

## Lecture 27: Metabolic Pathways Part I: Glycolysis

Reading in Campbell: Chapter 17.2-17.5

### Metabolic pathways are:

1. Conserved in different organisms.
2. Compartmentalized in Eukaryotes
3. Overall Irreversible (but most of the individual steps are not)
4. Usually committed after the first step
5. Regulated (usually at first step)

### What you need to know:

1. Input and Output metabolites
2. Steps that control flux
3. How flux is controlled
4. Cellular location of metabolic Steps
5. Selected Enzyme Mechanisms
6. Selected substrates/products

### Central Pathways of Energy Production:

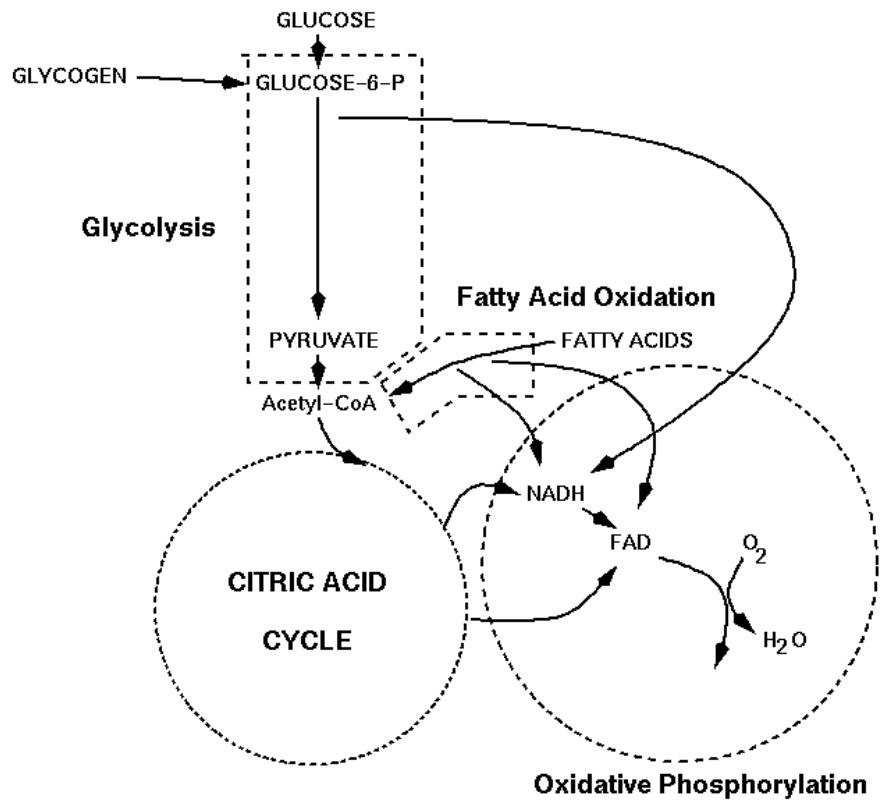
Intracellular locations:

- Glycolysis – cytosol
- Fatty Acid Oxidation: Inner matrix of mitochondria
- Citric Acid Cycle: Inner matrix of mitochondria
- Oxidative Phosphorylation: Inner membrane of mitochondria

### Energy Currency:

Stored in the following ways:

- High energy chemical species (i.e. phosphoanhydride bonds in ATP):
- High energy electron carriers
- Membrane potentials (concentration gradient and voltage difference)



**Glycolysis:**

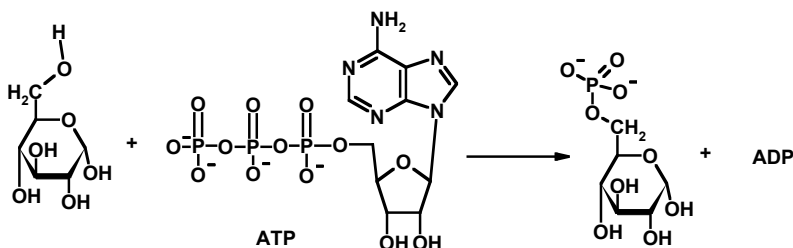
- **Location:** cytosol
- **Input:** Glucose
- **Output:** Pyruvate under aerobic, lactate under anaerobic
- **Energetics:** 2ATP, 2NADH (rapid production of ATP from glucose)
- **Key Controlling Step:** PFK
- **Substrate level phosphorylation**
- **Direct and Indirect Coupling**

**Key Enzyme Reactions**

**A: Hexokinase Reaction (step 1):** Glucose + ATP → Glucose-6-P + ADP

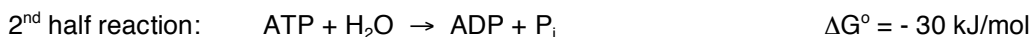
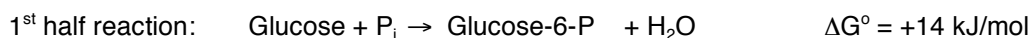
Group transfer reaction: Phosphate is transferred from ATP to glucose.

- Traps glucose in the cell as G-6-P
- Favorable hydrolysis of ATP directly coupled to phosphorylation of glucose. An example of direct coupling.



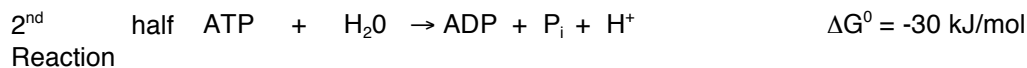
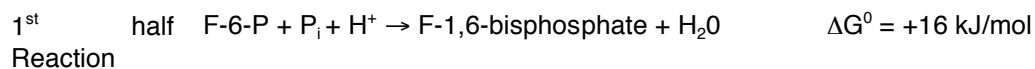
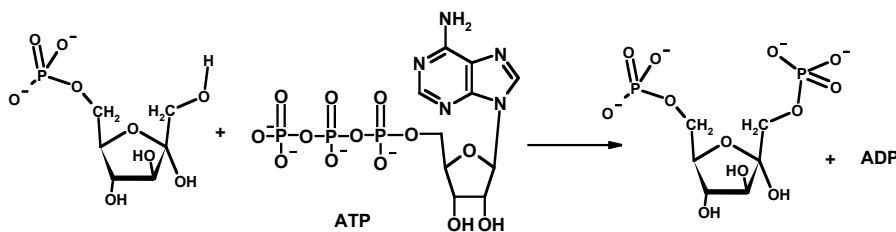
**Energetic coupling:** The high energy of ATP is often used to

drive unfavorable reactions by coupling the favorable hydrolysis of ATP to an unfavorable second reaction. Consider the following 1/2 reactions:



**Net sum:**

**B: Phosphofructokinase (PFK) step 3 [Key regulatory step]:**

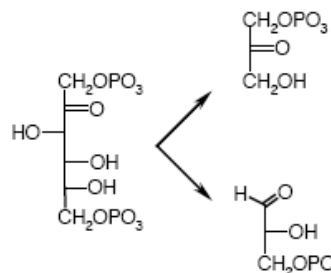


**C. Aldolase (step 4):** Fructose-1-6-bisP → dihydroxyacetone phosphate(DHAP)+glyceraldehyde-3-P (G-3-P).

This is the cleavage of a C6 sugar to give two C3 sugars.

The standard free energy for this reaction is extremely unfavorable -  $\Delta G^\circ = +23.8 \text{ kJ/mol!}$

But the Gibbs free energy is actually favorable,  $\Delta G = -6 \text{ kJ/mol}$ . Is this reaction spontaneous?



How is the concentration of G-3-P reduced to insure that the aldolase reaction is spontaneous?

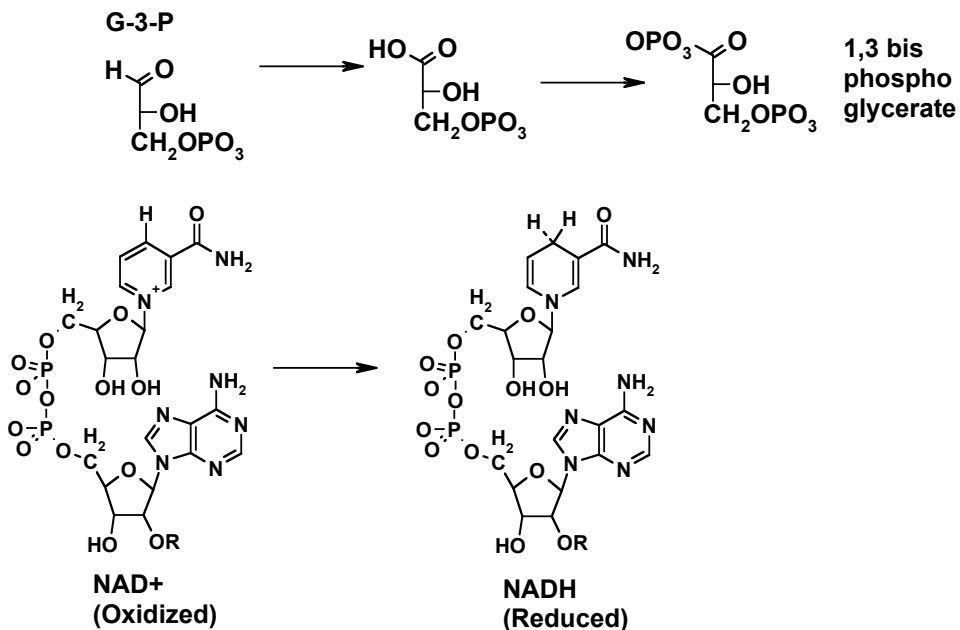
**This completes the “first stage” of glycolysis. Overall  $\Delta G$  for the first 5 steps under cellular conditions is  $-53 \text{ kJ/mol}$ . So far, 2 ATP molecules have been consumed.**

**D. Glyceraldehyde-3-P dehydrogenase (GAPDH) step 6 - Chemical to Redox Energy:**

Glyceraldehyde-3-P dehydrogenase: G-3-P +  $\text{NAD}^+$  → 1,3 bisphosphoglycerate + **NADH**

This reaction proceeds in two steps. The first step is the oxidation of the aldehyde to the carboxylic acid (thioester) using  $\text{NAD}^+$  as the electron acceptor. This results in the formation of a covalent enzyme intermediate. The second step is the phosphorylation of the carboxylic acid.

Both reactions are catalyzed by a single enzyme.



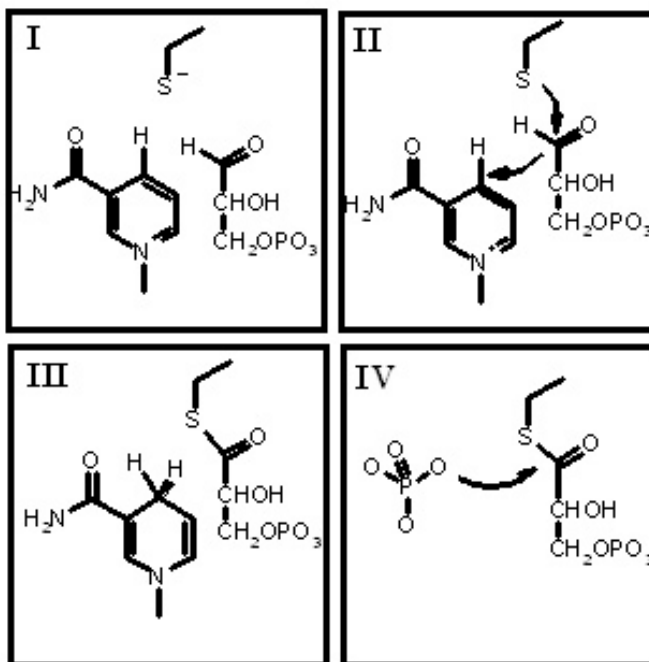
The Reaction Steps are (see diagram)

I. ES complex, Cys is deprotonated.

II. Thio group is a nucleophile, attacks aldehyde, H is transferred to NAD<sup>+</sup> as a hydride ion (H<sup>-</sup>), net transfer of 2 electrons.

III. Stable, thioester intermediate, NADH is in the reduced form and 3-P-G has been oxidized.

IV. Attack of phosphate on stable acyl-enzyme intermediate, regenerating enzyme, and producing 1,3-bis phosphoglycerate.



	Chemistry	Energetics
1st half Reaction	$G - 3 - P \rightarrow 3 \text{ phosphoglycerate} + 2e^- + H^+$ (aldehyde) $\rightarrow$ (carboxylic acid)	$\Delta G^\circ = -100 \text{ kJ/mol}$
2nd half Reaction	$NAD^+ + 2e^- + H^+ \rightarrow NADH$	$\Delta G^\circ = +60 \text{ kJ/mol}$
1st Reaction Sum	$NAD^+ + H^+ + G3P \rightarrow 3PGlycerate + NADH$	$\Delta G^\circ = -40 \text{ kJ/mol}$
2nd Reaction	$3PGlycerate + P_i \rightarrow 1,3\text{bisPGlycerate}$	$\Delta G^\circ = +50 \text{ kJ/mol}$
<b>Overall Sum</b>		<b><math>\Delta G^\circ = +10 \text{ kJ/mol}</math></b>

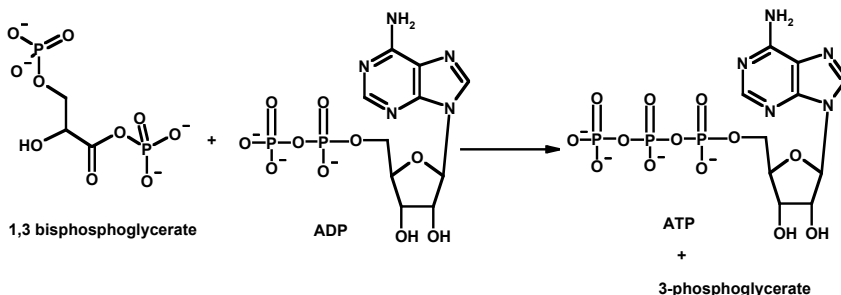
**E. Phosphoglycerate kinase**

(step 7):

Generation of 1<sup>st</sup> ATP by substrate level phosphorylation.

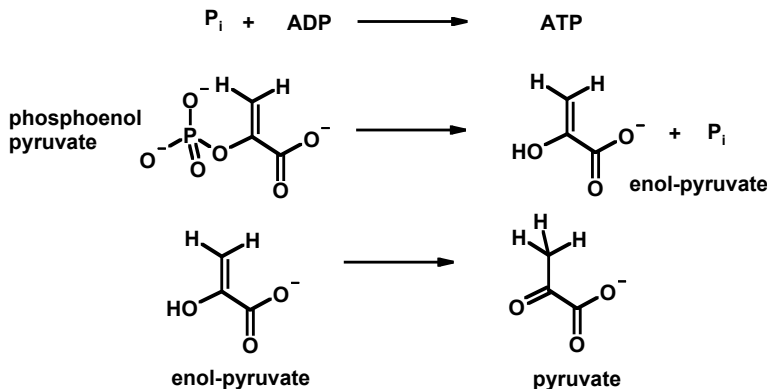
(Break-even reaction)

$\Delta G^\circ = -19 \text{ kJ/mol}$



**F. Pyruvate kinase (step 10):**

Generation of Second ATP by a second substrate level phosphorylation.



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

**Regulation of Biochemical Pathways:**

**General Properties of the Regulation of Biochemical Pathways:**

- Step below a convergence point is usually regulated.
- Usually steps that involve a large negative  $\Delta G$  are regulated, as these are usually irreversible.
- Opposing pathways are coordinately regulated, usually at a single key step.

**Mechanisms of Regulation:**

- Change in levels of enzymes by regulation of the synthesis/degradation. (slow)
- Change in the activity of enzymes by covalent modification (e.g. phosphorylation) of the enzyme (moderately fast)
- Change in the activity of enzymes by feedback inhibition by a chemical that is near the end of the pathway, or in another pathway (Usually allosteric activators/inhibitors). This is fast.
- Product inhibition. This is very fast.

**Regulation of Glycolysis:**

<b>Glucose <math>\leftrightarrow</math> G-6-P</b>	Hexokinase: Inhibited by G-6-P	
<b>Fructose-6-P <math>\leftrightarrow</math> F-1,6-P</b>	Phosphofructose kinase (PFK)	
	<u>Activated:</u> ADP, AMP F2,6-P cAMP	<u>Inhibited:</u> ATP Citrate (TCA) PEP (Glycolysis)
<b>PEP <math>\leftrightarrow</math> Pyr</b>	Pyruvate Kinase:	
	<u>Activated:</u> AMP, F-1,6-P	<u>Inhibited:</u> ATP, acetyl CoA, alanine

