Chapter 16

Bioenergetics and Oxidative Metabolism

Catabolic pathways- metabolic reactions involved in energy generation. Convert large, complex biomolecules to smaller molecules (CO₂, H₂O) with the production of storable energy. Often require O₂.

Anabolic pathways- metabolic pathways in the biosynthesis of large, complex molecules. Requires energy.

**Thermodynamics:**
- The first law of thermodynamics is that energy is conserved (can neither be created nor destroyed).
  - Example: Glucose → ATP then ATP → muscle contraction
- The second law of thermodynamics states that all processes progress towards the maximum entropy (S).
  - Entropy is a measure of the disorder of a system.

Free Energy (Gibb’s) - the portion of total energy in a system that is available for useful work.

\[ \Delta G = \Delta H - T \Delta S \]

where H is enthalpy of system, T is temp. in Kelvin, and S is entropy of system

→ If \( \Delta G = 0 \), then the system is at equilibrium
→ If \( \Delta G = - \), then the reaction is spontaneous, exothermic
→ If \( \Delta G = + \), then the reaction is not spontaneous, endothermic, requires energy

\[ A + B \leftrightarrow C + D \]

\[ K_{eq} = \frac{[C][D]}{[A][B]} \quad \text{and} \quad \Delta G^\circ = \Delta G^\circ + RT \ln \frac{[C][D]}{[A][B]} \]

at equilibrium, \( \Delta G = 0 \), so \( \Delta G^\circ = -RT \ln K_{eq} \) where R=8.314 J/K-mole and T is temp in Kelvin

→ \( \Delta G^\circ \) are additive: \( A \rightarrow B \rightarrow C \rightarrow D \)
\[ \Delta G_{AD}^\circ = \Delta G_{AB}^\circ + \Delta G_{BC}^\circ + \Delta G_{CD}^\circ \]

as long as \( \Delta G_{AD}^\circ \) is negative, the overall pathway will proceed

**Energy-rich Compounds**

The body uses energy rich compounds like ATP as a storable energy source in the body (like a battery). These compounds contain “high energy” bonds that when hydrolyzed release usable energy.

What makes a bond “high energy”?

1) Resonance Forms- the more possible resonance forms in which a molecule can exist stabilizes that molecule and lowers its energy.
   - Example: Pi has more resonance than when the phosphate is attached to ATP

2) A lot of “high energy” bonds have groups of similar charges near each other.

3) Hydrolysis of energy rich bonds leads to products which can isomerizes to a more stable compound.

4) Hydrolysis of bond leads to an acid → acid dissociation
**Acetyl-CoA:**
Produced in most of the major energy generating metabolic pathways. It is generated in the mitochondrial matrix.

Sources of Acetyl-CoA:
1. Glycogen → Glucose
2. Triglyceride → Free Fatty Acid
3. Protein → Amino Acid
4. Pyruvate → Acetyl-CoA

Fates of acetyl CoA:
1) oxidate acetyl groups in the TCA cycle for energy generation (primary fate)
2) convert to ketone bodies in the liver
3) transfer of acetyl units in cytosol in biosynthesis of sterols and long chain fatty acids

**Tricarboxylic Acid Cycle** (TCA, Kreb’s cycle, citric acid cycle)
Most of the enzymes in the TCA cycle are located inside of the mitochondria. The amount of mitochondria within a cell varies from tissue to tissue.

- Cardiac tissue-1/2 of cell is mitochondria, red blood cells
- Red blood cells, lens, cornea, testis-no mitochondria

Primary Function- To generate reducing equivalents (NADH, FADH₂) that are utilized to generate energy (ATP) in the electron transport-oxidative phosphorylation sequence.
The goal is to oxidize acetyl units to $2\text{CO}_2$ molecules, and capture the liberated metabolic energy in NADH, FADH$_2$, and GTP. For this process we must cleave a C-C bond, and this is most easily accomplished by carrying out a $\beta$ cleavage:

\[
\begin{array}{c}
O \\
\text{acetyl does not have a } \beta \text{ carbon, so the body adapts by adding the acetyl group to oxaloacetate (a } 4 \text{ C molecule) in the first step of the TCA cycle.}
\end{array}
\]

1) Step 1 - Acetyl-CoA + oxaloacetate + H$_2$O $\rightarrow$ citrate + CoA  
E=citrate synthase
Condensation reaction, Add the acetyl group of acetyl-CoA to oxaloacetate to form a 6C molecule (this step commits the acetyl group to the TCA cycle), $\Delta G= -31.4 \text{ kJ/mole}$ (highly regulated enzyme), oxaloacetate must bind first, then acetyl-CoA can bind to the enzyme (sequential type mechanism), oxaloacetate is used in other biochemical processes in the body.

2) Citrate is converted to isocitrate by the enzyme aconitase (mutase)
    Mutase reaction in which the OH group is moved from one carbon to another. A tertiary alcohol can not be oxidized (need a H on the carbon so that you can break a C-H bond), so the alcohol group is moved to another carbon to create a secondary alcohol. $\Delta G= +6.7 \text{ kJ/mole}$ (eq lies towards citrate)

3) Now the alcohol can be oxidized through a $\beta$ oxidation (lose CO$_2$).
   Isocitrate + NAD$^+$ $\rightarrow$ $\alpha$-ketoglutarate + NADH + H$^+$ + CO$_2$  
   E=isocitrate dehydrogenase
   This is a redox reaction with $\Delta G= -8.4 \text{ kJ/mole}$ (this is sufficient to pull the aconitase reaction forward). E is inhibited by high ATP, NADH levels.

4) $\alpha$-ketoglutarate + NAD$^+$ +CoA $\rightarrow$ succinyl-CoA + NADH + H$^+$ + CO$_2$
   E= $\alpha$-ketoglutarate dehydrogenase (multi enzyme complex)
   $\Delta G= -30 \text{ kJ/mole}$, this reaction creates a high energy intermediate.

5) Succinyl-CoA +Pi +GDP $\rightarrow$ Succinate + GTP + CoA  
   E=Succinyl-CoA synthetase
   This step is reversible with a $\Delta G= -3.3 \text{ kJ/mole}$ making this a good branch point for molecules to enter and leave the cycle. The energy in succinyl-CoA is used to generate a GTP molecule (equivalent to an ATP).

6) Succinate +FAD $\rightarrow$ Fumarate + FADH$_2$  
   E=Succinate dehydrogenase (part of e-transport)
   This reaction has $\Delta G= -8.4 \text{ kJ/mole} + 0.4 \text{ kJ/mole}$ (reversible). The enzyme is inhibited by oxaloacetate and malonate. This redox reaction does not produce enough energy to create an NADH molecule, but it is enough to create an FADH$_2$ molecule.

7) Fumarate + H$_2$O $\rightarrow$ Malate  
   E= Fumarate
   This reaction is a trans addition of H$_2$O across the double bond to introduce the oxygen needed in oxaloacetate and set up one more redox reaction. $\Delta G= -3.8 \text{ kJ/mole}$.

8) Malate + NAD$^+$ $\rightarrow$ Oxaloacetate + NADH + H$^+$  
   E= Malate dehydrogenase
   The reaction is very endergonic ($\Delta G= +30 \text{ kJ/mole}$); however, the reaction is pulled forward by the favorable citrate synthase reaction.

Summary:
Acetyl-CoA + 3NAD$^+$ + FAD + GDP + Pi + 2H$_2$O $\rightarrow$ 2CO$_2$ + 3NADH +3H$^+$ + FADH$_2$ + GTP + CoA
$\Delta G= -40 \text{ kJ/mole}$
In electron transport and oxidative phosphorylation:
   NADH produces 3ATP and FADH$_2$ produces 2ATP
Therefore TCA produces:
3 NADH x 3 ATP = 9 ATP
1 FADH2 x 2 ATP = 2 ATP
1 GTP = 1 ATP
12 ATP/cycle

**Note: The values of 3 and 2 are “consensus values.” Because they may not reflect actual values and because these ratios may change depending on metabolic conditions, these estimates of ATP yield are approximate.**

**TCA Intermediates**

See figure 16.16 in book

The TCA cycle provides intermediates for many biosynthetic pathways. Many of the intermediates are precursors in amino acid and nucleotide synthesis (example, α-ketoglutarate can be converted into glutamate and then to other amino acids). Oxaloacetate can be converted back to glucose. In addition, there are reactions to supply some of the TCA intermediates (Glu can be converted back to α-ketoglutarate to enter into the TCA cycle).

**Regulation of the TCA Cycle**

The TCA cycle must be carefully regulated by the cell. Enzymes with large, negative ΔG values are fairly irreversible. Therefore, these are the primary targets for regulation.

1) citrate synthase- ATP, NADH, and succinyl-CoA inhibit
2) isocitrate dehydrogenase- allosteric activation by ADP and NAD+ and inhibition by ATP and NADH
3) α-ketoglutarate dehydrogenase- inhibited by NADH and succinyl-CoA and activated by AMP

→ The NAD+/NADH ratio in a cell is an indication of the energy state of the cell, a high ratio will activate the TCA cycle to produce more ATP, while a high ratio will inhibit the cycle.

→ Likewise a high ADP/ATP ratio will activate the cycle, while a low ADP/ATP ratio will inhibit the cycle.

**Citrate Synthase Enzyme Mechanism:**

See figures 16.9 and 16.10 in book.

Oxaloacetate binds in the binding site, causing a conformational change in the E that allows acetyl-CoA to bind. The alpha-carbon of acetyl-CoA is deprotonated by a general base (amino acid in active site of enzyme) to form a nucleophilic carbanion, and a proton donation from a general acid (amino acid in active site of enzyme) activates the carbonyl carbon of oxaloacetate for nucleophilic attack by the carbanion. Then, the citroyl-CoA spontaneously hydrolyzes to form citrate.