Glycolysis

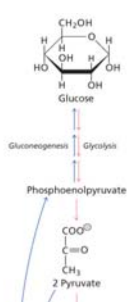
Chapter 16, Stryer Short Course

Glycolysis Expectations

- Memorize/learn Figure 16.1
  - Know overall reaction and stages
  - Explain chemical/physiological purpose of each step
    - Learn structures
    - Reversible/Irreversible step
    - Chemical names from structures
    - Enzyme names from structures

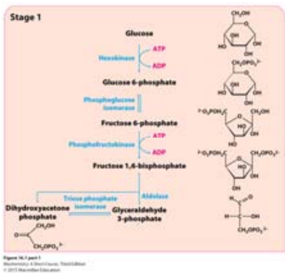
Glycolysis

- Ten enzymes that take glucose to pyruvate
- Cytosol
- Goal: ATP, NADH, pyruvate



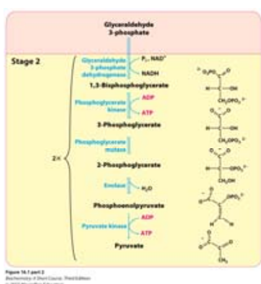
$$\text{Glucose} + 2\,\text{ADP} + 2\,\text{NAD}^{\oplus} + 2\,\text{P}_i \rightarrow 2\,\text{Pyruvate} + 2\,\text{ATP} + 2\,\text{NADH} + 2\,\text{H}^{\oplus} + 2\,\text{H}_2\text{O}$$

Energy Input Stage



- Expend 2 ATP
- Direct glucose toward appropriate metabolic path
- Regulation sites
- Make two identical 3-carbon units

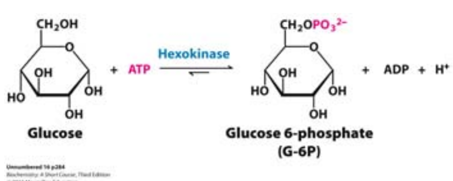
Energy Payoff Stage



- Recoup investment
  - Use energy of oxidation
  - Store high energy electrons as NADH
  - Produce 4 ATP (2 NET ATP)
- Produce pyruvate
  - Building block
  - Ready for further oxidation pathways

1. Hexokinase

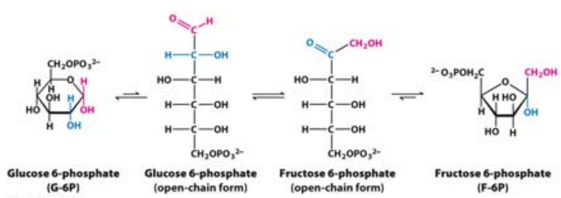
- Irreversible, regulation
- Physiological purposes:



Induced Fit

2. Phosphoglucose Isomerase

- Near-equilibrium
- Chemical purpose



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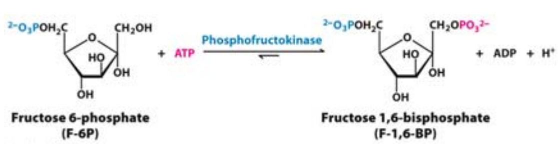
3. Phosphofructokinase-1

- Irreversible, regulation
- Physiological purpose:



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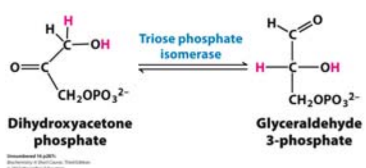
4. Aldolase

- $\Delta G^{o\prime}$ is +23kJ, but near equilibrium reaction
- Chemical logic:

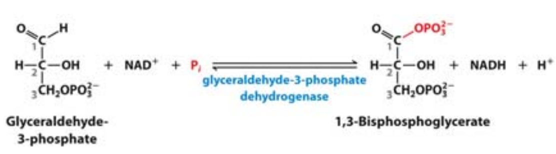
5. Triose Phosphate Isomerase

- Near equilibrium; Catalytic perfection
- Chemical logic:

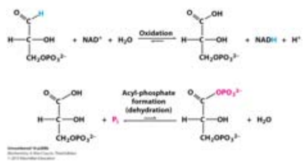


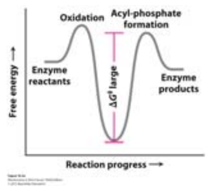
6. Glyceraldehyde-3-P DH

- Redox and dehydrogenases
- Chemical logic purpose



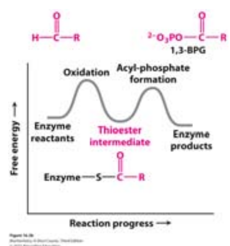
Uncoupled Reaction



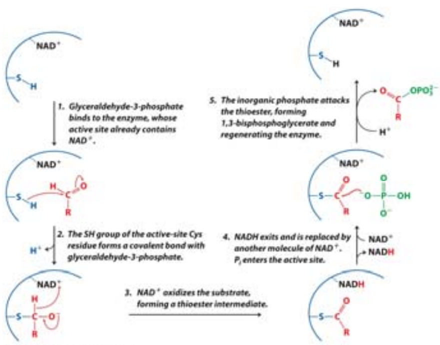


Coupled Reaction

- Coupled through covalent catalysis
- Potential conserved in high energy thioester
- Still slightly uphill reaction…

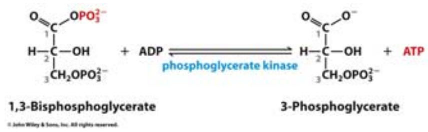


GAPDH Mechanism



7. Phosphoglycerate Kinase

- Substrate level phosphorylation
- Phosphoryl group transfer
- Coupled to reaction 6



Coupled Reactions

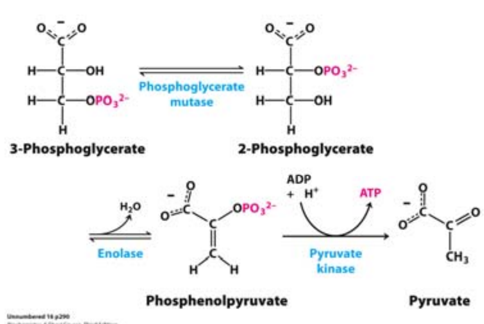
Glyceraldehyde 3-phosphate + $NAD^{\oplus}$ ⟶ 1,3-*Bis*phosphoglycerate + NADH + $H^{\oplus}$

1,3-*Bis*phosphoglycerate + ADP ⟶ 3-Phosphoglycerate + ATP

Glyceraldehyde 3-phosphate + $NAD^{\oplus}$ + P_i + ADP ⟶ 3-Phosphoglycerate + NADH + $H^{\oplus}$ + ATP

- GAPDH: $\Delta G^{o\prime}$ = 6.3 kJ/mol
- PG Kinase: $\Delta G^{o\prime}$ = -18.8 kJ/mol
- Overall:

Reactions 8-10



Thermodynamics

Table 16.1 Reactions of glycolysis

Step	Reaction	Enzyme	Reaction type	$\Delta G^{\circ\prime}$ in kJ mol^{-1} (kcal mol^{-1})	ΔG in kJ mol^{-1} (kcal mol^{-1})
1	Glucose + ATP → glucose 6-phosphate + ADP + H^+	Hexokinase	Phosphoryl transer	−16.7 (−4.0)	−33.5 (−8.0)
2	Glucose 6-phosphate ⇌ fructose 6-phosphate	Phosphoglucose isomerase	Isomerization	+1.7 (+0.4)	−2.5 (−0.6)
3	Fructose 6-phosphate + ATP → fructose 1,6-bisphosphate + ADP + H^+	Phosphofructokinase	Phosphoryl transfer	−14.2 (−3.4)	−22.2 (−5.3)
4	Fructose 1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	Aldolase	Aldol cleavage	+23.8 (+5.7)	−1.3 (−0.3)
5	Dihydroxyacetone phosphate ⇌ glyceraldehyde 3-phosphate	Triose Phosphate isomerase	Isomerization	+7.5 (+1.8)	+2.5 (+0.6)
6	Glyceraldehyde 3-phosphate + P_i + NAD^+ ⇌ 1,3-bisphosphoglycerate + NADH + H^+	Glyceraldehyde 3-phosphate dehydrogenase	Phosphorylation coupled to oxidation	+6.3 (+1.5)	−1.7 (−0.4)
7	1,3-Bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	Phosphoryl transfer	−18.8 (−4.5)	+1.3 (+0.3)
8	3-Phosphoglycerate ⇌ 2-phoshoglycerate	Phosphoglycerate mutase	Phosphoryl shift	+4.6 (+1.1)	+0.8 (+0.2)
9	2-Phosphoglycerate ⇌ Phosphoenolpyruvate + H_2O	Enolase	Dehydration	+1.7 (+0.4)	−3.3 (−0.8)
10	Phosphoenolpyruvate + ADP + H^+ → pyruvate + ATP	Pyruvate kinase	Phosphoryl transfer	−31.4 (−7.5)	−16.7 (−4.0)

Note: ΔG, the actual free-energy change, has been calculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiological conditions. Glycolysis can proceed only if the ΔG values of all reactions are negative. The small positive ΔG values of three of the above reactions indicate that the concentrations of metabolities in vivo in cells undergoing glycolysis are not precisely known.

Table 16.1
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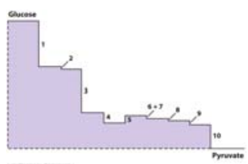
Overall Energetics

- Standard Free energies are up and down
- Free energies under cellular conditions are downhill
  - Three irreversible

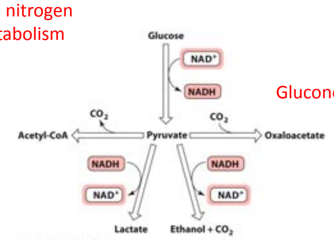


Fate of Pyruvate

Amino acid and nitrogen metabolism

Aerobic Energy

Gluconeogenesis



Anaerobic in higher organisms

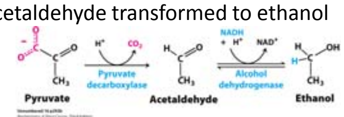
Anaerobic in microorganisms

The Problem of Anaerobic Metabolism

- With oxygen, the NADH produced in glycolysis is re-oxidized back to NAD^+
- NAD^+/NADH is a co-substrate which means…
- If there is no oxygen, glycolysis will stop because…
- The solution to the problem is to…

The solution in Yeast

- Pyruvate is decarboxylated to acetaldehyde
- Acetaldehyde transformed to ethanol



- NAD^+ is regenerated to be reused in GAPDH

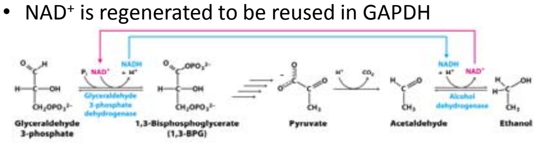


Figure 16.5
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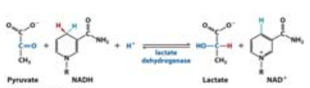
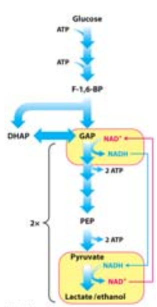
The Solution in Us

- Lactate formation



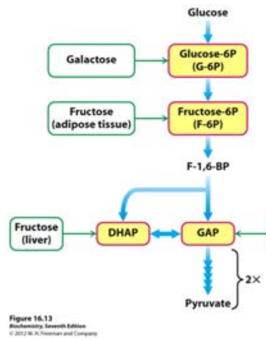


- Balanced equation

$$\text{Glucose} + 2\,P_i^{2-} + 2\,\text{ADP}^{3-} \longrightarrow 2\,\text{Lactate}^{-} + 2\,\text{ATP}^{4-} + 2\,H_2O$$

Other sugars enter glycolysis

Inability to process galactose is rare, but serious, genetic disorder



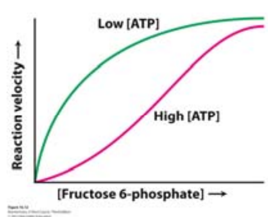
High fructose diet puts sugars through glycolysis while avoiding major regulation step

Regulation Overview

- Irreversible steps
  - **Phosphofructokinase (3)**
  - Hexokinase (1)
  - Pyruvate kinase (10)
- Tissue dependent
  - Muscle: regulated mainly by energy charge
  - Liver: regulated by building blocks, [glucose], hormones
  - Isozymes

Allosteric Regulation of PFK-1

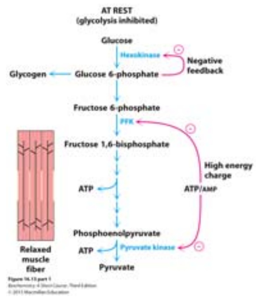
- Based on charge state
- ATP binding inactivates PFK
- AMP binding blocks inactivation
- Why AMP?



ADP + ADP ←→ AMP + ATP

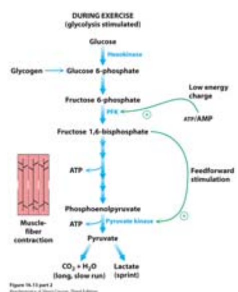
Resting Muscle

- PFK causes G-6-P buildup unless glucose is being stored
- Feedback inhibition
- Hexokinase NOT the committed step
  - If glycogen storage full, send glucose back to the liver!



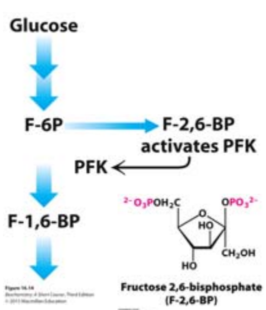
Active Muscle

- Low charge state activates glycolysis
- Feed forward activation to keep flux forward



Liver PFK Regulation

- ATP changes not as important in liver cells
- Citrate is inhibitor
  - Citrate is synthetic building block
  - No need to break down glucose to make precursors—store it!
- F-2,6-bP is activator
  - Signals full glycogen storage
  - Feedforward activation—store it as fat!

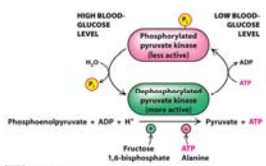


Liver Hexokinase and Glucokinase

- Hexokinase regulated in liver, too
  - But we cannot send glucose out of liver cell
  - Must be picked up even when liver cell does not need glucose and glycogen storage is full
  - Stored as fat
- Glucokinase: isozyme
  - Unregulated
    - Keeps activating glucose even when there is much G-6-P
    - If always active, blood sugar would crash.
    - How do we avoid depriving brain?
  - High Km
    - Only active at high [glucose]

Liver Pyruvate Kinase

- Also allosterically controlled
- Also hormonally controlled
- Low blood sugar = glucagon = inhibited glycolysis
- Allows for gluconeogenesis



General Tissue Glucose Transporters

- GLUT 1 & 3: Km below typical blood glucose (4-8 mM)—works constantly to import glucose
- GLUT 4: sent to cell surface by insulin
  - Increases uptake of glucose

Table 16.3 Family of glucose transporters

Name	Tissue location	K_M	Comments
GLUT1	All mammalian tissues	1 mM	Basal glucose uptake
GLUT2	Liver and pancreatic β cells	15–20 mM	In the pancreas, plays a role in the regulation of insulin In the liver, removes excess glucose from the blood
GLUT3	All mammalian tissues	1 mM	Basal glucose uptake
GLUT4	Muscle and fat cells	5 mM	Amount in muscle plasma membrane increases with endurance training
GLUT5	Small intestine	—	Primarily a fructose transporter

Pancreas and Liver Transporters

- High Km: glucose only taken into these tissues when at high concentration
- Glycolysis is sensor for high [glucose] in pancrease
  - Release of insulin