Glycolysis

Glucose is a valuable molecule. It can be used to generate **energy** (in red blood cells and in brain under normal conditions, glucose is the sole energy source), and it can be used to generate a wide variety of **intermediates** required for a range of biosynthetic purposes.

We will begin with a discussion of glycolysis, the catabolic conversion of glucose into energy with the production of either pyruvate or waste products. It is important to remember, however, that energy generation is **not** the only purpose of the pathway, and that in many cell types the formation of glucose metabolites with critical biological roles is as important as the energy produced during the pathway.

Overview of Glycolysis

Under **anaerobic** conditions, the glycolytic pathway present in most species results in a balanced reaction:

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{inorganic phosphate} \rightarrow 2\ \text{L-Lactic acid} + 2\ \text{ATP} + H_2O$$

In yeast and some other microorganisms, the final products are ethanol and carbon dioxide rather than lactate. The production of either ethanol or lactate is often termed fermentation.

Glycolysis can be a very fast pathway; under anaerobic conditions, glycolysis results in rapid generation of relatively small amounts of ATP and large amounts of waste products. This is of benefit to the organism when rapid ATP generation is required.

Under **aerobic** conditions, the reaction is slightly different:

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{phosphate} + 2\ \text{NAD} \rightarrow 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + H_2O$$

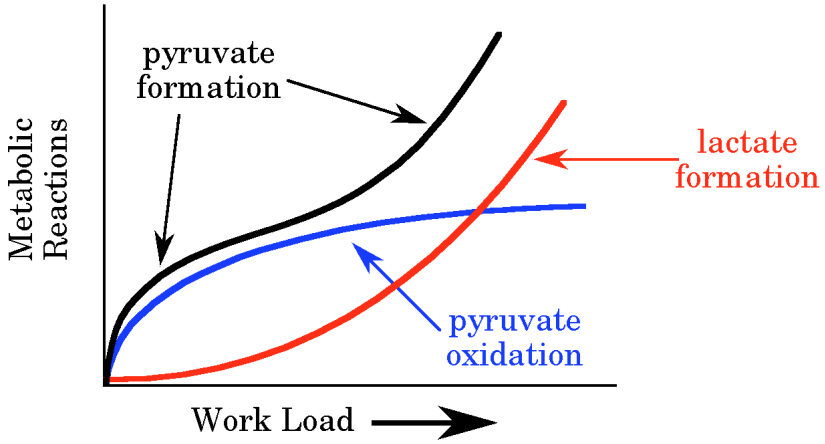
The pyruvate and NADH can be used to generate large amounts of additional energy via the TCA cycle and the oxygen-requiring pathway of oxidative phosphorylation.

This raises an interesting question: Why perform anaerobic glycolysis if the TCA cycle and oxidative phosphorylation pathways generate much more energy?

As mentioned above **glycolysis is a fast pathway**; in some situations the flow of glucose through the glycolytic pathway can greatly exceed the capacity of the TCA cycle and oxidative phosphorylation pathways to metabolize the pyruvate and NADH produced. This is true in skeletal muscle during heavy exercise; under these conditions, the muscle converts the excess pyruvate to lactate.

Lactate generation serves two purposes: it regenerates the NAD required for additional glycolysis, and it produces a byproduct (lactic acid) that can be exported from the cell. The release of lactic acid has a minor drawback: it also releases protons, and therefore decreases the pH in the environment surrounding the cell. This pH decrease is the cause of the “burn”: the sensation in muscles that signals

the limit of anaerobic exercise. (Note: it is not the lactate build-up, but rather the pH change that is responsible for necessity to terminate exercise.) Although it is not entirely beneficial to pollute the extracellular environment with protons, it is clearly better than allowing the protons to build up within the cell, which would happen if the lactate (or pyruvate) were not exported.



Regulation of glucose metabolism

Single-celled organisms usually take up and utilize glucose as rapidly as they possibly can. When exposed to large amounts of glucose they tend to shift to anaerobic metabolism, and therefore waste large amounts of the available energy. This process has its uses for the organism: the generation of energy is so rapid that cells using anaerobic glycolysis grow faster than cells using the more energy efficient, but much slower, oxidation of pyruvate. The fact that some microorganisms perform primarily anaerobic glycolysis under some conditions is also of benefit to humans: the production of ethanol and carbon dioxide from glucose is used for beer, wine, and bread production.

In multicellular organisms, cells must cooperate. If one cell uses up too much glucose, it may adversely affect other cells in the organism, possibly leading to the death of the entire organism, including the offending cell. As a result, glucose metabolism (and many other cell life-cycle functions) is tightly regulated in most multicellular organisms.

This regulation occurs at several points in the glucose metabolic pathways. In humans, the regulatory points include:

1) The transport of glucose into the cell
2) The conversion of glucose to glucose-6-phosphate
3) Two enzymatic steps in the glycolytic pathway (phosphofructokinase and pyruvate kinase)
4) The flow of glucose into or out of other pathways (glycogen and hexose-monophosphate pathway).

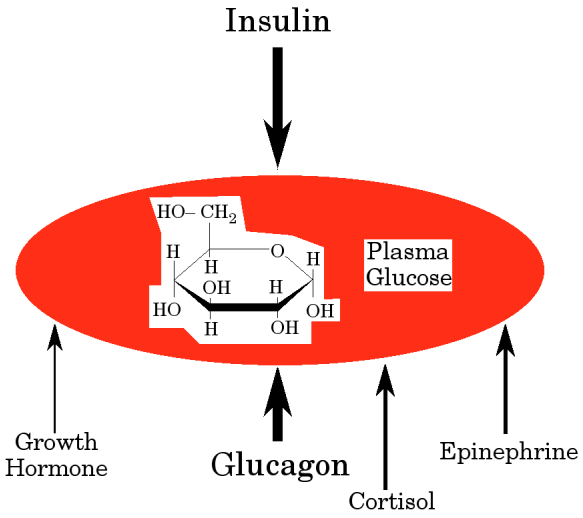
The coordinated regulation of these pathways requires an influence from outside the individual cell. Systemic hormones provide this influence: signaling molecules released into circulation that alter behavior of most cells throughout the organism.

Regulatory hormones

In mammals five hormones regulate plasma glucose levels and the rate of glycolysis: insulin, glucagon, epinephrine, cortisol, and growth hormone.

Insulin results in decreased plasma glucose; all of the other hormones listed raise plasma glucose.

A detailed description of the actions of these hormones is outside the scope of this course. (For those interested, the "Endocrine Notes" section of the course web site contains considerable information about the role of these hormones in the regulation of metabolism.) For our purposes, insulin and glucagon are the primary regulatory hormones that maintain normal plasma glucose levels; the other hormones alter plasma glucose for specific purposes, or attempt to counter excessive insulin action.



Insulin, **glucagon**, and **epinephrine** regulate the *activity* of *existing* control enzymes, usually by stimulating phosphorylation or dephosphorylation of proteins. **Insulin**, **cortisol**, and to a lesser extent, **glucagon**, alter the rates of gene transcription and of protein degradation, and therefore alter the *amounts* of enzymes.

Glucose transport

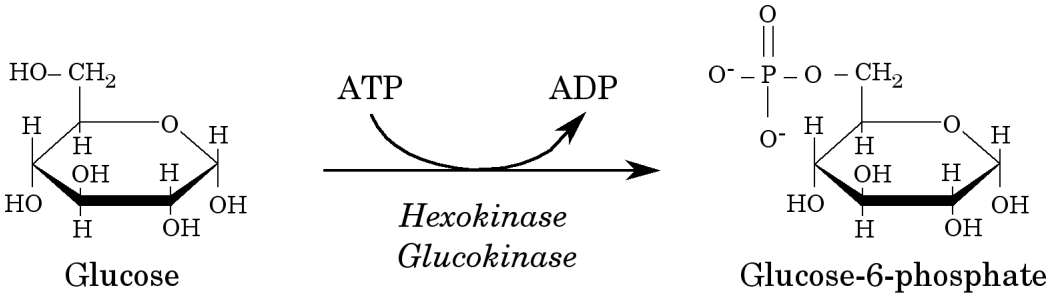
Glucose cannot diffuse through membranes. As a result, cells must have a transport protein that allows the glucose to cross the membrane. Most cells have passive transporters, which are proteins that act as enzymes to move the glucose from high to low concentration. For most cells, glucose transport is not limiting and is not tightly controlled.

Skeletal muscle and adipose tissue, however, have a specialized glucose transporter that is tightly regulated. The glucose transporter in these cells is normally sequestered in intracellular vesicles; stimulation of the cell (by insulin, or, in the

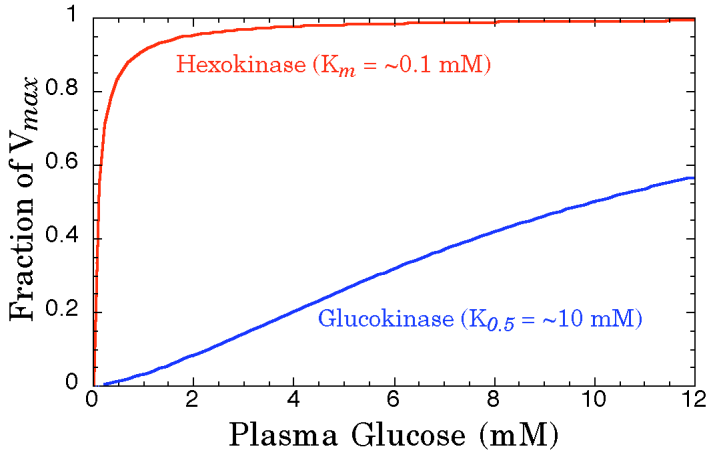
case of skeletal muscle, by muscle contraction) results in fusion of the vesicles with the plasma membrane, and therefore in a rapid increase in the number of transporters in the membrane. Assuming that the cellular concentration of glucose is lower than that outside the cell, an increase in the number of transporters results in an increase in the rate of transport of glucose (in the same way that an increase in enzyme concentration results in an increase in rate of product formation).

Glucose phosphorylation

The second site of regulation occurs inside the cell, when the glucose is phosphorylated to produce glucose-6-phosphate. The purpose of the reaction is to alter the concentration gradient (because once glucose has been phosphorylated, it is no longer glucose), and to lock glucose-6-phosphate in the cell (because glucose-6-phosphate cannot be transported by the glucose transporter). In all cells, the phosphorylation of glucose is a regulated step, although the regulation differs somewhat depending on the cell type.



The phosphorylation of glucose is performed by two different isozymes. One is hexokinase, which is present in most tissues. **Hexokinase has a high affinity for glucose**. Most tissues refrain from wasteful glucose uptake because hexokinase is inhibited by its product glucose-6-phosphate. Muscle cells have a high concentration of hexokinase; muscle cell phosphorylation of glucose is usually regulated by glucose uptake (*i.e.* by the glucose transporter), and not by hexokinase activity. This is especially obvious during muscle contraction, when glucose-6-phosphate utilization tends to be very rapid, and therefore prevents inhibition of hexokinase.



The liver and pancreas have a different isoenzyme, glucokinase. **Glucokinase has**

a low affinity for glucose, and is not inhibited by glucose-6-phosphate; instead, the concentration of glucokinase is increased by insulin. The activity of glucokinase is also regulated by glucose concentration. The enzyme has a $K_{0.5}$ for glucose of ~10 mM, which is higher than the typical blood concentration of 5 mM, and therefore the rate of the reaction increases significantly with increasing substrate concentration.

In the graph above, glucokinase activity is shown as exhibiting positive cooperativity (n = 1.5); this has been observed, but the reason for the cooperative behavior is not understood, because the protein is thought to be monomeric. Note that near the typical blood concentration of glucose (~5 mM), hexokinase is operating near V_{max}, while glucokinase activity changes essentially linearly with changes in glucose concentration.

The mechanism of hexokinase illustrates an important point about enzyme mechanisms. One potential problem with phosphorylation events, such as the hexokinase reaction, that use ATP as the phosphate donor is that ATP hydrolysis is more energetically favorable than phosphate transfer to the substrate. The enzyme must prevent ATP hydrolysis in spite of the high concentration of H_2O in aqueous solution. Hexokinase (and probably other, similar, kinases) does this by undergoing a conformational change upon binding to the carbohydrate substrate. The conformational change excludes water from the active site, and therefore prevents the reaction of ATP with water that would otherwise occur. Studies performed using hexokinase and alternate substrates show that steric exclusion of water is indeed necessary. Incubation of hexokinase with substrates lacking the carbon that protrudes from the ring results in ATP hydrolysis without carbohydrate phosphorylation.

The remaining sites of glucose metabolic regulation occur within the pathways, and will be discussed both in the context of the pathways, and in somewhat more detail at the end of the section on glucose metabolism.

Glycolytic pathway

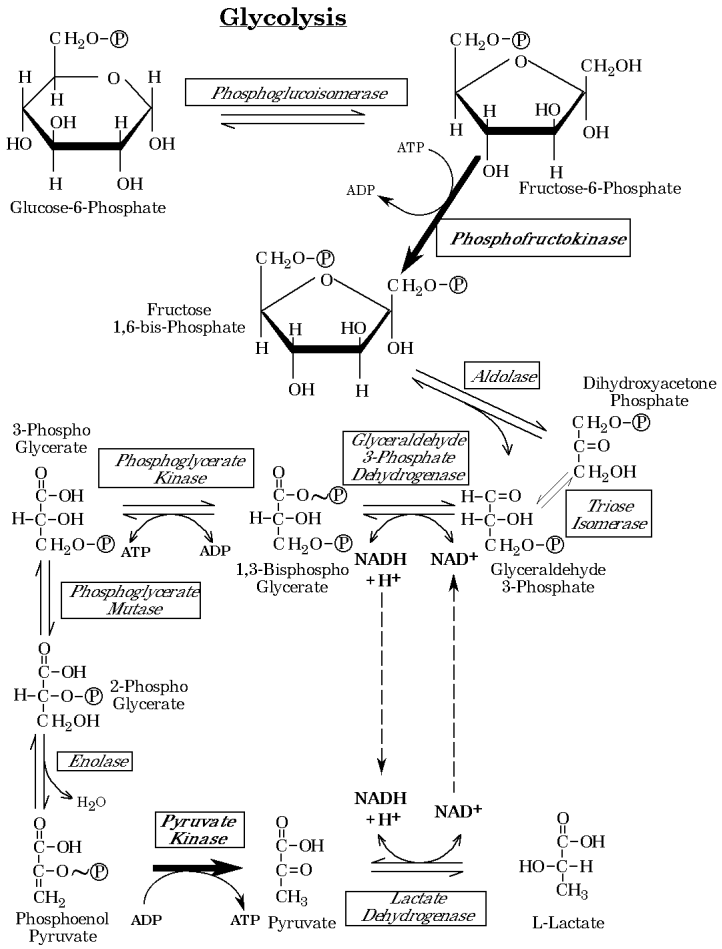
The glycolytic pathway has been extensively studied. Structures are available for at least one species variant for each of the enzymes, and many of the reaction mechanisms have been worked out in detail. In these notes, however, I will focus on the reactions, the intermediates, and the regulation of the pathway, rather than on the enzyme mechanisms or structure.

Enzymes and reactions

Phosphoglucoisomerase

The reaction pathway as drawn below begins with the branch-point compound glucose-6-phosphate. Phosphoglucoisomerase interconverts the aldohexose derivative glucose-6-phosphate and the ketohexose derivative fructose-6-phosphate. Note that fructose-6-phosphate is not chiral at C2; when using fructose-6-phosphate as substrate phosphoglucoisomerase stereospecifically forms glucose-6-phosphate rather than making a mixture of the C2 epimers mannose-6-phosphate and glucose-

6-phosphate. This is due to the specific properties of the enzyme; achieving similar results non-enzymatically would be very difficult.



Phosphofructokinase

Phosphofructokinase catalyzes the second energy-requiring step in glycolysis. As with hexokinase/glucokinase, the phosphate donor for the phosphofructokinase reaction is ATP.

The reaction catalyzed by phosphofructokinase is the **committed step** for glycolysis. While glucose-6-phosphate can be used for a number of other pathways, and the phosphoglucoisomerase reaction is freely reversible, the compound fructose-1,6-bisphosphate is committed to glycolysis. Phosphofructokinase is the **primary control point** for glycolysis, and is tightly regulated. (Note the thick arrow in the diagram.)

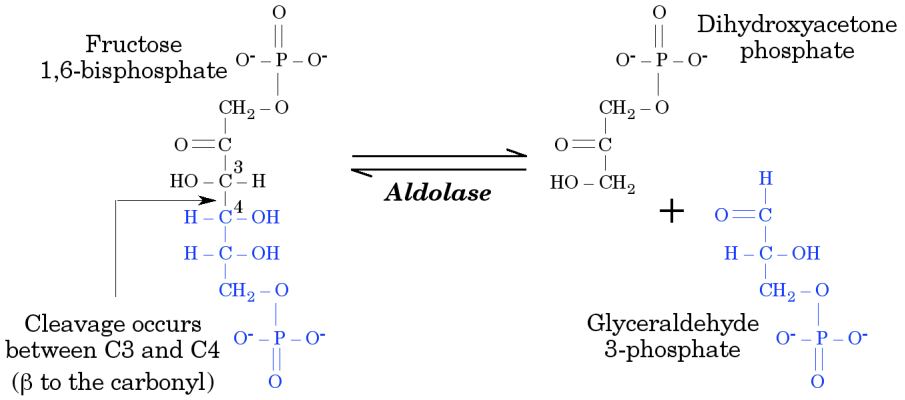
The most important regulator of phosphofructokinase is **fructose-2,6-bisphosphate**, which is produced by a separate enzyme. Fructose-2,6-bisphosphate increases the affinity of phosphofructokinase for fructose-6-phosphate, and thus acts as a critically important stimulator. **ATP** inhibits phosphofructokinase, because high levels of ATP indicate that the cell has sufficient energy. Phosphofructokinase is also inhibited by **citrate**. Citrate is a signal that the cell

has excess amounts of TCA cycle intermediates; this is an important point: one major role of glycolysis is to generate intermediates for other pathways, and high levels of these intermediates can regulate glycolysis.

Phosphofructokinase is a tetrameric enzyme; ATP binding to the regulatory site both increases the apparent Hill coefficient for the fructose-6-phosphate substrate and raises the $K_{0.5}$. The effect, under normal cellular conditions, is a considerable decrease in the amount of fructose-6-phosphate bound to the enzyme. The effect of ATP is reversed by fructose-2,6-bisphosphate and by AMP; AMP is a sensitive signal that the cellular ATP level is decreasing, and that ATP production is therefore necessary.

Aldolase

Aldolase catalyzes an aldol cleavage (or, in the reverse direction, an aldol condensation) reaction. This reaction requires the linear (keto) form of fructose. Note that glucose-1,6-bisphosphate cannot be used for this reaction, because it does not have a free carbonyl (the carbonyl cannot form because the oxygen is covalently bonded to the phosphate group). Note also that aldol cleavage of a linear glucose derivative would result in the formation of a two-carbon and a four-carbon compound, instead of two interconvertible triose phosphates that can both be utilized in the glycolytic pathway.



Triose Phosphate Isomerase

Triose phosphate isomerase is an extremely efficient enzyme (its rate limiting step is interaction of the enzyme with the substrate). Triose phosphate isomerase catalyzes the interconversion of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. This reaction is necessary because glyceraldehyde-3-phosphate is the only substrate for the remaining steps in the pathway, and because other processes produce or require dihydroxyacetone phosphate. Triose phosphate isomerase allows the entry of dihydroxyacetone phosphate into glycolysis, or exit of this metabolite from the glycolytic pathway.

Dihydroxyacetone phosphate is unstable; however, at equilibrium, dihydroxyacetone phosphate concentration is 21-times higher than glyceraldehyde-3-phosphate concentration. How is this possible? The activation energy for non-enzymatic breakdown of dihydroxyacetone phosphate into other molecules is much

smaller than activation energy for glyceraldehyde-3-phosphate breakdown, but ΔG´° for dihydroxyacetone phosphate is lower than for glyceraldehyde-3-phosphate.

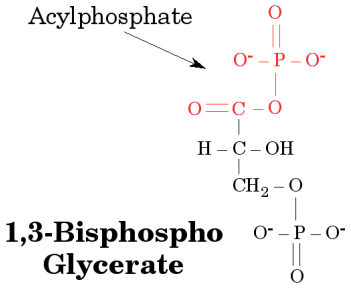
Note that for glycolysis to proceed, glyceraldehyde-3-phosphate must be formed, in spite of the fact that the ΔG´° for the formation of glyceraldehyde-3-phosphate is positive. During glycolysis, the formation of glyceraldehyde-3-phosphate occurs because the glyceraldehyde-3-phosphate is immediately used up by the next reaction. The subsequent reactions result in a low glyceraldehyde-3-phosphate concentration that creates a negative ΔG in spite of the positive ΔG´°.

The reactions to this point that began with glucose have resulted in formation of two glyceraldehyde-3-phosphate molecules, at a cost of two ATP.

Note that all of the remaining reactions occur "twice", because *two* 3 carbon compounds have been produced from each 6 carbon glucose molecule!

Glyceraldehyde-3-phosphate Dehydrogenase

Glyceraldehyde-3-phosphate dehydrogenase both reduces NAD and adds an inorganic phosphate to the glyceraldehyde-3-phosphate to make 1,3 bisphosphoglycerate. Although the reaction has a ΔG°´ = +6.3 kJ/mol, the reaction can run in either direction at physiological concentrations of substrates. 1,3-Bisphosphoglycerate contains an acyl phosphate, which is a high-energy phosphate bond.



Phosphoglycerate Kinase

The phosphoglycerate kinase reaction is sometimes called the "break-even point" because it regenerates the two molecules of ATP used in activating the glucose. In the reaction, the 1-position phosphate from 1,3-bisphosphoglycerate is donated to ADP. This process is referred to as **substrate-level phosphorylation**. This reaction occurs because the acyl phosphate contains sufficient energy to make phosphate transfer to ADP readily achievable.

In erythrocytes, a different enzyme, BPG mutase, can convert 1,3-bisphospho-glycerate into 2,3-bisphosphoglycerate, which is a regulator of hemoglobin oxygen binding. Doing so does not result in ATP synthesis, because the BPG mutase converts the high-energy acyl phosphate to a low energy phosphate ester.

Phosphoglycerate Mutase

Phosphoglycerate mutase transfers the phosphate from the 3-position to the 2-

position, converting 3-phosphoglycerate to 2-phosphoglycerate. Phosphoglycerate mutase has a fairly complicated reaction mechanism that uses 2,3-bisphosphoglycerate as an intermediate.

Enolase

Enolase catalyzes a dehydration reaction that results in the formation of phosphoenolpyruvate. 2-phosphoglycerate contains a low energy phosphate; the rearrangement of the molecule results in the high-energy phosphate present in phosphoenolpyruvate. (Note that the reaction does not create energy; the bond rearrangement allows the energy inherent in 2-phosphoglycerate to become more readily accessible.)

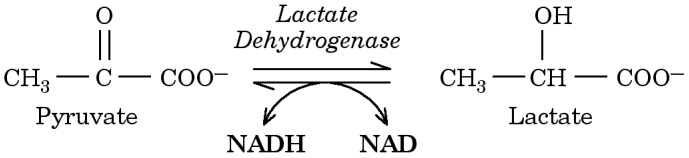
Pyruvate Kinase

The reaction catalyzed by pyruvate kinase includes the second substrate-level phosphorylation event; this is the "pay-off" point of glycolysis, which results in net ATP formation. Unlike phosphoglycerate kinase, pyruvate kinase has a large negative $\Delta G^{\circ\prime}$ in spite of the ATP production that occurs as part of the reaction. Pyruvate kinase is the second major control point in the glycolytic pathway: pyruvate kinase is activated by **AMP** and **fructose-1,6-bisphosphate**. The latter is a "feed-forward stimulator": production of fructose-1,6-bisphosphate by phosphofructokinase stimulates pyruvate kinase, and therefore results in increased pyruvate production, with the irreversible pyruvate kinase step acting to pull the other reactions to completion. The **flow of substrate through the glycolytic pathway is thus controlled by the irreversible enzymes at each end of the pathway**; if phosphofructokinase is active, it directly activates pyruvate kinase, and the action of both of these physiologically irreversible enzymes forces the reversible intervening steps to occur.

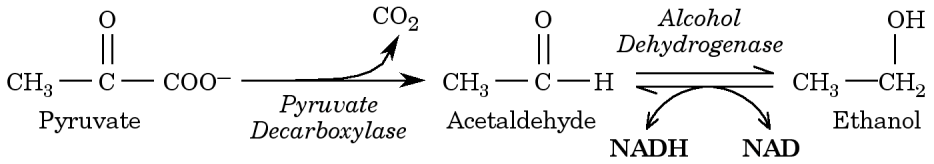
Pyruvate kinase is inhibited by acetyl-CoA and ATP and (in liver) by alanine and by phosphorylation of the protein. The alanine inhibition is due to the fact that pyruvate can be produced from alanine; if alanine is present, glycolysis is unnecessary.

Pyruvate reduction

Anaerobic glycolysis results in **reduction** of pyruvate to lactate:



or in decarboxylation of pyruvate to acetaldehyde followed by **reduction** of the acetaldehyde to ethanol:



Neither of these reduction methods allows recovery of the large amount of energy contained in the pyruvate molecule; in fact, the high-energy NADH is used to reduce the pyruvate. Extracting energy from pyruvate requires oxidation (rather than reduction) of the molecule. Oxidation of pyruvate requires oxygen, and must therefore be carried out under aerobic conditions. In addition, in eukaryotes, pyruvate oxidation must be carried out in the correct location: inside the mitochondrion.

Regeneration of NAD by one of these pyruvate reduction methods is required for maintenance of glycolysis under conditions in which conversion of NADH to NAD by the oxygen-dependent electron transport pathway is insufficient. This step is important because the amount of nicotinamide cofactor present in the cell is limited; without NAD, glycolysis would terminate at the level of glyceraldehyde-3-phosphate.

The ethanol produced by some microorganisms is purely a waste product; although ethanol contains some useful energy, organisms that produce ethanol cannot metabolize it. However, lactate can be converted back to pyruvate by the reversible lactate dehydrogenase reaction. Some tissues (especially the heart and liver) will take up lactate and use lactate dehydrogenase to regenerate the pyruvate and NADH, both of which can then be used to generate ATP.

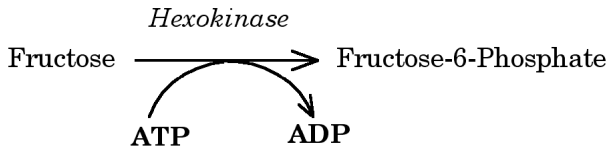
Energetics

The formation of fructose-1,6-bisphosphate from glucose requires the investment of two molecules of ATP. During the conversion of fructose-1,6-bis-phosphate to lactate, the pathway generates a total of four molecules of ATP, resulting in a net yield of two ATP per glucose consumed. This is not a very large amount of energy; as mentioned above, producing more energy requires use of the TCA cycle and oxidative phosphorylation pathways, processes that we will discuss later.

Entry of fructose into glycolysis

Monosaccharides other than glucose can also be used in the glycolytic pathway. In the US, the most important dietary monosaccharide other than glucose is fructose. Sucrose, also known as table sugar, is a disaccharide formed from glucose and fructose. In addition, many foods are sweetened with “high-fructose corn syrup”.

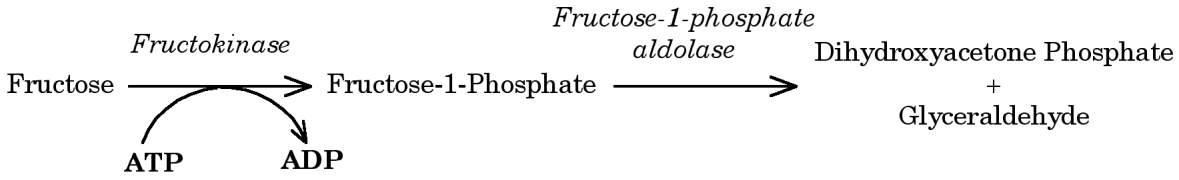
In most tissues, fructose can be phosphorylated by hexokinase to produce the glycolytic intermediate fructose-6-phosphate, which is then processed normally.



The potential problem with this pathway is that hexokinase has a much higher affinity for glucose than for fructose, and, if glucose is available, fructose will not be used as a substrate.

The liver has relatively little hexokinase. Instead, the liver uses a different enzyme, fructokinase, to phosphorylate fructose, resulting the in formation of fructose-1-phosphate. A specialized form of aldolase then cleaves the fructose-1-phosphate to dihydroxyacetone phosphate and glyceraldehyde. Dihydroxyacetone phosphate is a glycolytic intermediate; the glyceraldehyde must be phosphorylated to glyceraldehyde-3-phosphate (this can occur by two different pathways), which is also a glycolytic intermediate.

The use of fructokinase results in a bypassing of phosphofructokinase, and therefore bypasses the most important regulatory control point of glycolysis. In addition, fructose-1-phosphate aldolase is a slower enzyme than fructokinase; under conditions of high fructose availability, excessive fructose phosphorylation may result in trapping of so much phosphate in the fructose that ATP levels drop significantly. Normal individuals can usually compensate for the less well-regulated carbohydrate metabolism that is associated with fructose ingestion; individuals with certain metabolic abnormalities may find their physiological problems exacerbated by high dietary fructose.



Regulation of Glycolysis

Inhibition of reversible reactions tends to be less effective than inhibition of irreversible reactions. There are a number of reasons for this. One major reason is that, in many processes, the reversible steps are used for more than one purpose, while the irreversible steps, because of their irreversibility, are used for single purposes. Another major reason is that irreversible steps are typically far from equilibrium, and as a result, altering the activity of these enzymes has a significant effect on the flux of carbon compounds through the pathway.

Methods for regulating pathways include allosteric control of the regulated enzymes, temporary or permanent covalent modification of the enzymes, or alterations in the amount of the enzymes. In general, the allosteric and covalent modification regulation occurs on short time scales, while the alteration in the amount of enzyme is a longer-term regulator.

It is also possible to regulate pathways by altering the amount of the starting materials available. For glycolysis, availability of two starting materials can be important: glucose and NAD. If either of these is unavailable, the glycolytic process will halt. Depending on the cell type, glucose levels may depend on glucose availability in the extracellular medium (*e.g.*, in the blood stream in humans, or outside the cell in the case of prokaryotes). In some cells, glucose availability is dependent on transport of glucose into the cell, either as a result of direct glucose transport, or as a result of glucose phosphorylation. Finally, in some cells, glucose

can be released from intracellular storage molecules such as glycogen or starch. NAD availability depends on the oxidation of NADH, either by the glycolysis associated pyruvate reduction process (lactate dehydrogenase or alcohol dehydrogenase depending on the organism) or by the electron transport pathway.

In multicellular organisms, hormones frequently also have regulatory effects. In complex multicellular organisms (such as humans), the hormonal effects, and some of the other regulatory effects, very according to cell type. For example, glycolysis in human liver cells is regulated in ways that differ significantly from those in other cell types.

As mentioned earlier, glycolysis is regulated at several different steps. The first site of regulation is the entry of glucose into the cell. In muscle and adipose tissue, hormones that alter the amount of glucose transporter in the plasma membrane regulate glucose entry. In other cell types, the entry of glucose into the cell is regulated by the rate at which glucose is phosphorylated, while glucose transport is not directly regulated.

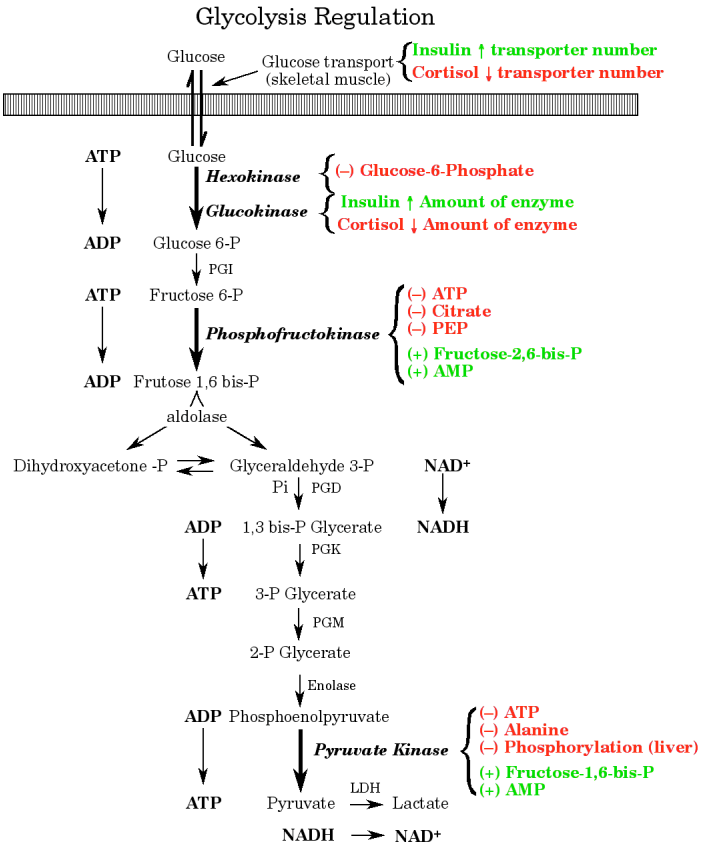
The second site of regulation is the phosphorylation of glucose. In most tissues, the phosphorylation is performed by hexokinase. Hexokinase, which has a high affinity for glucose, is inhibited by glucose-6-phosphate. The liver enzyme glucokinase, which has a low affinity for glucose, is essentially unaffected by its product. Instead the hormones insulin and cortisol alter the amount of the enzyme present. In addition glucokinase is also regulated by glucokinase regulatory protein, which inhibits glucokinase in the presence of fructose-6-phosphate.

Glycolysis is also regulated at two other sites: phosphofructokinase and pyruvate kinase.

ATP is both an effector and a substrate for phosphofructokinase. The effector role is necessary because ATP is a major product of the pathway. While substrates regulate enzyme activity, increasing concentrations of substrate increase the velocity of the reaction. Physiologically, however, high concentrations of ATP mean that the phosphofructokinase reaction is unnecessary. In other words, the high concentrations of ATP would have the “wrong” effect on a Michaelis-Menten enzyme, and as a result, phosphofructokinase is subject to allosteric inhibition by ATP.

Phosphofructokinase is also inhibited by phosphoenolpyruvate. This is logical, because if pyruvate kinase activity is low, phosphofructokinase should be inhibited also. In contrast, pyruvate kinase is stimulated by fructose-1,6-bisphosphate. This is also logical; if phosphofructokinase is active, pyruvate kinase should also be active.

The regulation of the glycolytic pathway, and the steps involved in ATP production and oxidation or reduction of NAD are summarized in the figure below.



The regulation of carbohydrate metabolism will be discussed further in the context of all of the carbohydrate metabolic pathways.