Glycolysis is the metabolic process of converting 1 molecule of glucose to 2 molecules of pyruvate through a series of 10 enzyme catalyzed reactions. All the steps of glycolysis occur in the cytosol of a cell and do not require \( O_2 \).

Note: converting pyruvate to acetyl-CoA and then acetyl-CoA entering the TCA cycle indirectly requires \( O_2 \).

Overall Reaction:

\[
\text{Glucose} + 2\text{ADP} + 2\text{NAD}^+ + 2\text{Pi} \rightarrow 2\text{pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O}
\]

**Glucose Facts:**

A) Main fuel source of brain. The brain uses about 120 g of glucose per day.

Only fuel source for red blood cells, cornea, lens (because these cells have no mitochondria).

Kidney, medulla, testes, leukocytes, white muscle fibers use a lot of glucose because these cells have very few mitochondria.

B) Starch is the storage form of glucose in plants, and glycogen is the storage form of glucose in animals. The liver and the muscles are the main storage sites for glycogen. We only store enough glycogen to survive on for about 1 day.

C) The body maintains a constant level of glucose in the blood.

1) Ingest Glucose
2) Glucose passes through cells of intestinal tract and enters portal blood → bloodstream
3) Pancreas monitors the level of glucose in the bloodstream and produces hormone regulators of blood glucose levels

↓ levels too low, produces glucagon which signals the liver to put more glucose into the bloodstream

↑ levels too high, produces insulin which signals cells to increase uptake of glucose and signals liver to take in glucose and store it as glycogen

**Glycolysis Pathway:**

See figure 14-1 for the glycolysis pathway.
**Gibbs Free Energy Changes for the Glycolytic Enzymes**

<table>
<thead>
<tr>
<th>Rxn#</th>
<th>Enzyme</th>
<th>$\Delta G$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexokinase</td>
<td>-33.5</td>
</tr>
<tr>
<td>2</td>
<td>Phosphoglucoisomerase</td>
<td>-2.5</td>
</tr>
<tr>
<td>3</td>
<td>Phosphofructokinase</td>
<td>-22.2</td>
</tr>
<tr>
<td>4</td>
<td>Aldolase</td>
<td>-1.3</td>
</tr>
<tr>
<td>5</td>
<td>Triose phosphate isomerase</td>
<td>+2.5</td>
</tr>
<tr>
<td>6</td>
<td>Glyceraldehyde-3-phosphate</td>
<td>-3.4</td>
</tr>
<tr>
<td>7</td>
<td>Glyceraldehyde-3-phosphate</td>
<td>+2.5</td>
</tr>
<tr>
<td>8</td>
<td>Phosphoglycerate mutase</td>
<td>+1.6</td>
</tr>
<tr>
<td>9</td>
<td>Enolase</td>
<td>-6.6</td>
</tr>
<tr>
<td>10</td>
<td>Pyruvate kinase</td>
<td>-33.4</td>
</tr>
</tbody>
</table>

**Step 1: 1. phosphoryl transfer** (phosphate group attached to the sixth carbon)

\[
\text{ATP-Mg}^{2+} \rightarrow \text{ADP} \quad \text{glucose} \rightarrow \text{glucose 6-phosphate} \quad \text{hexokinase}
\]

This reaction is coupled to the hydrolysis of ATP which results in an energetically favorable reaction (large, negative $\Delta G$= irreversible rxn). This reaction is not unique to glycolysis. In fact, all glucose is phosphorylated once it enters the cell, no matter what its ultimate fate is (glycolysis, glycogenesis, pentose phosphate pathway).

→ There are two enzymes which can carry out this reaction:

- **Hexokinase**: all cells, $K_M = 0.1$ mM, product inhibited, can phosphorylate all hexose sugars (glucose, mannose, fructose)
- **Glucokinase**: liver only, $K_M = 10$mM, not product inhibited, specific for only D-glucose, amt. of enzyme is controlled by insulin

→ Cells often have transport systems built into their membranes for uptake of fuel molecules such as glucose. Phosphorylated glucose is no longer recognized by the glucose transport system and is therefore trapped in the cell. There is no transport system for phosphorylated glucose. The glucose transporter is a passive transporter. Converting glucose to G6P depletes the level of glucose inside the cell, thus keeping glucose flowing inside the cell down its concentration gradient.

→ **Binding.** Substrates must be recognized by the active site of the enzyme which operates on them. There are two factors which affect this - shape and charge distribution. The strength of the interaction can depend much on the charges. Monosaccharide have polar bonds, allowing distinctive hydrogen bonds to form. This allows an enzyme to be highly specific. However, to provide a strong interaction
between substrate and enzyme a large, electrically charged functional group is needed. So a phosphate group is a useful handle on to the substrate. This is particularly true of three carbon sugars which are capable of forming only a small number of hydrogen bonds with the active site of an enzyme.

**Step 2: isomerization**

\[
\text{Glucose-6-phosphate} \rightleftharpoons \text{Fructose 6-phosphate}
\]

This reaction is an isomerization in which an aldose is converted to a ketose. The enzyme must first open the ring of G6P, catalyze the isomerizations, and then reclose the five-membered ring of F6P. This reaction is reversible and is also used in the gluconeogenesis pathway.

**Step 3: phosphoryl transfer** (phosphate group attached to the first carbon)

\[
\text{ATP- Mg}^{2+} \rightarrow \text{ADP}
\]

\[
\text{Fructose 6-phosphate} \rightarrow \text{Fructose 1,6-diphosphate}
\]

This reaction is coupled to the hydrolysis of ATP which results in an energetically favorable reaction (large, negative \(\Delta G\) = irreversible rxn). This reaction is unique to glycolysis and is considered the major rate-limiting step of the pathway. Phosphofructokinase is subject to a lot of regulation:

- ATP and citrate inhibits
- AMP activates

\[
\rightarrow \text{There are two ATP binding sites on this enzyme. One is a substrate binding site (since ATP is a cofactor for the reaction) with a small } K_M \text{ value (binds ATP even at low concentrations). The other site is an allosteric inhibitory site with a high } K_M \text{ (only binds ATP when the concentration is high).}
\]

\[
\rightarrow \text{The citrate binding site is an allosteric inhibitory site. The amount of citrate is indicative of the TCA cycle activity. When the citrate concentration is high, the cell is actively metabolizing other fuel sources (like fatty acids) , so glucose is conserved by inhibiting glycolysis.}
\]

**Step 4: Splitting of six-carbon compound** into two three-carbon compounds (phosphate group is attached to third carbon of each compound).

\[
\text{Aldolase}
\]

\[
\text{Fructose 1,6-bisphosphate} \rightleftharpoons \text{2 glyceraldehyde 3-phosphate + dihydroxyacetone phosphate}
\]

GAP \hspace{2cm} DHAP

This is a reversible reaction and is also used in gluconeogenesis. This reaction is a variant of an aldol cleavage and an aldol condensation, thus the enzyme is named aldolase. GAP (aldose) and DHAP (ketose) are isomers of one another and can be easily converted from one to the other.
**Step 5:** Conversion of DHAP into GAP

*Triose phosphate isomerase*

\[
\text{DHAP} \leftrightarrow \text{GAP}
\]

This reaction is rapid and reversible. The equilibrium actually lies to the left, however the reaction proceeds readily from DHAP to GAP because the GAP product is removed in the next reaction of glycolysis. → We now see why the conversion to fructose was necessary in step 2 of glycolysis. Had we cleaved glucose with aldose, a 2-carbon and a 4-carbon fragment would have resulted. These two fragments would not be interchangeable, thus we would need to separate paths to extract the energy in each fragment.

**Step 6:** reduction of NAD⁺

\[
\text{Pi} + \text{NAD}⁺ \longrightarrow \text{NADH} + \text{H}⁺
\]

*GAP dehydrogenase*

This is an oxidoreductase reaction in which GAP is oxidized and NAD⁺ is reduced. The energy obtained from this redox reaction (ΔG° is about -50 kJ/mole) is enough to attach an inorganic phosphate (not from ATP ***critical***) onto GAP (unfavorable reaction with a ΔG° of about +47 kJ/mole). The reaction can be viewed as the sum of two processes: the oxidation of the aldehyde to a carboxylic acid by NAD⁺, and the joining of the carboxylic acid and inorganic phosphate to form the acyl-phosphate product. The key to this reaction is the formation of a high energy thioester intermediate on the enzyme. See figure 14-9 in book for the enzyme mechanism.

→ Must have a supply of NAD⁺ in cytosol for glycolysis to keep going.

→ Under aerobic conditions, the electrons from the NADH produced in this step must be shuttled into the mitochondria using either the malate aspartate shuttle system or the α-glycerol phosphate shuttle system. The use of the shuttle systems result in NAD⁺ left in the cytosol and NADH (or FADH₂ depending on shuttle) in the mitochondria which enters the electron transport system.

→ Under anaerobic conditions, the electron transport system no longer functions; therefore, the pyruvate produced at the end of glycolysis is reduced to lactate and NADH is oxidized back to NAD⁺.
Step 7: formation of ATP

\[
1,3\text{-bisphosphoglycerate} + \text{ADP} \leftrightarrow 3\text{-phosphoglycerate} + \text{ATP}
\]

Phosphoglycerate kinase

Note - the enzyme is named for the reverse reaction.

1,3-bisphosphoglycerate is an energy-rich molecule with a greater phosphoryl-transfer potential than that of ATP. This formation of ATP is referred to as substrate-level phosphorylation and does not require any O₂.

In essence, the energy released during the redox reaction in step 6 is used to transfer an inorganic phosphate molecule onto ADP.

Step 8: phosphoryl shift

Phosphoglycerate mutase

\[
\text{3-phosphoglycerate} \leftrightarrow \text{2-phosphoglycerate}
\]

#-phosphoglycerate is not an energy-rich molecule that would be capable of transferring its phosphate group to ADP. Therefore, the molecule must undergo two rearrangements in order to form an energy-rich molecule. Step 8 is the first of these rearrangements. Here, the phosphate is simply moved from carbon 3 to carbon 2. This step is reversible, and the enzyme is used again in gluconeogenesis.

Step 9: dehydration

Enolase

\[
\text{2-phosphoglycerate} \leftrightarrow \text{phosphoenolpyruvate} + \text{H}_2\text{O}
\]

PEP

In this step, the dehydration of 2-phosphoglycerate introduces a double bond creating an enol. An enol phosphate has a high phosphoryl-transfer potential, because the phosphate group traps the molecule in its unstable enol form creating an energy-rich molecule. When the phosphate group is donated to ADP, the enol is converted to the more stable ketone (pyruvate).

Step 10: formation of ATP

\[
\text{PEP} + \text{ADP} + \text{H}^+ \rightarrow \text{pyruvate} + \text{ATP}
\]

Pyruvate kinase

In this step, the phosphate group is donated from PEP to ADP to form ATP. This step is irreversible. The enzyme is named for the reverse reaction, even though the reaction is irreversible (????).
**Lactic Acid Fermentation**

Reaction occurs in cells without mitochondria (RBC) or in cells when O$_2$ is limited (muscle cells during exercise). The entire purpose of this reaction is to convert the NADH produced in step 6 of glycolysis back to NAD$^+$ so that glycolysis can continue. This is simply a redox reaction in which pyruvate is reduced to lactate by the enzyme lactate dehydrogenase.

![Pyruvate to Lactate Reaction](image)

Pyruvate $\rightarrow$ Lactate

Lactate can then enter the bloodstream and travel to the liver where it is converted back to glucose through the gluconeogenesis pathway.

**Conversion of Pyruvate to Acetyl-CoA**

Much more energy can be obtained from glucose if it is oxidized completely to CO$_2$ and H$_2$O through the TCA cycle and electron transport system (requires O$_2$ and mitochondria). The entry to the TCA cycle is through acetyl-CoA. Pyruvate is converted into acetyl-CoA by the enzyme pyruvate dehydrogenase (multi-enzyme complex) which is located inside the mitochondria.

![Pyruvate to Acetyl-CoA Reaction](image)

**ATP Derived from Glucose**

1. No O$_2$ available or no mitochondria: Glucose + 2Pi + 2ADP $\rightarrow$ 2 lactate + 2ATP + 2 H$_2$O

Glucose must be converted to lactate, 2 ATP produced.

2. Complete oxidation of Glucose to CO$_2$ and H$_2$O:

<table>
<thead>
<tr>
<th>Conversion</th>
<th>Products</th>
<th>ATP formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose $\rightarrow$ 2 Pyruvate</td>
<td>2 ATP, 2 NADH</td>
<td>2ATP, 4 ATP (α-GP shuttle) or 6 ATP(M-A shuttle)</td>
</tr>
<tr>
<td>2 Pyruvate $\rightarrow$ 2 acetyl-CoA</td>
<td>2 NADH</td>
<td>6 ATP</td>
</tr>
<tr>
<td>2 acetyl-CoA into TCA cycle</td>
<td>2 GTP, 6 NADH, 2 FADH$_2$</td>
<td>2 ATP, 18 ATP, 4 ATP, 36 or 38 ATP</td>
</tr>
</tbody>
</table>
**Regulation of Glycolysis**

Glycolysis is a tightly regulated pathway. Only the irreversible enzymes (hexokinase, phosphofructokinase, pyruvate kinase) can be regulated. Phosphofructokinase is by far the most regulated enzyme. Regulation can occur in several ways:

1. allosteric modulators
2. hormone control (through phosphorylation/dephosphorylation)
3. de novo synthesis

**Inhibitor and Toxins**

1. Arsenate – affects GAP dehydrogenase
   Arsenate looks like Pi and is added to GAP by GAP dehydrogenase instead of Pi. The arsenate group is removed by phosphoglycerate kinase in step 7, but no ATP is generated in this step. Therefore, get 0 net ATP from glycolysis. Glycolysis is not halted, but no ATP is produced. This is detrimental to RBC.
2. Iodoacetate (iodoacetamide) – inhibits GAP dehydrogenase
   Inhibits GAP dehydrogenase by irreversibly binding to the Cys residue in the active site rendering the enzyme inactive.
3. Fluoride ion – inhibits enolase
   Fluoride ion complexes with the Mg+2 ion at the active site of the enzyme rendering the enzyme inactive.

**Pentose Phosphate Pathway (PPP)**

See Figure 14.29 in book for metabolic pathway.

The primary functions of this pathway are:
1. To generate reducing equivalents, in the form of NADPH, for reductive biosynthesis reactions within cells (ex: fatty acid synthesis, steroid synthesis).
2. To provide the cell with ribose-5-phosphate (R5P) for the synthesis of the nucleotides and nucleic acids.
3. Although not a significant function of the PPP, it can operate to metabolize dietary pentose sugars derived from the digestion of nucleic acids as well as to rearrange the carbon skeletons of dietary carbohydrates into glycolytic/gluconeogenic intermediates (GAP, fructose 6-phosphate)

→ about 30% of the oxidation of glucose in the liver occurs via the PPP  
→ The enzymes of the PPP are located in the cytoplasm.  
→ The oxidative steps (1-3) generate the NADPH: For every molecule of glucose that enters the PPP pathway, 2 molecules of NADPH are regenerated from NADP⁺.  
→ The nonoxidative steps of the PPP pathway are designed to generate R5P. If R5P is not needed in the cell, this 5 carbon sugar can be converted into a 6-carbon sugar (fructose-6-phosphate) and a 3-carbon sugar (GAP) which can be utilized in the glycolysis or gluconeogenesis pathways. (Note: it takes 3 molecules of R5P to make 2 molecules of F6P and one molecule of GAP)

Transketolase and transaldolase are enzymes involved in the rearrangement of carbon skeletons.

→ Transketolase is an enzyme which transfers C2 units and requires thiamine pyrophosphate (TPP) as a cofactor.
→ Transaldolase is an enzyme which transfers C3 units. The reaction involves a Schiff base formation between the substrate and an essential lysine residue in the active site of the enzyme.
→ These two enzymes can be reversed in times when R5P need is high in the cell.

**PPP Regulation:**

See figure 14.34

The glucose-6-phosphate dehydrogenase enzyme is the main site of regulation. This enzyme is regulated by the NADP+ concentration in the cell.