Chapter 11 - Glycolysis

• For centuries, bakeries and breweries have exploited the conversion of glucose to ethanol and CO₂ by glycolysis in yeast.

11.1 Glycolysis Is a Ubiquitous Pathway

Converts: 1 glucose  $\rightarrow$ 2 pyruvate

• Pyruvate can be further metabolized to:
  (1) Lactate or ethanol (anaerobic)
  (2) Acetyl CoA (aerobic)

• Acetyl CoA is further oxidized to CO₂ and H₂O via the citric acid cycle

• Much more ATP is generated from the citric acid cycle than from glycolysis.

Fig 11.1

• Catabolism of glucose via glycolysis and the citric acid cycle.

(continued next slide)
Net reaction of glycolysis

- Two molecules of ATP are produced
- Two molecules of NAD⁺ are reduced to NADH

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

Glycolysis (10 reactions) can be divided into two stages

- **Hexose stage**: 2 ATP are consumed per glucose
- **Triose stage**: 4 ATP are produced per glucose

**Net**: 2 ATP produced per glucose

Table 11.1

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Glucose + ATP → Glucose-6-phosphate + ADP + H⁺</td>
<td>Hexokinase, phosphoglucomutase</td>
</tr>
<tr>
<td>2. Glucose-6-phosphate + ATP → Glucose-6-phosphate + ADP + H⁺</td>
<td>6-Phosphofructokinase</td>
</tr>
<tr>
<td>3. Fructose-6-phosphate + ATP → Fructose-6-phosphate + ADP + H⁺</td>
<td>Aldolase</td>
</tr>
<tr>
<td>4. Fructose-1,6-bisphosphate → Two molecules of glyceraldehyde-3-phosphate</td>
<td>Triokinase</td>
</tr>
<tr>
<td>5. Glyceraldehyde-3-phosphate + NAD⁺ + 2 H⁺ → 2 NADH + 2 H⁺</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>6. Phosphoglycerate mutase</td>
<td></td>
</tr>
<tr>
<td>7. 3-Phosphoglycerate + ADP → 2 Phosphoglycerate + ATP</td>
<td>Phosphoglycerate kinase</td>
</tr>
<tr>
<td>8. 2-Phosphoglycerate + ATP → Phosphoenolpyruvate + H⁺</td>
<td>Enolase</td>
</tr>
<tr>
<td>9. Phosphoenolpyruvate + ATP → Pyruvate + ATP</td>
<td>Pyruvate kinase</td>
</tr>
</tbody>
</table>

Fig 11.2

- Glycolysis (next 4 slides)
11.2 Glycolysis Has 10 Enzyme-Catalyzed Steps

- Each chemical reaction prepares a substrate for the next step in the process
- A hexose is cleaved to two trioses
- Interconversion of the trioses allows both to be further metabolized via glycolytic enzymes
- ATP is both consumed and produced in glycolysis
1. Hexokinase

- Transfers the γ-phosphoryl of ATP to glucose C-6 oxygen to generate glucose 6-phosphate (G6P)
- Mechanism: attack of C-6 hydroxyl oxygen of glucose on the γ-phosphorous of MgATP\(^2\)- displacing MgADP\(^-\)
- Four kinases in glycolysis: steps 1, 3, 7, and 10
- All four kinases require Mg\(^{2+}\) and have a similar mechanism

Properties of hexokinases

- Broad substrate specificity - hexokinases can phosphorylate glucose, mannose and fructose
- Yeast hexokinase undergoes an induced-fit conformational change when glucose binds
- Conformational change helps prevent hydrolysis of ATP to ADP and P\(_i\) (Fig 6.13)

Isozymes of hexokinase

- Isozymes - multiple forms of hexokinase occur in mammalian tissues and yeast
- Hexokinases I, II, III are active at normal glucose concentrations (K\(_m\) values ~10\(^{-6}\) to 10\(^{-4}\)M)
- Hexokinase IV (Glucokinase, K\(_m\) ~10\(^{-2}\)M) is active at higher glucose levels, allows the liver to respond to large increases in blood glucose
2. Glucose 6-Phosphate Isomerase

- Converts glucose 6-phosphate (G6P) (an aldose) to fructose 6-phosphate (F6P) (a ketose)
- Enzyme preferentially binds the \( \alpha \)-anomer of G6P (converts to open chain form in the active site)
- Enzyme is highly stereospecific for G6P and F6P
- Isomerase reaction is near-equilibrium in cells

3. Phosphofructokinase-1 (PFK-1)

- Catalyzes transfer of a phosphoryl group from ATP to the C-1 hydroxyl group of F6P to form fructose 1,6-bisphosphate (F1,6BP)
- PFK-1 is metabolically irreversible and a critical regulatory point for glycolysis in most cells (PFK-1 is the first committed step of glycolysis)
- A second phosphofructokinase (PFK-2) synthesizes fructose 2,6-bisphosphate (F2,6BP)
4. Aldolase

- Aldolase cleaves the hexose F1,6BP into two triose phosphates: glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP)
- Reaction is near-equilibrium, not a control point
- Mechanism is common for cleaving C-C bonds in biological systems (and C-C bond formation in the reverse direction)
5. Triose Phosphate Isomerase (TPI)

- Conversion of DHAP into glyceraldehyde 3-phosphate (GAP)
- Reaction is very fast (diffusion controlled), and only the D-isomer of GAP is formed
- Radioisotopic tracer studies show:
  One GAP molecule: C1,2,3 from Glucose C4,5,6
  Second GAP: C1,2,3 from Glucose C3,2,1

6. Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH)

- Conversion of GAP to 1,3-bisphosphoglycerate (1,3BPG)
- Molecule of NAD+ is reduced to NADH
- Oxidation of the aldehyde group of GAP proceeds with large negative free-energy change
Conservation of oxidative energy

- Energy from oxidation of GAP aldehyde is conserved in acid-anhydride linkage of 1,3BPG
- Next step of glycolysis uses the high-energy phosphate of 1,3BPG to form ATP from ADP
- Mechanism of GAPDH shows how an energy-rich compound forms in an oxidation reaction

Reaction of GAPDH: GAP converted to 1,3BPG

\[
\begin{align*}
\text{Glyceraldehyde 3-phosphate} & \quad \rightarrow \\
\text{1,3-Bisphosphoglycerate} & \quad \text{NADH} \quad + \quad \text{H}^+ \quad + \quad \text{ADP} \quad \rightarrow \quad \text{ATP} \quad + \quad \text{NAD}^+ \quad + \quad \text{Pi}
\end{align*}
\]

Fig 11.7

- Mechanism of GAPDH (3 slides)

Fig 11.7 (continued)
7. Phosphoglycerate Kinase (PGK)

- Transfer of phosphoryl group from the energy-rich mixed anhydride 1,3BPG to ADP yields ATP and 3-phosphoglycerate (3PG)
- Substrate-level phosphorylation - Steps 6 and 7 couple oxidation of an aldehyde to a carboxylic acid with the phosphorylation of ADP to ATP

Phosphoglycerate kinase reaction

Box 11.2 Arsenate ($AsO_4^{3-}$) poisoning

- Arsenate can replace $P_i$ as a substrate for G3PDH
- Arseno analog which forms is unstable

Phosphoglycerate reaction
8. Phosphoglycerate Mutase

- Catalyzes transfer of a phosphoryl group from one part of a substrate molecule to another
- Reaction occurs without input of ATP energy
- Mechanism requires 2 phosphoryl-group transfer steps
- Enzymes from animal muscle and yeast have a different mechanism than does plant enzyme
9. Enolase: 2PG to PEP

- **3-Phosphoglycerate (3PG)** is dehydrated to **phosphoenolpyruvate (PEP)**
- Elimination of water from C-2 and C-3 yields the enol-phosphate PEP
- PEP has a **very high** phosphoryl group transfer potential because it exists in its unstable enol form

10. Pyruvate Kinase (PK)

- Catalyzes a substrate-level phosphorylation
- Metabolically irreversible reaction
- Regulation both by allosteric modulators and by **covalent modification**
- Pyruvate kinase gene can be regulated by various hormones and nutrients
11.3 The Fate of Pyruvate

1. **Aerobic conditions**: oxidized to acetyl CoA which enters the citric acid cycle for further oxidation.

2. **Anaerobic conditions (microorganisms)**: conversion to ethanol.

3. **Anaerobic conditions (muscles, red blood cells)**: conversion to lactate.

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**A. Metabolism of Pyruvate to Ethanol (yeast - anaerobic)**

- Two reactions required:
  1. Pyruvate carboxylase
  2. Alcohol dehydrogenase

Pyruvate $\xrightarrow{(1)}$ Acetaldehyde $\xrightarrow{(2)}$ Ethanol
Fig 11.11

- Anaerobic conversion of pyruvate to ethanol (yeast)

B. Reduction of Pyruvate to Lactate (muscles - anaerobic)

- Muscles lack pyruvate dehydrogenase and cannot produce ethanol from pyruvate
- Muscle lactate dehydrogenase converts pyruvate to lactate
- This reaction regenerates NAD⁺ for use by GAPDH in glycolysis

Recycling of lactate

- Lactate formed in skeletal muscles during exercise is transported to the liver
- Liver lactate dehydrogenase can reconvert lactate to pyruvate
- Lactic acidosis can result from insufficient oxygen (an increase in lactic acid and decrease in blood pH)
Reduction of pyruvate to lactate

Overall reactions for glucose degradation to lactate

• Two ATP per molecule glucose consumed
• No oxygen is required

Glucose + 2 P_{i} + 2 ADP^{3-} → 2 Lactate^{−} + 2 ATP^{4-} + 2 H_{2}O

11.4 Free-Energy Changes in Glycolysis

• Actual free-energy changes (ΔG) large only for:
  #1 (hexokinase)
  #3 (phosphofructokinase)
  #10 (pyruvate kinase)
• These steps are metabolically irreversible, and these enzymes are regulated
• ΔG for all other steps are close to zero (they are near-equilibrium in cells)
11.5 Regulation of Glycolysis

1. When ATP is needed, glycolysis is activated
   • AMP and fructose 2,6-bisphosphate (F2,6BP) relieve the inhibition of PFK-1 by ATP
2. When ATP levels are sufficient, glycolysis activity decreases
   • PFK-1 is inhibited by ATP and citrate
   • Hexokinase inhibited by excess glucose 6-phosphate

Fig 11.13 Metabolic regulation of glycolysis

A. Regulation of Hexose Transporters
   • Glucose enters mammalian cells by passive transport down a concentration gradient from blood to cells
   • GLUT is a family of six passive hexose transporters
   • Glucose uptake into skeletal and heart muscle and adipocytes by GLUT 4 is stimulated by insulin
   • Other GLUT transporters mediate glucose transport in and out of cells in the absence of insulin
B. Regulation of Hexokinase

- **Hexokinase** reaction is metabolically irreversible
- G6P (product) levels increase when glycolysis is inhibited at sites further along in the pathway
- G6P inhibits hexokinase isozymes I, II and III
- **Glucokinase** forms G6P in the liver (for glycogen synthesis) when glucose is abundant (activity is modulated by fructose phosphates and a regulatory protein)
C. Regulation of Phosphofructokinase-1

- ATP is a substrate and an allosteric inhibitor of PFK-1
- AMP allosterically activates PFK-1 by relieving the ATP inhibition (ADP is also an activator in mammalian systems)
- Changes in AMP and ADP concentrations can control the flux through PFK-1
- Elevated levels of citrate (indicate ample substrates for citric acid cycle) also inhibit PFK-1

Fig 11.16 Regulation of PFK-1 by ATP and AMP

- AMP relieves ATP inhibition of PFK-1

Regulation of PFK-1 by Fructose 2,6-bisphosphate (F2,6BP)

- F2,6BP is formed from F6P by the enzyme phosphofructokinase-2 (PFK-2)
- Fig 11.17 β-D-Fructose 2,6-bisphosphate

Formation and hydrolysis of F2,6BP

\[
\begin{align*}
\text{Fructose 6-phosphate} & \xrightarrow{\text{PFK-2}} \text{Fructose 2,6-bisphosphate} \\
\text{Fructose 2,6-bisphosphate} & \xrightarrow{\text{P}} \text{Fructose 6-phosphate} \\
\text{Fructose 2,6-bisphosphate} & \xrightarrow{\text{H}_2\text{O}} \text{P}_\text{i} \\
\end{align*}
\]
D. Regulation of Pyruvate Kinase (PK)

- Four PK isozymes exist in mammalian tissues
- PK is allosterically activated by F1,6BP, inhibited by ATP
- Glucagon stimulates protein kinase A which phosphorylates PK converting it to a less active form (liver and intestinal cells)
E. The Pasteur Effect

- Under **anaerobic conditions** the conversion of glucose to pyruvate is **much higher** than under aerobic conditions (yeast cells produce more ethanol and muscle cells accumulate lactate).
- The **Pasteur Effect** is the **slowing of glycolysis** in the presence of oxygen.
- More ATP is produced under **aerobic conditions** than under anaerobic conditions, therefore **less glucose** is consumed aerobically.

11.6 Other Sugars Can Enter Glycolysis

- Glucose is the main **metabolic fuel** in most organisms.
- Other sugars convert to glycolytic intermediates.
- Fructose and sucrose (contains fructose) are major sweeteners in many foods and beverages.
- Galactose from milk lactose (a disaccharide).
- Mannose from dietary polysaccharides, glycoproteins.

A. Fructose Is Converted to Glyceraldehyde 3-Phosphate

Fig 11.21

<table>
<thead>
<tr>
<th>Fructose</th>
<th>Fructose 1-phosphate</th>
</tr>
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<tbody>
<tr>
<td>CH₂OH</td>
<td>CH₂OPO₃²⁻</td>
</tr>
<tr>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>HO</td>
<td>HO</td>
</tr>
<tr>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

**Fig 11.21 (continued)**
B. Galactose is Converted to Glucose 1-Phosphate

Fig 11.22 (continued)

C. Mannose is Converted to Fructose 6-Phosphate

11.7 Formation of 2,3-Bisphosphoglycerate in Red Blood Cells

- 2,3-Bisphosphoglycerate (2,3BPG) allosterically regulates hemoglobin oxygenation (red blood cells)
- Erythrocytes contain bisphosphoglycerate mutase which forms 2,3BPG from 1,3BPG
- In red blood cells about 20% of the glycolytic flux is diverted for the production of the important oxygen regulator 2,3BPG
Fig 11.24

- Formation of 2,3BPG in red blood cells