Metabolism

Part I: Glycolysis or the Embden-Meyerhoff Pathway

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Metabolism (from the Greek *metabolas*, changeable) has been described as the sum total of the chemical reactions carried out by biological systems. It includes processes by which cells: 1) capture energy from their surroundings, 2) convert nutrients into the building blocks for synthesis of macromolecules such as polysaccharides, proteins, and nucleic acids, 3) synthesize the macromolecules needed for cell growth and replication, and 4) degrade macromolecules to obtain energy or to salvage their building blocks for future construction.

It is useful to divide metabolism into catabolic and anabolic sequences of reactions or pathways. *Catabolism* (from the Greek *katabole*, throwing down) refers to processes which break down or degrade compounds into smaller, simpler molecules such as the lactate ion, ethanol, CO_2, NH_3, etc. Catabolic pathways are invariably accompanied by a net release of free energy, and one of the goals of metabolism is to capture at least some of this energy in the form of "high energy" compounds such as adenosine triphosphate (ATP). *Anabolism* (from the Greek *anabole*, rising up) describes sequences of reactions in which increasingly more complex molecules are synthesized at the expense of ATP.

The adjective "intermediary" is often used to describe metabolism because both catabolic and anabolic processes occur in a series of small, discrete steps that pass through a number of intermediates on their way to the end product or products.

There are many reasons why metabolism involves long sequences of reactions that gradually modify the structure of a compound. It is much easier for an enzyme to catalyze small changes in the structure of a substrate such as glucose than it is to ask the enzyme to bind glucose and simultaneously oxidize all six carbon atoms. Breaking metabolism into a number of small steps also makes it easier to capture some of the energy given off in catabolic reactions. It allows the cell to synthesize the many intermediates which serve as precursors for the biosynthesis of the polysaccharides, proteins, and nucleic acids needed for cell growth. It provides a way of converting fats or proteins into carbohydrates, or vice versa, as conditions require. Finally, it provides a number of places where a reaction can be either stimulated or inhibited, and therefore allows the cell to exert finely tuned control over the rate of metabolic reactions.

Anaerobic versus Aerobic Processes

When you consider the number of reactions each cell carries out, the number of different cells within even the simplest plants and animals, and the vast differences between species of plants and animals, the amount of information needed to describe metabolism would seem virtually limitless. Fortunately, metabolism is simplified by two factors. First, metabolism exhibits only minor variations within the cells of a species, or even among the myriad cells of different species. Second, metabolic processes are coupled through a backbone of essential reactions that can be organized into pathways such as "glycolysis", and an understanding of a limited number of these pathways can yield a great deal of information about the entire process of metabolism.

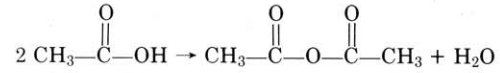
It is often useful to distinguish between anaerobic, aerobic, and facultative organisms when discussing metabolism. *Anaerobic* (literally: without air) organisms flourish in the absence of oxygen. They cannot utilize O_2 in their metabolism and are often poisoned by its presence. *Aerobic* organisms, on the other hand, need O_2 to survive. Organisms that have the ability to live under either aerobic or anaerobic conditions are called *facultative*.

This paper focuses on a sequence of reactions known as glycolysis or the Embden-Meyerhoff pathway. Glycolysis is the principal source of energy for anaerobic organisms and the first step in the degradation of glucose in aerobic organisms. This pathway breaks down glucose to either pyruvate, lactate or ethanol and captures part of the energy released to synthesize ATP from ADP and phosphate.

Biochemical Energetics

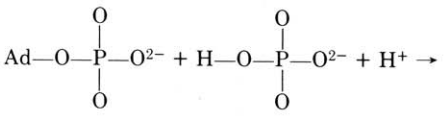
Adenosine triphosphate (ATP) is the primary source of chemical energy for a seemingly endless variety of biological processes. It fuels processes as diverse as bioluminescence; the transport of ions and molecules across cell membranes; the contraction of muscles; the synthesis of carbohydrates, fats, proteins, and nucleic acids; and both N_2 and CO_2 fixation.

Although we call ATP a "triphosphate", it is actually an acid anhydride like acetic anhydride.

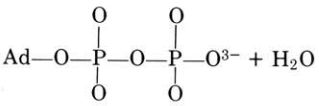


acetic acid — acetic anhydride

ATP is a mixed anhydride made by condensing adenosine monophosphate (AMP) with a phosphate ion (HPO_4^{2-} or $H_2PO_4^-$, depending on pH) to form adenosine diphosphate (ADP). If we let the symbol "Ad" stand for adenosine, this "phosphorylation" reaction can be written as follows,

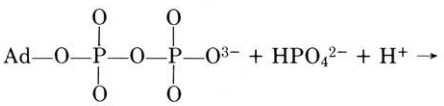


AMP

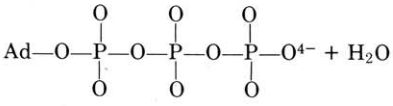


ADP

Phosphorylation of the diphosphate then gives the triphosphate (ATP),



ADP



ATP

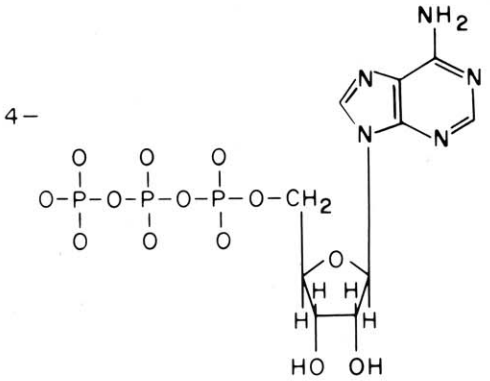


Figure 1. Structure of ATP.

whose complete structure is shown in Figure 1.

The key to understanding the role of ATP in metabolism is appreciating what is meant when ATP is referred to as a "high energy" phosphate. Phosphate esters such as glucose 6-phosphate and anhydrides such as ATP are unstable to hydrolysis. Before we can compare their free energies of hydrolysis, however, it is important to recognize that a strict adherence to the definition of standard state causes problems when dealing with biological systems. The standard state for reactions involving the H^+ ion would have a H^+ ion concentration of exactly 1.000 *M*. This is clearly unrealistic for biological reactions which are much more likely to occur near pH 7. The "standard state" for biochemical reactions is therefore defined as pH 7, and this convention is indicated by using the symbol $\Delta G°'$ instead of $\Delta G°$. The difference between $\Delta G°$ and $\Delta G°'$ for a reaction which liberates or consumes a single H^+ ion is 40.0 kJ/mol.

The standard free energy of hydrolysis of a typical phosphate ester such as glucose 6-phosphate or fructose 6-phosphate is roughly 15 kJ/mol. The free energy of hydrolysis of ATP to ADP (or ADP to AMP) is twice as large.

$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + HPO_4^{2-} + H^+$$

$$\Delta G°' = -31 \text{ kJ/mol}$$

ATP and ADP are therefore often referred to as "high-energy" phosphates because the energy stored in one of these "high-energy" phosphates is large enough to drive the phosphorylation of a sugar such as glucose or fructose.

$$\text{glucose} + ATP \longrightarrow \text{glucose 6-phosphate} + ADP$$

$$\Delta G°' = -16.7 \text{ kJ/mol}$$

There are several reasons why the standard free energy of hydrolysis of either ATP or ADP is unusually large. At a typical physiological pH, the polyphosphate backbone carries a number of negatively charged oxygen atoms, which repel each other strongly. Hydrolysis of ATP to ADP and phosphate ion, or ADP to AMP and phosphate, alleviates some of that repulsion (*1*). Hydrolysis also increases the total ionic charge,

$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + HPO_4^{2-} + H^+$$

$$ADP^{3-} + H_2O \longrightarrow AMP^{2-} + HPO_4^{2-} + H^+$$

and therefore the contribution of solvation to the free energy of the products (*2*). The free energy associated with the reaction between the H^+ ion given off during hydrolysis and the surrounding buffering medium also drives these reactions to completion (*3*). Finally, more resonance structures can be written for the products of these reactions than for the reactants, and hydrolysis therefore increases the resonance stabilization (*4*).

The only way for anaerobic organisms to synthesize ATP is to make "super high-energy" phosphates which are even less stable to hydrolysis than ATP or ADP. Most of the steps in glycolysis are therefore designed to build super high-energy phosphate intermediates such as phosphoenolpyruvate and 1,3-diphosphoglycerate which contain enough energy to convert ADP to ATP.

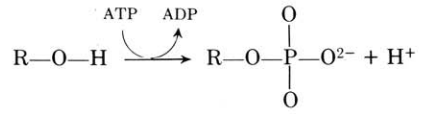
$$\text{phosphoenolpyruvate} + ADP + H^+ \longrightarrow \text{pyruvate} + ATP$$

$$\Delta G°' = -31 \text{ kJ/mol}$$

Enzyme Nomenclature

Before discussing the individual steps in glycolysis it might be useful to look at the nomenclature of enzymes. Most enzymes are named by listing the substrate and then the type of reaction they catalyze. An enzyme that oxidizes alcohols to aldehydes by removing hydrogen, for example, would be called an alcohol dehydrogenase.

An important class of enzymes for discussions of intermediary metabolism are the *kinase* enzymes, which catalyze the transfer of a phosphate from ATP to an alcohol to form a phosphate ester or phosphomonoester.



The phosphorylation of one of the -OH groups on glucose to form glucose 6-phosphate, for example, is catalyzed by enzymes known as hexokinase and glucokinase. *Mutase* enzymes, on the other hand, catalyze the internal transfer of a phosphate within a substrate, such as phosphoglyceromutase which converts 3-phosphoglycerate to 2-phosphoglycerate.

An *isomerase* catalyzes the conversion of one isomer to another. Isomerase enzymes may catalyze reactions that involve oxidation-reduction, such as the conversion of an aldehyde (glucose 6-phosphate) into a ketone (fructose 6-phosphate), but there is no net change in the oxidation state of the substrate. *Dehydrogenase* enzymes catalyze reactions in which there is a net change in the oxidation state of the substrate. Although the reactions catalyzed by a dehydrogenase are usually reversible, they are always named in the direction of oxidation. Reduction of acetaldehyde to ethanol, for example, is catalyzed by the enzyme alcohol dehydrogenase.

Glycolysis

Glycolysis (literally: glucose dissolution) is a sequence of 10 or 11 enzyme-catalyzed reactions shown in Figures 2 and 3 that degrade a molecule of glucose into two molecules of either pyruvate, lactate or ethanol, with the net synthesis of two molecules of ATP.

Step 1: Phosphorylation of Glucose

The first step is an enzyme-catalyzed phosphorylation of glucose at C_6 to form a phosphate ester.

$$\text{glucose} + ATP \xrightarrow{\text{hexokinase}} \text{glucose 6-phosphate} + ADP$$

$$\Delta G°' = -17 \text{ kJ/mol}$$

Although this step consumes a molecule of ATP, it has the advantage of labeling the glucose molecule with a highly charged phosphate group that helps subsequent enzymes recognize and bind the appropriate substrates (*5*).

Step 2: Isomerization of Glucose 6-Phosphate

Glucose 6-phosphate is then converted from an aldehyde (aldose) into a ketone (ketose).

$$\text{glucose 6-phosphate} \xrightarrow{\text{phosphoglucoisomerase}} \text{fructose 6-phosphate}$$

$$\Delta G^{\circ\prime} = +2 \text{ kJ/mol}$$

The equilibrium constant for this reaction slightly favors the starting material ($K_c = 0.4$), but the reaction readily proceeds to the right due to the relatively large quantities of glucose 6-phosphate which build up in the cell.

Step 3: Phosphorylation of Fructose 6-Phosphate

A second molecule of ATP is now consumed to phosphorylate fructose 6-phosphate at C_1 to form fructose 1,6-diphosphate.

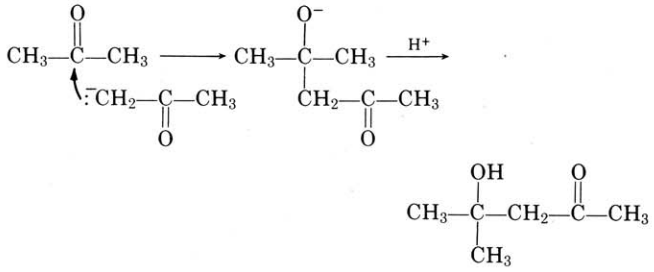
$$\text{fructose 6-phosphate} + \text{ATP} \xrightarrow{\text{phosphofructokinase}}$$

$$\text{fructose 1,6-diphosphate} + \text{ADP} \qquad \Delta G^{\circ\prime} = -14.2 \text{ kJ/mol}$$

Once again, the free energy stored in a molecule of ATP is used to drive what would otherwise be an unfavorable reaction.

Step 4: Cleavage of Fructose 1,6-Diphosphate

In the presence of base, aldehydes or ketones with an α-hydrogen can undergo a reversible reaction known as an aldol condensation.



Fructose 1,6-diphosphate can be thought of as the product of an aldol condensation of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.

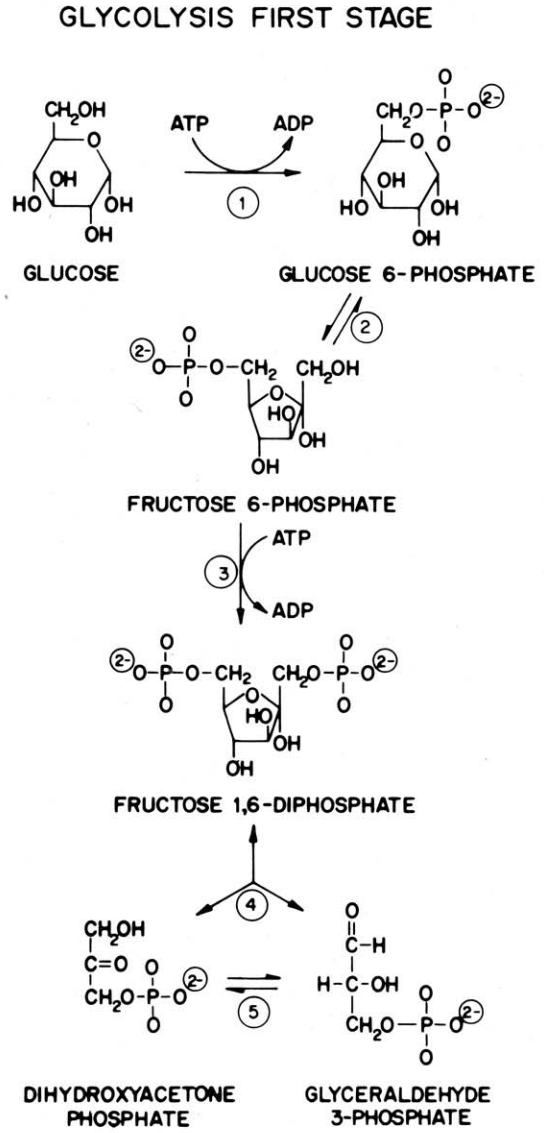


Figure 2. Reaction sequence for the first stage of glycolysis.

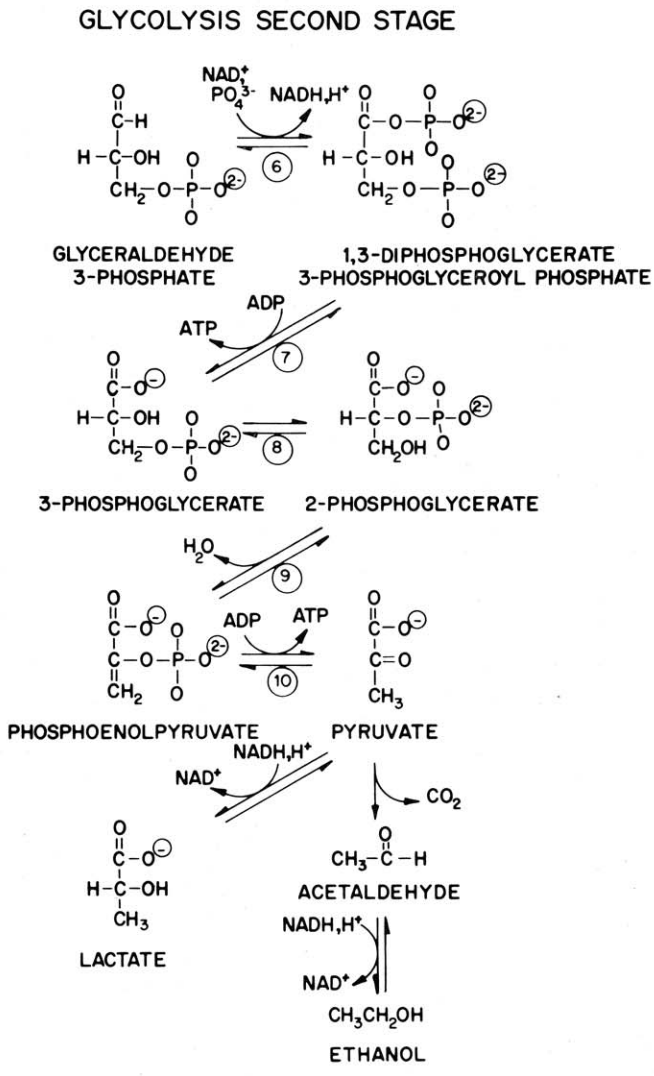
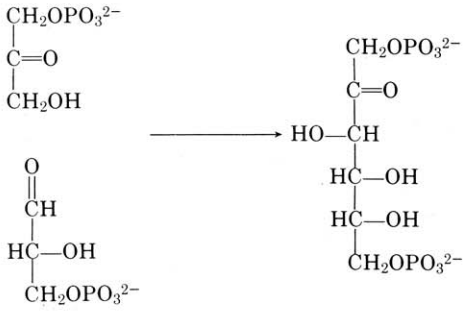


Figure 3. Reaction sequence for the second stage of glycolysis.

dihydroxyacetone phosphate



glyceraldehyde 3-phosphate fructose 1,6-diphosphate

The enzyme which cleaves fructose 1,6-diphosphate to form a mixture of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate is therefore commonly known as "aldolase".

fructose 1,6-diphosphate $\xrightarrow{\text{aldolase}}$ dihydroxyacetone phosphate +

glyceraldehyde 3-phosphate $\Delta G^{\circ\prime} = +24.0$ kJ/mol

The equilibrium for this reaction strongly favors the starting material ($K_c = 6.25 \times 10^{-5}$). The reaction is constantly pulled to the right, however, as the products of this reaction are consumed in subsequent steps.

Step 5: Interconversion of Triose Phosphates

Dihydroxyacetone phosphate and glyceraldehyde 3-phosphate are both triose (C_3 sugar) phosphates. Although DHAP cannot enter the second half of the glycolytic pathway, it can be converted into a second molecule of glyceraldehyde 3-phosphate by an isomerase enzyme.

dihydroxyacetone phosphate $\xrightarrow{\text{triose phosphate isomerase}}$

glyceraldehyde 3-phosphate $\Delta G^{\circ\prime} = +7.66$ kJ/mol

The net effect of the first five steps of glycolysis is to split a molecule of glucose into two molecules of glyceraldehyde 3-phosphate with no net change in the empirical formula, $(CH_2O)_n$, and therefore no net oxidation or reduction.

Step 6: Oxidation of Glyceraldehyde 3-Phosphate

Although the primary goal of glycolysis is the production of ATP, the first five steps consume two molecules of ATP. The sixth step sets the stage for a reaction that produces two molecules of ATP, and therefore brings us to the break-even point. In this step, glyceraldehyde is oxidized and then phosphorylated to give an acyl phosphate known as either 1,3-diphosphoglycerate or 3-phosphoglyceroyl phosphate.

glyceraldehyde 3-phosphate + NAD^+ + HPO_4^{2-}

$\xrightarrow{\text{triose phosphate dehydrogenase}}$ 1,3-diphosphoglycerate + NADH + H^+

$\Delta G^{\circ\prime} = +6.3$ kJ/mol

Enzymes do not oxidize or reduce a substrate directly, they only catalyze oxidation or reduction reactions. The oxidizing agent in this reaction is a coenzyme known as nicotinamide adenine dinucleotide (NAD^+) which binds to the triose phosphate dehydrogenase enzyme and picks up a hydride ($H:^-$) ion to form the reduced coenzyme NADH. The second hydrogen atom removed from the substrate in this oxidation is released into solution as a H^+ ion.

Step 7: Synthesis of ATP

1,3-Diphosphoglycerate is a "super high-energy" phosphate which transfers a phosphate to ADP to form ATP.

1,3-diphosphoglycerate + ADP $\xrightarrow{\text{phosphoglycerate kinase}}$

3-phosphoglycerate + ATP $\Delta G^{\circ\prime} = -18.8$ kJ/mol

It should not be surprising that 1,3-diphosphoglycerate has an unusually large free energy of hydrolysis. The repulsion between electrons in the acyl phosphate group at C_1 is very similar to the repulsion between electrons in the polyphosphate backbone of ATP that enhances the free energy of hydrolysis of that compound. Furthermore, the 1,3-diphosphoglycerate ion contains two highly charged phosphate groups in close proximity.

The net effect of steps 6 and 7 is to capture in the form of ATP some of the energy released when an aldehyde is oxidized. Because two 1,3-diphosphoglycerate ions are formed for each molecule of glucose consumed, this step generates two molecules of ATP which compensate for the ATP consumed in steps 1 and 3.

Step 8: Isomerization of 3-Phosphoglycerate

By transferring a phosphate group from C_3 to C_2, this step sets the stage for the synthesis of another super high-energy phosphate.

3-phosphoglycerate $\xrightarrow{\text{phosphoglyceromutase}}$ 2-phosphoglycerate

$\Delta G^{\circ\prime} = +4.44$ kJ/mol

Step 9: Dehydration of 2-Phosphoglycerate

Dehydration of 2-phosphoglycerate yields phosphoenolpyruvate (PEP).

2-phosphoglycerate $\xrightarrow{\text{enolase}}$ phosphoenolpyruvate + H_2O

$\Delta G^{\circ\prime} = +1.8$ kJ/mol

This is one of the most beautiful metabolic reactions. The overall free energy of reaction is negligibly small, and yet the reaction converts a low-energy phosphate ($\Delta G^{\circ\prime}_{hyd} = -18$ kJ/mol) into a super high-energy phosphate ($\Delta G^{\circ\prime}_{hyd} = -61.9$ kJ/mol) capable of transforming ADP to ATP.

Step 10: Synthesis of ATP

Keto-enol equilibria lie heavily on the side of the ketone. Rearrangement of phosphoenolpyruvate to the keto isomer therefore provides enough driving force to transfer the phosphate group from PEP to ADP to form ATP and the pyruvate ion.

phosphoenolpyruvate + ADP $\xrightarrow{\text{pyruvate kinase}}$ pyruvate + ATP

$\Delta G^{\circ\prime} = -31$ kJ/mol

Because two phosphoenolpyruvate ions are produced for every molecule of glucose that enters the glycolytic pathway, the net effect of this step is the synthesis of two molecules of ATP.

One molecule of ATP is consumed in each of steps 1 and 3 of glycolysis, and two molecules are produced in each of steps 7 and 10. The overall energy balance for glycolysis is therefore 2 ATP per glucose.

Step 11: Reduction of Pyruvate

One more step is needed to complete the glycolytic pathway. Oxidation of glyceraldehyde 3-phosphate in step 6 was accompanied by the reduction of NAD^+ to NADH. NAD^+, like other enzyme cofactors, is present at only low concentrations in the cell. Glycolysis must therefore contain a step in which NADH is oxidized back to NAD^+.

Aerobic organisms can recycle NADH to NAD^+ by reducing oxygen to water. Anaerobic organisms achieve the same goal by reducing the pyruvate ion to either the lactate ion or ethanol. Reduction of pyruvate to lactate occurs in anaero-

bic organisms such as the lactate bacteria responsible for turning milk sour. It can also occur in aerobic tissues such as skeletal muscles when the demand for oxygen exceeds the rate at which it can be supplied.

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{lactate dehydrogenase}} \text{lactate} + \text{NAD}^+$$

$$\Delta G^{\circ\prime} = -25 \text{ kJ/mol}$$

Reduction of pyruvate to ethanol occurs in alcoholic fermentation. The first step in this process involves an irreversible enzyme-catalyzed decarboxylation of pyruvate to form acetaldehyde,

$$\text{pyruvate} \xrightarrow{\text{pyruvate decarboxylase}} \text{acetaldehyde} + \text{CO}_2$$

which is then reduced by NADH to ethanol.

$$\text{acetaldehyde} + \text{NADH} + \text{H}^+ \xrightarrow{\text{alcohol dehydrogenase}} \text{ethanol} + \text{NAD}^+$$

The Efficiency of Glycolysis

The standard free energy of reaction for the degradation of glycose to the lactate ion (−197 kJ/mol glucose) is only a small fraction of the energy available when glucose is oxidized to CO_2 and H_2O (−2,870 kJ/mol). The beauty of glycolysis, however, is that it liberates this energy in the absence of any net oxidation or reduction. One end of the lactate ion ($-CO_2^-$) is oxidized relative to glucose, while the other end ($-CH_3$) is reduced.

Perhaps a better estimate of the efficiency of glycolysis can be obtained by calculating the fraction of the energy liberated during glycolysis that is captured in the form of ATP. Many biochemists do this calculation by dividing the standard free energy of hydrolysis of the ATP synthesized in the pathway (2 mol ATP × −31 kJ/mol) by the overall free energy of reaction (−197 kJ/mol) (*6*), which gives an efficiency of roughly 30%.

This calculation is misleading, however, because physiological concentrations of glucose, lactate, ATP, ADP, etc., are so far from the standard state. If we correct the free energy of reaction for the degradation of glucose to reflect concentrations of glucose (5 m*M*) and lactate (2.9 m*M*) in a typical cell, such as a red blood cell (*7*), we obtain a value of $\Delta G'$ for this reaction of approximately −210 kJ/mol. If we do the same correction for the free energy of hydrolysis of ATP, using typical red blood cell concentrations of ATP (1.85 m*M*), ADP (0.138 m*M*) and phosphate (1 m*M*) (*7*), we get estimates of $\Delta G'$ on the order of −55 kJ/mol. This corresponds to the capture in the form of ATP at physiological concentrations of more than 50% of the energy given off during glycolysis (*5*).

Other Carbohydrates as Sources of Intermediates for Glycolysis

Other carbohydrates besides glucose can enter the glycolytic pathway. Fructose, for example, can be phosphorylated to fructose 1-phosphate by a kinase and then split by aldolase into dihydroxyacetone phosphate and glyceraldehyde. DHAP can then be converted into glyceraldehyde 3-phosphate by the triose phosphate isomerase enzyme, while glyceraldehyde can be phosphorylated to glyceraldehyde 3-phosphate by a kinase. The first step in the degradation of galactose involves phosphorylation by a kinase to give galactose 1-phosphate, which is epimerized to glucose 1-phosphate and then converted to glucose 6-phosphate by a mutase enzyme.

In times of plenty, excess carbohydrates in plants and animals are converted into glucose that is stored as starch or glycogen. When needed as a source of energy, these polysaccharides enter glycolysis through the action of two enzymes, glycogen (or starch) phosphorylase, which cleaves individual glucose molecules from the polysaccharide chain to form glucose 1-phosphate, and phosphoglucomutase, which transforms this intermediate into glucose 6-phosphate.

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