Metabolic Pathways and Energy Metabolism
Last Week...

[Chemical structures and graphs related to enzymes and inhibitors]
Energy Metabolism

- The first thing a living organism has got to be able to do is harness energy from the environment

- Plants do it by absorbing sunlight

- We do it by eating food

- I suppose this explains the focus of every biochemistry textbook on the processes of energy metabolism:

  1) Glycolysis

  2) The citric acid cycle

  3) Oxidative Phosphorylation
Sugar to Energy Overview

- **Glucose** is the most direct input into the energetics metabolic pathway

- As evidenced by the behavior of a typical 7 year old after he/she has a slushy.

- The first step is **glycolysis**, in which we directly **break the sugar up**.
Glycolysis: The Playas!

- The overall reaction

We start with:

α-D-Glucose

2 (Oxidized) NAD⁺

2 ADP

We end up with:

2 Pyruvate

2 NADH

2 ATP
The Whole Shebang!

- The whole enchilada!

- El toto!

- The long and the short of it!
Feeder Pathways: It Ain’t Always Glucose!
Glycolysis Step 1

- Glucose isn’t going to hydrolyze itself! We need to activate it
- Also prevents glucose from leaking out of the cell, promotes glucose uptake
- The enzyme? Hexokinase: The reaction is Mg$^{2+}$ dependent
Glycolysis Step 2

- Now we need to isomerize the five membered pyranose ring to a four membered furanose ring

\[ \Delta G^\circ = 1.7 \text{ kJ/mol} \]

- The enzyme: G6P Isomerase

His388
Glycolysis Step 3

- How much glycolysis we gonna have?

- As the first irreversible step in glycolysis, PFK-1 is tightly controlled.
- Allosterically inhibited by citrate, glucose 1,6-bisphosphate and ATP (two active sites)!
- Glucagon ↑ PFK-1 Expression ↓
- Allosteric activation: AMP (low energy)
Finally, we bust up the ring for good!!

Aldolase works through a complex, multistep mechanism involving... two Schiff Bases!

Needs for there to be a carbonyl on C₂ and an alcohol on C₄ so that the Schiff base can do its business!
Aldolase mechanism (lyase)

- Makes:
  Dihydroxyacetone phosphate (DHAP)

\[
\text{HO-} \overset{\text{O}}{\text{O}} \overset{\text{P}}{\text{O}} \text{HO}
\]

and...

Glyceraldehyde-3-phosphate

\[
\text{HO} \overset{\text{P}}{\text{O}} \text{O} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{OH}}
\]
Glycolysis Step 5

- GHAP → GAP with Triose Phosphate Isomerase (TIM). Diffusion controlled!

- Basically His and Glu playing with a couple of protons
Glycolysis Step 6

- We now have a conveniently placed carbonyl carbon... guess what we’re going to do next...

Glyceraldehyde-3-phosphate dehydrogenase

- We get our first reduced NAD! (NADH)

Quite Unstable! Is going to move on
Glycolysis Step 7

- We’ve got our high energy phosphoester linkage, now we can use it to make ATP!

![Chemical reaction diagram]

- Nucleophilic attack by Mg$^{2+}$ activated oxygen

\[ \text{1,3-Bisphosphoglycerate} \xrightarrow{\text{Mg}^{2+}} \text{phosphoglycerate kinase} \xrightarrow{\text{Mg}^{2+}} \text{3-Phosphoglycerate} \]

\[ \text{3-Phosphoglycerate} \xrightarrow{\text{ADP}} \text{3-Phosphoglycerate} \xrightarrow{\text{Mg}^{2+}} \text{ADP} \]

\[ \Delta G^{\circ} = -18.5 \text{ kJ/mol} \]
Glycolysis Step 8

- We need to activate the phosphate group on 3-phosphoglycerate by putting it near some ‘mobile’ electrons

\[
\begin{align*}
\text{3-Phosphoglycerate} & \quad \text{2-Phosphoglycerate} \\
\text{\(\Delta G^\circ = 4.4 \, \text{kJ/mol}\)}
\end{align*}
\]

- Mechanism requires phosphohistidine! (His 8).
Glycolysis Step 9

- In case the carbonyl wasn’t enough, we’re going to add a Carbon-Carbon double bond adjacent to the phosphate:

\[ \text{2-Phosphoglycerate} \rightleftharpoons \text{Phosphoenolpyruvate} \]

\( \Delta G^\circ = 7.5 \text{ kJ/mol} \)
Glycolysis Step 10

- Time to reap the reward!

\[ \Delta G^\circ = +14.4 \text{ kJ}\cdot\text{mol}^{-1} \quad \Delta G^\circ = -46 \text{ kJ}\cdot\text{mol}^{-1} \]

Overall \(\Delta G^\circ = -31.4 \text{ kJ}\cdot\text{mol}^{-1}\)
Glycolysis: The end?... Of Course Not!

- And what do we do with this?
- Sure, as long as we have a way to get rid of this!

- Well, of we need to, we can oxidize NADH back to NAD$^+$ by reducing pyruvate to lactate
Lactate dehydrogenase (Homolactic Fermentation)

\[
\Delta G^\circ = -25.1 \text{ kJ/mol}
\]

Isozyme Control!
Alcoholic Fermentation

Pyruvate

Acetaldehyde

Ethanol

TPP, Mg$^{2+}$

Hydroxyethylthiamine pyrophosphate

Resonance-stabilized carbanion

NADH + H$^+$

NAD$^+$

C$\equiv$O

CH$_3$

C$\equiv$O

CH$_3$

C$\equiv$O

CH$_3$

O

H$^+$

H$^+$

H$^+$

H$_3$C

OH

H$_3$C

OH
Summing up Glycolysis

Free energy change with conc.

Standard free energy change

**TABLE 14-2** Free-Energy Changes of Glycolytic Reactions in Erythrocytes

<table>
<thead>
<tr>
<th>Glycolytic reaction step</th>
<th>$\Delta G^{\circ}$ (kJ/mol)</th>
<th>$\Delta G$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Glucose + ATP $\rightarrow$ glucose 6-phosphate + ADP</td>
<td>$-16.7$</td>
<td>$-33.4$</td>
</tr>
<tr>
<td>2. Glucose 6-phosphate $\leftrightarrow$ fructose 6-phosphate</td>
<td>$1.7$</td>
<td>0 to 25</td>
</tr>
<tr>
<td>3. Fructose 6-phosphate + ATP $\rightarrow$ fructose 1,6-bisphosphate + ADP</td>
<td>$-14.2$</td>
<td>$-22.2$</td>
</tr>
<tr>
<td>4. Fructose 1,6-bisphosphate $\leftrightarrow$ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate</td>
<td>$23.8$</td>
<td>0 to $-6$</td>
</tr>
<tr>
<td>5. Dihydroxyacetone phosphate $\leftrightarrow$ glyceraldehyde 3-phosphate</td>
<td>$7.5$</td>
<td>0 to 4</td>
</tr>
<tr>
<td>6. Glyceraldehyde 3-phosphate + $P_i$ + NAD$^+$ $\leftrightarrow$ 1,3-bisphosphoglycerate + NADH + H$^+$</td>
<td>$6.3$</td>
<td>$-2$ to 2</td>
</tr>
<tr>
<td>7. 1,3-Bisphosphoglycerate + ADP $\leftrightarrow$ 3-phosphoglycerate + ATP</td>
<td>$-18.8$</td>
<td>0 to 2</td>
</tr>
<tr>
<td>8. 3-Phosphoglycerate $\leftrightarrow$ 2-phosphoglycerate</td>
<td>$4.4$</td>
<td>0 to 0.8</td>
</tr>
<tr>
<td>9. 2-Phosphoglycerate $\leftrightarrow$ phosphoenolpyruvate + $H_2O$</td>
<td>$7.5$</td>
<td>0 to 3.3</td>
</tr>
<tr>
<td>10. Phosphoenolpyruvate + ADP $\rightarrow$ pyruvate + ATP</td>
<td>$-31.4$</td>
<td>$-16.7$</td>
</tr>
</tbody>
</table>

Note: $\Delta G^{\circ}$ is the standard free-energy change, as defined in Chapter 13 (p. 491). $\Delta G$ is the free-energy change calculated from the actual concentrations of glycolytic intermediates present under physiological conditions in erythrocytes, at pH 7. The glycolytic reactions bypassed in gluconeogenesis are shown in red. Biochemical equations are not necessarily balanced for H or charge (p. 506).
Gluconeogenesis

- Alternative pathway for last step of glycolysis

- This step occurs in mitochondria. PEP then needs to be transported out.
Gluconeogenesis

- Bypassing step 3...

Fructose 1,6-bisphosphate + H2O → Fructose 6-phosphate + Pi

\[ \Delta G^\circ = -16.3 \text{ kJ mol}^{-1} \]

- Mediated by Fructose 1,6-bisphosphatase

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Linearized Michaelis-Menten Kinetics

- Bypassing step 1...

Glucose 6-phosphate + H2O → glucose + Pi

\[ \Delta G^\circ = -13.8 \text{ kJ mol}^{-1} \]

- Mediated by Glucose 6-phosphatase
Gluconeogenesis Overall

Overall:

\[ 2 \text{ pyruvate} + 4 \text{ ATP} + 2 \text{ GTP} + 2 \text{ NADPH} + 2\text{H}^+ + 4 \text{ H}_2\text{O} \rightarrow \]
\[ \text{Glucose} + 4 \text{ ADP} + 2 \text{ GDP} + 6 \text{ P}_i + 2\text{NAD}^+ \]
\[ \Delta G^\circ = -16 \text{ kJ mol}^{-1} \]

- Gluconeogenesis happens mostly in the Liver when there’s lots of ATP around.
- Excess sugar is stored as glycogen
The Citric Acid Cycle

- Where does the pyruvate go?

2 Pyruvate

- Glycolysis (10 successive reactions)

2 Ethanol + 2CO₂
Fermentation to alcohol in yeast

2 Lactate
Fermentation to lactate in vigorously contracting muscle, erythrocytes, some other cells, and in some microorganisms

2 Acetyl-CoA

Citric acid cycle

4CO₂ + 4H₂O
Animal, plant, and many microbial cells under aerobic conditions
Some New Playas!

Flavin Adenine Dinucleotide

Acetyl-coenzyme A (acetyl-CoA)

β-Mercaptoethylamine residue

Pantothenic acid residue

Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

GTP
Pyruvate Dehydrogenase (Multienzyme Complex)

**Advantages of Complex:**
- Product release close to next active site
- Coordinated Control
Step 1: Making Citrate

- Citrate is made from Acetyl CoA and Oxaloacetate

- Oxaloacetate feeds in from the ‘end’ of the cycle
Step 2: Making iso-Citrate

- This sets up reduction of NAD⁺
- Uses a catalytic Iron / Sulphur cluster (4Fe-4S)
Step 3: Making $\alpha$-Ketoglutarate

- Uses NAD$^+$ to oxidize isocitrate

- Detailed mechanism unknown

- Our second oxidative decarboxylation!
Step 4: Making Succinyl CoA

- Another enzyme complex
- Similar mechanism to Pyruvate Dehydrogenase when we made Acetyl CoA

\[ \Delta G^\circ = -33.5 \text{ kJ/mol} \]

- Our third oxidative decarboxylation
Step 5: Releasing Succinate

- Transfer of high energy thioester to high energy phosphoester
- Mammals make GTP, plants make ATP
- GTP can be converted to ATP by nucleotide diphosphate kinase

$\Delta G^\circ = -2.9 \text{ kJ/mol}$
Step 6: Oxidation of Succinate to Fumarate

- We’ve done about all the oxidative decarboxylation we can handle. Time to start moving back towards oxaloacetate

\[
\Delta G^\circ = 0 \text{ kJ/mol}
\]

- This step requires a strong oxidant

- This reaction is run in reverse for oxidative phosphorylation; FAD is **covalently linked** to the enzyme
Step 7: Hydration of Fumarate to Malate

- Detailed mechanism unknown

\[
\Delta G^\circ = -3.8 \text{ kJ/mol}
\]
Step 8: Oxidation of Malate to Oxaloacetate

- Detailed mechanism unknown, though it must involve a hydride transfer to NAD$^+$ in a manner similar to the other dehydrogenases.

- This reaction is highly endergonic.
Energetics of the Citric Acid cycle

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$\Delta G^\circ$</th>
<th>est. $\Delta G$ heart, liver</th>
<th>$K_{eq}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (citrate synthase)</td>
<td>-31.4</td>
<td>-53.9</td>
<td>$3.2 \times 10^5$</td>
</tr>
<tr>
<td>2 (aconitase)</td>
<td>+6.7</td>
<td>+0.8</td>
<td>0.0067</td>
</tr>
<tr>
<td>3 (isocitrate dehydrogenase)</td>
<td>-8.4</td>
<td>-17.5</td>
<td>29.7</td>
</tr>
<tr>
<td>4 ($\alpha$-ketoglutarate dehydrogenase)</td>
<td>-30</td>
<td>-43.9</td>
<td>$1.8 \times 10^5$</td>
</tr>
<tr>
<td>5 (succinyl CoA synthetase)</td>
<td>-3.3</td>
<td>~0</td>
<td>3.8</td>
</tr>
<tr>
<td>6 (succinate dehydrogenase)</td>
<td>+0.4</td>
<td>~0</td>
<td>0.85</td>
</tr>
<tr>
<td>7 (fumarase)</td>
<td>-3.8</td>
<td>~0</td>
<td>4.6</td>
</tr>
<tr>
<td>8 (malate dehydrogenase)</td>
<td>+29.7</td>
<td>~0</td>
<td>$6.2 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

Note how many $\Delta G$ in vivo are close to zero. This should give an idea of the concentrations of intermediates.
Regulation of Citric Acid cycle
Anapleroticity

- The citric acid cycle is both catabolic and anabolic

- Intermediates can feed in and out to keep their concentrations constant
Linearized Michaelis-Menten Kinetics
Linearized Michaelis-Menten Kinetics