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 MCB 102, Spring 2008, Metabolism Lecture 3

Reading: Ch. 14 of *Principles of Biochemistry*, “Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway.”

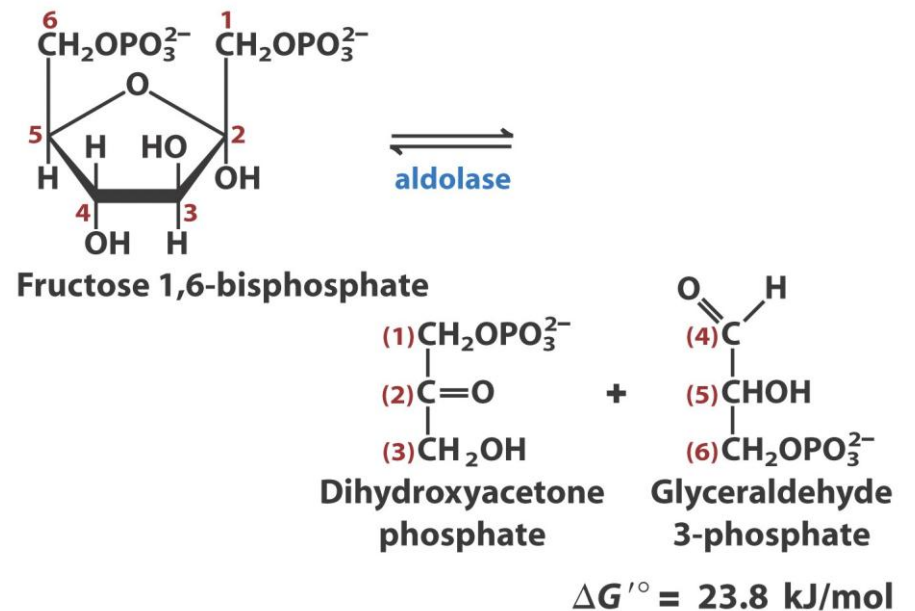
Preparatory Phase of Glycolysis (continued)
[STEP 4] Splitting Fructose 1,6-bisphosphate

Fructose 1,6-bisphosphate \leftrightarrow Dihydroxyacetone phosphate + Glyceraldehyde 3-phosphate

Aldolase. We used 2 ATP to generate fructose-1,6-bisphosphate. The six-carbon sugar molecule is split in the middle, producing two three-carbon molecules. Note: C-C bond cleavage is rare in biochemistry.

Mechanism. “**Aldolase**” name is derived from “aldol condensation” reaction in organic chemistry, and involves the formation of a covalently-linked, substrate-enzyme intermediate.

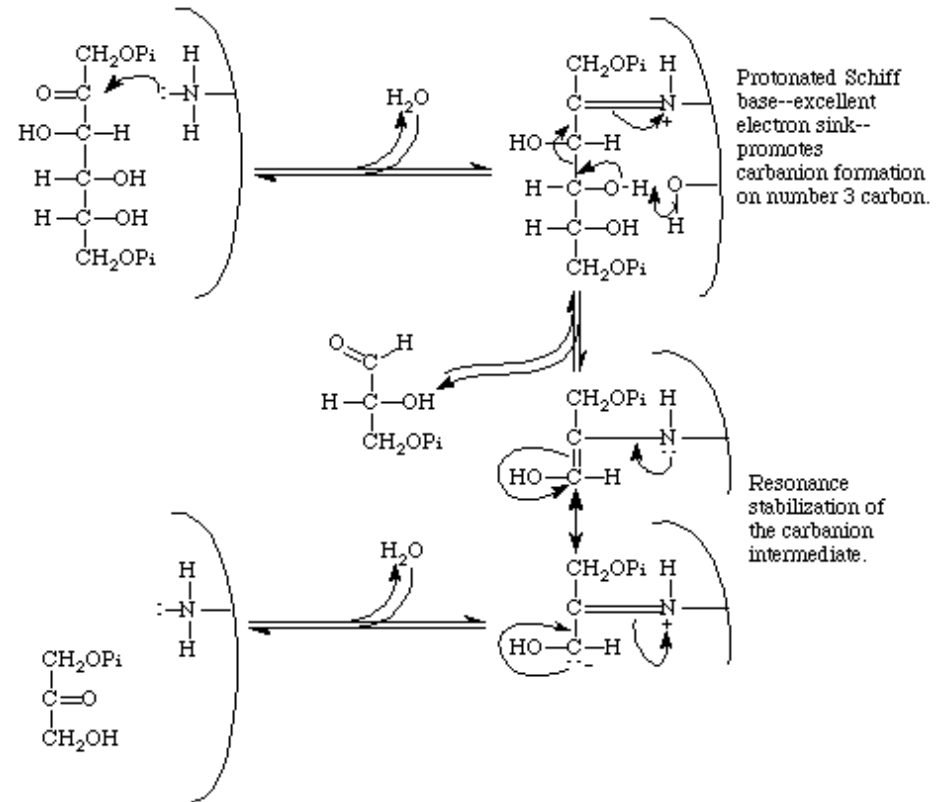
The epsilon amino group of lysine in the enzyme active site makes a nucleophilic attack on the carbonyl carbon, forms a protonated imine at C2, or a **protonated Schiff base**, water is eliminated, **glyceraldehyde-3-phosphate** leaves, and water comes into to release the enzyme-linked DHAP (by a reversal of the original Schiff base formation step).



[Draw out mechanism in detail.]

How do we know this mechanism? Proof of the mechanism comes from use of a reducing agent, sodium borohydride, to produce a reduced & stable secondary-amine-enzyme adduct. Thus the fructose-1,6-bisphosphate stably links to the enzyme.

How is catalysis of the C-C bond possible? The quaternary nitrogen has tremendous electron pulling power. A **negatively charged carbanion intermediate** stabilizes the strong electron pulling power of the nitrogen to generate the strong electron-pulling center.



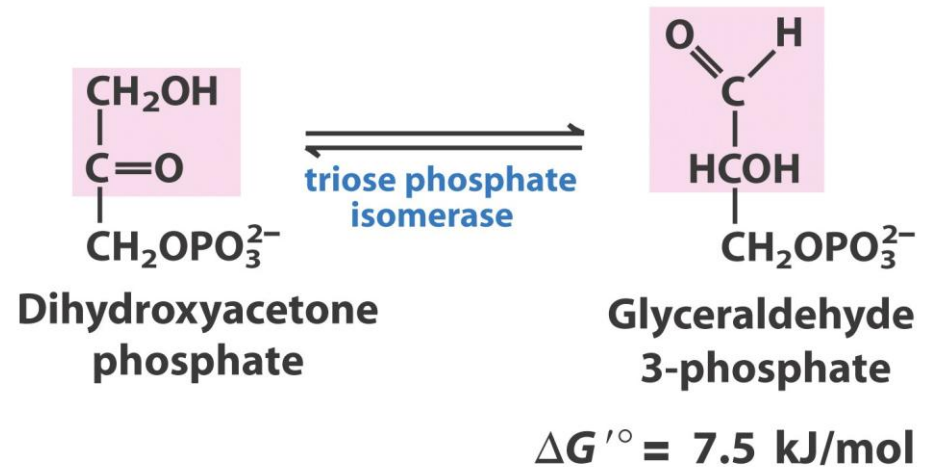
[STEP 5] Triose Phosphate Isomerase.



Mechanism. Parallels phosphoglucosomerase, *i.e.*, converting an aldehyde sugar into a ketose—a typical general acid/base catalyzed reaction. An enediol intermediate forms.

Energetics. The reaction of course, as suggested by the small positive $\Delta G'^{\circ}$ of 7.5 kJ/mol, is reversible and near equilibrium.

Symmetry. Basically the 3-carbon halves of the original 6-carbon glucose have now been isomerized into identical 3-carbon units.



[End of the Preparatory Phase.]

Pay-off Phase (STEPS 6-10)

[STEP 6] Glyceraldehyde Phosphate Dehydrogenase. Example of a reaction coupled to oxidation/reduction.



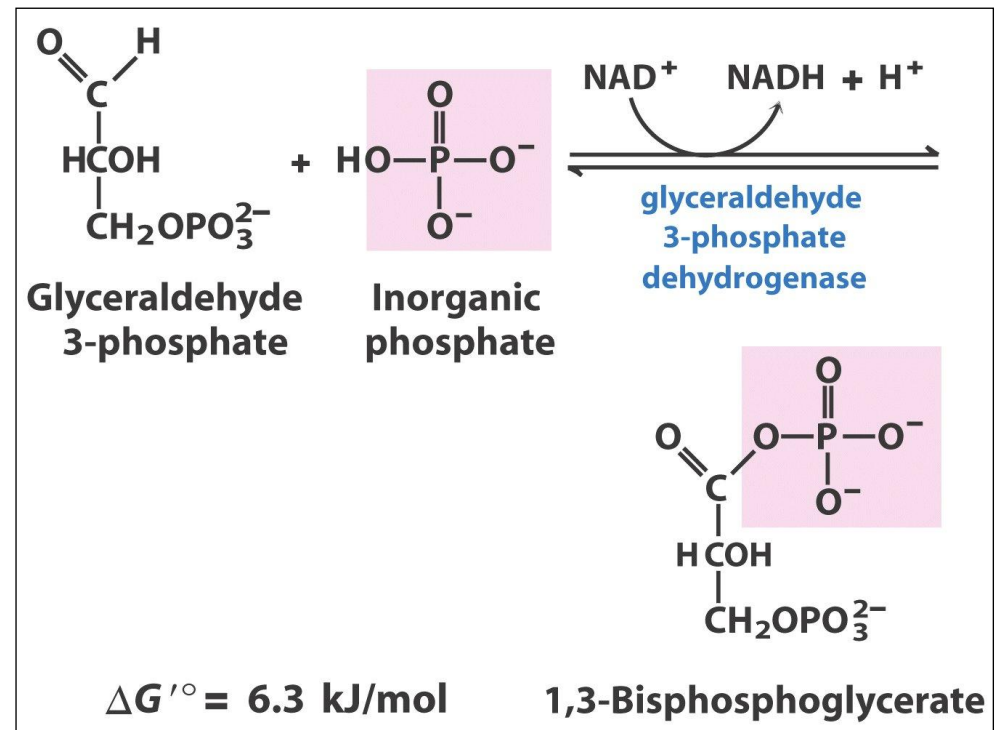
Energetics. Adding inorganic phosphate onto an organic compound is generally an uphill reaction. But the reaction is not. *Why?*



A downhill reaction is coupled to the uphill reaction of adding P_i onto GAP, forming **acyl phosphate** (i.e. **ΔE° > 0**). A very high energy bond is made.

Mechanism. Glyceraldehyde phosphate dehydrogenase has one crucial cysteine residue. You can chemically modify the -SH group of the cysteine. When you chemically modify the residue, the enzyme activity is eliminated. The -SH side chain is essential for activity.

The cysteine sulfhydryl makes a nucleophilic attack on the electron-poor carbonyl carbon of the aldehyde. Upon release of the proton, a **thiohemiacetal** is formed between the compound the -SH.



A hydride moiety, the proton plus two electrons, is transferred onto NAD^+ . This is a strongly downhill reaction. The oxidation is on the carbon. This is aided by the abstraction of the proton on the -OH group, ending up with a **thioester**.

Thioesters. The hydrolysis of thioesters is much more strongly downhill than the hydrolysis of simple esters. Oxygen-based esters like this give resonance stabilization so that both of the oxygen atoms carry a somewhat similar partial negative charge. With thioesters, because of the size difference between oxygen and sulfur, there is not much of this resonance stabilization. When hydrolyzed, thioesters, therefore, generate higher amounts of free energy.

Phosphorolysis. In this final reaction to release product, instead of water, P_i splits the thioester bond. This carbon in the thioester bond is the most electron-poor atom here. As a result, the **mixed acid anhydride** product is released—and the phosphate bond is *super* high-energy.

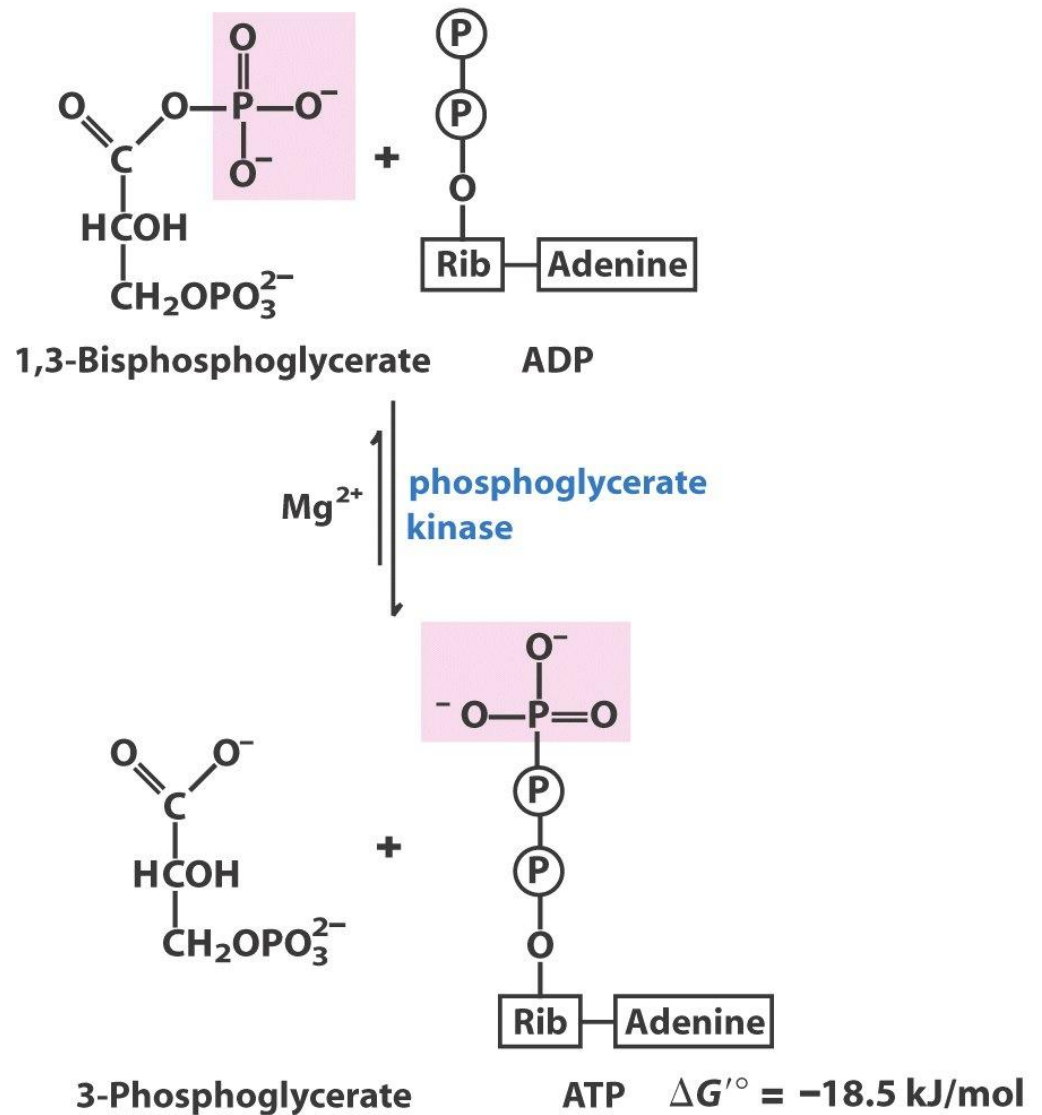
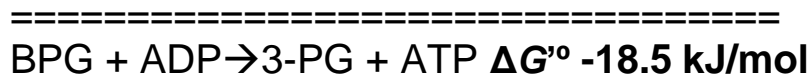
Arsenate. When you do glycolysis and you break open yeast cells, glycolysis does not happen in the absence of phosphate. What would happen if you were to add arsenate instead of phosphate? Arsenic is right under phosphorus in the Periodic Table. Arsenate looks almost exactly like inorganic phosphate. Arsenate will react in the glyceraldehydes phosphate dehydrogenase reaction. You get an analog of 1,3-bisphosphoglycerate, except that the phosphate on the one-carbon is replaced by arsenate. This gets the system into trouble. Unlike 1,3-bisphosphoglycerate, this compound with the arsenate analog is unstable. It spontaneously gets hydrolyzed, and you lose this analog of the energy- rich phosphate. You cannot generate ATP. If you are doing this with phosphate, then you have a super high-energy phosphate bond here, so you can take advantage of it.

[STEP 7] Phosphoglycerate Kinase. A payoff step.

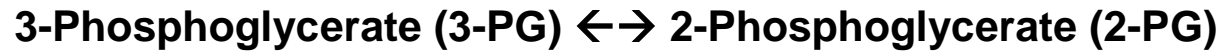
Mechanism. The reaction uses the high-energy phosphate bond and ADP as an acceptor of this phosphate on C1 of BPG to generate 3-phosphoglycerate & ATP.

Energetics. The backward reaction does not occur easily because this phosphoglycerate kinase reaction has a reasonably negative standard free energy change of -18.5 kJ/mol. Thermodynamically, the reaction is spontaneous. The initial phosphate bond at the one position is high-energy phosphate bond.

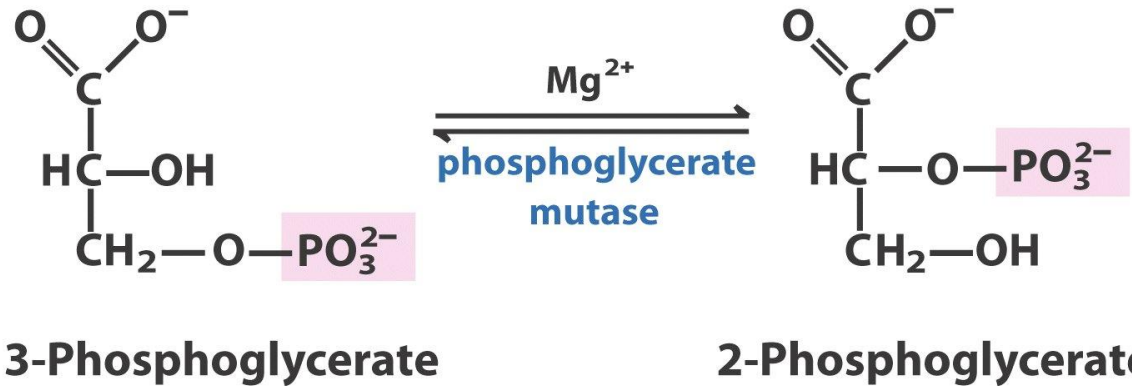
How much was the BPG phosphoanhydride bond worth?



[STEP 8] Phosphoglycerate Mutase.



A “**mutase**” is a class of enzymes that move a -R group from one position in the molecule to another position in that molecule. Here the -R group is P_i .



$\Delta G'^{\circ} = 4.4 \text{ kJ/mol}$

Mechanism. The oddity is that the reaction starts with phosphorylated Phosphoglycerate Mutase enzyme (E-Pi). E-Pi donates its P_i onto the C2 of the substrate, 3-PG, creating the intermediate, **2,3-bisphosphoglycerate (2,3-bisPG)**. Next, the 2,3-BPG will donate its 3- P_i onto the enzyme to generate 2-phosphoglycerate (2-PG) and then regenerate E-Pi.



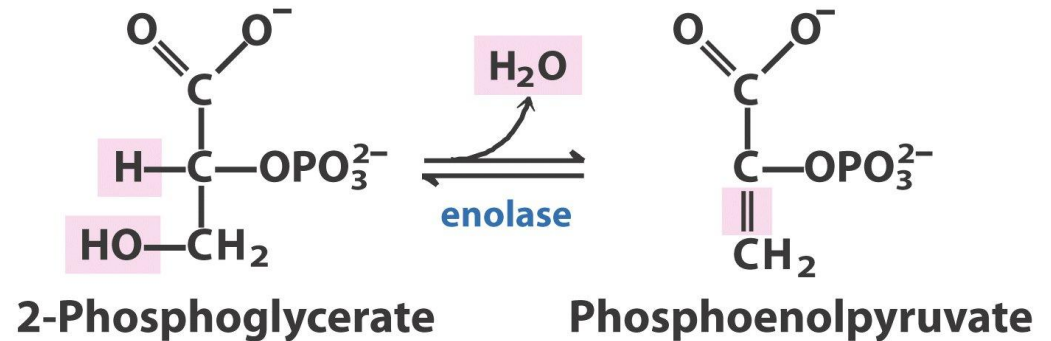
Energetics. $\Delta G'^{\circ}$ is near zero and near equilibrium, since the substrate was remodeled subtly, such that a similar phosphanhydride bond was broken and then remade.

Phosphoglucomutase. An analogous enzyme, which regulates entry into glycolytic pathway in muscle, phosphoglucomutase, uses a similar mechanism. [\[More soon...\]](#)

[STEP 9] Enolase. By removing water, this step increases the hydrolysis potential of the phosphate.



Energetics. Hydrolysis of the phosphate on 2-phosphoglycerate cannot generate much energy. But enolase does an internal rearrangement (a dehydration reaction) to make PEP. Now the same phosphate bond behaves as a super high-energy bond.



$$\Delta G'^{\circ} = 7.5 \text{ kJ/mol}$$

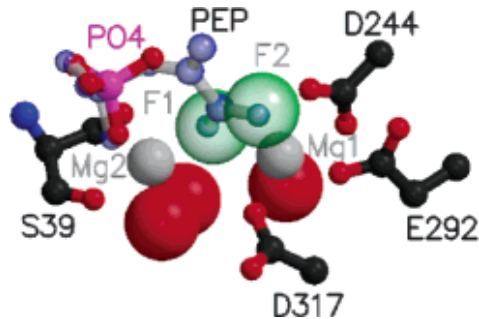
Mechanism. You abstract the proton from C2 of 2-PG that is followed by an elimination of the hydroxyl group. Abstraction of the C2 proton is not easy, because it is not very acidic. Enolase uses a magnesium ion (Mg²⁺). The reactive center of the enzyme contains a couple of Mg²⁺'s to help abstract the proton, generating PEP.

[STEP 10] Pyruvate Kinase. Again, the name of the enzyme does not tell you the direction in which the reaction proceeds.

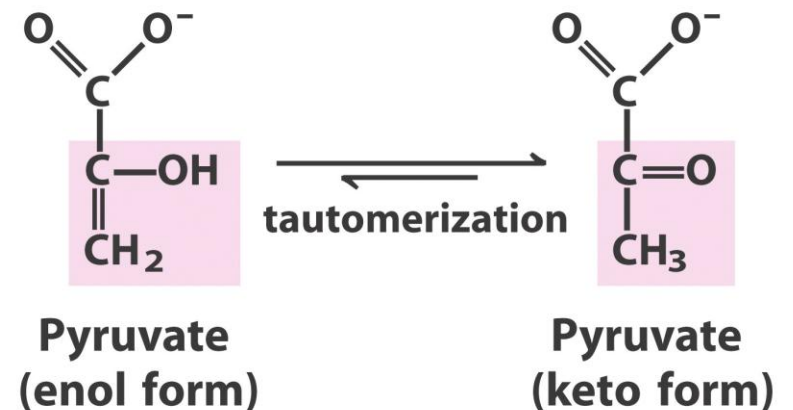
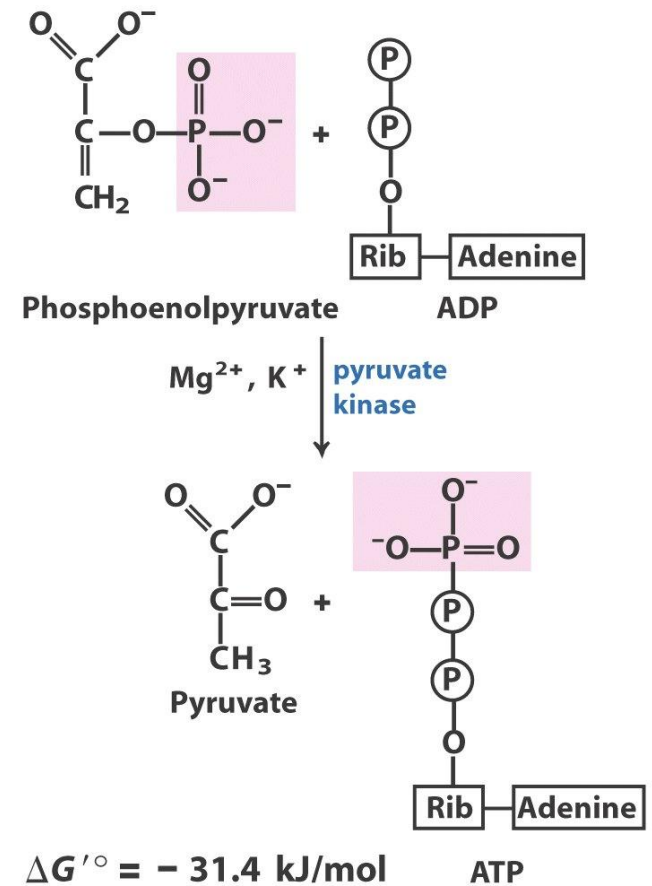


Energetics. Although two molecules of ATP were spent, a total of four molecules of ATP were recovered.

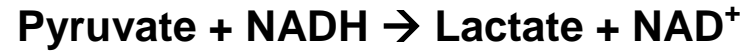
Mechanism. Type of substrate phosphorylation mechanism. Physiological Mg^{2+} in the active site is required. (Mn^{2+} also works.)



Fluoride inhibition. Fluoride is a classic inhibitor of glycolysis (discovered by Otto Warburg in 1941). It modifies the phosphate and magnesium in enolase, leading to controversy regarding the fluorination of drinking water.



Glycolysis in anaerobic conditions.



The enzyme is called **lactate dehydrogenase**. This gets rid of the NADH that was generated during the glyceraldehyde phosphate dehydrogenase step and regenerates NAD^+ . At this point, there would be no net oxidation or net reduction during glycolysis. Muscle cells do this to maintain levels of NAD^+ in the absence of O_2 .