
◉ Sheet ○ Slides

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| Subject: | TCA cycle |
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| Number: | 7 |

Minutes 00:00 – 7:30 is written in sheet #6

## Regulation of Citric Acid Cycle

-Regulation of Citric Acid Cycle depends on two major concepts:

1) NADH/NAD+ ratio

more NAD+ >>> cycle will be activated

more NADH >>> Cycle will be inhibited

The enzymes that are affected by this regulation method are the ones that produce NADH, which are isocitrate dehydrogenase, a-ketoglutarate dehydrogenase, and malate dehydrogenase. NADH will inhibit these enzymes by feedback inhibition.

2) ATP/ADP ratio

more ATP >>> cycle will be inhibited

more ADP >>> cycle will be activated

One enzyme is affected by this method, which is the isocitrate dehydrogenase (the rate-limiting enzyme of the cycle).

*Why isn't $FADH_2$ a regulator of the cycle?

Because it is always bound to a protein and it isn't free to activate or inhibit enzymes other than the one it is bound to.

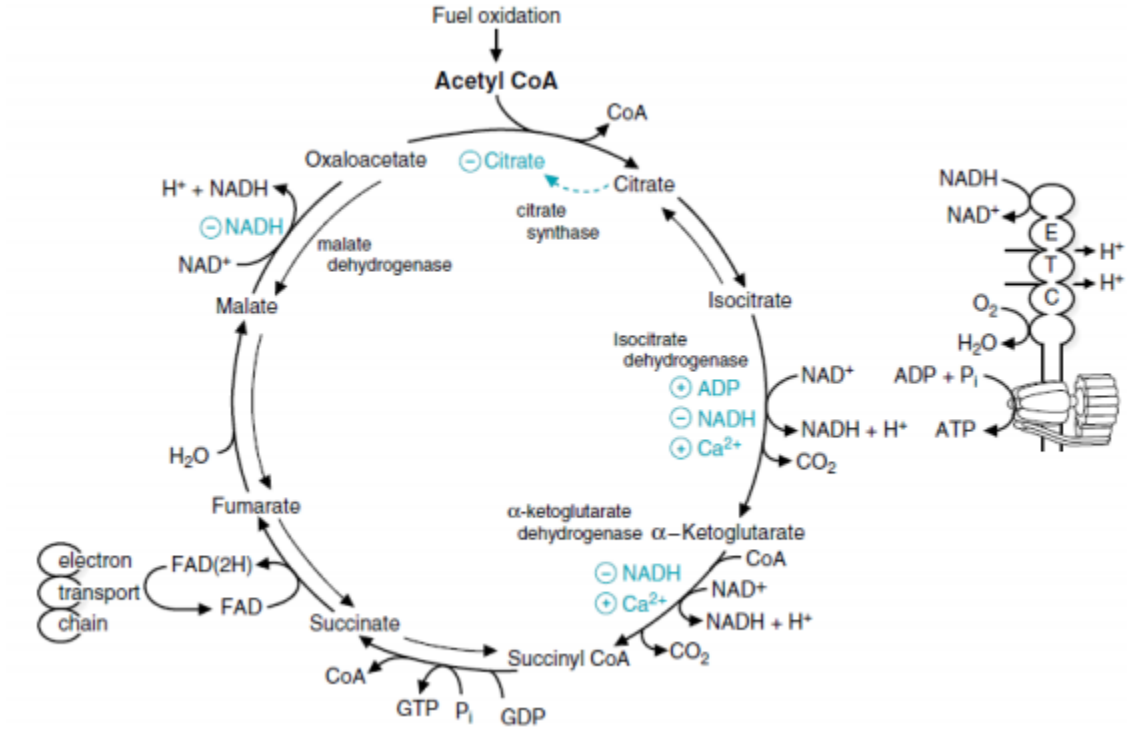
-All enzymes that have to do with energy production (a-ketoglutarate dehydrogenase, isocitrate dehydrogenase, pyruvate dehydrogenase, etc...) are activated by Calcium.

*Why Calcium?

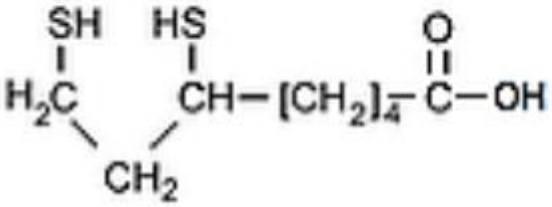
Because when muscles move, calcium concentrations increase and thus activate energy production enzymes to provide energy for the movement of the muscles.

-Another enzyme that is regulated is citrate synthase, because it is logical to have control on the first enzyme in a pathway, because you don't want to waste energy on intermediates you don't need. (feedback inhibition by citrate)

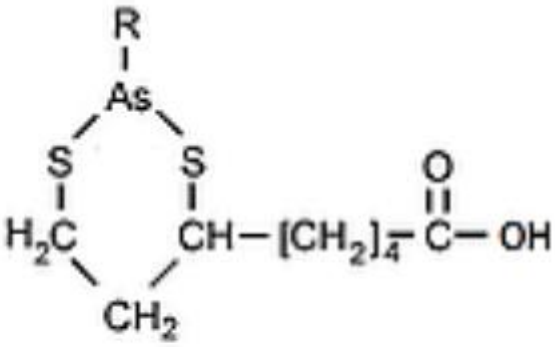
Ps: citrate synthase is not an allosteric enzyme like the others, but rather a simple enzyme that undergoes competitive inhibition. Also the equilibrium of the citrate-isocitrate is shifted towards the citrate, and the equilibrium of the malate-oxaloacetate is shifted towards the malate, so usually we have a low concentration of oxaloacetate and a high concentration of citrate. Which means high concentration of the product and low concentration of the reactant, so the reaction will be inhibited.




-Arsenic is highly toxic and fatal, because it binds to lipoic acid that is located in pyruvate dehydrogenase and a-ketoglutarate dehydrogenase. Lipoic acid is made up of 2 thiol groups or a disulfide bond. Arsenic forms bonds with the 2 sulfurs of the lipoic acid preventing them from making neither thiol groups or disulfide bridges, and accordingly neither the pyruvate dehydrogenase nor the a-ketoglutarate will be able to produce energy, and the citric acid cycle can no longer occur.



Dihydro lipoic acid



Arsenic-ringed lipoic acid

-Genetic deficiencies in different enzymes can occur. The most common deficiency in pyruvate dehydrogenase or a-ketoglutarate dehydrogenase is in enzyme #1 in the complex, it has no treatment and it is fatal.

## TCA cycle intermediates

-The intermediates of the citric acid cycle are not limited in their function to the citric acid cycle, and can participate in other processes, for example:

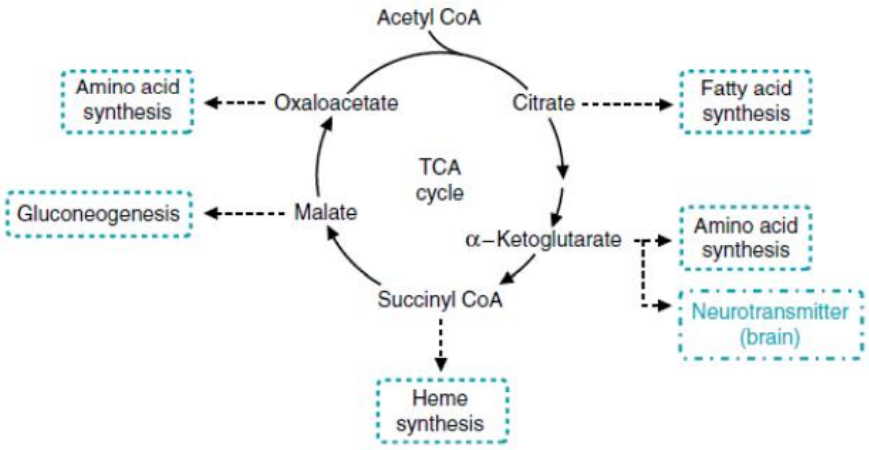
1)Citrate: when found in high concentrations moves to the cytosol and inhibits glycolysis, and participates in fatty acid and lipid biosynthesis.

2)a-ketoglutarate: when found in high concentrations it participates in amino acid synthesis (a-ketoglutarate ››› glutamate/glutamic acid), and neurotransmitter synthesis. (a-ketoglutarate ››› glutamate ››› gamma-aminobutyric acid/GABA)

3)Succinyl CoA can be converted to propionyl CoA and participate in heme synthesis.

4) Malate participates in gluconeogenesis, which is the formation of glucose from non-carbohydrate sources.

5)Oxaloacetate: amino acid synthesis (aspartic acid)




Minutes 7:30 - 17:38

## Anaplerotic Reactions

-When these intermediates are found in low concentrations, other reactions replenish their shortage, those interactions are called <u>anaplerotic reactions</u> (opposite of the 5 reactions mentioned above):

- ↑Glutamate ›› a-ketoglutarate
- ↑Aspartic acid ›› oxaloacetate
- Degradation of certain amino acids, heme products, fatty acids ›› propionyl CoA ›› succinyl CoA

-The most important anaplerotic enzyme is pyruvate carboxylase that requires biotin (as a Coenzyme) to produce oxaloacetate. Pyruvate has 3 carbons, pyruvate carboxylase adds carboxylic group to it forming oxaloacetate. Oxaloacetate deficiency occurs mostly in the liver and kidneys in which there are high rates of gluconeogenesis, so there are high concentrations of pyruvate carboxylase, why? ↑gluconeogenesis ›› ↓malate ›› ↓oxaloacetate, so we need more pyruvate carboxylase to make more oxaloacetate. This enzyme is activated by Acetyl CoA.

*Why acetyl CoA?

Because when we have more of it we need more oxaloacetate to bind to it, so it activates its production by pyruvate carboxylase.

## Oxidative phosphorylation

-Oxidative phosphorylation is the process of phosphorylation by oxidation-reduction reactions, to make ATP. It is the fourth and last stage of energy production.

-After the citric acid cycle, electrons are produced and then taken to the electron transport chain (the oxidative part, no ATP), which is coupled to ATP synthase (the phosphorylation part, production of ATP).

-Oxidative phosphorylation occurs in the mitochondria. Most energy processes occur in the matrix (inner mitochondrial membrane), except for glycolysis which occurs in the cytosol.

-The Mitochondria has two membranes:

1)Outer mitochondrial membrane: Permeable to small molecules (MW ≤ 5000), and ions and porins.

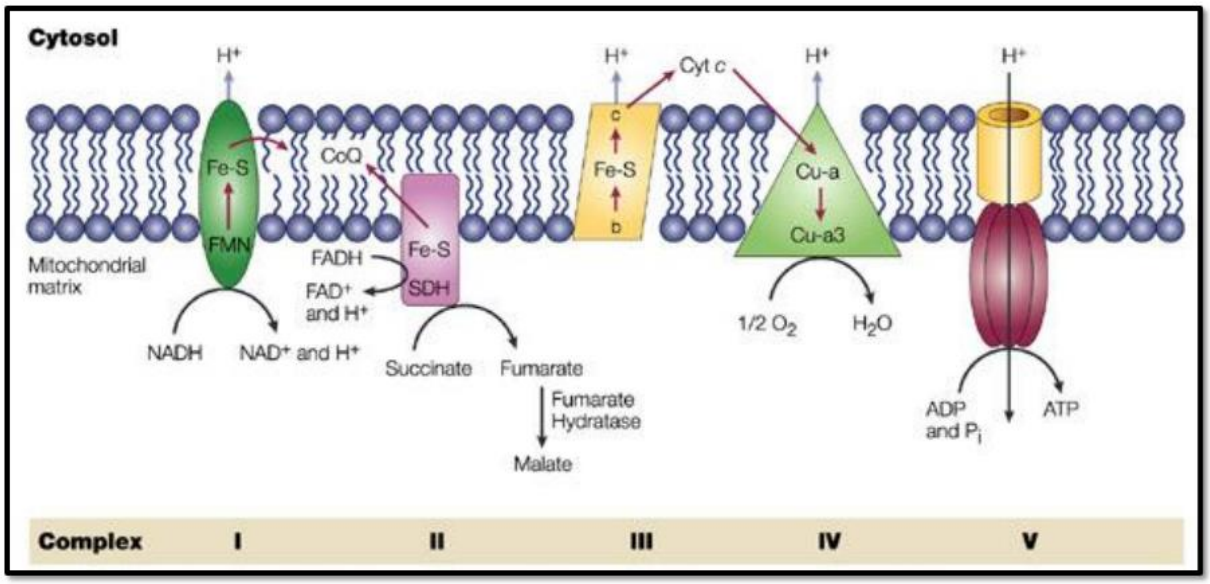
2)Inner mitochondrial membrane: impermeable to anything even H+, which means anything that is going to go across it needs a channel.

-Oxidative phosphorylation was explained by Peter Mitchell (1961) in 3 major aspects:

1) Flow of electrons (from the TCA cycle) through a chain of molecules that have the capacity to move them. The electrons move according to the reduction potential (from high energy state to lower energy state).

2) The energy drop caused by the movement of electrons is used to pump protons from the matrix to the intermembranous space (movement against their electrochemical gradient).

3) Flow of protons according to their electrochemical gradient through ATP synthase, to provide the energy needed for the synthesis of ATP by the direct phosphorylation of ADP.




Minutes 17:38 - 28:48

*What is the source of electrons for the Oxidative phosphorylation?

The Citric Acid cycle.

*What are the forms of the electrons coming from the citric acid cycle?

NADH and $FADH_2$

-Three enzymes make NADH, which then swims through the matrix until it reaches a transmembrane protein in the inner mitochondrial membrane, binds to its binding site and becomes NAD+. The enzyme that takes H+ from NADH is NADH dehydrogenase (or complex 1).

*What is the difference between NADH and $FADH_2$?

NADH is a free molecule, while $FADH_2$ is a bound molecule and can't possibly swim to the inner mitochondrial membrane. That's why the succinate dehydrogenase, in which $FADH_2$ is found, is part of the inner mitochondrial membrane because the only direct connection between the Krebs cycle and the oxidative phosphorylation is through succinate dehydrogenase, which is also called complex 2.

-Electrons from the Krebs cycle enter the electron transport chain through 2 points of entry: either complex 1 and the source is NADH, or through complex 2 and the source is $FADH_2$. (2 electrons in complex 1, and 2 in complex 2).

***There is NO direct connection between complex 1 and complex 2

-Electrons will move from complex 1 to 3, and from complex 2 to 3, and then from complex 3 to complex 4, in which oxygen is reduced to water.

*What does oxygen need to be reduced to water?

It needs electrons and protons.

*How do electrons move?

1) Direct electron movement in the Heme.

2) Move as Hydrogens in FADH2 and $FMNH_2$.

3) Move as hydride ions in NADH and NADPH.

*What moves electrons in the transport electron chain?

1) Cytochromes are proteins that have heme groups responsible for electron movement (heme moves 1 electron at a time). The mitochondria contain three classes of cytochromes (a, b and c). Proteins with FAD and FMN can be called cytochromes (moves 2 electrons at a time).

2) Iron-sulfur clusters (moves 1 electron at a time)

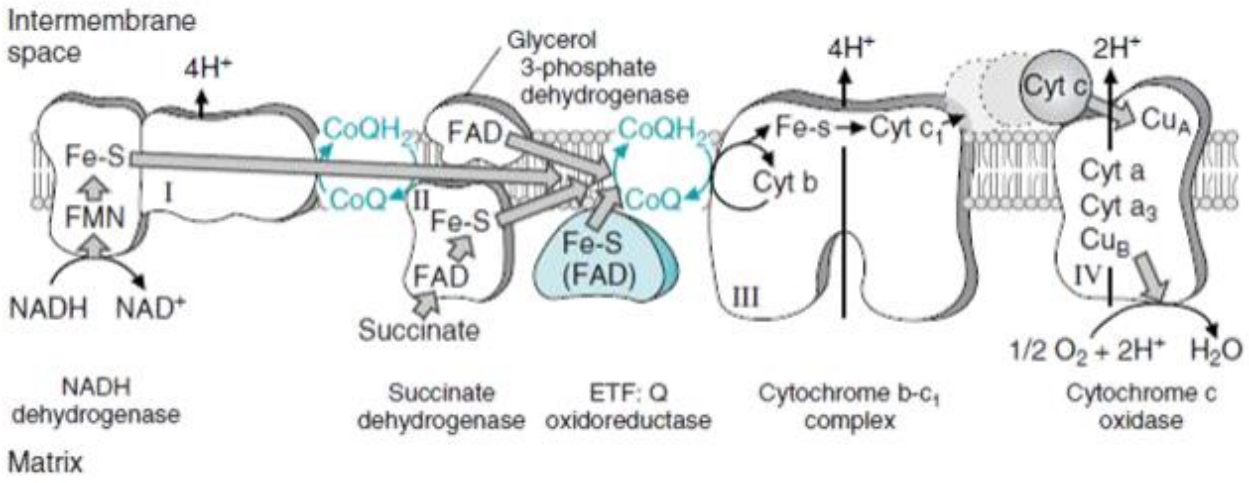
Minutes 28:48 - 38:51

-Complex 1 (NADH dehydrogenase) has FMN and Iron-sulfur clusters.

-Complex 2 (succinate dehydrogenase) has FAD and Iron-sulfur clusters

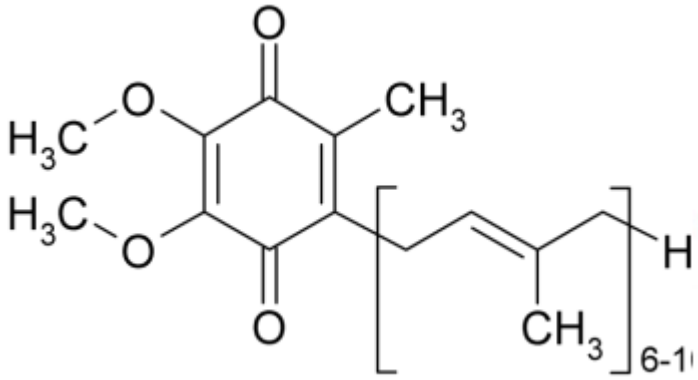
-Complex 3 (cytochrome b-c1 complex) has heme b, heme c, and Iron-sulfur clusters.

-Complex 4 (cytochrome c oxidase) has two coppers (a and b) and two hemes (A and A3).




-Proteins can't move in the membrane because they are water soluble proteins, so electrons need carriers to transport the electrons. The carrier which is needed to move electrons from complex 1 and 2 to complex 3 is called Coenzyme Q (ubiquinone), and from complex 3 to 4 is cytochrome C (peripheral to the membrane).

-Ubiquinone: Lipid-soluble benzoquinone with a long isoprenoid side chain (ring with 2 double bonds and an oxygen and long hydrocarbon chain that is replicated 6-10 times)




-The structure function relation:

1) the ketone groups receive electrons and get reduced to hydroxyl groups, for electron transport. It can take 2 electrons (CoQ becomes CoQH, $CoQH_2$)

2)Long Hydrocarbon chain (isoprenoid chain): makes the molecule lipid soluble so it can move through the membrane.

-Ubiquinone is commercially available, and is prescribed for recovering Myocardial infarction patients, to speed up the electron movement, and in turn increase ATP concentrations.

-cytochrome C: a peripheral protein that moves electrons from complex 3 to complex 4. It contains heme c which has the ability of transferring electrons (1 electron at a time), so to move the 2 electrons coming from complex 3 to 4 we need 2 successive molecules of cytochrome C.

-complex 4 contains heme and copper. Why heme?? Because reduced heme (ferrous/Fe+2) can bind to oxygen. Electrons move to complex 4 reducing heme which then binds $O_2$ reducing it to water.

Minutes 38:51 – 46:21

Sorry for any mistakes. ✌