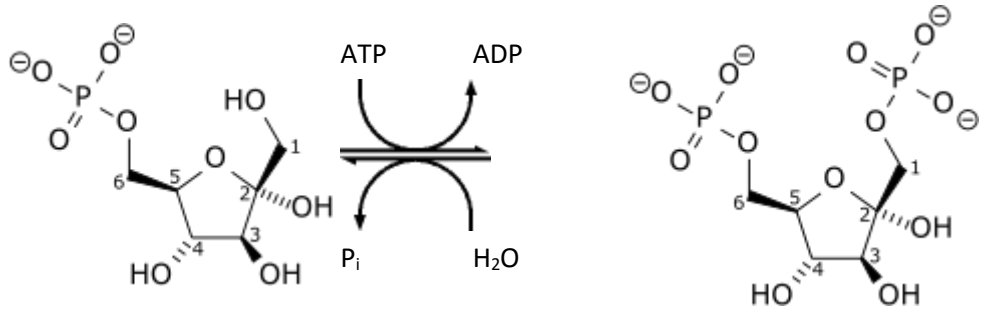
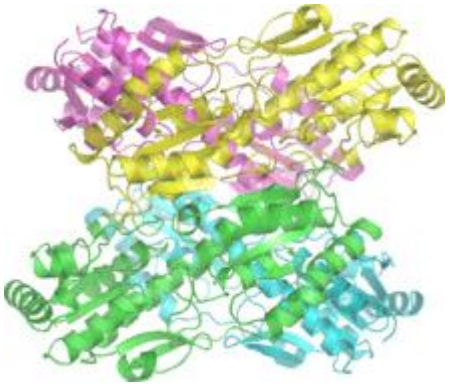
Phosphofructokinase 1

Phosphofructokinase-1 (PFK-1) is one of the most important regulatory enzymes (EC 2.7.1.11) of glycolysis. It is an allosteric enzyme made of 4 subunits and controlled by many activators and inhibitors.
PFK-1 catalyzes the important "committed" step of glycolysis, the conversion of fructose 6-phosphate and ATP to fructose 1,6-bisphosphate and ADP.
Because of that, it is one of the key regulatory and rate limiting steps of glycolysis.
PFK is able to regulate glycolysis through allosteric inhibition, and in this way, the cell can increase or decrease the rate of glycolysis in response to the cell's energy requirements.

Structure and Mechanism





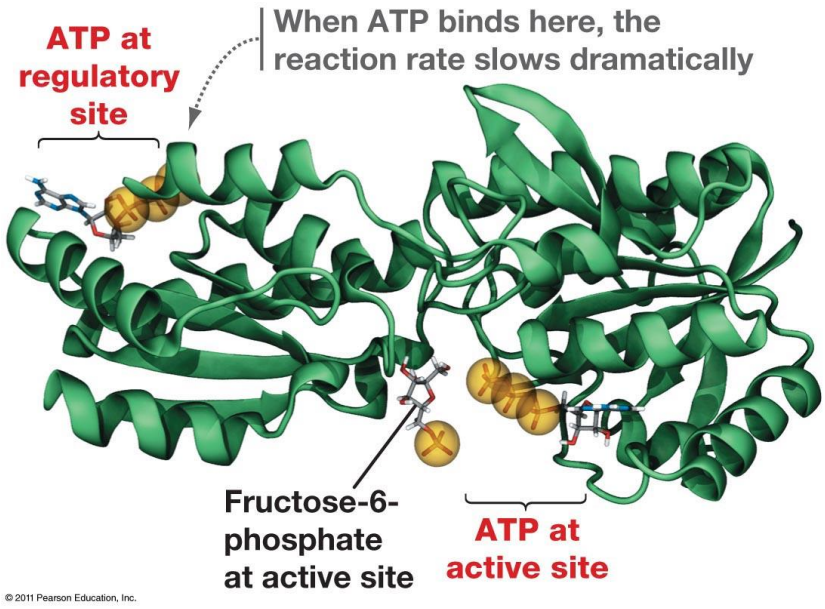
Mammalian PFK1 is a 340kd tetramer composed of three types of subunit: muscle (M), liver (L), and platelet (P).
The composition of the PFK1 tetramer differs according to the tissue type it is present in.
For example, mature muscle expresses only the M isozyme, therefore the muscle PFK1 is composed solely of homotetramers of M4.
The liver and kidneys express predominantly the L isoform.
Erythrocytes express both M and L subunits, which randomly tetramerize to form M4, L4 and the three hybrid forms of the enzyme (ML3, M2L2, M3L).

As a result, the kinetic and regulatory properties of the various isoenzymes pools are dependent on subunit composition. Tissue-specific changes in PFK activity and isoenzymic content contribute significantly to the diversities of glycolytic and gluconeogenic rates observed for different tissues.

PFK1 activity can be described using the symmetry model of allosterism whereby there is a concerted transition from an enzymatically inactive T-state to the active R-state.
F6P binds with a high affinity to the R state but not the T state enzyme. For every molecule of F6P that binds to PFK1, the enzyme progressively shifts from T state to the R state. Thus a graph plotting PFK1 activity against increasing F6P concentrations would adopt the sigmoidal curve shape traditionally associated with allosteric enzymes.

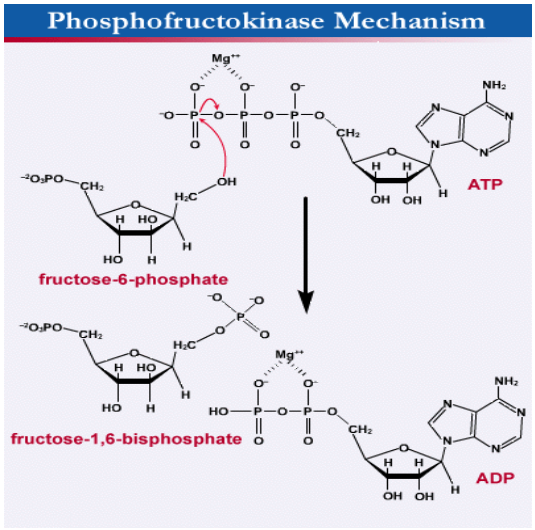
PFK1 belongs to the family of phosphotransferases and it catalyzes the transfer of γ-phosphate from ATP to fructose-6-phosphate.
The PFK1 active site comprises both the ATP-Mg2+ and the F6P binding sites.
The positively charged side chain of Arg in the active site forms a hydrogen-bonded salt bridge with the negatively charged phosphate group of F6P. This interaction stabilizes the R state relatively to the T state.

In the T state, enzyme conformation shifts slightly such that the space previously taken up by the Arg is replaced with Glu. This swap in positions between adjacent amino acid residues inhibits the ability of F6P to bind the enzyme.



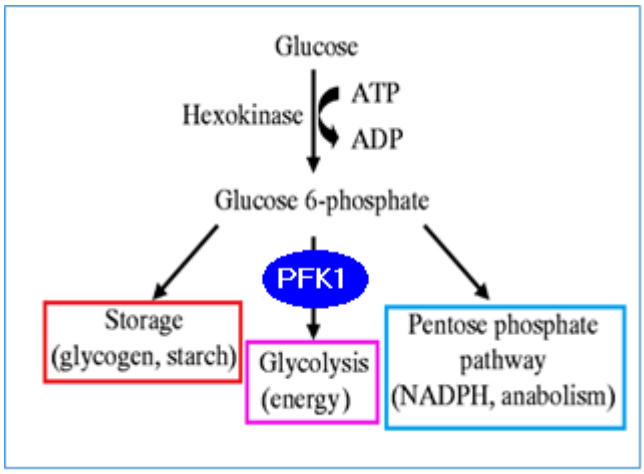
PFK1 is an allosteric enzyme and has a structure similar to that of hemoglobin insofar as it is a dimer of a dimer. Each subunit of the tetramer is 319 amino acids and consists of two domain, one that binds the substrate ATP, and the other that binds fructose-6-phosphate.
On the opposite side of the each subunit from each active site is the allosteric site, at the interface between subunits in the dimer. ATP and AMP compete for this site.
The N-terminal domain has a catalytic role binding the ATP, and the C-terminal has a regulatory role.

Allosteric activators, such as AMP, bind to the allosteric site as to facilitate the formation of the R state by inducing structural changes in the enzyme. Similarly, inhibitors such as ATP bind to the same allosteric site and facilitate the formation of the T state, thereby inhibiting enzyme activity.

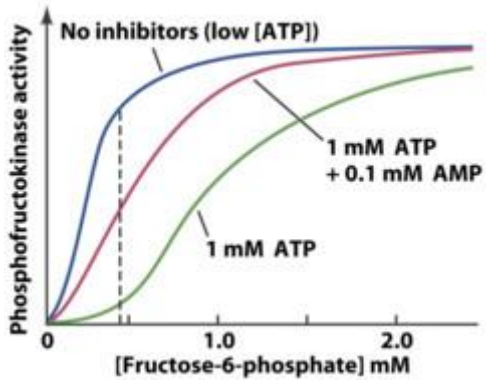


The reaction mechanism implies the hydroxyl oxygen of carbon 1 doing a nucleophilic attack on the gamma phosphate of ATP. The P-O bond electrons are pushed to the anhydride oxygen between the beta and gamma phosphates of ATP.

Regulation



PFK1 is the most important control site in the mammalian glycolytic pathway.
This step is subject to extensive regulation since it is not only highly exergonic under physiological conditions, but also because it is a committed step - the first irreversible reaction unique to the glycolytic pathway.
This leads to a precise control of glucose (and the other monosaccharides galactose and fructose going down the glycolytic pathway).
Before this enzyme's reaction, glucose-6-phosphate can potentially travel down the pentose phosphate pathway (to form sugars for nucleotides), or be converted to glucose-1-phosphate for glycogenesis.



PFK1 is allosterically inhibited by high levels of **ATP** but **AMP** reverses the inhibitory action of ATP. Therefore, the activity of the enzyme increases when the cellular ATP/AMP ratio is lowered.
Glycolysis is thus stimulated when energy charge falls.
PFK1 has two sites with different affinities for ATP, which is both a substrate and an inhibitor.
ATP is the final product of glucose catabolism (glycolysis – Krebs cycle – oxidative phosphorilation). ATP concentration build up indicates an excess of energy and glycolysis must slow down.

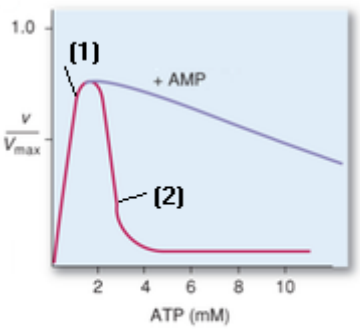
Anyway, ATP concentration has not a great range of variation in the cells. The difference in ATP concentration between a rest state and a full activity state is only 10% for an organism.
On the other hand, glycolysis rate undergoes much greater variations. This is due to the AMP role, antagonist to ATP inhibition.
AMP concentration is mainly linked to the following reaction, catalyzed by adenilate kinase enzyme:

$$ADP + ADP \leftrightarrow AMP + ATP$$

The reaction constant is

$$k = \frac{[ATP][AMP]}{[ADP]^2} = 0{,}44$$

[ADP] is generally about 10% [ATP] in a cell, while [AMP] is about 1% [ATP].
More specifically, in eritrocites, we have:
[ATP] = 1,850 mM [ADP] = 0,145 mM [AMP] = 0,005 mM Total conc. = 2,000 mM

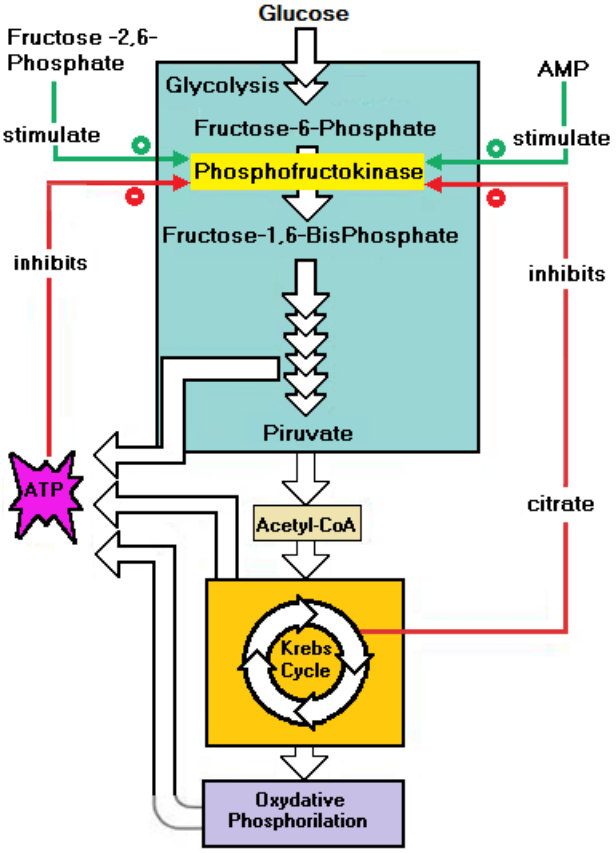


ATP is both a reagent (1)and an inhibitor (2) for PFK-1

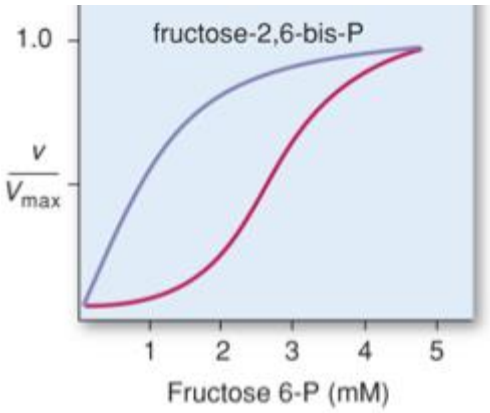
Let's say that during physical exercise we have an 8% [ATP] drop.
This leads to:
[ATP] = 1,702 mM
[ADP] + [AMP] = 2,000 – 1,702 = 0,298 mM
And, substituting these values in the above k,
[ADP] = 0,278 mM
[AMP] = 0,020 mM.
That means [AMP] increases 4 times during physical exercise and as a result [ATP]/[AMP] decreases 4 times and we have the same decrease for inhibition.

PFK1 is also inhibited by low **pH** levels, which augment the inhibitory effect of ATP. The pH falls when muscle is functioning anaerobically and producing excessive quantities of **lactic acid**.
This inhibitory effect serves to protect the muscle from damage that would result from the accumulation of too much acid.

Finally, PFK1 is allosterically inhibited by **citrate**, the first acid in Krebs Cycle.
Although citrate does build up when the Krebs Cycle enzymes approach their maximum velocity, it is questionable whether citrate accumulates to a sufficient concentration to inhibit PFK-1 under normal physiological conditions.

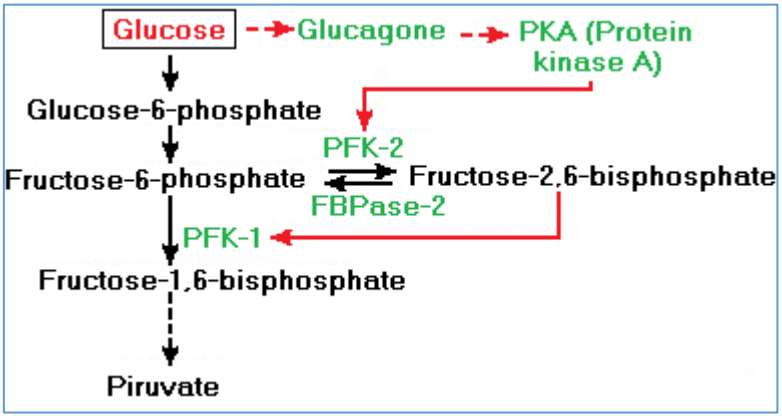


However the most potent activator is **fructose 2,6-bisphosphate**, which is also produced from fructose-6-phosphate by a different enzyme, PFK2.

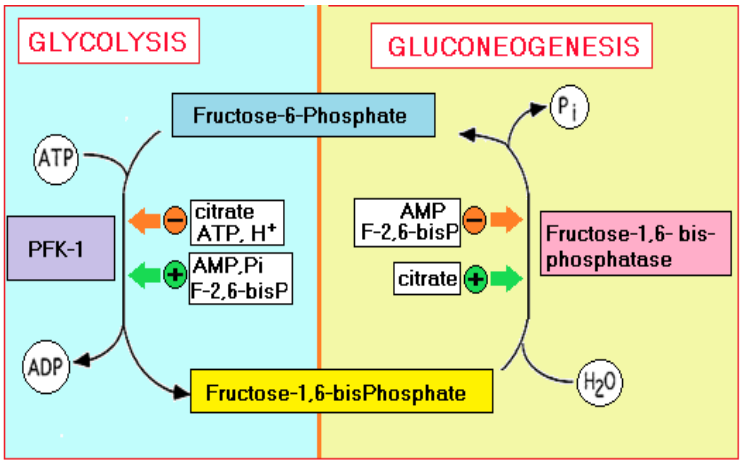


Hence, an abundance of F6P results in a higher concentration of fructose 2,6-bisphosphate (F-2,6-BP). The binding of F-2,6-BP increases the affinity of PFK1 for F6P and diminishes the inhibitory effect of ATP. This is an example of feedforward stimulation as glycolysis is accelerated when glucose is abundant.

PFK-2 is inhibited by **glucagon** through repression of synthesis. Glucagon activates protein kinase A which, in turn, shuts off the kinase activity of PFK2. This reverses any synthesis of F-2,6-BP from F6P and thus inhibits PFK1 activity.



Glucagon is a peptide hormone, produced by the pancreas. It raises the concentration of glucose in the bloodstream. Its effect is opposite that of insulin, which lowers the glucose concentration. The pancreas releases glucagon when the concentration of glucose in the bloodstream falls too low. Glucagon causes the liver to convert stored glycogen into glucose, which is released into the bloodstream. High blood glucose levels stimulate the release of insulin. Insulin allows glucose to be taken up and used by tissues. Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level.



Summarizing…

The precise regulation of PFK1 prevents glycolysis and gluconeogenesis from occurring simultaneously.

However, there is substrate cycling between F6P and F-1,6-BP.

Fructose-1,6-bisphosphatase (FBPase) catalyzes the hydrolysis of F-1,6-BP back to F6P, the reverse reaction catalyzed by PFK1. There is a small amount of FBPase activity during glycolysis and some PFK1 activity during gluconeogenesis. This cycle allows for the amplification of metabolic signals as well as the generation of heat by ATP hydrolysis