects, which are all rare, typically affect the central nervous system, causing symptoms such as movement disorders and neurodegeneration. A rare form of fumarase deficiency results in brain malformation and developmental disabilities. Individuals who have a different fumarase defect show a higher risk of developing leiomyomas, noncancerous tumors such as uterine fibroids. A small percentage of these do become malignant. The other citric acid cycle enzyme mutations are also associated with cancer. One possible explanation is that a defective enzyme contributes to **carcinogenesis** (the development of cancer, or uncontrolled cell growth) by directly interfering with the cell's vital pathways for energy metabolism. Another possibility is that a defective enzyme causes the accumulation of particular metabolites, which are responsible for altering the cell's developmental fate.

Fumarase appears to be linked to cancer through the second mechanism. Normal cells respond to a drop in oxygen availability (hypoxia) by activating transcription factors known as hypoxia-inducible factors (HIFs). These proteins interact with DNA to turn on the expression of genes for glycolytic enzymes and a growth factor that promotes the development of new blood vessels. When the fumarase gene is defective, fumarate accumulates and inhibits a protein that destabilizes HIFs. As a result, the fumarase deficiency promotes glycolysis (an anaerobic pathway) and the growth of blood vessels. These two adaptations would favor tumors, whose growth, although characteristically rapid, may be limited by the availability of oxygen and other nutrients delivered by the bloodstream.

Defects in isocitrate dehydrogenase also promote cancer in an indirect fashion. Many cancerous cells exhibit a mutation in one of the two genes for the enzyme, suggesting that the unaltered copy is necessary for maintaining the normal activity of the citric acid cycle, while the mutated copy plays a role in carcinogenesis. Interestingly, the mutation usually converts an active-site Arg residue to His, indicating some strong selective pressure for a gain-of-function mutation (most mutations in proteins lead to loss of function). The mutated isocitrate dehydrogenase no longer carries out the usual reaction (converting isocitrate to α-ketoglutarate) but instead converts α-ketoglutarate to 2-hydroxyglutarate in an NADPH-dependent manner. The mechanism whereby 2-hydroxyglutarate contributes to carcinogenesis is not clear, but its involvement is bolstered by the observation that individuals who harbor other mutations that lead to 2-hydroxyglutarate accumulation have an increased risk of developing brain tumors.

Questions:

1. How would a fumarase deficiency affect the levels of pyruvate, fumarate, and malate?
2. Why does a succinate dehydrogenase deficiency produce the same symptoms as a fumarase deficiency?
3. Why would a deficiency of succinate dehydrogenase lead to a shortage of free coenzyme A?
4. Individuals who are deficient in fumarase develop lactic acidosis. Explain.
5. Draw the structure of 2-hydroxyglutarate, the product of the reaction catalyzed by the mutated isocitrate dehydrogenase.
6. Describe the single-nucleotide changes that could convert the arginine in isocitrate dehydrogenase to histidine.
7. How does operation of the mutated isocitrate dehydrogenase affect the cell's supply of reduced cofactors?

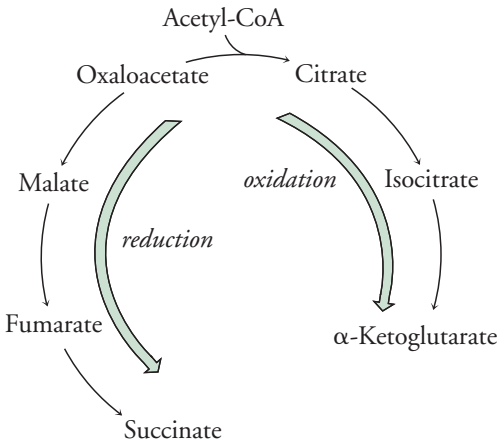


Figure 14-14 Pathways that might have given rise to the citric acid cycle. The pathway starting from oxaloacetate and proceeding to the right is an oxidative biosynthetic pathway, whereas the pathway that proceeds to the left is a reductive pathway. The modern citric acid cycle may have evolved by connecting these pathways.

the metabolism of organisms that resemble earlier life-forms. Such organisms emerged before atmospheric oxygen was available and may have used sulfur as their ultimate oxidizing agent, reducing it to H_2S. Their modern-day counterparts are anaerobic autotrophs that harvest free energy by pathways that are independent of the pathways of carbon metabolism. These organisms therefore do not use the citric acid cycle to generate reduced cofactors that are subsequently oxidized by molecular oxygen. However, all organisms must synthesize small molecules that can be used to build proteins, nucleic acids, carbohydrates, and so on.

Even organisms that do not use the citric acid cycle contain genes for some citric acid cycle enzymes. For example, the cells may condense acetyl-CoA with oxaloacetate, leading to α-ketoglutarate, which is a precursor of several amino acids. They may also convert oxaloacetate to malate, proceeding to fumarate and then to succinate. Together, these two pathways resemble the citric acid cycle, with the right arm following the usual oxidative sequence of the cycle and the left arm following a reversed, reductive sequence **(Fig. 14-14)**. The reductive sequence of reactions might have evolved as a way to regenerate the cofactors reduced during other catabolic reactions (for example, the NADH produced by the glyceraldehyde-3-phosphate dehydrogenase reaction of glycolysis; see Section 13-1).

It is easy to theorize that the evolution of an enzyme to interconvert α-ketoglutarate and succinate could have created a cyclic pathway similar to the modern citric acid cycle. Interestingly, *E. coli*, which uses the citric acid cycle under aerobic growth conditions, uses an interrupted citric acid cycle like the one diagrammed in Figure 14-14 when it is growing anaerobically.

Since the final four reactions of the modern citric acid cycle are metabolically reversible, the primitive citric acid cycle might easily have accommodated one-way flux in the clockwise direction, forming an oxidative cycle. If the complete cycle proceeded in the counterclockwise direction, the result would have been a reductive biosynthetic pathway **(Fig. 14-15)**. This pathway, which would incorporate, or "fix," atmospheric CO_2 into biological molecules, may have preceded the modern CO_2-fixing pathway found in green plants and some photosynthetic bacteria (described in Section 16-3).

CONCEPT REVIEW

- Describe the sources of the acetyl groups that enter the citric acid cycle.
- List the substrates and products for each of the cycle's eight reactions.
- Which products of the citric acid cycle represent forms of energy currency for the cell?
- Which substrates and products of the citric acid cycle regulate flux through the pathway?
- Describe how primitive oxidative and reductive biosynthetic pathways might have been combined to generate a circular metabolic pathway.

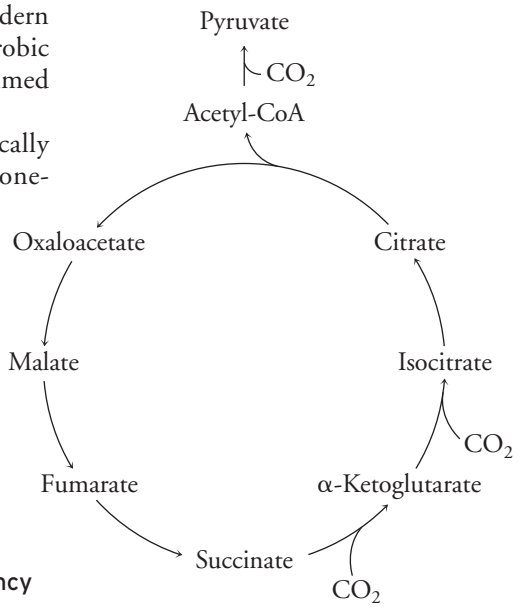


Figure 14-15 A proposed reductive biosynthetic pathway based on the citric acid cycle. This pathway might have operated to incorporate CO_2 into biological molecules.

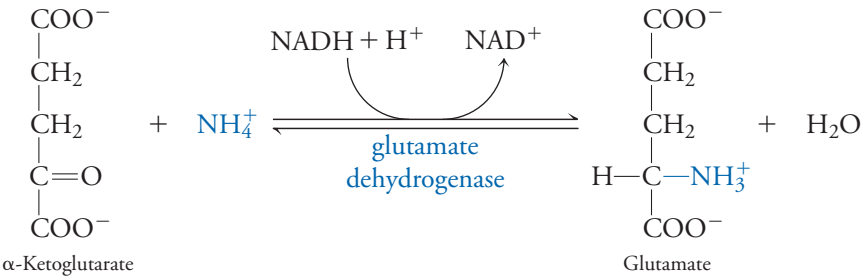
14-3 Anabolic and Catabolic Functions of the Citric Acid Cycle

KEY CONCEPTS
- The citric acid cycle supplies precursors for the synthesis of other compounds.
- Citric acid cycle intermediates can be replenished.

In mammals, six of the eight citric acid cycle intermediates (all except isocitrate and succinate) are the precursors or products of other pathways. For this reason, it is impossible to designate the citric acid cycle as a purely catabolic or anabolic pathway.

Citric acid cycle intermediates are precursors of other molecules

Intermediates of the citric acid cycle can be siphoned off to form other compounds (Fig. 14-16). For example, succinyl-CoA is used for the synthesis of heme. The five-carbon α-ketoglutarate (sometimes called 2-oxoglutarate) can undergo reductive amination by glutamate dehydrogenase to produce the amino acid glutamate:



Glutamate is a precursor of the amino acids glutamine, arginine, and proline. Glutamine in turn is a precursor for the synthesis of purine and pyrimidine nucleotides. We have already seen that oxaloacetate is a precursor of monosaccharides (Section 13-2). Consequently, *any of the citric acid cycle intermediates, which can be converted by the cycle to oxaloacetate, can ultimately serve as gluconeogenic precursors.*

Citrate produced by the condensation of acetyl-CoA with oxaloacetate can be transported out of the mitochondria to the cytosol. ATP-citrate lyase then catalyzes the reaction

$$\text{ATP} + \text{citrate} + \text{CoA} \rightarrow \text{ADP} + P_i + \text{oxaloacetate} + \text{acetyl-CoA}$$

The resulting acetyl-CoA is used for fatty acid and cholesterol synthesis, which take place in the cytosol. Note that the ATP-citrate lyase reaction undoes the work of the

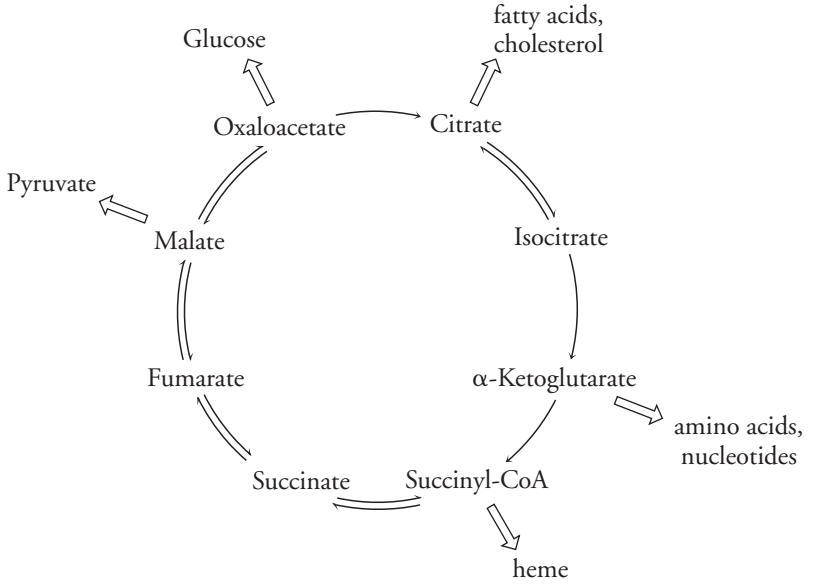
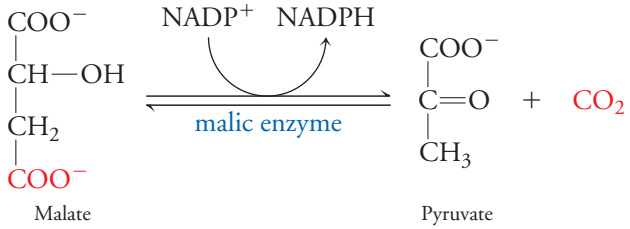


Figure 14-16 **Citric acid cycle intermediates as biosynthetic precursors.**

exergonic citrate synthase reaction. This seems wasteful, but cytosolic ATP-citrate lyase is essential because acetyl-CoA, which is produced in the mitochondria, cannot cross the mitochondrial membrane to reach the cytosol, whereas citrate can. The oxaloacetate product of the ATP-citrate lyase reaction can be converted to malate by a cytosolic malate dehydrogenase operating in reverse. Malate is then decarboxylated by the action of malic enzyme to produce pyruvate:



Pyruvate can reenter the mitochondria and be converted back to oxaloacetate to complete the cycle shown in **Figure 14-17**. In plants, isocitrate is diverted from the citric acid cycle in a biosynthetic pathway known as the **glyoxylate pathway** (Box 14-B).

Anaplerotic reactions replenish citric acid cycle intermediates

Intermediates that are diverted from the citric acid cycle for other purposes can be replenished through **anaplerotic reactions** (from the Greek *ana*, "up," and *plerotikos*, "to fill"; **Fig. 14-18**). One of the most important of these reactions is catalyzed by pyruvate carboxylase (this is also the first step of gluconeogenesis; Section 13-2):

$$\text{pyruvate} + CO_2 + \text{ATP} + H_2O \rightarrow \text{oxaloacetate} + \text{ADP} + P_i$$

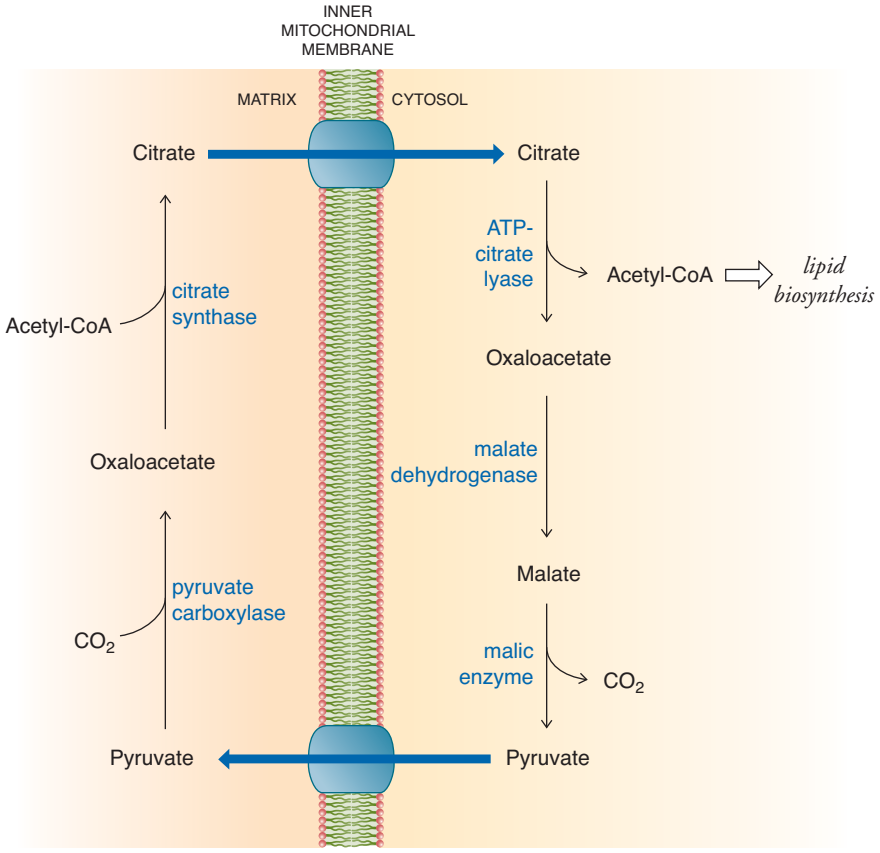


Figure 14-17 The citrate transport system. Both citrate and pyruvate cross the inner mitochondrial membrane via specific transport proteins. This system allows carbon atoms from mitochondrial acetyl-CoA to be transferred to the cytosol for the synthesis of fatty acids and cholesterol.

BOX 14-B BIOCHEMISTRY NOTE

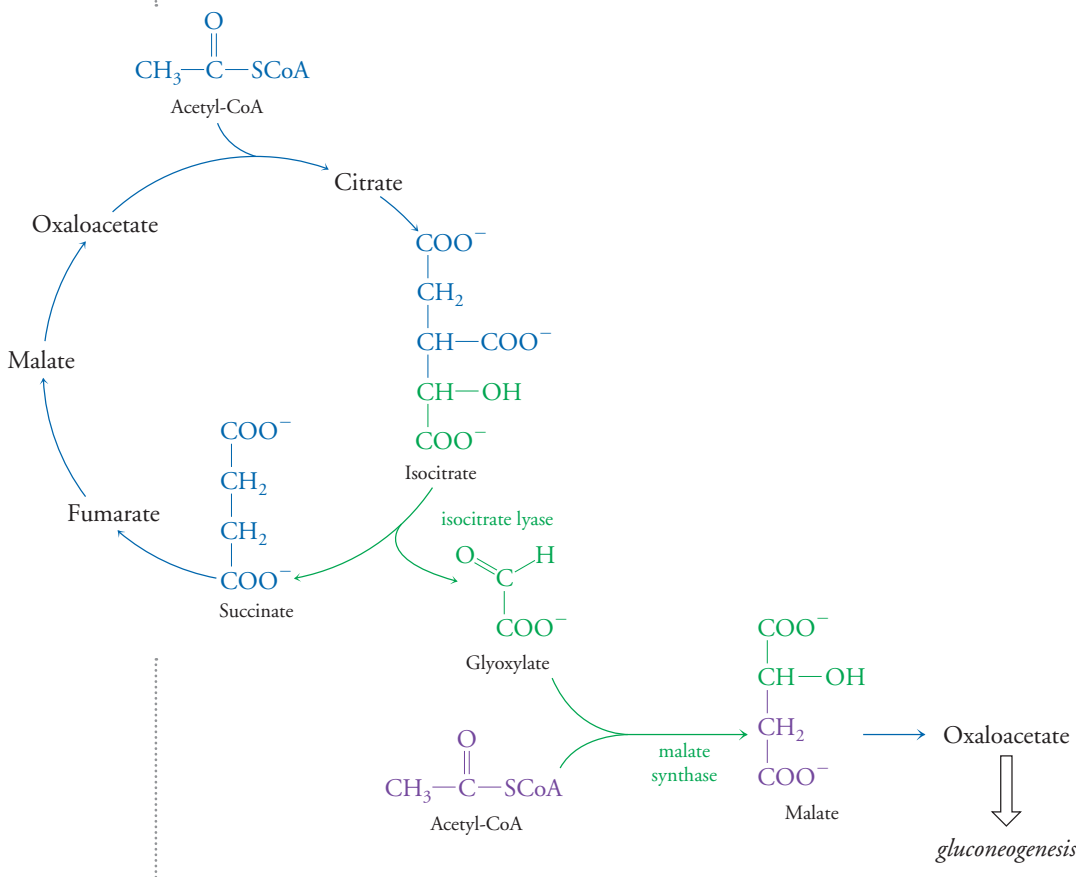
The Glyoxylate Pathway

Plants and some bacterial cells contain certain enzymes that act together with some citric acid cycle enzymes to convert acetyl-CoA to oxaloacetate, a gluconeogenic precursor. Animals lack the enzymes to do this and therefore cannot undertake the net synthesis of carbohydrates from two-carbon precursors. In plants, the glyoxylate pathway includes reactions that take place in the mitochondria and the **glyoxysome,** an organelle that, like the peroxisome, contains enzymes that carry out some essential metabolic processes.

In the glyoxysome, acetyl-CoA condenses with oxaloacetate to form citrate, which is then isomerized to isocitrate, as in the citric acid cycle. However, the next step is not the isocitrate dehydrogenase reaction but a reaction catalyzed by the glyoxysome enzyme isocitrate lyase, which converts isocitrate to succinate and the two-carbon compound glyoxylate. Succinate continues as usual through the mitochondrial citric acid cycle to regenerate oxaloacetate.

In the glyoxysome, the glyoxylate condenses with a second molecule of acetyl-CoA in a reaction catalyzed by the glyoxysome enzyme malate synthase to form the four-carbon compound malate. Malate can then be converted to oxaloacetate for gluconeogenesis. The two reactions that are unique to the glyoxylate pathway are shown in green in the figure below; reactions that are identical to those of the citric acid cycle are shown in blue.

In essence, the glyoxylate pathway bypasses the two CO_2-generating steps of the citric acid cycle (catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase) and incorporates a second acetyl unit (at the malate synthase step). The net result of the glyoxylate pathway is the production of a four-carbon compound that can be used to synthesize glucose. This pathway is highly active in germinating seeds, where stored oils (triacylglycerols) are broken down to acetyl-CoA. The glyoxylate pathway thus provides a route for synthesizing glucose from fatty acids. Because animals lack isocitrate lyase and malate synthase, they cannot undertake the net synthesis of carbohydrates from fat.



Question: Write the net equation for the glyoxylate cycle as shown here.

Acetyl-CoA activates pyruvate carboxylase, so when the activity of the citric acid cycle is low and acetyl-CoA accumulates, more oxaloacetate is produced. The concentration of oxaloacetate is normally low since the malate dehydrogenase reaction is thermodynamically unfavorable and the citrate synthase reaction is highly favorable. The replenished oxaloacetate is converted to citrate, isocitrate, α-ketoglutarate, and so on, so the concentrations of all the citric acid cycle intermediates increase and the cycle can proceed more quickly. *Since the citric acid cycle acts as a catalyst, increasing the concentrations of its components increases flux through the pathway.*

The degradation of fatty acids with an odd number of carbon atoms yields the citric acid cycle intermediate succinyl-CoA. Other anaplerotic reactions include the pathways for the degradation of some amino acids, which produce α-ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate. Some of these reactions are transaminations, such as

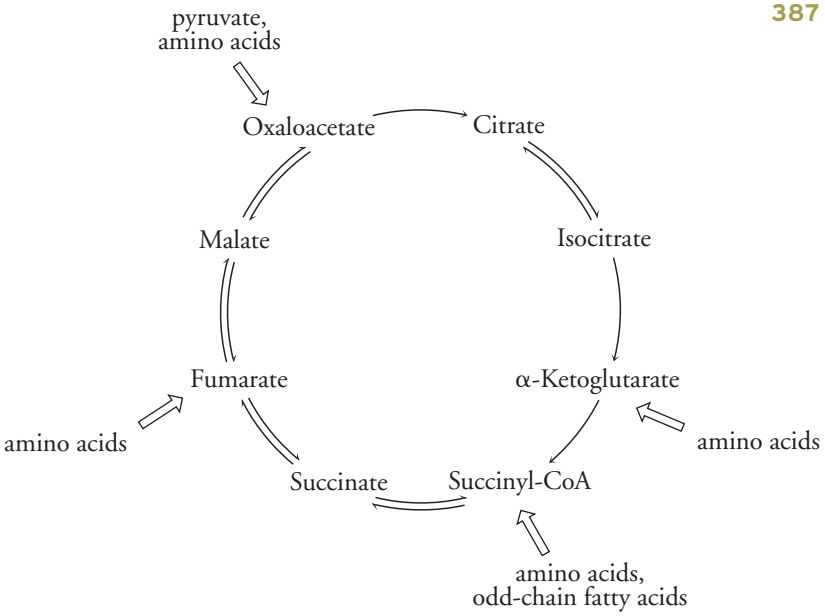
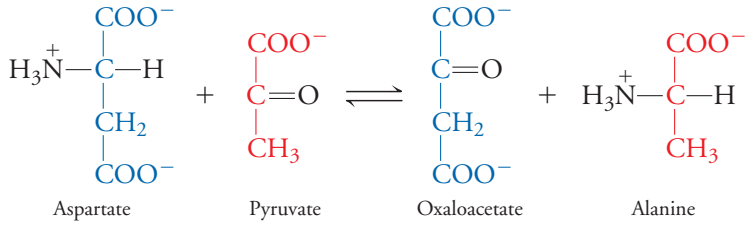
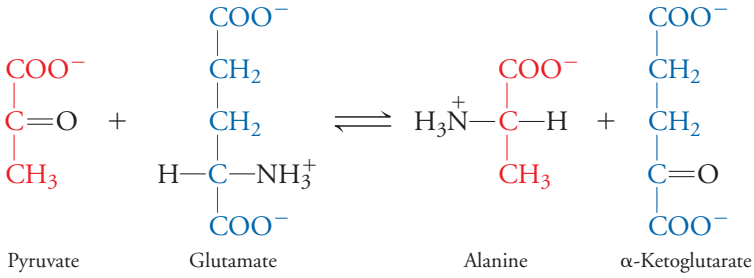


Figure 14-18 **Anaplerotic reactions of the citric acid cycle.**



Because transamination reactions have ΔG values near zero, the direction of flux into or out of the pool of citric acid cycle intermediates depends on the relative concentrations of the reactants.

In vigorously exercising muscle, the concentrations of citric acid cycle intermediates increase about three- to fourfold within a few minutes. This may help boost the energy-generating activity of the citric acid cycle, but it cannot be the sole mechanism, since flux through the citric acid cycle actually increases as much as 100-fold due to the increased activity of the three enzymes at the control points: citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. The increase in citric acid cycle intermediates may actually be a mechanism for accommodating the large increase in pyruvate that results from rapid glycolysis at the start of exercise. Not all the pyruvate is converted to lactate (Section 13-1); some is shunted into the pool of citric acid cycle intermediates via the pyruvate carboxylase reaction. Some pyruvate also undergoes a reversible reaction catalyzed by alanine aminotransferase



The resulting α-ketoglutarate then augments the pool of citric acid cycle intermediates, thereby increasing the ability of the cycle to oxidize the extra pyruvate.

Note that any compound that enters the citric acid cycle as an intermediate is not itself oxidized; it merely boosts the catalytic activity of the cycle, whose net reaction is still the oxidation of the two carbons of acetyl-CoA.

CONCEPT REVIEW

- Describe how the citric acid cycle supplies the precursors for the synthesis of amino acids, glucose, and fatty acids.
- What does the ATP-citrate lyase reaction accomplish?
- Why is the concentration of oxaloacetate low?
- Why does synthesizing more oxaloacetate increase flux through the citric acid cycle?

SUMMARY

14-1 The Pyruvate Dehydrogenase Reaction

- In order for pyruvate, the product of glycolysis, to enter the citric acid cycle, it must undergo oxidative decarboxylation catalyzed by the multienzyme pyruvate dehydrogenase complex, which yields acetyl-CoA, CO_2, and NADH.

14-2 The Eight Reactions of the Citric Acid Cycle

- The eight reactions of the citric acid cycle function as a multistep catalyst to convert the two carbons of acetyl-CoA to 2 CO_2.
- The electrons released in the oxidative reactions of the citric acid cycle are transferred to 3 NAD^+ and to ubiquinone. The reoxidation of the reduced cofactors generates ATP by oxidative phosphorylation. In addition, succinyl-CoA synthetase yields one molecule of GTP or ATP.
- The regulated reactions of the citric acid cycle are its irreversible steps, catalyzed by citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.
- The citric acid cycle most likely evolved from biosynthetic pathways leading to α-ketoglutarate or succinate.

14-3 Anabolic and Catabolic Functions of the Citric Acid Cycle

- Six of the eight citric acid cycle intermediates serve as precursors of other compounds, including amino acids, monosaccharides, and lipids. Anaplerotic reactions convert other compounds into citric acid cycle intermediates, thereby allowing increased flux of acetyl carbons through the pathway.

GLOSSARY TERMS

citric acid cycle
mitochondrial matrix
multienzyme complex
substrate-level phosphorylation
Pasteur effect
carcinogenesis
glyoxysome
glyoxylate pathway
anaplerotic reaction

PROBLEMS

14-1 The Pyruvate Dehydrogenase Reaction

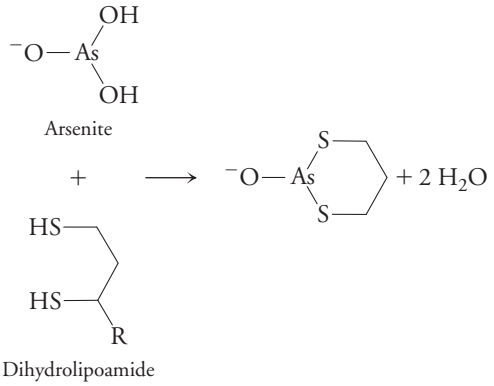
1. What are four possible transformations of pyruvate in mammalian cells?

2. Determine which one of the five steps of the pyruvate dehydrogenase complex reaction is metabolically irreversible and explain why.

3. The product of the pyruvate dehydrogenase complex, acetyl-CoA, is released in step 3 of the overall reaction. What is the purpose of steps 4 and 5?

4. Beriberi is a disease that results from a dietary lack of thiamine, the vitamin that serves as the precursor for thiamine pyrophosphate (TPP). There are two metabolites that accumulate in individuals with beriberi, especially after ingestion of glucose. Which metabolites accumulate and why?

5. Arsenite is toxic in part because it binds to sulfhydryl compounds such as lipoamide, as shown in the figure. What effect would the presence of arsenite have on the citric acid cycle?



6. Using the pyruvate dehydrogenase complex reaction as a model, reconstruct the TPP-dependent yeast pyruvate decarboxylase reaction in alcoholic fermentation (see Section 13-1).

7. How is the activity of the pyruvate dehydrogenase complex affected by **(a)** a high [NADH]/[NAD^+] ratio or **(b)** a high [acetyl-CoA]/[CoASH] ratio?

8. The activity of the pyruvate dehydrogenase complex is also controlled by phosphorylation. Pyruvate dehydrogenase kinase catalyzes the phosphorylation of a specific Ser residue on the E1 subunit of the enzyme, rendering it inactive. The pyruvate dehydrogenase phosphatase enzyme reverses the inhibition by catalyzing the removal of this phosphate group. The kinase and the phosphatase enzymes themselves are controlled by cytosolic Ca^{2+} levels. In the muscle, Ca^{2+} levels rise when the muscle contracts. Which of these two enzymes is inhibited by Ca^{2+} and which is activated by Ca^{2+}?

9. Most cases of pyruvate dehydrogenase deficiency disease that have been studied to date involve a mutation in the E1 subunit of the enzyme. The disease is extremely difficult to treat successfully, but physicians who identify patients with a pyruvate dehydrogenase deficiency will administer thiamine as a first course of treatment. Explain why.

10. A second strategy to treat a pyruvate dehydrogenase deficiency disease (see Problem 9) involves administering dichloroacetate, a compound that inhibits pyruvate dehydrogenase kinase (see Problem 8). How might this strategy be effective?

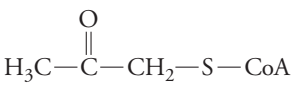
14-2 The Eight Reactions of the Citric Acid Cycle

11. Why is it advantageous for citrate, the product of Reaction 1 of the citric acid cycle, to inhibit phosphofructokinase, which catalyzes the third reaction of glycolysis (see Section 13-1)?

12. Animals that have ingested the leaves of the poisonous South African plant *Dichapetalum cymosum* exhibit a 10-fold increase in levels of cellular citrate. The plant contains fluoroacetate, which is converted to fluoroacetyl-CoA. Describe the mechanism that leads to increased levels of citrate in animals that have ingested this poisonous plant. (*Note:* Fluoroacetyl-CoA is not an inhibitor of citrate synthase.)

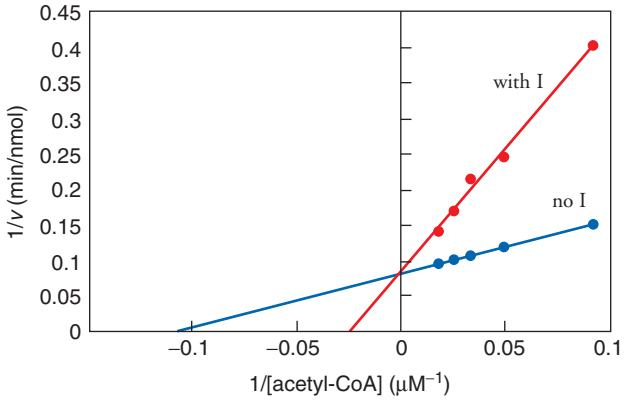
13. Site-directed mutagenesis techniques were used to synthesize a mutant citrate synthase enzyme in which the active-site histidine was converted to an alanine. Why did the mutant citrate synthase enzyme exhibit decreased catalytic activity?

14. The compound *S*-acetonyl-CoA can be synthesized from 1-bromoacetone and coenzyme A.



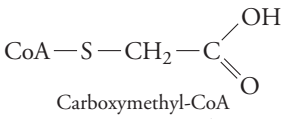
S-Acetonyl-CoA

(a) Write the reaction for the formation of *S*-acetonyl-CoA.
(b) The Lineweaver–Burk plot for the citrate synthase reaction with and without *S*-acetonyl-CoA is shown. What type of inhibitor is *S*-acetonyl-CoA? Explain.



(c) Acetyl-CoA acts as an allosteric activator of pyruvate carboxylase. *S*-acetonyl CoA does not activate pyruvate carboxylase, and it cannot compete with acetyl-CoA for binding to the enzyme. What does this tell you about the binding requirements for an allosteric activator of pyruvate carboxylase?

15. The compound carboxymethyl-CoA (shown below) is a competitive inhibitor of citrate synthase and is a proposed transition state analog. Use this information to propose a structure for the reaction intermediate derived from acetyl-CoA in the rate-limiting step of the reaction, just prior to its reaction with oxaloacetate.



Carboxymethyl-CoA
(transition state analog)

16. Citrate competes with oxaloacetate for binding to citrate synthase. Isocitrate dehydrogenase is activated by Ca^{2+} ions, which are released when muscle contracts. How do these two regulatory strategies assist the cell in making the transition from the rested state (low citric acid cycle activity) to the exercise state (high citric acid cycle activity)?

17. Administration of high concentrations of oxygen (hyperoxia) is effective in the treatment of lung injuries but at the same time can also be quite damaging.
(a) It has been shown that lung aconitase activity is dramatically decreased during hyperoxia. How would the concentration of citric acid cycle intermediates be affected?
(b) The decreased aconitase activity and decreased mitochondrial respiration in hyperoxia are accompanied by elevated levels of glycolysis and the pentose phosphate pathway. Explain why.

18. The scientists who carried out the hyperoxia experiments described in Problem 17 noted that they could mimic this effect by administering either fluoroacetate or fluorocitrate to cells in culture. Explain. (*Hint:* See Solution 12.)

19. Kinetic studies with aconitase in the 1970s revealed that *trans*-aconitate is a competitive inhibitor of the enzyme if *cis*-aconitate is used as the substrate. But if citrate is used as the substrate, *trans*-aconitate is a noncompetitive inhibitor. Propose a hypothesis that explains this observation.

20. A yeast mutant is isolated in which the gene for aconitase is nonfunctional. What are the consequences for the cell, particularly with regard to energy production?

21. The $\Delta G^{\circ\prime}$ value for the isocitrate dehydrogenase reaction is -21 kJ · mol^{-1}. What is K_{eq} for this reaction?

22. The crystal structure of isocitrate dehydrogenase shows that there is a cluster of highly conserved amino acids in the substrate binding pocket—three arginines, a tyrosine, and a lysine. Why are these residues conserved and what is a possible role for these amino acid side chains in substrate binding?

23. In bacteria, isocitrate dehydrogenase is regulated by phosphorylation of a specific Ser residue in the enzyme active site. X-ray structures of the phosphorylated and the nonphosphorylated enzyme show no significant conformational differences.

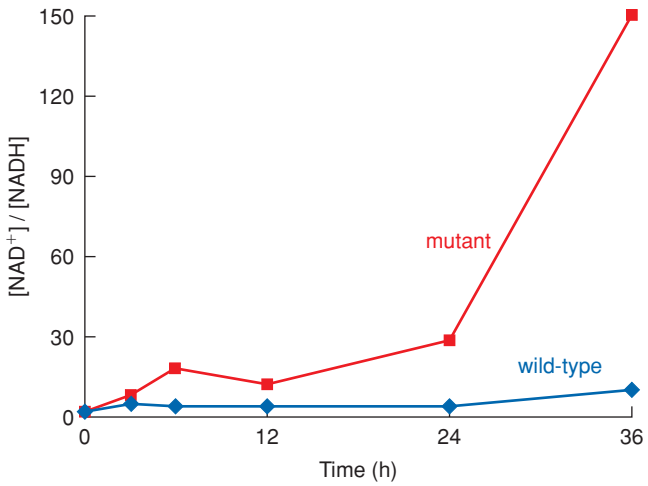
(a) How does phosphorylation regulate isocitrate dehydrogenase activity?

(b) To confirm their hypothesis, the investigators constructed a mutant enzyme in which the Ser residue was replaced with an Asp residue. The mutant was unable to bind isocitrate. Are these results consistent with the hypothesis you proposed in part (a)?

24. The expression of several enzymes changes when yeast grown on glucose are abruptly shifted to a two-carbon food source such as acetate.

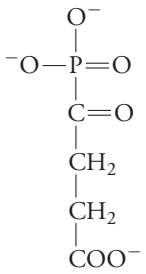
(a) Why does the level of expression of isocitrate dehydrogenase increase when the yeast are shifted from glucose to acetate?

(b) The metabolism of a yeast mutant with a nonfunctional isocitrate dehydrogenase enzyme was compared to that of a wild-type yeast. The yeast were grown on glucose and then abruptly shifted to acetate as the sole carbon source. The [NAD$^+$]/[NADH] ratio was measured over a period of 48 hours. The results are shown below. Why does the ratio increase slightly at 36 hours for the wild-type yeast? Why is there a more dramatic increase in the ratio for the mutant?



25. Using the pyruvate dehydrogenase complex reaction as a model, draw the intermediates of the α-ketoglutarate dehydrogenase reaction. Describe what happens in each of the five reaction steps.

26. Using the mechanism you drew for Problem 25, explain how succinyl phosphonate (above right) inhibits α-ketoglutarate dehydrogenase.



Succinyl phosphonate

27. Succinyl-CoA inhibits both citrate synthase and α-ketoglutarate dehydrogenase. How is succinyl-CoA able to inhibit both enzymes?

28. A patient with an α-ketoglutarate deficiency exhibits a small increase in blood pyruvate level and a large increase in blood lactate level, resulting in a [lactate]/[pyruvate] ratio that is many times greater than normal. Explain the reason for these symptoms.

29. Succinyl-CoA synthetase is also called succinate thiokinase. Why is the enzyme considered to be a kinase?

30. Succinyl-CoA synthetase is a dimer composed of an α subunit and a β subunit. A single gene codes for the α subunit protein. Two genes code for two different β subunit proteins—one subunit is specific for GDP, and one is specific for ADP.

(a) The β subunit specific for ADP is expressed in "catabolic tissues" such as brain and muscles, whereas the β subunit specific for GDP is expressed in "anabolic tissues" such as liver and kidneys. Propose a hypothesis to explain this observation.

(b) Individuals who are born with a mutation in the gene coding for the α subunit of the enzyme experience severe lactic acidosis and usually die within a few days of birth. Why is this mutation so deleterious?

(c) Individuals who are born with a mutation in the gene coding for the ADP-specific β subunit of the enzyme experience normal to moderately elevated concentrations of lactate and usually survive to their early 20s. Why is the prognosis for these patients better than for patients with a mutation in the gene for the α subunit?

31. Malonate is a competitive inhibitor of succinate dehydrogenase. What citric acid cycle intermediates accumulate if malonate is present in a preparation of isolated mitochondria?

32. Succinate dehydrogenase is not considered to be part of the glyoxylate pathway, yet it is vital to the proper functioning of the pathway. Why?

33. The $\Delta G^{\circ\prime}$ for the fumarase reaction is 23.4 kJ · mol^{-1}, but the ΔG value is close to zero. What is the ratio of fumarate to malate under cellular conditions at 37°C? Is this reaction likely to be a control point for the citric acid cycle?

34. A mutant bacterial fumarase was constructed by replacing the Glu (E) at position 315 with Gln (Q). The kinetic parameters of the mutant and wild-type enzymes were compared, and the results are shown in the table below. Explain the significance of the changes.

	Wild-type enzyme	**E315Q mutant enzyme**
V_{max} (μmol · min^{-1} · mg^{-1})	345	32
K_M (mM)	0.21	0.25
k_{cat} (s^{-1})	1150	107
k_{cat}/K_M (M^{-1} · s^{-1})	5.6×10^6	4.3×10^5

35. Reaction 8 and Reaction 1 of the citric acid cycle can be considered to be coupled because the exergonic cleavage of the thioester bond of acetyl-CoA in Reaction 1 drives the regeneration of oxaloacetate in Reaction 8.

(a) Write the equation for the overall coupled reaction and calculate its $\Delta G^{\circ\prime}$.

(b) What is the equilibrium constant for the coupled reaction? Compare this equilibrium constant with the equilibrium constant of Reaction 8 alone.

36. Malate dehydrogenase is more active in cells oxidizing glucose aerobically than in cells oxidizing glucose anaerobically. Explain why.

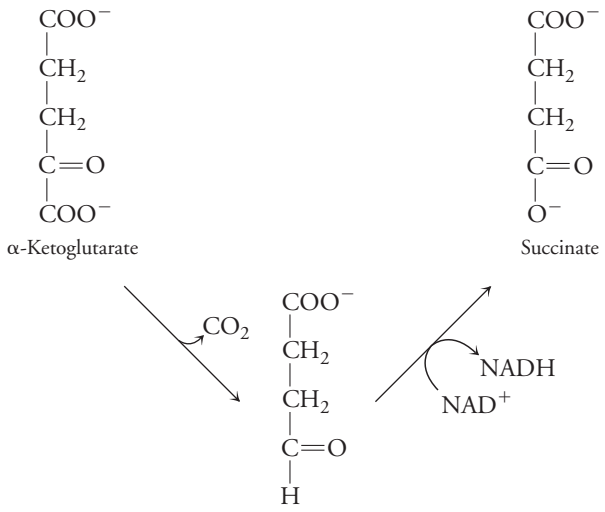
37. **(a)** Oxaloacetate labeled at C4 with ^{14}C is added to a suspension of respiring mitochondria. What is the fate of the labeled carbon?

(b) Acetyl-CoA labeled at C1 with ^{14}C is added to a suspension of respiring mitochondria. What is the fate of the labeled carbon?

38. When leavened bread is made, the bread dough is "punched" down and then put in a warm place to "rise" to increase its volume. Give a biochemical explanation for this observation.

39. Flux through the citric acid cycle is regulated by the simple mechanisms of **(a)** substrate availability, **(b)** product inhibition, and **(c)** feedback inhibition. Give examples of each.

40. Certain microorganisms with an incomplete citric acid cycle decarboxylate α-ketoglutarate to produce succinate semialdehyde. A dehydrogenase then converts succinate semialdehyde to succinate. These reactions can be combined with other standard citric acid cycle reactions to create a pathway from citrate to oxaloacetate. How does this alternative pathway compare to the standard citric acid cycle in its ability to make free energy available to the cell?



14-3 Anabolic and Catabolic Functions of the Citric Acid Cycle

41. Why is the reaction catalyzed by pyruvate carboxylase the most important anaplerotic reaction of the citric acid cycle?

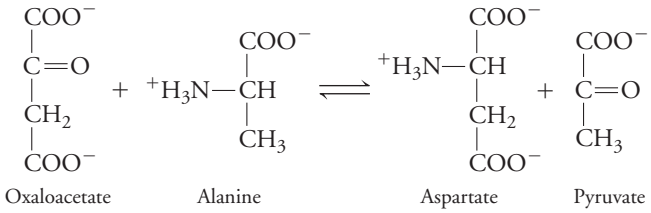
42. Why is the activation of pyruvate carboxylase by acetyl-CoA a good regulatory strategy?

43. Many amino acids are broken down to intermediates of the citric acid cycle.

(a) Why can't these amino acid "remnants" be completely oxidized to CO_2 by the citric acid cycle?

(b) Explain why amino acids that are broken down to pyruvate can be completely oxidized by the citric acid cycle.

44. Describe how the transamination reaction below could function as an anaplerotic reaction for the citric acid cycle.



45. Is net synthesis of glucose in mammals possible from the following compounds?

(a) The fatty acid palmitate (16:0), which is degraded to eight acetyl-CoA

(b) The fatty acid pentadecanoate (15:0), which is degraded to six acetyl-CoA and one propionyl-CoA

(c) Glyceraldehyde-3-phosphate

(d) Leucine, which is degraded to acetyl-CoA and acetoacetate (a compound that is metabolically equivalent to two acetyl-CoA groups)

(e) Tryptophan, which is degraded to alanine and acetoacetate

(f) Phenylalanine, which is degraded to acetoacetate and fumarate

46. Pancreatic islet cells cultured in the presence of 1–20 mM glucose showed increased activities of pyruvate carboxylase and the E1 subunit of the pyruvate dehydrogenase complex proportional to the increase in glucose concentration. Explain why.

47. A physician is attempting to diagnose a neonate with a pyruvate carboxylase deficiency. An injection of alanine normally leads to a gluconeogenic response, but in the patient no such response occurs. Explain.

48. The physician treats the patient described in Problem 47 by administering glutamine. Explain why glutamine supplements are effective in treating the disease.

49. Physicians often attempt to treat a pyruvate carboxylase deficiency by administering biotin. Explain why this strategy might be effective.

50. Patients with a pyruvate dehydrogenase deficiency and patients with a pyruvate carboxylase deficiency (see Problems 47–49) both have high blood levels of pyruvate and lactate. Explain why.

51. Metabolites in rat muscle were measured before and after exercising. After exercise, the rat muscle showed an increase in oxaloacetate concentration, a decrease in phosphoenolpyruvate concentration, and no change in pyruvate concentration. Explain.

52. Oxygen does not appear as a reactant in any of the citric acid cycle reactions, yet it is essential for the proper functioning of the cycle. Explain why.

53. The activity of isocitrate dehydrogenase in *E. coli* is regulated by the covalent attachment of a phosphate group to the enzyme. Phosphorylated isocitrate dehydrogenase is inactive. When acetate is the food source for a culture of *E. coli,* isocitrate dehydrogenase is phosphorylated.

(a) Draw a diagram showing how acetate is metabolized in *E. coli.*

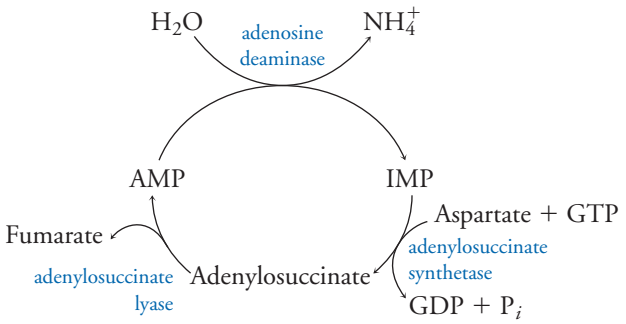
(b) When glucose is added to the culture, the phosphate group is removed from isocitrate dehydrogenase. How does flux through the metabolic pathways change in *E. coli* when glucose is the food source instead of acetate?

54. Yeast are unusual in that they are able to use ethanol as a gluconeogenic substrate. Ethanol is converted to glucose using the assistance of the glyoxylate pathway. Describe how the ethanol → glucose conversion takes place.

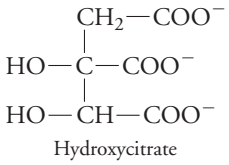
55. Animals lack a glyoxylate pathway and cannot convert fats to carbohydrates. If an animal is fed a fatty acid with all its carbons replaced by the isotope ^{14}C, some of the labeled carbons later appear in glucose. How is this possible?

56. A bacterial mutant with low levels of isocitrate dehydrogenase is able to grow normally when the culture medium is supplemented with glutamate. Explain why.

57. The purine nucleotide cycle (shown below) is an important pathway in muscle cells. The activity of the cycle increases during periods of high muscle activity. Explain how the purine nucleotide cycle contributes to the ability of the muscle cell to generate energy during intense exercise. IMP is inosine monophosphate.



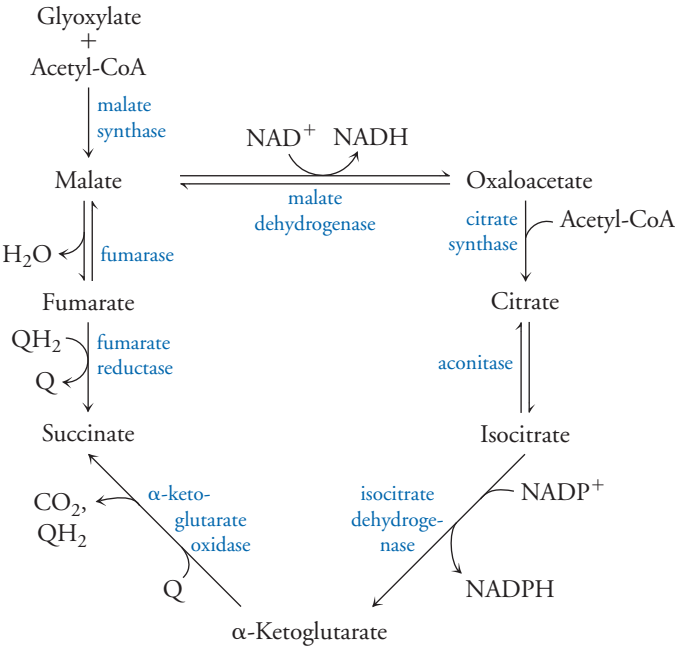
58. The plant metabolite hydroxycitrate is advertised as an agent that prevents fat buildup.



Hydroxycitrate

(a) How does this compound differ from citrate?
(b) Hydroxycitrate inhibits the activity of ATP-citrate lyase. What kind of inhibition is likely to occur?
(c) Why might inhibition of ATP-citrate lyase block the conversion of carbohydrates to fats?
(d) The synthesis of what other compounds would be inhibited by hydroxycitrate?

59. *Helicobacter pylori* is a bacterium that colonizes the upper gastrointestinal tract in humans and is the causative agent of chronic gastritis, ulcers, and possibly gastric cancer. Knowledge of the intermediary metabolism of this organism will be helpful in the development of effective drug therapies to treat these diseases. The citric acid "cycle" in *H. pylori* is a noncyclic, branched pathway that is used to produce biosynthetic intermediates instead of metabolic energy. Succinate is produced in the "reductive branch," whereas α-ketoglutarate is produced in the "oxidative branch." The two branches are linked by the α-ketoglutarate oxidase reaction. The pathway is shown in the diagram below.



(a) Compare and contrast the citric acid cycle in *H. pylori* with the citric acid cycle in mammals.
(b) The K_M values for the enzymes listed in the table below are higher than the K_M values for the corresponding enzymes in other species of bacteria. What does this tell you about the conditions under which the citric acid cycle operates in *H. pylori*?
(c) Compare the properties of *H. pylori* citrate synthase with mammalian citrate synthase.
(d) What enzymes might serve to regulate the citric acid cycle in *H. pylori*?
(e) What enzymes might be used as drug targets for persons suffering from gastritis, ulcers, or gastric cancer?

Enzyme	Substrate	Inhibitors	Activators
Citrate synthase	Acetyl-CoA, oxaloacetate	ATP	
Aconitase	Citrate		
Isocitrate dehydrogenase ($NADP^+$-dependent)	Isocitrate, $NADP^+$	Higher concentrations of $NADP^+$, isocitrate	AMP (slight)
α-ketoglutarate oxidase	α-ketoglutarate, FAD		CoASH
Malate dehydrogenase	Oxaloacetate, NADH		
Fumarase	Malate		
Fumarate reductase	Fumarate, QH_2		
Malate synthase	Glyoxylate, acetyl-CoA		

60. *H. pylori,* whose citric acid cycle has an oxidative branch and a reductive branch (see Problem 59), uses amino acids and fatty acids present in the gastrointestinal tract as a source of biosynthetic intermediates.

(a) Describe how *H. pylori* uses the acetyl-CoA derived from fatty acid breakdown to synthesize glucose and glutamate.

(b) Describe how *H. pylori* converts aspartate to glutamate.

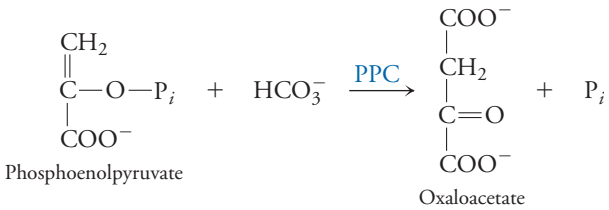
61. Yeast cells that are grown on nonfermentable substrates and then abruptly switched to glucose exhibit substrate-induced inactivation of several enzymes. Which enzymes would glucose cause to be inactivated and why?

62. Phagocytes such as macrophages and neutrophils are cellular components of the immune system that protect the host against damage caused by invading microorganisms. Phagocytes engulf and internalize a foreign microbe, forming a membrane-bound structure called a phagosome. The phagosome then fuses with the lysosome, a cellular organelle that contains a wide variety of proteolytic enzymes that destroy the pathogen if the host is fortunate. But some microbes can survive the harsh conditions of the phagolysosome. An example is *Mycobacterium tuberculosis,* which can reside in the macrophage in a dormant state for a prolonged period of time. Investigators noted that following engulfment by the macrophage, the levels of bacterial isocitrate lyase, malate synthase, citrate synthase, and malate dehydrogenase increased to levels as much as 20 times above normal.

(a) What pathway(s) does *M. tuberculosis* employ while in the phagosome and why are these pathways essential to its survival?

(b) What might be good drug targets for treating a patient infected with *M. tuberculosis*?

63. Bacteria and plants (but not animals) possess the enzyme phosphoenolpyruvate carboxylase (PPC), which catalyzes the reaction shown here.



Phosphoenolpyruvate — Oxaloacetate

(a) What is the importance of this reaction to the organism?

(b) PPC is allosterically activated by both acetyl-CoA and fructose-1,6-bisphosphate. Explain these regulatory strategies.

64. Succinic acid is an important compound used by the pharmaceutical, cosmetics, and food industries. "Green" production of succinic acid by fermenting bacteria is a more environmentally responsible way to produce the compound, which has traditionally been synthesized from petrochemicals. Investigators interested in optimizing bacterial succinic acid production noted that malate dehydrogenase activity increased under anaerobic conditions.

(a) Draw a reaction scheme outlining how succinic acid can be produced from phosphoenolpyruvate. Include the names of all reactants, products, and enzymes.

(b) Why is it essential that the production of succinic acid take place under anaerobic conditions?

65. Experiments with cancer cells grown in culture show that glutamine is consumed at a high rate and used for biosynthetic reactions, aside from protein synthesis. One possible pathway involves the conversion of glutamine to glutamate and then to α-ketoglutarate. The α-ketoglutarate can then be used to produce pyruvate for gluconeogenesis.

(a) Describe the types of reactions that convert glutamine to α-ketoglutarate.

(b) Give the sequence of enzymes that can convert α-ketoglutarate to pyruvate.

66. Many cancer cells carry out glycolysis at a high rate but convert most of the resulting pyruvate to lactate rather than to acetyl-CoA. Acetyl-CoA, however, is required for the synthesis of fatty acids, which are needed in large amounts by rapidly growing cancer cells. In these cells, the isocitrate dehydrogenase reaction apparently operates in reverse. Explain why this reaction could facilitate the conversion of amino acids such as glutamate into fatty acids.

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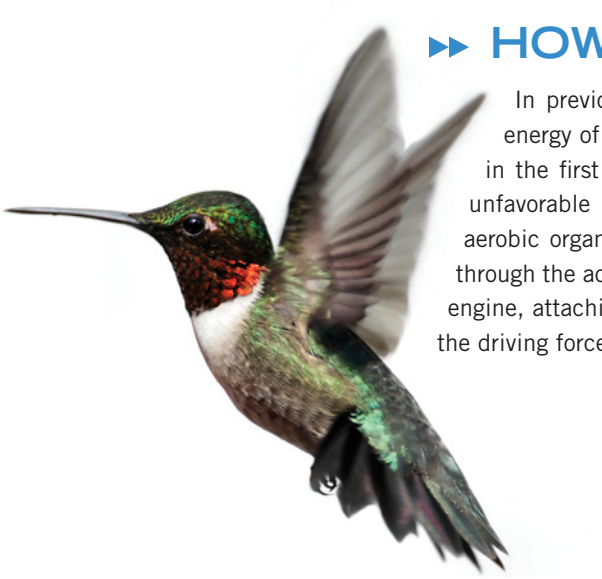
chapter 15

OXIDATIVE PHOSPHORYLATION



[Steve Byland/iStockphoto]

▸▸ HOW does ATP synthase carry out an unfavorable reaction?

In previous chapters, we have seen numerous examples of cells using the free energy of the ATP reaction to carry out cellular work. But in order to produce ATP in the first place, cells must add a third phosphate to ADP, a thermodynamically unfavorable reaction. Glycolysis and the citric acid cycle provide some ATP, but in aerobic organisms, such as the hummingbird shown here, most of the ATP is made through the action of a mitochondrial ATP synthase. This enzyme functions like a rotary engine, attaching a phosphoryl group to ADP as it spins. In this chapter, we'll examine the driving force for this unusual molecular machine.

THIS CHAPTER IN CONTEXT

Do You Remember?

- **Living organisms obey the laws of thermodynamics (Section 1-3).**
- **Transporters obey the laws of thermodynamics, providing a way for solutes to move down their concentration gradients or using ATP to move substances against their gradients (Section 9-1).**
- **Coenzymes such as NAD^+ and ubiquinone collect electrons from compounds that become oxidized (Section 12-2).**
- **A reaction that breaks a phosphoanhydride bond in ATP occurs with a large change in free energy (Section 12-3).**