CHAPTER 17: Citric Acid Cycle
(Krebs cycle)
(TCA or Tri Carboxylic Acid cycle)

Glycolysis: glucose $\rightarrow$ pyruvate

Intermediate step: pyruvate + CoA $\rightarrow$ acetyl CoA

Citric acid cycle: acetyl CoA $\rightarrow$ CO$_2$ + CoA

The stoichiometry of the citric acid cycle is:

$$\text{Acetyl CoA} + 3 \text{NAD}^+ + \text{FAD} + \text{GDP} + \text{Pi} + 2 \text{H}_2\text{O} \rightarrow$$

$$\rightarrow 2 \text{CO}_2 + 3 \text{NADH} + \text{FADH}_2 + \text{GTP} + 2 \text{H}^+ + \text{CoA}$$
Stages in the extraction of energy from foodstuffs:

- **FATS**
  - Fatty acids and glycerol

- **POLYSACCHARIDES**
  - Glucose and other sugars

- **PROTEINS**
  - Amino acids

  - Acetyl CoA

  - **Stage I**

  - CoA

  - **Stage II**

  - Oxidative phosphorylation
    - ATP, ADP

  - **Stage III**
    - Citric acid cycle
      - $2 \text{ CO}_2$

  - Energy charge
  - ATP-generating pathway
  - ATP-utilizing pathway

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Figure 17-15, page 455; Figure 17-16, page 457
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Pyruvate + CoA + NAD$^+$ → acetyl CoA + CO$_2$ + NADH

pyruvate
dehydrogenase
complex

(1) pyruvate + TPP $\xrightarrow{E_1} \text{hydroxyethyl-TPP} + \text{CO}_2$

(2) hydroxyethyl-TPP + lipoamide $\xrightarrow{E_2} \text{carbanion} + \text{acetyllipoamide of TPP}$

(3) acetyllipoamide + CoA $\xrightarrow{E_2} \text{dihydrolipoamide} + \text{acetyl CoA}$

(4) dihydrolipoamide + NAD$^+$ $\xrightarrow{E_3} \text{lipoamide} + \text{NADH} + \text{H}^+$
Table 20-2

Pyrurate dehydrogenase complex of _E. coli_

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Abbreviation</th>
<th>Number of chains</th>
<th>Prosthetic group</th>
<th>Reaction catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate dehydrogenase component</td>
<td>E₁</td>
<td>24</td>
<td>TPP</td>
<td>Oxidative decarboxylation of pyruvate</td>
</tr>
<tr>
<td>Dihydrolipoyl transacetylase</td>
<td>E₂</td>
<td>24</td>
<td>Lipoamide</td>
<td>Transfer of the acetyl group to CoA</td>
</tr>
<tr>
<td>Dihydrolipoyl dehydrogenase</td>
<td>E₃</td>
<td>12</td>
<td>FAD</td>
<td>Regeneration of the oxidized form of lipoamide</td>
</tr>
</tbody>
</table>

Pyruvate + TPP $\rightarrow$ hydroxyethyl-TPP + CO₂

![](image1)

Thiamine pyrophosphate (TPP)

![](image2)

Addition compound

![](image3)

Resonance forms of ionized hydroxyethyl-TPP
(2) Hydroxyethyl-TPP (ionized form) + Lipoamide \rightarrow \text{Carbanion of TPP} + \text{Acetyl lipoamide}

(3) Acetyl lipoamide + HS-CoA \rightarrow \text{Dihydrolipoamide} + \text{Acetyl CoA}

(4) Dihydrolipoamide + NAD\(^+\) \rightarrow \text{Lipoamide} + \text{NADH} + \text{H}^+
Structure of the Ez transacetylase core of the pyruvate dehydrogenase complex
Figure 20-5, page 513
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1. oxaloacetate condenses with acetyl CoA to form citrate

\[
\begin{align*}
\text{Oxaloacetate} + \text{Acetyl CoA} &\rightarrow \text{Citrate} \\
\text{NADH} \quad \text{FADH}_2 &\quad \text{GTP}
\end{align*}
\]

2. citrate is isomerized into isocitrate

\[
\begin{align*}
\text{Citrate} &\rightarrow \text{Isocitrate} \\
\text{Cis-Aconitate}
\end{align*}
\]

3. isocitrate is oxidized and decarboxylated to α-ketoglutarate

\[
\begin{align*}
\text{Isocitrate} \quad \text{NAD}^+ \quad \text{NADH} &\rightarrow \text{Oxaloacetate} \\
\text{Fumarate} \quad \text{Malate} \quad \text{CoA} &\quad \text{Oxaloacetate}
\end{align*}
\]

4. generation of GTP

\[
\begin{align*}
\text{GDP} \quad \text{GTP} \quad \text{Pi} \quad \text{CoA}
\end{align*}
\]

5. oxidative decarboxylation

\[
\begin{align*}
\text{NAD}^+ \quad \text{NADH} &\rightarrow \text{CO}_2 \\
\text{CH}_2 \quad \text{C=O} \quad \text{S-CoA}
\end{align*}
\]

6. oxidation of succinate to oxaloacetate

\[
\begin{align*}
\text{Succinate} \quad \text{FAD} \quad \text{FADH}_2 &\rightarrow \text{Oxaloacetate}
\end{align*}
\]

7-9. oxidation of succinate to oxaloacetate
Figure 20-5, page 513

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### Citric acid cycle

<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction</th>
<th>Enzyme</th>
<th>Prosthetic group</th>
<th>Type*</th>
<th>ΔG °'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetyl CoA + oxaloacetate + H₂O → citrate + CoA + H⁺</td>
<td>Citrate synthase</td>
<td></td>
<td>a</td>
<td>-7.5</td>
</tr>
<tr>
<td>2</td>
<td>Citrate → cis-aconitate + H₂O</td>
<td>Aconitase</td>
<td>Fe-S</td>
<td>b</td>
<td>+2.0</td>
</tr>
<tr>
<td>3</td>
<td>cis-Aconitate + H₂O → isocitrate</td>
<td>Aconitase</td>
<td>Fe-S</td>
<td>c</td>
<td>-0.5</td>
</tr>
<tr>
<td>4</td>
<td>Isocitrate + NAD⁺ → α-ketoglutarate + CO₂ + NADH</td>
<td>Isocitrate dehydrogenase</td>
<td></td>
<td>d + e</td>
<td>-2.0</td>
</tr>
<tr>
<td>5</td>
<td>α-Ketoglutarate + NAD⁺ + CoA → succinyl CoA + CO₂ + NADH</td>
<td>α-Ketoglutarate dehydrogenase complex</td>
<td>Lipoic acid, FAD, TPP</td>
<td>d + e</td>
<td>-7.2</td>
</tr>
<tr>
<td>6</td>
<td>Succinyl CoA + P_i + GDP → succinate + GTP + CoA</td>
<td>Succinyl CoA synthetase</td>
<td></td>
<td>f</td>
<td>-0.8</td>
</tr>
<tr>
<td>7</td>
<td>Succinate + FAD (enzyme-bound) → fumarate + FADH₂ (enzyme-bound)</td>
<td>Succinate dehydrogenase</td>
<td>FAD, Fe-S</td>
<td>e</td>
<td>~0</td>
</tr>
<tr>
<td>8</td>
<td>Fumarate + H₂O → L-malate</td>
<td>Fumarase</td>
<td></td>
<td>c</td>
<td>-0.9</td>
</tr>
<tr>
<td>9</td>
<td>L-Malate + NAD⁺ → oxaloacetate + NADH + H⁺</td>
<td>Malate dehydrogenase</td>
<td></td>
<td>e</td>
<td>+7.1</td>
</tr>
</tbody>
</table>

*Reaction type: (a) condensation; (b) dehydration; (c) hydration; (d) decarboxylation; (e) oxidation; (f) substrate-level phosphorylation.
α-ketoglutarate Dehydrogenase Complex

- It catalyzes a reaction similar to the one catalyzed by the pyruvate dehydrogenase complex.
- The same cofactors are involved: (TPP, lipoamide, CoA, FAD, NAD^+).
- $E_1', E_2', E_3'$
- "Homologous enzyme assemblies"
Figure 20-13, page 519
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Citrate synthase undergoes a large conformational change on binding oxaloacetate. Then it can bind acetyl CoA.

- dimer of two identical 49 kDa subunits
- each subunit has two domains (small - yellow, large - blue)

A  open form of enzyme alone
B  closed form of the liganded enzyme
Mechanism of synthesis of citryl CoA by citrate synthase

OAA + Acetyl CoA → Enol intermediate → Citryl CoA

His 274
Asp 375
His 320
Acetyl CoA + Oxaloacetate → Citrate → Isocitrate

Energy generation

\( \alpha \)-Ketoglutarate

Biosyntheses

Glyoxylate + Succinate

Pyruvate

\(-\) ATP, acetyl CoA, and NADH

Acetyl CoA

\(-\) ATP

Oxaloacetate

\(\Rightarrow\) Citrate

cis-Aconitate

\(-\) ATP, NADH

\(-\) ADP

Malate

Fumarate

Succinate

\(\Rightarrow\) Succinyl CoA

\(-\) Succinyl CoA, ATP and NADH

\(\Rightarrow\) \(\alpha\)-Ketoglutarate

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Figure 20-19, page 524; Figure 20-22, page 525

CONTROL OF THE TCA CYCLE
Beri-Beri

- a neurologic and cardiovascular disorder
- dietary deficiency of thiamine (vitamin B1)
- major problem in the Far East
  (rice has low content of thiamine)
- TPP is the prosthetic group of
  (i) pyruvate dehydrogenase
  (ii) α-ketoglutarate dehydrogenase
  (iii) transketolase
- TPP utilizes the transfer of an activated aldehyde unit
- it causes increased levels of pyruvate and α-ketoglutarate in blood

CHAPTER 17

PROBLEMS

from textbook # 1, 9, 12, 16

from companion # 1, 3, 8, 9, 13