Chapter 18
Glycolysis
Biochemistry
by
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18.1 – What Are the Essential Features of Glycolysis?
The Embden-Meyerhof (Warburg) Pathway
• Consists of two phases:
  1. First phase converts glucose to two Glyceraldehyde-3-P
     – Energy investment phase
     – Consumes 2 molecules of ATP
  2. Second phase produces two pyruvates
     – Energy generation phase
     – Produces 4 molecules of ATP
• Products are 2 pyruvate, 2 ATP and 2 NADH
• Essentially all cells carry out glycolysis
• Ten reactions - same in all cells - but rates differ
• Three possible fates for pyruvate

18.2 – Why Are Coupled Reactions Important in Glycolysis?
• Coupled reactions convert some, but not all, of the metabolic energy of glucose into ATP
• Under cellular conditions, approximately 5% of the energy of glucose is released in glycolysis
Glucose + 2 ADP + 2 Pi → 2 lactate + 2 ATP + 2H+ + 2 H2O
\[ \Delta G^0^\prime = -122.6 \text{ kJ/mol} \]
2 ADP + 2 Pi → 2 ATP + 2 H2O \[ \Delta G^0^\prime = 61 \text{ kJ/mol} \]
18.3 – What Are the Chemical Principles and Features of the First Phase of Glycolysis?

1. Phosphorylation (Hexokinase)
2. Isomerization (Phosphoglucoisomerase)
3. Phosphorylation (Phosphofructokinase)
4. Cleavage (Aldolase)
5. Isomerization (Triose phosphate isomerase)

Rx 1: Hexokinase

The first reaction - phosphorylation of glucose
- Hexokinase (or glucokinase in liver)
- This is a priming reaction - ATP is consumed here in order to get more later
- ATP makes the phosphorylation of glucose spontaneous
- Mg\(^{2+}\) is required
Under cellular condition

\[ \Delta G = \Delta G^0 + RT \ln \left( \frac{[G-6-P][ADP]}{[Glu][ATP]} \right) \]

(p582)

- The cellular advantages of phosphorylating glucose
  1. Phosphorylation keeps the substrate in the cell
  2. Keeps the intracellular concentration of glucose low, favoring diffusion of glucose into the cell
  3. Makes it an important site for regulation

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>mM</th>
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<tbody>
<tr>
<td>Glucose</td>
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<tr>
<td>Glucose-6-phosphate</td>
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</tr>
<tr>
<td>Fructose-1,6-bisphosphate</td>
<td>0.011</td>
</tr>
<tr>
<td>Glucose-1-phosphate</td>
<td>0.030</td>
</tr>
<tr>
<td>1,6-Bisphosphoglycerate</td>
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<td>2,3-Bisphosphoglycerate</td>
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<tr>
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<tr>
<td>3-Phosphoglycerate</td>
<td>0.0003</td>
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<tr>
<td>Phosphoenolpyruvate</td>
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<td>Pyruvate</td>
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<td>ATP</td>
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<tr>
<td>ADP</td>
<td>0.04</td>
</tr>
<tr>
<td>F</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Hexokinase*

1st step in glycolysis; \( \Delta G \) large, negative

- \( K_m \) for glucose is 0.1 mM; cell has 4 mM glucose, so hexokinase is normally active
- Hexokinase is regulated -- allosterically inhibited by (product) glucose-6-P -- but is not the most important site of regulation of glycolysis
- Can phosphorylate a variety of hexose sugars, including glucose, mannose, and fructose

*Glucokinase*

- Glucokinase (\( K_m ^{\text{glucose}} = 10 \text{ mM} \)) only turns on when cell is rich in glucose
- Is not product inhibited
- Is an inducible enzyme by insulin
Figure 18.5
Glucose-6-phosphate is the branch point for several metabolic pathways.

Rx 2: Phosphoglucoisomerase

Glucose-6-P (aldose) to Fructose-6-P (ketose)

- Why does this reaction occur
  - next step (phosphorylation at C-1) would be tough for hemiacetal -OH, but easy for primary -OH
  - isomerization activates C-3 for cleavage in aldolase reaction

- Ene-diol intermediate in this reaction
- Be able to write a mechanism!

Figure 18.6
The phosphogluco-isomerase mechanism involves opening of the pyranose ring (Step A), proton abstraction leading to enediol formation (Step B), and proton addition to the double bond, followed by ring closure (Step C).
Rx 3: Phosphofructokinase

PFK is the committed step in glycolysis
- The second priming reaction of glycolysis
- Committed step and large, negative ΔG -- means PFK is highly regulated

Fructose-6-P + Pi → Fructose-1,6-bisP  ΔG°' = 16.3 kJ/mol

Regulation of Phosphofructokinase
- ATP is a allosteric inhibitor
  - Has two distinct binding sites for ATP
  - A high-affinity substrate site and a low-affinity regulatory site
- AMP reverses the inhibition due to ATP
  - Raise dramatically when ATP decrease (p586)
- PFK increases activity when energy status is low
- PFK decreases activity when energy status is high
- Citrate is also an allosteric inhibitor
- Fructose-2,6-bisphosphate is allosteric activator

Figure 18.8
At high [ATP], phosphofructokinase (PFK) behaves cooperatively and the plot of enzyme activity versus [fructose-6-phosphate] is sigmoid. High [ATP] thus inhibits PFK, decreasing the enzyme’s affinity for fructose-6-phosphate.

Figure 18.9
Fructose-2,6-bisphosphate activates phosphofructokinase, increasing the affinity of the enzyme for fructose-6-phosphate and restoring the hyperbolic dependence of enzyme activity on substrate.
Fructose-2,6-bisphosphate decreases the inhibition of phosphofructokinase due to ATP.

Rx 4: Aldolase

C₆ is cleaved to 2 C₃s (DHAP, Gly-3-P)
- Fructose bisphosphate aldolase cleaves fructose-1,6-bisphosphate between the C-3 and C-4 carbons to yield two triose phosphates
- Dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G-3-P)

- Animal aldolases are Class I aldolases
- Class I aldolases form covalent Schiff base intermediate between substrate and active site lysine
- Class II aldolase are produced mainly in bacteria and fungi
- Understand the evidence for Schiff base intermediate (box on page 590)
Figure 18.13
(a) A mechanism for the fructose-1,6-bisphosphate aldolase reaction. The Schiff base formed between the substrate carbonyl and an active-site lysine acts as an electron sink, increasing the acidity of the $\beta$-hydroxyl group and facilitating cleavage as shown. (b) In Class II aldolases, an active-site Zn$^{2+}$ stabilizes the enolate intermediate, leading to polarization of the substrate carbonyl group.

**Rx 5: Triose Phosphate Isomerase**

*DHAP is converted to G3-P*

- An ene-diol mechanism
- Active site Glu acts as general base
- Triose phosphate isomerase is a near-perfect enzyme
18.4 – What Are the Chemical Principles and Features of the Second Phase of Glycolysis?

**Metabolic energy produces 4 ATP**

- Net ATP yield for glycolysis is two ATP
- Second phase involves two very high energy phosphate intermediates
  - 1,3 BPG
  - Phosphoenolpyruvate
Rx 6: Glyceraldehyde-3-Phosphate Dehydrogenase

G-3-P is oxidized to 1,3-BPG

- Energy yield from converting an aldehyde to a carboxylic acid is used to make 1,3-BPG and NADH

G3PDH (or GAPDH)

G-3-P is oxidized to 1,3-BPG

- Oxidation (aldehyde to carboxylic acid) and phosphorylation
- Mechanism is one we saw in Chapter 14 (see Figure 14.10)
- Mechanism involves covalent catalysis and a nicotinamide coenzyme

Rx 7: Phosphoglycerate Kinase

ATP synthesis from a high-energy phosphate

- This is referred to as "substrate-level phosphorylation"
- Coupled reactions

\[
\text{Glyceraldehyde-3-P + ADP + Pi + NAD}^+ \rightarrow \text{3-phosphoglycerate + ATP + NADH + H}^+ \Delta G^{\circ} = -12.6 \text{ kJ/mol}
\]
ATP is synthesized by three major routes:

1. **Substrate-level phosphorylation** (Glycolysis, Citric acid cycle)

2. **Oxidative phosphorylation** (Driven by electron transport)

3. **Photophosphorylation** (Photosynthesis)

2,3-BPG (for hemoglobin) is made by circumventing the PGK reaction
- Bisphosphoglycerate mutase
- Erythrocytes contain 4-5 mM 2,3-BPG

**Rx 8: Phosphoglycerate Mutase**

*Phosphoryl group from C-3 to C-2*
- Repositions the phosphate
- Mutase
**Rx 8: Phosphoglycerate Mutase**

- Phosphoenzyme intermediates
- A bit of 2,3-BPG is required as a cofactor

Figure 18.24. A mechanism for the phosphoglycerate mutase reaction in rabbit muscle and in yeast. Zelda Rose of the Institute for Cancer Research in Philadelphia showed that the enzyme requires a small amount of 2,3-BPG to phosphorylate the histidine residue before the mechanism can proceed. Prior to her work, the role of the phosphohistidine in this mechanism was not understood.

**Rx 9: Enolase**

- 2-PG to PEP
- Make a high-energy phosphate product
- Dehydration

2-Phosphoglycerate (2-PG)

\[ \text{CH}_2 \text{OH} \]

Phosphoenolpyruvate (PEP)

\[ \text{CH}_2 \]

\[ \Delta G^\circ = +1.8 \text{ kJ/mol} \]

Figure 18.26. The enolase reaction.

**Rx 10: Pyruvate Kinase**

**PEP to Pyruvate makes ATP**

- Substrate-level phosphorylation
- Another key control point for glycolysis
- Enol-keto tautomer

\[ \text{COO}^- \]

\[ \text{CH}_2 \text{O} \]

\[ \text{ADP}^+ \]

\[ \text{K}^+ \]

\[ \text{Mg}^{2+} \]

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

Figure 18.27. The pyruvate kinase reaction.
Figure 18.28 The conversion of phosphoenolpyruvate (PEP) to pyruvate may be viewed as involving two steps: phosphoryl transfer followed by an enol-keto tautomerization. The tautomerization is spontaneous ($\Delta G^\circ = -35-40 \text{ kJ/mol}$) and accounts for much of the free energy change for PEP hydrolysis.

Rx 10: Pyruvate Kinase
- Large, negative $\Delta G$ -- regulation
- Allosterically activated by AMP, F-1,6-bisP
- Allosterically inhibited by ATP, acetyl-CoA, and alanine
- Responsive to hormonally-regulated phosphorylation in the liver (glucagon) - the phosphorylated form of the enzyme is less active.

18.5 – What Are the Metabolic Fates of NADH and Pyruvate Produced in Glycolysis?

Aerobic or anaerobic?
- NADH must be recycled to NAD$^+$
  - If $O_2$ is available, NADH is re-oxidized in the electron transport pathway, making ATP in oxidative phosphorylation (chapter 20)
  - In anaerobic conditions, NADH is re-oxidized by lactate dehydrogenase (LDH), providing additional NAD$^+$ for more glycolysis.
Pyruvate also has two possible fates:
- aerobic: into citric acid cycle (chapter 19) where it is oxidized to CO₂ with the production of additional NADH (and FADH₂)
- anaerobic: (fermentation)
  1. In yeast: reduced to ethanol
     - Pyruvate decarboxylase (TPP)
     - Alcohol dehydrogenase (Reoxidized NADH to NAD⁺)
  2. In animals: reduced to lactate
     - Lactate dehydrogenase (Reoxidized NADH to NAD⁺)

18.6 – How Do Cells Regulate Glycolysis?

The elegant evidence of regulation (See Figure 18.31)
- Standard state ΔG values are scattered: + and -
- ΔG in cells is revealing:
  - Most values near zero
  - 3 of 10 Reactions have large, negative ΔG
- Large negative ΔG Reactions are sites of regulation
  1. Hexokinase
  2. Phosphofructokinase
  3. Pyruvate kinase

Overview of the regulation of glycolysis.
18.7 – Are Substrates Other Than Glucose Used in Glycolysis?

Fructose, mannose and galactose

• Fructose and mannose are routed into glycolysis by fairly conventional means. See Figure 18.32

18.7 – Are Substrates Other Than Glucose Used in Glycolysis?

• Fructose

1. In liver
   • Fructokinase
     Fructose + ATP → fructose-1-phosphate + ADP + H^+  
   • Fructo-1-phosphate aldolase
     fructose-1-phosphate → glyceraldehyde + DHAP  
   • Triose kinase
     glyceraldehyde → glyceraldehyde-3-phosphate

2. In kidney and muscle
   • Hexokinase
     Fructose + ATP → fructose-6-phosphate + ADP + H^+

• Mannose

• Hexokinase
  mannose + ATP → mannose-6-phosphate + ADP + H^+

• Phosphomannomutase
  mannose-6-phosphate → fructose-6-phosphate

18.7 – Are Substrates Other Than Glucose Used in Glycolysis?

• Galactose is more interesting - the Leloir pathway "converts" galactose to glucose

1. Galactokinase
   Galactose + ATP → galactose-1-phosphate + ADP + H^+

2. Galactose-1-phosphate uridylyltransferase

3. Phosphoglucomutase

4. UDP-galactose-4-epimerase
18.7 – Are Substrates Other Than Glucose Used in Glycolysis?

- Glycerol can also enter glycolysis
  - Glycerol is produced by the decomposition of triacylglycerols (chapter 23)
  - Converted to glycerol-3-phosphate by the action of glycerol kinase
  - Then oxidized to DHAP by the action of glycerol phosphate dehydrogenase
  - NAD⁺ as the required coenzyme
The glycerol kinase reaction

\[
\text{Glycerol} \quad \text{CH}_2\text{OH} \quad + \quad \text{ATP} \quad \overset{\text{Mg}^{2+}}{\longrightarrow} \quad \text{Glycerol-3-phosphate} \quad \text{CH}_2\text{OP}O_4^- \\
\text{HOCH} \quad \text{HOCH} \quad \text{ADP}
\]

The glycerol phosphate dehydrogenase reaction

\[
\text{Glycerol-3-phosphate} \quad \text{CH}_2\text{OP}O_4^- \quad + \quad \text{NAD}^+ \quad \overset{\text{oxidation}}{\longrightarrow} \quad \text{Dihydroxyacetone phosphate} \quad \text{CH}_2\text{OPO}_4^- \\
\text{CH}_2\text{OH} \quad \text{HOCH} \quad \text{NADH} \quad + \quad \text{H}^+
\]