Citric Acid Cycle

Pratt & Cornely, Ch 14

Overview

• Compartmentalization
  – Glycolysis: Cytosol
  – Citric Acid Cycle: mitochondria
Overview

- Glycolysis
- Pyruvate dehydrogenase complex
  - Commitment of carbon away from carbohydrates
- Citric acid cycle

Pyruvate Dehydrogenase Complex

\[
\begin{align*}
\text{Pyruvate} & \quad + \quad \text{HS-CoA} \quad + \quad \text{NAD}^+ \\
\text{Acetyl CoA} & \quad + \quad \text{CO}_2 \quad + \quad \text{NADH}
\end{align*}
\]

- Three distinct enzymes—in a massive complex
- Five chemical steps
- What cofactors needed?
Pyruvate Dehydrogenase (E₁)

- TPP cofactor: Decarboxylation of α carboxyketones
- Stabilization of “acyl anion”
- Draw mechanism of decarboxylation
Dihydrolipoamide Acyltransferase (E2)

- Transfer catalyzed by E1
- Serves as an linker to “swing” substrate through subunits
- Mechanism of redox
Step 3: transfer

- Maintenance of high energy bond
- Acetyl CoA product is made
- Lipoamide still reduced—not catalytically viable at this point

\[
\begin{align*}
\text{Acetyl-dihydrolipoamide} & \quad + \quad \text{HS-CoA} \quad \rightarrow \quad \text{Dihydrolipoamide} & \quad + \quad \text{Acetyl CoA}
\end{align*}
\]

Dihydrolipoamide dehydrogenase (E3)

- Redox of prosthetic FAD/FADH₂
- Still not a regenerated catalyst!
**Step 5: NADH produced**

- Prosthetic group is restored by NAD⁺
- Step 1 uses proton, step 5 regenerates
- Oxidation energy of one carbon atom used to
  - Produce high energy thioester
  - Produce NADH

**Overall Reaction**

[Diagram of metabolic pathway involving pyruvate, TDP, dihydrolipoamide acyltransferase, NAD⁺, and NADH]
Problem 6

- Using pyruvate DH as an example, propose a mechanism for the TPP dependent yeast pyruvate decarboxylate reaction in alcohol fermentation.

Fate of Acetyl CoA

- Storage of energy as fatty acid
- ATP production (harvest of high potential electrons)
- Formal reaction:

\[
\text{C} = \text{O} + 2 \text{H}_2\text{O} + \text{OH}^- \rightarrow 2 \text{CO}_2 + \text{HS-CoA} + 7 \text{H}^+ + 8\text{e}^- 
\]
Citric Acid Cycle

- Cyclic pathway
  - CO₂ production
- Substrate level NTP
- NADH stores high energy electrons
  - Oxidation of alcohol or oxidative decarboxylation
- QH₂ stores high energy electrons
  - Alkane reduction to π bond

Overview
Carbon Flow

- Each cycle is **net** oxidation of acetyl CoA
  - Not actual loss of carbon from acetyl CoA
- C-13 incorporation experiments
- 4-carbon compounds act “catalytically” in oxygen consumption
  - Cyclic pathway!

1. Citrate Synthase

- Highly exothermic—lysis of high energy bond
- Used to drive reaction in presence of small [oxaloacetate]
2. Aconitase

- Citrate is achiral and prochiral
- Green represents carbon from acetyl CoA
  - How can enzyme distinguish prochirality?
Prochirality

- Only one compound produced

3. Isocitrate Dehydrogenase

- Oxidative decarboxylation
- Spontaneous in β-ketoacids
- NADH
- α-ketoglutarate is a key intermediate
4. \(\alpha\)-Ketoglutarate Dehydrogenase Complex

- Analogous to pyruvate dehydrogenase complex
- Second decarboxylation, but this is \(\alpha\)-decarboxylation
- High energy bond retained

Problem 28

- A patient with a aKG DH deficiency exhibits a small increase in [pyruvate] and a large increase in [lactate] so that the [lactate]/[pyruvate] ratio is many times larger than normal. Explain.
5. Succinyl CoA Synthetase

- Synthetase means ATP (GTP) involved
- High energy bond used to do substrate-level phosphorylation
  - Good leaving group to activate Pi
  - Covalent catalysis
  - GDP $\rightarrow$ GTP

\[
\text{Succinyl CoA} + \text{P}_i + \text{GDP} \rightarrow \text{Succinate} + \text{HS-CoA} + \text{GTP}
\]
Notice: symmetrical Product! We lose track of which carbons are from acetyl CoA!

6. Succinate Dehydrogenase

- Oxidation to form C=C releases less energy
- FAD is bound redox reagent
- In turn, Q is reduced
• Q is membrane bound cofactor—revisit in chapter 15!

7. Fumarase

• Another prochiral molecule—makes L-malate
• Hydration reaction sets up another oxidation
8. Malate Dehydrogenase

- Large standard free energy
- Driven by low [oxaloacetate]
  - Coupled back to reaction #1

![Malate Dehydrogenase Reaction](image)

Carbon Flow

- Practice C-14 labeling problems using this basic chart

![Carbon Flow Chart](image)
ATP Harvest: By Enzyme

Table 13.2 Energy production in the citric acid cycle

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Energy-yielding product</th>
<th>ATP equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>NADH</td>
<td>2.5</td>
</tr>
<tr>
<td>α-Ketoglutarate dehydrogenase complex</td>
<td>NADH</td>
<td>2.5</td>
</tr>
<tr>
<td>Succinyl-CoA synthetase</td>
<td>GTP or ATP</td>
<td>1.0</td>
</tr>
<tr>
<td>Succinate dehydrogenase complex</td>
<td>QH$_2$</td>
<td>1.5</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>NADH</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>10.0</strong></td>
</tr>
</tbody>
</table>

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![Diagram of ATP production from glucose to CO$_2$](image)
Net ATP Harvest from Glucose

- Glycolysis = 5-7 ATP
  - 3 or 5 ATP from cytosolic NADH
  - In humans, cytosolic NADH transport costs 2 ATP equivalents
- Pyruvate DH = 5 ATP
- Citric Acid Cycle = 20 ATP
- Total: 30 ATP/glucose in humans

Regulation

- Flux is generated through three irreversible steps
- NADH inhibits
- Citrate: product inhibition
- Succinyl CoA is last irreversible product—feedback inhibition
- Ca^{2+}: hormone mediated signal for need for energy
- ADP: need of energy
Anabolic Roles for CAC

- Intermediates can be used for building
  - Amino acids
    - aKG $\rightarrow$ glutamate
  - Gluconeogenesis
    - Through oxaloacetate
    - Glucogenic amino acid
    - Acetyl CoA and ketogenic precursors cannot be used to make net glucose
  - Fatty acids
    - Require transport of citrate

Citrate Transport System

- Fatty acid biosynthesis happens in the cytosol
- Acetyl-CoA cannot get across the mitochondrial membrane
- At cost of 2 ATP, acetyl-CoA gets across membrane in citrate form
Anaplerotic Reactions

- Replenish CAC intermediates
- “Filling up” reactions
  - Enhanced aerobic respiration (increase flux)
  - Gluconeogenesis pathway
- Key Reaction: Formation of oxaloacetate by pyruvate carboxylase
- Some amino acids can also serve if in high concentration

Problem 42

- Why is the activation of pyruvate carboxylase by acetyl-CoA a good regulatory strategy?
Pyruvate Stimulates CAC

- Some amino acids boost flux by making more CAC intermediates
- Transamination
- High [pyruvate] at beginning of glycolysis boosts flux through CAC

Glyoxylate Pathway

- Makes acetyl-CoA into oxaloacetate in non-cycle
- Allows plants (seeds) to use stored fat to make net glucose
- At expense of bypassing oxidation reactions (NADH production)
Problem 55

- Animals lack a glyoxylate pathway and cannot convert fats to carbohydrates. If an animal is fed a fatty acid with all its carbons replaced by C-14, some of the labeled carbons later appear in glucose. How is this possible?