GLYCOLYSIS

Generation of ATP from Metabolic Fuels

- **Catabolic process – degradative pathway**
- Energy stored in sugars (carbohydrates) released to perform biological work
- Transforms GLUCOSE to PYRUVATE under ANAEROBIC conditions
- Glucose enters the cell via a specific transporter protein
- **Uses:**
  - Glucose
  - ATP
  - ADP + Pi
  - NAD⁺ (necessary co-factor)

- **Produces:**
  - Pyruvate
  - ATP
  - NADH – can be further oxidized under aerobic conditions to make ATP
Reactions of glycolysis occur in the CYTOSOL

- THREE FATES OF PYRUVATE
  - **Aerobic conditions**
    - conversion to acetyl CoA (pyruvate dehydrogenase) for use in TCA cycle and oxidative phosphorylation for ATP production
  - **Anaerobic conditions**
    - Lactate (animal muscles)
    - Ethanol (yeast)

**TABLE 13-1 | Standard Free Energy Changes for Glucose Catabolism**

<table>
<thead>
<tr>
<th>Catabolic Process</th>
<th>( \Delta G^{\circ} ) (kJ · mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_6H_{12}O_6 \rightarrow 2C_3H_6O_5 + 2H^+ ) (lactate)</td>
<td>(-196)</td>
</tr>
<tr>
<td>( C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6H_2O ) (glucose)</td>
<td>(-2850)</td>
</tr>
</tbody>
</table>

- ANABOLIC PROCESS: GLUCONEOGENESIS
  - Synthesize glucose FROM pyruvate or lactate
  - Increases free glucose concentration
OVERALL REACTION FOR GLYCOLYSIS:

\[
\text{Glucose} + 2 \text{ ADP} + 2 \text{ NAD}^+ + 2 \text{ P}_i \\
2 \text{ Pyruvate} + 2 \text{ ATP} + 2 \text{ NADH} + 2 \text{ H}^+ + 2 \text{ H}_2\text{O}
\]

- 10 Step Process – some steps tightly regulated
- Each glucose (6 carbons) split into TWO pyruvates (3 carbons each)
- Two molecules of ATP are produced
- Two molecules of NAD+ are reduced to NADH

- TWO PHASES:
  - INVESTMENT PHASE
    - First 5 reactions
    - Glucose is activated by phosphorylation
      - “Priming reactions” – need to invest energy to get more out
    - Uses 2 ATP’s per glucose
    - Glucose is converted to TWO molecules of glyceraldehyde 3-phosphate (G3P)
  - DIVIDEND PHASE
    - Second set of 5 reactions
    - Each glyceraldehyde 3-phosphate (G3P) \( \rightarrow \) pyruvate
    - Get FOUR ATP’s out
    - Net gain of 2 ATP’s

- Modest return of energy! Will see big return once pyruvates enter TCA cycle and oxidative phosphorylation.

HANDOUT:
- Not necessary to memorize structures except glucose and pyruvate
- Know types of enzymes and recognize names of intermediates and enzymes
- Know regulatory steps
- Be able to count ATP’s and follow what is made or used when and where.
INVESTMENT

DIVIDEND

NET:
2 ATP per GLUCOSE
2 NADH per GLUCOSE

Table 15.1
The reactions of glycolysis with common enzyme names and reaction type

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Reaction</th>
<th>Enzyme&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reaction Type&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose + ATP → glucose-6-phosphate + ADP</td>
<td>Hexokinase</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Glucose-6-phosphate → fructose-6-phosphate</td>
<td>Phosphoglucomutase</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP</td>
<td>Phosphofructokinase</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Fructose-1,6-bisphosphate → dihydroxyacetone phosphate + glyceraldehyde-3-phosphate</td>
<td>Aldolase</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Dihydroxyacetone phosphate → glyceraldehyde-3-phosphate</td>
<td>Triose phosphate isomerase</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Glyceraldehyde-3-phosphate + P, + NAD⁺ → 1,3-bisphospho-3-phosphate</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>1,3-Bisphosphoglycerate + ADP → 2-phosphoglycerate</td>
<td>Phosphoglycerate kinase</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>3-Phosphoglycerate → 2-phosphoglycerate</td>
<td>Phosphoglycerate mutase</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>2-Phosphoglycerate → phosphoenolpyruvate + H₂O</td>
<td>Enolase</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>Phosphoenolpyruvate + ADP → pyruvate + ATP</td>
<td>Pyruvate kinase</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Enzymes are listed by common names.

<sup>b</sup>Reaction type: (1) oxidation–reduction, (2) phosphoryl group transfer, (3) hydrolysis, (4) nonhydrolytic cleavage (addition or elimination), (5) isomerization–rearrangement, and (6) bond formation coupled to ATP cleavage (see Section 14.2).

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Figure 15-2 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons
REACTIONS OF GLYCOLYSIS IN DETAIL

Investment Phase

In the first five steps of glycolysis, one six-carbon molecule of glucose is split into two 3-carbon compounds. 2 molecules of ATP are required for these reactions.
STEP 1: Glucose to glucose-6-phosphate

Phosphorylation of glucose by HEXOKINASE
- **KINASE** – Enzymes that catalyze the transfer of a phosphoryl group from ATP to an acceptor substrate
- Type of **TRANSFERASE** enzyme
- Regulated but not the committed step
  - Glucose-6-phosphate can form glycogen or other pathways

**ATP COUNT**
- -1 (one ATP used)
- 1st Investment of Energy

**Phosphorylation keeps glucose in the cell**

STEP 2: Glucose-6-phosphate to fructose-6-phosphate

**ENZYME:** Phosphoglucone Isomerase

Type of **ISOMERASE** – Rearrangement of functional groups to form the isomer; \( \Delta G \) near zero; concentration of reactants and products affect direction.

- Convert Glucose-6-phosphate to fructose-6-phosphate
- Not a regulated or committed step

**ATP COUNT:**
- This step – 0
- Overall count – -1
STEP 3: Fructose-6-phosphate to fructose-1,6-bisphosphate

\[ \text{Fructose-6-phosphate} + \text{ATP} \xrightarrow{\text{Phosphofructokinase (PFK)}} \text{Fructose-1,6-bisphosphate} + \text{ADP} \]

- **ENZYME:** Phosphofructokinase
  - **KINASE** – same as first step; **TRANSFERASE** reaction
  - 2\(^{nd}\) Investment of energy – one more ATP used

- **ATP COUNT:**
  - This step – -1
  - Overall count – -2

- **KEY CONTROL STEP – IRREVERSIBLE!!**
  - Committed step
  - Note HIGHLY negative \( \Delta G^\circ' \) – means not reversible

STEP 4:
Fructose-1,6-bisphosphate \( \rightarrow \) glyceraldehyde-3-phosphate & dihydroxyacetone phosphate

\[ \text{Fructose-1,6-bisphosphate} \rightarrow \text{glyceraldehyde-3-phosphate} + \text{dihydroxyacetone phosphate} \]

- **ENZYME:** Aldolase
  - **Non-hydrolytic Cleavage** reaction (type of lyase)
  - Cleaves glucose molecule into 2 molecules
  - \( \Delta G^\circ = +22.8 \text{ kJ/mol} \); \textit{in vivo} \( \Delta G \) is less than zero – products are quickly consumed. Rapid consumption of products pulls reaction forward.

- **ATP COUNT:**
  - This step – 0
  - Overall count – -2

- **Not a regulatory step**
STEP 5: Dihydroxyacetone phosphate to glyceraldehyde-3-phosphate

- **ENZYME:** Triose phosphate isomerase
  - Isomerization – rearrangement reaction
  - Isomerase enzyme

- **ATP COUNT:**
  - This step – 0
  - Overall count – -2

- **Not a regulatory step**

Through 1st 5 steps (Investment Phase) we’ve **USED 2 ATP molecules**
Steps 6-10 \(\rightarrow\) Dividend Phase where the investment pays off!!

**Sum:** Glucose + 2 ATP \(\rightarrow\) 2 glyceraldehyde-3-phosphate +2 ADP + 2 Pi
**Dividend Phase**

In the second phase of glycolysis, glyceraldehyde-3-phosphate is converted to pyruvate.

These reactions yield 4 molecules of ATP, 2 for each molecule of pyruvate produced.
STEP 6: 2 Glyceraldehyde-3-phosphate to 2 1,3-bisphosphoglycerate

- **ENZYME:** Glyceraldehyde-3-phosphate dehydrogenase
  - DEHYDROGENASE reaction
  - Oxidation – Reduction enzymes (also called oxidoreductases)
  - Reactions generate either NADH, FADH$_2$ or NADPH
  - This reaction produces NADH

- **ATP COUNT:**
  - This step – 0
  - Overall count – -2
  - +2 NADH produced
  - Not a regulatory step

STEP 7: (2) 1,3-bisphosphoglycerate to (2) 3-phosphoglycerate

- **ENZYME:** Phosphoglycerate Kinase
  - Group Transfer reaction – KINASE reaction (same as 1 and 3)
  - STEP WHERE ATP IS MADE!!

- **ATP COUNT:**
  - This step – +2
  - Overall count – -2 + 2 = 0
  - +2 NADH overall
  - Not a regulatory step
STEP 8: (2) 3-phosphoglycerate to (2) 2-phosphoglycerate

- **ENZYME:** Phosphoglycerate Mutase
  - Isomerization – Rearrangement reaction – Mutase reaction (same as 2 and 5)

- **ATP COUNT:**
  - This step – 0
  - Overall count – -2 + 2 = 0
  - +2 NADH overall

- **Not a regulatory step**

STEP 9: (2) 2-phosphoglycerate to (2) phosphoenolpyruvate

- **ENZYME:** Enolase
  - Non-hydrolytic cleavage reaction (lyase)

- **ATP COUNT:**
  - This step – 0
  - Overall count – -2 + 2 = 0
  - +2 NADH overall

- **Not a regulatory step**
STEP 10: (2) phosphoenolpyruvate to (2) pyruvate

\[
\begin{align*}
\text{CH}_2 & \text{COO}^- \\
\text{C} & - \text{O} \quad \text{PO}_4^{3-} + \text{H}^+ + \text{ADP}^3- & \xrightarrow{\text{Mg}^{2+}, \text{K}^+} & \text{CH}_3 \\
\text{PEP} & & \text{C} & - \text{O} + \text{ATP}^{4-} \\
\end{align*}
\]

\[\Delta G^{\text{rxn}} = -31.7 \text{ kJ/mol}\]

- **ENZYME**: Pyruvate Kinase
  - Group Transfer reaction – KINASE reaction (same as 1 and 3)
  - STEP WHERE ATP IS MADE!!

- **ATP COUNT**:
  - This step – +2
  - Overall count – -2 + 2 + 2 = 2
  - +2 NADH overall

- This is a REGULATED step – Not Reversible

**GLYCOLYSIS ANIMATION**:
http://www.northland.cc.mn.us/biology/Biology1111/animations/glycolysis.html

**Table 15.2**
The ATP and NADH balance sheet for glycolysis

<table>
<thead>
<tr>
<th>Number(^a)</th>
<th>Reaction per Glucose</th>
<th>ATP Change per Glucose(^b)</th>
<th>NADH Change per Glucose(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose → glucose-6-phosphate</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Fructose-6-phosphate → fructose-1,6-bisphosphate</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2 Glyceraldehyde-3-phosphate ↔ 2 1,3-bisphosphoglycerate</td>
<td>0</td>
<td>+2</td>
</tr>
<tr>
<td>7</td>
<td>2 1,3-Bisphosphoglycerate ↔ 2 3-phosphoglycerate</td>
<td>+2</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>2 Phosphoenolpyruvate → 2 pyruvate</td>
<td>+2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total change</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) The number corresponds to the reaction number in Table 15.1.
\(^{b}\) A minus sign indicates loss of ATP by cleavage of a phosphoanhydride bond; a plus sign indicates formation of ATP (from ADP) or NADH (from NAD\(^+\)).

**GLYCOLYSIS THERMODYNAMICS**

Can regulate flux through a pathway by adjusting the rate of a reaction with a large free energy change.

- Increase the amount of enzyme
- Alter activity of the enzyme by small molecules

Three steps in glycolysis have large negative \(\Delta G\) values (1, 3, 10)

Remaining steps are near equilibrium (\(\Delta G \sim = 0\))
As soon as the substrate has gotten past the “dam”, the near-equilibrium reactions go with the flow, allowing the pathway intermediates to move toward the final product.

Usually multiple control points (dams) in a metabolic pathway.

The height of each step corresponds to its $\Delta G$ value in heart muscle glycolysis. The numbers correspond to the glycolytic enzymes.
REGULATION OF GLYCOLYSIS

- Glycolysis is a highly regulated process
  o Need to maintain constant levels of energy in cells
  o Regulation UP and DOWN depends on the cell’s need for ATP and NADH
  o Steps 2, 4-9 have $\Delta G^\circ'$ values close to zero, therefore are essentially operating at equilibrium
    - Can go in either direction
    - These steps are common to the GLUCONEOGENESIS pathway
  o Steps 1, 3 and 10 have large negative $\Delta G^\circ'$ values (not at equilibrium) and are the sites of regulation.

THREE KEY REGULATED STEPS

1. Hexokinase (Step #1)
   a. Regulates entry of free glucose into glycolysis
   b. Controlled by FEEDBACK INHIBITION
      i. Inhibited by product – glucose-6-phosphate
   c. NOT the committed step
   d. Regulates the concentration of glucose-6-phosphate

2. Phosphofructokinase (PFK) (Step #3)
   a. Catalyzes phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate (FBP)

b. KEY REGULATORY POINT OF GLYCOLYSIS
   c. Valve that controls glycolysis
   d. 1st major committed step – can’t go back
   e. PFK is INACTIVE when [ATP] cell is HIGH
      i. Makes good sense – when ATP is high, glycolysis no necessary so turned down at PFK
   f. If [AMP] (low energy precursor of ATP) HIGH, tells cell energy is LOW and to make more ATP
   g. Inhibited by CITRATE – physiological form of citric acid
      i. Citrate formed in TCA cycle from pyruvate
      ii. Therefore, if cellular [citrate] is sufficient, glycolysis is slowed
   h. ACTIVATED by fructose-2,6-bisphosphate (made when blood glucose conc. high)
3. **Pyruvate Kinase** (Step #10)
   a. Regulates formation of pyruvate from phosphoenolpyruvate
   b. Increase [ATP] inhibits pyruvate kinase and slows pyruvate formation

   - Red blood cells depend on a constant energy supply to maintain structural integrity
     - Remember that they don’t have nuclei or mitochondria
     - Therefore, **glycolysis is the primary source of ATP for red blood cells**
   - If energy needs are not met, the RBC’s can rupture (called hemolysis) and the blood loss called **hemolytic anemia**

   - 2nd most common form of **hemolytic anemia** is due to deficiency in **pyruvate kinase**
     - Autosomal recessive trait (carriers have no disease)
     - Treated with transfusions and/or splenectomies
     - No simple treatment

<table>
<thead>
<tr>
<th>Number</th>
<th>Reaction</th>
<th>Enzyme</th>
<th>$\Delta G^{\circ}$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose + ATP $\rightarrow$ glucose-6-phosphate + ADP</td>
<td>Hexokinase</td>
<td>-16.7</td>
</tr>
<tr>
<td>3</td>
<td>Fructose-6-phosphate + ATP $\rightarrow$ fructose-1,6-bisphosphate + ADP</td>
<td>Phosphofructokinase</td>
<td>-14.2</td>
</tr>
<tr>
<td>10</td>
<td>Phosphoenolpyruvate + ADP $\rightarrow$ pyruvate + ATP</td>
<td>Pyruvate kinase</td>
<td>-31.4</td>
</tr>
</tbody>
</table>

*a The number corresponds to the reaction number in Table 15.1.

**ENTRY OF OTHER CARBOHYDRATES INTO GLYCOLYSIS:**

1. **Dietary Starch**
   a. Hydrolyzed in mouth by amylases to glucose monomers
   b. Hydrolyzed in stomach by acid to glucose monomers
   c. Glucose absorbed through intestinal walls to blood and transported
      i. 1/3 goes to skeletal muscle and heart
      ii. 1/3 goes to BRAIN – needs 100g glucose/day; can’t use fatty acids
      iii. 1/3 goes to liver for storage as glycogen

2. **Disaccharides:**
   a. Maltose $\rightarrow$ 2 glucose
   b. Sucrose $\rightarrow$ fructose and glucose
   c. Lactose $\rightarrow$ glucose and galactose
Fructose and galactose enter glycolysis differently!

- **Fructose:**
  - In muscle, hexokinase phosphorylates fructose and enters pathway as fructose-6-phosphate. One step!
  - In liver, multiple steps needed.
    - Fructokinase phosphorylates at position 1
    - Aldolase cleavage
    - Additional phosphorylation
    - Enters as 1 molecule of DHAP that isomerizes to glyceraldehyde-3-phosphate
    - Glyceraldehyde product gets phosphorylated and then enters glycolysis as well
    - All 6 carbons enter as two molecules.

- **Galactose:**
  - C4 epimer of glucose
  - Requires 5 reactions to transform it to *glucose-6-phosphate* where it can enter glycolytic pathway

- **Glycerol:**
  - Released during degradation of TAG’s
  - 2 Reactions:
    - Phosphoryl transfer
    - Oxidation
  - Turns glycerol into dihydroxyacetone phosphate which isomerizes in glycolysis to glyceraldehyde-3-phosphate
Figure 15-3 Concepts in Biochemistry, 3/e
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