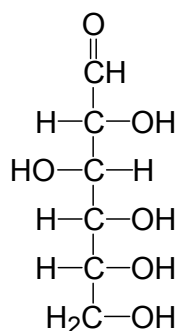


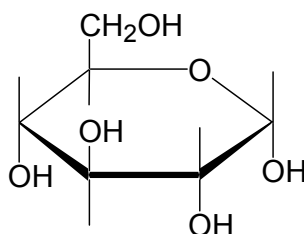
Glycolysis

January 22, 2003

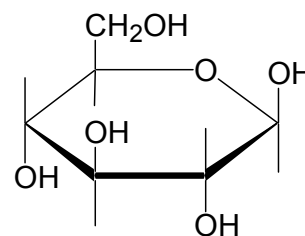
Bryant Miles



D-glucose



α -D-glucose



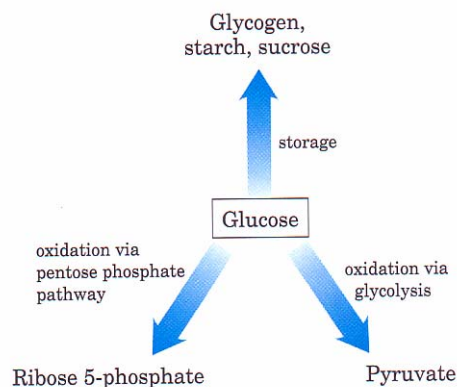
β -D-glucose

D-Glucose is a major fuel for most organisms. D-Glucose metabolism occupies the center position for all metabolic pathways. Glucose contains a great deal of potential energy. The complete oxidation of glucose yields $-2,840 \text{ kJ/mol}$ of energy.

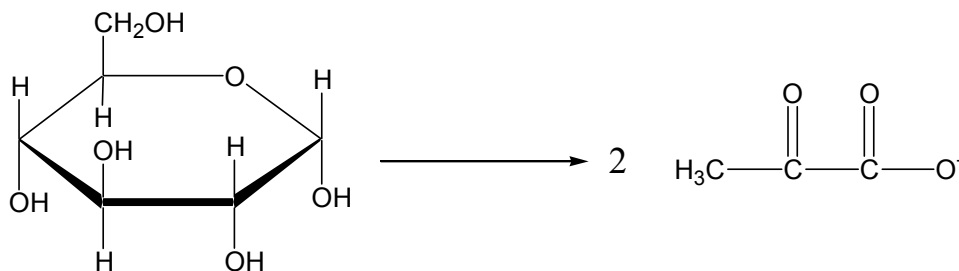


Glucose also provides metabolic intermediates for biosynthetic reactions. Bacteria can use the skeletal carbon atoms obtained from glucose to synthesize every amino acid, nucleotide, cofactor and fatty acid required for life.

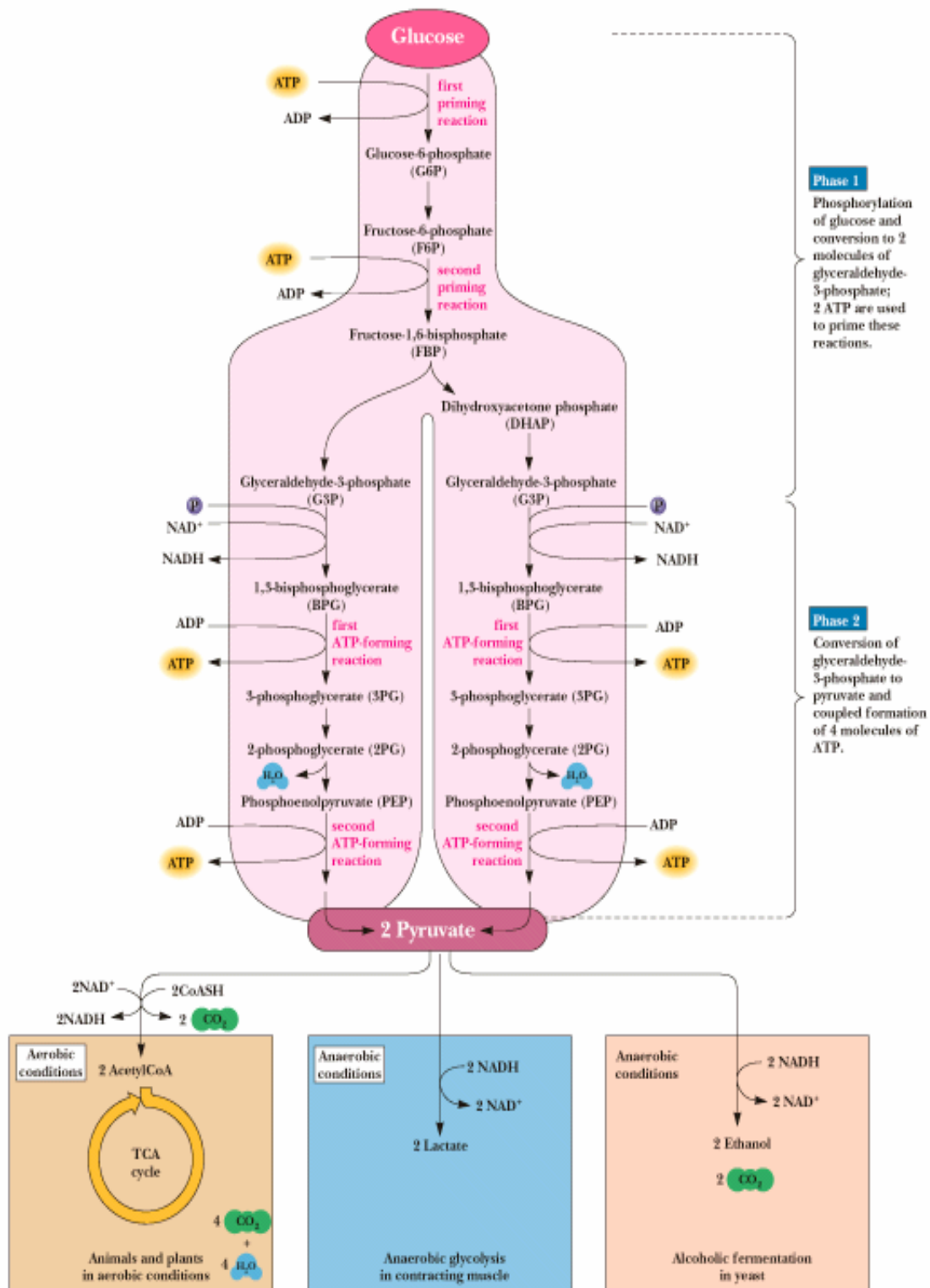
For higher plants and animals there are three major metabolic fates for glucose.



Nearly every living cell catabolizes glucose and other simple sugars by a process called glycolysis. Glycolysis differs from one species to another only in the details of regulation and the fate of pyruvate. Glycolysis is the metabolic pathway that catabolizes glucose into two molecules of pyruvate.



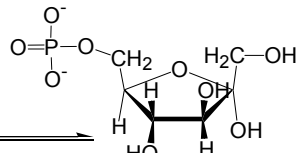
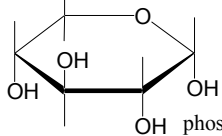
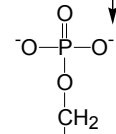
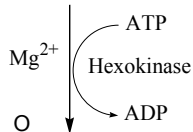
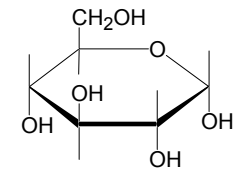
Glycolysis occurs in the cytosol of cells and is essentially an anaerobic process since the pathway's principle steps do not require oxygen. The glycolytic pathway is often referred to as the **Embden-Meyerhof pathway** in honor of the two of the three biochemical pioneers (What about Jacob Parnas?) who discovered it. The glycolytic pathway is shown below.



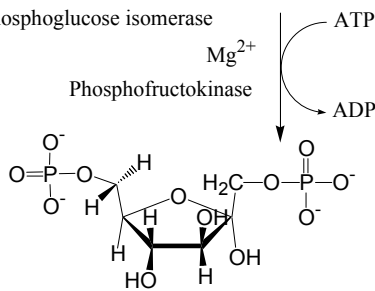
Glycolysis consists of 10 enzyme catalyzed reactions. The pathway can be broken down into two phases. The first phase encompasses the first five reactions to the point that glucose is broken down into 2 molecules of glyceraldehyde 3-phosphate. Phase 1 consumes two molecules of ATP. The second phase includes the last five reactions converting glyceraldehyde 3-phosphate into pyruvate. Phase 2 produces 4 molecules of ATP and 2 molecules of NADH. The net reaction (Phase 1 + Phase 2) produces 2 molecules of ATP and 2 molecules of NADH per molecule of glucose.

Phase 1 Preparatory Phase

The first reaction of glycolysis is the transfer of a phosphoryl group from ATP to glucose.



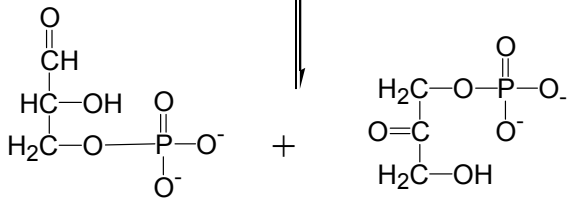
The second reaction is the isomerization of glucose 6-phosphate to fructose 6-phosphate.



The third step is the first committed step of glycolysis. This reaction is the transfer of a second phosphoryl group from ATP to fructose 6-phosphate.

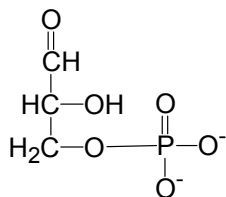
The fourth step of glycolysis involves the aldolytic cleavage of the C3-C4 bond to yield two triose phosphates.

Fructobisphosphate aldolase

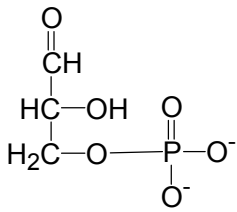


The 5th Step of glycolysis is the rapid isomerization of the triose phosphates

Triose Phosphate Isomerase

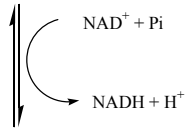


Phase II
Payoff Phase

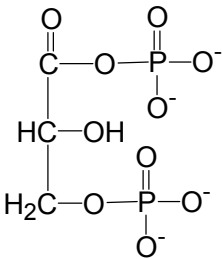


Note: two glyceraldehydes produced per 1 glucose.

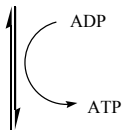
Step six: Oxidation and phosphorylation yielding a high-energy mixed anhydride bond.



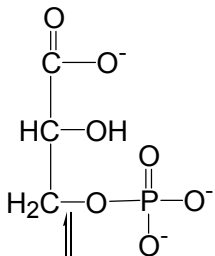
Glyceraldehyde 3-phosphate dehydrogenase



Step 7: transfer of the high-energy phosphoryl group to ADP to produce ATP.

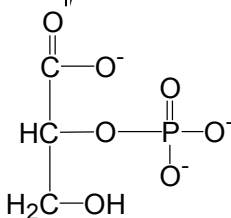


Phosphoglycerate kinase



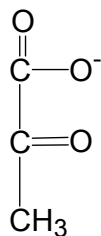
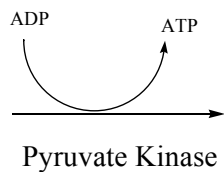
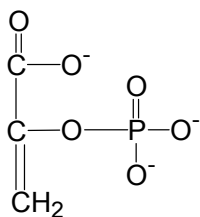
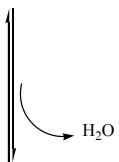
Step 8: Intramolecular phosphoryl-group transfer

Phosphoglycerate kinase



Step 9: Dehydration to yield an energy rich enol phosphodiester.

Enolase



Step 10: Transfer of a high energy phosphoryl group to ADP to yield ATP.

Net Overall



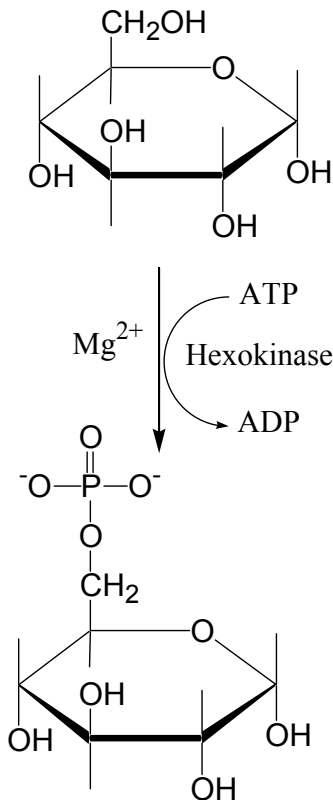
$$\Delta G^{\circ\prime} = -85 \text{ kJ/mol}$$

9 of the ten metabolites of glycolysis are phosphorylated. Phosphorylated intermediates serve 3 functions.

1. The phosphoryl groups are ionized at physiological pH giving them a net negative electrostatic charge. Biological membranes are impermeable to charged molecules. Intermediates are held within the cell.
2. The transfer of phosphoryl groups conserves metabolic energy. The energy released in breaking the phosphoanhydride bonds of ATP is partially conserved in the formation of phosphate esters. High-energy phosphate compounds formed in glycolysis donate phosphoryl groups to ADP to form ATP.
3. The enzymes of glycolysis use the binding energy of phosphate groups to lower the activation energy and increase the specificity of the enzyme reactions.

The Reactions of Glycolysis

Step 1: Phosphorylation of Glucose

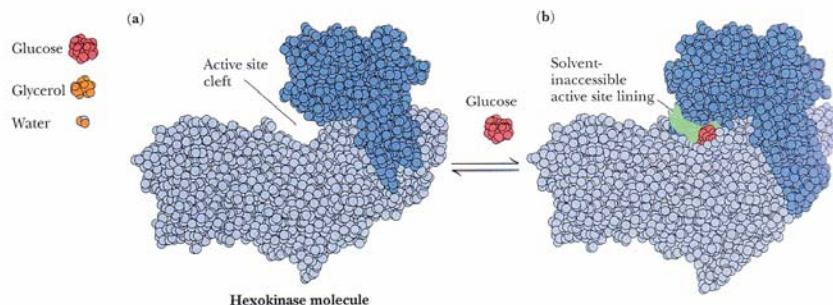


Hexokinase transfers the γ -phosphate of ATP to the C-6 hydroxyl group of glucose to yield glucose 6-phosphate. The phosphorylation of glucose commits it to the cell.

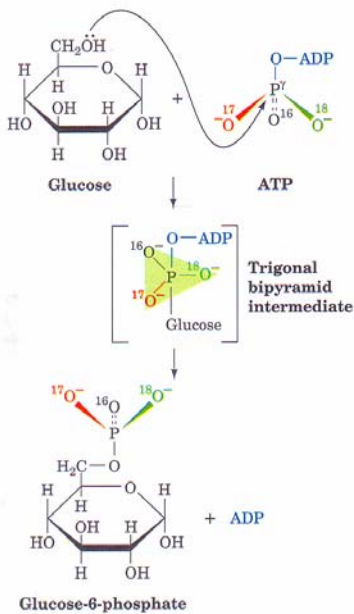
A **Kinase** is an enzyme that catalyzes the transfer of the terminal phosphoryl group of ATP to some acceptor nucleophile. The acceptor in the case of hexokinase is the C-6 hydroxyl group of D-glucose, D-mannose or D-fructose.

Hexokinase requires Mg^{2+} for activity. The true substrate for this enzyme is not ATP^{4-} but MgATP^{2-} .

Hexokinase is the poster enzyme for the induced fit mechanism where substrate binding induces conformational changes in the enzyme.

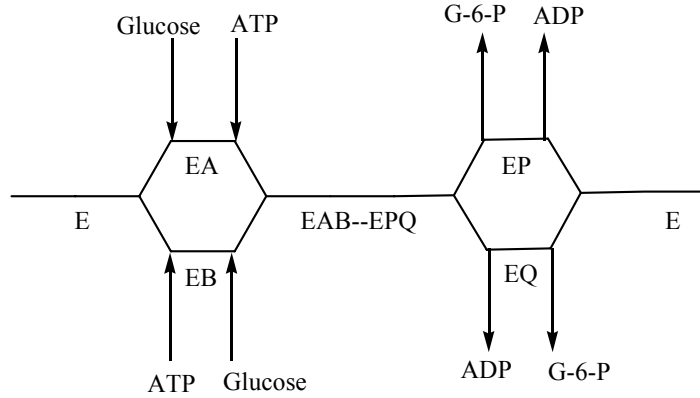


The C-6 hydroxyl group of D-glucose has similar reactivity as water. The enzyme active site is fully accessible by water. Hexokinase discriminates against water by a factor greater than 10^6 . The ability to discriminate comes from the conformational changes in hexokinase when the correct substrates are bound. Only when a hexose is bound does the conformation change and the enzyme becomes active.

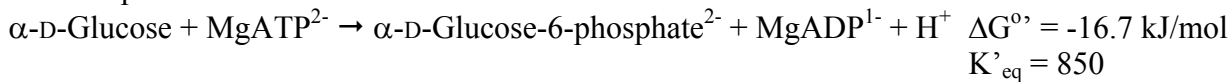


The mechanism of the phosphoryl group transfer is the typical S_N2 nucleophilic substitution reaction. By using a chiral phosphate shown to the left, it can be shown that transfer of the γ -phosphate of ATP proceeds with inversion of configuration at the electrophilic center of the phosphorous.

The kinetic mechanism of hexokinase is a random bi bi



The complete reaction for the hexokinase reaction is:



Under cellular conditions, the first step of glycolysis is even more favorable than the standard state. Take erythrocytes for example. The steady state concentration of $[\text{ATP}] = 1.85 \text{ mM}$, $[\text{Glucose}] = 5.0 \text{ mM}$, $[\text{G-6-P}] = 0.083 \text{ mM}$, and $[\text{ADP}] = 0.14 \text{ mM}$. The free energy change under these concentrations is given by:

$$\Delta G = \Delta G^{\circ} + RT \ln \left(\frac{[\text{ADP}][\text{G-6P}]}{[\text{ATP}][\text{Glc}]} \right) = -16.7 \frac{\text{kJ}}{\text{mol}} + 8.3145 \frac{\text{kJ}}{\text{mol}^{\circ} \text{K}} (298^{\circ} \text{K}) \ln \left(\frac{[0.14 \times 10^{-3} \text{ M}][0.083 \times 10^{-3} \text{ M}]}{[1.85 \times 10^{-3} \text{ M}][5.0 \times 10^{-3} \text{ M}]} \right)$$

$$\Delta G = -16.7 \frac{\text{kJ}}{\text{mol}} - 16.6 \frac{\text{kJ}}{\text{mol}} = -33.3 \frac{\text{kJ}}{\text{mol}}$$

Hexokinase is found in all cells of all organisms.

Isozymes of hexokinase are found in yeast and mammals. Glucokinase is an isozyme of hexokinase sometimes called Hexokinase IV (other times called Hexokinase D). Glucokinase is found in hepatocytes (liver cells). The hexokinase isozyme found in skeletal muscle has a K_m for glucose of 0.1 mM . The skeletal muscle maintains steady state concentration of glucose around 4 mM . This isozyme is allosterically inhibited by high concentrations of glucose-6-phosphate.

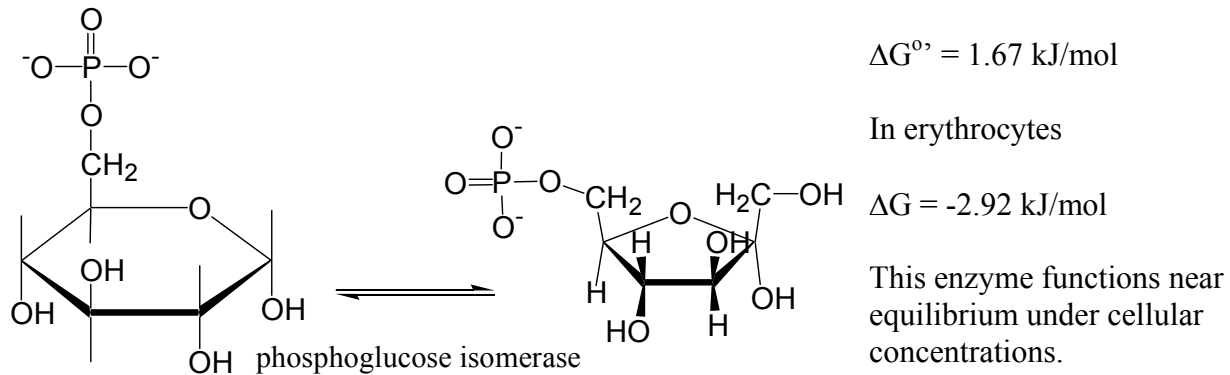
The isozyme glucokinase catalyzes the same reaction but has a very high K_m for glucose of 10.0 mM . This enzyme is not allosterically regulated by high glucose-6-phosphate concentrations. The high K_m , means that glucokinase only becomes metabolically important when glucose levels are high. This enzyme produces glucose-6-phosphate which is then stored by the liver in the form of glycogen. Glucokinase is an **inducible enzyme**. The amount of this enzyme present in hepatocyte cells is regulated by insulin.

Step 2: Isomerization of Glucose-6-Phosphate

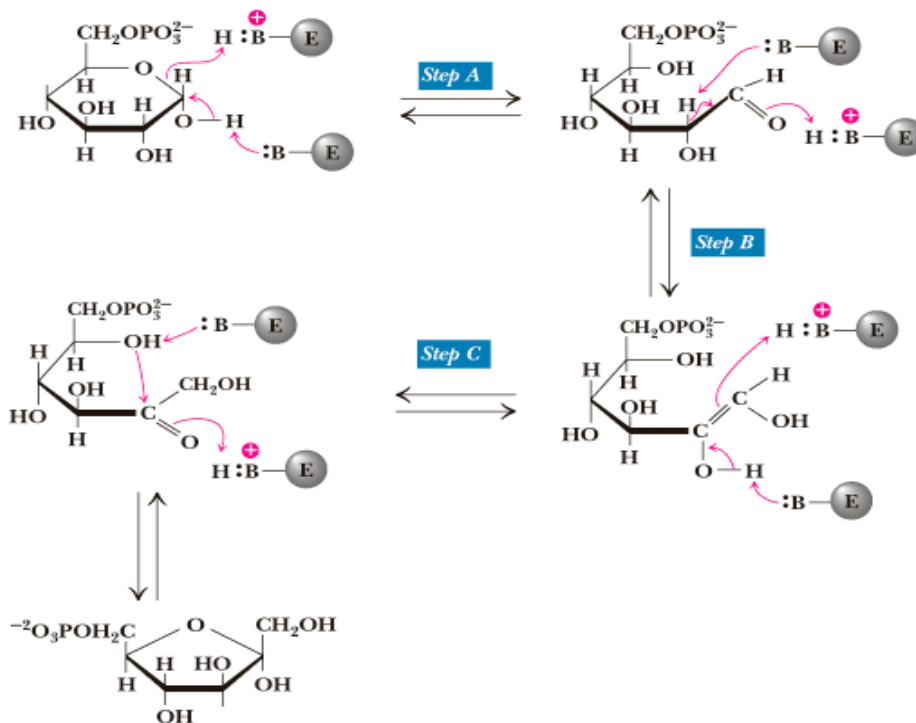
In the second reaction of glycolysis the aldose glucose-6-phosphate is isomerized into the ketose, fructose-6-phosphate. The carbonyl is shifted from the C1 of glucose to the C2 of fructose. This reaction serves two functions. Function 1: The next step of glycolysis is phosphorylation at the C1 position. The

hemiacetal hydroxyl group of glucose is a poor nucleophile, while the primary hydroxyl group of fructose is a good nucleophile. Function 2: The isomerization to fructose puts the carbonyl at the C2 position which activates the C3 carbon for aldolytic cleavage.

The isomerization is catalyzed by the enzyme phosphoglucose isomerase.



The predominant forms of glucose and fructose in solution are the ring forms shown above. Phosphoglucose isomerase must begin with the opening of the ring to interconvert G-6-P to F-6-P.



Every enzyme mechanism begins with the binding of the substrate.

Step A. A general acid, presumably a lys ϵ -amino group catalyzes the ring opening.

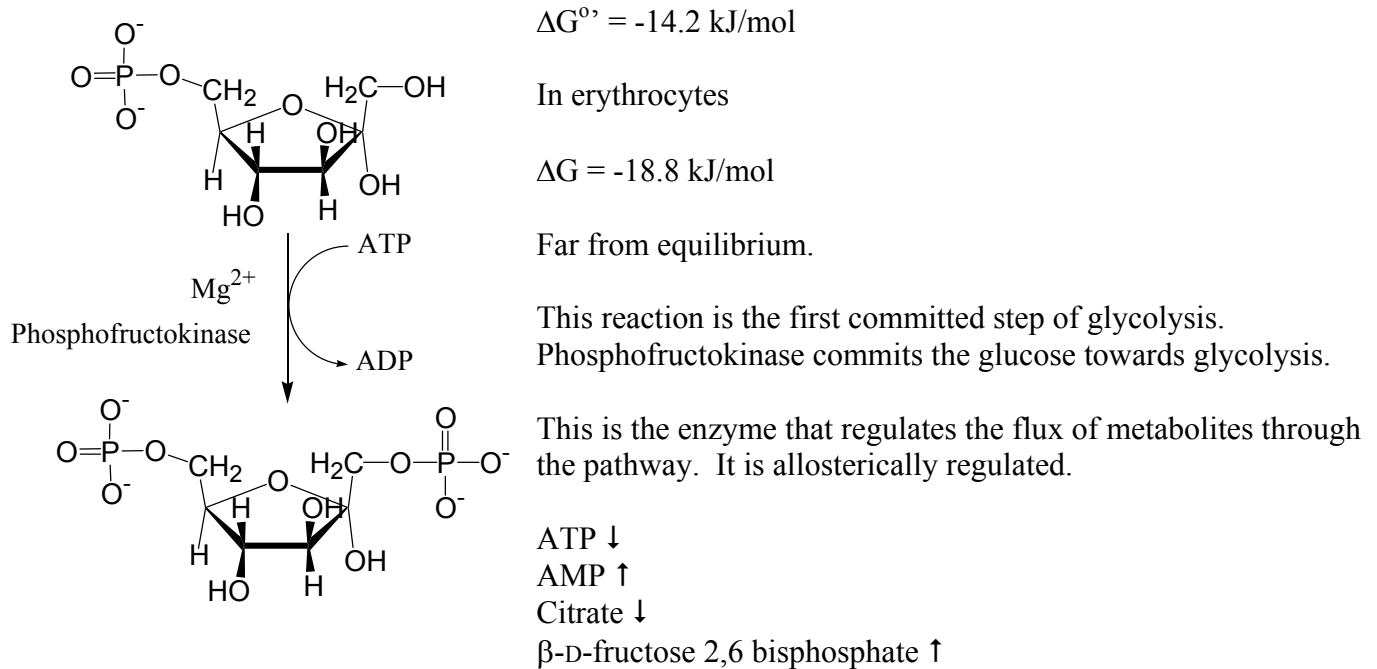
Step B. A base presumably a carboxylate of glutamate abstracts the proton of C2 to form a cis-enediolate intermediate,

Step C: The proton abstracted from C2 is replaced on the C1 carbon.

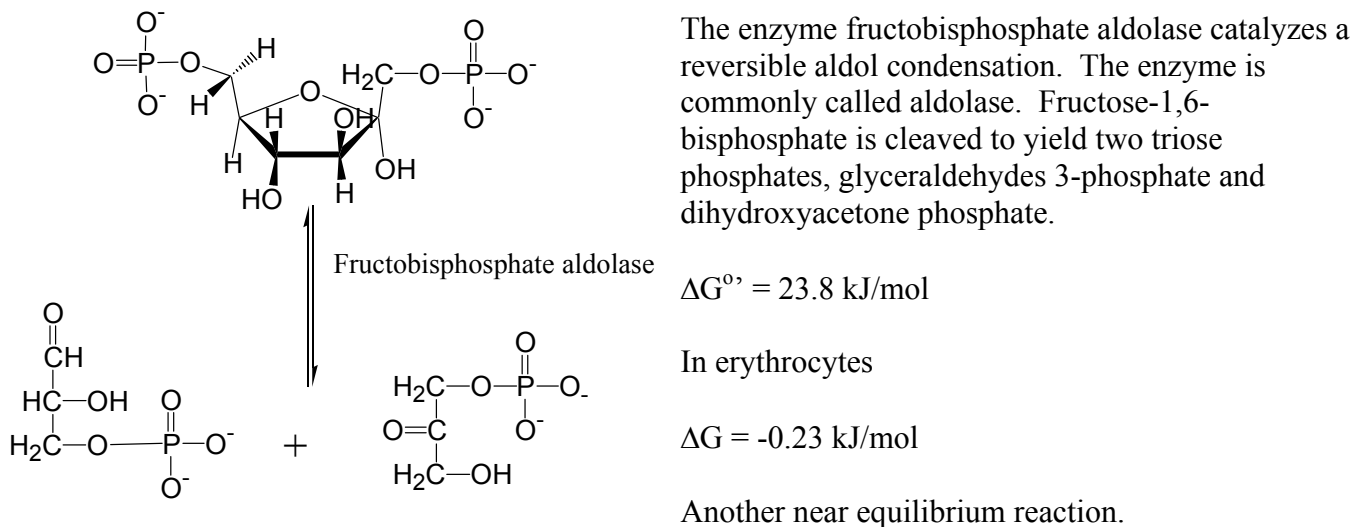
Ring closure then produces the products.

Reaction 3: Phosphorylation of Fructose-6-Phosphate

This is the second of the two priming reactions of glycolysis. The phosphorylation is catalyzed by the enzyme phosphofructokinase-1.

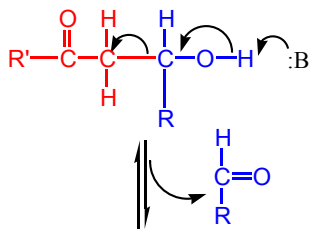


Reaction 4: Cleavage of Fructose-1,6-bisphosphate.



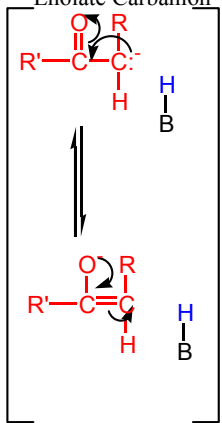
This reaction is an aldol cleavage reaction, mechanism shown on the following page. The bond cleave is the one between the C3 and C4 carbons. The aldol cleavage of fructose-1-6-bisphosphate results in two interconvertible C3 compounds that can enter a common degradative pathway. The enolate intermediate is stabilized by resonance.

Two classes of aldolase enzymes are found in nature. Animal tissues have Class I aldolase. Class I aldolases form a covalent Schiff base between the carbonyl of the substrate and an active site lysine.

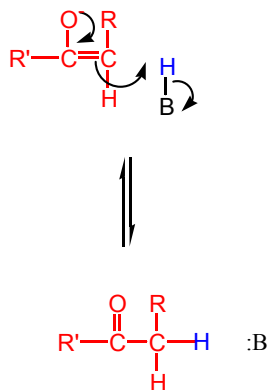


The mechanism for base catalyzed aldol cleavage reaction is shown to the left. The reverse of this reaction is an aldol condensation.

Resonance Stabilized Enolate Carbanion

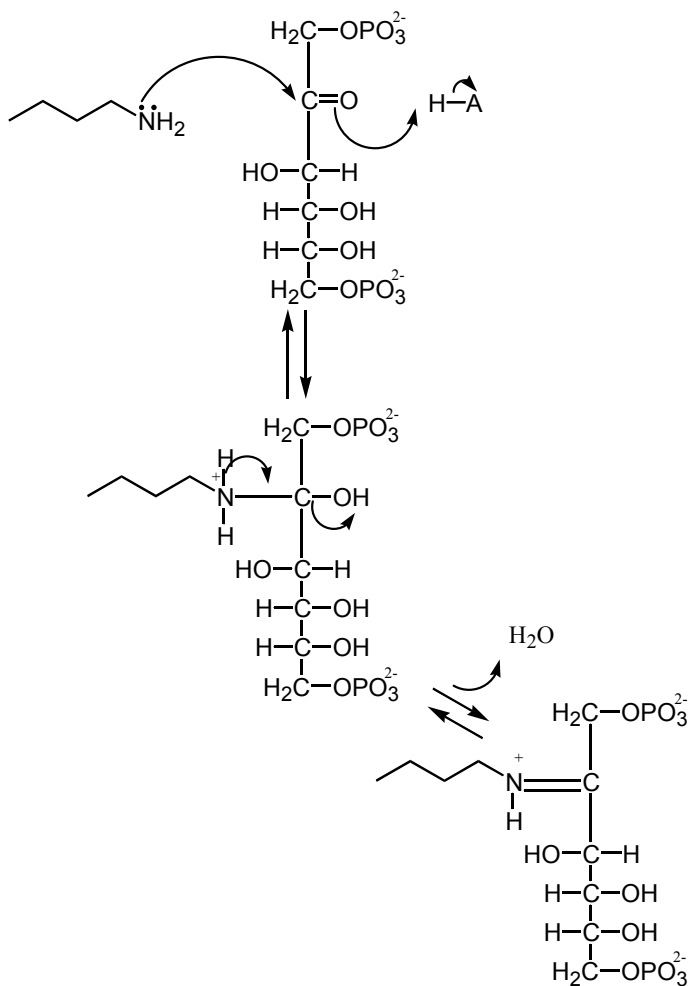


Shiff base formation

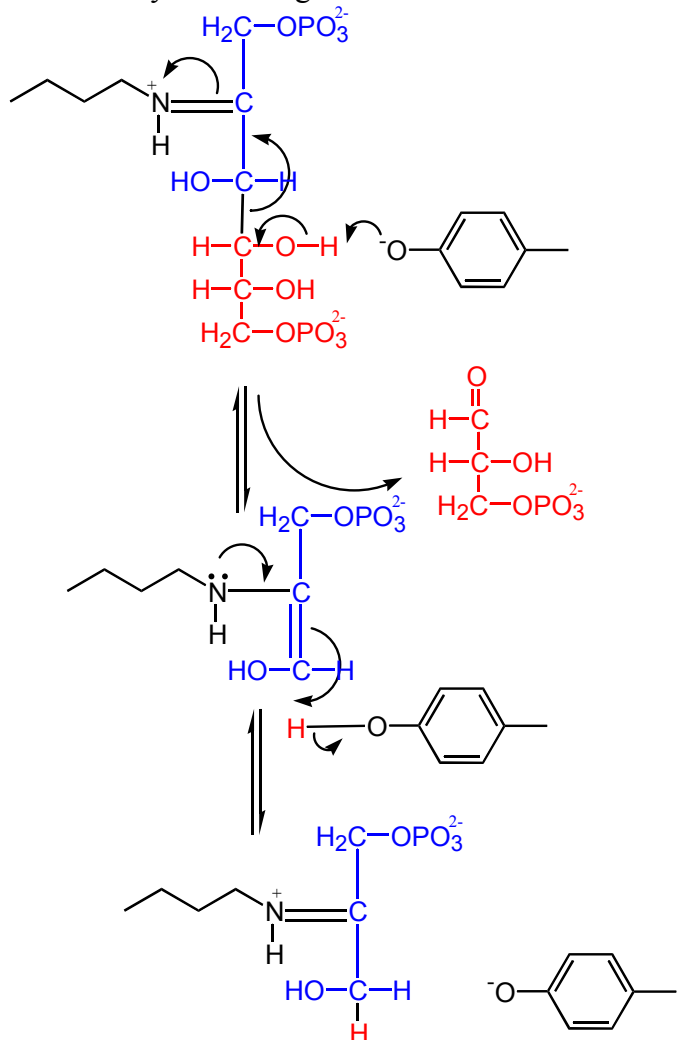


This reaction has a high activation energy because of the high energy carbanion character of the transition state. There are two types of aldolase enzymes that catalyze this reaction.

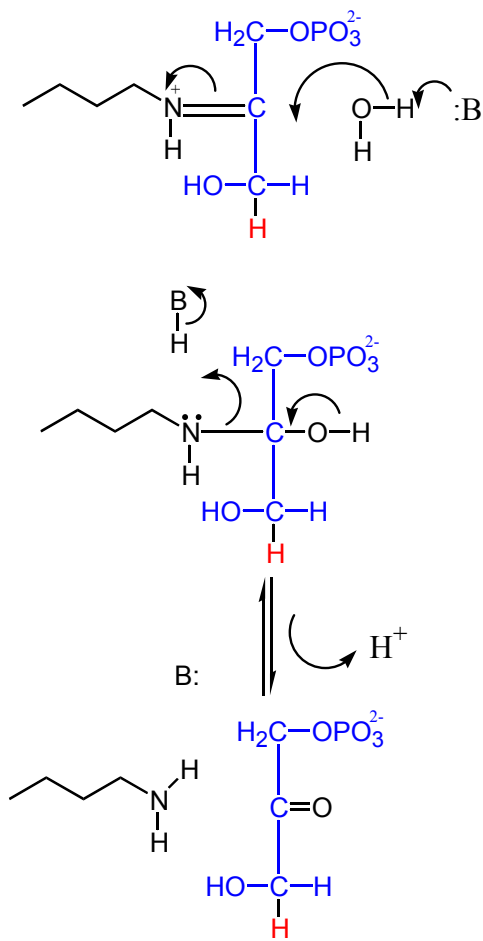
Class I aldolases are found in plants and animals. To lower the activation energy they use covalent catalysis. An active site lysine attacks the carbonyl to form a **Schiff base**. The Schiff base withdraws electrons from the reaction center stabilizing the carbanion character of the transition state and lowering the activation energy.



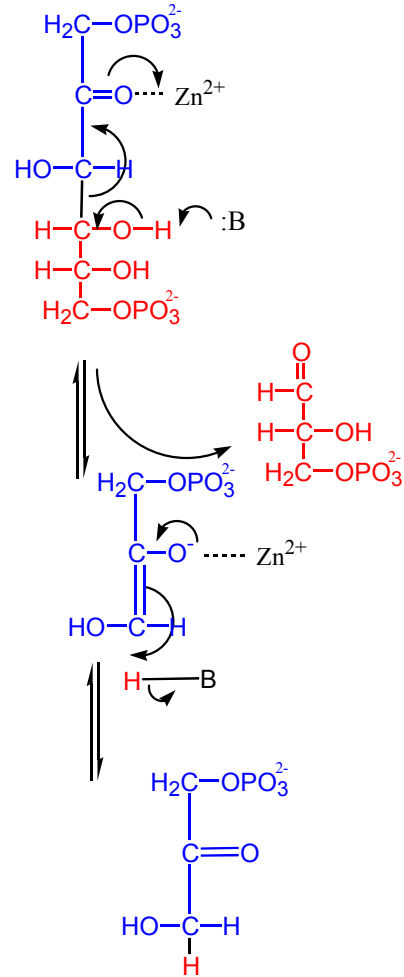
Adolytic Cleavage



Hydrolysis of the Schiff base.

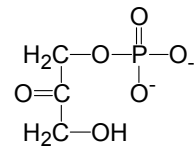


Class II aldolases



Class II aldolases are found in fungi, algae and some bacteria. They do not form a Schiff base with the substrate. Instead they lower the activating energy by polarizing the carbonyl bond and providing electrostatic stabilization of the carbanion character of the transition state with divalent metal ions such as Zn^{2+} or Fe^{2+} .

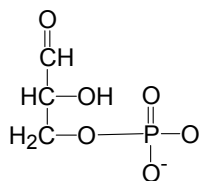
Reaction 5: Triose Phosphate Isomerization.



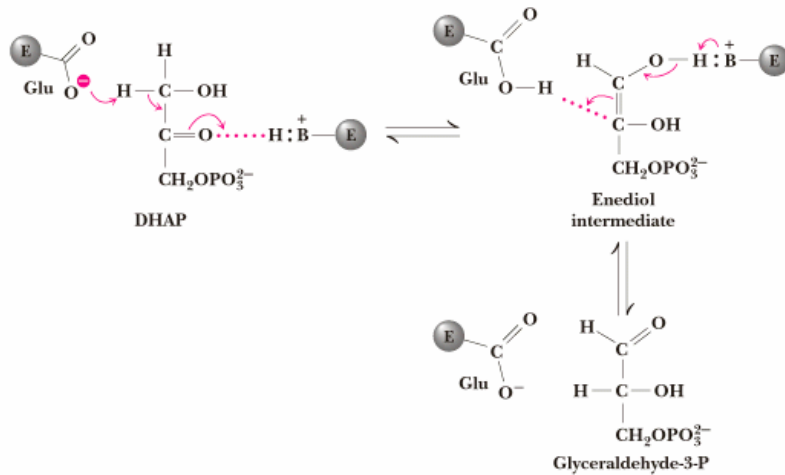
Of the two products formed in the aldolase reaction, only glyceraldehydes goes on into the second phase of glycolysis. The other triose phosphate, dihydroxyacetone phosphate must be converted into glyceraldehydes 3-phosphate by the enzyme **triose phosphate isomerase**.

By this reaction, C1,C2 and C3 of the starting glucose become indistinguishable from C6,C5 and C4 respectively.

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



In erythrocytes $\Delta G = 2.7 \text{ kJ/mol}$
Another reaction near equilibrium.



The mechanism of triose phosphate isomerase (TIM) is shown above. The mechanism proceeds with the formation of an enediol or enediolate intermediate similar to that of phosphoglucose isomerase. Glu-165 has been shown to function as a general acid/base catalyst. The pKa of Glu-165 has been perturbed to 6.5. In the first step of the reaction Glu-165 acts as a general base abstracting the proton from the C3 position of DHAP. The general acid catalyst protonating the carbonyl group is His-95. The result is the enediol intermediate. His-95 then functions as a general base abstracting the hydrogen from the hydroxyl group with Glu-165 functioning as a general acid donating its proton to the C2 carbon forming G-3-P.

TIM was the first enzyme discovered to have an α/β barrel tertiary structure. A cylinder of 8 parallel β -strands is surrounded by eight α -helices. Since the discovery numerous other proteins have been found to have this tertiary structure including the glycolytic enzymes, aldolase, enolase and pyruvate kinase.

Triose phosphate isomerase is an enzyme that has evolved into catalytic perfection. It catalyzes the interconversions at the diffusion limit. Meaning the rate determining step is the diffusion of the substrate to the active site.