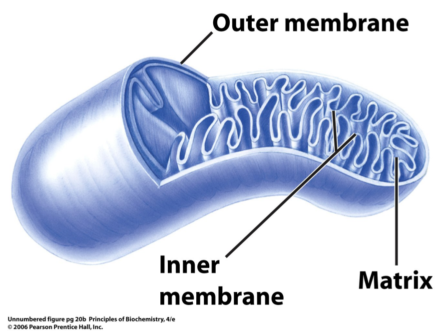
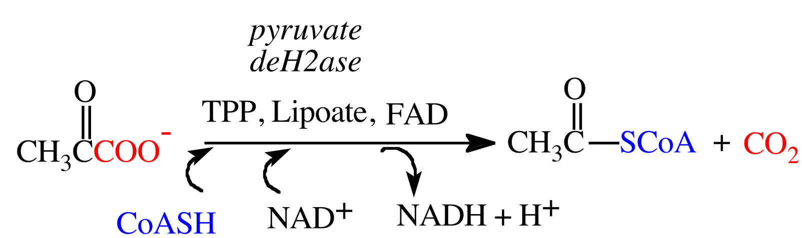
Chapter 13 - TCA Cycle

The third fate of glucose/pyruvate is complete oxidation to CO_2 + H_2O in the matrix of the mitochondrion.



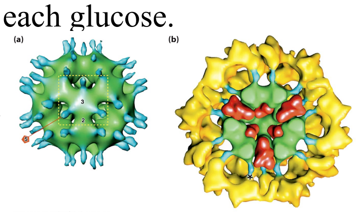
The 1st step is the **oxidation** and **decarboxylation** of pyruvate to Acetyl-CoA, a form of **activated acetate**:



$\Delta G'^{o} = -33.4$ kJ/mol $K_{eq} = 7 \times 10^5$

Remember, there are 2 pyruvates from each glucose.

In *E. coli, pyruvate dehydrogenase* is a large complex of 3 enzymes:



E_1 = *pyruvatedeH₂ase* – 24 copies
E_2 = *dihydrolipoyltransacetylase* – 24 copies
E_3 = *dihydrolipoyldeH₂ase* – 12 copies

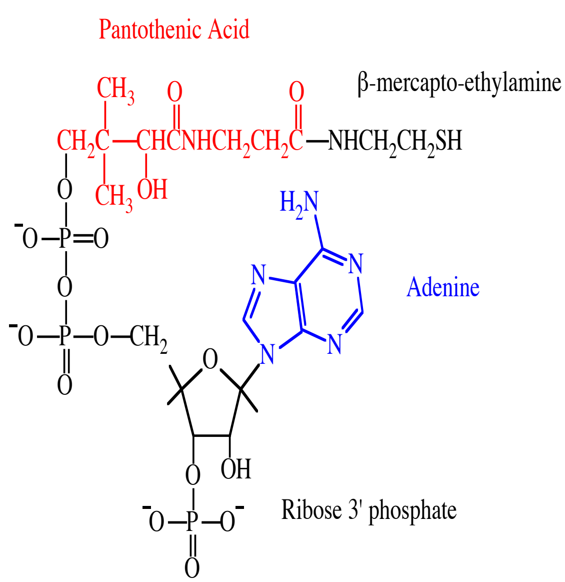
It uses 5 co-enzymes; 4 are derived from Vitamins:

TPP → Thiamin = Vitamin B_1 FAD → Riboflavin = Vitamin B_2

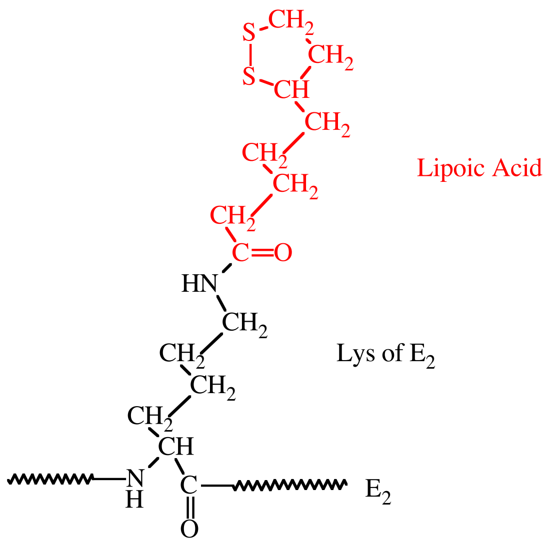
NAD → Niacin = Vitamin B_3 CoA → Pantothenate = Vitamin B_5

Lipoate

Coenzyme A



Lipoic Acid



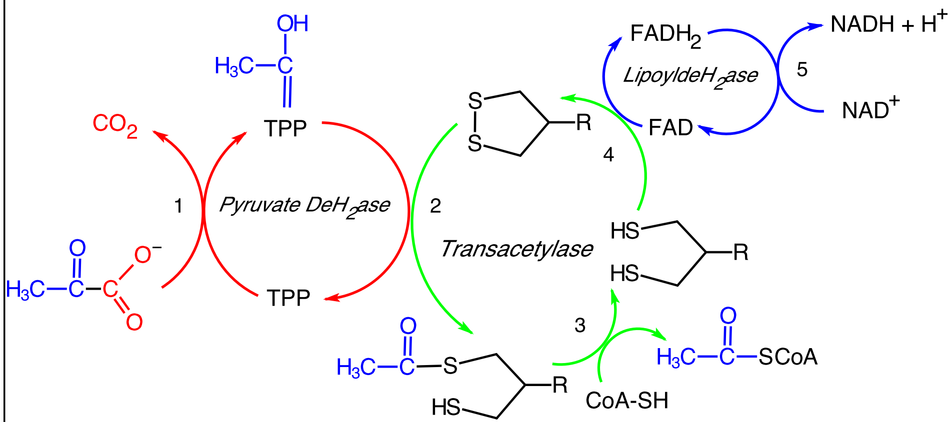
The reaction starts on E_1 and ends on E_3. The long flexible lipoic acid arm carries $2e^-$ from E_1 to E_3.

E_1 uses TPP to decarboxylate pyruvate exactly as for *pyruvate decarboxylase.*

Next, the lipoic acid on E_2 transfers the acetate from E_1 to CoA.

Then, the lipoic acid is re-oxidized by the FAD on E_3.

Finally, the $FADH_2$ is re-oxidized by NAD^+ and NADH carries the electrons away.



The reaction is irreversible and an important control point linking glycolysis and the TCA Cycle.

It is **inhibited** by **ATP, acetyl-CoA, NADH, fatty acids, CO_2** - "*high-energy signals*"

It is **activated** by **Pyruvate, AMP, CoA, NAD^+** - "*low-energy signals*"

In eukaryotes, the **Citric Acid Cycle / Krebs Cycle / Tricarboxylic Acid Cycle** acetate is oxidized to CO_2 and H_2O.

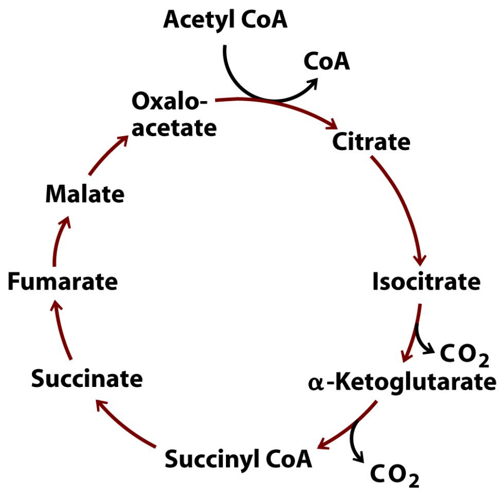


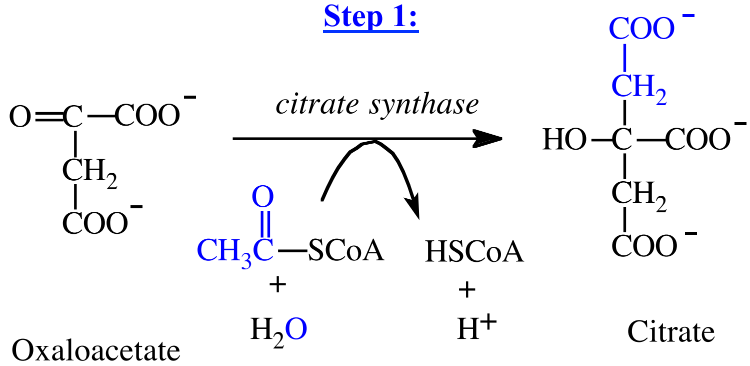
Figure 10-2b Principles of Biochemistry, 4/e

The acetate may come from oxidation of glucose, amino acids, or lipids.

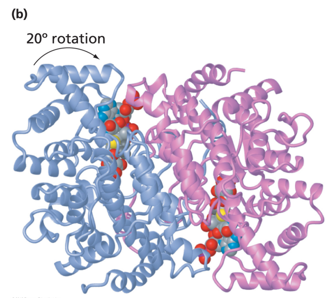
The intermediates are used in AA, carbohydrate, pyrimidine nucleotide and lipid synthesis.

Many of these enzymes are found in bacteria but bacteria rarely have the full cycle.

Step 1:



$\Delta G'^{o} = -32.2$ kJ/mol $K_{eq} = 4 \times 10^{5}$

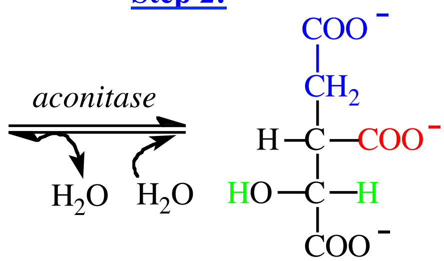


[OAA] is normally quite low so the *G* of thioester hydrolysis is used to drive the reaction forward.

Citrate is a **tricarboxylic acid**.

Citrate synthase is **inhibited** by **ATP**, **NADH**, **Acetyl-CoA**, **Succinyl-CoA and Citrate**.

Step 2:



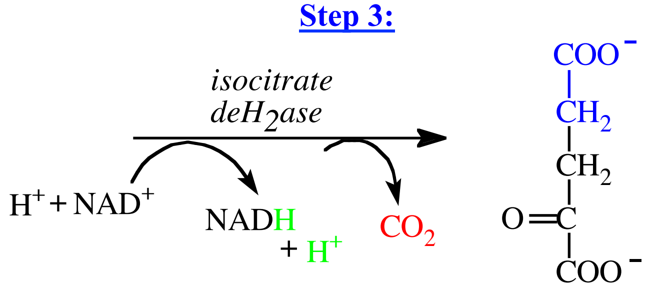
Isocitrate

$\Delta G'^{o}$ = +13.3 kJ/mol K_{eq} = 5x10^{-3}

Aconitase catalyses 2 reactions that result in the **isomerization** of citrate to isocitrate: $C_6H_5O_7 \rightarrow C_6H_5O_7$

The reaction is pulled forward by the following exergonic steps which consume isocitrate.

Step 3:

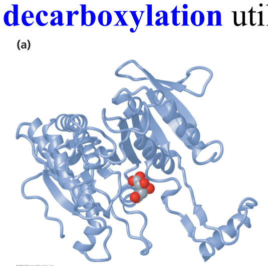


α-ketoglutarate

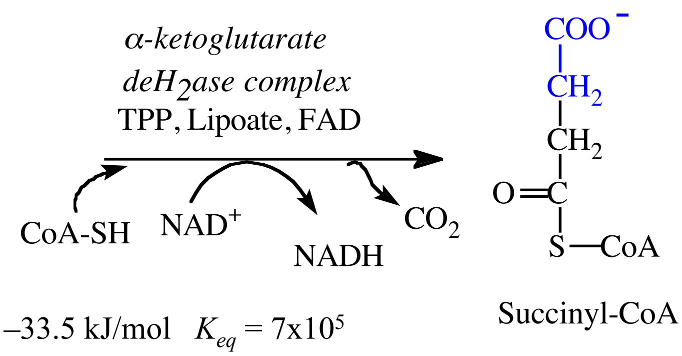
$\Delta G'^{o}$ = –20.9 kJ/mol K_{eq} = 5x10^{3}

This reaction is an **oxidation** and a **decarboxylation** utilizing NAD^+ or $NADP^+$.

It is **inhibited** by **ATP** and **activated** by **ADP**.



Step 4:



$\Delta G'^{o} = -33.5$ kJ/mol $K_{eq} = 7 \times 10^5$

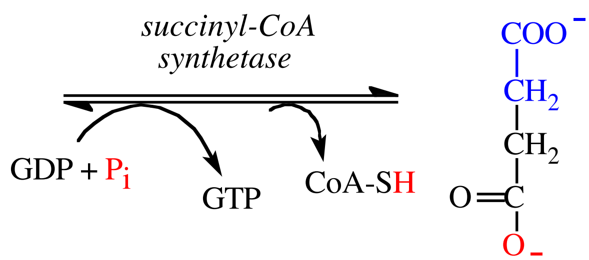
The mechanism is identical to the *pyruvate dehydrogenase* reaction.

This reaction is an **oxidation** and a **decarboxylation**.

Some of the G of oxidation is conserved in the formation of a thioester bond of succinyl-CoA.

This enzyme is **inhibited** by **NADH** and **succinyl-CoA**.

Step 5:



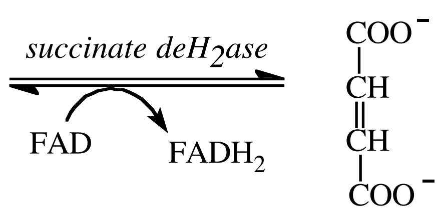
Succinate

$\Delta G'^o = -2.9$ kJ/mol $K_{eq} = 3$

The *G* released when the high-energy thioester is hydrolysed is conserved in the formation of GTP.

"**Substrate Level Phosphorylation**" Remember, GTP and ATP are energetically equivalent.

Step 6:

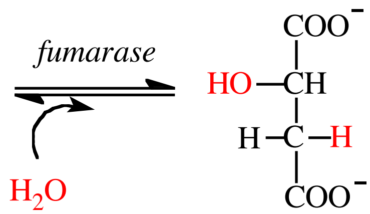


Fumarate

$\Delta G'^o = 0$ kJ/mol $K_{eq} = 1$

The *G* of oxidation is stored in reduced FAD which is covalently attached to the enzyme.

Step 7:

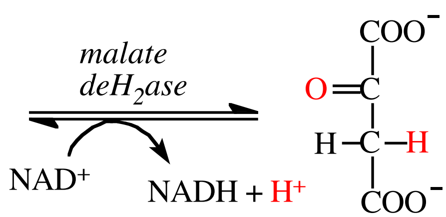


L-Malate

$\Delta G'^{o} = -3.8$ kJ/mol $K_{eq} = 5$

Fumarase stereospecifically adds water across the C=C bond.

Step 8:



Oxaloacetate

$\Delta G'^{o} = +29.7$ kJ/mol $K_{eq} = 6\text{x}10^{-6}$

This oxidation is highly endergonic so [OAA] is always low.
Exergonic reaction 1 pulls this forward.

About –50 kJ /mol of *G* is released by the cycle and drives it in the direction of products.

Energy and Mass Balance

Acetyl-CoA + $2H_2O$ + $3NAD^+$ + FAD + GDP + P_i → $2CO_2$ + CoA-SH + 3NADH + $2H^+$ + $FADH_2$ + GTP

Input

1 acetate = 2 C + 1 O

2 H_2O = 2 O

P_i = 1O

Output

2 CO_2 *i.e.* 2 C and 4 O;

- from OAA, **not** from the 1 acetate added at the beginning of the cycle.

4 steps involve oxidations that conserve *G* by reducing electron carriers (3 NADH + 1 $FADH_2$) plus 1 high energy phosphate is formed (GTP).

Note that reactions 6-8 regenerate OAA so there is no net consumption or production of intermediates. The cycle functions as a **catalyst**.

Why is O_2 required?

The cycle would stop if NAD^+ were not regenerated:

$$NADH + H^+ + \frac{1}{2} O_2 \rightarrow H_2O + NAD^+$$

The transport of electrons from NADH to O_2 is coupled to ATP formation.

$$ADP + P_i + H^+ \longrightarrow ATP + H_2O$$

The process is called **oxidative phosphorylation** and is the subject of Chapter 14.

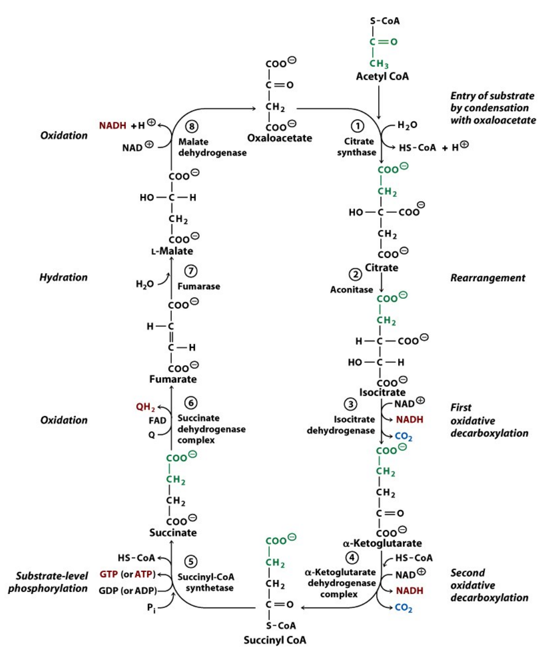


Figure 13-3 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.