

## GLYCOLYSIS

The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate occurs in ten steps, the first five of which constitute the *preparatory phase*. In these reactions, glucose is first phosphorylated at the hydroxyl group on C-6 (step 1). The D-glucose 6-phosphate thus formed is converted to D-fructose 6-phosphate (step 2), which is again phosphorylated, this time at C-1, to yield D-fructose 1,6-bisphosphate (step 3). For both phosphorylations, ATP is the phosphoryl group donor. As all sugar derivatives in glycolysis are the D isomers, we will usually omit the D designation except when emphasizing stereochemistry. Fructose 1,6-bisphosphate is split to yield two three-carbon molecules, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (step 4); this is the “lysis” step that gives the pathway its name. The dihydroxyacetone phosphate is isomerized to a second molecule of glyceraldehyde 3-phosphate (step 5), ending the first phase of glycolysis. From a chemical perspective, the isomerization in step 2 is critical for setting up the phosphorylation and C-C bond cleavage reactions in steps 3 and 4. Note that two molecules of ATP are invested before the cleavage of glucose into two three-carbon pieces; later there will be a good return on this investment.

**To summarize:** in the preparatory phase of glycolysis the energy of ATP is invested, raising the free-energy content of the intermediates, and the carbon chains of all the metabolized hexoses are converted into a common product, glyceraldehyde 3-phosphate.

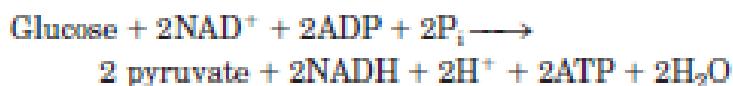
The energy gain comes in the *payoff phase* of glycolysis. Each molecule of glyceraldehydes 3-phosphate is oxidized and phosphorylated by inorganic phosphate (*not* by ATP) to form 1,3-bisphosphoglycerate (step 6). Energy is then released as the two molecules of 1,3-bisphosphoglycerate are converted to two molecules of pyruvate (steps 7 through 10). Much of this energy is conserved by the coupled phosphorylation of four molecules of ADP to ATP. The net yield is two molecules of ATP per molecule of glucose used, because two molecules of ATP were invested in the preparatory phase. Energy is also conserved in the payoff phase in the formation of two molecules of NADH per molecule of glucose.

In the sequential reactions of glycolysis, three types of chemical transformations are particularly noteworthy:

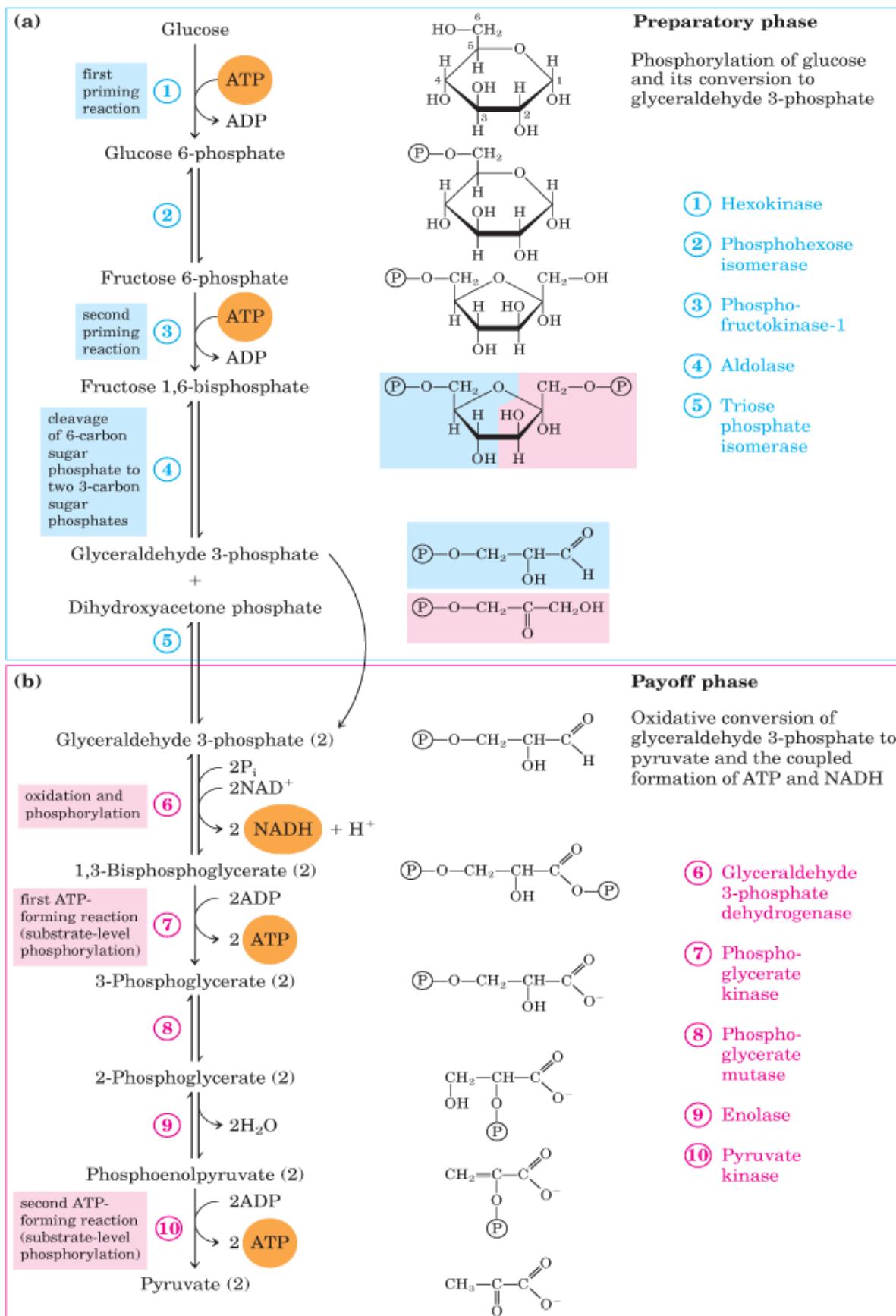
- (1) degradation of the carbon skeleton of glucose to yield pyruvate,
- (2) phosphorylation of ADP to ATP by high-energy phosphate compounds formed during glycolysis,
- (3) transfer of a hydride ion to NAD<sup>+</sup>, forming NADH.

**ATP Formation Coupled to Glycolysis** During glycolysis some of the energy of the glucose molecule is conserved in ATP, while much remains in the product, pyruvate.

The overall equation for glycolysis is



## Overview of Glycolysis



## STEPS IN GLYCOLYSIS

### The Preparatory Phase of Glycolysis Requires ATP

#### 1 Phosphorylation of Glucose

In the first step of glycolysis, glucose is activated for subsequent reactions by its phosphorylation at C-6 to yield **glucose 6-phosphate**, with ATP as the phosphoryl donor. This reaction, which is irreversible under intracellular conditions, is catalyzed by **hexokinase**. Kinases are enzymes that catalyze the transfer of the terminal phosphoryl group from ATP to an acceptor nucleophile.

#### 2 Conversion of Glucose 6-Phosphate to Fructose 6-Phosphate

The enzyme **phosphohexose isomerase** (**phosphoglucose isomerase**) catalyzes the reversible isomerisation of glucose 6-phosphate, an aldose, to **fructose 6-phosphate**, a ketose. The reaction proceeds readily in either direction, as might be expected from the relatively small change in standard free energy. This isomerization has a critical role in the overall chemistry of the glycolytic pathway, as the rearrangement of the carbonyl and hydroxyl groups at C-1 and C-2 is a necessary prelude to the next two steps. The phosphorylation that occurs in the next reaction (step 3) requires that the group at C-1 first be converted from a carbonyl to an alcohol, and in the subsequent reaction (step 4) cleavage of the bond between C-3 and C-4 requires a carbonyl group at C-2.

#### 3 Phosphorylation of Fructose 6-Phosphate to Fructose 1,6-Bisphosphate

In the second of the two priming reactions of glycolysis, **phosphofructokinase-1** (**PFK-1**) catalyzes the transfer of a phosphoryl group from ATP to fructose 6-phosphate to yield **fructose 1,6-bisphosphate**. The PFK-1 reaction is essentially irreversible under cellular conditions, and it is the first “committed” step in the glycolytic pathway; glucose 6-phosphate and fructose 6-phosphate have other possible fates, but fructose 1,6-bisphosphate is targeted for glycolysis. Phosphofructokinase-1 is a regulatory enzyme, one of the most complex known. It is the major point of regulation in glycolysis. The activity of PFK-1 is increased whenever the cell’s ATP supply is depleted or when the ATP breakdown products, ADP and AMP (particularly the latter), are in excess. The enzyme is inhibited whenever the cell has ample ATP and is well supplied by other fuels such as fatty acids.

#### 4 Cleavage of Fructose 1,6-Bisphosphate

The enzyme **fructose 1,6-bisphosphate aldolase**, often called simply **aldolase**, catalyzes a reversible aldol condensation. Fructose 1,6-bisphosphate is cleaved to yield two different triose phosphates, **glyceraldehydes 3-phosphate**, an aldose, and **dihydroxyacetone phosphate**, a ketose. Although the aldolase reaction has a strongly positive standard free-energy change in the direction of fructose 1,6-bisphosphate cleavage, at the lower concentrations of reactants present in cells, the actual free-energy change is small and the aldolase reaction is readily reversible.

#### 5 Interconversion of the Triose Phosphates

Only one of the two triose phosphates formed by aldolase, glyceraldehydes 3-phosphate, can be directly degraded in the subsequent steps of glycolysis. The other product, dihydroxyacetone phosphate, is rapidly and reversibly converted to glyceraldehyde 3-phosphate by the fifth enzyme of the sequence, **triose phosphate isomerase**. The reaction mechanism is similar to the reaction promoted by phosphohexose isomerase in step 2 of glycolysis. This reaction completes the preparatory phase of glycolysis. The hexose molecule has been phosphorylated at C-1 and C-6 and then cleaved to form two molecules of glyceraldehyde 3-phosphate.

### The Payoff Phase of Glycolysis Yields ATP and NADH

The payoff phase of glycolysis includes the energy-conserving phosphorylation steps in which some of the free energy of the glucose molecule is conserved in the form of ATP. One molecule of glucose yields two molecules of glyceraldehyde 3-phosphate; both halves of the glucose molecule follow the same pathway in the second phase of glycolysis. The conversion of two molecules of glyceraldehyde 3-phosphate to two molecules of pyruvate is accompanied by the formation of four molecules of ATP from ADP. However, the net yield of ATP per molecule of glucose degraded is only two, because two ATP were invested in the preparatory phase of glycolysis to phosphorylate the two ends of the hexose molecule.

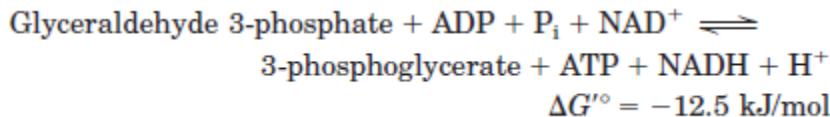
### 6 Oxidation of Glyceraldehyde 3-Phosphate to 1,3-Bisphosphoglycerate

The first step in the payoff phase is the oxidation of glyceraldehyde 3-phosphate to **1,3-bisphosphoglycerate**, catalyzed by **glyceraldehyde 3-phosphate dehydrogenase**. This is the first of the two energy-conserving reactions of glycolysis that eventually lead to the formation of ATP. The aldehyde group of glyceraldehyde 3-phosphate is oxidized, not to a free carboxyl group but to a carboxylic acid anhydride with phosphoric acid. This type of anhydride, called an **acyl phosphate**, has a very high standard free energy of hydrolysis. Much of the free energy of oxidation of the aldehyde group of glyceraldehyde 3-phosphate is conserved by formation of the acyl phosphate group at C-1 of 1,3-bisphosphoglycerate.

The acceptor of hydrogen in the glyceraldehyde 3-phosphate dehydrogenase reaction is  $\text{NAD}^+$ . The reduction of  $\text{NAD}^+$  proceeds by the enzymatic transfer of a hydride ion ( $:\text{H}^+$ ) from the aldehyde group of glyceraldehyde 3-phosphate to the nicotinamide ring of  $\text{NAD}^+$ , yielding the reduced coenzyme NADH. The other hydrogen atom of the substrate molecule is released to the solution as  $\text{H}^+$ .

### 7 Phosphoryl Transfer from 1,3-Bisphosphoglycerate to ADP

The enzyme **phosphoglycerate kinase** transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and **3-phosphoglycerate**. Steps 6 and 7 of glycolysis together constitute an energy-coupling process in which 1,3-bisphosphoglycerate is the common intermediate; it is formed in the first reaction (which would be endergonic in isolation), and its acyl phosphate group is transferred to ADP in the second reaction (which is strongly exergonic). The sum of these two reactions is



Thus the overall reaction is exergonic.

The outcome of these coupled reactions, both reversible under cellular conditions, is that the energy released on oxidation of an aldehyde to a carboxylate group is conserved by the coupled formation of ATP from ADP and  $\text{P}_i$ . The formation of ATP by phosphoryl group transfer from a substrate such as 1,3-bisphosphoglycerate is referred to as a **substrate-level phosphorylation**.

### 8 Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

The enzyme **phosphoglycerate mutase** catalyzes a reversible shift of the phosphoryl group between C-2 and C-3 of glycerate;  $\text{Mg}^{2+}$  is essential for this reaction.

### 9 Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

In the second glycolytic reaction that generates a compound with high phosphoryl group transfer potential, **enolase** promotes reversible removal of a molecule of water from 2-phosphoglycerate to yield **phosphoenolpyruvate (PEP)**. Although 2-phosphoglycerate and

phosphoenolpyruvate contain nearly the same *total* amount of energy, the loss of the water molecule from 2-phosphoglycerate causes a redistribution of energy within the molecule, greatly increasing the standard free energy of hydrolysis of the phosphoryl group.

### 10 Transfer of the Phosphoryl Group from Phosphoenolpyruvate to ADP

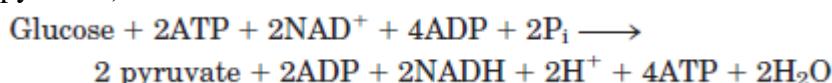
The last step in glycolysis is the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, catalyzed by **pyruvate kinase**, which requires  $K^+$  and either  $Mg^{2+}$  or  $Mn^{2+}$ . The overall reaction has a large, negative standard free energy change, due in large part to the spontaneous conversion of the enol form of pyruvate to the keto form. The pyruvate kinase reaction is essentially irreversible under intracellular conditions and is an important site of regulation

### The Overall Balance Sheet Shows a Net Gain of ATP

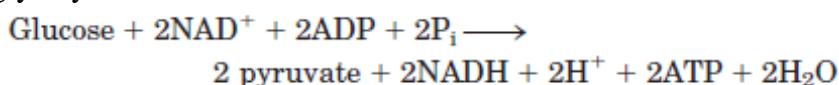
We can now construct a balance sheet for glycolysis to account for

- (1) the fate of the carbon skeleton of glucose,
- (2) the input of Pi and ADP and the output of ATP, and

(3) the pathway of electrons in the oxidation-reduction reactions. The left-hand side of the following equation shows all the inputs of ATP,  $NAD^+$ , ADP, and Pi, and the right-hand side shows all the outputs (keep in mind that each molecule of glucose yields two molecules of pyruvate):



Cancelling out common terms on both sides of the equation gives the overall equation for glycolysis under aerobic conditions:



In the overall glycolytic process, one molecule of glucose is converted to two molecules of pyruvate (the pathway of carbon). Two molecules of ADP and two of Pi are converted to two molecules of ATP (the pathway of phosphoryl groups). Four electrons, as two hydride ions, are transferred from two molecules of glyceraldehydes 3-phosphate to two of  $NAD^+$  (the pathway of electrons).

### Energy Yield

Further, 2 moles of ATP are generated in glycolysis. A summary of the steps in which ATP is consumed or formed is given in Table

Table 21-2. Energy yield of glycolysis

Step	Reaction	Consumption of ATP	Gain of ATP
1	Glucose $\longrightarrow$ Glucose 6-phosphate	1	
3	Fructose 6-phosphate $\longrightarrow$ Fructose 1, 6-diphosphate	1	
7	1, 3-diphosphoglycerate $\longrightarrow$ 3-phosphoglycerate		$1 \times 2 = 2$
10	Phosphoenolpyruvate $\longrightarrow$ Pyruvate		$1 \times 2 = 2$
		2	4
		Net gain of ATP = 4 - 2 = 2	