**Lec 32: Citric Acid Cycle & Fatty Acid Oxidation.**

Expectations:

i) Input

ii) Output

iii) Location

iv) Energy Gen Steps

v) Regulation

**General Features;**

* Also known as the TCA cycle (tricarboxylic acid) or the Krebs cycle.
* The enzymes that participate in the citric acid cycle are found in the **mitochondrial matrix.**
* **Catabolic role**: Amino acids, fats, and sugars enter the TCA cycle to produce energy. **Acetyl CoA** is a central intermediate
* **Anabolic role**: TCA cycle provides starting material for fats and amino acids. Note: carbohydrates cannot be synthesized from acetyl-CoA by humans. Pyruvate→Acetyl CoA is one way!
* In contrast to glycolysis, none of the intermediates are phosphorylated; but all are either di- or tricarboxylic acids.
* Regulation is largely by sensing energy levels.

**1: Overall Carbon Flow:**

All of the carbons that are input as **pyruvate** are released as **CO2**. This is as highly oxidized as carbon can get. Each time a CO2 is produced one NADH is produced. This reaction is called oxidative decarboxylation.

*Locations of CO2 release:*

1. Pyruvate Dehydrogenase: Pyruvate to Acetyl-CoA
2. Isocitrate dehydrogenase: Isocitrate to α-ketoglutarate
3. α-ketoglutarate dehydrogenase:α-ketoglutarate to succinyl-CoA

The largest change in the carbon structure occurs at step 1: The citrate synthase reaction catalyzes the following reaction:

 C2 (acetate) + C4 (oxaloacetate) → C6 (Citrate). Subsequent reactions remove two carbons from citrate to generate the C4 compound. Note that these carbons are not the same as those added.

**2. Energetics of the TCA Cycle:**

In contrast to glycolysis, most of the energetic currency is in the form of redox reactions, only a single ATP is produced/pyruvate while four NADH and one FADH2 are produced. Most of the energy from oxidation is of glucose is harvested in the TCA cycle. The TCA cycle is a slower but richer source of energy.

**2a: Oxidative decarboxylations:** These occur at three locations, leading to the loss of the three carbons from pyruvate.

1. Pyruvate dehydrogenase
2. Isocitrate dehydrogenase
3. α-ketoglutarate dehydrogenase

**Pyruvate dehydrogenase**: This reaction, as with α-ketoglutarate dehydrogense, produces a thio-ester as the oxidized species. The reaction proceeds intwo steps, loss of the CO2 group, followed by oxidation of the aldehyde and formation of the thio-ester (The latter step is equivalent to the scheme for oxidation of glyceraldehyde-3-P in glycolysis, but occurs by a different mechanism here.)

The thio-ester is formed between the oxidized product and Coenzyme A, to form acetyl-CoA. The thio-ester is the same oxidation state as a carboxylate.

**Thioesters in Biochemical Reactions:**

i) The relatively weak thioester bond facilitates the transfer of the acetyl group to other compounds, such as transfer of the acetyl group to oxaloacetate in the **citrate synthase** reaction.

ii) Acetyl-CoA or succinyl CoA are high energy compounds, capable of driving the synthesis of GTP, such as in the succinyl CoA synthetase in the citric acid cycle. GTP can be converted to ATP at no energy cost.



**2b.** The remaining section of the pathway, from **succinate** to **oxaloacetate** follows a classic three step oxidation scheme that is also seen in fatty acid oxidation:

***Alkane → Alkene → Alcohol → Ketone***

***REDOX REDOX***

1. Oxidation of succinate to fumarate reduces FAD to FADH2. ***Alkane → Alkene***

2. Addition of water to the double bond, to make the alcohol. ***Alkene → Alcohol***

3. Oxidation of Malate to Oxaloacetate reduces NAD+ to NADH. ***Alcohol → Ketone***

**3. Regulation of the TCA Cycle:**

1. High energy, irreversible steps are regulated.
2. Regulated reactions are at the "top" of the pathway.

Examples of:

1. Product Inhibition.
2. Allosteric inhibition by feedback inhibition by products 'down stream' in the pathway.

**Energy sensing is the most important regulatory control of the TCA cycle (I=inhibited)**

|  |  |  |
| --- | --- | --- |
|  | **High Energy** | **OTHER** |
| **Step** | **NADH** | **ATP** | **Compound** | **Prod. Inh** | **Feedback Inh** |
| Pyruvate Dehydrogenase | **I** | **I** | * Inhibited by Acetyl Co-A
 |  |  |
| Citrate Synthase | **I** | **I** | * Inh. by succinyl-CoA
 |  |  |
| * Inhibited by citrate
 |  |  |

**Role of citrate in regulation of glycolysis**: Citrate stabilizes the tense-form of PFK, shutting down glycolysis.

**Fatty Acid Oxidation (β-Oxidation):**

**A. Formation of Acyl-CoA (Cytosol):** The fatty acids in the cytosol are coupled to coenzyme A to form acyl-CoA. The activation reaction is catalyzed by *acyl-CoA synthetase* and involves the hydrolysis of ATP to AMP, i.e. the equivalent of two high energy ATP molecules (60 kJ/mol). The released pyrophosphate is hydrolyzed to inorganic phosphate, making the overall ΔG negative for the rection (indirect coupling) **Note:** it is only necessary to utilize ATP once in the activation of the fatty acid.

**B. Transport into mitochondria:** The acyl-CoA is transported into the mitochondrial matrix for oxidation. This location is ideal for funneling the products of β-oxidation (NADH and FADH2) to E. transport.

**C. β-Oxidation** (**Mito. matrix):**

***Alkane → Alkene → Alcohol → Ketone***

***REDOX REDOX***

Acyl-CoA is shortened **2 carbons at a time** from the carboxyl end of the fatty acid using the following steps:

1. Formation of trans α-β double bond by **acyl-CoA dehydrogenase**, an FAD enzyme.

2. Addition of water to the newly formed double bond to generate the alcohol by **enoyl-CoA hydratase**

3. Oxidation of the alcohol by NAD+ to give the ketone, catalyzed by **3-L-hydroxyacyl-CoA dehydrogenase.**

4. Cleavage reaction by **β-ketoacy-CoA thiolase** (thiolysis), generates acetyl-CoA and an acyl-CoA that is two carbons shorter. The acetyl-CoA enters the TCA cycle.

5. Steps 1-4 are repeated until only acetyl-CoA remains.

