

# LECTURE2-GLYCOLYSIS

Monday, April 14, 2008  
9:16 AM

Metabolism Lecture 2 — GLYCOLYSIS — Restricted for students enrolled in MCB102, UC Berkeley, Spring 2008 ONLY

Bryan Krantz: University of California, Berkeley  
MCB 102, Spring 2008, Metabolism Lecture 2

Reading: Chs. 13 & 14 of *Principles of Biochemistry*, "Bioenergetics and Biochemical Reaction Types" & "Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway."

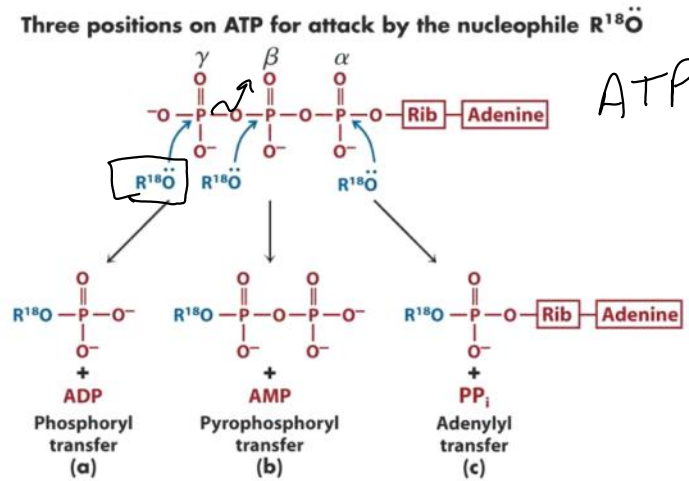
## Phosphate Transfer Potentials

ATP can make various types of modifications to metabolites

- [A] phosphorylation
- [B] pyrophosphorylation
- [C] Adenylylation

Transfer chemically activate the metabolite for future metabolic steps.

Three positions on ATP for attack by the nucleophile  $R^{18}O$



### Phosphate Transfer Potentials

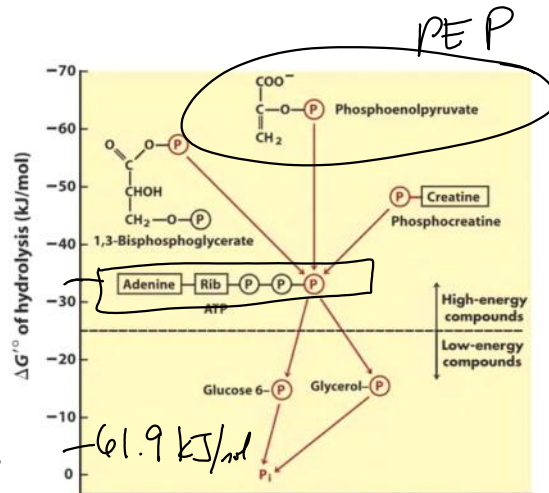
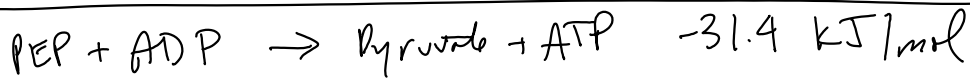
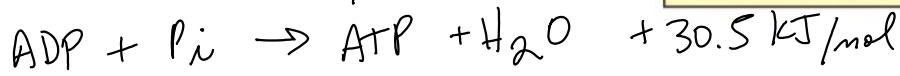
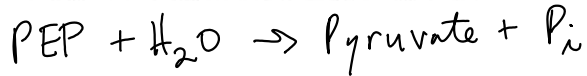
Later these Phosphoryl groups may be removed (hydrolyzed).

Energy of hydrolysis may be thermodynamically coupled to ATP formation.

Phosphate transfer demonstrates why ATP is the central energy currency in the cell.

Quantitative example of phosphoryl transfer & thermodynamic coupling:

Phosphoenolpyruvate (PEP) → Pyruvate



**WHY Phosphorylated Intermediates?**

[1] Keeps intermediates inside cell, P-intermediate negatively charged. This a barrier to transport across the membrane.

[2] Conserve the energy stored in the original ATP

phosphoanhydride bond energy is conserved.

[3] Increased binding energy

ENZYMES ↓ efficiently recognize catalyze RXNS. Use  $Mg^{2+}$ .  
↓ Activation Energy Barrier

**REDOX: Quantifying Metabolic Energy Transduction by Electron Flow**

Reduction/oxidation reaction

Nernst Equation

$$E = E^{\circ} - (RT / nF) \ln[e^{-} \text{ acceptor}]/[e^{-} \text{ donor}]$$

$n$  = number of moles of electrons ( $e^{-}$ ) in reaction

$F$  = Faraday constant 96485 Coulombs / mole of  $e^{-}$

Half reaction (by convention) compared to std. hydrogen electrode (SHE).

$E$  (the redox potential) is basically the voltage required to stop a chemical reaction

Relation of Standard Redox Potential Change to  $\Delta G^{\circ}$

$$\Delta G^{\circ} = -nF \Delta E^{\circ}$$

Std. Redox Potentials in Table 13-7 of Lehninger.

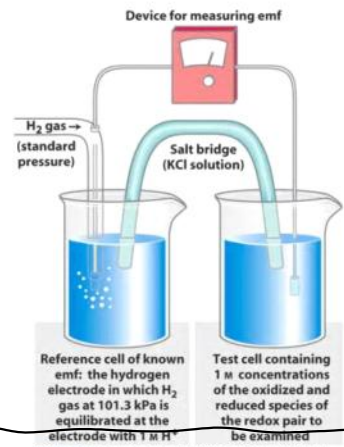


TABLE 13-7 Standard Reduction Potentials of Some Biologically Important Half-Reactions, at pH 7.0 and 25 °C (298 K)

Half-reaction	$E^{\circ}$ (V)
$1/2 O_2 + 2H^{+} + 2e^{-} \rightarrow H_2O$	0.816
$Fe^{3+} + e^{-} \rightarrow Fe^{2+}$	0.771
$NO_2^{-} + 2H^{+} + 2e^{-} \rightarrow NO_2 + H_2O$	0.421
Cytochrome <i>f</i> ( $Fe^{3+}$ ) + $e^{-} \rightarrow$ cytochrome <i>f</i> ( $Fe^{2+}$ )	0.365
$Fe(OH)_3$ (ferricyanide) + $e^{-} \rightarrow Fe(OH)_2$	0.36
Cytochrome $a_3$ ( $Fe^{3+}$ ) + $e^{-} \rightarrow$ cytochrome $a_3$ ( $Fe^{2+}$ )	0.35
$O_2 + 2H^{+} + 2e^{-} \rightarrow H_2O_2$	0.295
Cytochrome <i>a</i> ( $Fe^{3+}$ ) + $e^{-} \rightarrow$ cytochrome <i>a</i> ( $Fe^{2+}$ )	0.29
Cytochrome <i>c</i> ( $Fe^{3+}$ ) + $e^{-} \rightarrow$ cytochrome <i>c</i> ( $Fe^{2+}$ )	0.254
Cytochrome $c_1$ ( $Fe^{3+}$ ) + $e^{-} \rightarrow$ cytochrome $c_1$ ( $Fe^{2+}$ )	0.22
Cytochrome <i>b</i> ( $Fe^{3+}$ ) + $e^{-} \rightarrow$ cytochrome <i>b</i> ( $Fe^{2+}$ )	0.077
Ubiquinone + $2H^{+} + 2e^{-} \rightarrow$ ubiquinol + $H_2$	0.045
Fumarate $^{2-}$ + $2H^{+} + 2e^{-} \rightarrow$ succinate $^{2-}$	0.031
$2H^{+} + 2e^{-} \rightarrow H_2$ (at standard conditions, pH 0)	0.000

Source: Data mostly from Leibel, EA (1976) in *Handbook of Biochemistry and Molecular Biology*, 3rd edn (Fleckenstein, GJ, ed.), Plenum and Chemical Data, Vol. 1, pp. 132-133; CRC Press, Boca Raton, FL.

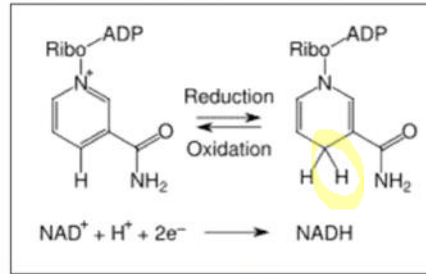
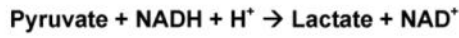
\* This is the value for heme 480-580 bound to a specific hemoprotein (the average nonheme cytochromes has a different  $E^{\circ}$  that depends on its protein environment).

**Nicotinamide adenine dinucleotide (NAD<sup>+</sup>)**

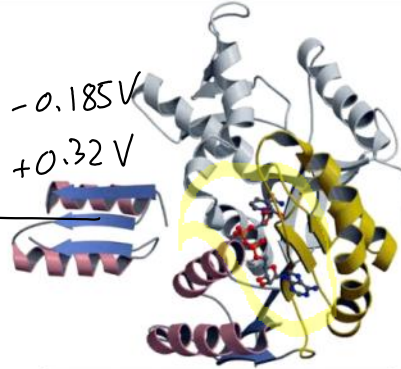
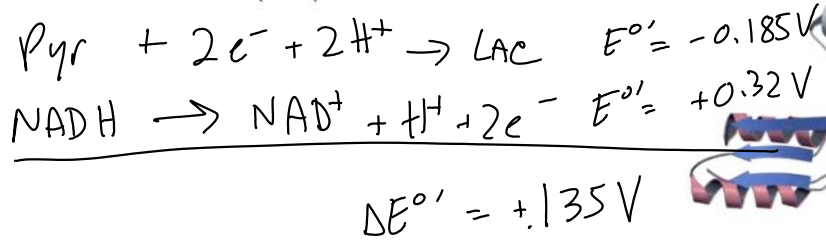
Business end is the nicotinamide ring, where the electrons (and proton) are added.

“Electron carrier” (Others are, e.g., FMN, FAD and NADP.)

Consider a quantitative example:



Think of the two half reactions, where the std. values are obtained from the Table (13-7)



Reaction will go forward and is spontaneous. Remember  $\Delta E^{\circ'}$  is related to the  $\Delta G$  by:

$$\Delta G^{\circ'} = -nF\Delta E^{\circ'}$$

**Rossmann fold** – usual protein fold that recognizes NAD in enzyme active sites.

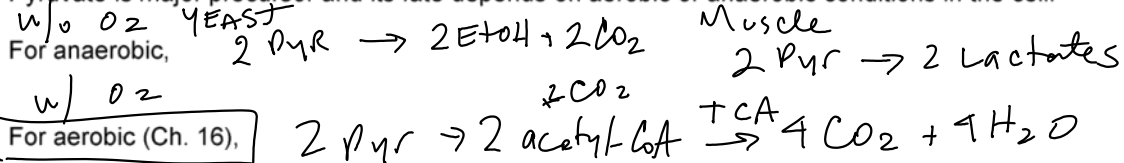
## GLYCOLYSIS

Glucose metabolism is central in all kingdoms of life, as it is an energy rich fuel.

Literally, "splitting sugar"; it takes 10 steps (divided into 2 phases):

### Glucose → 2 Pyruvate

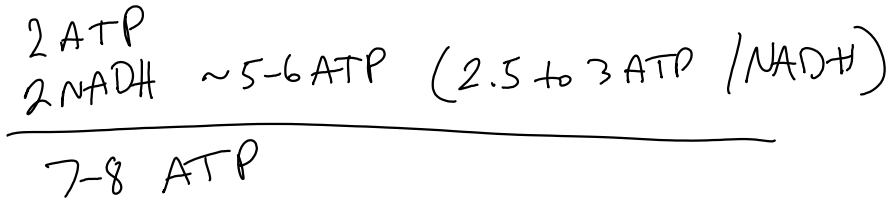
Pyruvate is major precursor and its fate depends on aerobic or anaerobic conditions in the cell.



The basic energy balance for glycolysis in terms of the major energy carriers is:



Under aerobic conditions that's worth:

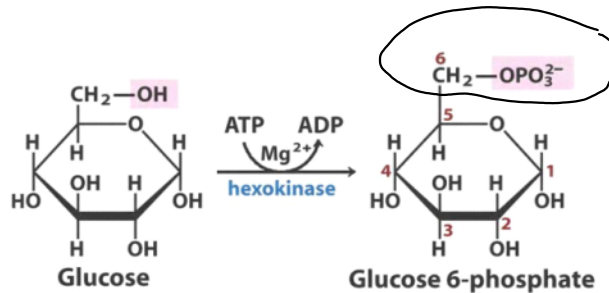
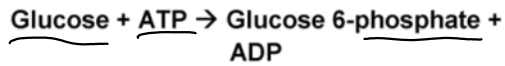


## TWO PHASES OF GLYCOLYSIS:

[A] Preparatory (5 steps) and [B] Pay-off (5 steps)

### [PHASE A] Preparatory Phase

#### [STEP 1] Phosphorylation of Glucose



**Hexokinase** uses high phosphoryl donor potential of ATP to phosphorylate the six position of D-glucose.

$$\Delta G'^{\circ} = -16.7 \text{ kJ/mol}$$

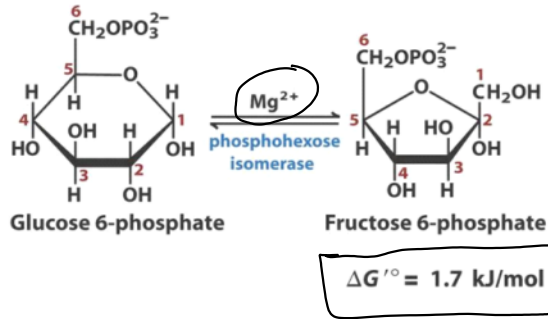
**Mechanism.** Evidence shows ATP→ADP hydrolysis activity so gamma Pi of ATP is transferred to glucose with the aid of the divalent metal ion, Mg<sup>2+</sup>. Big conformational change—induced fit—of 8 Å.

**Other notes.** Multiple isozymes exist in different tissues with different regulatory properties. The enzyme can transfer to other sugars, D-mannose and D-fructose in other tissues.

**[STEP 2] Phosphoglucosomerase**

**Glucose-6-phosphate → Fructose-6-phosphate**

**Mechanism.** Glucose-6-phosphate is an **aldose**, where carbon-one is an aldehyde. Fructose-6-phosphate is a **ketose** in which carbon-one is an alcohol and carbon-two is a ketone. You start from carbon-one of glucose, which is the aldehyde. Carbon-two is an alcohol. This is carbon-one and two of glucose-6-phosphate.

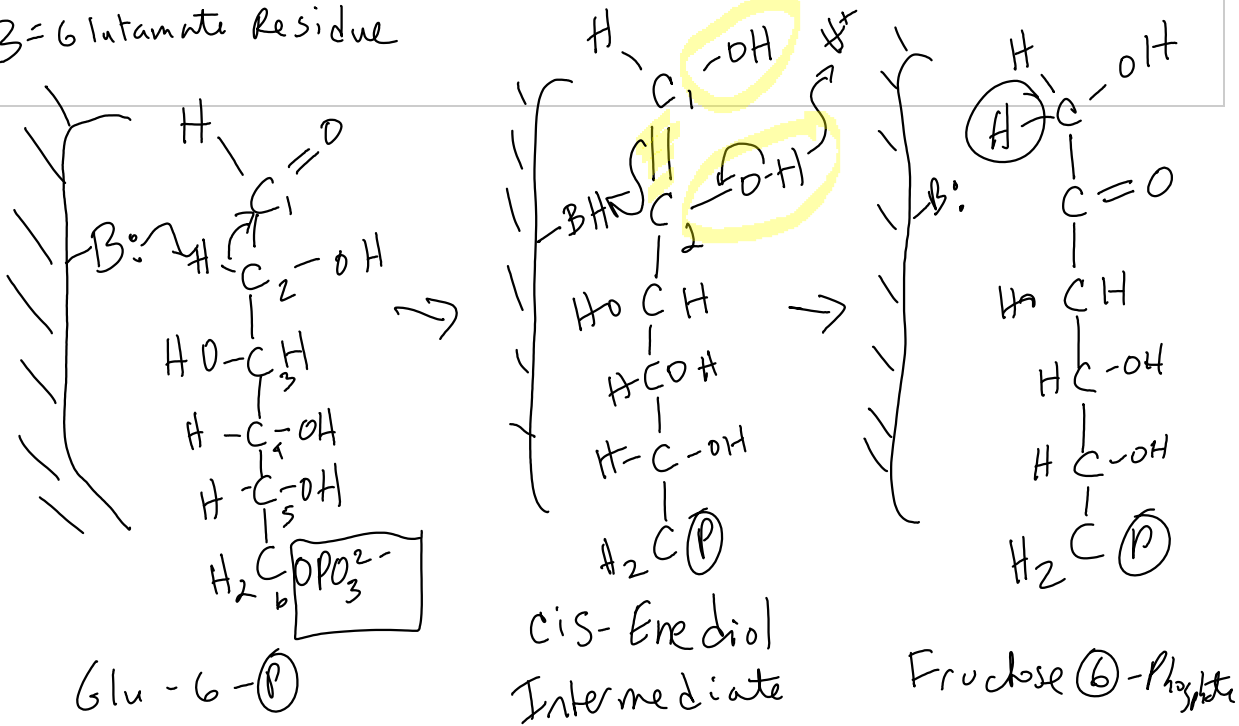


- (i) A base in the enzyme comes in to abstract this proton on carbon-two. Then, the electron moves around and the aldehyde oxygen captures a proton. You get an intermediate that people call **enediol**.
- (ii) In that intermediate, you would have an alcohol on carbon-one and a double bond between carbons one and two (alkene group). The double bond attacks a proton from the original base, which is now an acid. You get this proton attached to carbon-one. Carbon two becomes a ketone, giving fructose-6-phosphate.

**Classic example of general acid/base catalysis.** A general base (glutamate residue in enzyme) comes in to abstract the proton and becomes an acid. Then the acid donates the proton.

**Energetics.** The energy level of the glucose-6-phosphate is similar to the energy level of fructose-6-phosphate  $\Delta G^\circ = 1.7 \text{ kJ/mol}$ . The standard free energy is even unfavorable. *Is this a big deal?*

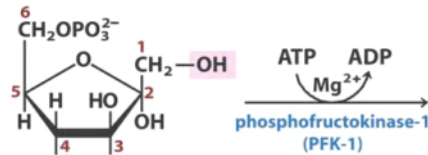
B = Glutamate Residue





**[STEP 3] Phosphofructokinase (PFK).** Adds second phosphate group onto the 1 position.

**Fructose 6-phosphate + ATP → fructose-1,6-bisphosphate**



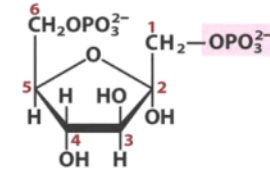
**Mechanism.** Typical kinase reaction.

**Energetics.** Large free energy change typical of kinase due to the phosphoryl transfer potential of ATP, generating a low energy phosphate bond at about 12 kJ/mol. Downhill reaction.

Fructose 6-phosphate

Committed step

$\Delta G'^{\circ} = -14.2 \text{ kJ/mol}$



**PFK Regulation & The Pasteur Effect.** The

fact that PFK is important in regulation came from the old experiments of **Louis Pasteur**, and it is still called the **Pasteur Effect**. Yeast often convert glucose into two molecules of ethanol and two molecules of  $\text{CO}_2$  under anaerobic conditions, but **when Pasteur added oxygen to this system, the generation of ethanol and  $\text{CO}_2$  stopped.**



**Regulation.** Why does PFK become inhibited? With  $\text{O}_2$ , yeast cells metabolize the product of glycolysis in the citric acid cycle and oxidative phosphorylation. That will generate far more ATP than glycolysis can. The citric acid cycle generates about 30 molecules of ATP per glucose. ATP concentration goes up in the yeast cells. There is another site far away from the substrate-binding site, where another ATP binds. This is an **allosteric regulatory site**. When ATP binds here it changes the conformation of the enzyme.

**Regulation in Muscle.** *What happens with muscle?* PFK is the most important regulatory point of the glycolytic pathway in muscle. Unlike in yeast, the ATP concentration is regulated very carefully by other mechanisms. During exercise, ATP levels in your muscle do not go down very far, that is, not enough to affect PFK.

*How do we regulate it?* Muscle cells use an enzyme adenylate kinase (or myokinase). Myokinase catalyzes the interconversion between two ADP to AMP plus ATP.



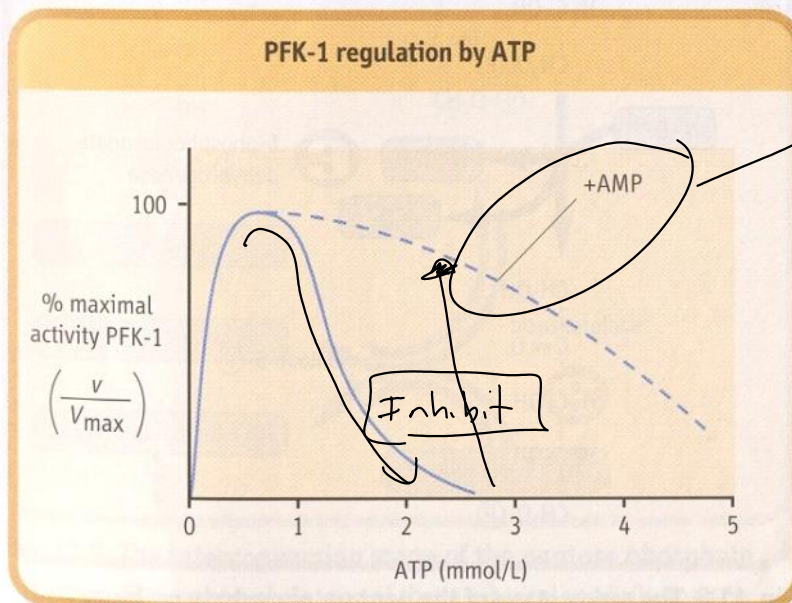
$$K_{eq} = \frac{[\text{AMP}][\text{ATP}]}{[\text{ADP}]^2}$$

In muscle, [ATP] matters indirectly, and [AMP] is a much more important in regulating PFK. When AMP concentration goes up, the activity of the enzyme also goes up. The kinetics will become a more normal saturation curve.

During exercise, the ATP concentration goes down slightly, but that increases the [AMP] tremendously, activating PFK and initiating glycolysis. PFK will perform saturation kinetics when AMP is high in active muscle, and it will perform sigmoidal kinetics when AMP is low in resting muscle.

**AMP is the allosteric effector in muscle.**

**PFK activity catalyzes the committed (irreversible) step in glycolysis, suggesting why it is so regulated. (\*\*MORE types of regulation in Ch. 15\*\*)**



**Fig. 11.6 Allosteric regulation of phosphofructokinase-1 (PFK-1) by ATP. AMP is a potent activator of PFK-1 in the presence of ATP.**