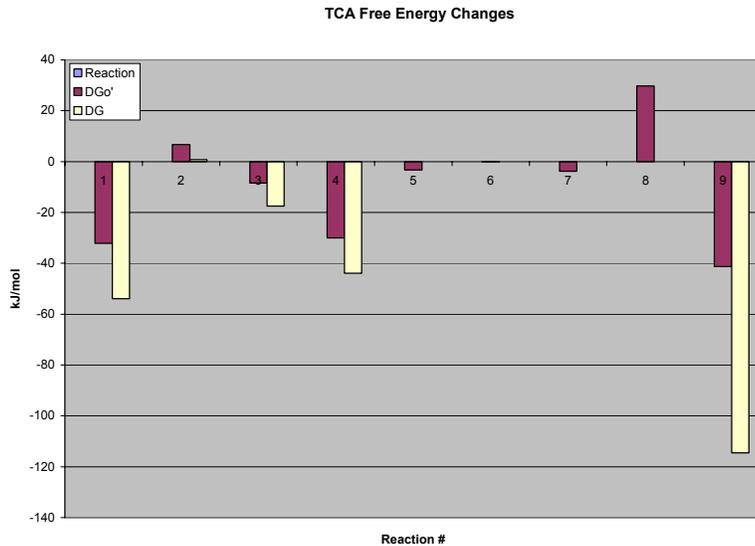


# Regulation of the Citric Acid Cycle

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## I. Changes in Free Energy



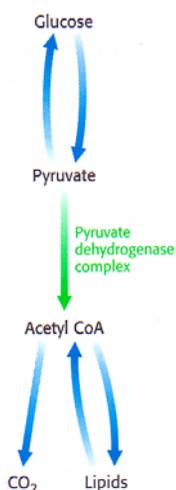
- 1.) Citrate Synthase
- 2.) Aconitase
- 3.) Isocitrate Dehydrogenase
- 4.)  $\alpha$ -Ketoglutarate Dehydrogenase
- 5.) Succinyl-CoA Synthetase
- 6.) Succinate Dehydrogenase
- 7.) Fumarase
- 8.) Malate Dehydrogenase
- 9.) Overall reaction

$\Delta G^{\circ'}$   
 $\Delta G$

3 Molecules of NADH and 1 molecule of FADH<sub>2</sub> are generated each turn of the Citric acid cycle. The eight electrons captured are transported by electron carriers to O<sub>2</sub> generating a proton gradient that drives the oxidative phosphorylation of ADP to generate ATP. The stoichiometry of electron transport and oxidative phosphorylation is 2.5 ATP per NADH and 1.5 ATP per FADH<sub>2</sub>. As a result 9 ATP are generated by electron transport-oxidative phosphorylation per turn of the cycle.

Molecular oxygen does not participate in the citric acid cycle. However, the cycle operates only under aerobic conditions because NAD<sup>+</sup> and FAD cannot be regenerated in the absence of oxygen. Glycolysis has an aerobic and anaerobic mode, but the citric acid cycle only operates under aerobic conditions.

## II. Regulation of Pyruvate Dehydrogenase



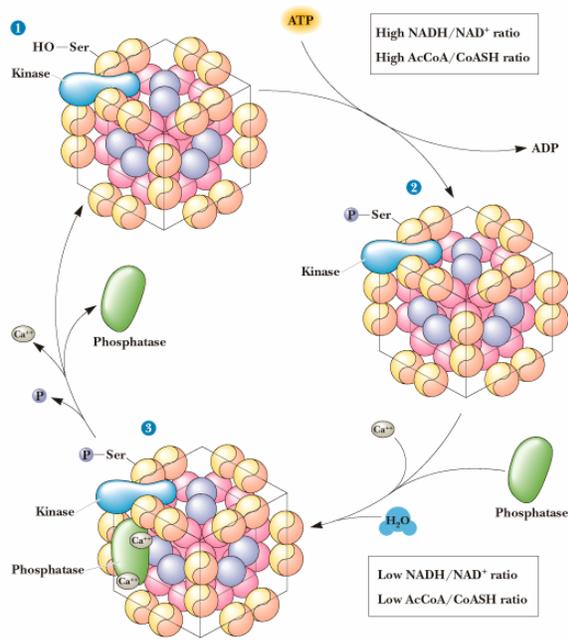
Pyruvate is an important metabolite. It is the product of glycolysis and a substrate for gluconeogenesis. Under anaerobic conditions, pyruvate is fermented into lactate or alcohol to regenerate NAD<sup>+</sup>. Under aerobic conditions pyruvate is converted into acetyl-CoA by pyruvate dehydrogenase. This is an irreversible step in animals. Animals are unable to directly convert acetyl-CoA back to pyruvate. The oxidative decarboxylation of pyruvate to form acetyl-CoA commits the acetyl group to either complete oxidation by the citric acid cycle into CO<sub>2</sub> or incorporation in fatty acids and lipids. This irreversible branch point is regulated by several means.

This enzyme is allosterically regulated.

High concentrations of acetyl-CoA inhibit the transacetylase component of E<sub>2</sub>.

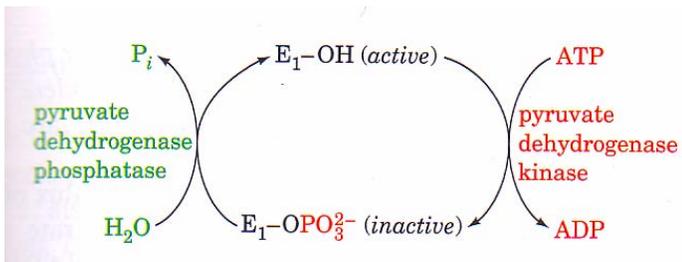
High concentrations of NADH inhibits the dihydrolipoyl dehydrogenase component of E<sub>3</sub>.

In Mammals, pyruvate dehydrogenase is regulated by phosphorylation by **pyruvate**



**dehydrogenase kinase** a regulatory enzyme that is part of the mammalian pyruvate dehydrogenase complex. Pyruvate dehydrogenase kinase is allosterically activated by NADH and acetyl-CoA such that when the mitochondrial matrix concentrations of these two effectors rises, they stimulate the phosphorylation of a serine residue on the pyruvate dehydrogenase E<sub>1</sub> subunit, which blocks the first step of catalysis, the decarboxylation of pyruvate. Pyruvate allosterically inhibits the kinase's activity.

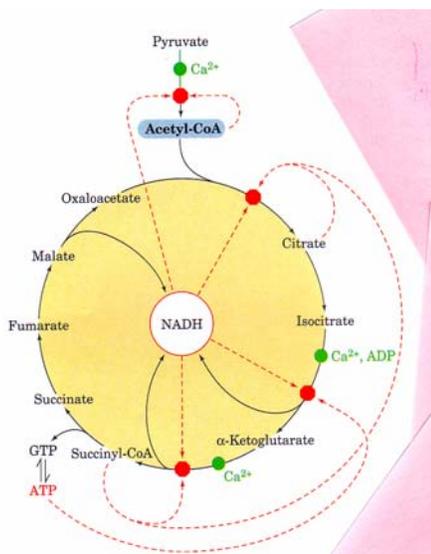
Eventually the mitochondrial matrix concentrations of NADH and acetyl-CoA will come down and the enzyme will need to be reactivated. The reactivation of this enzyme is accomplished by another enzyme, **pyruvate dehydrogenase phosphatase**, a Ca<sup>2+</sup> activated enzyme that binds to the dehydrogenase complex, hydrolyzes the phosphorylated serine, reactivating the enzyme. This phosphatase will remain associated with the dehydrogenase complex as long as the NADH/NAD<sup>+</sup> and the acetyl-CoA/CoA ratios remain low. High concentrations of NADH or acetyl-CoA inactivate the phosphatase and cause it to dissociate from the pyruvate dehydrogenase



complex. Insulin and Ca<sup>2+</sup> ions activate the phosphatase.

Important to note that the cAMP dependent phosphorylation cascade has no effect on the phosphorylation of pyruvate kinase. The cascade produced by insulin however does activate the phosphatase.

### III. Regulation of the Citric Acid Cycle



The citric acid cycle must be carefully regulated by the cell. If the citric acid cycle were permitted to run unchecked, large amounts of metabolic energy would be wasted in the over production of reduced coenzymes and ATP. Conversely if the citric acid cycle ran too slowly, ATP would not be generated fast enough to sustain the cell.

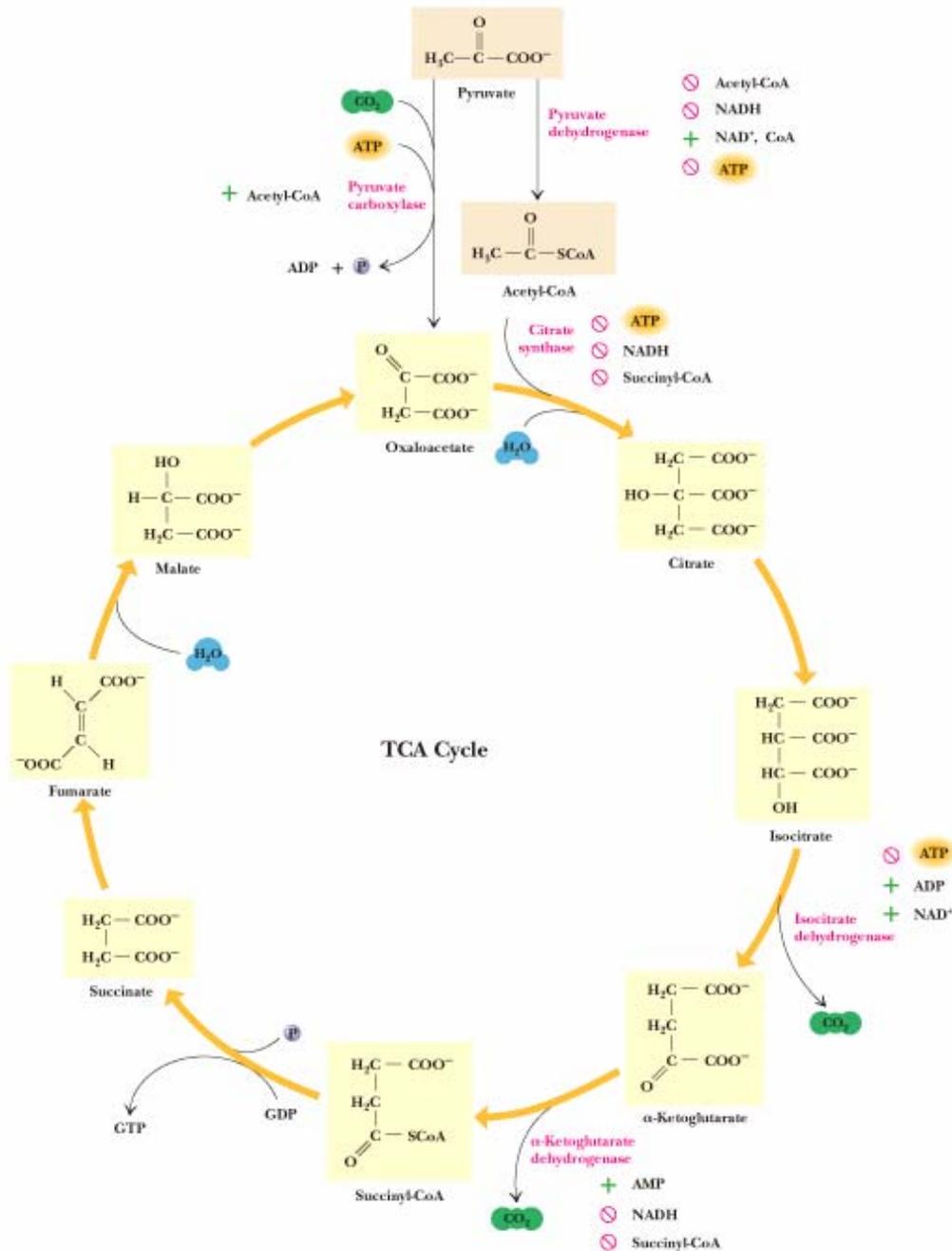
By looking at the changes in free energy of the reactions of the citric acid cycle, it is clear that there are three irreversible steps. These three reactions of the cycle, citrate synthase, isocitrate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase operate with large negative free energy changes under the concentrations of products and reactants in the matrix of the mitochondria.

Because the citric acid cycle is linked to oxygen consumption to regenerate NAD<sup>+</sup>, the citric acid cycle is regulated primarily by product feedback inhibition. Glycolysis and glycogen metabolism are

under complex systems of allosteric and hormonal control. The citric acid cycle in contrast is regulated by three simple mechanisms.

1. Substrate availability
2. Product inhibition
3. Competitive feedback inhibition.

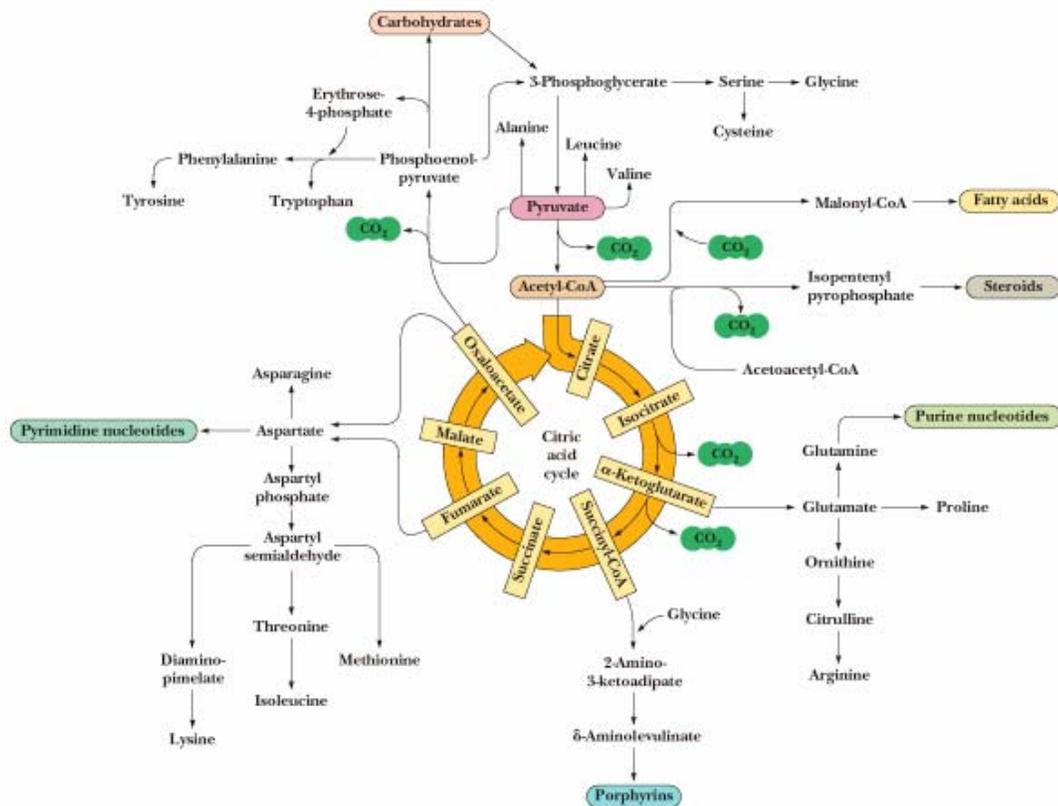
The allosteric effectors that control the flux of metabolites through the citric acid cycle are shown on the next page. The principle signals are acetyl-CoA, succinyl-CoA, ATP, ADP, AMP, NAD<sup>+</sup> and NADH. All of the regulatory enzymes of the citric acid cycle including pyruvate dehydrogenase are allosterically inhibited by NADH. The combination of the electron transport chain and oxidative phosphorylation produce ATP from NADH, consequently ATP is an allosteric inhibitor of pyruvate dehydrogenase and isocitrate dehydrogenase. The TCA cycle is turned on however by high ratios of either ADP/ATP or NAD<sup>+</sup>/NADH which indicate that the cell has run low of NADH or ATP.



#### IV. Citric Acid Cycle Intermediates Are Precursors for Biosynthetic Reactions.

It is easy to think of the citric acid cycle as a catabolic pathway oxidizing acetate into CO<sub>2</sub> and generating ATP. Many of the intermediates (α-ketoglutarate, succinyl-CoA, fumarate and oxaloacetate) produced in the citric acid cycle are precursors for important biomolecules. A Transamination reaction converts glutamate into α-ketoglutarate or vice versa. Glutamate is a precursor for the synthesis of other amino acids and purine nucleotides. Succinyl-CoA is a precursor for porphyrins. Oxaloacetate can be transaminated to form aspartate. Aspartate itself is a precursor for other amino acids and pyrimidine nucleotides. Oxaloacetate is a substrate for gluconeogenesis.

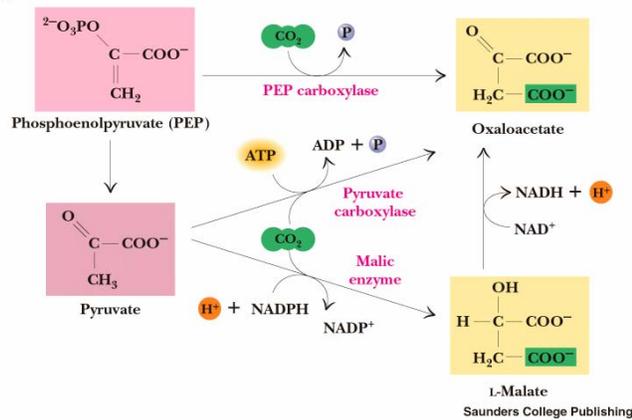
Garrett & Grisham: Biochemistry, 2/e  
Figure 20.22



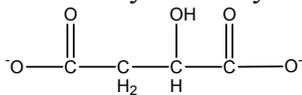
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Finally, citrate can be exported out of the mitochondria into the cytosol where it is broken down by ATP-citrate lyase to yield oxaloacetate and acetyl-CoA. The acetyl-CoA produced is a precursor for fatty acids. The oxaloacetate produced in this reaction is reduced to malate which can be either be transported back into the matrix of the mitochondria where it is reoxidized into oxaloacetate or malate can be oxidatively decarboxylated to pyruvate by malic enzyme which can then be transported back to the mitochondria.

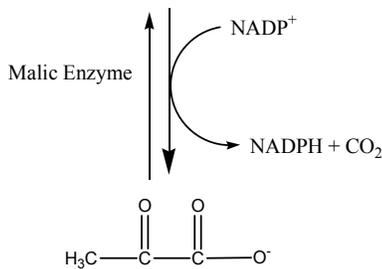




Malic enzyme catalyzes the following reversible reaction.



The malic enzyme is found in the cytosol or mitochondria of many plants and animals and is a  $\text{NADP}^+$  dependent enzyme.

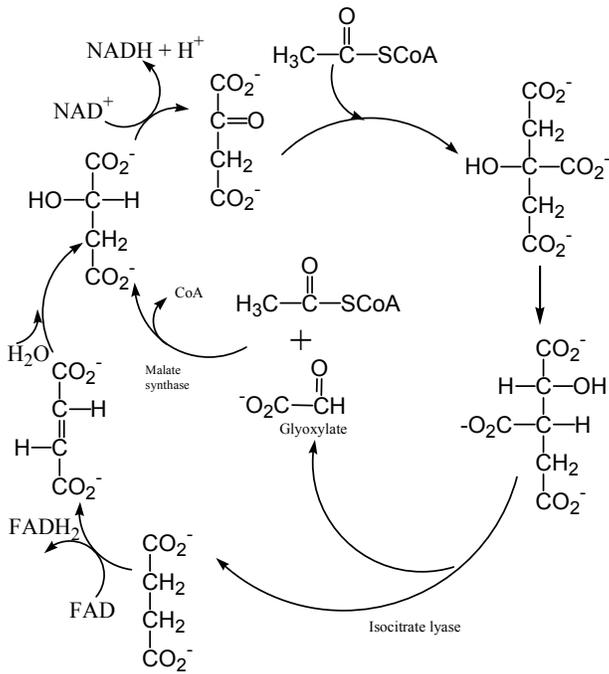


Other metabolites fed into the citric acid cycle are succinyl-CoA from the degradation of odd-chain fatty acids,  $\alpha$ -ketoglutarate and oxaloacetate from the transamination reactions of glutamate and aspartate.

## VI. The Glyoxylate Cycle of Plants, Yeast and Bacteria

Plants, fungi, algae, protozoans and bacteria can thrive on two carbon compounds such as acetate, ethanol and acetyl-CoA, as their sole carbon source. In the citric acid cycle, we have seen how acetyl-CoA is oxidized into 2 molecules of  $\text{CO}_2$  to generate ATP. There is no net synthesis in the TCA cycle since we end up with oxaloacetate, the exact same compound we began with. The TCA cycle has evolved to produce ATP from acetyl-CoA. This cycle cannot produce massive amounts of biosynthetic precursors needed for growth on acetate unless alternative reactions are available. In order to grow and thrive on acetate, the two  $\text{CO}_2$  producing reactions of the citric acid cycle need to be bypassed. The living organisms listed above can grow on acetate by employing a modification of the citric acid cycle called the **glyoxylate cycle** which takes two carbon compounds and converts them into four carbon compounds. This cycle bypasses the oxidative decarboxylations of the citric acid cycle by using two alternative enzymes. Isocitrate lyase and malate synthase. Isocitrate lyase cleaves isocitrate into glyoxylate and succinate. Malate synthase takes glyoxylate and condenses it with another acetyl-CoA to form Malate and CoA. The net effect is the preservation of carbon units using two molecules of acetyl-CoA to generate malate which is then converted by malate dehydrogenase into oxaloacetate which can be then converted into PEP and on through gluconeogenesis. The enzymes of the glyoxylate cycle are contained in glyoxysomes which are organelles devoted to this cycle. There are enzymes common to both the TCA and glyoxylate cycles as well as isozymes and functionally distinct enzymes allowing these two organelles to operate independently in these two cycles.

## Glyoxylate Cycle



The glyoxylate cycle allows seeds to grow in the dark where photosynthesis is impossible. Many seeds are rich in lipids allowing these organisms to degrade fatty acids to generate acetyl-CoA. The glyoxylate cycle then can take two of these acetyl-CoA's to generate oxaloacetate and from there other intermediates.

The glyoxysomes lack succinate dehydrogenase, fumarase and malate dehydrogenase. Consequently intermediates must be shuttle back and forth between the glyoxysome and the mitochondria. Succinate is transported to the mitochondria where it is converted into oxaloacetate. The transamination of aspartate is necessary because oxaloacetate cannot be transported out of the mitochondria. Aspartate then moves from the mitochondria back the glyoxysome where it is transaminated back to oxaloacetate and completes the shuttle.

