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CHAPTER 17

Citric Acid Cycle

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Michael P. Gadomski/Photo Researchers, Inc.

The metabolic activity of this germinating seed is geared to generate the energy and carry out the biosynthetic reactions necessary for growth. Among the most active pathways in this and most organisms is the citric acid cycle, a series of reactions that participate in both catabolic and anabolic processes.

In the preceding two chapters, we examined the catabolism of glucose and its biosynthesis, storage, and mobilization. Although glucose is a source of energy for nearly all cells, it is not the only metabolic fuel, nor is glycolysis the only energy-yielding catabolic pathway. Cells that rely exclusively on glycolysis to meet their energy requirements actually waste most of the chemical potential energy of carbohydrates. When glucose is converted to lactate or ethanol, a relatively reduced product leaves the cell. If the end product of glycolysis is instead further oxidized, the cell can recover considerably more energy.

The oxidation of an organic compound requires an electron acceptor, such as NO_3^-, SO_4^{2-}, Fe^{3+}, or O_2, all of which are exploited as oxidants in different organisms. In aerobic organisms, the electrons produced by oxidative metabolism are ultimately transferred to O_2. Oxidation of metabolic fuels is carried out by the citric acid cycle, a sequence of reactions that arose sometime after levels of atmospheric oxygen became significant, about 3 billion years ago. As the reduced carbon atoms of metabolic fuels are oxidized to CO_2, electrons are transferred to electron carriers that are subsequently reoxidized by O_2. In this chapter, we examine the oxidation reactions of the citric acid cycle itself. In the following chapter, we examine the fate of the electrons and see how their energy is used to drive the synthesis of ATP.

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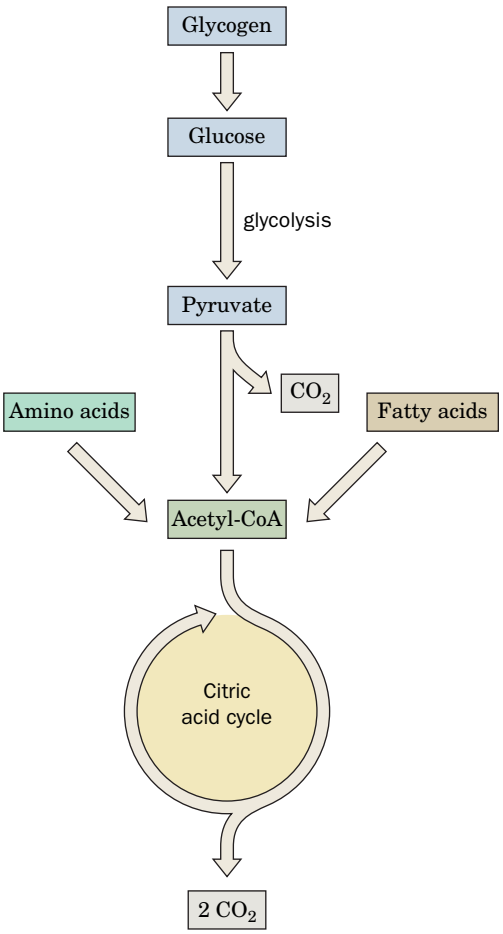


FIG. 17-1 Overview of oxidative fuel metabolism. Acetyl groups derived from carbohydrates, amino acids, and fatty acids enter the citric acid cycle, where they are oxidized to CO_2. **WPLS See the Animated Figures.**

It is sometimes convenient to think of the citric acid cycle as an addendum to glycolysis. Pyruvate derived from glucose can be split into CO_2 and a two-carbon fragment that enters the cycle for oxidation as acetyl-CoA (Fig. 17-1). However, it is really misleading to think of the citric acid cycle as merely a continuation of carbohydrate catabolism. *The citric acid cycle is a central pathway for recovering energy from several metabolic fuels, including carbohydrates, fatty acids, and amino acids, that are broken down to acetyl-CoA for oxidation.* In fact, under some conditions, the principal function of the citric acid cycle is to recover energy from fatty acids. We will also see that the citric acid cycle supplies the reactants for a variety of biosynthetic pathways.

We begin this chapter with an overview of the citric acid cycle. Next, we explore how acetyl-CoA, its starting compound, is formed from pyruvate. After discussing the reactions catalyzed by each of the enzymes of the cycle, we consider the regulation of these enzymes. Finally, we examine the links between citric acid cycle intermediates and other metabolic processes.

1 Overview of the Citric Acid Cycle

KEY CONCEPT

- The citric acid cycle is a multistep catalytic process that converts acetyl groups derived from carbohydrates, fatty acids, and amino acids to CO_2, and produces NADH, $FADH_2$, and GTP.

The **citric acid cycle** (Fig. 17-2) is an ingenious series of eight reactions that oxidizes the acetyl group of acetyl-CoA to two molecules of CO_2 in a manner that conserves the liberated free energy in the reduced compounds NADH and $FADH_2$. The cycle is named after the product of its first reaction, **citrate**. One complete round of the cycle yields two molecules of CO_2, three NADH, one $FADH_2$, and one "high-energy" compound (GTP or ATP).

The citric acid cycle first came to light in the 1930s, when Hans Krebs, building on the work of others, proposed a circular reaction scheme for the interconversion of certain compounds containing two or three carboxylic acid groups (that is, di- and tricarboxylates). At the time, many of the citric acid cycle intermediates were already well known as plant products: **citrate** from citrus fruit, **aconitate** from monkshood (*Aconitum*), **succinate** from amber (succinum), **fumarate** from the herb *Fumaria,* and **malate** from apple (*Malus*). Two other intermediates, **α-ketoglutarate** and **oxaloacetate,** are known by their chemical names because they were synthesized before they were identified in living organisms. Krebs was the first to show how the metabolism of these compounds was linked to the oxidation of metabolic fuels. His discovery of the citric acid cycle, in 1937, ranks as one of the most important achievements of metabolic chemistry (Box 17-1). Although the enzymes and intermediates of the citric acid cycle are now well established, many investigators continue to explore the molecular mechanisms of the enzymes and how the enzymes are regulated for optimal performance under varying metabolic conditions in different organisms.

Before we examine each of the reactions in detail, we should emphasize some general features of the citric acid cycle:

1. The circular pathway, which is also called the **Krebs cycle** or the **tricarboxylic acid (TCA) cycle**, oxidizes acetyl groups from many

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Citric cycle overview

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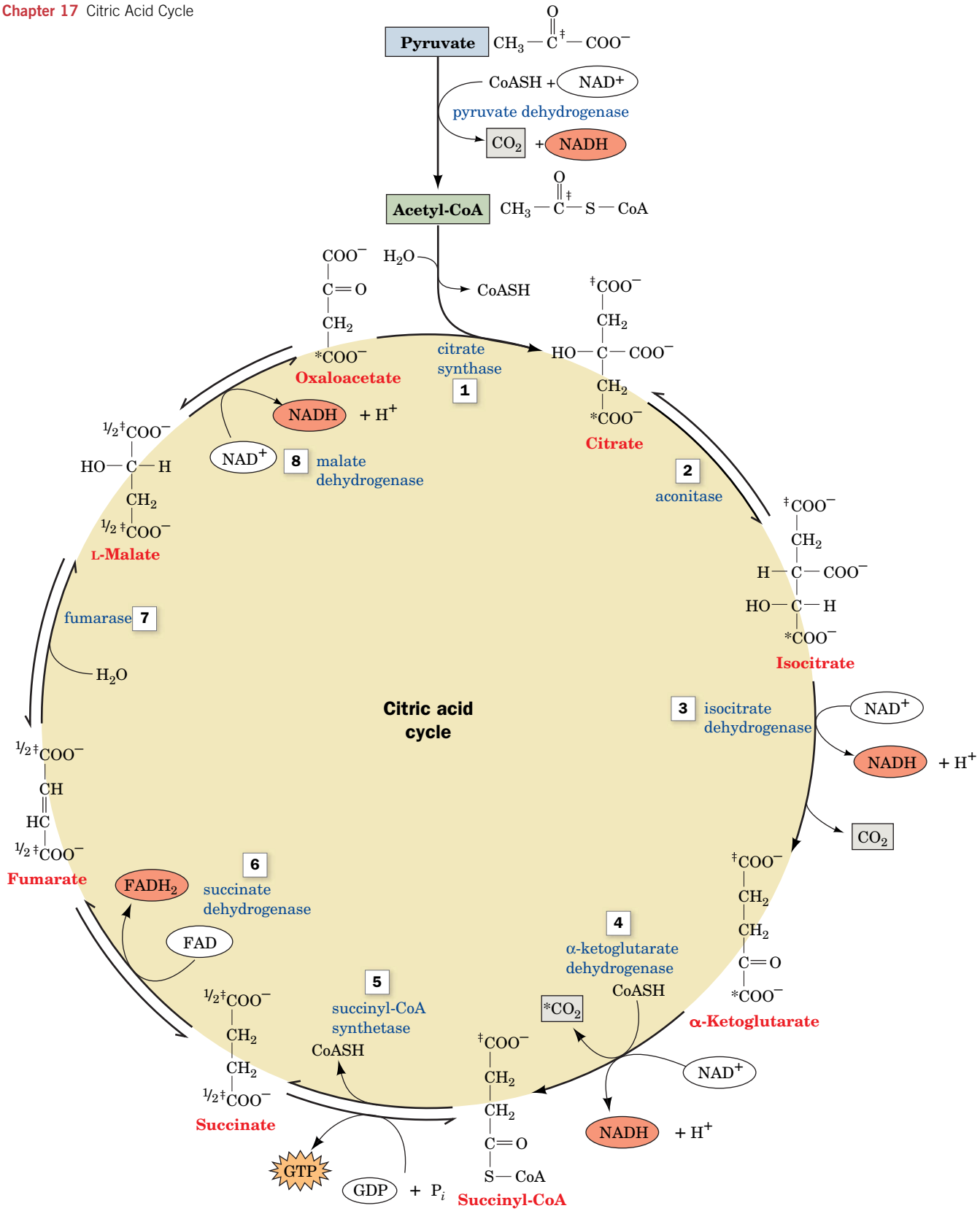


FIG. 17-2 The reactions of the citric acid cycle. The reactants and products of this catalytic cycle are boxed. The pyruvate → acetyl-CoA reaction (*top*) supplies the cycle's substrate via carbohydrate metabolism but is not considered to be part of the cycle. An isotopic label at C4 of oxaloacetate (*) becomes C1 of α-ketoglutarate and is released as CO_2 in Reaction 4. An isotopic label at C1 of acetyl-CoA (‡) becomes C5 of α-ketoglutarate and is scrambled in Reaction 5 between C1 and C4 of succinate ($^1/_2$‡). **WPLS See the Animated Figures.**

? Without looking at the text, write an equation for the citric acid cycle.

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sources, not just pyruvate. Because it accounts for the major portion of carbohydrate, fatty acid, and amino acid oxidation, the citric acid cycle is often considered the "hub" of cellular metabolism.

2. The net reaction of the citric acid cycle is

$$3\,NAD^+ + FAD + GDP + P_i + \text{acetyl-CoA} \rightarrow 3\,NADH + FADH_2 + GTP + CoA + 2\,CO_2$$

The oxaloacetate that is consumed in the first step of the citric acid cycle is regenerated in the last step of the cycle. Thus, *the citric acid cycle acts as a multistep catalyst that can oxidize an unlimited number of acetyl groups.*

3. In eukaryotes, all the enzymes of the citric acid cycle are located in the mitochondria, so all substrates, including NAD^+ and GDP, must be generated in the mitochondria or be transported into mitochondria from the

Box 17-1 Pathways of Discovery — Hans Krebs and the Citric Acid Cycle



Hans Krebs (1900–1981) Hans Krebs worked in Otto Warburg's laboratory from 1926 until 1930 and later declared that he had learned more from Warburg (see Box 15-1) than from any other teacher. Krebs applied Warburg's tissue-slice technique to the study of biosynthetic reactions (Warburg himself was interested primarily in oxidative and degradative reactions). Over a period of years, Krebs investigated synthetic pathways for urea, uric acid, and purines, as well as oxidative pathways. He was forced to leave his native Germany for England in 1933, but unlike many other German emigrant scientists, he was able to bring much of his laboratory equipment with him. In England, Krebs continued to work on a series of metabolic reactions that he named the citric acid cycle.

The cycle was discovered not through sudden inspiration but through a series of careful experiments spanning the years 1932 to 1937. Krebs became interested in the "combustion" phase of fuel use, namely, what occurs after the fermentation of glucose to lactate. Until the 1930s, the mechanism of glucose oxidation and its relationship to cellular respiration (oxygen uptake) was a mystery. Krebs understood that the stoichiometry of the overall process (glucose + $6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$) required a multistep pathway. He was also aware that other researchers had examined the ability of muscle tissue to rapidly oxidize various dicarboxylates (α-ketoglutarate, succinate, and malate) and a tricarboxylate (citrate), but none of these substances had a clear relationship to any foodstuffs.

In 1935, Albert Szent-Györgyi found that cellular respiration was dramatically accelerated by small amounts of succinate, fumarate, malate, or oxaloacetate. In fact, the addition of any of these compounds stimulated O_2 uptake and CO_2 production far in excess of what would be expected for their oxidation. In other words, the compounds acted catalytically to boost the combustion of other compounds in the cell. At about the same time, Carl Martius and Franz Knoop showed that citrate could be converted to α-ketoglutarate. Soon, Krebs had an entire sequence of reactions for converting citrate to oxaloacetate: citrate → aconitate → isocitrate → α-ketoglutarate → succinate → fumarate → malate → oxaloacetate. However, for this sequence of reactions to work catalytically, it must repeatedly return to its starting point; that is, the first compound must be regenerated. And there was still no obvious link to glucose metabolism!

Krebs believed that citrate and the other intermediates were involved in glucose combustion because they appeared to burn at the same rate as foodstuffs and were the only substances that did so. In addition, earlier work had shown that the three-carbon compound malonate not only blocks the conversion of succinate to fumarate, it blocks all combustion by living cells. In 1937, Martius and Knoop provided Krebs with a key piece of information: Oxaloacetate and pyruvate could be converted to citrate in the presence of hydrogen peroxide.

Krebs now had the missing link: Pyruvate is a product of glucose metabolism, and its reaction with oxaloacetate to form citrate closed off the linear series of reactions to form a cycle. The idea of a circular pathway was not new to Krebs. He and Kurt Henseleit had elucidated the four-step urea cycle in 1932 (Section 21-3). Krebs quickly showed that the reaction of pyruvate with oxaloacetate to form citrate took place in living tissue and that the rates of citrate synthesis and breakdown were high enough to account for the observed fuel combustion in a variety of tissue types.

Remarkably, Krebs' initial report on the citric acid cycle was rejected by *Nature,* a leading journal, before being accepted for publication in the less prestigious *Enzymologia.* In his report, Krebs established the major outlines of the pathway, although some details were later revised. For example, the mechanism of citrate formation (which involves acetyl-CoA rather than pyruvate) and the participation of succinyl-CoA in the cycle were not immediately appreciated. Coenzyme A was not discovered until 1945, and only in 1951 was acetyl-CoA shown to be the intermediate that condenses with oxaloacetate to form citrate. Work by Krebs and others established that the citric acid cycle plays a major role in the oxidation of amino acids and fatty acids. In fact, the pathway accounts for approximately two-thirds of the energy derived from metabolic fuels. Krebs also recognized the role of the citric acid cycle in supplying precursors for synthetic reactions.

[Krebs, H.A. and Johnson, W.A., The role of citric acid in intermediate metabolism in animal tissues, *Enzymologia* **4,** 148–156 (1937).]

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cytosol. Similarly, all the products of the citric acid cycle must be consumed in the mitochondria or transported into the cytosol.

4. The carbon atoms of the two molecules of CO_2 produced in one round of the cycle are not the two carbons of the acetyl group that began the round (Fig. 17-2). These acetyl carbon atoms are lost in subsequent rounds of the cycle. However, the net effect of each round of the cycle is the oxidation of one acetyl group to 2 CO_2.
5. Citric acid cycle intermediates are precursors for the biosynthesis of other compounds (e.g., oxaloacetate for gluconeogenesis; Section 16-4).
6. The oxidation of an acetyl group to 2 CO_2 requires the transfer of four pairs of electrons. The reduction of 3 NAD^+ to 3 NADH accounts for three pairs of electrons; the reduction of FAD to $FADH_2$ accounts for the fourth pair. Much of the free energy of oxidation of the acetyl group is conserved in these reduced coenzymes. Energy is also recovered as GTP (or ATP). In Section 18-3C, we will see that approximately 10 ATP are formed when the four pairs of electrons are eventually transferred to O_2.

CHECKPOINT

- Explain why the citric acid cycle is considered to be the hub of cellular metabolism.
- What are the substrates and products of the net reaction corresponding to one turn of the citric acid cycle?

2 Synthesis of Acetyl-Coenzyme A

KEY CONCEPTS

- Pyruvate dehydrogenase is a multienzyme complex that catalyzes a five-part reaction in which pyruvate releases CO_2 and the remaining acetyl group becomes linked to coenzyme A.
- The reaction sequence requires the cofactors TPP, lipoamide, coenzyme A, FAD, and NAD^+.

Acetyl groups enter the citric acid cycle as part of the "high-energy" compound acetyl-CoA (recall that thioesters have high free energies of hydrolysis; Section 14-2D). Although acetyl-CoA can also be derived from fatty acids (Section 20-2) and certain amino acids (Section 21-4), we focus here on the production of acetyl-CoA from pyruvate derived from carbohydrates.

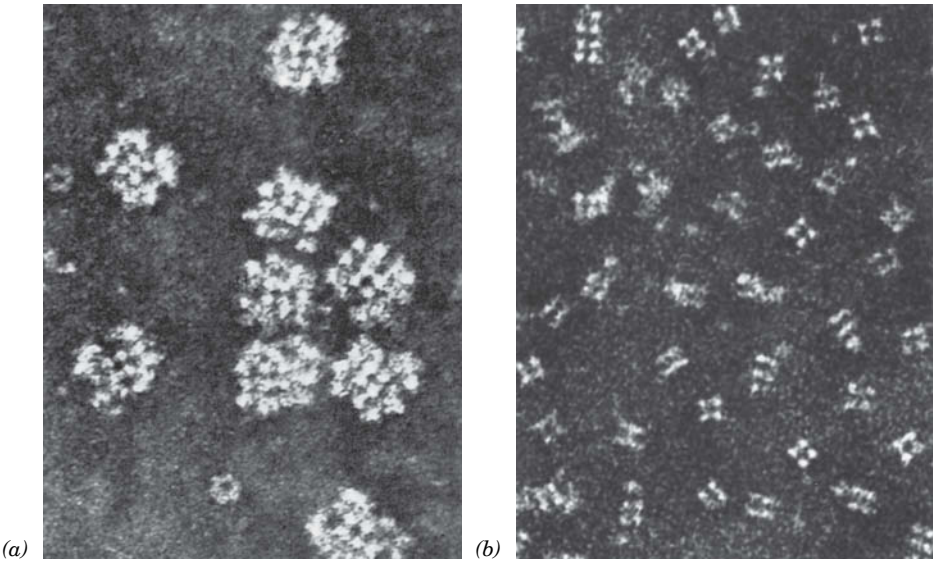
As we saw in Section 15-3, the end product of glycolysis under anaerobic conditions is lactate or ethanol. However, under aerobic conditions, when the NADH generated by glycolysis is reoxidized in the mitochondria, the final product is pyruvate. A transport protein imports pyruvate along with H^+ (i.e., a pyruvate–H^+ symport) into the mitochondrion for further oxidation.

A Pyruvate Dehydrogenase Is a Multienzyme Complex

Multienzyme complexes are groups of noncovalently associated enzymes that catalyze two or more sequential steps in a metabolic pathway. Virtually all organisms contain multienzyme complexes, which represent a step forward in the evolution of catalytic efficiency because they offer the following advantages:

1. Enzymatic reaction rates are limited by the frequency with which enzymes collide with their substrates (Section 11-3D). When a series of reactions occurs within a multienzyme complex, the distance that substrates must diffuse between active sites is minimized, thereby enhancing the reaction rate.
2. The channeling (passing) of metabolic intermediates between successive enzymes in a metabolic pathway reduces the opportunity for these intermediates to react with other molecules, thereby minimizing side reactions.
3. The reactions catalyzed by a multienzyme complex can be coordinately controlled.

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Courtesy of Lester Reed, University of Texas at Austin

FIG. 17-3 Electron micrographs of the *E. coli* pyruvate dehydrogenase multienzyme complex. (*a*) The intact complex. (*b*) The dihydrolipoyl transacetylase (E2) core complex.

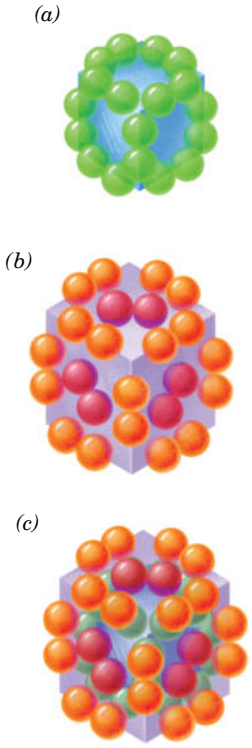
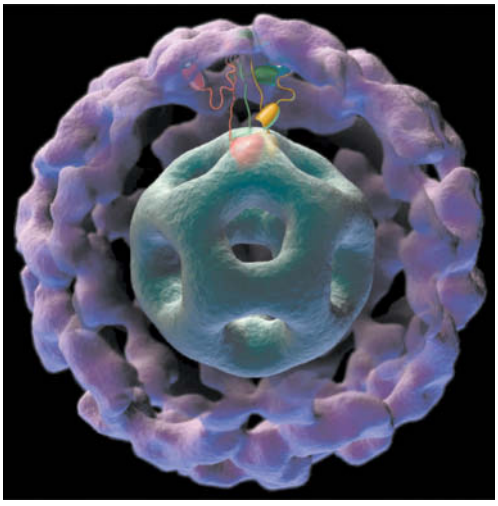


FIG. 17-4 Structural organization of the *E. coli* pyruvate dehydrogenase multienzyme complex. (*a*) The dihydrolipoyl transacetylase (E2) core. The 24 E2 proteins (*green spheres*) associate as trimers at the corners of a cube. (*b*) The 24 pyruvate dehydrogenase (E1) proteins (*orange spheres*) form dimers that associate with the E2 core (*shaded cube*) along its 12 edges. The 12 dihydrolipoyl dehydrogenase (E3) proteins (*purple spheres*) form dimers that attach to the six faces of the E2 cube. (*c*) Parts *a* and *b* combined form the entire 60-subunit complex.

Acetyl-CoA is formed from pyruvate through oxidative decarboxylation by a multienzyme complex named **pyruvate dehydrogenase.** This complex contains multiple copies of three enzymes: **pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2),** and **dihydrolipoyl dehydrogenase (E3).**

The *E. coli* pyruvate dehydrogenase complex is an ~4600-kD particle with a diameter of about 300 Å (Fig. 17-3*a*). The core of the particle is made of 24 E2 proteins arranged in a cube (Figs. 17-3*b* and 17-4*a*), which is surrounded by 24 E1 proteins and 12 E3 proteins (Fig. 17-4*b,c*). In mammals, yeast, and some bacteria, the pyruvate dehydrogenase complex is even larger and more complicated, although it catalyzes the same reactions using homologous enzymes and similar mechanisms. In these ~10,000-kD complexes, the largest known multienzyme complexes, the E2 core consists of 60 subunits arranged with dodecahedral symmetry [Fig. 17-5; a dodecahedron is a regular polyhedron with *I* symmetry (Fig. 6-34*c*) that has 20 vertices and 12 pentagonal faces] surrounded by a shell consisting of ~45 E1 $\alpha_2\beta_2$ heterotetramers and ~9 E3 homodimers. E1 and E3 competitively bind to mutually exclusive sites on E2 in a random distribution. Mammalian complexes, in addition, contain ~12 copies of **E3 binding protein,** which facilitates the binding of E3 to the E2 core, and several copies of a kinase and a phosphatase that function to regulate the activity of the complex (Section 17-4A).



Courtesy of Jacqueline Milne, National Institutes of Health, Bethesda, MD

FIG. 17-5 Model of the *Bacillus stearothermophilus* pyruvate dehydrogenase complex. This ~500-Å-diameter cutaway surface diagram is based on cryoelectron microscopic images of a complex consisting of a core of 60 E2 subunits (*gray-green*) surrounded by an outer shell of 60 E1 subunits (*purple*) separated by an ~90-Å-wide annular gap. Three full-length E2 subunits are schematically represented in red, green, and yellow to indicate their catalytic domains (embedded in the E2 core), their peripheral subunit-binding domains (embedded in the E3 shell) that bind the E1 (and E3) subunits, and their lipoyl domains, all connected by flexible polypeptide linkers (Section 17-2B). Here, the red lipoyl domain is shown visiting an E1 active site (*white dot*), whereas the green and yellow lipoyl domains occupy intermediate positions in the annular region between the E2 core and E1 shell. In a normal pyruvate dehydrogenase complex, E3 subunits would take the place of some of the E1 subunits. Indeed, a complex of 60 E3 subunits and 60 E2 subunits closely resembles the E1–E2 complex.

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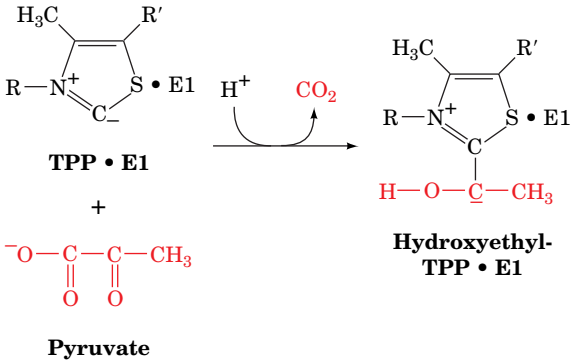
B The Pyruvate Dehydrogenase Complex Catalyzes Five Reactions

The pyruvate dehydrogenase complex catalyzes five sequential reactions with the overall stoichiometry

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

Five different coenzymes are required: thiamine pyrophosphate (TPP; Section 15-3B), **lipoamide,** coenzyme A (Fig. 14-11), FAD (Fig. 14-13), and NAD^+ (Fig. 11-4). The coenzymes and their mechanistic functions are listed in Table 17-1. The sequence of reactions catalyzed by the pyruvate dehydrogenase complex is as follows (Fig. 17-6):

1. Pyruvate dehydrogenase (E1), a TPP-requiring enzyme, decarboxylates pyruvate with the formation of a hydroxyethyl-TPP intermediate:



   This reaction is identical to that catalyzed by yeast pyruvate decarboxylase (Fig. 15-20). Recall (Section 15-3B) that the ability of TPP's thiazolium ring to add to carbonyl groups and act as an electron sink makes it the coenzyme most utilized in α-keto acid decarboxylation reactions.
2. Unlike pyruvate decarboxylase, however, pyruvate dehydrogenase does not convert the hydroxyethyl-TPP intermediate into acetaldehyde

TABLE 17-1 The Coenzymes and Prosthetic Groups of Pyruvate Dehydrogenase

Cofactor	Location	Function
Thiamine pyrophosphate (TPP)	Bound to E1	Decarboxylates pyruvate, yielding a hydroxyethyl–TPP carbanion
Lipoic acid	Covalently linked to a Lys on E2 (lipoamide)	Accepts the hydroxyethyl carbanion from TPP as an acetyl group
Coenzyme A (CoA)	Substrate for E2	Accepts the acetyl group from lipoamide
Flavin adenine dinucleotide (FAD)	Bound to E3	Reduced by lipoamide
Nicotinamide adenine dinucleotide (NAD^+)	Substrate for E3	Reduced by $FADH_2$

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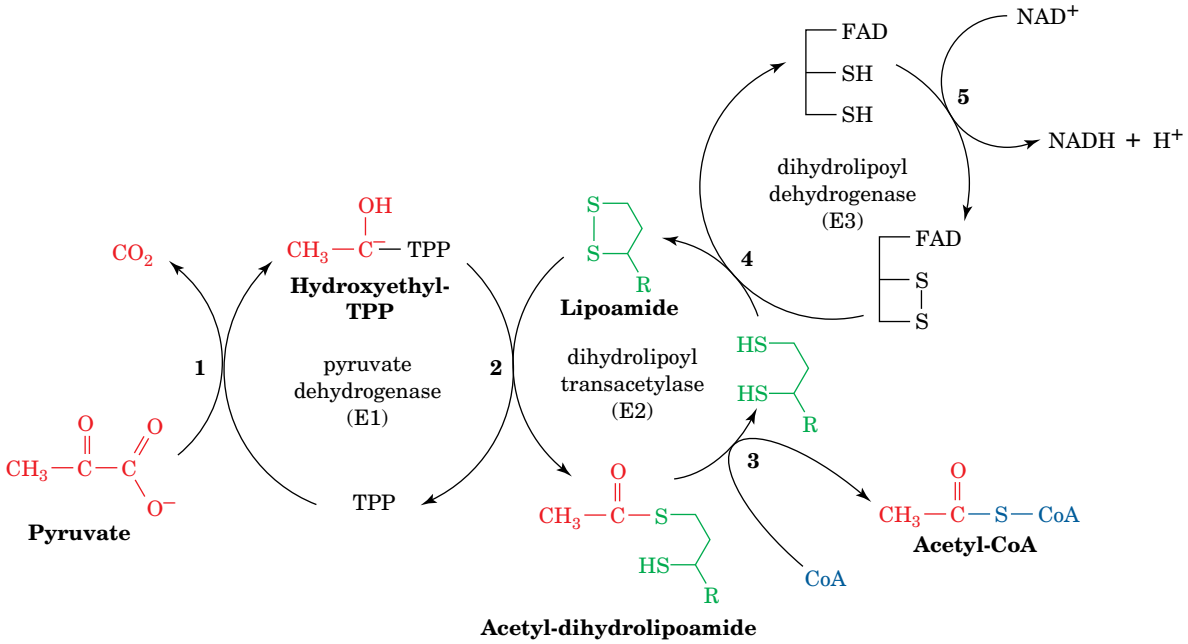


FIG. 17-6 The five reactions of the pyruvate dehydrogenase multienzyme complex. E1 (pyruvate dehydrogenase) contains TPP and catalyzes Reactions 1 and 2. E2 (dihydrolipoyl transacetylase) contains lipoamide and catalyzes Reaction 3. E3 (dihydrolipoyl dehydrogenase) contains FAD and a redox-active disulfide and catalyzes Reactions 4 and 5.

? What type of reaction occurs in each of the five steps?

and TPP. Instead, the hydroxyethyl group is transferred to the next enzyme, dihydrolipoyl transacetylase (E2), which contains a lipoamide group. Lipoamide consists of **lipoic acid** linked via an amide bond to the ε-amino group of a Lys residue (Fig. 17-7). The reactive center of lipoamide is a cyclic disulfide that can be reversibly reduced to yield **dihydrolipoamide.** The hydroxyethyl group derived from pyruvate attacks the lipoamide disulfide, and TPP is eliminated, thus regenerating

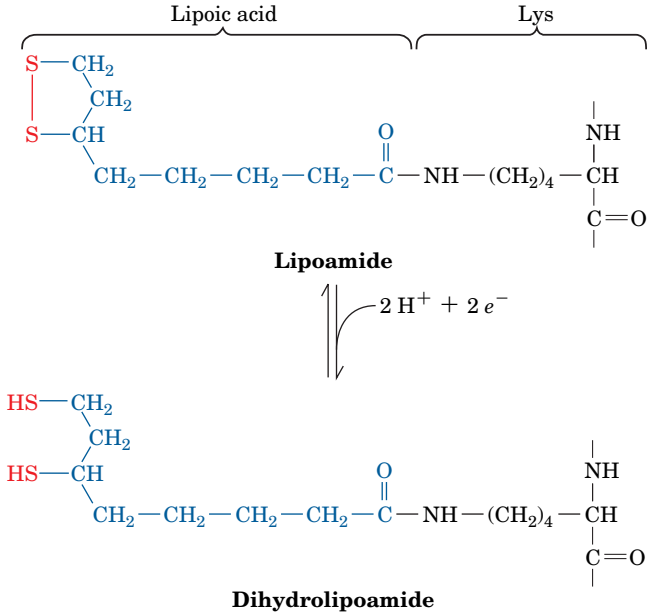
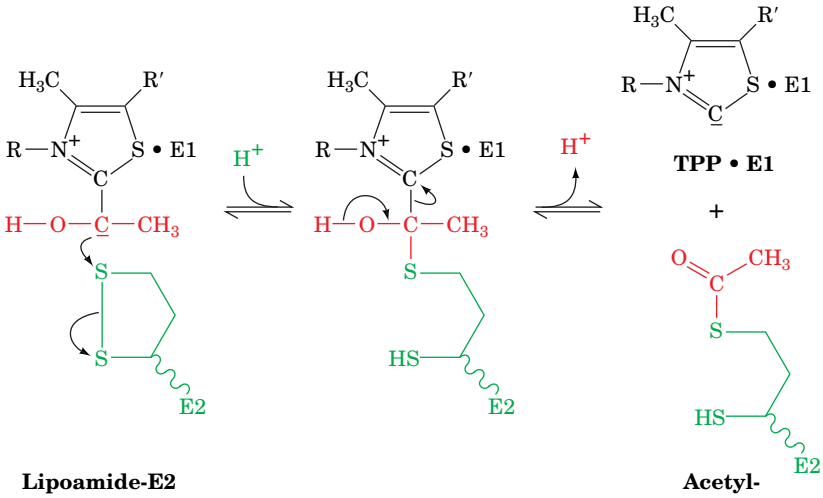


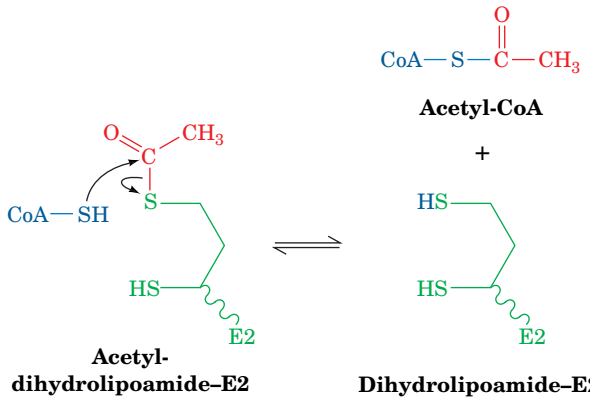
FIG. 17-7 Interconversion of lipoamide and dihydrolipoamide. Lipoamide consists of lipoic acid covalently joined to the ε-amino group of a Lys residue via an amide bond.

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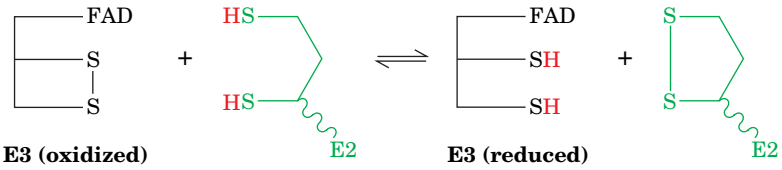
active E1. The hydroxyethyl carbanion is oxidized to an acetyl group as the lipoamide disulfide is reduced:



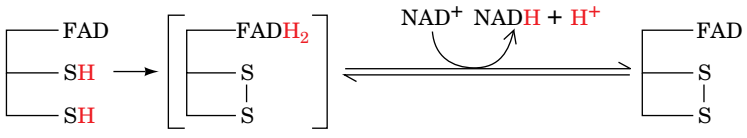
3. E2 then catalyzes a transesterification reaction in which the acetyl group is transferred to CoA, yielding acetyl-CoA and dihydrolipoamide-E2:



4. Acetyl-CoA has now been formed, but the lipoamide group of E2 must be regenerated. Dihydrolipoyl dehydrogenase (E3) reoxidizes dihydrolipoamide to complete the catalytic cycle of E2. Oxidized E3 contains a reactive Cys—Cys disulfide group and a tightly bound FAD. The oxidation of dihydrolipoamide is a disulfide interchange reaction:



5. Finally, reduced E3 is reoxidized. The sulfhydryl groups are reoxidized by a mechanism in which FAD funnels electrons to NAD^+, yielding NADH:



The X-ray structure of dihydrolipoyl dehydrogenase together with mechanistic information indicate that the reaction catalyzed by dihydrolipoamide

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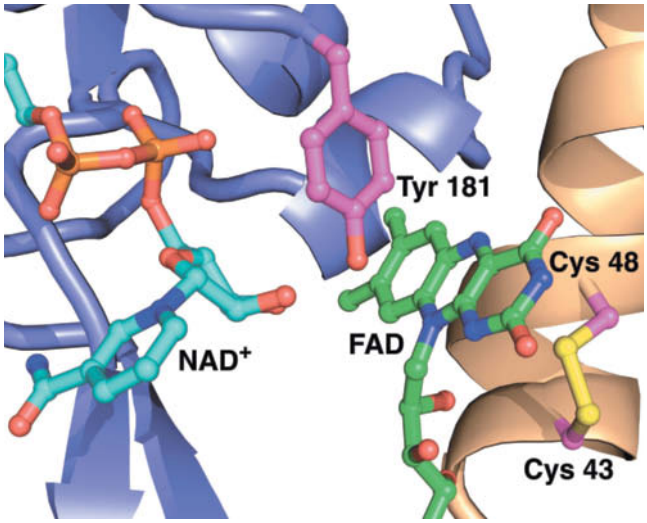


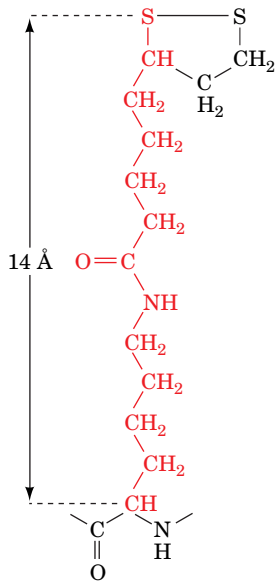
FIG. 17-8 Active site of dihydrolipoamide dehydrogenase (E3). In this X-ray structure of the enzyme from *Pseudomonas putida,* the redox-active portions of the bound NAD^+ and FAD cofactors, the side chains of Cys 43 and Cys 48 forming the redox-active disulfide bond, and the side chain of Tyr 181 are shown in ball-and-stick form with FAD C green, NAD^+ C cyan, protein side chain C magenta, N blue, O red, P orange, and S yellow. Note that the side chain of Tyr 181 is interposed between the flavin and the nicotinamide rings. Portions of the homodimeric protein's two subunits are drawn in ribbon form in purple and light orange. [Based on an X-ray structure by Wim Hol, University of Washington. PDBid 1LVL.]

? Compare the reduction potentials of NAD^+, FAD, and cysteine disulfide (Table 14-4). What do these values predict about the flow of electrons among the three groups?

dehydrogenase (E3) is more complex than Reactions 4 and 5 in Fig. 17-6 suggest. The enzyme's redox-active disulfide bond occurs between Cys 43 and Cys 48, which reside on a highly conserved segment of the enzyme's polypeptide chain. The disulfide bond links successive turns in a distorted segment of an α helix (in an undistorted helix, the C_α atoms of Cys 43 and Cys 48 would be too far apart to permit the disulfide bond to form). The enzyme's flavin group is almost completely buried in the protein, which prevents the surrounding solution from interfering with the electron-transfer reaction catalyzed by the enzyme. The nicotinamide ring of NAD^+ binds on the side of the flavin opposite the disulfide. In the absence of NAD^+, the phenol side chain of Tyr 181 covers the nicotinamide-binding pocket to shield the flavin from contact with the solution (Fig. 17-8). The Tyr side chain apparently moves aside to allow the nicotinamide ring to bind near the flavin ring.

FAD prosthetic groups in proteins have standard reduction potentials of around 0 V (Table 14-4), which makes $FADH_2$ unsuitable for donating electrons to NAD^+ ($\mathscr{E}°' = -0.315$ V). Evidence suggests that the FAD group in dihydrolipoamide dehydrogenase never becomes fully reduced as $FADH_2$. Due to the precise positioning of the flavin and nicotinamide ring, electrons are rapidly transferred from the enzyme disulfide through FAD to NAD^+, so a reduced flavin anion ($FADH^-$) has but a transient existence. Thus, *FAD appears to function more as an electron conduit than as a source or sink of electrons.*

A Swinging Arm Transfers Intermediates. How are reaction intermediates channeled between E2 (the core of the pyruvate dehydrogenase complex) and the E1 and E3 proteins on the outside? The key is the lipoamide group of E2. The lipoic acid residue and the side chain of the Lys residue to which it is attached have a combined length of about 14 Å. This **lipoyllysyl arm** (*at right*) is linked to the rest of the E2 protein by a highly flexible and >140-Å-long, Pro- and Ala-rich segment, which together function as a long tether that swings the disulfide group from E1 (where it acquires a hydroxyethyl group), to the E2 active site (where the hydroxyethyl group is transferred to form acetyl-CoA), and from there to E3 (where the reduced disulfide is reoxidized). Because of the flexibility and reach of this assembly, one E1 protein can acetylate numerous E2 proteins, and one E3 protein can reoxidize several dihydrolipoamide groups. The flexible linkers protrude into the space between the E2 core and the E1–E3 outer shell (Fig. 17-5) and swing around in order to "visit" the active sites of E1, E2, and E3. The entire pyruvate dehydrogenase complex can be inactivated by the reaction of the lipoamide group with certain arsenic-containing compounds (see Box 17-2).



Lipoyllysyl arm (fully extended)

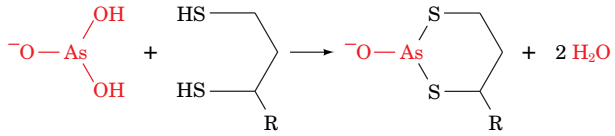
CHECKPOINT

- Summarize the advantages of multienzyme complexes.
- Write an equation for the pyruvate dehydrogenase reaction.
- Describe the five reactions of the pyruvate dehydrogenase multienzyme complex.
- What cofactors are required? Which of these are prosthetic groups?

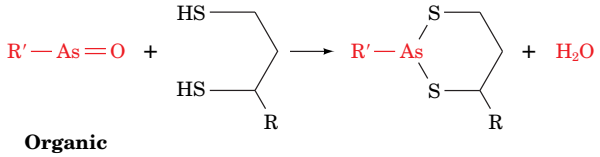
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Box 17-2 Biochemistry in Health and Disease — Arsenic Poisoning

The toxicity of arsenic has been known since ancient times. As(III) compounds such as **arsenite** (AsO_3^{3-}) and **organic arsenicals** are toxic because they bind to sulfhydryl compounds (including lipoamide) that can form bidentate adducts:



Arsenite **Dihydro-lipoamide**



Organic arsenical

The inactivation of lipoamide-containing enzymes by arsenite, especially the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes, brings respiration to a halt. However, organic arsenicals are more toxic to microorganisms than they are to humans, apparently because of differences in the sensitivities of their various enzymes to the compounds. This differential toxicity is the basis for the early twentieth century use of organic arsenicals in the treatment of **syphilis** (a bacterial disease) and trypanosomiasis (a parasitic disease). These compounds were actually the first antibiotics, although, not surprisingly, they produced severe side effects.

Arsenic is often suspected as a poison in untimely deaths. It was long thought that Napoleon Bonaparte died from arsenic poisoning while in exile on the island of St. Helena, a suspicion that is strongly supported by the recent finding that a lock of his hair contains high levels of arsenic. But was it murder or environmental pollution? Arsenic-containing dyes were used in wallpaper at the time, and it was eventually determined that in damp weather, fungi convert the arsenic to a volatile compound. Surviving samples of the wallpaper from Napoleon's room in fact contain arsenic. Napoleon's arsenic poisoning may therefore have been unintentional.



© Heritage Images/Corbis

Napoleon Bonaparte.

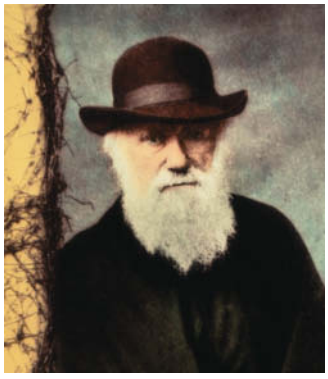


Photo Researchers

Charles Darwin.

Charles Darwin may also have been an unwitting victim of chronic arsenic poisoning. In the years following his epic voyage on the *Beagle,* Darwin was plagued by eczema, vertigo, headaches, gout, and nausea–all symptoms of arsenic poisoning. Fowler's solution, a widely used nineteenth century "tonic," contained 10 mg of arsenite per mL. Many individuals, quite possibly Darwin himself, took this "medication" for years.

3 Enzymes of the Citric Acid Cycle

KEY CONCEPTS

- The eight enzymes of the citric acid cycle catalyze condensation, isomerization, oxidation–reduction, phosphorylation, and hydration reactions.
- Two reactions produce CO_2, one reaction produces GTP, and four reactions generate the reduced coenzymes NADH or $FADH_2$.

In this section, we discuss the eight enzymes of the citric acid cycle. The elucidation of the mechanisms for each of these enzymes is the result of an enormous amount of experimental work. Even so, there remain questions about the mechanistic details of the enzymes and their regulatory properties.

A Citrate Synthase Joins an Acetyl Group to Oxaloacetate

Citrate synthase catalyzes the condensation of acetyl-CoA and oxaloacetate. This initial reaction of the citric acid cycle is the point at which carbon atoms (from carbohydrates, fatty acids, and amino acids) are "fed into the furnace" as acetyl-CoA. The citrate synthase reaction proceeds with an Ordered Sequential kinetic mechanism in which oxaloacetate binds before acetyl-CoA.

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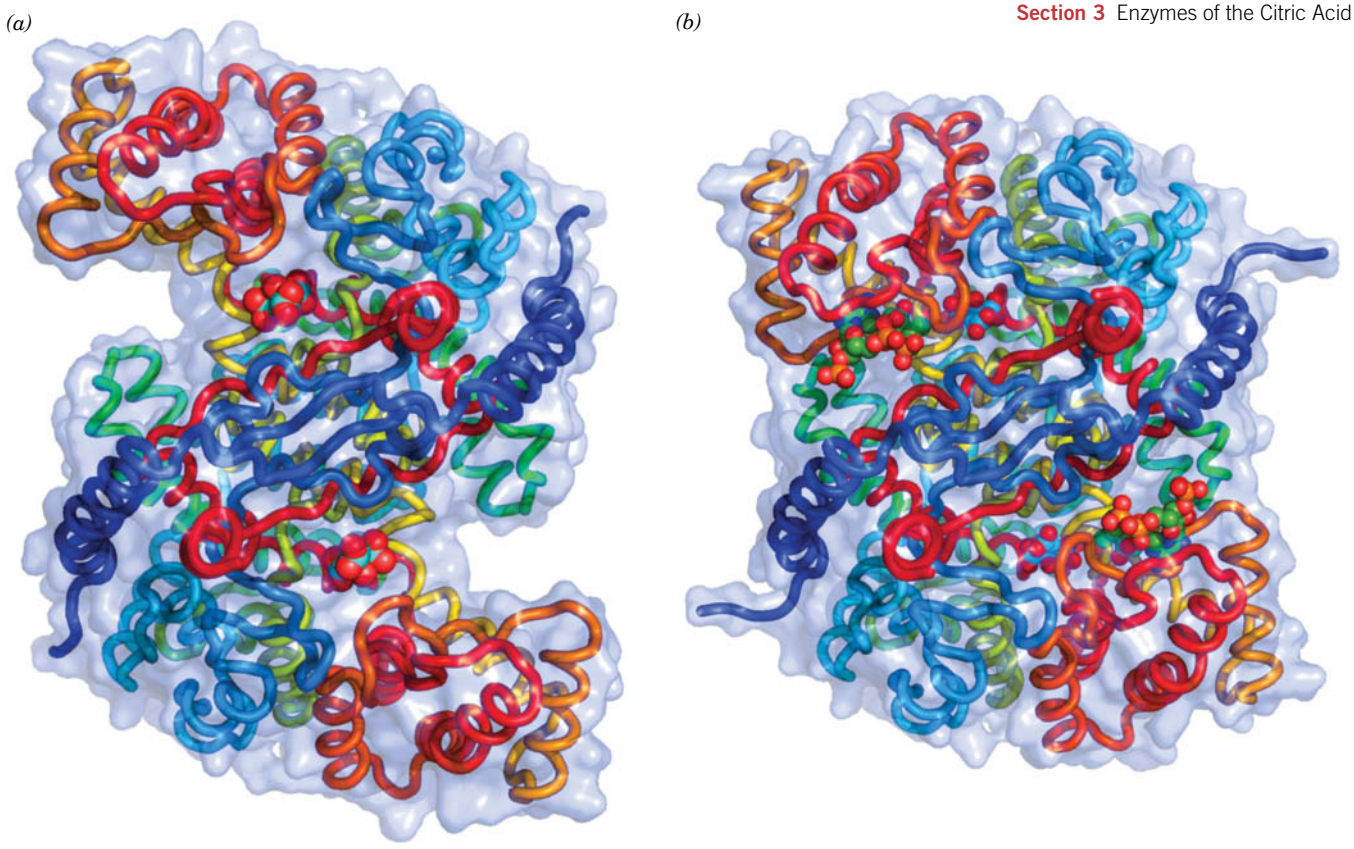


FIG. 17-9 Conformational changes in citrate synthase. (*a*) The open conformation. (*b*) The closed, substrate-binding conformation. In both forms, the homodimeric protein is viewed along its twofold axis and is represented by its transparent molecular surface with its polypeptide chains drawn in worm form colored in rainbow order from their N-termini (*blue*) to their C-termini (*red*). The reaction product citrate, which is bound to both enzymatic forms, and coenzyme A, which is also bound to the closed form, are shown in space-filling form with citrate C cyan, CoA C green, N blue, O red, and P orange. The large conformational shift between the open and closed forms entails 18° rotations of the small domains (*upper left and lower right*) relative to the large domains resulting in relative interatomic movements of up to 15 Å. [Based on X-ray structures by James Remington and Robert Huber, Max-Planck-Institut für Biochemie, Martinsried, Germany. PDBids 1CTS and 2CTS.]

X-Ray studies show that the free enzyme (a homodimer) is in an "open" form, with two domains that form a cleft containing the substrate-binding site (**Fig. 17-9*a***). When substrate binds, the smaller domain undergoes a remarkable 18° rotation, which closes the cleft (**Fig. 17-9*b***). The existence of the "open" and "closed" forms explains the enzyme's Ordered Sequential kinetic behavior. *The conformational change generates the acetyl-CoA binding site and seals the oxaloacetate binding site so that solvent cannot reach the bound substrate.* We have seen similar conformational changes in adenylate kinase (Fig. 14-10) and hexokinase (Fig. 15-2), which prevent ATP hydrolysis.

In the reaction mechanism proposed by James Remington, three ionizable side chains of citrate synthase participate in catalysis (**Fig. 17-10**):

1. The enol of acetyl-CoA is generated in the rate-limiting step of the reaction when Asp 375 (a base) removes a proton from the methyl group. His 274 forms a hydrogen bond with the enolate oxygen.
2. **Citryl-CoA** is formed in a concerted acid–base catalyzed step, in which the acetyl-CoA enolate (a nucleophile) attacks oxaloacetate. His 320 (an acid) donates a proton to oxaloacetate's carbonyl group. The citryl-CoA intermediate remains bound to the enzyme. Citrate synthase is one of the few enzymes that directly forms a carbon–carbon bond without the assistance of a metal ion cofactor.

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PROCESS DIAGRAM

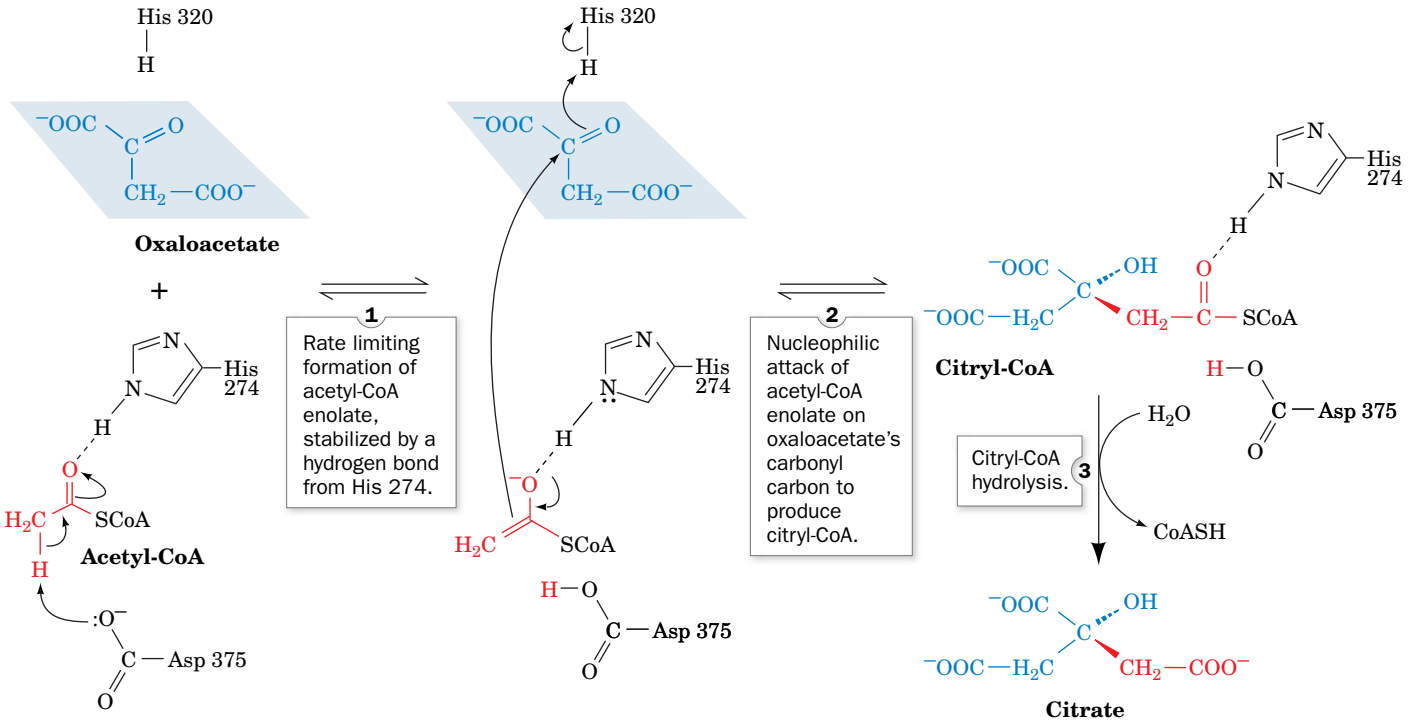


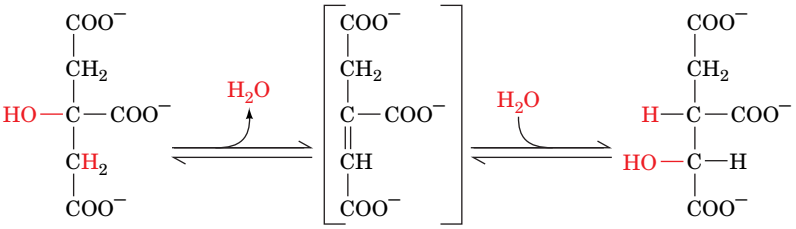
FIG. 17-10 **The mechanism of the citrate synthase reaction.** His 274 and His 320 in their neutral forms and Asp 375 have been implicated as general acid–base catalysts. [Mostly after Remington, J.S., *Curr. Opin. Struct. Biol.* **2,** 732 (1992).] **WPLS See the Animated Process Diagrams.**

? **Would citrate synthase activity be more sensitive to a slight increase in pH or a slight decrease in pH?**

3. Citryl-CoA is hydrolyzed to citrate and CoA. This hydrolysis provides the reaction's thermodynamic driving force ($\Delta G^{\circ\prime} = -31.5$ kJ · mol^{-1}). We will see later why this reaction requires such a large, seemingly wasteful, expenditure of free energy.

B Aconitase Interconverts Citrate and Isocitrate

Aconitase catalyzes the reversible isomerization of citrate and **isocitrate,** with ***cis*-aconitate** as an intermediate:



The reaction begins with a dehydration step in which a proton and an OH group are removed. Since citrate has two carboxymethyl groups substituent to its central C atom, it is prochiral rather than chiral. Thus, although water might conceivably be eliminated from either of the two carboxymethyl arms, aconitase removes water only from citrate's lower (*pro-R*) arm (i.e., such that the product molecule has the *R* configuration; Box 4-2).

Aconitase contains a **[4Fe−4S] iron–sulfur cluster** (an arrangement of four iron atoms and four sulfur atoms, Section 18-2C) that presumably coordinates

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the OH group of citrate to facilitate its elimination. Iron–sulfur clusters normally participate in redox processes; aconitase is an intriguing exception.

The second stage of the aconitase reaction is rehydration of the double bond of *cis*-aconitate to form isocitrate. Although addition of water across the double bond of *cis*-aconitate could potentially yield four stereoisomers, aconitase catalyzes the stereospecific addition of OH^- and H^+ to produce only one isocitrate stereoisomer. The ability of an enzyme to differentiate its substrate's *pro-R* and *pro-S* groups was not appreciated until 1948, when Alexander Ogston pointed out that aconitase can distinguish between the two $—CH_2COO^-$ groups of citrate when it is bound to the enzyme (Section 11-1B).

Aconitase Functions as an Iron Sensor. Nature is opportunistic in that it often finds more than one use for a protein, a phenomenon dubbed moonlighting (after the practice of taking a second job, often at night, when the moon often shines). Aconitase is such a multifunctional protein. Cells require a balanced amount of iron. Too little and their many iron-containing proteins will lose function, whereas too much is toxic, particularly to the liver, heart, and pancreas, causing a life-threatening condition known as **hemochromatosis.** Dietary iron is transported through bloodstream by the protein **transferrin**, enters cells through receptor-mediated endocytosis (Section 20-1B) in which the receptor is **transferrin receptor,** and any excess iron is stored by the protein **ferritin**. When a cell has insufficient iron, its aconitase is inactivated through the loss of its [4Fe–4S] iron–sulfur cluster. The resulting protein, which undergoes extensive conformational changes to become known as **iron regulatory protein-1 (IRP1),** binds to specific sequences on the mRNAs encoding the transferrin receptor and ferritin. This inhibits the degradation of the transferrin receptor mRNA, resulting in an increased rate of synthesis of transferrin receptor, whereas it blocks the translation of the ferritin mRNA. A cell's rate of iron uptake is thereby increased, whereas its amount of ferritin-bound iron is decreased.

C NAD^+-Dependent Isocitrate Dehydrogenase Releases CO_2

Isocitrate dehydrogenase catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (also known as **2-oxoglutarate**). This reaction produces the first CO_2 and NADH of the citric acid cycle. Note that this CO_2 began the citric acid cycle as a component of oxaloacetate, not of acetyl-CoA (Fig. 17-2). (Mammalian tissues also contain an isocitrate dehydrogenase isozyme that uses $NADP^+$ as a cofactor.)

NAD^+-dependent isocitrate dehydrogenase, which also requires a Mn^{2+} or Mg^{2+} cofactor, catalyzes the oxidation of a secondary alcohol (isocitrate) to a ketone **(oxalosuccinate)** followed by the decarboxylation of the carboxyl group β to the ketone (Fig. 17-11). Mn^{2+} helps polarize the newly formed carbonyl group. The isocitrate dehydrogenase reaction mechanism is similar to that of phosphogluconate dehydrogenase in the pentose phosphate pathway (Section 15-6A).

The oxalosuccinate intermediate of the isocitrate dehydrogenase reaction exists only transiently, and its existence was therefore difficult to confirm.

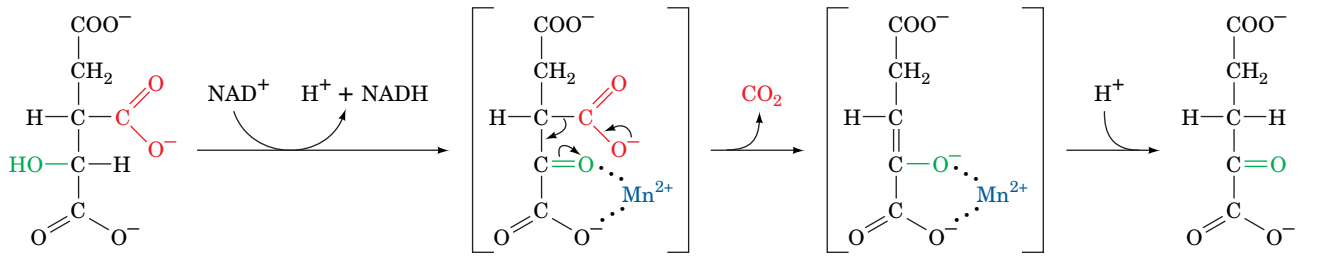


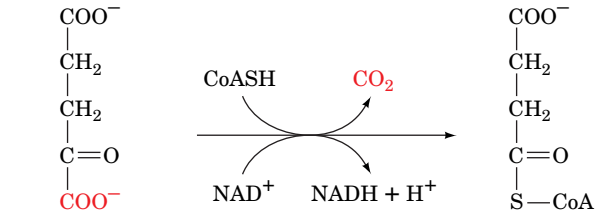
FIG. 17-11 The reaction mechanism of isocitrate dehydrogenase. Oxalosuccinate is shown in brackets because it does not dissociate from the enzyme.

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However, an enzymatic reaction can be slowed by mutating catalytically important residues—in this case, Tyr 160 and Lys 230—to create kinetic "bottlenecks" so that reaction intermediates accumulate. Accordingly, crystals of the mutant isocitrate dehydrogenase were exposed to the substrate isocitrate and immediately visualized via X-ray crystallography using rapid measurement techniques that require the highly intense X-rays generated by a synchrotron. These studies revealed the oxalosuccinate intermediate in the active site of the enzyme.

D α-Ketoglutarate Dehydrogenase Resembles Pyruvate Dehydrogenase

α-Ketoglutarate dehydrogenase catalyzes the oxidative decarboxylation of an α-keto acid (α-ketoglutarate). This reaction produces the second CO_2 and NADH of the citric acid cycle:



α-Ketoglutarate **Succinyl-CoA**

Again, this CO_2 entered the citric acid cycle as a component of oxaloacetate rather than of acetyl-CoA (Fig. 17-2). Thus, although each round of the citric acid cycle oxidizes two C atoms to CO_2, the C atoms of the entering acetyl groups are not oxidized to CO_2 until subsequent rounds of the cycle.

The α-ketoglutarate dehydrogenase reaction chemically resembles the reaction catalyzed by the pyruvate dehydrogenase multienzyme complex. α-Ketoglutarate dehydrogenase is a multienzyme complex containing **α-ketoglutarate dehydrogenase (E1), dihydrolipoyl transsuccinylase (E2),** and **dihydrolipoyl dehydrogenase (E3).** Indeed, this E3 is identical to the E3 of the pyruvate dehydrogenase complex (a third member of the **2-keto acid dehydrogenase** family of multienzyme complexes is **branched-chain α-keto acid dehydrogenase,** which participates in the degradation of isoleucine, leucine, and valine; Section 21-4). The reactions catalyzed by the α-ketoglutarate dehydrogenase complex occur by mechanisms identical to those of the pyruvate dehydrogenase complex. Again, the product is a "high-energy" thioester, in this case, **succinyl-CoA.**

E Succinyl-CoA Synthetase Produces GTP

Succinyl-CoA synthetase (also called **succinate thiokinase**) couples the cleavage of the "high-energy" succinyl-CoA to the synthesis of a "high-energy" nucleoside triphosphate (both names for the enzyme reflect the reverse reaction). Mammalian enzymes usually synthesize GTP from GDP + P_i, whereas plant and bacterial enzymes usually synthesize ATP from ADP + P_i. These reactions are nevertheless energetically equivalent since ATP and GTP are rapidly interconverted through the action of nucleoside diphosphate kinase (Section 14-2C):

$$\text{GTP} + \text{ADP} \rightleftharpoons \text{GDP} + \text{ATP} \qquad \Delta G^{\circ\prime} = 0$$

How does succinyl-CoA synthetase couple the exergonic cleavage of succinyl-CoA ($\Delta G^{\circ\prime} = -32.6$ kJ · mol^{-1}) to the endergonic formation of a nucleoside triphosphate ($\Delta G^{\circ\prime} = +30.5$ kJ · mol^{-1}) from the corresponding nucleoside diphosphate and P_i? This question was answered by an experiment with isotopically labeled ADP. In the absence of succinyl-CoA, the spinach enzyme catalyzes the transfer of the γ-phosphoryl group from ATP to [^{14}C]ADP, producing [^{14}C]ATP. Such an isotope-exchange reaction suggests the

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