Citric Acid Cycle

Chapter 19
Stryer Short Course

Overview of Cycle

• Oxidation of acetyl group
  – Production of ATP
  – High energy electrons for ATP

Fate of Acetyl CoA

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

\[
\begin{align*}
\text{S-CoA} & \\
\text{C} = \text{O} + 2 \text{H}_2\text{O} + \text{OH}^{\ominus} & \rightarrow 2 \text{CO}_2 + \text{HS-CoA} + 7 \text{H}^{\ominus} + 8\text{e}^{\ominus} \\
\text{CH}_3 & \\
\end{align*}
\]
Where we are going...

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

Stage 2: regeneration
Stage 1: Oxidation

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

- Overall purpose: oxidative decarboxylation
- This step: put hydroxyl in correct position

3. Isocitrate Dehydrogenase

- Oxidative decarboxylation
- Spontaneous in β-ketoacids
- NADH production
- α-ketoglutarate is a key intermediate
4. α-Ketoglutarate Dehydrogenase Complex

- Analogous to pyruvate dehydrogenase complex
- Second decarboxylation, but this is α-decarboxylation
- Forms NADH and high energy bond

![α-Ketoglutarate to Succinyl CoA](image)

Carbon Review

![Carbon cycle](image)

5. Succinyl CoA Synthetase

- Synthetase means ATP (GTP) involved
- High energy bond used to do substrate-level phosphorylation

![Succinyl CoA Synthetase](image)
Reactions 6-8

- Common motif
- DH and cofactors

6. Succinate Dehydrogenase

- Oxidation to form C=C releases less energy
- FAD is bound redox reagent
7. Fumarase

- Hydration reaction sets up another oxidation

\[ \text{Fumarate} + \text{H}_2\text{O} \rightarrow \text{Malate} \]

8. Malate Dehydrogenase

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

\[ \text{NAD}^+ + \text{NADH} + \text{H}^+ \rightarrow \text{NAD}^+ \]

Recognize Reaction Type

<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction</th>
<th>Enzyme</th>
<th>Prosthetic group</th>
<th>Type</th>
<th>ΔG° (kJ/mol)</th>
<th>ΔG° (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pyruvate + NADH + H+ → Lactate + NAD+</td>
<td>Lactate dehydrogenase</td>
<td>NADH</td>
<td>559</td>
<td>-23.5</td>
<td>-31.4</td>
</tr>
<tr>
<td>2</td>
<td>Lactate + NAD+ → Pyruvate + NADH + H+</td>
<td>Lactate dehydrogenase</td>
<td>NADH</td>
<td>-12.4</td>
<td>-7.3</td>
<td>-7.3</td>
</tr>
<tr>
<td>3</td>
<td>Citrate + NADH + H+ → Isocitrate + NAD+</td>
<td>Isocitrate dehydrogenase</td>
<td>NADH</td>
<td>-40.1</td>
<td>-7.3</td>
<td>-7.3</td>
</tr>
<tr>
<td>4</td>
<td>Isocitrate + NADH + H+ → α-Ketoglutarate + NAD+</td>
<td>α-Ketoglutarate dehydrogenase</td>
<td>NADH</td>
<td>-17.3</td>
<td>-17.3</td>
<td>-17.3</td>
</tr>
<tr>
<td>5</td>
<td>α-Ketoglutarate + NADH + H+ → Glutamate + NAD+</td>
<td>Glutamate dehydrogenase</td>
<td>NADH</td>
<td>-5.9</td>
<td>-5.9</td>
<td>-5.9</td>
</tr>
<tr>
<td>6</td>
<td>Glutamate + NADH + H+ → Citrate + NAD+</td>
<td>Glutamate dehydrogenase</td>
<td>NADH</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>7</td>
<td>Pyruvate + NADH + H+ → Malate + NAD+</td>
<td>Pyruvate dehydrogenase</td>
<td>NADH</td>
<td>-2.9</td>
<td>-2.9</td>
<td>-2.9</td>
</tr>
<tr>
<td>8</td>
<td>Malate + NADH + H+ → Pyruvate + NAD+</td>
<td>Malate dehydrogenase</td>
<td>NADH</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Overview

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>Lactate dehydrogenase</td>
<td>NADH</td>
<td>2.5</td>
</tr>
<tr>
<td>α-Ketoglutarate dehydrogenase</td>
<td>NADH</td>
<td>2.5</td>
</tr>
<tr>
<td>Succinyl-CoA synthetase</td>
<td>CTP or ATP</td>
<td>3.0</td>
</tr>
<tr>
<td>Succinol-CoA synthetase</td>
<td>CTP</td>
<td>3.5</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>NADH</td>
<td>2.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10.0</td>
</tr>
</tbody>
</table>
**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
- Product inhibition
- Energy charge

**Regulation: Between Pathways**

- Shutdown of CAC leads to citrate buildup
- Citrate goes into cytoplasm
  - Begins fatty acid synthesis
  - Inactivates glycolysis
Regulation: High NADH

- Buildup of acetyl CoA
- Shuts down PDH and CAC
- Pyruvate builds up in mitochondria
- Acetyl CoA activates PEPCK: pyruvate to oxaloacetate
- High [NADH] helps shuttle oxaloacetate into cytoplasm for gluconeogenesis

Anabolic Roles for CAC

- Not just for degradation
- Intermediates can be used for building
  - Amino acids
  - Gluconeogenesis
  - Fatty acids

Anaplerotic Reactions

- Problem with dual role: if intermediates used in biosynthesis, how can we oxidize acetyl CoA?
  - "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
Key Anaplerotic Reaction

• If energy charge is high, citrate is backed up—oxaloacetate sent out for gluconeogenesis
• If energy charge is low, citrate is used up, and oxaloacetate builds up flux of cycle

Problem

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

No Net Carb Production

• In animals, acetyl CoA cannot produce NET glucose
• To make an oxaloacetate in the CAC requires the use of an oxaloacetate!
Glyoxylate Pathway

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