Glycolysis

Glucose utilization in cells of higher plants and animals
Glycolysis

What is it?
10-step metabolic pathway
Starts the breakdown of glucose to get energy

Diagram:

- Glycogen breakdown to glucose
- Glycogen synthesis from glucose
- Gluconeogenesis from glutamate
- Gluconeogenesis from pyruvate
- glucose to glycogen conversion
- Acetyl CoA formation from pyruvate
- Acetyl CoA to citric acid cycle
- Citric acid cycle to ATP
- ATP to ADP and P_i
- ADP and P_i to ATP
- ATP to oxidative phosphorylation
- Oxidative phosphorylation to H_2O and CO_2
- H_2O and CO_2 to Fatty acids
- Fatty acids to Fats
- Fats to Fat synthesis
- Fat synthesis to Fats
- Fatty acids to Fat oxidation
- Fat oxidation to Fatty acids
- Fatty acids to glucose
Glycolysis

Glycolysis consists of reactions that convert glc to pyruvate
Under aerobic conditions: pyruvate $\rightarrow$ acetyl CoA
Under anaerobic conditions: pyruvate $\rightarrow$ ethanol + CO$_2$ or pyruvate $\rightarrow$ lactate
Glycolysis

Glucose → Glucose 6-phosphate via hexokinase

Glucose 6-phosphate → Fructose 6-phosphate via phosphofructokinase

Fructose 6-phosphate → Triose phosphate via aldolase

Triose phosphate → Dihydroxyacetone phosphate via triose phosphate isomerase

Dihydroxyacetone phosphate → Glyceraldehyde 3-phosphate via glyceraldehyde 3-phosphate dehydrogenase

Glyceraldehyde 3-phosphate → 1,3-Bisphosphoglycerate via phosphoglycerate kinase

Net Reaction:

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

ATP formation coupled to glycolysis

Glucose + 2NAD$^+$ → 2pyruvate + 2NADH + 2H$^+$
Δ$G^\circ$ = -146 kJ/mol

2ADP + 2P$_i$ → 2ATP + 2H$_2$O
Δ$G^\circ$ = 2 x 30.5 kJ/mol = 61 kJ/mol

Net reaction:
Glc + 2NAD$^+$ + 2ADP + 2P$_i$ → 2pyr + 2NADH + 2H$^+$ + 2ATP + 2H$_2$O
ΔΔ$G^\circ$ = -146 kJ/mol + 61 kJ/mol = **-85 kJ/mol**

After glycolysis, pyruvate goes on to be oxidized completely to CO$_2$ and H$_2$O which has a total standard Δ$G^\circ$ = **-2840 kJ/mol**
Glycolysis

**Phosphorylated Intermediates**
Each of the 9 intermediates between glc and pyr are phosphorylated

Functions of intermediates

1. Role of charged phosphate

2. Energy storage
Glycolysis

**Phosphorylated Intermediates**
Each of the 9 intermediates between glc and pyr are phosphorylated

**Functions of intermediates**

1. They are ionized (-) at pH 7, because membrane is impermeable to charged species, intermediates cannot diffuse out of cell

2. Conserve metabolic energy, energy released in breaking phoshoanhydride bonds (ATP) is partially conserved in phosphate ester (glc-6-phosphate)
**Glycolysis - Prep**

**Reaction 1 - Hexokinase**

Kinases transfer phosphoryl group from ATP to other molecules

\[ \Delta G^{\circ} = -16.7 \text{ kJ/mol} \quad \text{irreversible} \]

Glc enters cell - how?
glc-6-phosphate is not a substrate for transporter
HK in all cells
Glucokinase (isozyme of HK) in hepatocytes, more specific for glc
Glycolysis - Prep

Reaction 2 - Glucose 6-phosphate isomerase
Move carbonyl to carbon 2
\[ \Delta G^\circ = 1.7 \text{ kJ/mol (reversible)} \]
Glycolysis - Prep

Reaction 3 - Phosphofructokinase-1
Adds another phosphoryl group to sugar

**Regulatory enzyme, major point of regulation in glycolysis**

Activity of PFK-1 regulated how?
- by ATP levels
- by other allosteric effectors - F2,6BP

\[ \Delta G^\circ = -14.2 \text{ kJ/mol (irreversible)} \]
Glycolysis - Prep

Reaction 4 - Aldolase
Breaks the 6-carbon compound into two 3-carbon compounds
An aldot cleavage (an aldehyde and an alcohol are generated)

ΔG° = 23.8 kJ/mol (irreversible or reversible - why??)

During glycolysis the products are removed quickly by next two steps, pulling the rxn in the direction of cleavage even though +ΔG°
Glycolysis - Prep

Reaction 5 - Triose phosphate isomerase
Get two identical 3-carbon units
Need glyceraldehyde 3-phosphate to go to next step
End of prep phase

\[ \Delta G^\circ = 7.5 \text{ kJ/mol (reversible)} \]
Glycolysis - Payoff

Reaction 6: Glyceraldehyde 3-phosphate dehydrogenase
Loss of 2e\(^-\) and 2H\(^+\)
First of 2 energy-conserving rxns that form ATP
\(\Delta G^\circ = 6.3 \text{ kJ/mol (reversible)}\)

Biochemists use “~” to indicate bonds with high phosphoryl group transfer potentials

Principle 3 - few important molecules carry the “currencies” of metabolism, NAD\(^+\) = Reducing packet
Glycolysis - Payoff

Reaction 7: Phosphoglycerate kinase (named for reverse rxn)
Harvest the high phosphoryl group transfer potential of 1,3-bisphosphoglycerate to make ATP
$\Delta G^\circ = -18.5$ kJ/mol

Sum of last 2 rxns (steps 6/7)
Glyceraldehyde 3-phosphate + ADP + P$_i$ + NAD$^+$ $\Leftrightarrow$
3-phosphoglycerate + ATP + NADH + H$^+$
$\Delta G^\circ = -12.5$ kJ/mol

Prin. 4  coupled rxns drive energy-requiring processes
Glycolysis - Payoff

Reaction 8 and 9: Phosphoglycerate mutase and Enolase
Rearrange the molecule to produce a form with a high phosphoryl group transfer potential ("~")
Step 8: $\Delta G^\circ = 4.4 \text{ kJ/mol}$
Step 9: $\Delta G^\circ = 7.5 \text{ kJ/mol}$

Large differences in free energies of hydrolysis:
- 2-phosphoglycerate $\rightarrow -17.6 \text{ kJ/mol}$
- Phosphoenolpyruvate $\rightarrow -61.9 \text{ kJ/mol}$
Glycolysis - Payoff

Reaction 10: Pyruvate kinase
Harvest the high phosphoryl group transfer potential of PEP to make ATP
\[ \Delta G^\circ = -31.4 \text{ kJ/mol (irreversible)} \]
Glycolysis - Enzyme mechanisms

1. Same few chemical transformations get used over & over
Glycolysis has:
   - 2 isomerases (+ 1 mutase)
   - 4 kinases
   - dehydrogenase

2. Breaking a carbon-carbon bond: aldolase
Glycolysis - Enzyme mechanisms

Fructose 1,6-bisphosphate

binding and ring opening

Aldolase

In steps 1 and 2 an amine is converted to a Schiff base (imine).

Dihydroxyacetone phosphate

Proton exchange with solution restores enzyme.

second product released

Schiff base is hydrolyzed in reverse of Schiff base formation.

Protonated Schiff base

Enamine intermediate

Glyceraldehyde 3-phosphate

first product released

Protonated Schiff base
Glycolysis - Enzyme mechanisms

Aldolase type I mechanism

Protonated Schiff base acts as an electron sink

Protonated Schiff base (again)

Enamine intermediate
3. Energy coupling: Glyceraldehyde 3-phosphate dehydrogenase

His - general base
Cys - nucleophile
Glycolysis - Enzyme mechanisms

3. Energy coupling: Glyceraldehyde 3-phosphate dehydrogenase

His - general base
Cys - nucleophile