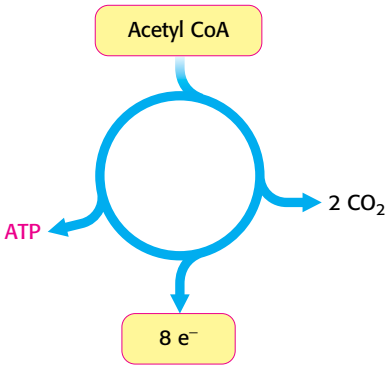
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CHAPTER

14

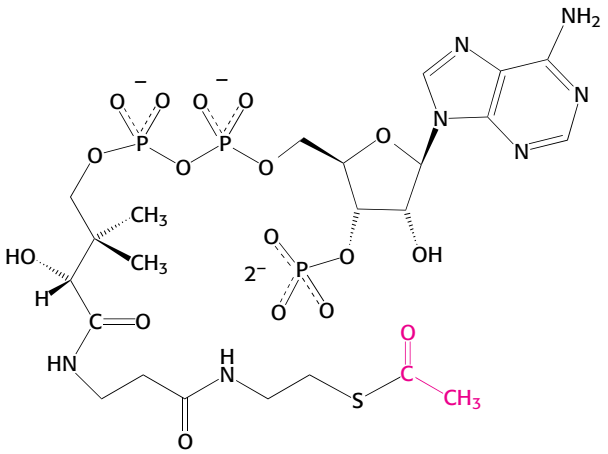
The Citric Acid Cycle





Roundabouts, or traffic circles, function as hubs to facilitate traffic flow. The citric acid cycle is the biochemical hub of the cell, oxidizing carbon fuels, usually in the form of acetyl CoA, as well as serving as a source of precursors for biosynthesis. [(Left) Lynn Saville/Getty Images.]

The metabolism of glucose to pyruvate in glycolysis, an anaerobic process, harvests but a fraction of the ATP available from glucose. Most of the ATP generated in metabolism is provided by the *aerobic* processing of glucose. This process starts with the complete oxidation of glucose derivatives to carbon dioxide. This oxidation takes place in a series of reactions called the *citric acid cycle*, also known as the *tricarboxylic acid* (TCA) *cycle* or the *Krebs cycle*. The citric acid cycle is the *final common pathway for the oxidation of fuel molecules*—carbohydrates, fatty acids, and amino acids. Most fuel molecules enter the cycle as *acetyl coenzyme A*.



Acetyl coenzyme A (Acetyl CoA)

OUTLINE

17.1 The Pyruvate Dehydrogenase Complex Links Glycolysis to the Citric Acid Cycle

17.2 The Citric Acid Cycle Oxidizes Two-Carbon Units

17.3 Entry to the Citric Acid Cycle and Metabolism Through It Are Controlled

17.4 The Citric Acid Cycle Is a Source of Biosynthetic Precursors

17.5 The Glyoxylate Cycle Enables Plants and Bacteria to Grow on Acetate

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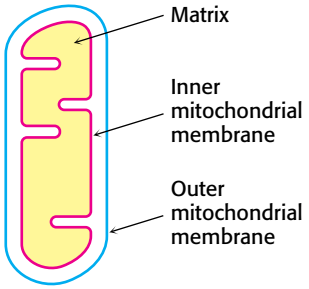


FIGURE 17.1 Mitochondrion. The double membrane of the mitochondrion is evident in this electron micrograph. The numerous invaginations of the inner mitochondrial membrane are called cristae. The oxidative decarboxylation of pyruvate and the sequence of reactions in the citric acid cycle take place within the matrix. [(Left) Omikron/Photo Researchers.]

Under aerobic conditions, the pyruvate generated from glucose is oxidatively decarboxylated to form acetyl CoA. In eukaryotes, the reactions of the citric acid cycle take place in the matrix of the mitochondria (Figure 17.1), in contrast with those of glycolysis, which take place in the cytoplasm.

The citric acid cycle harvests high-energy electrons

The citric acid cycle is the central metabolic hub of the cell. It is the gateway to the aerobic metabolism of any molecule that can be transformed into an acetyl group or a component of the citric acid cycle. The cycle is also an important source of precursors for the building blocks of many other molecules such as amino acids, nucleotide bases, and porphyrin (the organic component of heme). The citric acid cycle component, oxaloacetate, is also an important precursor to glucose (Section 16.3).

What is the function of the citric acid cycle in transforming fuel molecules into ATP? Recall that fuel molecules are carbon compounds that are capable of being oxidized—that is, of losing electrons (Chapter 15). The citric acid cycle includes a series of oxidation–reduction reactions that result in the oxidation of an acetyl group to two molecules of carbon dioxide. This oxidation generates high-energy electrons that will be used to power the synthesis of ATP. *The function of the citric acid cycle is the harvesting of high-energy electrons from carbon fuels.*

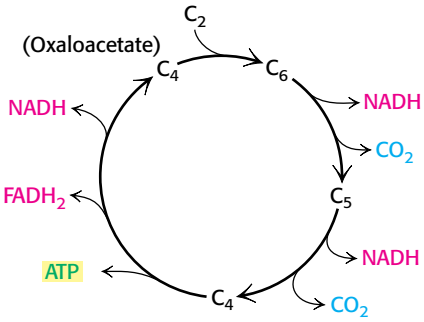


FIGURE 17.2 Overview of the citric acid cycle. The citric acid cycle oxidizes two-carbon units, producing two molecules of CO_2, one molecule of ATP, and high-energy electrons in the form of NADH and $FADH_2$.

The overall pattern of the citric acid cycle is shown in Figure 17.2. A four-carbon compound (oxaloacetate) condenses with a two-carbon acetyl unit to yield a six-carbon tricarboxylic acid. The six-carbon compound releases CO_2 twice in two successive oxidative decarboxylations that yield high-energy electrons. A four-carbon compound remains. This four-carbon compound is further processed to regenerate oxaloacetate, which can initiate another round of the cycle. Two carbon atoms enter the cycle as an acetyl unit and two carbon atoms leave the cycle in the form of two molecules of CO_2.

Note that the citric acid cycle itself neither generates a large amount of ATP nor includes oxygen as a reactant (Figure 17.3). Instead, the citric acid

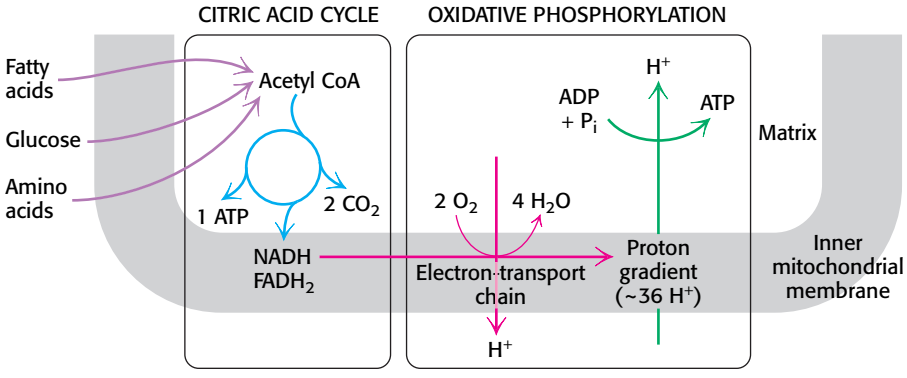


FIGURE 17.3 Cellular respiration. The citric acid cycle constitutes the first stage in cellular respiration, the removal of high-energy electrons from carbon fuels in the form of NADH and $FADH_2$ (blue pathway). These electrons reduce O_2 to generate a proton gradient (red pathway), which is used to synthesize ATP (green pathway). The reduction of O_2 and the synthesis of ATP constitute oxidative phosphorylation.

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cycle removes electrons from acetyl CoA and uses these electrons to reduce NAD^+ and FAD to form NADH and $FADH_2$. Three hydride ions (hence, six electrons) are transferred to three molecules of nicotinamide adenine dinucleotide (NAD^+), and one pair of hydrogen atoms (hence, two electrons) is transferred to one molecule of flavin adenine dinucleotide (FAD). Electrons released in the reoxidation of NADH and $FADH_2$ flow through a series of membrane proteins (referred to as the *electron-transport chain*) to generate a proton gradient across the inner mitochondrial membrane. These protons then flow through ATP synthase to generate ATP from ADP and inorganic phosphate. These electron carriers yield nine molecules of ATP when they are oxidized by O_2 in *oxidative phosphorylation* (Chapter 18).

The citric acid cycle, in conjunction with oxidative phosphorylation, provides the preponderance of energy used by aerobic cells—in human beings, greater than 90%. It is highly efficient because the oxidation of a limited number of citric acid cycle molecules can generate large amounts of NADH and $FADH_2$. Note in Figure 17.2 that the four-carbon molecule, oxaloacetate, that initiates the first step in the citric acid cycle is regenerated at the end of one passage through the cycle. Thus, one molecule of oxaloacetate is capable of participating in the oxidation of many acetyl molecules.

17.1 The Pyruvate Dehydrogenase Complex Links Glycolysis to the Citric Acid Cycle

Carbohydrates, most notably glucose, are processed by glycolysis into pyruvate (Chapter 16). Under anaerobic conditions, the pyruvate is converted into lactate or ethanol, depending on the organism. Under aerobic conditions, the pyruvate is transported into mitochondria by a specific carrier protein embedded in the mitochondrial membrane. In the mitochondrial matrix, pyruvate is oxidatively decarboxylated by the *pyruvate dehydrogenase complex* to form acetyl CoA.

$$\text{Pyruvate} + \text{CoA} + NAD^+ \longrightarrow \text{acetyl CoA} + CO_2 + \text{NADH} + H^+$$

This irreversible reaction is the link between glycolysis and the citric acid cycle (Figure 17.4). Note that the pyruvate dehydrogenase complex produces CO_2 and captures high-transfer-potential electrons in the form of NADH. Thus, the pyruvate dehydrogenase reaction has many of the key features of the reactions of the citric acid cycle itself.

The pyruvate dehydrogenase complex is a large, highly integrated complex of three distinct enzymes (Table 17.1). Pyruvate dehydrogenase complex is a member of a family of homologous complexes that include the citric acid cycle enzyme α-ketoglutarate dehydrogenase complex (p. 505). These complexes are giant, larger than ribosomes, with molecular masses ranging from 4 million to 10 million kDa (Figure 17.5). As we will see, their

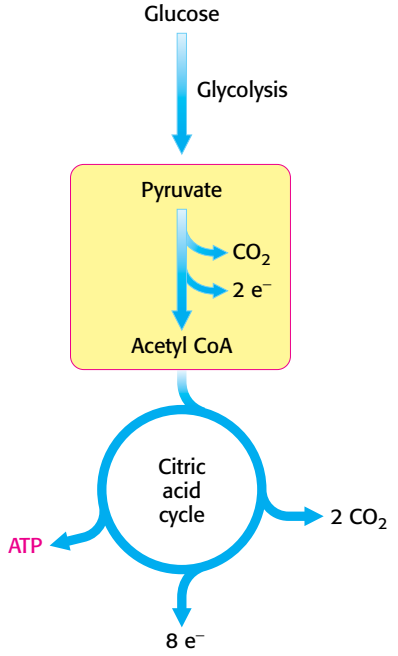


FIGURE 17.4 The link between glycolysis and the citric acid cycle. Pyruvate produced by glycolysis is converted into acetyl CoA, the fuel of the citric acid cycle.

TABLE 17.1 Pyruvate dehydrogenase complex of *E. coli*

Enzyme	Abbreviation	Number of chains	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E_1	24	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E_2	24	Lipoamide	Transfer of acetyl group to CoA
Dihydrolipoyl dehydrogenase	E_3	12	FAD	Regeneration of the oxidized form of lipoamide

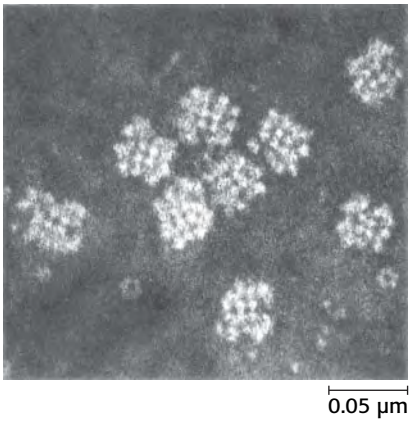


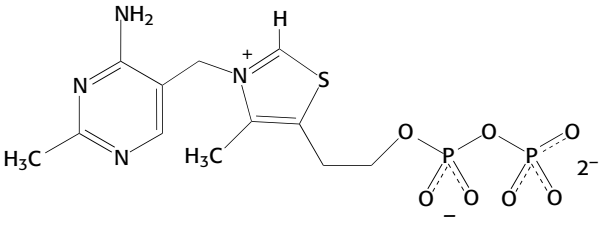
FIGURE 17.5 Electron micrograph of the pyruvate dehydrogenase complex from *E. coli*. [Courtesy of Dr. Lester Reed.]

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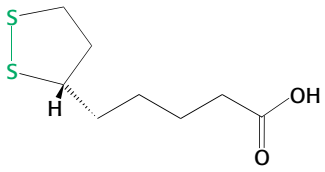
elaborate structures allow groups to travel from one active site to another, connected by tethers to the core of the structure.

Mechanism: The synthesis of acetyl coenzyme A from pyruvate requires three enzymes and five coenzymes

The mechanism of the pyruvate dehydrogenase reaction is wonderfully complex, more so than is suggested by its simple stoichiometry. The reaction requires the participation of the three enzymes of the pyruvate dehydrogenase complex and five coenzymes. The coenzymes *thiamine pyrophosphate* (TPP), *lipoic acid,* and *FAD* serve as catalytic cofactors, and *CoA* and NAD^+ are stoichiometric cofactors, cofactors that function as substrates.

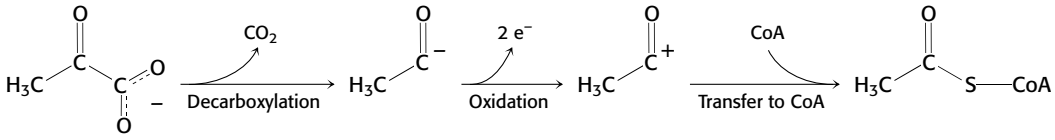


Thiamine pyrophosphate (TPP)



Lipoic acid

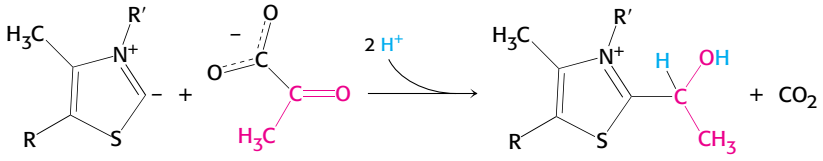
The conversion of pyruvate into acetyl CoA consists of three steps: decarboxylation, oxidation, and transfer of the resultant acetyl group to CoA. A fourth step is required to regenerate the active enzyme.



Pyruvate **Acetyl CoA**

These steps must be coupled to preserve the free energy derived from the decarboxylation step to drive the formation of NADH and acetyl CoA:

1. *Decarboxylation*. Pyruvate combines with TPP and is then decarboxylated to yield hydroxyethyl-TPP (Figure 17.6).



Carbanion of TPP **Pyruvate** **Hydroxyethyl-TPP**

This reaction, the rate limiting step in acetyl CoA synthesis, is catalyzed by the *pyruvate dehydrogenase component* (E_1) of the multienzyme complex. TPP is the prosthetic group of the pyruvate dehydrogenase component.

2. *Oxidation*. The hydroxyethyl group attached to TPP is *oxidized* to form an acetyl group while being simultaneously transferred to lipoamide, a derivative of lipoic acid that is linked to the side chain of a lysine residue by

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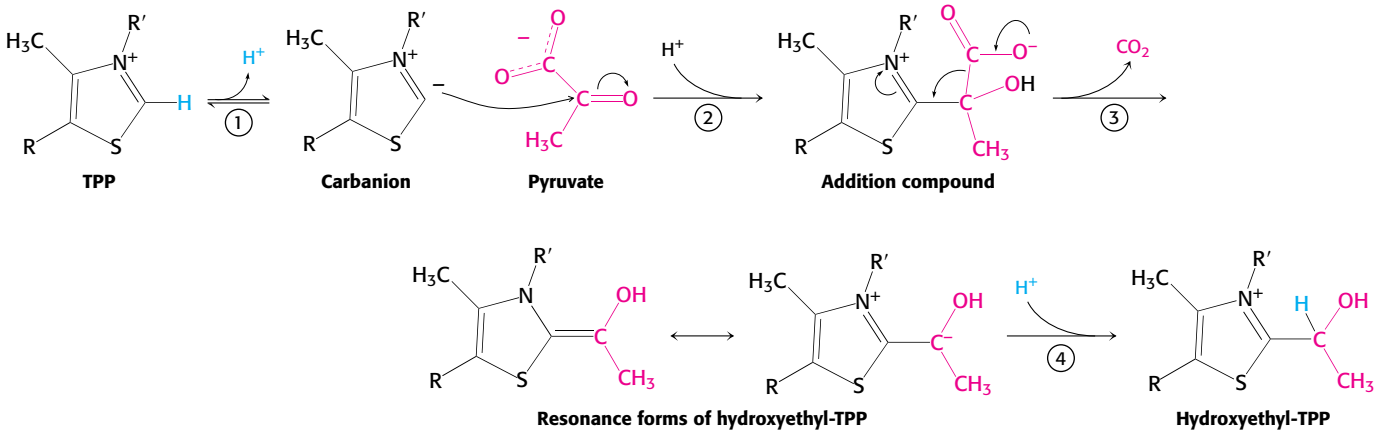
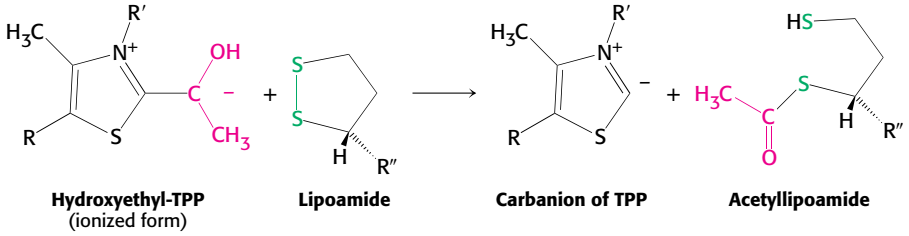


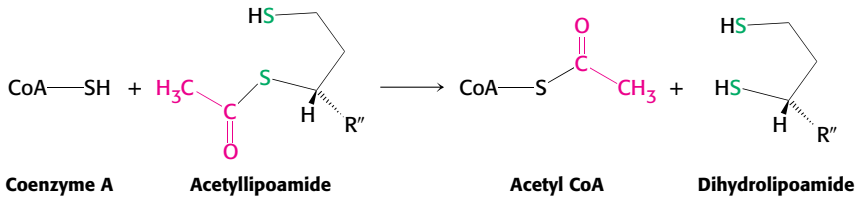
FIGURE 17.6 Mechanism of the E_1 decarboxylation reaction. E_1 is the pyruvate dehydrogenase component of the pyruvate dehydrogenase complex. A key feature of the prosthetic group, TPP, is that the carbon atom between the nitrogen and sulfur atoms in the thiazole ring is much more acidic than most ═C— groups, with a pK_a value near 10. (1) This carbon center ionizes to form a *carbanion*. (2) The carbanion readily adds to the carbonyl group of pyruvate. (3) This addition is followed by the decarboxylation of pyruvate. The positively charged ring of TPP acts as an electron sink that stabilizes the negative charge that is transferred to the ring as part of the decarboxylation. (4) Protonation yields hydroxyethyl-TPP.

an amide linkage. Note that this transfer results in the formation of an energy-rich thioester bond.



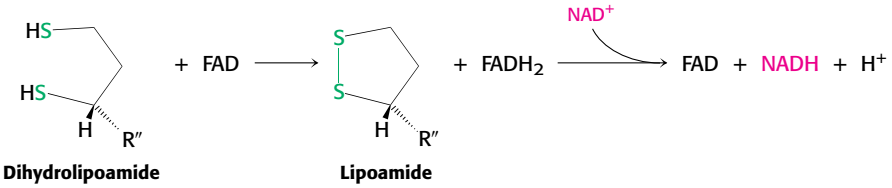
The oxidant in this reaction is the disulfide group of lipoamide, which is reduced to its disulfhydryl form. This reaction, also catalyzed by the pyruvate dehydrogenase component E_1, yields *acetyllipoamide*.

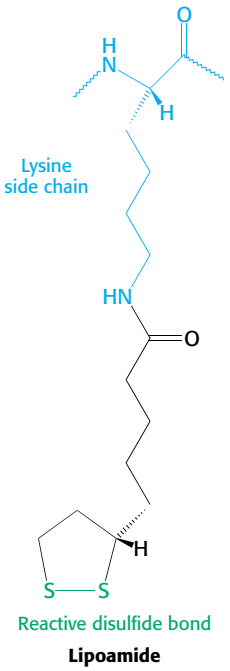
3. *Formation of Acetyl CoA. The acetyl group is transferred from acetyllipoamide to CoA to form acetyl CoA.*



Dihydrolipoyl transacetylase (E_2) catalyzes this reaction. The energy-rich thioester bond is preserved as the acetyl group is transferred to CoA. Recall that CoA serves as a carrier of many activated acyl groups, of which acetyl is the simplest (Section 15.3). Acetyl CoA, the fuel for the citric acid cycle, has now been generated from pyruvate.

4. *Regeneration of oxidized lipoamide*. The pyruvate dehydrogenase complex cannot complete another catalytic cycle until the dihydrolipoamide is oxidized to lipoamide. In the fourth step, *the oxidized form of lipoamide is regenerated by dihydrolipoyl dehydrogenase* (E_3). Two electrons are transferred to an FAD prosthetic group of the enzyme and then to NAD^+.





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This electron transfer from FAD to NAD^+ is unusual because the common role for FAD is to receive electrons from NADH. The electron-transfer potential of FAD is increased by its chemical environment within the enzyme, enabling it to transfer electrons to NAD^+. Proteins tightly associated with FAD or flavin mononucleotide (FMN) are called *flavoproteins.*

Flexible linkages allow lipoamide to move between different active sites

The structures of all of the component enzymes of the bacterial pyruvate dehydrogenase complex are known, albeit from different complexes and species. Thus, it is now possible to construct an atomic model of the complex to understand its activity (Figure 17.7).

The core of the complex is formed by the transacetylase component E_2. Transacetylase consists of eight catalytic trimers assembled to form a hollow cube. Each of the three subunits forming a trimer has three major domains (Figure 17.8). At the amino terminus is a small domain that contains a bound flexible lipoamide cofactor attached to a lysine residue. This domain is homologous to biotin-binding domains such as that of pyruvate carboxylase (Figure 16.26). The lipoamide domain is followed by a small domain that interacts with E_3 within the complex. A larger transacetylase domain completes an E_2 subunit. E_1 is an $\alpha_2\beta_2$ tetramer, and E_3 is an $\alpha\beta$ dimer. Multiple copies of E_1 and E_3 surround the E_2 core. In mammals, this core contains another protein, E_3-binding protein (E_3-BP), which facilitates the interaction between E_2 and E_3. If E_3-BP is missing, the complex has greatly reduced activity.

How do the three distinct active sites work in concert (Figure 17.9)? The key is the long, flexible lipoamide arm of the E_2 subunit, which carries substrate from active site to active site:

1. Pyruvate is decarboxylated at the active site of E_1, forming the hydroxyethyl-TPP intermediate, and CO_2 leaves as the first product. This active site lies deep within the E_1 complex, connected to the enzyme surface by a 20-Å-long hydrophobic channel.

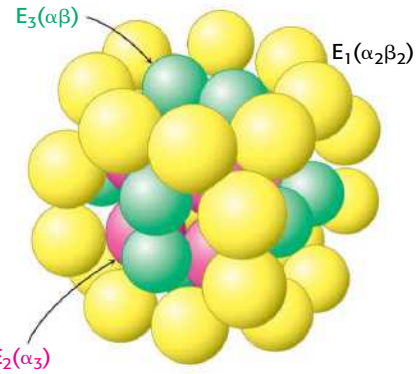


FIGURE 17.7 Schematic representation of the pyruvate dehydrogenase complex. The transacetylase core (E_2) is shown in red, the pyruvate dehydrogenase component (E_1) in yellow, and the dihydrolipoyl dehydrogenase (E_3) in green.

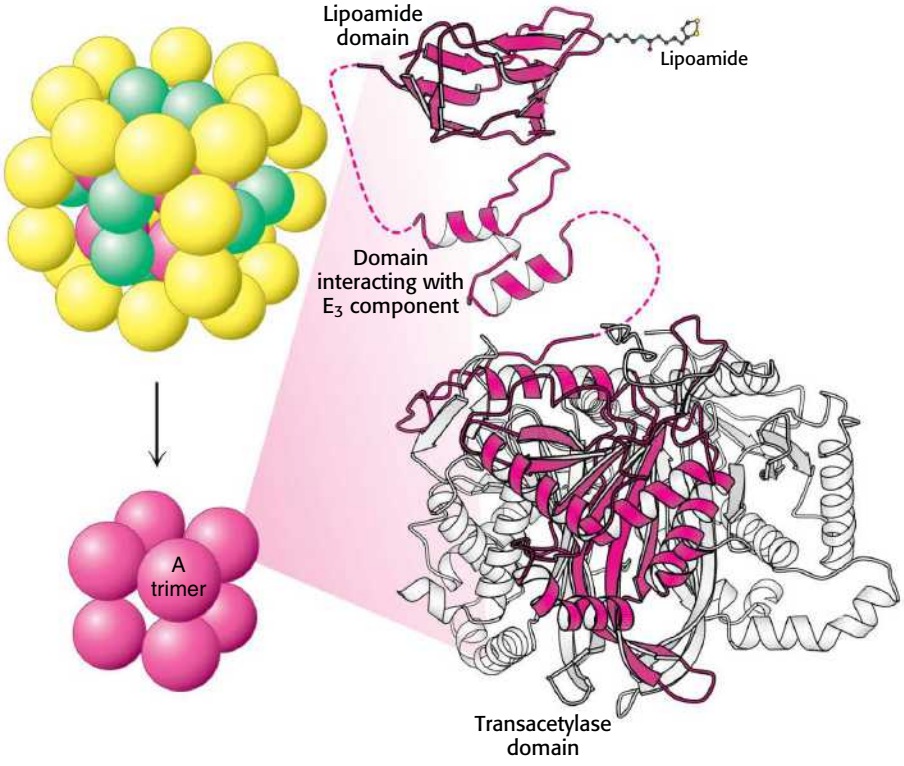


FIGURE 17.8 Structure of the transacetylase (E_2) core. Each red ball represents a trimer of three E_2 subunits. *Notice* that each subunit consists of three domains: a lipoamide-binding domain, a small domain for interaction with E_3, and a large transacetylase catalytic domain. The transacetylase domains of three identical subunits are shown, with one depicted in red and the others in white in the ribbon representation.

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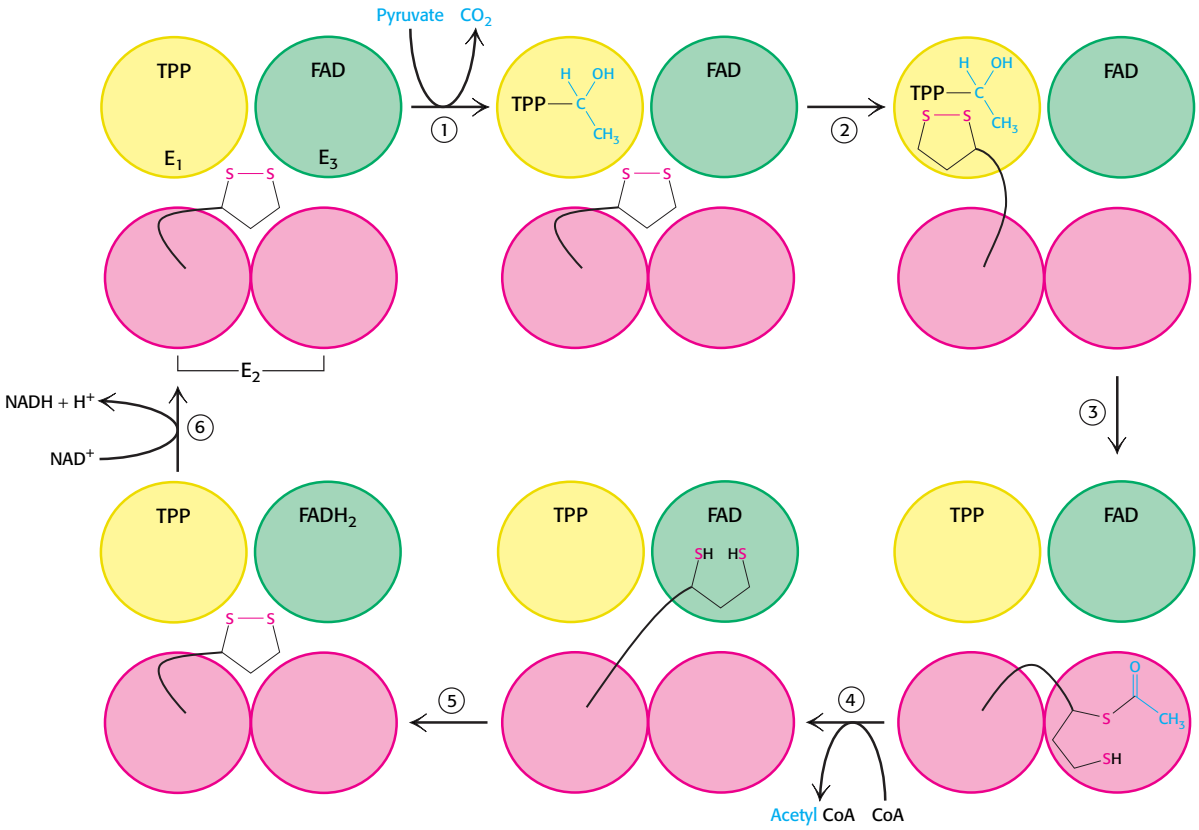


FIGURE 17.9 Reactions of the pyruvate dehydrogenase complex. At the top (left), the enzyme (represented by a yellow, a green, and two red spheres) is unmodified and ready for a catalytic cycle. (1) Pyruvate is decarboxylated to form hydroxyethyl-TPP. (2) The lipoamide arm of E_2 moves into the active site of E_1. (3) E_1 catalyzes the transfer of the two-carbon group to the lipoamide group to form the acetyl–lipoamide complex. (4) E_2 catalyzes the transfer of the acetyl moiety to CoA to form the product acetyl CoA. The dihydrolipoamide arm then swings to the active site of E_3. E_3 catalyzes (5) the oxidation of the dihydrolipoamide and (6) the transfer of the protons and electrons to NAD^+ to complete the reaction cycle.

2. E_2 inserts the lipoamide arm of the lipoamide domain into the deep channel in E_1 leading to the active site.

3. E_1 catalyzes the transfer of the acetyl group to the lipoamide. The acetylated arm then leaves E_1 and enters the E_2 cube to visit the active site of E_2, located deep in the cube at the subunit interface.

4. The acetyl moiety is then transferred to CoA, and the second product, acetyl CoA, leaves the cube. The reduced lipoamide arm then swings to the active site of the E_3 flavoprotein.

5. At the E_3 active site, the lipoamide is oxidized by coenzyme FAD. The reactivated lipoamide is ready to begin another reaction cycle.

6. The final product, NADH, is produced with the reoxidation of $FADH_2$ to FAD.

The structural integration of three kinds of enzymes and the long, flexible lipoamide arm make the coordinated catalysis of a complex reaction possible. The proximity of one enzyme to another *increases the overall reaction rate and minimizes side reactions.* All the intermediates in the oxidative decarboxylation of pyruvate remain bound to the complex throughout the reaction sequence and are readily transferred as the flexible arm of E_2 calls on each active site in turn.

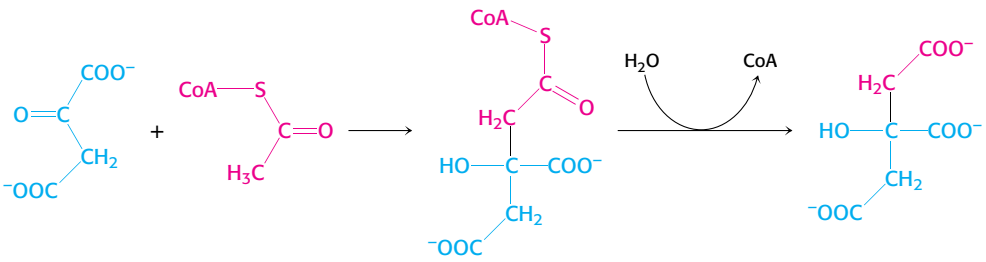
17.2 The Citric Acid Cycle Oxidizes Two-Carbon Units

The conversion of pyruvate into acetyl CoA by the pyruvate dehydrogenase complex is the link between glycolysis and cellular respiration because *acetyl CoA is the fuel for the citric acid cycle*. Indeed, all fuels are ultimately metabolized to acetyl CoA or components of the citric acid cycle.

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Citrate synthase forms citrate from oxaloacetate and acetyl coenzyme A

The citric acid cycle begins with the condensation of a four-carbon unit, oxaloacetate, and a two-carbon unit, the acetyl group of acetyl CoA. Oxaloacetate reacts with acetyl CoA and H_2O to yield citrate and CoA.



Oxaloacetate **Acetyl CoA** **Citryl CoA** **Citrate**

This reaction, which is an aldol condensation followed by a hydrolysis, is catalyzed by *citrate synthase.* Oxaloacetate first condenses with acetyl CoA to form *citryl CoA,* a molecule that is energy rich because it contains the thioester bond that originated in acetyl CoA. The hydrolysis of citryl CoA thioester to citrate and CoA drives the overall reaction far in the direction of the synthesis of citrate. In essence, the hydrolysis of the thioester powers the synthesis of a new molecule from two precursors.

Synthase
An enzyme catalyzing a synthetic reaction in which two units are joined usually without the direct participation of ATP (or another nucleoside triphosphate).

Mechanism: The mechanism of citrate synthase prevents undesirable reactions

Because the condensation of acetyl CoA and oxaloacetate initiates the citric acid cycle, it is very important that side reactions, notably the hydrolysis of acetyl CoA to acetate and CoA, be minimized. Let us briefly consider how the citrate synthase prevents the wasteful hydrolysis of acetyl CoA.

Mammalian citrate synthase is a dimer of identical 49-kDa subunits. Each active site is located in a cleft between the large and the small domains of a subunit, adjacent to the subunit interface. X-ray crystallographic studies of citrate synthase and its complexes with several substrates and inhibitors have revealed that the enzyme undergoes large conformational changes in the course of catalysis. Citrate synthase exhibits sequential, ordered kinetics: oxaloacetate binds first, followed by acetyl CoA. The reason for the ordered binding is that *oxaloacetate induces a major structural rearrangement leading to the creation of a binding site for acetyl CoA.* The binding of oxaloacetate converts the open form of the enzyme into a closed form (Figure 17.10). In each subunit, the small domain rotates 19 degrees relative to the large domain. *Movements as large as 15 Å are produced by the rotation of α helices elicited by quite small shifts of side chains around bound oxaloacetate.* These structural changes create a binding site for acetyl CoA.

Citrate synthase catalyzes the condensation reaction by bringing the substrates into close proximity, orienting them, and polarizing certain bonds (Figure 17.11). The donation and removal of protons transforms acetyl CoA into an *enol intermediate*. The enol attacks oxaloacetate to form a carbon−carbon double bond linking acetyl CoA and oxaloacetate. The newly formed citryl CoA induces additional structural changes in the enzyme, causing the active site to become completely enclosed. The enzyme cleaves the citryl CoA thioester by hydrolysis. CoA leaves the enzyme, followed by citrate, and the enzyme returns to the initial open conformation.

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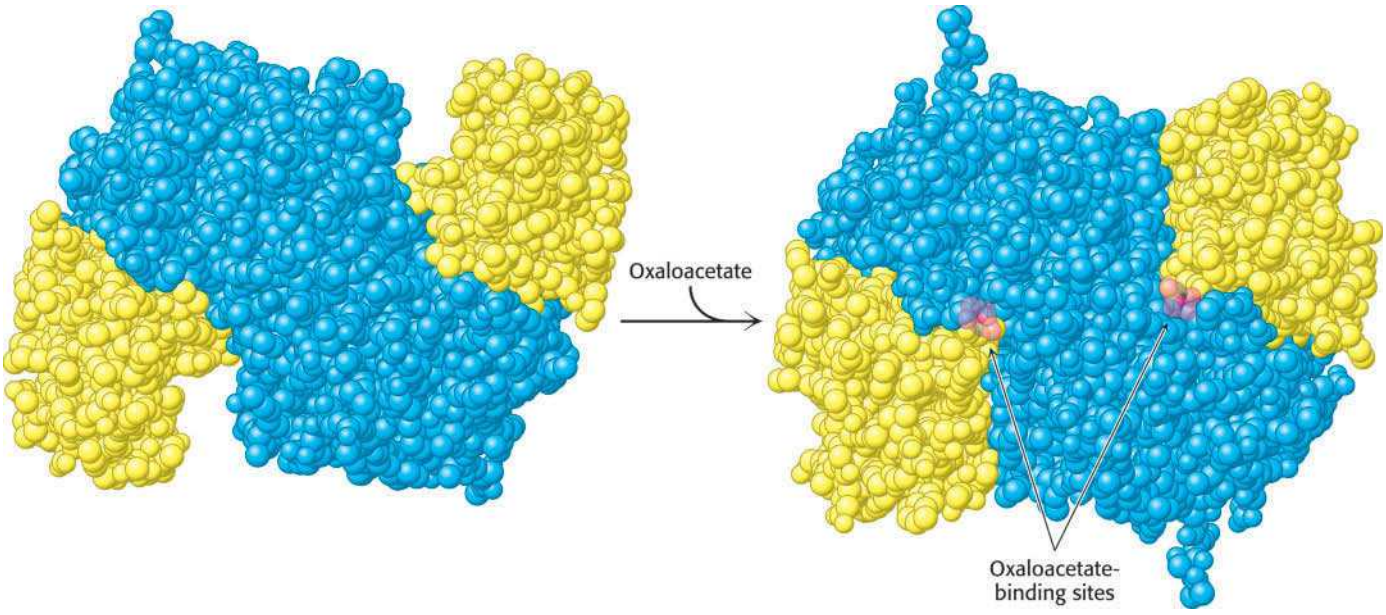


FIGURE 17.10 Conformational changes in citrate synthase on binding oxaloacetate. The small domain of each subunit of the homodimer is shown in yellow; the large domains are shown in blue. (Left) Open form of enzyme alone. (Right) Closed form of the oxaloacetate-enzyme complex. [Drawn from 5CSC.pdb and 4CTS.pdb.]

We can now understand how the wasteful hydrolysis of acetyl CoA is prevented. Citrate synthase is well suited to hydrolyze *citryl* CoA but not *acetyl* CoA. How is this discrimination accomplished? First, acetyl CoA does not bind to the enzyme until oxaloacetate is bound and ready for condensation. Second, the catalytic residues crucial for the hydrolysis of the thioester linkage are not appropriately positioned *until citryl CoA is formed.* As with hexokinase and triose phosphate isomerase (Section 16.1), *induced fit prevents an undesirable side reaction.*

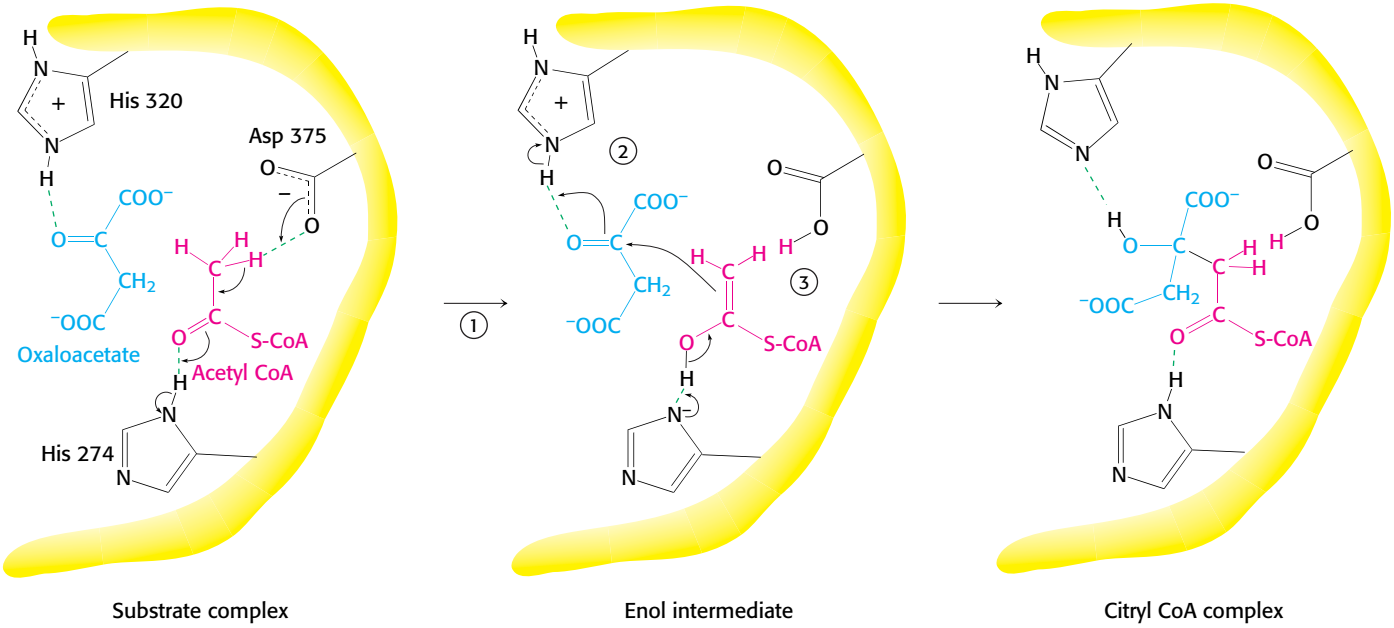
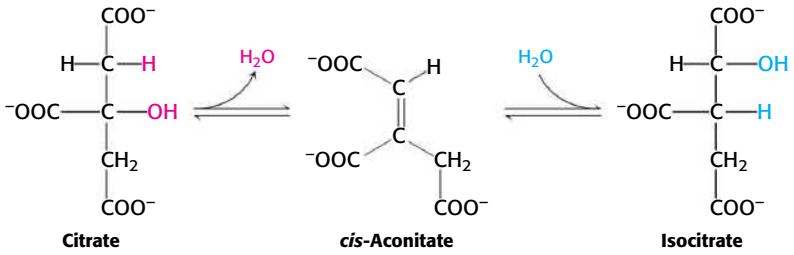


FIGURE 17.11 Mechanism of synthesis of citryl CoA by citrate synthase. (1) In the substrate complex (left), His 274 donates a proton to the carbonyl oxygen of acetyl CoA to promote the removal of a methyl proton by Asp 375 to form the enol intermediate (center). (2) Oxaloacetate is activated by the transfer of a proton from His 320 to its carbonyl carbon atom. (3) Simultaneously, the enol of acetyl CoA attacks the carbonyl carbon of oxaloacetate to form a carbon–carbon bond linking acetyl CoA and oxaloacetate. His 274 is reprotonated. Citryl CoA is formed. His 274 participates again as a proton donor to hydrolyze the thioester (not shown), yielding citrate and CoA.

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Citrate is isomerized into isocitrate

The hydroxyl group is not properly located in the citrate molecule for the oxidative decarboxylations that follow (Problem 27). Thus, citrate is isomerized into isocitrate to enable the six-carbon unit to undergo oxidative decarboxylation. The isomerization of citrate is accomplished by a *dehydration* step followed by a *hydration* step. The result is an interchange of an H and an OH. The enzyme catalyzing both steps is called *aconitase* because cis-*aconitate* is an intermediate.



Aconitase is an *iron–sulfur protein,* or *nonheme-iron protein,* in that it contains iron that is not bonded to heme. Rather, its four iron atoms are complexed to four inorganic sulfides and three cysteine sulfur atoms, leaving one iron atom available to bind citrate through one of its COO^- groups and an OH group (Figure 17.12). This Fe-S cluster participates in dehydrating and rehydrating the bound substrate.

Isocitrate is oxidized and decarboxylated to alpha-ketoglutarate

We come now to the first of four oxidation–reduction reactions in the citric acid cycle. The oxidative decarboxylation of isocitrate is catalyzed by *isocitrate dehydrogenase.*

$$\text{Isocitrate} + NAD^+ \longrightarrow \alpha\text{-ketoglutarate} + CO_2 + NADH$$

The intermediate in this reaction is oxalosuccinate, an unstable β-ketoacid. While bound to the enzyme, it loses CO_2 to form α-ketoglutarate.

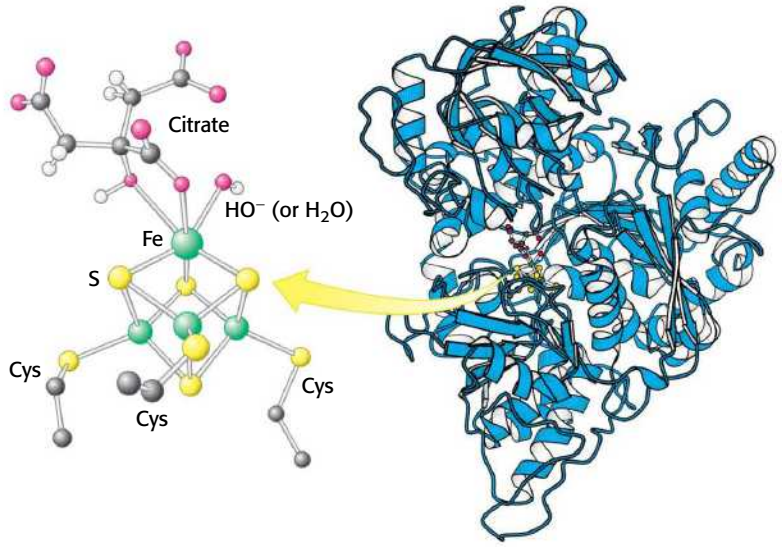
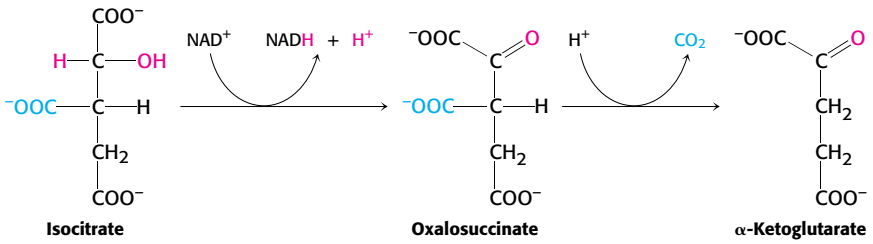


FIGURE 17.12 Binding of citrate to the iron–sulfur complex of aconitase. A 4Fe-4S iron–sulfur cluster is a component of the active site of aconitase. *Notice* that one of the iron atoms of the cluster binds to a COO^- group and an OH group of citrate. [Drawn from 1C96.pdb.]

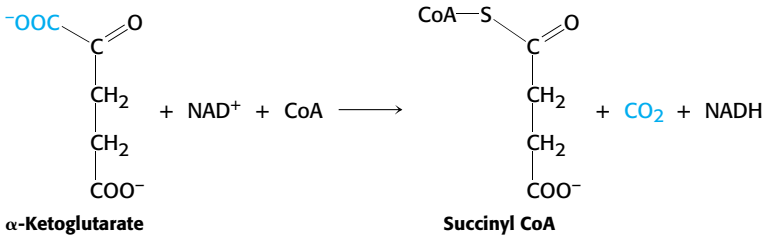
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This oxidation generates the first high-transfer-potential electron carrier, NADH, in the cycle.

Succinyl coenzyme A is formed by the oxidative decarboxylation of alpha-ketoglutarate

The conversion of isocitrate into α-ketoglutarate is followed by a second oxidative decarboxylation reaction, the formation of succinyl CoA from α-ketoglutarate.



This reaction is catalyzed by the α-*ketoglutarate dehydrogenase complex,* an organized assembly of three kinds of enzymes that is homologous to the pyruvate dehydrogenase complex. In fact, the E_3 component is identical in both enzymes. The oxidative decarboxylation of α-ketoglutarate closely resembles that of pyruvate, also an α-ketoacid.

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \xrightarrow{\text{Pyruvate dehydrogenase complex}} \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

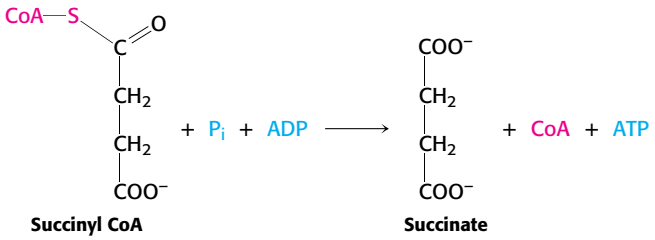
$$\alpha\text{-Ketoglutarate} + \text{CoA} + \text{NAD}^+ \xrightarrow{\alpha\text{-Ketoglutarate dehydrogenase complex}} \text{succinyl CoA} + \text{CO}_2 + \text{NADH}$$

Both reactions include the decarboxylation of an α-ketoacid and the subsequent formation of a thioester linkage with CoA that has a high transfer potential. The reaction mechanisms are entirely analogous (p. 497).

A compound with high phosphoryl-transfer potential is generated from succinyl coenzyme A

Succinyl CoA is an energy-rich thioester compound. The $\Delta G^{\circ\prime}$ for the hydrolysis of succinyl CoA is about -33.5 kJ mol^{-1} (-8.0 kcal mol^{-1}), which is comparable to that of ATP (-30.5 kJ mol^{-1}, or -7.3 kcal mol^{-1}). In the citrate synthase reaction, the cleavage of the thioester bond powers the synthesis of the six-carbon citrate from the four-carbon oxaloacetate and the two-carbon fragment. *The cleavage of the thioester bond of succinyl CoA is coupled to the phosphorylation of a purine nucleoside diphosphate, usually ADP.* This reaction, which is readily reversible, is catalyzed by *succinyl CoA synthetase* (succinate thiokinase).

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This reaction is the only step in the citric acid cycle that directly yields a compound with high phosphoryl-transfer potential. In mammals, there are two isozymic forms of the enzyme, one specific for ADP and one for GDP. In tissues that perform large amounts of cellular respiration, such as skeletal and heart muscle, the ADP-requiring isozyme predominates. In tissues that perform many anabolic reactions, such as the liver, the GDP-requiring enzyme is common. The GDP-requiring enzyme is believed to work in reverse of the direction observed in the TCA cycle; that is, GTP is used to power the synthesis of succinyl CoA, which is a precursor for heme synthesis. The *E. coli* enzyme uses either GDP or ADP as the phosphoryl-group acceptor.

Note that the enzyme *nucleoside diphosphokinase,* which catalyzes the following reaction,

$$\text{GTP} + \text{ADP} \rightleftharpoons \text{GDP} + \text{ATP}$$

allows the γ phosphoryl group to be readily transferred from GTP to form ATP, thereby allowing the adjustment of the concentration of GTP or ATP to meet the cell's need.

Mechanism: Succinyl coenzyme A synthetase transforms types of biochemical energy

The mechanism of this reaction is a clear example of an energy transformation: energy inherent in the thioester molecule is transformed into phosphoryl-group-transfer potential (Figure 17.13). The first step is the displacement of coenzyme A by orthophosphate, which generates

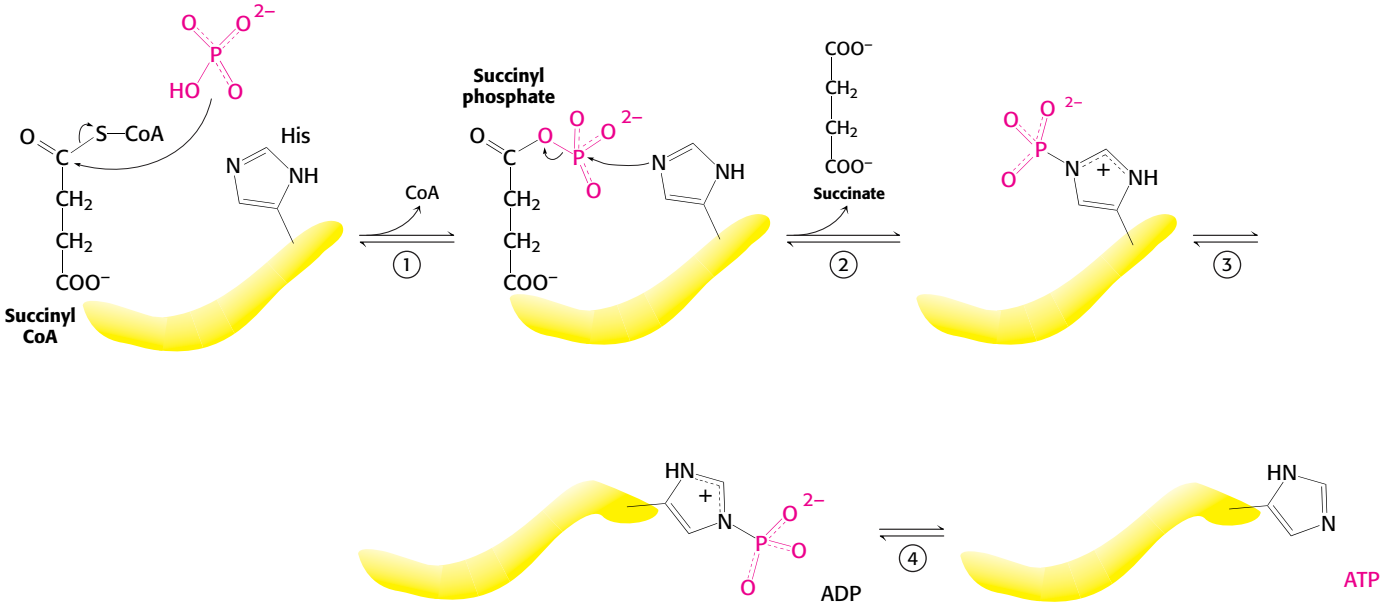


FIGURE 17.13 Reaction mechanism of succinyl CoA synthetase. The reaction proceeds through a phosphorylated enzyme intermediate. (1) Orthophosphate displaces coenzyme A, which generates another energy-rich compound, succinyl phosphate. (2) A histidine residue removes the phosphoryl group with the concomitant generation of succinate and phosphohistidine. (3) The phosphohistidine residue then swings over to a bound ADP, and (4) the phosphoryl group is transferred to form ATP.

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another energy-rich compound, succinyl phosphate. A histidine residue plays a key role as a moving arm that detaches the phosphoryl group, then swings over to a bound ADP and transfers the group to form ATP. The participation of high-energy compounds in all the steps is attested to by the fact that the reaction is readily reversible: $\Delta G^{\circ\prime} = -3.4$ kJ mol^{-1} (-0.8 kcal mol^{-1}). The formation of ATP at the expense of succinyl CoA is an example of substrate-level phosphorylation (Section 16.1).

Succinyl CoA synthetase is an $\alpha_2\beta_2$ heterodimer; the functional unit is one $\alpha\beta$ pair. The enzyme mechanism shows that a phosphoryl group is transferred first to succinyl CoA bound in the α subunit and then to a nucleoside diphosphate bound in the β subunit. Examination of the three-dimensional structure of succinyl CoA synthetase reveals that each subunit comprises two domains (Figure 17.14). The amino-terminal domains of the two subunits have different structures, each characteristic of its role in the mechanism. The amino-terminal domain of the α subunit forms a Rossmann fold (Section 16.1), which binds the ADP substrate of succinyl CoA synthetase. The amino-terminal domain of the β subunit is an ATP-grasp domain, found in many enzymes, which here binds and activates ADP. Succinyl CoA synthetase has evolved by adopting these domains and harnessing them to capture the energy associated with succinyl CoA cleavage, which is used to drive the generation of a nucleoside triphosphate.

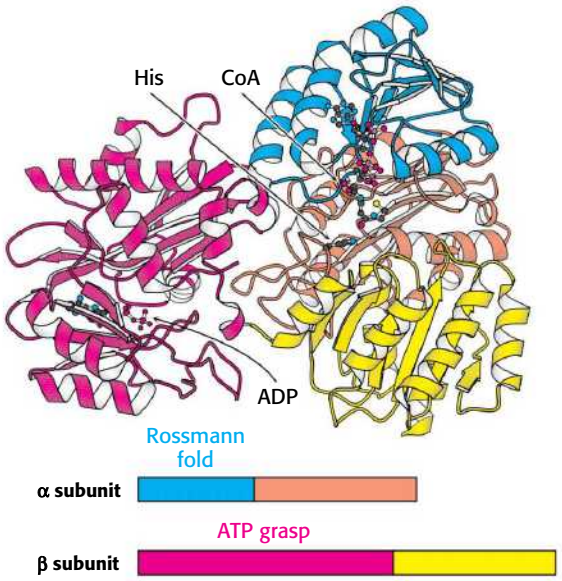
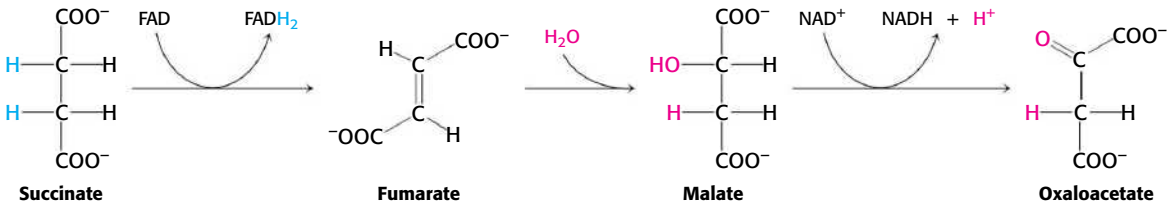


FIGURE 17.14 Structure of succinyl CoA synthetase. The enzyme is composed of two subunits. The α subunit contains a Rossmann fold that binds the ADP component of CoA, and the β subunit contains a nucleotide-activating region called the ATP-grasp domain. The ATP-grasp domain is shown here binding a molecule of ADP. *Notice* that the histidine residue is between the CoA and the ADP. This histidine residue picks up the phosphoryl group from near the CoA and swings over to transfer it to the nucleotide bound in the ATP-grasp domain. [Drawn from 1CQI.pdb.]

Oxaloacetate is regenerated by the oxidation of succinate

Reactions of four-carbon compounds constitute the final stage of the citric acid cycle: the regeneration of oxaloacetate.



The reactions constitute a metabolic motif that we will see again in fatty acid synthesis and degradation as well as in the degradation of some amino acids. A methylene group (CH_2) is converted into a carbonyl group C=O in three steps: an oxidation, a hydration, and a second oxidation reaction. Oxaloacetate is thereby regenerated for another round of the cycle, and more energy is extracted in the form of $FADH_2$ and NADH.

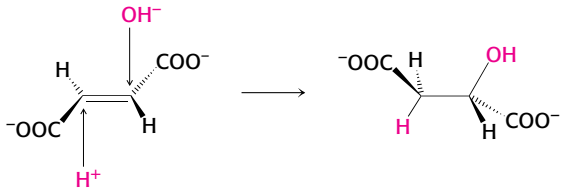
Succinate is oxidized to fumarate by *succinate dehydrogenase*. The hydrogen acceptor is FAD rather than NAD^+, which is used in the other three oxidation reactions in the cycle. FAD is the hydrogen acceptor in this reaction because the free-energy change is insufficient to reduce NAD^+. FAD is nearly always the electron acceptor in oxidations that remove two hydrogen *atoms* from a substrate. In succinate dehydrogenase, the isoalloxazine ring of FAD is covalently attached to a histidine side chain of the enzyme (denoted E-FAD).

$$\text{E-FAD} + \text{succinate} \rightleftharpoons \text{E-FADH}_2 + \text{fumarate}$$

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Succinate dehydrogenase, like aconitase, is an iron–sulfur protein. Indeed, succinate dehydrogenase contains three different kinds of iron–sulfur clusters: 2Fe-2S (two iron atoms bonded to two inorganic sulfides), 3Fe-4S, and 4Fe-4S. Succinate dehydrogenase—which consists of a 70-kDa and a 27-kDa subunit—differs from other enzymes in the citric acid cycle in being embedded in the inner mitochondrial membrane. In fact, *succinate dehydrogenase is directly associated with the electron-transport chain, the link between the citric acid cycle and ATP formation.* $FADH_2$ produced by the oxidation of succinate does not dissociate from the enzyme, in contrast with NADH produced in other oxidation–reduction reactions. Rather, two electrons are transferred from $FADH_2$ directly to iron–sulfur clusters of the enzyme, which in turn passes the electrons to coenzyme Q (CoQ). Coenzyme Q, an important member of the electron-transport chain, passes electrons to the ultimate acceptor, molecular oxygen, as we shall see in Chapter 18.

The next step is the hydration of fumarate to form L-malate. *Fumarase* catalyzes a stereospecific trans addition of H^+ and OH^-. The OH^- group adds to only one side of the double bond of fumarate; hence, only the L isomer of malate is formed.



Fumarate **L-Malate**

Finally, malate is oxidized to form oxaloacetate. This reaction is catalyzed by *malate dehydrogenase,* and NAD^+ is again the hydrogen acceptor.

$$\text{Malate} + NAD^+ \rightleftharpoons \text{oxaloacetate} + NADH + H^+$$

The standard free energy for this reaction, unlike that for the other steps in the citric acid cycle, is significantly positive ($\Delta G^{\circ\prime} = +29.7$ kJ mol^{-1}, or $+7.1$ kcal mol^{-1}). The oxidation of malate is driven by the use of the products—oxaloacetate by citrate synthase and NADH by the electron-transport chain.

The citric acid cycle produces high-transfer-potential electrons, ATP, and CO_2

The net reaction of the citric acid cycle is

$$\text{Acetyl CoA} + 3\,NAD^+ + FAD + ADP + P_i + 2\,H_2O \longrightarrow 2\,CO_2 + 3\,NADH + FADH_2 + ATP + 2\,H^+ + CoA$$

Let us recapitulate the reactions that give this stoichiometry (Figure 17.15 and Table 17.2):

1. Two carbon atoms enter the cycle in the condensation of an acetyl unit (from acetyl CoA) with oxaloacetate. Two carbon atoms leave the cycle in the form of CO_2 in the successive decarboxylations catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.

2. Four pairs of hydrogen atoms leave the cycle in four oxidation reactions. Two NAD^+ molecules are reduced in the oxidative decarboxylations of isocitrate and α-ketoglutarate, one FAD molecule is reduced in the oxidation

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