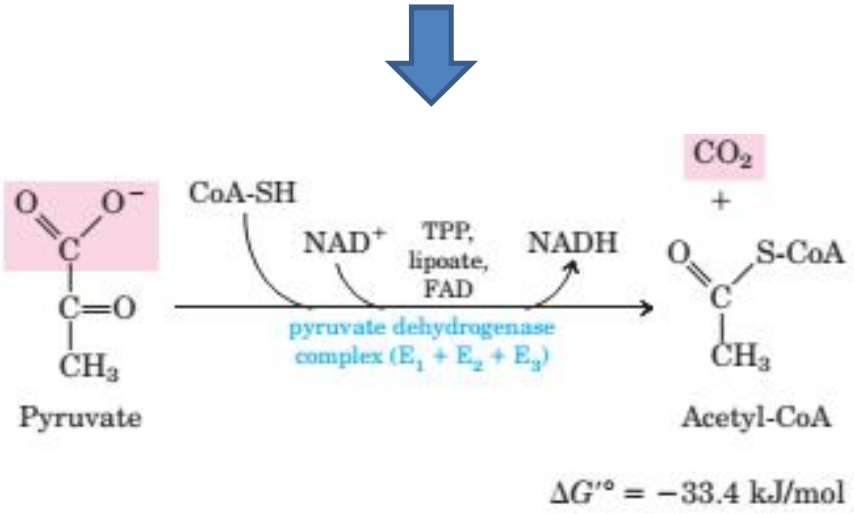
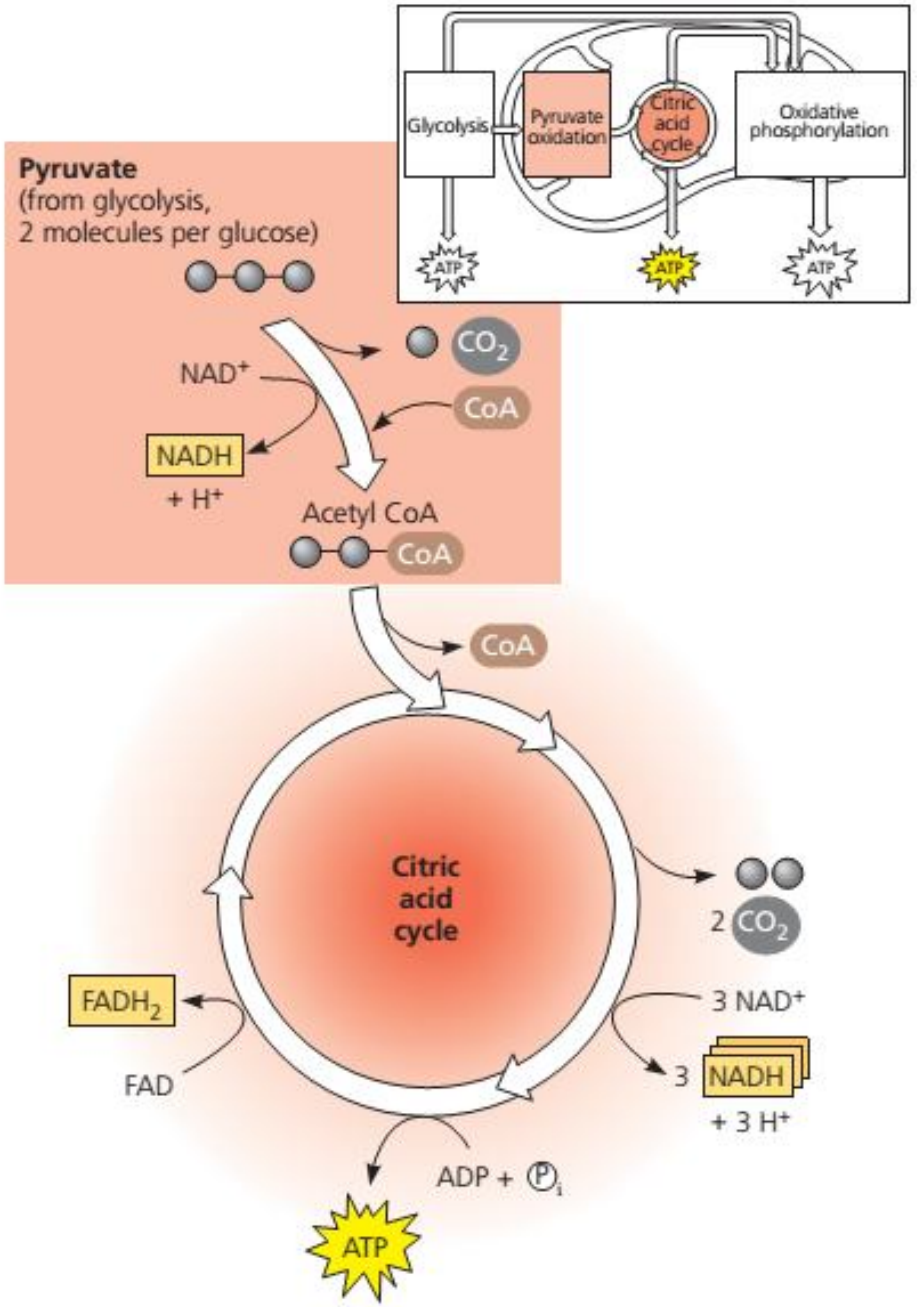

The Citric
Acid Cycle

•The citric acid cycle is also called the tricarboxylic acid cycle or the Krebs cycle, the latter honoring Hans Krebs, the German-Britis scientist.

•The cycle functions as a metabolic furnace that oxidizes organic fuel derived from pyruvate.

•Upon entering the mitochondrion via active transport, pyruvate is first converted to a compound called acetyl coenzyme A, or **acetyl CoA**

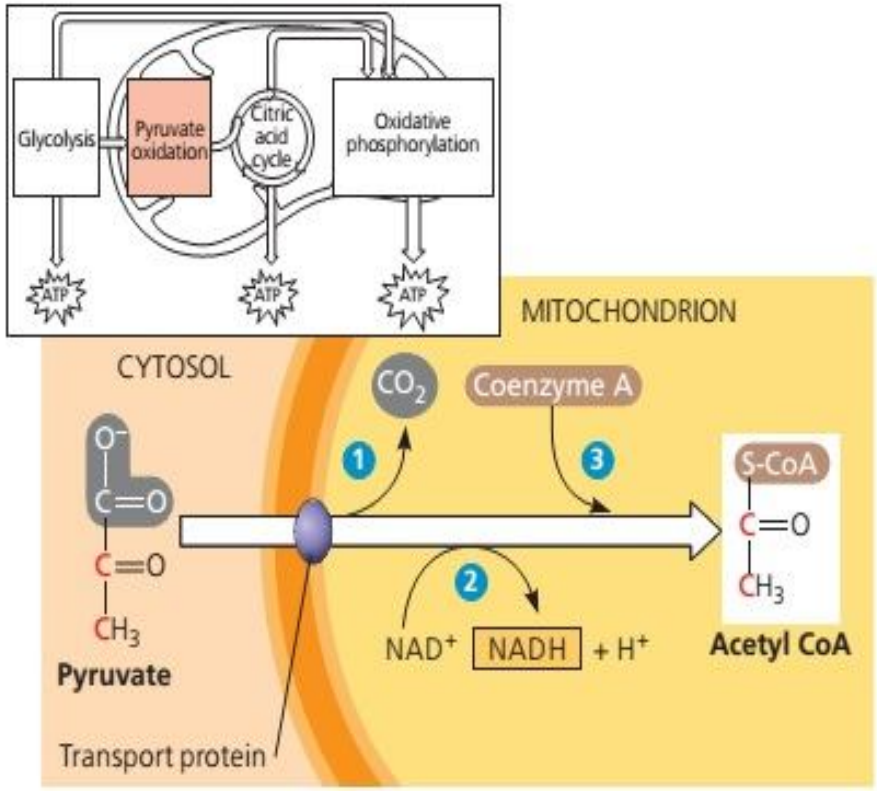




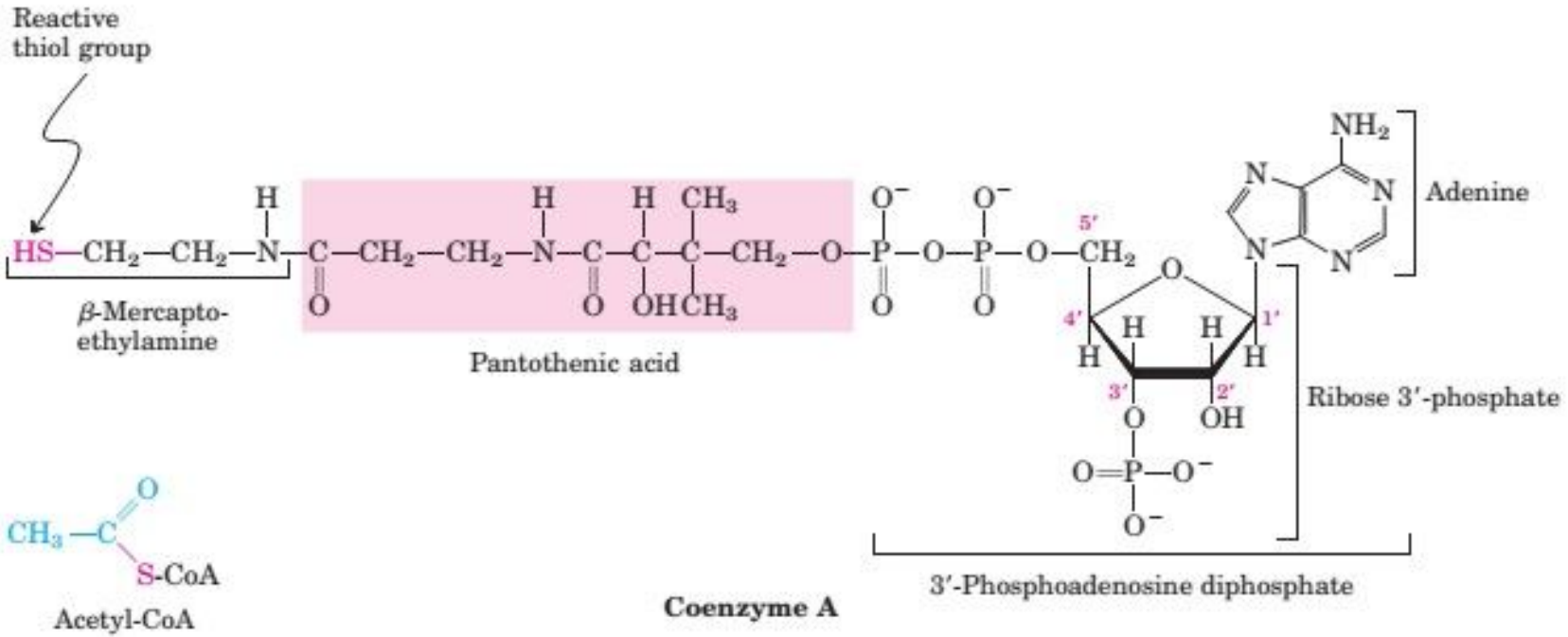
•This step, linking glycolysis
and the citric acid cycle, is carried out by a multienzyme complex that catalyzes three reactions:

1. Pyruvate's carboxyl group (—COO+), which is already fully oxidized and thus has little chemical energy, is removed and given off as a molecule of CO2. (This is the first step in which CO2 is released during respiration.)
2. The remaining two-carbon fragment is oxidized, forming acetate (CH3COO+, the ionized form of
acetic acid). The extracted electrons are transferred to NAD+ storing energy in the form of NADH.
3. Finally, coenzyme A (CoA), a sulfur-containing compound derived from a B vitamin, is attached via its sulfur atom to the acetate, forming acetyl CoA, which has a high potential energy; in other words, the reaction of acetyl CoA to yield lower energy products is highly exergonic.

This molecule will now feed its acetyl group into the citric acid cycle for further oxidation.



Oxidation of pyruvate to acetyl CoA, the step before the citric acid cycle. Pyruvate is a charged molecule, so in eukaryotic cells it must enter the mitochondrion via active transport, with the help of a transport protein. Next, a complex of several enzymes (the pyruvate dehydrogenase complex) catalyzes the three numbered steps, which are described in the text. The acetyl group of acetyl CoA will enter the citric acid cycle. The CO_2 molecule will diffuse out of the cell. By convention, coenzyme A is abbreviated S-CoA when it is attached to a molecule, emphasizing the sulfur atom (S).



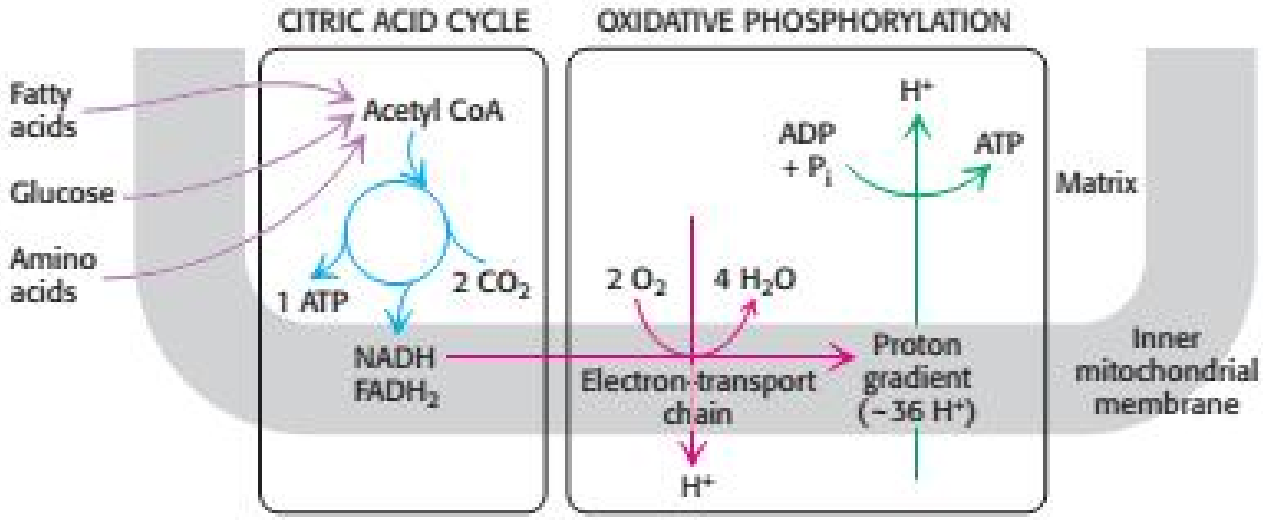
Coenzyme A

Coenzyme A (CoA). A hydroxyl group of pantothenic acid is joined to a modified ADP moiety by a phosphate ester bond, and its carboxyl group is attached to β-mercaptoethylamine in amide linkage. The hydroxyl group at the 3′ position of the ADP moiety has a phosphoryl group not present in free ADP. The —SH group of the mercaptoethylamine moiety forms a thioester with acetate in acetyl-coenzyme A (acetyl-CoA) (lower left).

•Note that the citric acid cycle itself neither generates a large amount of ATP nor includes oxygen as a reactant.

•Instead, the citric acid cycle removes electrons from acetyl CoA and uses these electrons to form NADH and FADH2.

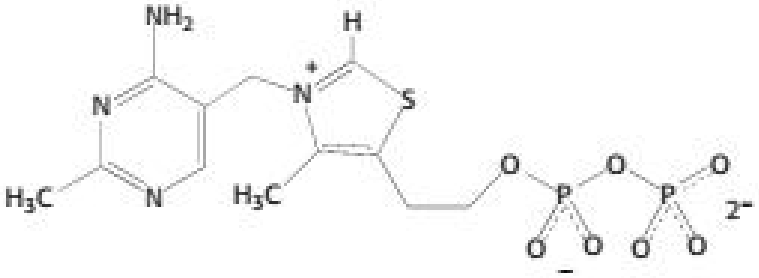
•Three hydride ions (hence, six electrons) are transferred to three molecules of nicotinamide adenine dinucleotide (NAD1), and one pair of hydrogen atoms (hence, two electrons) is transferred to one molecule of flavin adenine dinucleotide (FAD). These electron carriers yield nine molecules of ATP when they are oxidized by O2 in *oxidative phosphorylation*



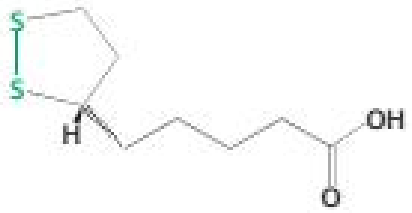
Mechanism: The synthesis of acetyl coenzyme A from pyruvate requires three enzymes and five coenzymes

• 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 NAD1 are stoichiometric cofactors, cofactors that function as substrates.

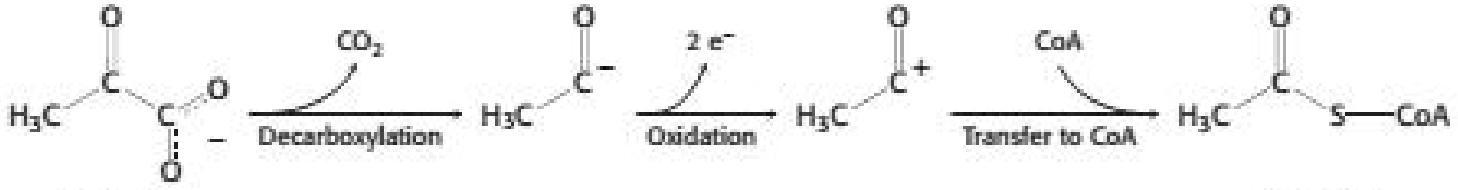


Thiamine pyrophosphate (TPP)



Lipoic acid

The conversion of pyruvate into acetyl CoA consists of three steps: decarboxylation, oxidation, and transfer of the resultant acetyl group to CoA.

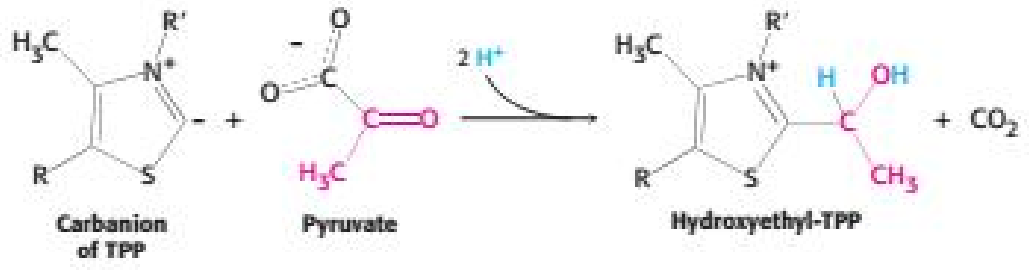


Pyruvate

Acetyl CoA

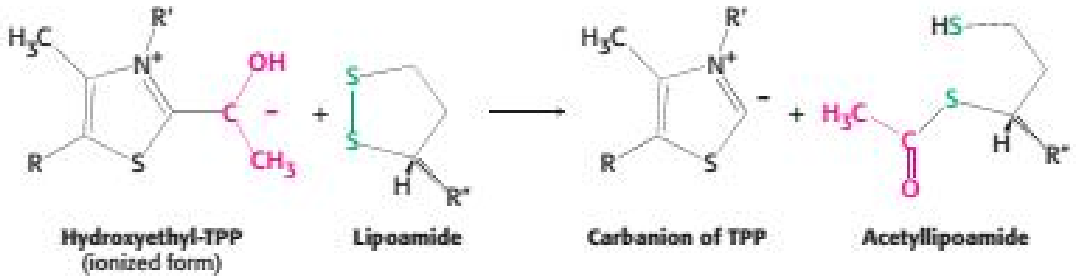
These steps must be coupled to preserve the free energy derived from the decarboxylation step to drive the formation of NADH and acetyl CoA.

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



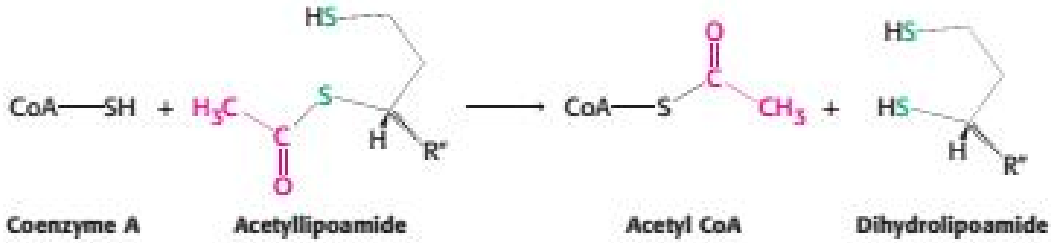
This reaction is catalyzed by the *pyruvate dehydrogenase component* (E1) of the multienzyme complex. TPP is the prosthetic group of the pyruvate dehydrogenase component.

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



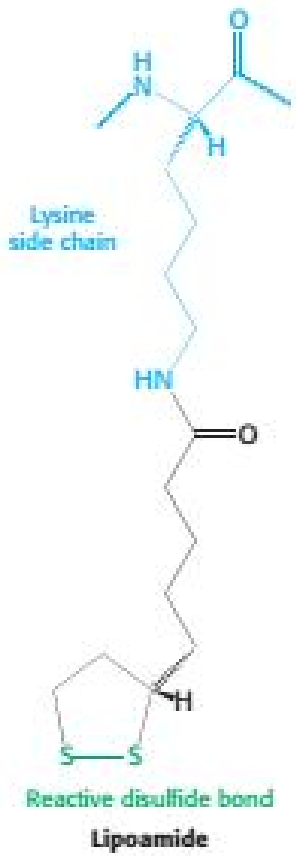
The oxidant in this reaction is the disulfide group of lipoamide, which is reduced to its disulfhydryl form. This reaction, also catalyzed by the pyruvate dehydrogenase component E1, yields *acetyllipoamide.*

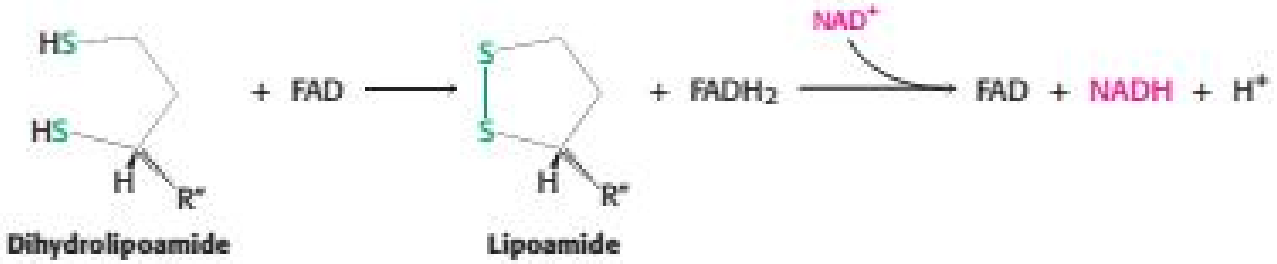
3. *Formation of Acetyl CoA. The acetyl group is transferred from acetyllipoamide to CoA to form acetyl CoA.*



Dihydrolipoyl transacetylase (E2) 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. Acetyl CoA, the fuel for the citric acid cycle, has now been generated from pyruvate.

The pyruvate dehydrogenase complex cannot complete another catalytic cycle until the dihydrolipoamide is oxidized to lipoamide. In a fourth step, *the oxidized form of lipoamide is regenerated by dihydrolipoyl dehydrogenase* (E3). Two electrons are transferred to an FAD prosthetic group of the enzyme and then to NAD1.





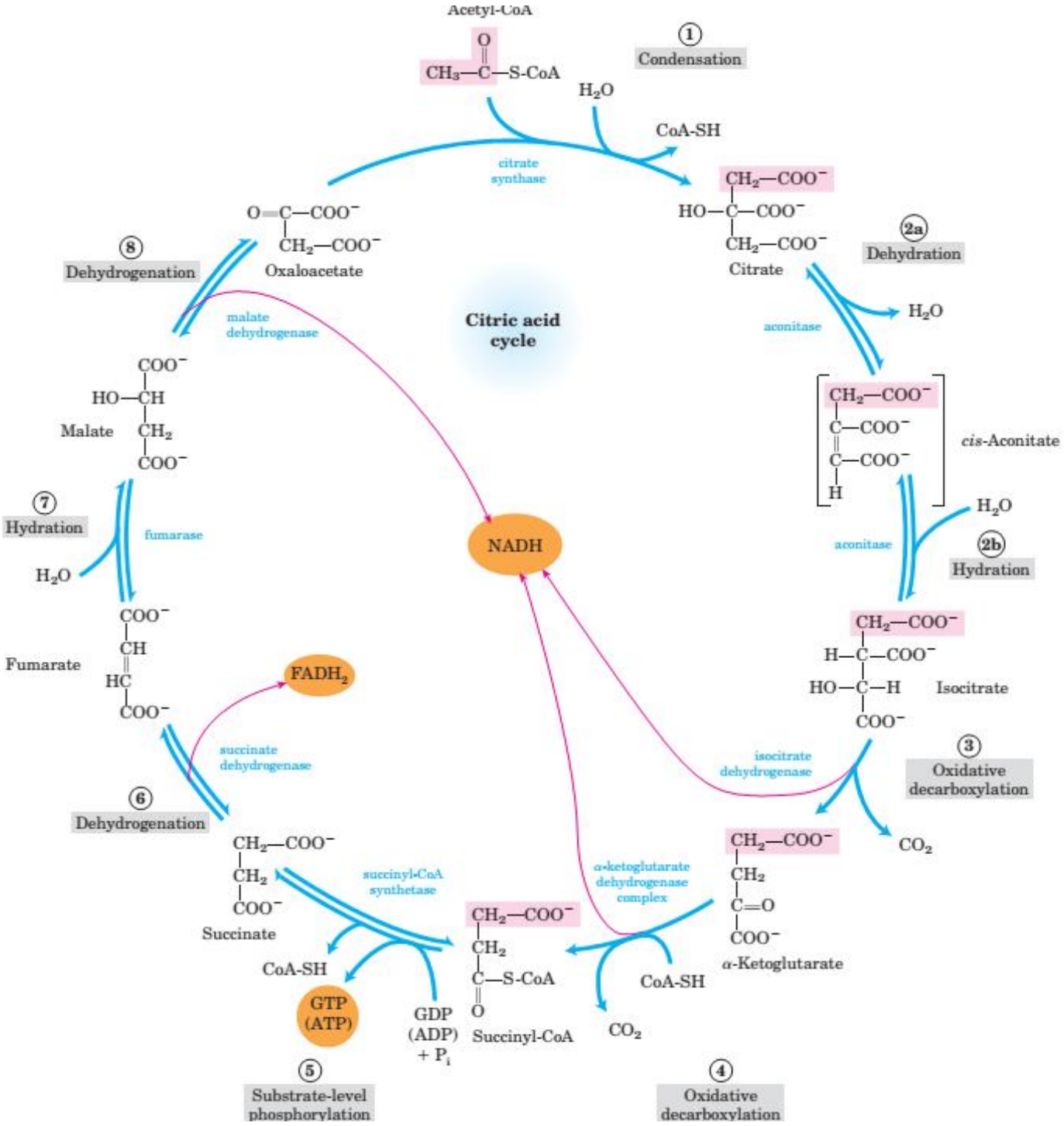
This electron transfer from FAD to NAD1 is unusual because the common role for FAD is to receive electrons from NADH. The electron-transfer potential of FAD is increased by its chemical environment within the enzyme, enabling it to transfer electrons to NAD1. Proteins tightly associated with FAD or flavin mononucleotide (FMN) are called *flavoproteins.*

Reactions of the Citric Acid Cycle

1. Citrate synthase catalyzes the condensation of acetyl-CoA and **oxaloacetate** to yield **citrate,** giving the cycle its name.

2. The strategy of the cycle's next two steps is to rearrange citrate to a more easily oxidized isomer and then oxidize it. **Aconitase** isomerizes citrate, a not readily oxidized tertiary alcohol, to the easily oxidized secondary alcohol **isocitrate.** The reaction sequence involves a dehydration, producing enzyme-bound ***cis*-aconitate, followed by a hydration, so that citrate's hydroxyl group is, in effect, transferred to an adjacent carbon atom.**

3. Isocitrate dehydrogenase oxidizes isocitrate to the β-keto acid intermediate **oxalosuccinate** with the coupled reduction of NAD+ to NADH; oxalosuccinate is then decarboxylated, yielding **α-ketoglutarate.** This is the first step in which oxidation is coupled to NADH production and also the first CO2-generating step.


Acetyl-CoA
CH3—C—S-CoA
O
1
Condensation
H2O
CoA-SH
citrate synthase
CH2—COO−
HO—C—COO−
CH2—COO−
Citrate
2a
Dehydration
H2O
aconitase
CH2—COO−
C—COO−
C—COO−
H
cis-Aconitate
H2O
2b
Hydration
aconitase
CH2—COO−
H—C—COO−
HO—C—H
COO−
Isocitrate
3
Oxidative decarboxylation
CO2
isocitrate dehydrogenase
CH2—COO−
CH2
C=O
COO−
α-Ketoglutarate
α-ketoglutarate dehydrogenase complex
CoA-SH
CH2—COO−
CH2
C—S-CoA
O
Succinyl-CoA
CO2
4
Oxidative decarboxylation
succinyl-CoA synthetase
CH2—COO−
CH2
COO−
Succinate
CoA-SH
GTP (ATP)
GDP (ADP) + Pi
5
Substrate-level phosphorylation
6
Dehydrogenation
succinate dehydrogenase
FADH2
COO−
CH
HC
COO−
Fumarate
7
Hydration
H2O
fumarase
COO−
HO—CH
CH2
COO−
Malate
8
Dehydrogenation
malate dehydrogenase
O=C—COO−
CH2—COO−
Oxaloacetate
NADH
Citric acid cycle

4. The multienzyme complex **-ketoglutarate dehydrogenase** oxidatively decarboxylates α-ketoglutarate to **succinyl-coenzyme A.** The reaction involves the reduction of a second NAD+ to NADH and the generation of a second molecule of CO2. At this point in the cycle, two molecules of CO2 have been produced, so that the net oxidation of the acetyl group is complete. Note, however, that it is not the carbon atoms of the entering acetyl-CoA that have been oxidized.

5. Succinyl-CoA synthetase converts succinyl-coenzyme A to **succinate.** The free energy of the thioester bond is conserved in this reaction by the formation of "high-energy" GTP from GDP + P*i*.

6. The remaining reactions of the cycle serve to oxidize succinate back to oxaloacetate in preparation for another round of the cycle. **Succinate dehydrogenase** catalyzes the oxidation of succinate's central single bond to a trans double bond, yielding **fumarate** with the concomitant reduction of the redox coenzyme FAD to FADH2 (the molecular formulas of FAD and FADH2.

7. Fumarase then catalyzes the hydration of fumarate's double bond to yield **malate.**

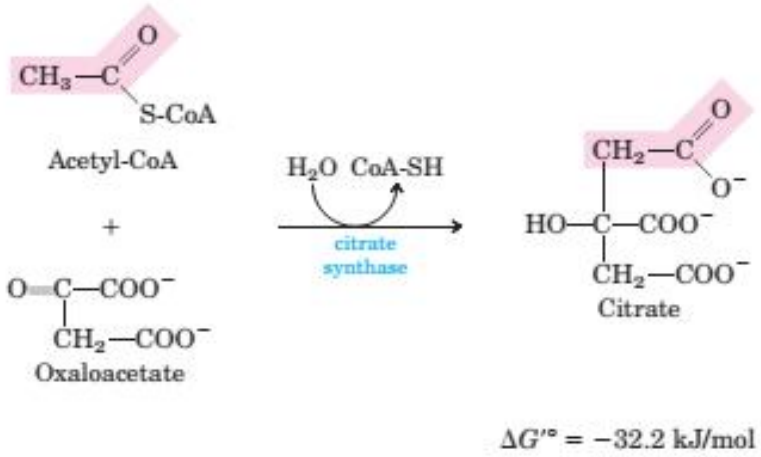
8. Finally, **malate dehydrogenase** reforms oxaloacetate by oxidizing malate's secondary alcohol group to the corresponding ketone with concomitant reduction of a third NAD+ to NADH. Acetyl groups are thereby completely oxidized to CO2 with the following stoichiometry:

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

The citric acid cycle functions catalytically as a consequence of its regeneration of oxaloacetate: An endless number of acetyl groups can be oxidized through the agency of a single oxaloacetate molecule.

Mechanism of Action

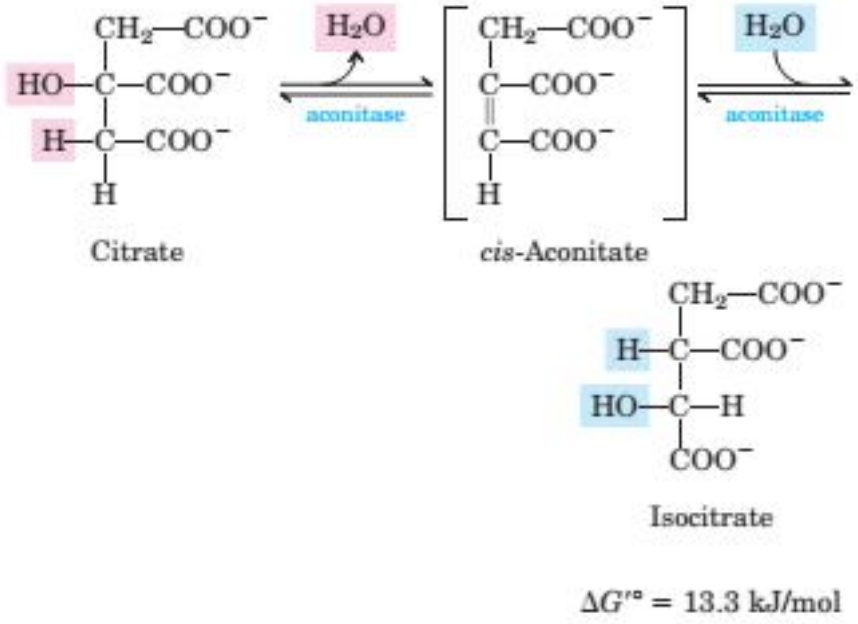
1 Formation of Citrate The first reaction of the cycle is the condensation of acetyl-CoA with **oxaloacetate** to form **citrate**, catalyzed by **citrate synthase**:



- In this reaction the methyl carbon of the acetyl group is joined to the carbonyl group (C-2) of oxaloacetate.

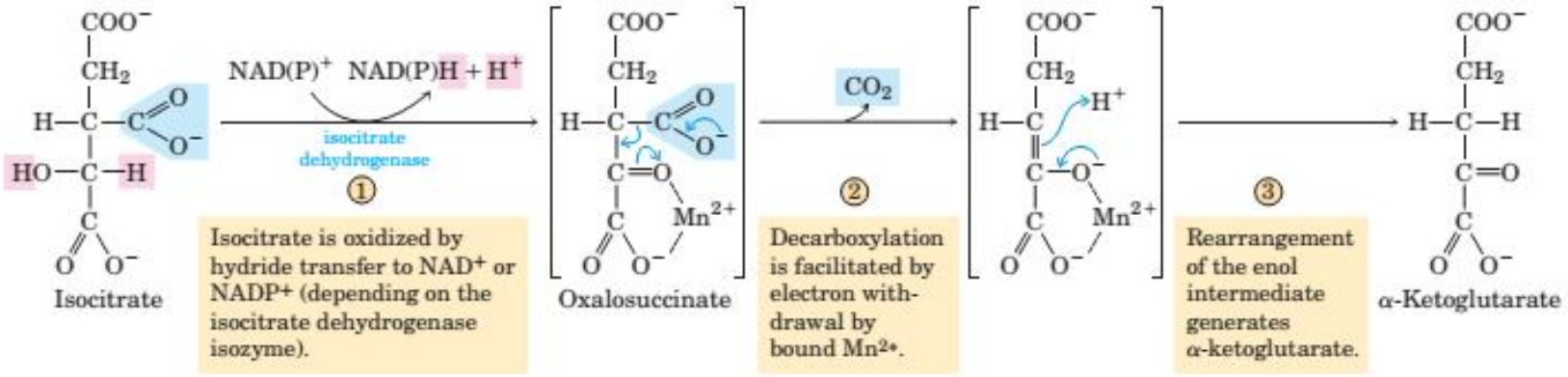
- CitroylCoA is a transient intermediate formed on the active site of the enzyme. It rapidly undergoes hydrolysis to free CoA and citrate, which are released from the active site.

2 Formation of Isocitrate via cis-Aconitate The enzyme **aconitase** (more formally, **aconitate hydratase**) catalyzes the reversible transformation of citrate to **isocitrate**, through the intermediary formation of the tricarboxylic acid ***cis*-aconitate, which normally does not dissociate from the active site.**



• Aconitase can promote the reversible addition of H2O to the double bond of enzyme-bound *cis*-aconitate in two different ways, one leading to citrate and the other to isocitrate.

3 Oxidation of Isocitrate to α-Ketoglutarate and CO2 In the next step, **isocitrate dehydrogenase** catalyzes oxidative decarboxylation of isocitrate to form **α-ketoglutarate**

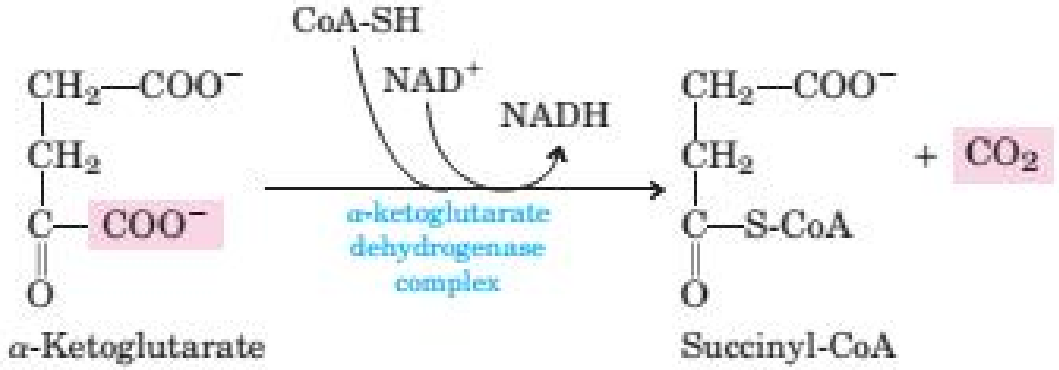


• Mn2+ in the active site interacts with the carbonyl group of the intermediate oxalosuccinate, which is formed transiently but does not leave the binding site until decarboxylation converts it to α-ketoglutarate.

• Mn2+ also stabilizes the enol formed transiently by decarboxylation.

4 Oxidation of α-Ketoglutarate to Succinyl-CoA and CO2

The next step is another oxidative decarboxylation, in which α-ketoglutarate is converted to **succinylCoA** and CO2 by the action of the **α-ketoglutarate dehydrogenase complex**

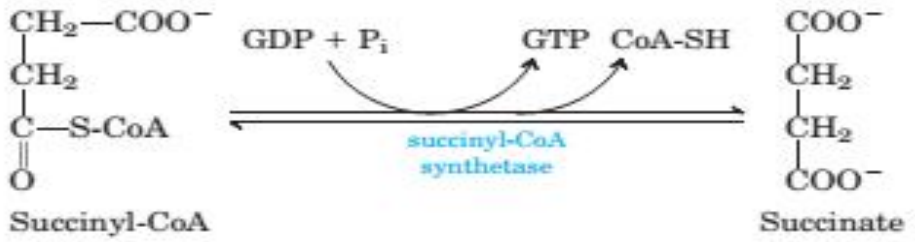


$\Delta G'^{\circ} = -33.5$ kJ/mol

• NAD+ serves as electron acceptor and CoA as the carrier of the succinyl group. The energy of oxidation of α-ketoglutarate is conserved in the formation of the thioester bond of succinyl-CoA.

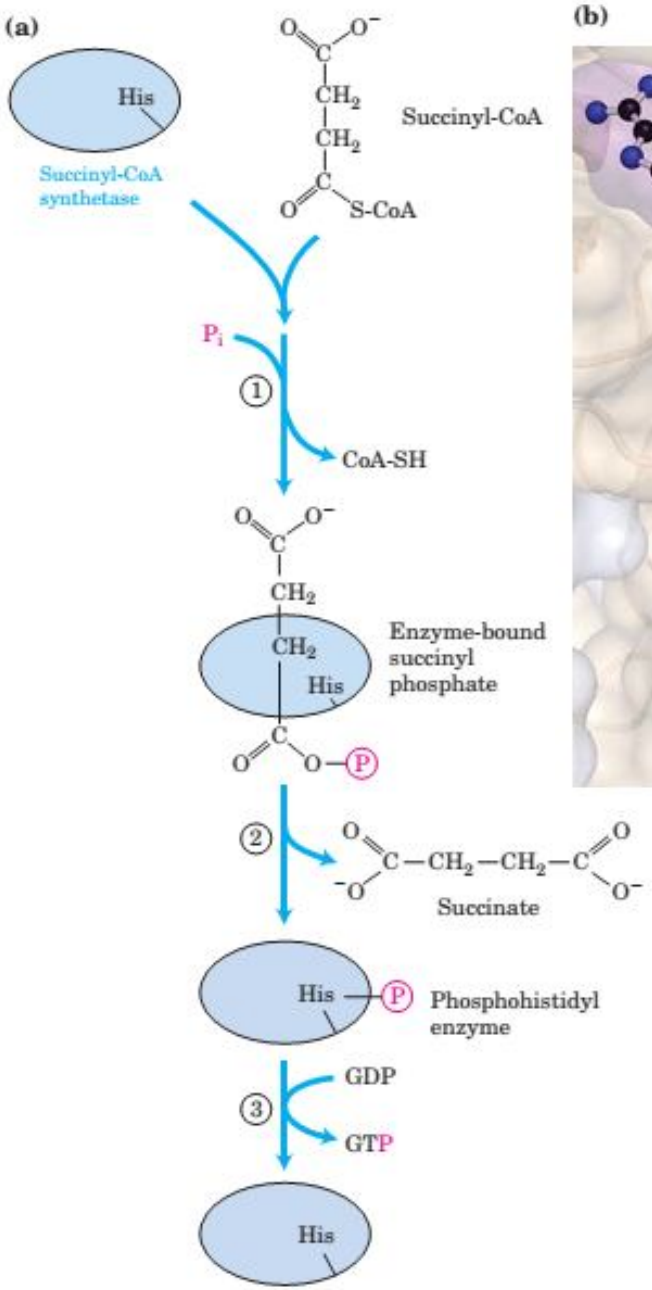
5 Conversion of Succinyl-CoA to Succinate , Succinyl-CoA, like acetyl-CoA, has a thioester bond with a strongly negative standard free energy of hydrolysis $(\Delta G'^{\circ} \approx -36$ kJ/mol).

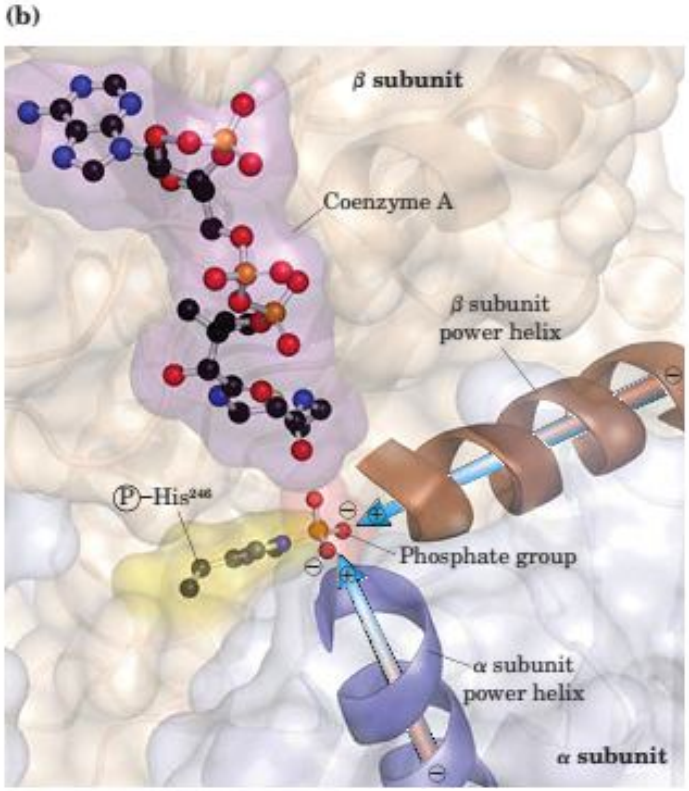
• In the next step of the citric acid cycle, energy released in the breakage of this bond is used to drive the synthesis of a phosphoanhydride bond in either GTP or ATP, with a net Δ*G'0* of only =2.9 kJ/mol. **Succinate** is formed in the process:



$\Delta G'^{\circ} = -2.9$ kJ/mol

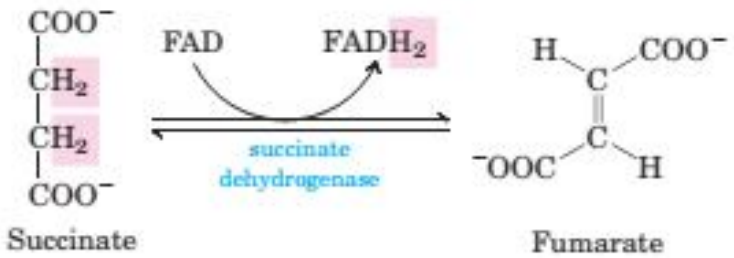
• The enzyme that catalyzes this reversible reaction is called **succinyl-CoA synthetase** or **succinic thiokinase**; both names indicate the participation of a nucleoside triphosphate in the reaction.





The succinyl-CoA synthetase reaction. **(a)** In step 1 a phosphoryl group replaces the CoA of succinyl-CoA bound to the enzyme, forming a high-energy acyl phosphate. In step 2 the succinyl phosphate donates its phosphoryl group to a His residue of the enzyme, forming a high-energy phosphohistidyl enzyme. In step 3 the phosphoryl group is transferred from the His residue to the terminal phosphate of GDP (or ADP), forming GTP (or ATP). **(b)** Active site of succinyl-CoA synthetase of *E. coli* (derived from PDB ID 1SCU). The active site includes part of both the α (blue) and the β (brown) subunits. The power helices (blue, brown) place the partial positive charges of the helix dipole near the phosphate group of P–His^{246} in the α chain, stabilizing the phosphohistidyl enzyme. The bacterial and mammalian enzymes have similar amino acid sequences and three-dimensional structures.

6 Oxidation of Succinate to Fumarate The succinate formed from succinyl-CoA is oxidized to **fumarate** by the flavoprotein **succinate dehydrogenase**:



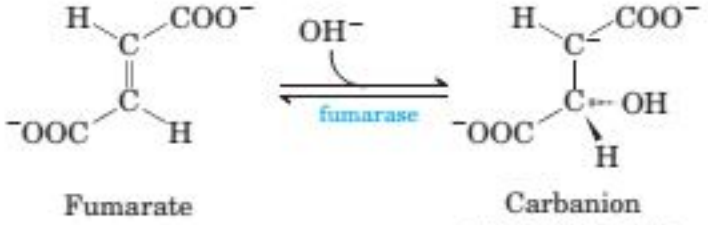
$\Delta G'^{\circ} = 0$ kJ/mol

• In eukaryotes, succinate dehydrogenase is tightly bound to the mitochondrial inner membrane; in bacteria, to the plasma membrane.

•The enzyme contains three different iron-sulfur clusters and one molecule of covalently bound FAD.

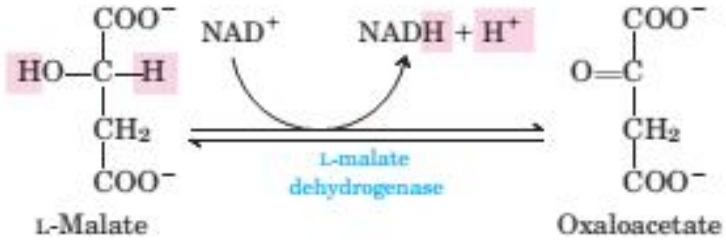
• Electron flow from succinate through these carriers to the final electron acceptor, O2, is coupled to the synthesis of about 1.5 ATP molecules per pair of electrons (respiration-linked phosphorylation).

7 Hydration of Fumarate to Malate The reversible hydration of fumarate to **L-malate** is catalyzed by **fumarase** (formally, **fumarate hydratase**). The transition state in this reaction is a carbanion:

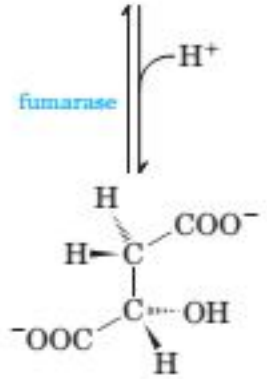


Fumarate — Carbanion transition state

8 Oxidation of Malate to Oxaloacetate In the last reaction of the citric acid cycle, NAD-linked **L-malate dehydrogenase** catalyzes the oxidation of L-malate to oxaloacetate:

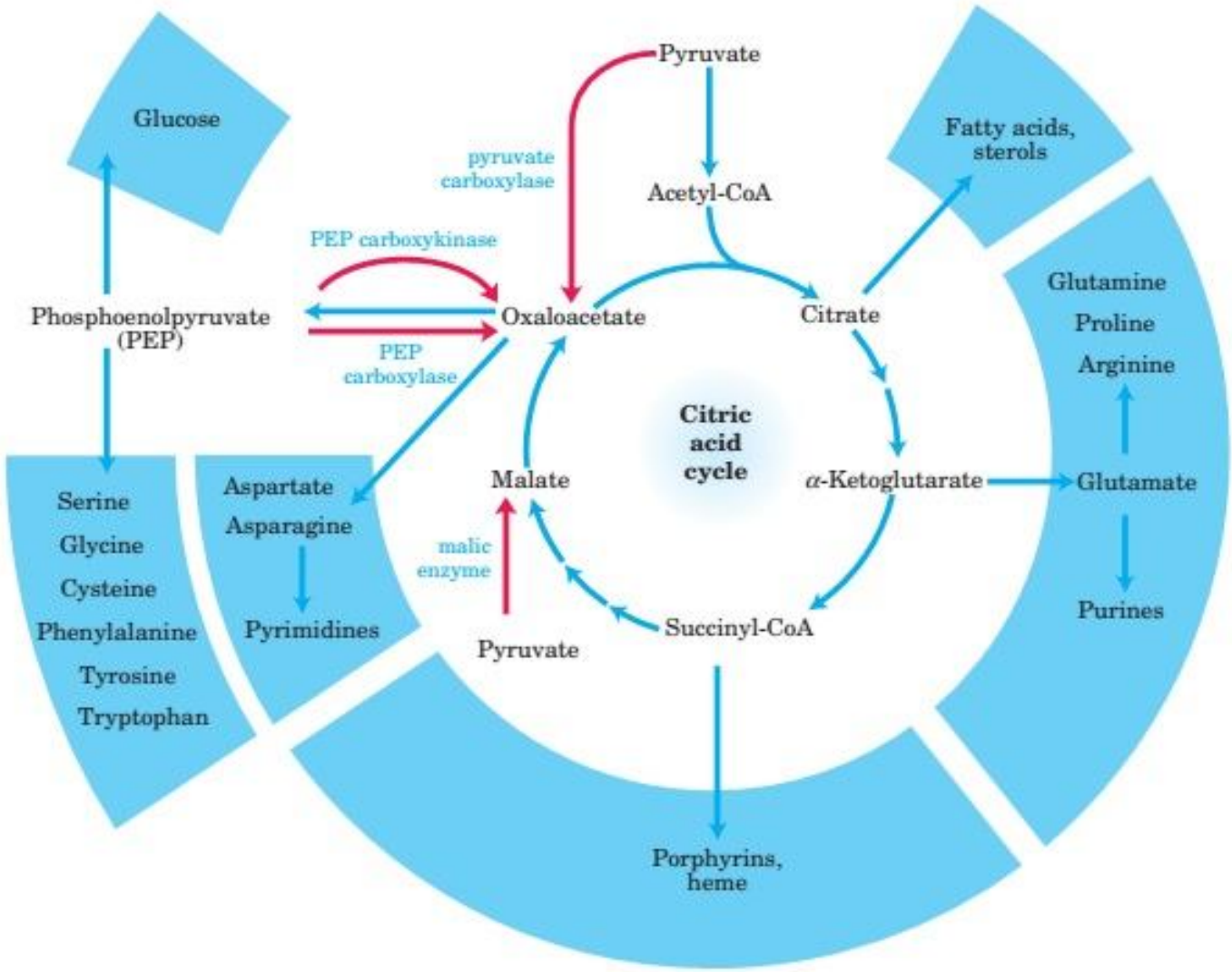


$\Delta G'^{\circ} = 29.7$ kJ/mol



L-Malate

$\Delta G'^{\circ} = -3.8$ kJ/mol



Role of the citric acid cycle in anabolism. Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways. Shown in red are four anaplerotic reactions that replenish depleted cycle intermediates (see Table 16–2).