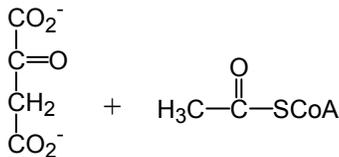


# The Citric Acid Cycle

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## I. Citrate Synthase



The first reaction of the citric acid cycle is the condensation of acetyl-CoA and oxaloacetate to form citrate and CoA-SH.

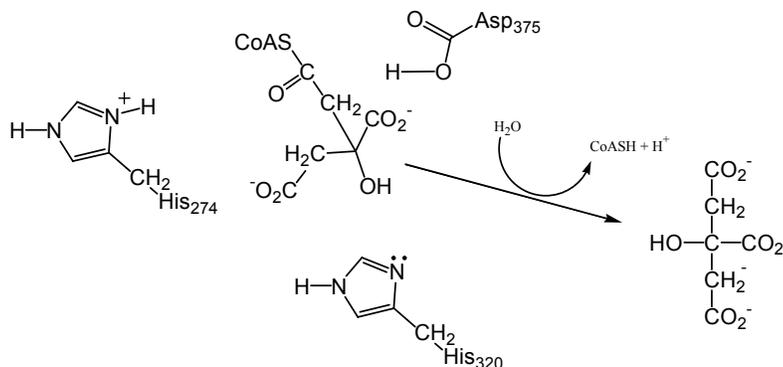
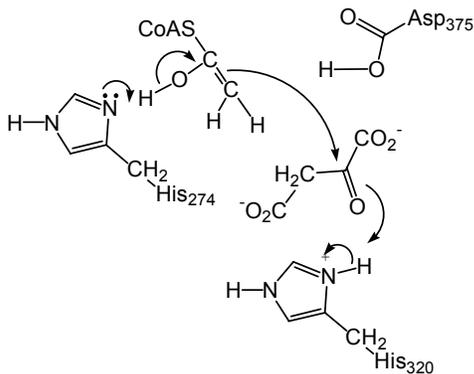
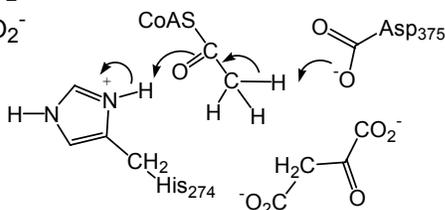
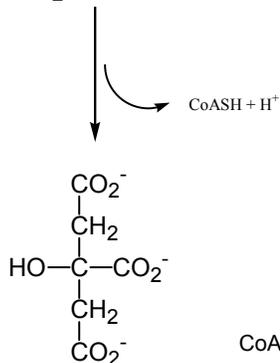
The enzyme that catalyzes this reaction is called citrate synthase.

$$\Delta G^{0'} = -32.2 \text{ kJ/mol}$$

The change in free energy based on the steady state concentrations of oxaloacetate, acetyl-CoA and citrate in the mitochondria of isolated from pig hearts is:

$$\Delta G = -53.9 \text{ kJ/mol}$$

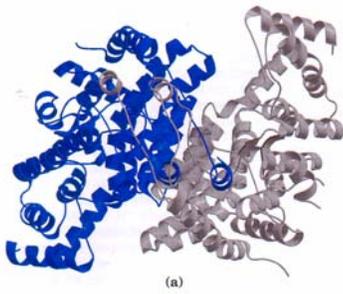
A very exergonic reaction and irreversible.



The mechanism of citrate synthase is shown to the left. In the active site of the enzyme we have two histidines and an aspartate which function as general acids and bases during catalysis.

The first step is to generate an enol of acetyl-CoA. Asp-375 functions as a general base abstracting a proton for the methyl group of Acetyl-CoA. His-274 concertedly functions as a general acid donating a proton to the carbonyl to form the enol.

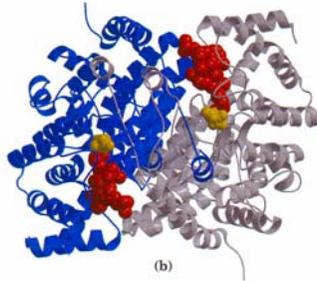
The enol generated in the first step is converted into a nucleophile by the abstraction of the enol hydrogen by His-274 now functioning as a general base. The electrons of the double bond attack the electrophilic center of the ketone of oxaloacetate. His-320 functions in concert as a general acid donating its proton to the carbonyl oxygen of oxaloacetate to form citryl-CoA. Citryl-CoA spontaneously hydrolyses while it is still bound to the active site to generate coenzyme A and citrate.



Citrate synthase is a homodimer with symmetry as you can see to the left.

It has a sequential order kinetic mechanism.

First the enzyme binds **oxaloacetate** which induces the large conformational change shown (b). This is yet another example of induced fit. The conformation change induced by oxaloacetate binding creates the **acetyl-CoA** binding site and seals oxaloacetate from the aqueous solvent.



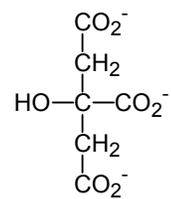
Now that you are no longer novices regarding metabolic strategies, you probably already have a sense that citrate synthase is going to be allosterically regulated.

- It is the first step of a metabolic pathway.
- It catalyzes an irreversible step in the pathway.
- It is a homodimer with symmetry.

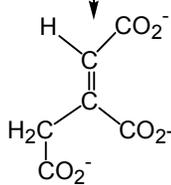
And your intuitions are correct. This enzyme is a site for allosteric regulation.

One of the products of the citric acid cycle is NADH which allosterically inhibits the enzyme. Succinyl-CoA, the product of the fifth step of the cycle also inhibits.

## II. Aconitase



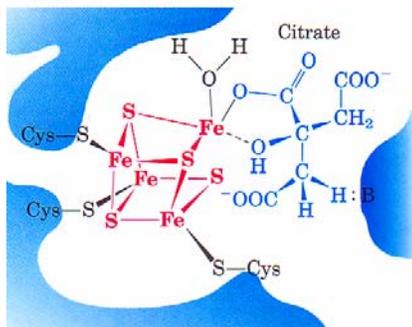
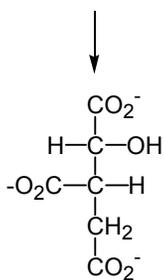
Citrate is a tertiary alcohol. It is difficult to oxidize tertiary alcohols because forming the ketone would involve breaking a carbon-carbon bond. To get around this problem, citrate is isomerized into isocitrate. Isocitrate is a secondary alcohol which can be easily oxidized to the ketone by  $\text{NAD}^+$ . The enzyme that catalyzes this migration of a hydroxyl group is aconitase. This enzyme catalyzes the dehydration of citrate to form cis-aconitate and then rehydrating the double bond to form isocitrate.



$$\Delta G^{\circ'} = +6.7 \text{ kJ/mol}$$

The change in free energy based on the steady state concentrations of citrate and isocitrate in the mitochondria of isolated pig hearts is:

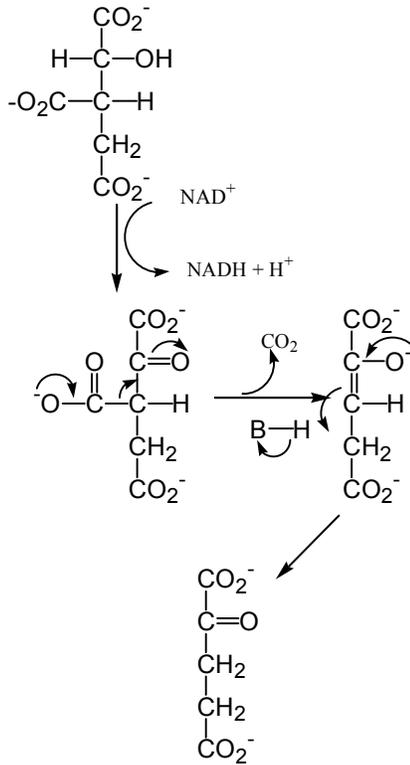
$$\Delta G = +0.8 \text{ kJ/mol ; near equilibrium.}$$



Aconitase is an iron sulfur protein.

It contains four iron atoms complexed to four inorganic sulfides and three cysteine sulfur atoms called a 4Fe-4S iron-sulfur cluster. One of the iron atoms has an open coordination site that complexes with the carboxylate group of C3 and the hydroxyl group of citrate. This iron residue facilitates the dehydration and rehydration reaction and accounts for the stereospecificity of the reaction.

### III. Isocitrate Dehydrogenase



This is the first oxidation step of the pathway.

Isocitrate dehydrogenase catalyzes the oxidation of isocitrate to oxalosuccinate which is a  $\beta$ -keto acid. This  $\beta$ -keto acid spontaneously decarboxylates to form  $\alpha$ -ketoglutarate. This is the second time we have oxidized a compound to form a  $\beta$ -keto acid which spontaneously decarboxylated itself. In the pentose phosphate pathway, 6-phosphogluconate dehydrogenase oxidized 6-phosphogluconate to 3-keto-6-phosphogluconate, a  $\beta$ -keto acid, which spontaneously decarboxylated to form ribulose-5-phosphate.

$$\Delta G^{\circ'} = -8.4 \text{ kJ/mol}$$

The change in free energy based on the steady state concentrations of isocitrate and  $\alpha$ -ketoglutarate in the mitochondria of isolated from pig hearts is:

$$\Delta G = -17.5 \text{ kJ/mol ;}$$

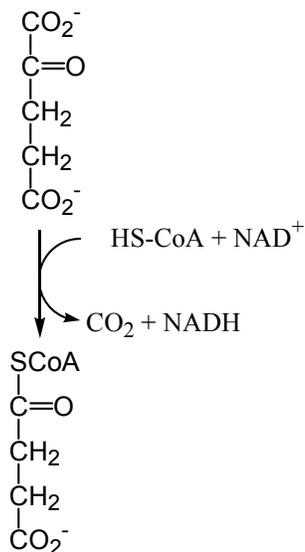
A very exergonic, irreversible reaction.

As you expect, this irreversible reaction is allosterically regulated.

NADH and ATP are allosteric inhibitors.

ADP is an allosteric activator which lowers the  $K_m$  for isocitrate by a factor of 10. When the concentration of ADP is low, the  $K_m$  for citrate is well above the physiological concentration making the enzyme essentially inactive. With ADP bound in the allosteric binding site, the  $K_m$  is lowered by a factor of ten making the enzyme active at physiological concentrations.

### IV. $\alpha$ -Ketoglutarate Dehydrogenase



The next step in the TCA cycle is the oxidative decarboxylation of  $\alpha$ -ketoglutarate to form succinyl-CoA and NADH.

$$\Delta G^{\circ'} = -30.0 \text{ kJ/mol}$$

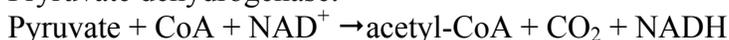
The change in free energy based on the steady state concentrations of  $\alpha$ -ketoglutarate and succinyl-CoA in the mitochondria of isolated from pig hearts is:

$$\Delta G = -43.9 \text{ kJ/mol ;}$$

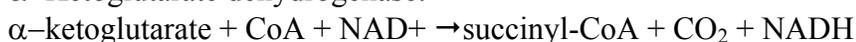
A very exergonic, irreversible reaction

If you look at the chemical transformation involved, you will note the similarity between this enzymatic reaction and the one catalyzed by pyruvate dehydrogenase.

Pyruvate dehydrogenase:



$\alpha$ -Ketoglutarate dehydrogenase:



Both of these reactions include the decarboxylation of an  $\alpha$ -keto acid and the subsequent formation of an high energy thioester linkage with coenzyme A.

$\alpha$ -Ketoglutarate dehydrogenase is a humongous multienzyme complex that is very similar to the pyruvate dehydrogenase multienzyme complex.

Pyruvate Dehydrogenase Complex of *E. coli*.

Enzyme	#	Prosthetic Groups	Reaction Catalyzed.
Pyruvate Dehydrogenase	E <sub>1</sub> 24	TPP	Oxidative Decarboxylation
Dihydrolipoyl transacetylase	E <sub>2</sub> 24	Lipoamide	Transfer of acetyl group
Dihydrolipoyl Dehydrogenase	E <sub>3</sub> 12	FAD	Regenerate lipoamide.

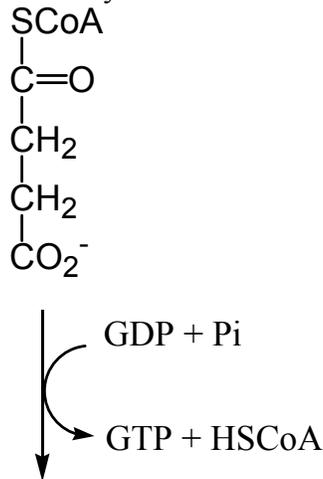
$\alpha$ -Ketoglutarate Dehydrogenase Complex of *E. coli*.

Enzyme	#	Prosthetic Groups	Reaction Catalyzed.
$\alpha$ -Ketoglutarate Dehydrogenase	E <sub>1</sub> 24	TPP	Oxidative Decarboxylation
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Dihydrolipoyl Dehydrogenase	E <sub>3</sub> 12	FAD	Regenerate lipoamide.

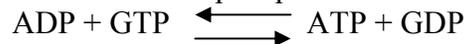
The mechanism for  $\alpha$ -Ketoglutarate dehydrogenase is identical to that of pyruvate dehydrogenase.

**V. Succinyl-CoA Synthetase**

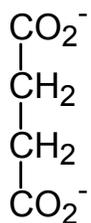
So far we have generated 2 molecules of CO<sub>2</sub> and 2 molecules of NADH. The electrons of NADH will be routed through the electron transport chain and ultimately generated 2.5 equivalents of ATP by oxidative phosphorylation. The succinyl-CoA contains a high energy bond which is going to be utilized in this step of the cycle.



The enzyme succinyl-CoA synthetase couples the conversion of succinyl-CoA into succinate with the synthesis of GTP from GDP and Pi. The standard free energy change for hydrolyzing succinyl-CoA into succinate and coenzyme A is -33.8 kJ/mole. The standard free energy required to synthesize GTP from GDP and Pi is +30.5 kJ/mole. If we couple these two reactions together than the standard free energy change is -3.3 kJ/mole. This enzyme catalyzes a substrate level phosphorylation to generate the only NTP produced directly in the citric acid cycle. The GTP produced is converted into ATP by the enzymatic activity of nucleoside diphosphate kinase:



For succinyl-CoA synthetase:

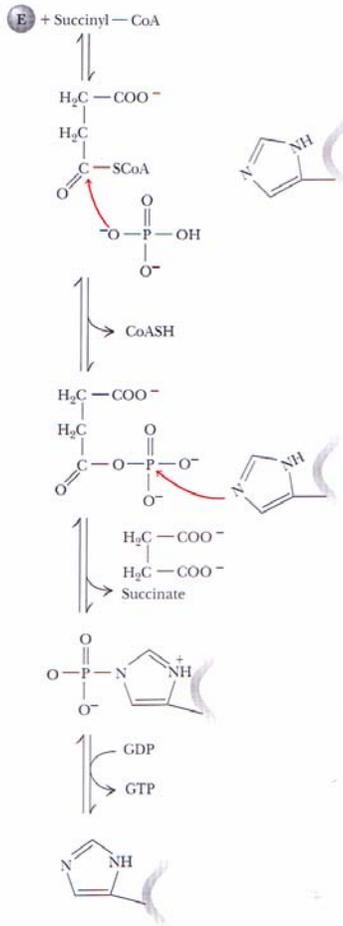


$$\Delta G^{\circ\prime} = -3.3 \text{ kJ/mol}$$

The change in free energy based on the steady state concentrations of all the reactants and products in the mitochondria of isolated from pig hearts is:

$$\Delta G \approx 0;$$

A near equilibrium reaction.



The mechanism for this enzyme is shown to the left.

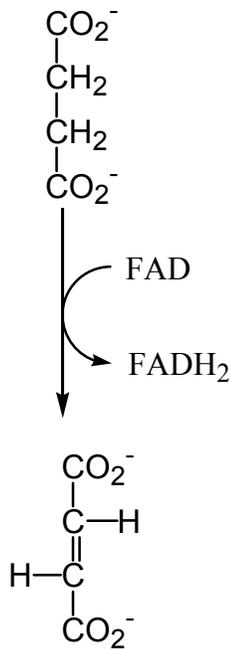
In the first step an enzyme bound inorganic phosphate group attacks the thioester to convert the high energy thioester bond into a high energy acyl-phosphate bond.

This enzyme bound succinyl-phosphate then undergoes nucleophilic attack by an active site histidine residue to form succinate and a phosphorylated histidine intermediate.

In the last step of this mechanism, GDP is bound and the phosphate group is transferred to GDP forming GTP.

The potential energy of the thioester bond has been conserved first by the formation of succinyl-phosphate, then by the formation of the phosphorylated histidine residue and finally by the substrate level phosphorylation of GDP to form GTP.

## VI. Succinate Dehydrogenase



The final stages of the citric acid cycle all have four carbon atoms. In the next step succinate dehydrogenase is going to oxidize succinate into fumarate.

Why FAD is the electron acceptor rather than NAD<sup>+</sup>?

The oxidation of an alkane is not sufficiently exergonic to reduce NAD<sup>+</sup>  $E^{\circ} = -0.315$ .

The oxidation of an alkane is sufficiently exergonic to reduce FAD.  $E^{\circ} = 0.0308$

Fumarate + 2e<sup>-</sup> + 2H<sup>+</sup> → Succinate  $E^{\circ} = 0.031$

$\Delta E^{\circ} = -0.0002 \text{ V} = \Delta G^{\circ} = +0.04 \text{ kJ/mol}$

The change in free energy based on the steady state concentrations of all the reactants and products in the mitochondria of isolated from pig hearts is:

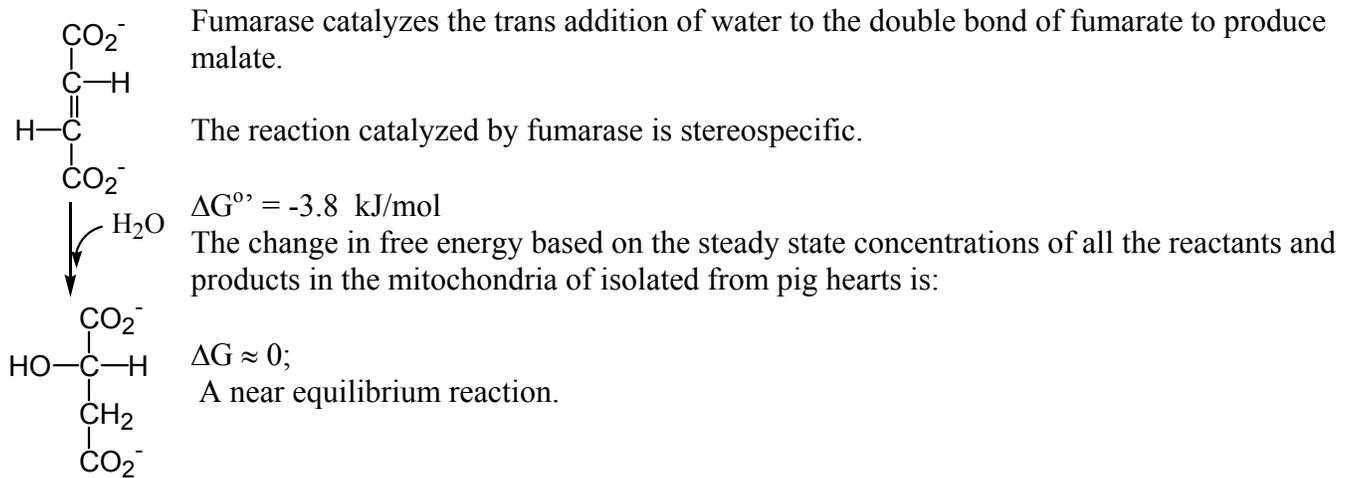
$\Delta G \approx 0$ ;

A near equilibrium reaction.

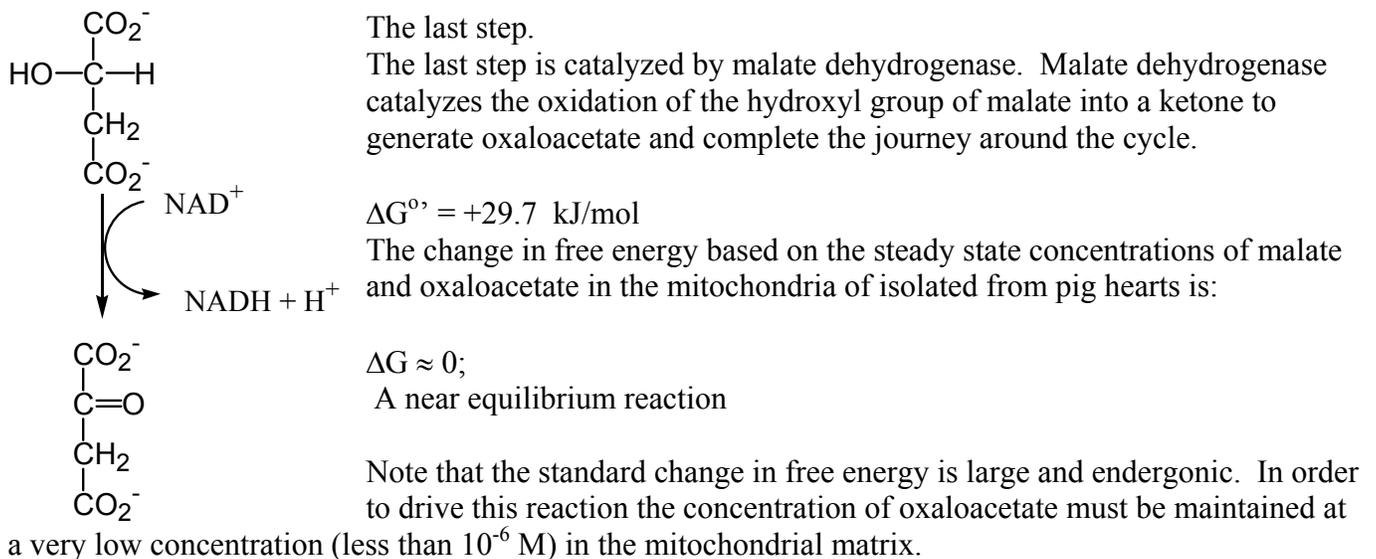
Succinate dehydrogenase is an integral membrane bound enzyme that is part of the electron transport chain. All of the other enzymes of the citric acid cycle are soluble proteins found in the mitochondrial matrix. Succinate dehydrogenase also contains three iron sulfur clusters as part of its electron transport chain. The electrons captured

by the FAD are passed to the iron sulfur clusters to coenzyme Q (quinone) to produce the reduced form QH<sub>2</sub> (dihydroquinone).

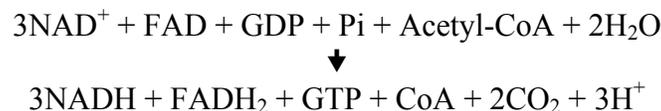
### VII. Fumarase



### VIII. Malate Dehydrogenase



### IX The Net reaction of the citric acid cycle is:



$\Delta G^{\circ\prime} = -40 \text{ kJ/mol}$

The change in free energy based on the steady state concentrations of all the reactants and products in the mitochondria of isolated from pig hearts is:

$\Delta G = -115 \text{ kJ/mol};$