

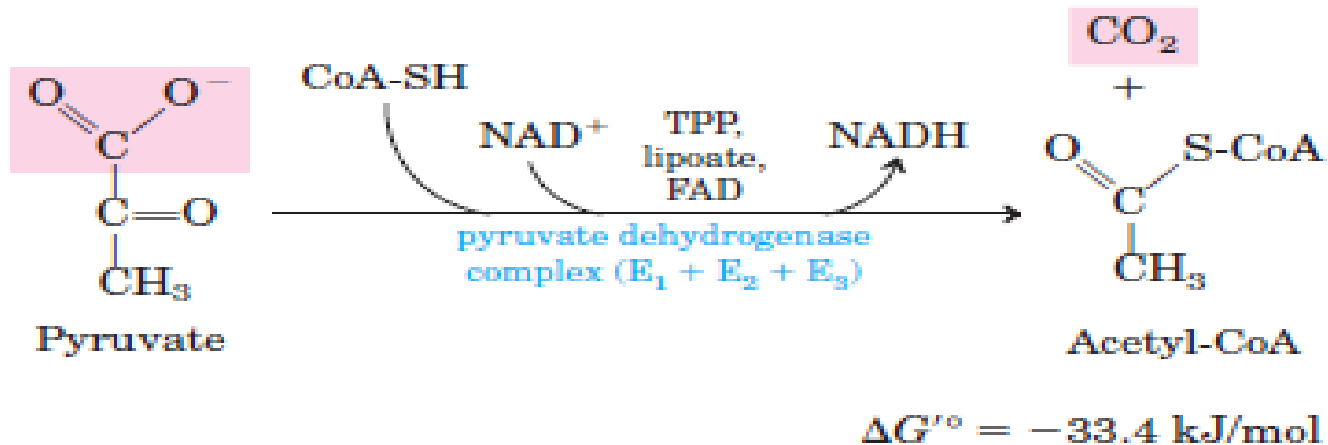
# **Krebs Cycle**

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# Tricarboxylic acid (TCA) cycle, citric acid cycle, or Krebs cycle

- The multienzyme system called the pyruvate dehydrogenase complex first oxidizes pyruvate generated during glycolysis, to form  $\text{CO}_2$  and acetyl coenzyme A (acetyl-CoA), an energy-rich molecule composed of coenzyme A and acetic acid joined by a high energy thiol ester bond (figure 1).



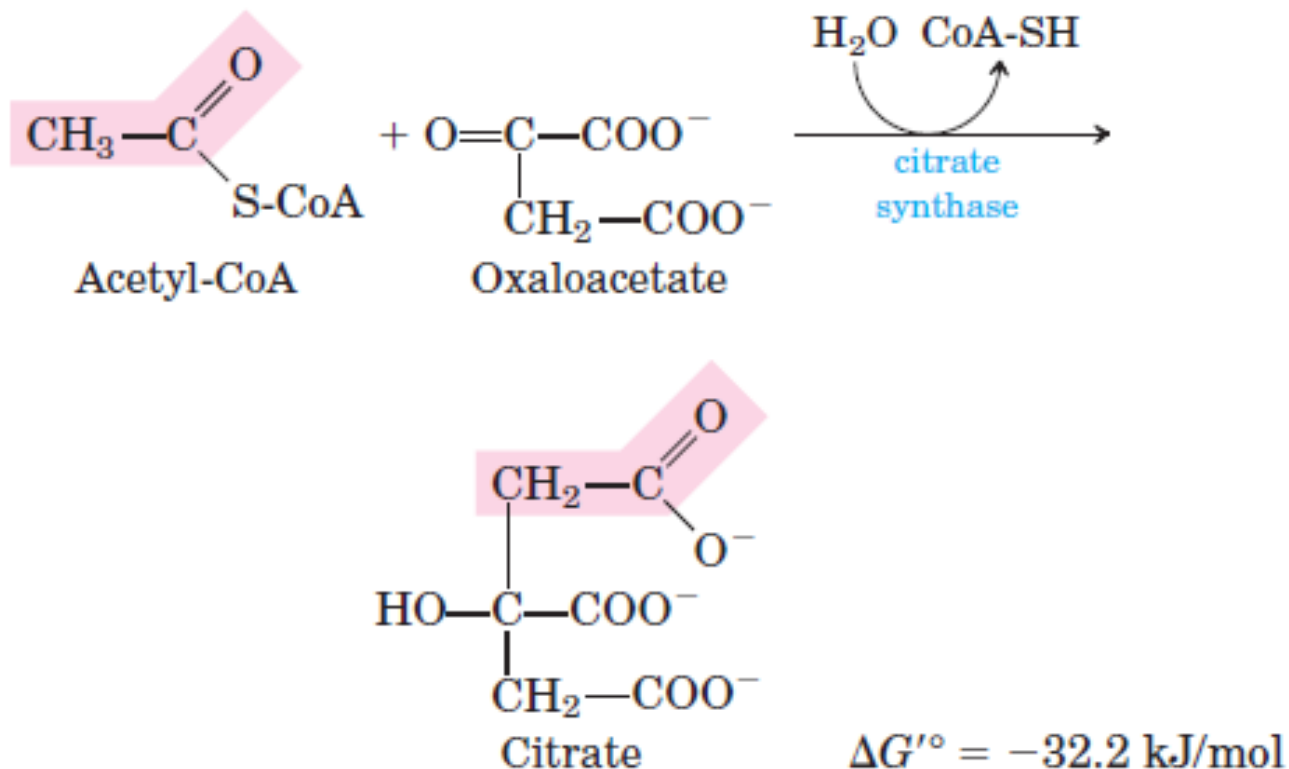
**FIGURE 16-2 Overall reaction catalyzed by the pyruvate dehydrogenase complex.** The five coenzymes participating in this reaction, and the three enzymes that make up the enzyme complex,

# Krebs Cycle

- Acetyl-CoA can be further degraded in the tricarboxylic acid cycle.
- In the first reaction acetyl-CoA is condensed with a four carbon intermediate, oxaloacetate, to form citrate and to begin the six-carbon stage.
- Citrate (a tertiary alcohol) is rearranged to give isocitrate, a more readily oxidized secondary alcohol.
- Isocitrate is subsequently oxidized and decarboxylated twice to yield  $\alpha$ -ketoglutarate, then succinyl-CoA.
- At this point two NADHs are formed and two carbons are lost from the cycle as  $\text{CO}_2$ .
- The cycle now enters the four-carbon stage during which two oxidation steps yield one  $\text{FADH}_2$  and one NADH per acetyl-CoA.
- In addition, GTP is produced from succinyl-CoA by substrate-level phosphorylation.
- Eventually oxaloacetate is reformed and ready to join with another acetyl-CoA.
- The TCA cycle generates two  $\text{CO}_2$ , three NADH, one  $\text{FADH}_2$ , and one GTP for each acetyl-CoA molecule oxidized.

# TCA Cycle

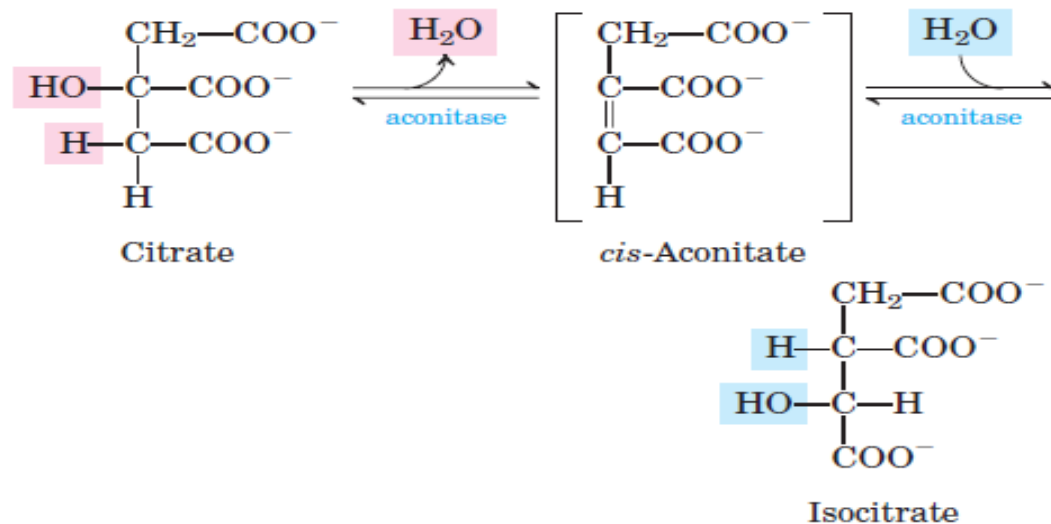
① **Formation of Citrate** The first reaction of the cycle is the condensation of acetyl-CoA with **oxaloacetate** to form **citrate**, catalyzed by **citrate synthase**:



# ...TCA Cycle

## (2) Formation of Isocitrate via cis-Aconitate

- The enzyme aconitase 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 H<sub>2</sub>O to the double bond of enzyme-bound cis-aconitate in two different ways, one leading to citrate and the other to isocitrate:

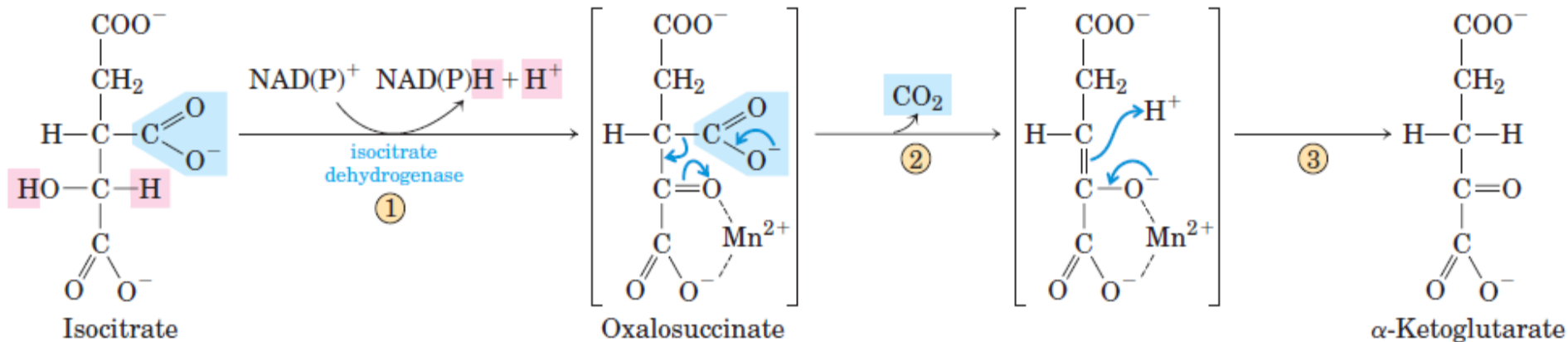


$$\Delta G'^{\circ} = 13.3 \text{ kJ/mol}$$

# ...TCA Cycle

## (3) Oxidation of Isocitrate to $\alpha$ -Ketoglutarate and $\text{CO}_2$

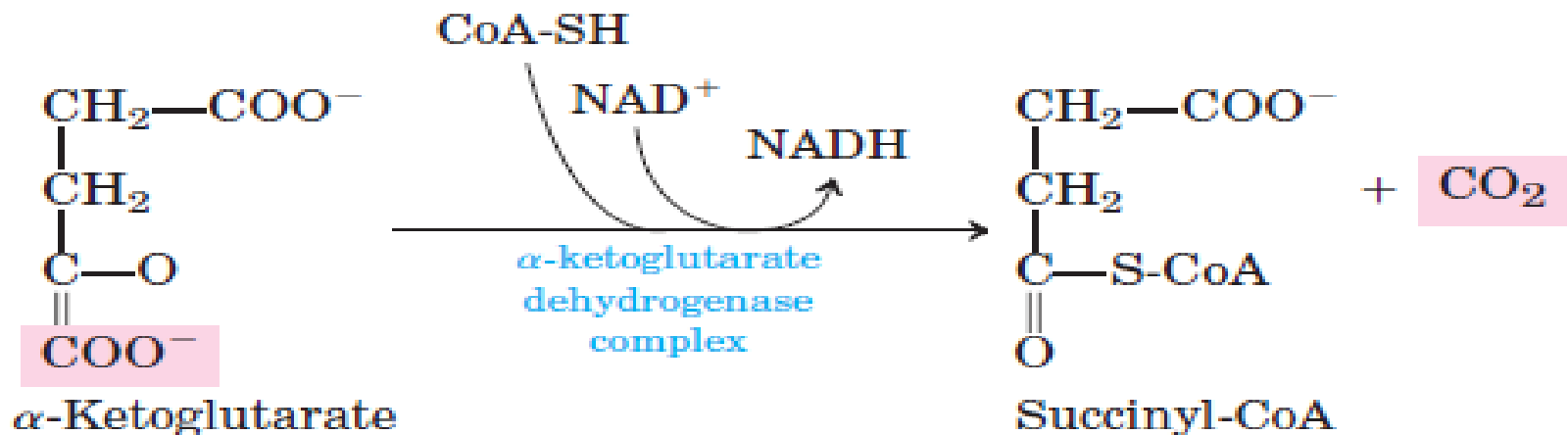
- In the next step, isocitrate dehydrogenase catalyzes oxidative decarboxylation of isocitrate to form  $\alpha$ -ketoglutarate.
- $\text{Mn}^{2+}$  in the active site interacts with the carbonyl group of the intermediate oxalosuccinate, which is formed transiently but does not leave the binding site until decarboxylation converts it to  $\alpha$ -ketoglutarate.
- $\text{Mn}^{2+}$  also stabilizes the enol formed transiently by decarboxylation.



# ...TCA Cycle

## ④ Oxidation of $\alpha$ -Ketoglutarate to Succinyl-CoA and $\text{CO}_2$

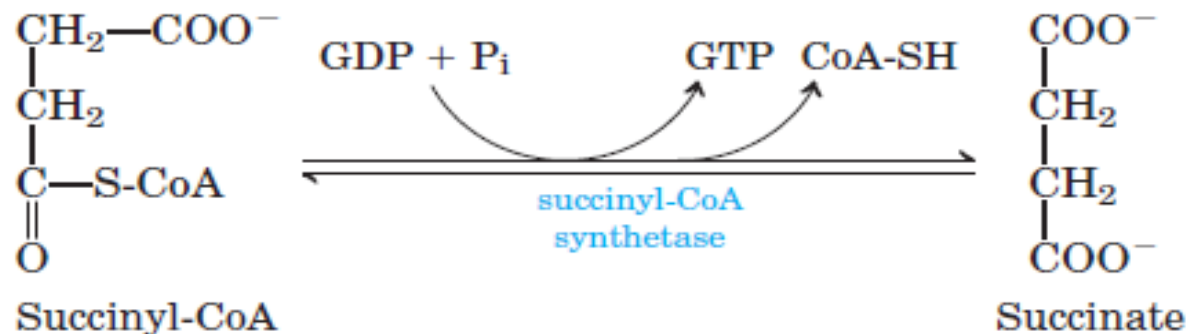
The next step is another oxidative decarboxylation, in which  $\alpha$ -ketoglutarate is converted to succinyl-CoA and  $\text{CO}_2$  by the action of the  **$\alpha$ -ketoglutarate dehydrogenase complex**;  $\text{NAD}^+$  serves as electron acceptor and CoA as the carrier of the succinyl group. The energy of oxidation of  $\alpha$ -ketoglutarate is conserved in the formation of the thioester bond of succinyl-CoA:



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

# ...TCA Cycle

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  $\Delta G'^{\circ}$  of only  $-2.9$  kJ/mol. **Succinate** is formed in the process:

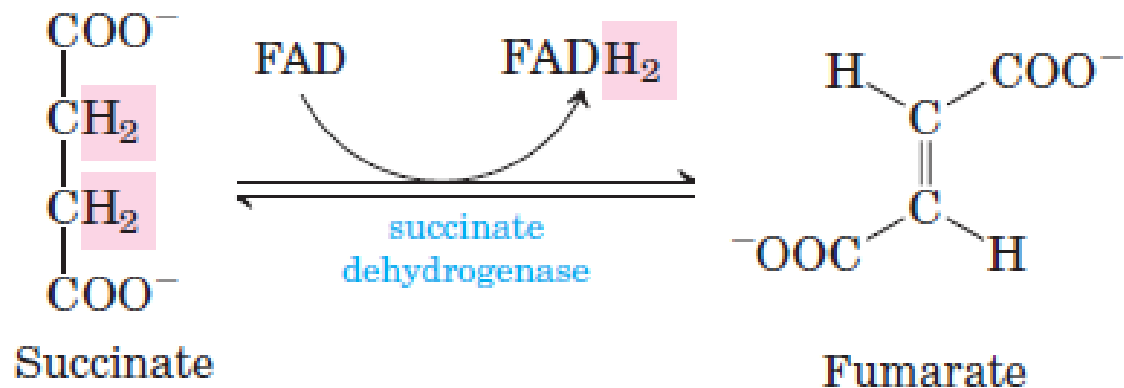


$$\Delta G'^{\circ} = -2.9 \text{ kJ/mol}$$



# ...TCA Cycle

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

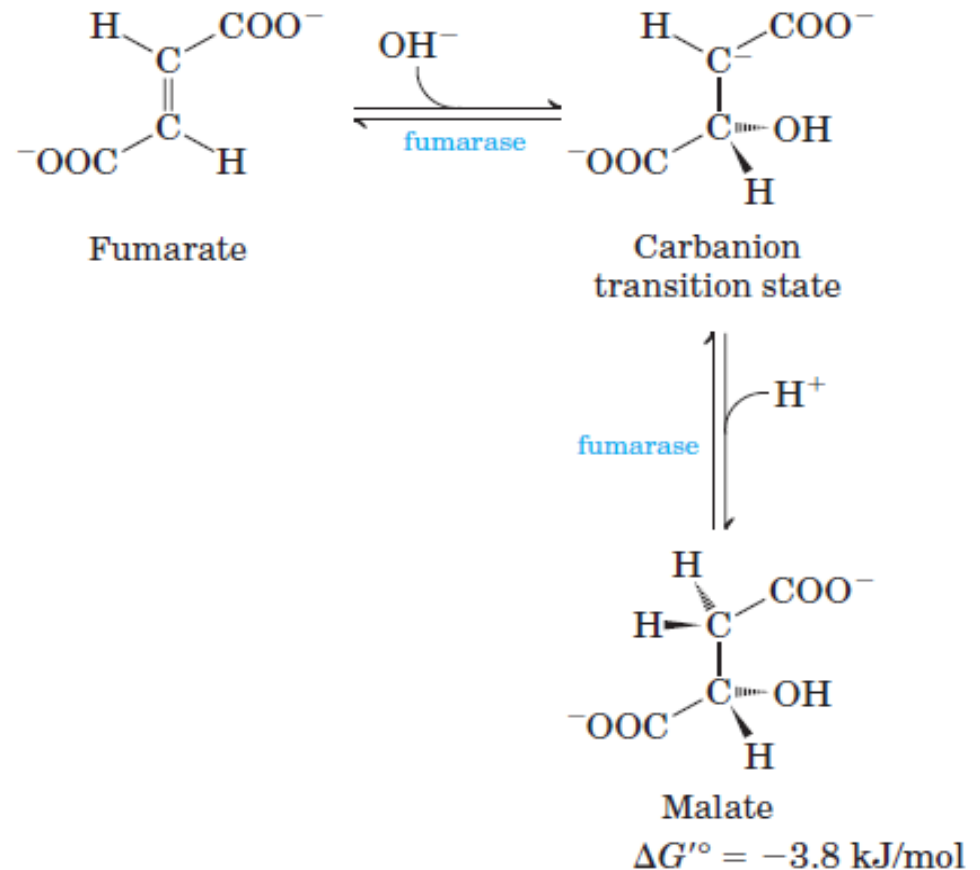


$$\Delta G'^{\circ} = 0 \text{ kJ/mol}$$

# ...TCA Cycle

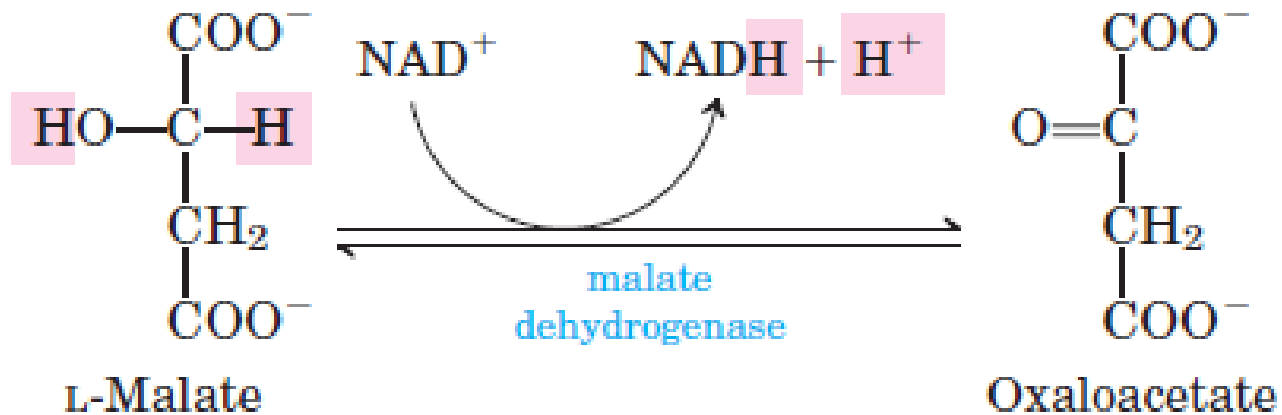
## (7) Hydration of Fumarate to Malate

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

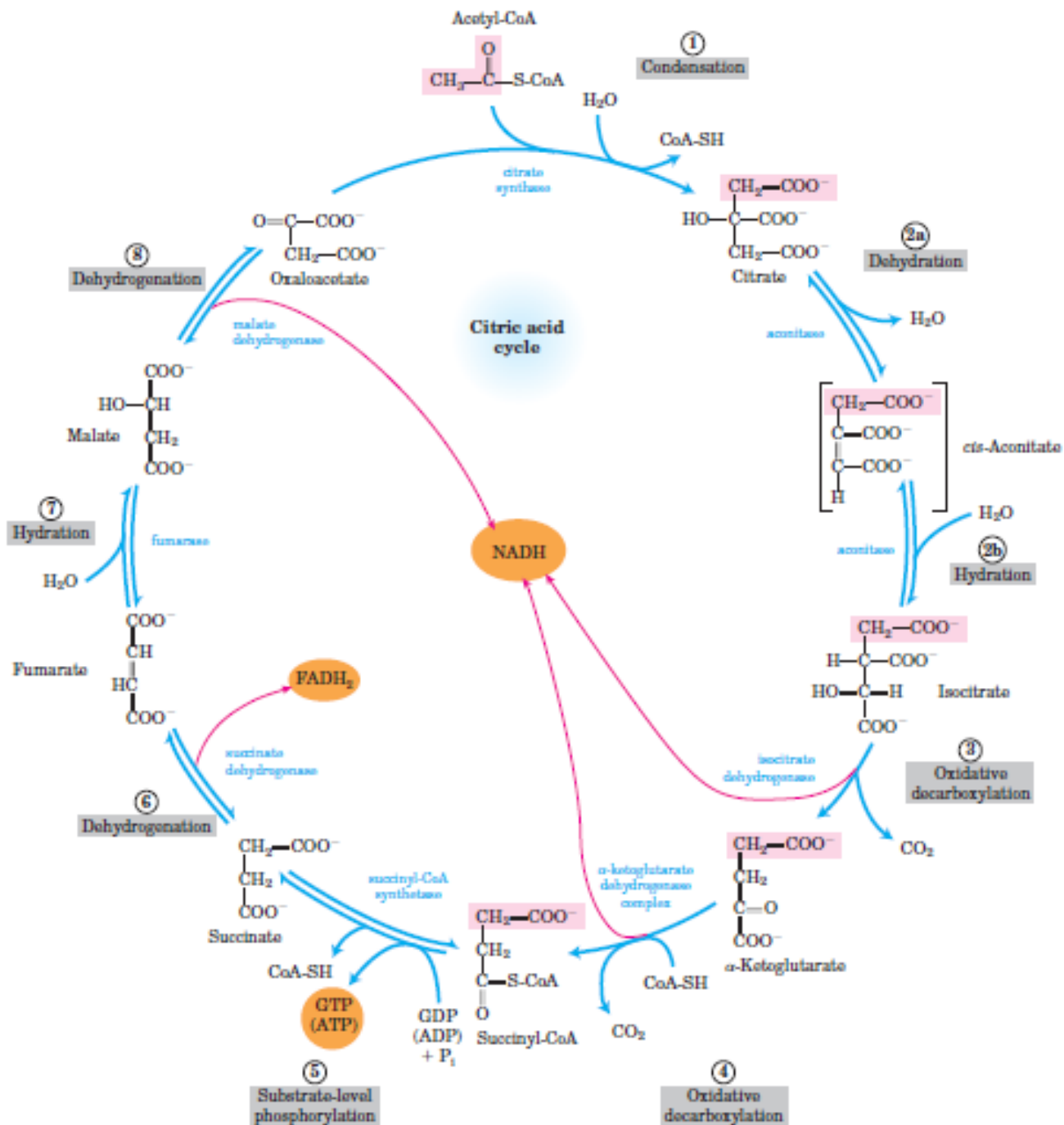


# ...TCA Cycle

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:



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



- TCA cycle enzymes are widely distributed among microorganisms.
- The complete cycle appears to be functional in many aerobic bacteria, free-living protozoa, and most algae and fungi.
- However, the facultative anaerobe *E. coli* does not use the full TCA cycle under anaerobic conditions or when the glucose concentration is high but does at other times.
- Even those microorganisms that lack the complete TCA cycle usually have most of the cycle enzymes, because one of TCA cycle's major functions is to provide carbon skeletons for use in biosynthesis.

# Questions

- Write an essay on citric acid (TCA) cycle.
- Where is the tricarboxylic acid cycle located in procaryotes? Diagrammatically explain the substrate and products of TCA cycle.