BioChem 330 - Course Outline

• Metabolism and Bioenergetics (II)
  – ENZYME CATALYSIS:
    • kinetic constants $k_{\text{cat}}$, $K_m$
    • Catalytic strategies, the serine proteases
  – CATABOLISM (breakdown)
    • Carbohydrates
      – Glycolysis
      – Tricarboxylic Acid Cycle
      – Electron Transport
      – Chemiosmosis and ATPase
    • Fatty acids and amino acids
• Linkage of cytosolic events (glycolysis) to mitochondrial events (tricarboxylic acid cycle - TCA) in the digestion of carbohydrates

• The linker: pyruvate dehydrogenase
What’s happening to the substrate?

• ..in the pyruvate dehydrogenase complex
  – Conversion of pyruvate to Acetyl CoA and CO$_2$
    • Unlike action of LDH, there is no regeneration of NAD$^+$ for glycolysis
    • No going back

• ... then in the Krebs cycle
  – Converting acetylCoA to 2 CO$_2$
  – The Cycle: origin and properties
A Bedtime Story by PLW

Eubacterium

Electron transport to acceptor ($O_2$)

Meets...

Curly:
Type: ?
Motility a specialty

Mo is eaten by Curly!!

1. Protozoa
2. Fungi
3. Plants
4. & Animals

Curly/Mo Jrs I & II
• **Curly**
  - Cytoplasm
  - Glycolysis

• **Mo**
  - Mitochondria...

...where, for our purposes...

And now...
The Coupling Machinery is Complex

- Pyruvate -> Acetyl CoA
  - (Oxidative Decarboxylation)
  - $\text{CH}_3\text{-CO-CO}_2^- + \text{NAD}^+ + \text{CoA-SH} \rightarrow \text{CH}_3\text{-CO-S-CoA} + \text{NADH} + \text{CO}_2$
  - $\Delta G^o' = -8 \text{ kcal/mole}$

- Catalyzed by pyruvate dehydrogenase complex
  - $E_1$ (24 $\alpha_2\beta_2$ tetramers), $E_2$ (8 $\alpha_3$ trimers), and $E_3$ (6 $\alpha\beta$ dimers)

- “Stoichiometric” cofactors
  - NAD$^+$
  - CoA-SH

- “Catalytic” cofactors
  - Thiamine pyrophosphate
  - Lipoic Acid (fatty acid)
  - FAD
How it starts...

• First the E1 bound TPP (related to vitamin B1, absence of which causes beriberi)

  ...does some carbanion chemistry on pyruvate...

  \[
  \text{Pyruvate} \quad \text{Carbanion of TPP} \quad \text{Addition compound} \quad \text{Resonance forms of ionized hydroxyethyl-TPP}
  \]

  ... and now the “aldehyde” gets oxidized by running into oxidized lipoate on E2...

  \[
  \text{Lysine side chain} \quad \text{Lipoamide}
  \]
...and then the dramatic wrap-up

While the electrons from substrate stay with the lipoamide and ...

...go first to a bound FAD

....and finally end up in a NADH mobile e- carrier
The components of which can be broken down into...

- THE ENZYMES:
  - E1: pyruvate dehydrogenase
  - E2: Dihydrolipoyl transacetylase
  - E3: Dihydrolipoyl dehydrogenase

**TABLE 17.1  Pyruvate 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>
All of which occurs in a factory which is enormous...

- Located in the matrix of the mitochondrion and larger than ribosomes
• The 24 dehydrogenase proteins (E1) on the outside of the complex bind pyruvate and release CO₂
• The 24 transacetylases (E2) proteins shuttle remaining atoms to CoA and the electrons to E3,
• The 12 dehydrogenases E3 takes electrons from E2 through the FAD and ultimately to NAD+
To what end?

cube. Electron microscopic studies of the interactions of \( E_1 \) and \( E_3 \) with the \( E_2 \) core indicate that their active sites are far apart (\( \sim 40 \, \text{Å} \)).

The structural integration of three kinds of enzymes makes possible the coordinated catalysis of a complex reaction (Figure 20-12). All the intermediates in the oxidative decarboxylation of pyruvate are tightly bound to the complex. The proximity of one enzyme to another increases the overall reaction rate and minimizes side reactions. The activated intermediates are transferred from one active site to another by the lipoamide prosthetic group of the transacetylase (see Figure 20-8). The attachment of the lipoyl group to the \( \varepsilon \)-amino group of a lysine residue on the transacetylase provides a flexible arm for the reactive ring. The high degree of mobility of the lipoyl domain and its 14-Å molecular string enables the lipoyl moiety of a transacetylase subunit \( (E_2) \) to interact with the thiamine pyrophosphate unit of an adjacent pyruvate dehydrogenase subunit \( (E_1) \) and with the flavin unit of an adjacent dihydrolipoyl dehydrogenase \( (E_3) \). Further-

1. Active aldehyde
2. Aceyllipoamide
3. Reduced/oxidized lipoamide
4. Reduced/oxidized FAD
Rxns TCA cycle

1. Citrate synthase:  - 8 kcal/mol
2. Aconitase:  + 3 kcal/mol
3. Isocitrate dehyd.:  - 5 kcal/mol
4. α-ketoglutarate dehyd.:  -8 kcal/mol
5. Succinyl CoA synthetase:  -1 kcal/mole
6. Succinate dehyd:  0 kcal/mol
7. Fumarase:  -1 kcal/mol
8. Malate dehydro.:  +7.5 kcal/mol
Some bookkeeping...

- The overall reaction -

The net reaction of the cycle is:

\[
\text{Acetyl CoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + P_i + 2\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 3\ \text{NADH} + \text{FADH}_2 + \text{GTP} + 2\text{H}^+ + \text{CoA}
\]

2 carbons in Oxidn state
-CH\textsubscript{3} - 3
-COS + 3

2 carbons out Oxidn state
CO\textsubscript{2} 2 x (+4)

---

0

Overall: 8 e\textsuperscript{-} oxidation

All e\textsuperscript{-} leave in hydrogenations:

FAD → FADH\textsubscript{2} (2 e\textsuperscript{-})
3 NAD\textsuperscript{+} → 3NADH (3 x 2 e\textsuperscript{-})
What is the significance of the “cycle”? 

• All intermediates are “cofactors” in a broad sense
  – That fact was how the cycle was discovered
    (superstoichiometric stimulation of pyruvate oxidation by the
    various intermediates, and particularly citrate, succinate, α-KG)

• Pulled forward by hydrolysis of bound intermediate
  (citrylCoA)

This reaction, which is an aldol condensation followed by a hydrolysis, is catalyzed by citrate synthase. Oxaloacetate first condenses with acetyl CoA to form citryl CoA, which is then hydrolyzed to citrate and CoA. Hydrolysis of citryl CoA pulls the overall reaction far in the direction of the synthesis of citrate.
Biosynthetic considerations...

• Duties:
Implications of those duties

- They may betray the origins of the cycle in anaerobic bacteria
Mitochondrial function in normal and diabetic β-cells

PIERRE MAECHLER AND CLAES B. WOLLHEIM

Figure 1 A map of human mitochondrial DNA indicating diabetes-associated mutations. The mito genome encodes 37 genes (16,569 bp): 13 polypeptides, 22 tRNAs, 2 ribosomal RNAs.

The polypeptides are constituents of the respiratory-chain complexes: 7 complex I subunits, 1 complex III subunits, 3 subunits of complex IV, 2 subunits of complex V.

The genes for tRNAs are presented as one-letter symbols. Mutations in four of these tRNA genes are associated with diabetes: those for leucine (L), serine (S), lysine (K) and glutamic acid (E) tRNAs.
Mitochondrial function in normal and diabetic β-cells
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Figure 2 The TCA cycle and respiratory chain in a mitochondrion. Substrate oxidation in the TCA cycle activates the respiratory chain, leading to the generation of ATP, which is subsequently translocated to the cytosol. ANT, adenine nucleotide translocator; GDH, glutamate dehydrogenase; Glu, glutamate; KG, ketoglutarate; OAA, oxaloacetate; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; Suc-CoA, succinyl-CoA; SDH, succinate dehydrogenase; TCA, tricarboxylic acid; UCP2, uncoupling protein 2.
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Figure 3 Model for coupling of glucose metabolism to insulin secretion in the β-cell. Glucose is phosphorylated by glucokinase (GK) and converted to pyruvate (Pyr) by glycolysis. Pyruvate preferentially enters the mitochondria and fuels the TCA cycle, resulting in the transfer of reducing equivalents to the respiratory chain, leading to hyperpolarization of the mitochondrial membrane ($\Delta \psi_m$) and generation of ATP. Subsequently, closure of K ATP-channels depolarizes the cell membrane ($\Delta \psi_c$).

This opens voltage-gated Ca$^{2+}$ channels, raising the cytosolic Ca$^{2+}$ concentration ($[\text{Ca}^{2+}]_c$), which triggers insulin exocytosis. Several putative messengers, or additive signals, proposed to participate in the metabolism–secretion coupling are indicated.