

Regulation of Glycolysis

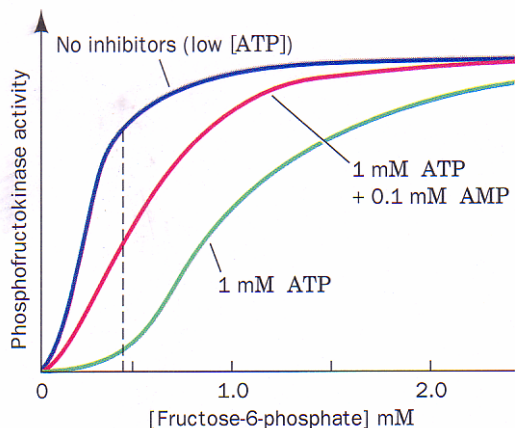
January 27, 2003

Bryant Miles

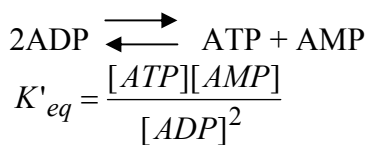
Because the principle function of glycolysis is to produce ATP, it must be regulated so that ATP is generated only when needed. The enzyme which controls the flux of metabolites through the glycolytic pathway is phosphofructokinase (PFK-1). PFK-1 is an allosteric enzyme that occupies the key regulatory position for glycolysis. PFK-1 has a tetrameric enzyme composed of four identical subunits. Like other allosteric proteins (hemoglobin) and enzymes (ATCase) the binding of allosteric effectors and substrates is communicated to each of the active sites. Quaternary changes are concerted and preserve the symmetry of the tetramer. PFK-1 has two sets of alternative interactions between subunits which are stabilized by hydrogen bonds and electrostatic interactions. The two set of conformations are called the T and R states.

These two conformational states are in equilibrium: $T \rightleftharpoons R$

I. ATP feedback inhibition. The function of the glycolytic pathway is to generate ATP. ATP is both a substrate and an allosteric inactivator. The enzyme has two binding sites for ATP. One is the substrate binding site and the other one is an inhibitory site. The PFK-1 substrate binding site binds ATP equally well in both the T and R states. The inhibitory ATP binding site only binds ATP when the enzyme is in the T conformation. The other substrate fructose-6-phosphate binds only to the R state. High concentrations of ATP shift the equilibrium towards the T conformation which decreases the affinity of the enzyme for F-6-P.



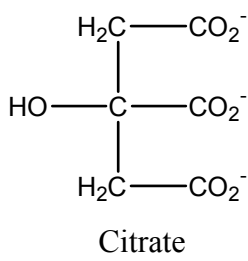
II. AMP reverses inhibition. AMP reverses the inhibition due to high concentrations of ATP. AMP binds preferentially to the R state of PFK. This is important the concentration of ATP drops only 10 % during vigorous exercising. AMP concentration levels in the cell can rise dramatically due to the enzymatic activity of adenylate kinase.



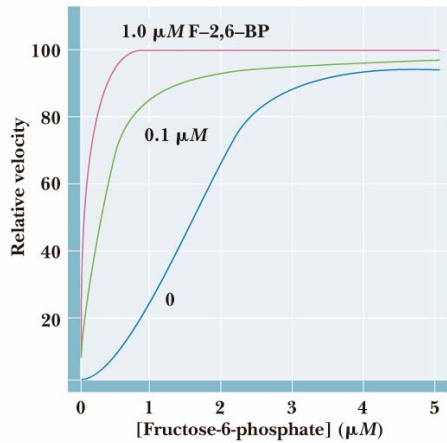
The steady state concentration of ATP in the cell is 10 times greater than the concentration of ADP, and 50 times the concentration of AMP. As a result of the activity of adenylate kinase, a 10% decrease in the concentration of ATP results in 400 % increase in the concentration of AMP.

III Other allosteric effectors of PFK-1

ADP is another allosteric effector of PFK-1. ADP like AMP reverses the inhibitory effects of ATP and is therefore considered an allosteric activator. The activity of PFK-1 is dependent on the ATP, ADP and AMP concentrations which are all functions of the cellular energy status.

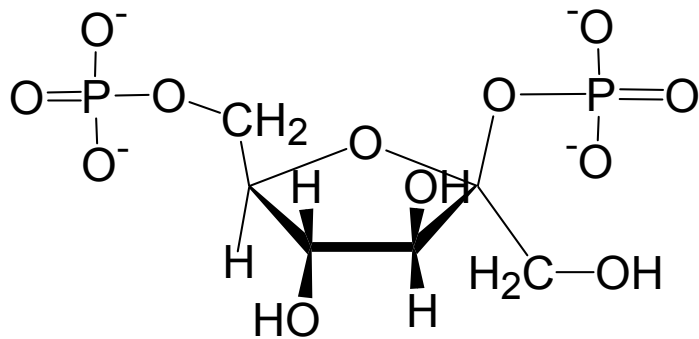
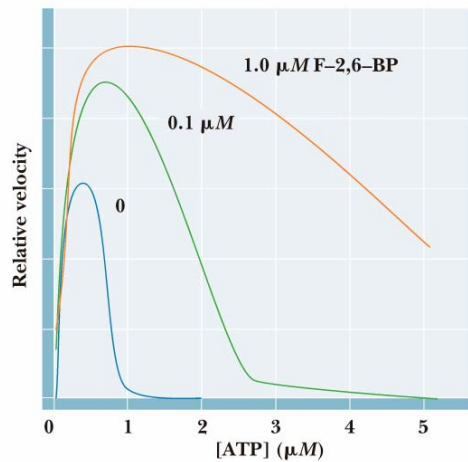


Under aerobic conditions, the pyruvate formed by glycolysis is fed into the citric acid cycle where it is completely oxidized into CO_2 and H_2O . **Citrate** is a metabolite of the citric acid cycle. When the citric acid cycle is saturated, glycolysis needs to be slowed down. When the citric acid cycle is saturated, the citrate concentration in the cytosol increases. Citrate binds preferentially to the T-state of PFK-1. Thus high concentrations of citrate inactivate the enzyme.

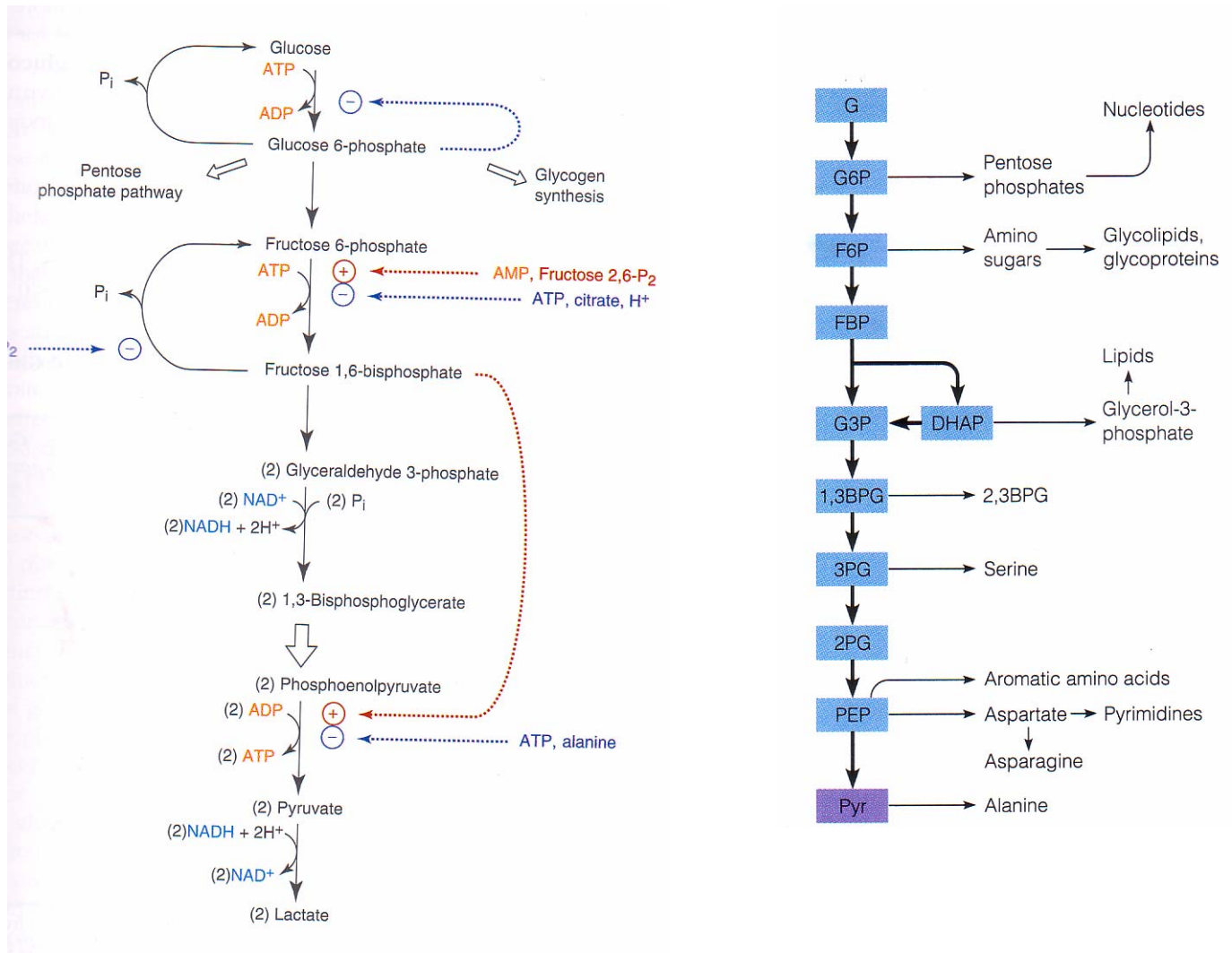


PFK-1 is also regulated by **β -D-fructose 2-6-bisphosphate** which is a potent allosteric activator of the enzyme. β -D-fructose 2-6-bisphosphate binds to the R-state of the enzyme and increases the affinity of the enzyme for fructose-6-phosphate.

β -D-fructose 2-6-bisphosphate also decreases the inhibitory effects of ATP



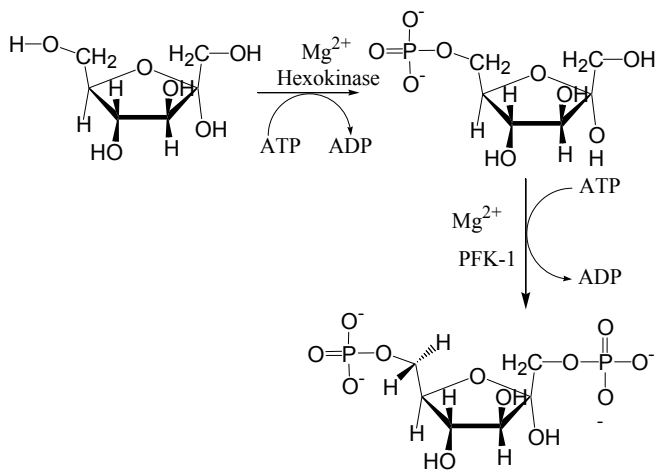
β -D-Fructose-2,6-bisphosphate



Metabolism of Hexoses other than glucose.

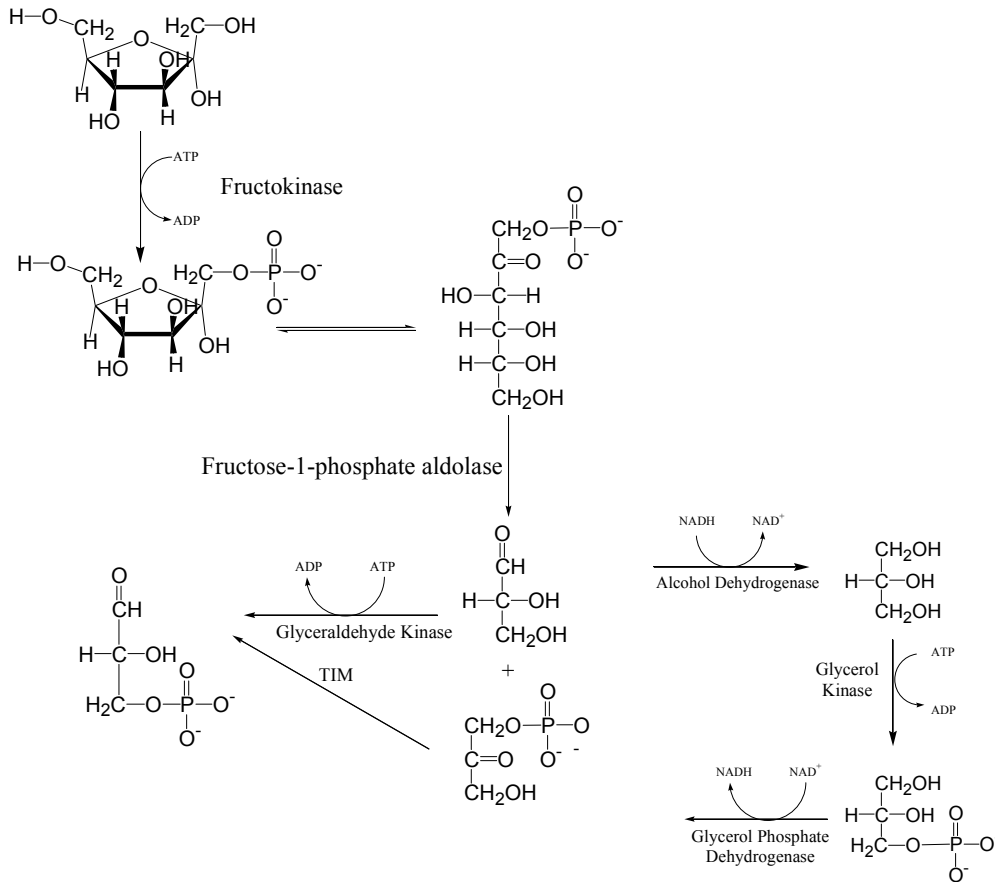
I. Fructose.

Sucrose is a major fuel source of our diets. Sucrose is a disaccharide of fructose and glucose. There are two pathways for the metabolism of fructose.



The first and simplest pathway for fructose catabolism occurs in the muscle cells where fructose is a substrate for hexokinase which produces fructose-6-phosphate, a substrate for PFK-1.

The liver, however, contains little hexokinase. Instead it contains glucokinase which specifically phosphorylates glucose. The liver catabolizes fructose through a pathway that involves six enzymes.



Step 1:

In the liver the first step of fructose catabolism is the phosphorylation of fructose by fructokinase to form fructose-1-phosphate. Note that neither hexokinase or phosphofructokinase can phosphorylate fructose-1-phosphate into fructose-1,6-bisphosphate.

Step 2:

In the liver we find a class I aldolase which is an isozyme of fructose bisphosphate aldolase (aldolase Type A). The Type A aldolase is specific for the substrate fructose-1,6-bisphosphate. The isozyme of aldolase found in the liver is called a Type B aldolase. It can utilize fructose-1-phosphate as well as fructose-1,6-bisphosphate as a substrate. So the Type B aldolase found in the liver catalyzes the aldolytic cleavage of fructose-1-phosphate into glyceraldehydes and dihydroxyacetone phosphate.

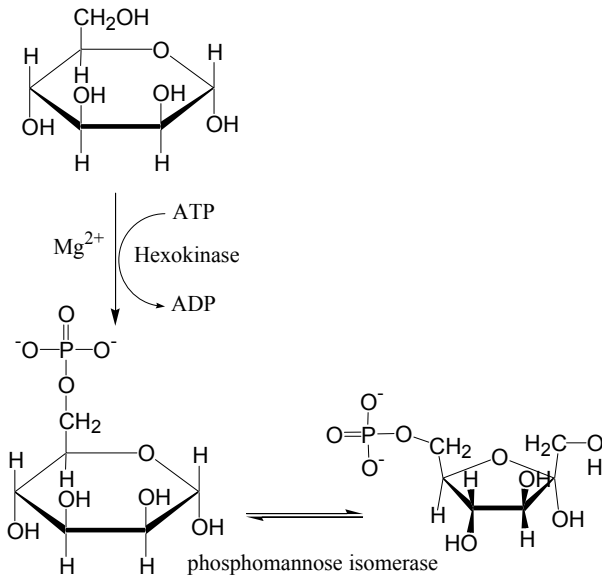
Step 3:

The dihydroxyacetone phosphate produced can be converted into glyceraldehyde 3-phosphate by the reaction catalyzed by triose phosphate isomerase. The other product of the aldolytic cleavage, glyceraldehydes can be directly phosphorylated by glyceraldehyde kinase to form glyceraldehydes 3-phosphate.

Step 4: There is an alternative pathway where the glyceraldehydes is reduced by alcohol dehydrogenase into glycerol. Glycerol is then phosphorylated by glycerol kinase to form glycerol-3-phosphate. The glycerol 3-phosphate is then oxidized by glycerol phosphate dehydrogenase into dihydroxyacetone

phosphate which of course is converted into glyceraldehydes 3-phosphate by triose phosphate isomerase.

II Mannose



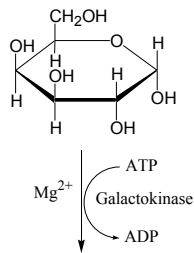
Mannose is the C2 epimer of glucose.

Mannose is a substrate for hexokinase which converts it into Mannose 6-phosphate.

An enzyme similar to phosphoglucose isomerase, phosphomannose isomerase isomerizes mannose 6-phosphate into fructose-6-phosphate.

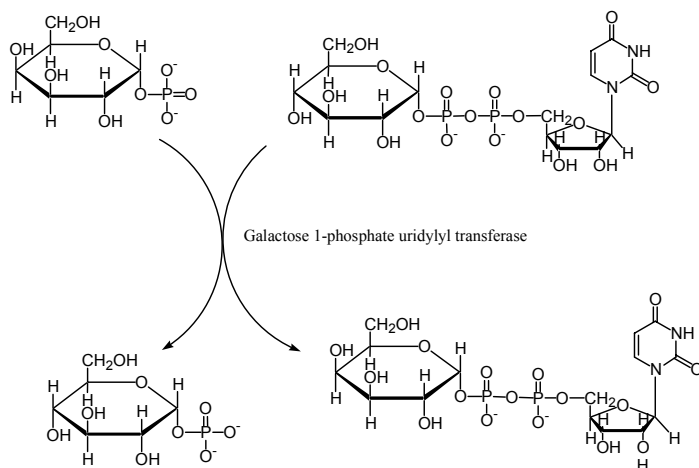
Fructose 6-phosphate is the substrate for PFK-1.

III Galactose



Galactose is the C4 epimer of glucose.

The entry of galactose into glycolysis begins with the phosphorylation of galactose by galactokinase to form galactose 1-phosphate.



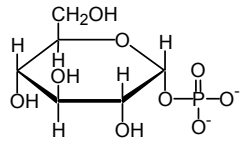
Galactose 1-phosphate is then converted into UDP-galactose by the enzyme galactose-1-phosphate uridylyltransferase with the concurrent formation of glucose-1-phosphate from UDP-glucose.

This enzyme has a ping pong kinetic mechanism. UDP-glucose binds to the enzyme first. A covalent enzyme-UMP intermediate is formed liberating glucose-1-phosphate which dissociates from the enzyme. Galactose-1-phosphate then binds and reacts with the covalent UMP-enzyme intermediate to form

UDP-galactose which then dissociates from the enzyme.

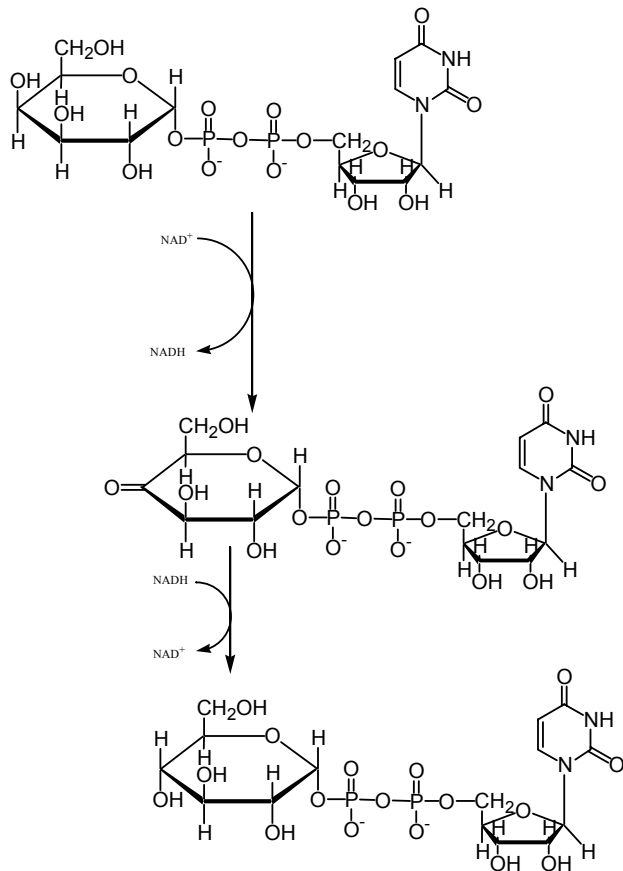
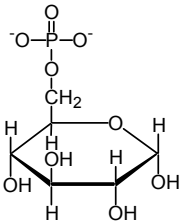
Galactosemia is the genetic disease caused by the inability of the individual to convert galactose into glucose. The symptoms of it are mental retardation, liver damage and cataracts. This genetic disease is

caused by a deficiency of the galactose-1-phosphate uridylyltransferase enzyme. Galactosemia is treated by a galactose free diet which reverses all of the symptoms except for the mental retardation.



The glucose 1-phosphate formed in the first step of this reaction is converted into glucose-6-phosphate by the enzyme phosphoglucomutase.

Phosphoglucomutase



The UDP-galactose is converted into UDP-glucose by the enzyme UDP-glucose-4-epimerase. This enzyme has an active site NAD⁺ bound and functions through the sequential oxidation and reduction of the C4 atom.

The net result of UDP-glucose uridylyltransferase and UDP-glucose-4-epimerase is the conversion of galactose-1-phosphate into glucose-1-phosphate.