

# Lecture Summary Citric Acid II

1. The only substrate level phosphorylation in the citric acid cycle is catalyzed by succinyl-CoA synthetase. The products of this reaction in the citric acid cycle are GTP and succinate. Note that the enzyme is named for the reverse reaction.
2. Succinate dehydrogenase contains a covalently-linked FAD electron carrier. The  $\Delta G$  of zero allows the reaction to be readily reversed to produce succinate, when needed. The products of this reaction in the forward direction of the citric acid cycle are  $\text{FADH}_2$  and fumarate (trans double bond). This reaction is similar to the first oxidation reaction for a fatty acid.
3. Addition of water to fumarate (catalyzed by fumarase) yields L-malate.
4. Oxidation of L-malate by malate dehydrogenase yields NADH and oxaloacetate. This reaction is a rare oxidation reaction that is energetically unfavorable. Conversion of malate to oxaloacetate is the only energy "bump" to be gotten over in the citric acid cycle and that is readily accomplished thanks to the 'pulling' of the citrate synthase reaction, which keeps oxaloacetate concentrations low.
5. The citric acid cycle can be regulated allosterically in several places, *but the most important regulation of the cycle is probably the amount of  $\text{NAD}^+$  and FAD that is available*.  $\text{NAD}^+$  (and FAD) is essential for the cycle to operate and it is essential for the pyruvate dehydrogenase complex reaction to occur. This relates to metabolic control, as we shall see in discussions later of electron transport and oxidative phosphorylation.
6. When all of the NADHs and  $\text{FADH}_2$ s of the citric acid cycle are converted to ATP, the cycle yields 30- 38 ATPs per molecule of glucose (depending on how you count them - we'll talk about this later), compared to 2 for glycolysis (under anaerobic conditions). The citric acid cycle is thus an incredibly efficient producer of energy for the cell.
7. Many factors combine to regulate metabolism through the citric acid cycle. All of these ultimately come down to energy needs. Most are manifested through the availability or lack of  $\text{NAD}^+$ . When  $\text{NAD}^+$  is lacking (high NADH levels), the cycle will be inhibited. When  $\text{NAD}^+$  levels are high (low NADH levels), the cycle is favored. Oxygen is a limiting reagent needed to keep the citric acid cycle turning. This is because oxygen is required ultimately for the conversion of NADH back to  $\text{NAD}^+$ . Remember that  $\text{NAD}^+$  is required for three reactions of the citric acid cycle. If any one of these reactions is stopped, the cycle grinds to a halt. While glycolysis can use fermentation to get around conditions lacking oxygen, the citric acid cycle cannot.
8. The pyruvate dehydrogenase complex is regulated by substrate level regulation ( $\text{NAD}^+/\text{NADH}$ ) and allosteric regulation (ATP inactivates, AMP and ADP activate). The complex is also regulated by covalent modification - phosphorylation/dephosphorylation. The

relevant enzymes are a kinase, which phosphorylates pyruvate dehydrogenase (inactivating it) and a phosphatase, which dephosphorylates pyruvate dehydrogenase, activating it.

9. Pyruvate dehydrogenase is sensitive to arsenite and mercury, due to the fact that these compounds will react with the sulfur atoms in lipoamide of E3 of the pyruvate dehydrogenase complex. Treatment of lipoamide with BAL extracts arsenite from the lipoamide, due to the fact that BAL also contains sulfurs to which the arsenite binds.

10. Anaplerotic reactions are involved in "filling up" the intermediates of metabolism, which are needed for multiple purposes. For example, oxaloacetate may be used up in making aspartic acid and must be replenished. One mechanism for this may be conversion of glutamic acid into alpha ketoglutarate, with subsequent conversion to oxaloacetate. Citric acid cycle intermediates are involved in metabolism of amino acids, fatty acids, nucleotides, and sugars.

11. Oxaloacetate is important in many metabolic pathways. It can be converted to glucose in gluconeogenesis, to aspartate by transamination, and to citrate in the citric acid cycle. Both oxaloacetate and alpha ketoglutarate are important anaplerotic intermediates.

12. The glyoxylate cycle is a pathway related to the citric acid cycle that occurs in plants and bacteria. It requires two enzymes not found in animals (in addition to the normal enzymes of the citric acid cycle). The enzymes unique to the glyoxylate cycle are isocitrate lyase (catalyzes cleavage of isocitrate to glyoxylate and succinate) and malate synthase (catalyzes linkage of acetyl-CoA to glyoxylate to form malate).

13. Because of decarboxylation in the citric acid cycle of animals, no net synthesis of glucose can occur via the citric acid cycle in this organisms. On the other hand, plants and bacteria that have the enzymes of the glyoxylate cycle are able to form glucose in net amounts from acetyl-CoA because they can bypass the decarboxylation reactions of the citric acid cycle and convert acetyl-CoA into useful material.