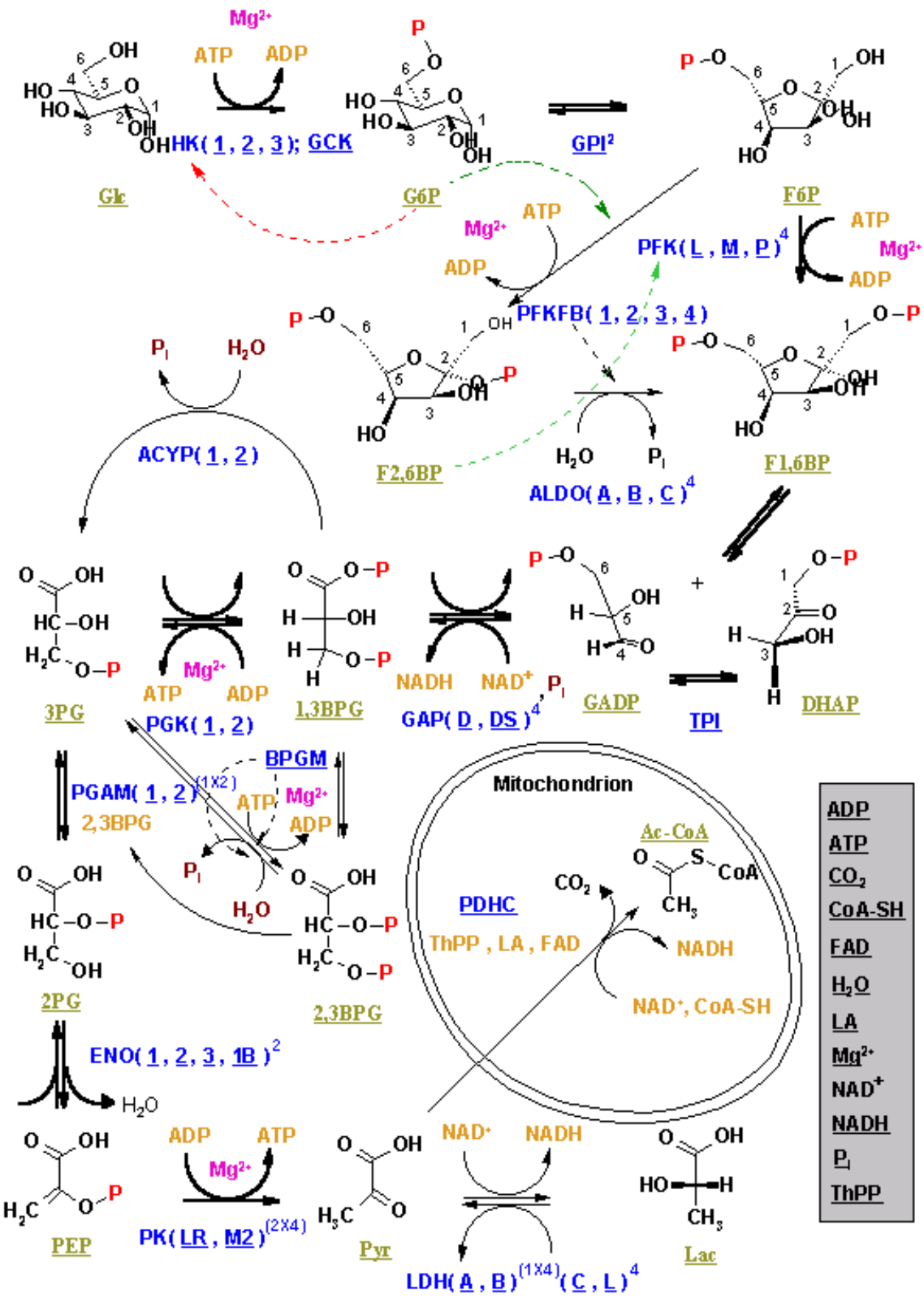
Glycolysis : Splitting of sugar for energy



Glycolysis is one of the catabolic paths found in cells. As defined, Catabolism is a pathway, which breaks down more complex molecules into simpler ones with energy release. This particular metabolic pathway, glycolysis, is a series of reactions in which a molecule of glucose can be oxidized forming two molecules of pyruvic acid. It is the initial process of many pathways of carbohydrate catabolism, and serves to:

- Generate high-energy ATP molecules
- Produce a variety of 6 or 3 carbon intermediate metabolites.

Glycolysis can operate under aerobic or anaerobic conditions, and supplies 3C compounds for further mitochondrial degradation in the presence of Oxygen. Glycolysis can be observed in nearly all types of organisms, however since it produces less energy per glucose molecule than the complete aerobic oxidation, it is used more often in anaerobic conditions.

In aerobic conditions, a considerable amount of ATP is made in mitochondria, and some is made during glycolysis. In cases where there is a lack of O2, glycolysis creates an expanding pool of 3C compounds (mainly lactate). This results in a decrease of pH, creating more acidic conditions. This can occur in cases where blood supply to tissue is blocked, or with poor muscle tone. During anaerobic glycolysis there is a production of only 2 ATP per Glucose, opposed to the production of 2ATP + 2NADH + Pyruvate (3C) per glucose in aerobic conditions. The extra NADHs can serve as a source of energy.

Aerobic Glycolysis is divided in 2 parts, and can be represented by:

1- Glucose → 2 Pyruvate (Energy Release)

2- 2 ADP + 2 Pi + 2 NAD+ → 2 ATP + $2H_20$ + 2 NADH + $2H^+$ (Energy Capture)

Note that the Energy Release and Energy Capture balance each other.

Stage I (investment of ATP)

1) Hexokinase (HK)

It is the 1st Investment of ATP. It is an enzyme that phosphorylates a hexose, to a hexose phosphate. Like many other kinases, this reaction requires the magnesium salt form of ATP, in order to balance the charges, neutralizing the negative charges of ATP.

α-D-Glucose + MgATP → α-D-Glucose-6-P + $MgADP^-$ + H^+

This is an irreversible reaction, due to the considerable release of energy.

$\Delta G^{o'}$ = -16 KJ/mol

HK shows induced fit as glucose binds to it. HK is able to handle a variety of different hexoses (α-D-glucose, α-D-fructose, α-D-mannose). The glucose molecule in this reaction is sheltered on the active site, in order to prevent water from coming in contact. Aldohexoses are the preferred substrates, glucose and mannose. Also know that there is allosteric inhibition by the enzyme product α-D-Glucose-6-P, which slows down these reactions.

There are also other Isozymes of HK, for example GK, glucokinase. GK is an enzyme specific for glucose. I should also point out that Km (Michaelis Constant) for HK varies from 0.001 - 0.1 mM, and for GK Km = 10mM. Therefore, when high levels of

glucose are present in the body, GK plays an important role in order to maximize glucose metabolism. GK however, is not inhibited by Glucose-6-P.

2) Glucose-6-P Isomerase

It consists of the conversion of aldohexose-6-P into ketohexose-6-P. It is a reversible enzyme, which means it works in both directions.

α-D-Glucose-6-P $\rightarrow^{Mg^{++}}\rightarrow$ α-D-Fructose-6-P $\Delta G^{o'} = 1.7$ KJ/mol

This conversion of an aldohexose-6-P into a ketohexose-6-P is reversible. $\Delta G^{o'}$ is close enough to zero in order to have a fairly even energetic plane to go back and forth. This isomerase works by converting α-D-Glucose-6-P into its chain aldehyde, which is then converted into a ketohexose-6-P, which is then converted back to a cyclic molecule, α-D-Frucose-6-P.

3) Phosphofructokinase-1 (PFK-1)

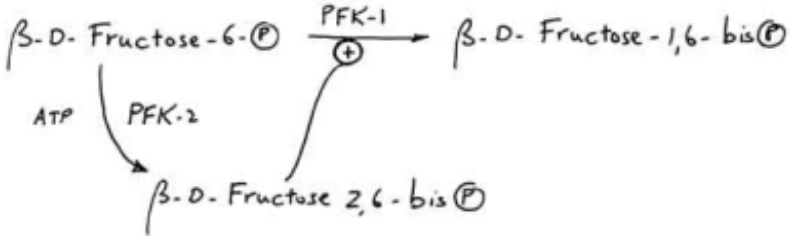
This is the 2nd ATP investment, and its an important control point, since it is a rate limiting step in glycolysis. It has relatively slow v_o Also note the use of β-D-Frucose-1,6-bisP. This molecule comes from the inter conversion of β-D-Frucose-1,6-bisP and α -D-Frucose-1,6-bisP, these two isomers are able to go from one to another without the help of any enzyme.

ATP + β-D-Frucose-6-P $\rightarrow^{Mg^{++}}\rightarrow$ ADP + β-D-Frucose-1,6-bisP $\Delta G^{o'} = -14.3$ KJ/mol

So in order to boost glycolysis, you can boost PFK-1. This is an irreversible reaction, as its $\Delta G^{o'}$ is not close to zero, and the reaction only goes to the right. Largely by the breakdown of the ATP, which releases a lot of energy. This reaction is inhibited by ATP, but ATP is the substrate, so what's going on? Well, if ATP levels are high, you are in good shape; you want to turn off the production. If ATP levels are down, then you want to turn it on. Also note that AMP and ADP relieve this inhibition, which makes sense since they are a sign that ATP levels are going down.

Citrate is another inhibitor of PFK-1. Citrate is an intermediate of the Krebs cycle in the mitochondria. The idea here is that if citrate is in the cytoplasm, the Krebs cycle must be working well, which is a good indicator that we have a healthy ATP production.

This multi-regulated enzyme is however, it is regulated by β-D-fructose-2,6-bisP, which is the most important regulator. β-D-fructose-2,6-bisP made from β-D-fructose-6-P by an enzyme called PFK-2. This enzyme activity rises when [fructose-6-P] increases. This [fructose-6-P] indicates PFK-1 is moving too slow (i.e. Substrate buildup). β-D-fructose-2,6-bisP stimulates PFK-1 to relieve the buildup of fructose-6-P, this mechanism is called feed-forward control of PFK-1.



4) Aldolase

β-D-fructose-1,6-bisP ↔ Dihydroxyacetone-P + D-Glyceraldehyde-3-P
ΔG°' = 24Kj/mol

This enzyme splits a β-D-fructose-1,6-bisP into 2 parts, producing two 3C sugars. However ΔG°' is rather unfavorable, there is a big hill, so how does it work? We know that ΔG°' reflects the energies of S and P, but the [P] and [S] can also be very important for a reaction. It really depends on how close to equilibrium the actual [P] and [S] are, in the cytoplasm.

$\Delta G = \Delta G^{\circ\prime} + 2.3RT*\log([DHAP][G3P])/([Fr\text{-}1,6\text{-}BP])$

In cytoplasm: [DHAP], [G3P] and [Fr-1,6-BP] are close to their equilibrium concentration. At equilibrium $\Delta G^{\circ\prime} = -2.3RT*\log(K_{eq})$, thus at equilibrium the actual ΔG for the reaction is close to zero. The unfavorable ΔG°' is offset by the actual cell concentrations of S and P.

5) Triose P Isomerase

DHAP → D-Glyceraldehyde-3-P ΔG°'= 7.6KJ/mol

This particular reaction, inter converts the two 3C products of aldolase. ΔG°' is unfavorable in this reaction, but the concentration of DHAP and G3P in the cytoplasm are close to the equilibrium values, so ΔG is close to 0 and a conversion from DHAP to G3P is possible. This step concludes the first stage of Glycolysis, with the production of the two molecules of G3P.

<u>Stage 2 (Production of ATP)</u>

6) Glyceraldehyde-3-Phospohate Dehydrogenase (G3PDH)

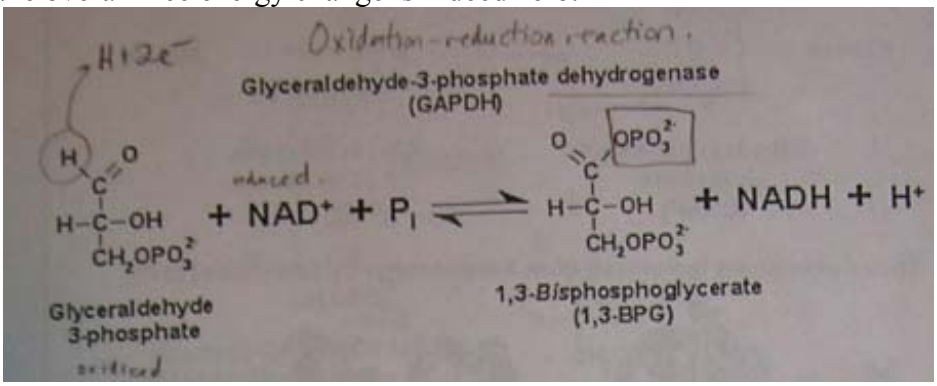
This is where G3P is oxidized, there is a conversion of aldehyde into a high energy phosphoanhydride. In this reaction ΔG°' is unfavourable, but [S] and [P] are close to equilibrium. Therefore ΔG overall is close to zero.

During aldehyde oxidation NAD^+ accepts H^- (proton + $2e^-$)

Phosphate forms a mixed anhydride with the carboxyl at C-1

D-Glyceraldehyde-3-P ↔ 1,3-bisphosphoglycerate + NADH + H^+ ΔG°'=6.3KJ/mol

This is a near equilibrium reaction. Standard free energy change is somewhat positive, but the overall free energy change is indeed zero.



The mechanism of this reaction involves a Cys residue on the active site. The S acts like an O molecule (for more info on the mechanism check lecture 29 handout notes on BCH210 website). This cysteine is critical, and it can be blocked in a reaction with iodoacetate.

$ICH_2\text{-}COO^- + \text{-}SH \rightarrow \text{-}S\text{-}CH_2\text{-}COO^-$

1,3-BPGA has a higher phosphate transfer potential than ATP. This step is the main energy payoff of glycolysis, since it makes two NADH molecules.

7) Phosphoglycreate Kinase : 1st formation of ATP

On this step, a molecule of ATP is formed using the energy stored on the 1,3-BPGA. This is a favorable reaction with $\Delta G^{o\prime} = -19$ kJ/mol.

1,3-bisphosphoglycerate + ADP $\rightarrow^{Mg^{++}}\rightarrow$ 3-Phosphoglycerate

Again on this reaction, the concentrations of S and P in the cytoplasm is important, they are close to their equilibrium values, thus ΔG is close to 0 and this reaction is reversible within the cell.

The PGK and the GAPDH are intimately associated in the cell. They are actually bound to each other. The product of GAPDH is channeled to the active site of PGK. Therefore there aren't many free 1,3-BPG found inside cells.

In arsenic poisoning, arsenate can compete with phosphate in the G3PDH reaction. Arsenate forms unstable arsenoanhydride. Thus, there is a loss of the phosphoglycerate kinase step, and a loss of ATP formation in arsenic-poisoned cells.

8) Phosphoglycerate mutase

This involves the conversion of 3-PGA to 2-PGA, it is a reversible reaction due to the concentrations of S and P.

9) Enolase

Step where 2-PGA is dehydrated and forms PEP. PEP is "peppy" (energetic), has very high phosphate transfer potential. The reversible elimination of H_2O produces unstable phosphoenol (enol= double bond with hydroxyl group).

2-PGA $\leftrightarrow^{H_2O}\leftrightarrow$ Phosphoenolpyruvate (PEP) $\Delta G^{o\prime} = -19$ kJ/mol.

Enolase is inhibited by fluoride (sold as rat poison)

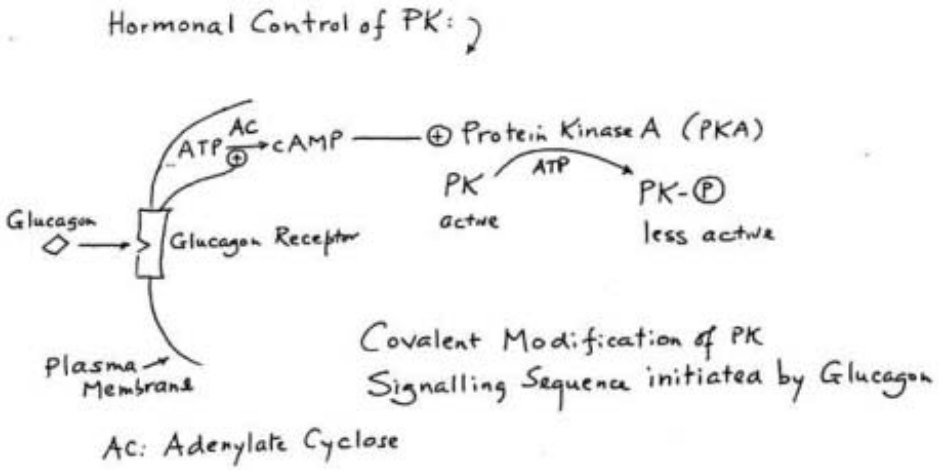
10) Pyruvate Kinase (PK)

Phosphoenolpyruvate(PEP) + ADP $\rightarrow^{Mg^{++}}\rightarrow$ Pyruvate + ATP $\Delta G^{o\prime} = -32$ kJ/mol.

This is where PEP transfers its P to ADP, and forms Pyruvate and ATP. This is an irreversible reaction, but it is also a control point. PK is a regulatory enzyme. ATP is an allosteric inhibitor of PK, and Fructose-1,6-BP is an allosteric activator. This makes sense, since if there is a build up of Fru-1,6-BP, that means aldolase is not working fast enough, and activating PK pulls the reaction, speeding up aldolase. Also note that PK can be regulated by covalent modification (phosphorylation by PKA). As a general rule,

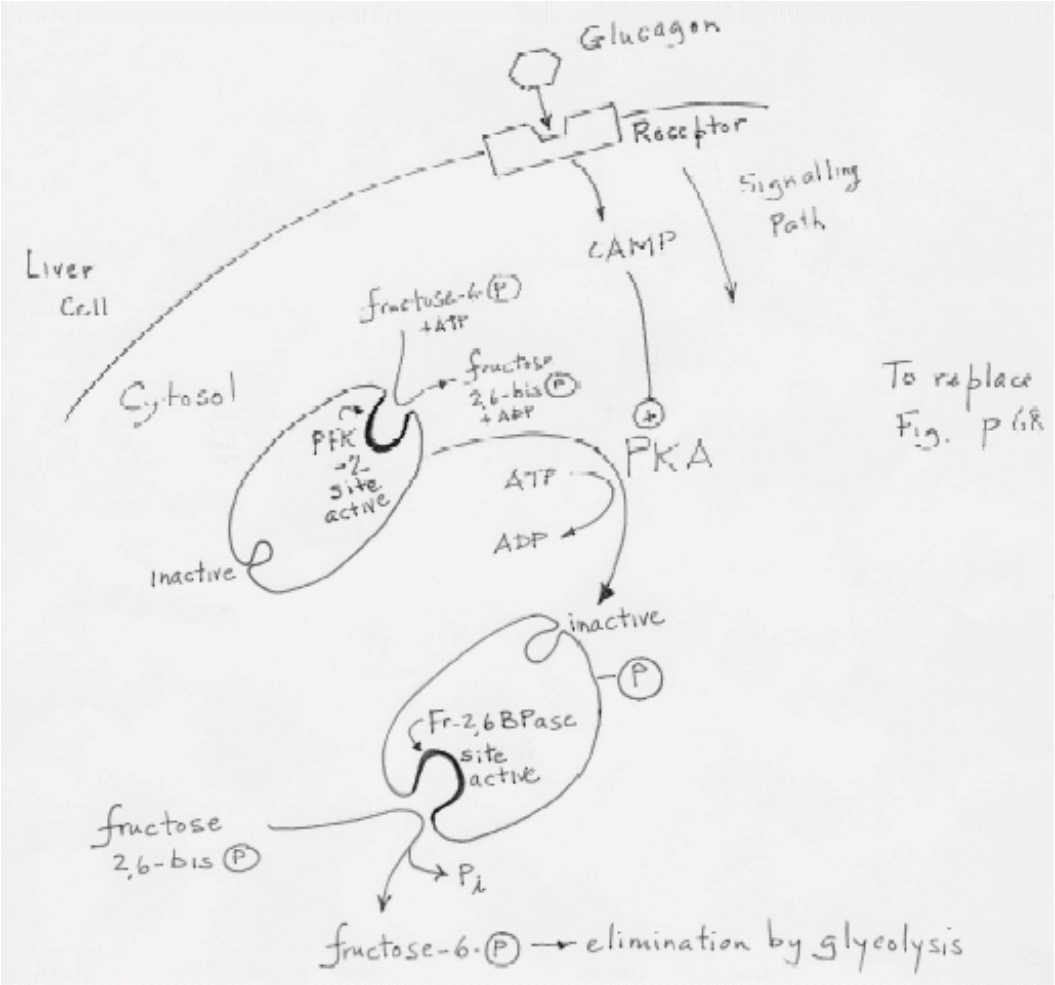
metabolically irreversible reactions occur with regulated enzymes. The reason why this is a metabolically irreversible reaction is because the enzyme is regulated, so the reaction is never able to reach a steady state equilibrium. In this case, the enzyme is quite low. In some instances you might want to turn off glycolysis, so that's where this kind of regulation is important.

This is done by hormonal control of the enzyme by glucagons, a peptide hormone secreted by the pancreas. Glucagon attempts to preserve blood glucose levels, which is vital for brain function. Neurons are very sensitive to reduced glucose and oxygen supply and to pH. This can happen by the reduction of the use of glucose by the liver.



Another way to shut down glycolysis in liver is by the hormonal control of PFK-1. Glucagon from the pancreas is able to depress glycolysis. Remember how PFK-2 produces Fru-2,6-BP which is the main activator of PFK-1. Well, as Glucagon activates PKA, PFK-2 is phosphorylated and becomes "inactive", which prevents the activation of PFK-1.

Glucagon leads to loss of PFK-2 activity and loss of fructose 2,6-BP. However this is not as simple as that. PFK-2 also has a second activity, which is turned on, upon the phosphorylation of the enzyme. This second function is a Fuctose-2,6-bisphosphatase, which breaks down fructose-2,6-BP into fructose-6-P and a Pi.



Anaerobic Glycolysis and Fermentation

In the absence of O_2, yeast for example, can process Pyruvate in fermentation. The two important steps in this process are Pyruvate decarboxylase and alcohol dehydrogenase. As a result you get ethanol and CO_2 and ATP in anaerobic glycolysis (fermentation).

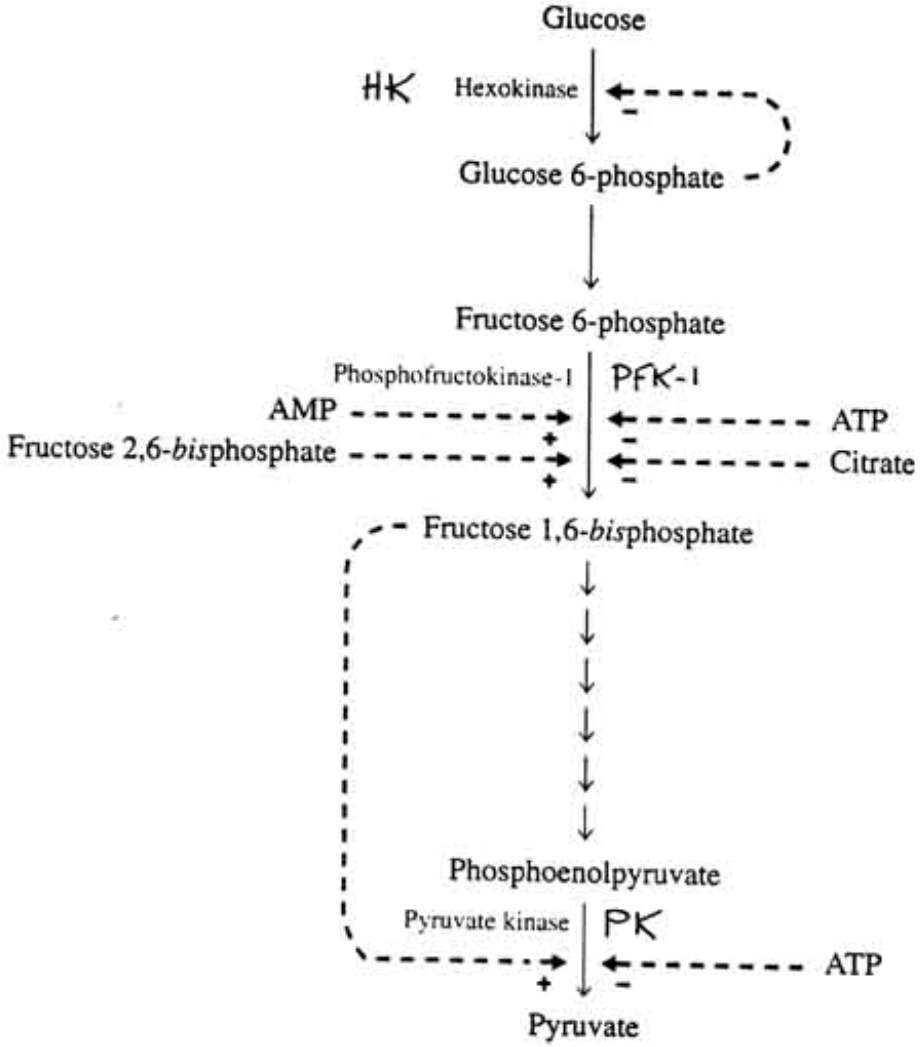
Glycogenolysis

It is very important that the body holds the blood glucose level above 3mM. If these levels go lower, the person will have hypoglycemia, causing loss of consciousness and brain damage. Therefore, the body can use glycogen as a precursor of glucose in our body. This glycogen is mainly found in the liver. Hormones can interact with cells (glucagons in liver and epinephrine in muscle) via receptors, which initiate signaling pathways. Upon activation, glycogen phosphorylase starts to break down the glycogen and produce free α-D-Glucose-1-P and leaving the glycogen 1 residue shorter. Then, with the action of a Phosphoglycomutase, this molecule is converted to α-D-Glucose-6-P. This molecule can now be used in glycolysis or it can be converted to glucose and be used by other tissues.

PKA inactivates glycogen synthesis, which makes sense, in order to allow remaining glucose to be used in other areas of the body. Insulin in the other hand, is a

hormone that opposes the actions of glucagons and epinephrine. Insulin is made by the pancreas, and is secreted when blood glucose levels are high. Insulin, once it is bound to its receptor, activates Protein Phosphatase-1. This enzyme is responsible for activating glycogen synthase via dephosphorylation of the enzyme. This allows the synthesis of more glycogen, which will then be used to store glucose. Insulin also inactivates glycogen breakdown and favours glycolysis, which makes logical sense, as you have lots of glucose available.

This is just a picture illustrating the different regulation sites of glycolysis.



- Leonardo Silenieks