**Lecture 32: Citric Acid Cycle & Fatty Acid Metabolism.**

**Expectations for Citric Acid Cycle:**

i) Input - pyruvate

ii) Output CO2, NADH, FADH2, GTP

iii) Location mitochondrial matrix

iv) Energy Generating Steps- oxidative decarboxylations

v) Regulation – energy sensing (NADH, ATP).

vi) Biosynthesis of amino acids.

**Features of Citric Acid Cycle:**

* 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:*

* Pyruvate Dehydrogenase: Pyruvate to acetyl-CoA
* Isocitrate dehydrogenase: Isocitrate to α-ketoglutarate
* α-ketoglutarate dehydrogenase:α-ketoglutarate to succinyl-CoA

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

 **C2 (acetate) + C4 (oxaloacetate) → C6 (Citrate)**

Subsequent reactions remove two carbons from citrate to generate the C4 compound, oxaloacetate at the end of the cycle.

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

* Most of the energetic currency is in the form of redox reactions, only a single ATP (GTP) 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 (step 0)
2. Isocitrate dehydrogenase (step 3)
3. α-ketoglutarate dehydrogenase (step 4)

**Pyruvate dehydrogenase (decarboxylase)**(Step 0)

1. loss of the CO2 group.

2. oxidation of the aldehyde and formation of the thio-ester. (The thio-ester is the same oxidation state as a carboxylate.)

The thio-ester is formed between the oxidized product and Coenzyme A, to form acetyl-CoA.

**Thioesters in Biochemical Reactions:** The relatively weak thioester bond facilitates the transfer of the attached group to other compounds.

**i) Citrate synthase mechanism** (Step 1).

Asp-375 – general base His-274 – general acid

A) proton abstraction by Asp375, proton donation by His274

B) nucleophilic attack of –ene to C=O on oxaloacetate.

C) hydrolysis of thioester.

**ii) Succinate thiokinase** (Step 5): succinyl CoA can provide enough energy to driving the synthesis of GTP.

A) phosphorlysis of thio-CoA ester.

B) Transfer of phosphate to His

C) Transfer from phosphoryl-His to GDP, forming GTP.

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

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

***REDOX REDOX***

Step 6. Oxidation of succinate to fumarate reduces FAD to FADH2. ***Alkane → Alkene***

Step 7. Addition of water to the double bond, to make the alcohol. ***Alkene → Alcohol***

Step 8. Oxidation of Malate to Oxaloacetate reduces NAD+ to NADH. ***Alcohol → Ketone***

**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 'downstream' in the pathway.

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

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

**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 reaction (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 -ideal for funneling the products of β-oxidation (NADH and FADH2) to E. transport.

**C. β-Oxidation** (**Mito. matrix):** 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. The last cycle produces two acetylCoA.



**Fatty Acid Synthesis:** Occurs in the **cytosol** using acetyl CoA (derived from citrate), elongating by 2 carbons at a time, each pair of Cs is added from malonyl-CoA. Electron **donor** is NADPH.

