CONTENTS

Fermentation

(*a*) **Sprinters at the end of a race.** A runner's respiratory and circulatory systems cannot supply oxygen to her leg muscles fast enough to keep up with the demand for energy, so glycolysis and lactate fermentation must provide the ATP. Panting after the race brings in the oxygen needed to remove the lactate through cellular respiration.

(*b*) **Bread.** Bread rises as CO_2 is liberated by fermenting yeast, which converts glucose to ethanol *via* the alcoholic fermentation pathway.

C H A P T E R

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Glycolysis

Carbohydrates are the first cellular constituents formed by photosynthetic organisms and result from the fixation of CO_2 on absorption of light. The carbohydrates are metabolized to yield a vast array of other organic compounds, many of which are subsequently utilized as dietary constituents by animals.The animals ingest great quantities of carbohydrates that can be either stored, or oxidized to obtain energy as ATP, or converted to lipids for more efficient energy storage or used for the synthesis of many cellular constituents.

The major function of carbohydrates in metabolism is as a fuel to be oxidized and provide energy for other metabolic processes. *The carbohydrate is utilized by cells mainly as glucose.* The 3 principal monosaccharides resulting from digestive processes are glucose, fructose and galactose. Much of the glucose is derived from starch which accounts for over half of the fuel in the diets of most humans. Glucose is also produced from other dietary components by the liver and, to a lesser extent, by the kidneys. *Fructose* results on large intake of sucrose while *galactose* is produced when lactose is the principal carbohydrate of the diet. Both fructose and galactose are easily converted to glucose by the liver. It is thus apparent that glucose is the major fuel of most organisms and that it can be quickly metabolized from glycogen stores when there arises a sudden need for energy.

Pentose sugars such as arabinose, ribose and xylose may be present in the diet. But their fate after absorption is, however, obscure.

GENERAL CONSIDERATIONS OF GLYCOLYSIS

Glycolysis ($glycos^G$ = sugar (sweet); $lysis^G$ = dissolution) is the sequence of 10 enzyme-catalyzed reactions that converts glucose into pyruvate with the simultaneous production of ATP. Moreover, glycolysis also includes the formation of lactate from pyruvate. The glycolytic sequence of reactions differs from one species to the other only in the mechanism of its regulation and in the subsequent metabolic fate of the pyruvate formed. In aerobic organisms, glycolysis is the prelude to the citric acid cycle and the electron transport chain which together harvest most of the energy contained in glucose. In fact, glycolysis is the central pathway of glucose catabolism.

Glycolysis takes place in the extramitochondrial part of the cell (or the soluble cytoplasm). It is frequently referred to as Embden-Meyerhof-Parnas or EMP pathway, in honour of these poineer workers in the field, and still represents one of the greatest achievements in the field of biochemistry. Other illustrious investigators, who contributed significantly to the final elucidation of glycolytic pathway, include Fritz A. Lipmann, Harden and Young, A.V. Hill, Carl Neuberg, Otto Warburg, and Carl F. Cori and his wife Gerty T. Cori.

Gustave Embden (LT,1874-1933)– A German biochemist, one of the great poineers of metabolic studies.
Otto Meyerhof (LT, 1883-1951)– Another German biochemist, a Nobel Laureate of 1992; sought refuge in the United States in 1938.
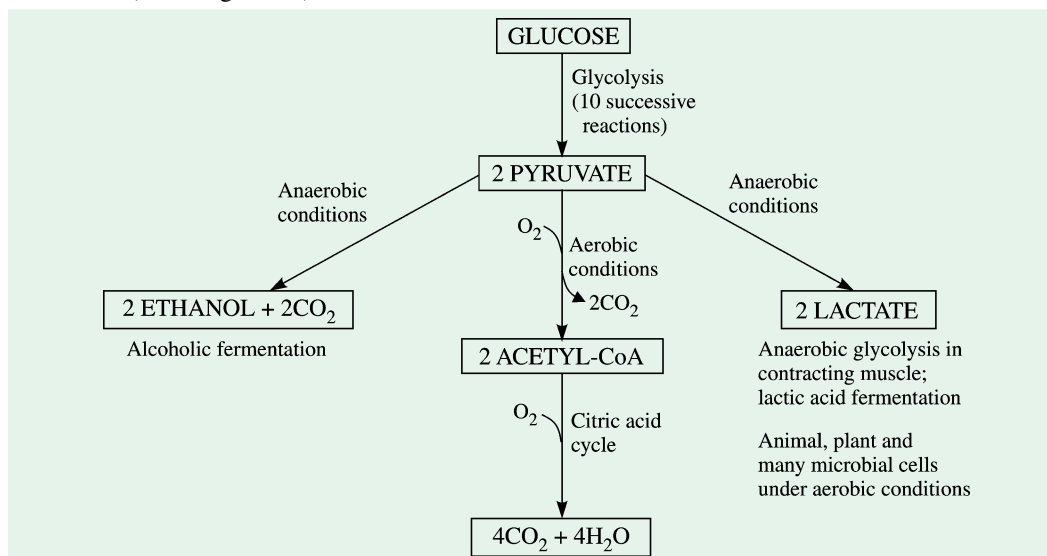**Jacob Parnas**– Another leading biochemist on cell respiration.

There are 3 important routes taken by pyruvate after glycolysis, depending on the organism and the metabolic conditions (refer Fig. 21–1) :

Fig. 21–1. Some important fates of glucose

(*a*) In aerobic organisms, the pyruvate so formed then enters mitochondria where it is oxidized, with the loss of its carboxyl group as CO_2, to form the acetyl group of acetyl-coenzyme A. Later, the acetyl group is completely oxidized to CO_2 and H_2O by the citric acid cycle with the intervention of molecular oxygen. This pathway is followed by aerobic animal and plant cells.

(*b*) If the supply of oxygen is insufficient, as in vigorously contracting skeletal muscles, the pyruvate cannot be oxidized further for lack of oxygen. Under such conditions, it is then reduced to lactate, a process called *anaerobic glycolysis*. Lactate is also produced from glucose in anaerobic microorganisms that carry out *lactic acid fermentation*.

CARL FERDINAND CORI AND GERTY THERESA CORI

Carl, a Czech-born American biochemist at Washington State University, shared the **1947 Nobel Prize for Medicine or Physiology** with his wife **Gerty** and **B.A. Houssay** from Argentina for their work on the metabolism of glycogen. They showed that the cells used an enzyme called *phosphorylase* to convert the stored glycogen to glucose, the sugar form which normally meets energy requirements of all cells in the body including muscle cells. They also found that the enzyme exists in either of its 2 forms: active or inactive. *The breakdown of glycogen to glucose is, in fact, a classic biochemical reaction that has bred three separate Nobel Prizes.* Both Carl and Gerty Cori discovered the Cori cycle. In essence, in the Cori cycle (adjoining figure) there is cycling of glucose due to glycolysis in muscle and gluconeogenesis in liver. In fact, lactate produced in muscles by glycolysis is transported by the blood to the liver. Gluconeogenesis in the liver converts the lactate back to glucose, which can be carried back to the muscles by the blood. Glucose can be stored in the muscles as glycogen until it is degraded by glycogenolysis.
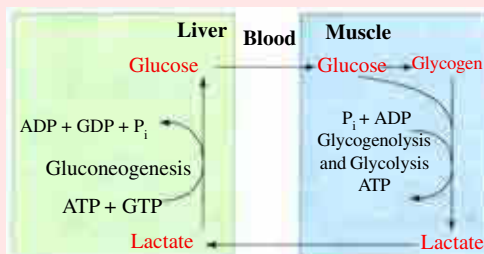
(*c*) In some microorganisms (*e.g.*, brewer's yeast), the pyruvate formed from glucose is transformed anaerobically into ethanol and CO_2, a process called *alcoholic fermentation*. Since living organisms first arose in an atmosphere devoid of oxygen, anaerobic breakdown of glucose is the most ancient type of biological mechanism for obtaining energy from organic fuel molecules (Lehninger AL, 1984).

OTTO H. WARBURG (LT, 1883-1970) Warburg, a student of Emil Fischer, is considered by many to be the greatest biochemist of the first half of twentieth century. His first publication (with Fischer) appeared in 1904, and his last in 1970, the year of his death at age 87.

Two Phases of Glycolysis

During glycolysis, the 6-carbon glucose is broken down into two moles of 3-carbon pyruvate *via* 10 enzyme-catalyzed sequential reactions. These reactions are grouped under 2 phases, phase I and II (refer Figs. 21–2 and 21-3).

A. **Phase I** or **Preparatory Phase.** It consists of the first 5 steps. In these reactions, glucose is enzymatically phosphorylated by ATP (first at carbon 6 and later at carbon 1) to yield fructose 1,6-diphosphate which is then split in half to yield 2 moles of the 3-carbon compound, glyceraldehyde 3-phosphate. The first

Fig 21-2.   Glycolysis is a two-stage process

The 'uphill' part involves raising glucose to a higher energy level by using ATP. In the 'downhill' part, the products are oxidized, yielding 2 molecules of pyruvate, 2 molecules of reduced coenzyme and a net gain in ATP.

phase of glycolysis, thus, results in cleavage of the hexose chain. This phase requires an investment of 2ATP moles to activate (or *prime*) the glucose mole and prepare it for its cleavage into two 3-carbon pieces. Besides glucose, other hexoses such as D-fructose, D-galactose and D-mannose may also convert into glyceraldehyde 3-phosphate.
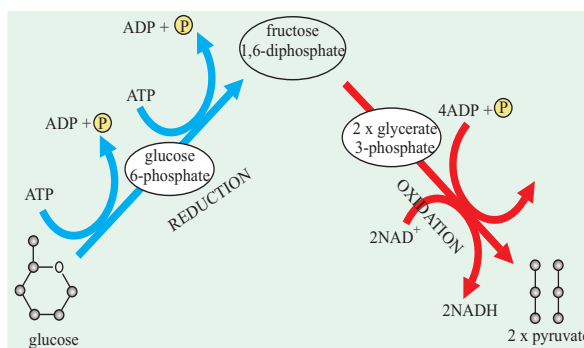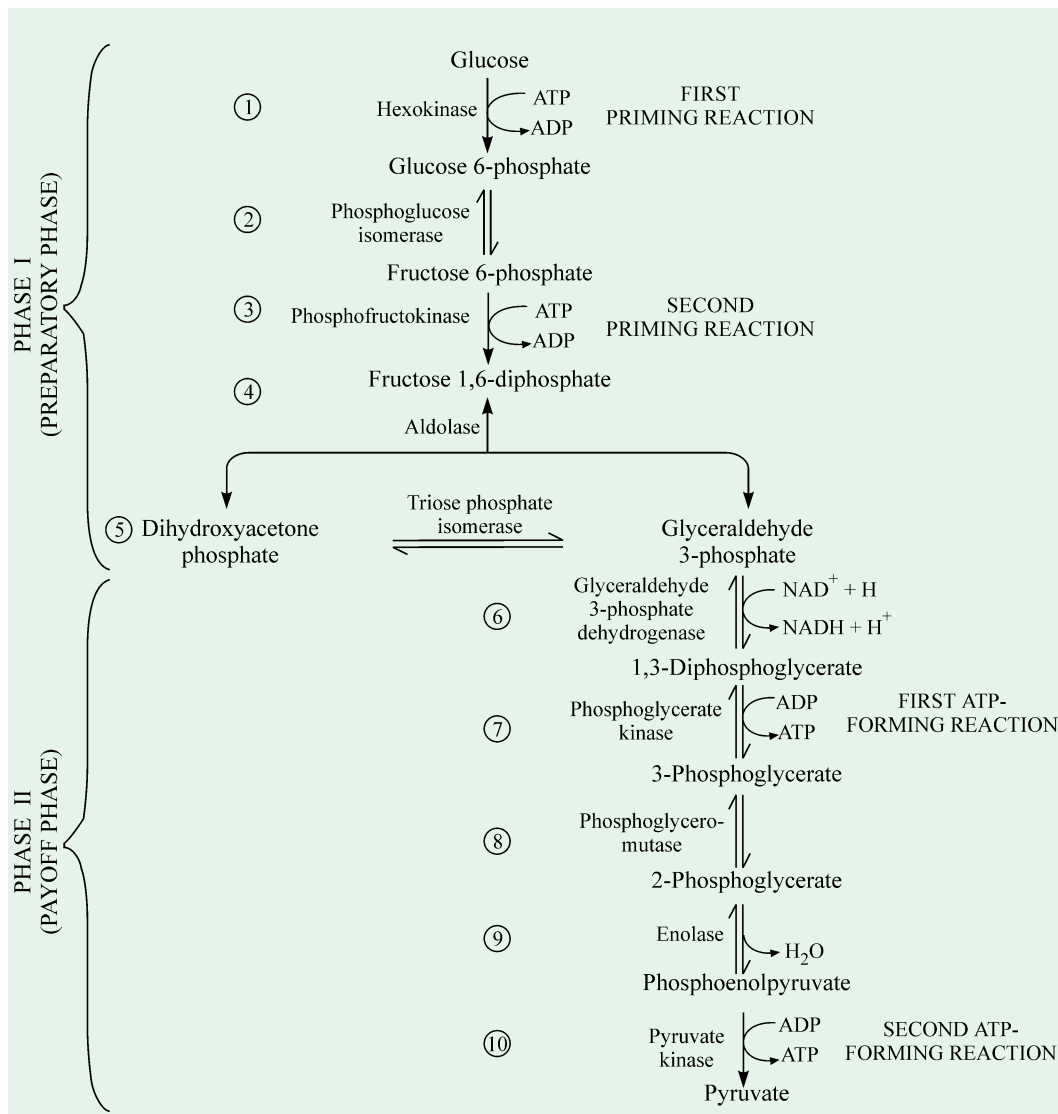
Fig. 21–3. Two phases of glycolysis

B. Phase II or **Payoff Phase.** The last 5 reactions of glycolysis constitute this phase. This phase represents the payoff of glycolysis, in which the energy liberated during conversion of 3 moles of glyceraldehyde 3-phosphate to 2 moles of pyruvate is converted by the coupled phosphorylation of 4 moles of ADP to ATP. Although 4 moles of ATP are formed in phase II, the net overall yield is only 2 moles of ATP per mole of glucsoe oxidized, since 2 moles of ATP are invested in phase I. The phase II is, thus, energy conserving.

A noticeable feature of glycolysis is that each of the 9 metabolic intermediates between glucose and pyruvate is a phosphorylated compound. The phosphoryl groups in these compounds are in either *ester* or *anhydride* likage. The phosphoryl or phosphate groups perform the following 3 functions :

1. The phosphate groups are completely ionized at *p*H 7, so that each of the 9 intermediates of glycolysis gains a net negative charge. Since cell membranes are, in general, impermeable to charged molecules, the glycolytic intermediates cannot escape from the cell. Only

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glucose can enter cells and pyruvate or lactate can leave cells because cell membranes have specific transport systems that allow these molecules to pass.

2. The phosphate groups are essential components in the conservation of energy since they are ultimately transferred to ADP to produce ATP.

3. The phosphate groups act as recognition or binding groups required for the proper fit of the glycolytic intermediates to the active site of their corresponding enzymes.

Enzymes Involved in Glycolysis

In most kinds of cells, the enzymes that catalyze glycolytic reactions (refer Table 21–1) are present in the extramitochondrial soluble fraction of the cell, the *cytosol*. On the other hand, the enzymes involved in citric acid cycle are located in the mitochondrial membrane in eukaryotes and in the plasma membrane in prokaryotes. *A remarkable feature of the glycolytic enzymes is that nearly all of them require Mg^{2+} for activity.*
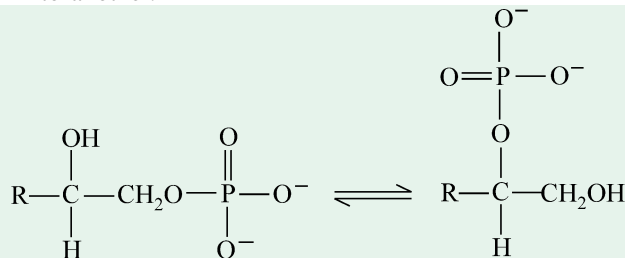
Kinds of Reactions in Glycolysis

For a better understanding of the various reactions of glycolysis, some of the kinds of reactions that occur in glycolysis are listed below :
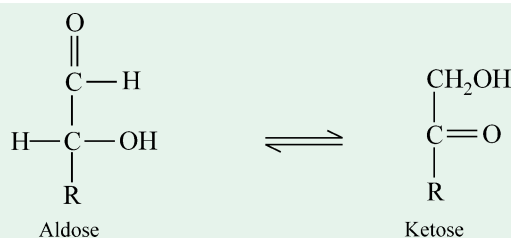
1. **Phosphoryl transfer.** A phosphate group is transferred from ATP to a glycolytic intermediate or *vice versa*.

$$R-OH + ATP \rightleftharpoons R-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O^- + ADP + H^+$$
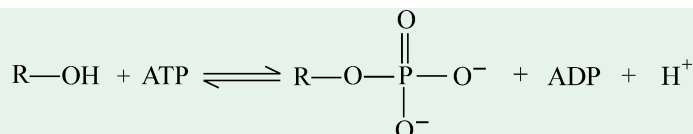
2. **Phosphoryl shift.** A phosphate group is shifted within a molecule from one oxygen atom to another.

$$R-\overset{\overset{OH}{|}}{\underset{\underset{H}{|}}{C}}-CH_2O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O^- \rightleftharpoons R-\overset{\overset{O=\overset{O^-}{\overset{|}{P}}-O^-}{\overset{|}{O}}}{\underset{\underset{H}{|}}{C}}-CH_2OH$$

3. **Isomerization.** An aldose is converted into a ketose or *vice versa*.

$$\begin{array}{c} \overset{\overset{O}{\|}}{C}-H \\ | \\ H-\overset{}{C}-OH \\ | \\ R \\ \text{Aldose} \end{array} \rightleftharpoons \begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ R \\ \text{Ketose} \end{array}$$

4. **Dehydration.** A molecule of water is eliminated.

$$\begin{array}{c} | \\ H-C- \\ | \\ H-C-OH \\ | \\ H \end{array} \rightleftharpoons \begin{array}{c} | \\ C- \\ \| \\ H-C \\ | \\ H \end{array} + H_2O$$
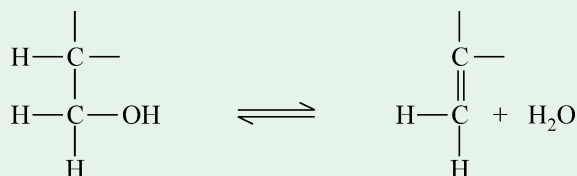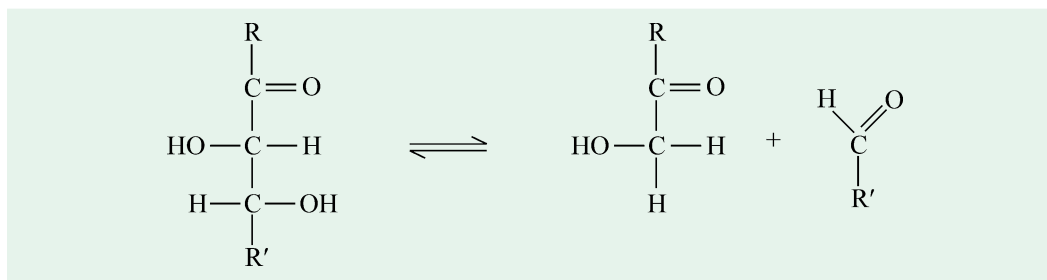
Table 21-1. Enzymes and reaction types of glycolysis

Step No.	Enzyme	Enzyme Commission Number	Coenzyme (s) and Cofactor (s)	Activator (s)	Inhibitor (s)	Kind of Reaction Catalyzed	$\Delta G^{\circ\prime}$ kcal/mol	$\Delta G^{\circ}*$ kcal/mol
1	Hexokinase	2.7.1.1	Mg^{2+}	ATP4–, Pi	Glucose 6-phosphate	Phosphoryl transfer	– 4.00	–8.0
2	Phosphoglucoisomerase	5.3.1.9	Mg2+	—	2-deoxyglucose 6-phosphate	Isomerization	+ 0.40	–0.6
3	Phosphofructokinase	2.7.1.11	Mg^{2+}	Fructose 2, 6-diphosphate, AMP, ADP, cAMP, K^+	ATP4–, citrate	Phosphoryl transfer	– 3.40	–5.3
4	Aldolase	4.1.2.7	Zn^{2+} (in microbes)	—	Chelating agents	Aldol cleavage	+ 5.73	–0.3
5	Phosphotriose isomerase	5.3.1.1	Mg^{2+}	—	—	Isomerization	+ 1.83	+ 0.6
6	Glyceraldehyde 3-phosphate dehydrogenase	1.2.1.12	NAD	—	Iodoacetate	Phosphorylation coupled to oxidation	+ 1.50	+ 0.6
7	Phosphoglycerate kinase	2.7.2.3	M^{2+}	—	—	Phosphoryl transfer	– 4.50	+ 0.3
8	Phosphoglycerate mutase	5.4.2.1	Mg^{2+} 2, 3-diphos-phoglycerate Mg^{2+}, Mn^{2+} Zn^{2+}, Cd^{2+}	—	—	Phosphoryl shift	+ 1.06	+ 0.2
9	Enolase	4.2.1.11	Kg^{2+}, K^+	—	Fluoride + phosphate	Dehydration	+ 0.44	– 0.8
10	Pyruvate kinase	2.7.1.40	Kg^{2+}, K^+	—	Acetyl CoA, alanine, Ca^{2+}	Phosphoryl transfer	– 7.50	– 4.0

*ΔG, the actual free energy change, has been calculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiologic conditions. Glycolysis can proceed only if the ΔG values of all reactions are negative. The small positive ΔG values of 3 of the above reactions indicate that the concentrations of metabolites *in vivo* in cells undergoing glycolysis are not precisely known.

5. **Aldol cleavage.** A C–C bond is split in a reversal of an aldol condensation.

$$
\begin{array}{c}
R \\
|\\
C\!=\!O \\
|\\
HO\!-\!C\!-\!H \\
|\\
H\!-\!C\!-\!OH \\
|\\
R'
\end{array}
\quad \rightleftharpoons \quad
\begin{array}{c}
R \\
|\\
C\!=\!O \\
|\\
HO\!-\!C\!-\!H \\
|\\
H
\end{array}
\quad + \quad
\begin{array}{c}
H \quad O \\
\diagdown \;\; \diagup \\
C \\
|\\
R'
\end{array}
$$

REACTION STEPS OF GLYCOLYSIS

The various reaction steps of glycolysis are schematically represented in Fig. 21–3. The details of these reactions and those of the enzymes, which catalyze them, are given below :

Step 1 : *Phosphorylation of Glucose*

In the first step, glucose is activated (or *primed*) for subsequent reactions by its phosphorylation at C_6 to yield glucose 6-phosphate, using ATP as phosphate donor. It is phosphoryl transfer type of reaction and is catalyzed by the inducible enzyme *hexokinase*, found in most animal, plant and microbial cells, and by an additional enzyme in the liver, *glucokinase*. The reaction is accompanied by considerable loss of free energy as heat. It is physiologically

> The terms used for glyceric acid, pyruvic acid and lactic acid are respectively, glycerate, pyruvate and lactate. These terms are used to emphasize that at pH of the cell, the acid involved in the reaction is largely in dissociated form.
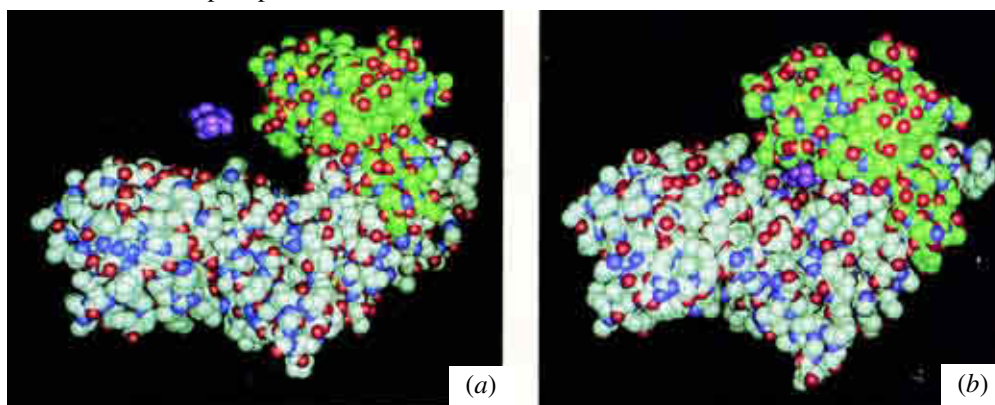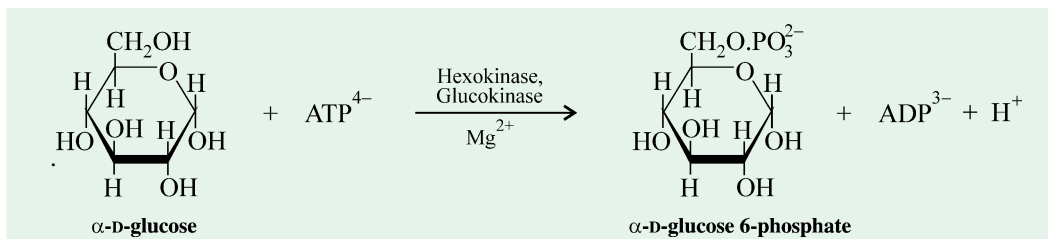
irreversible reaction because of the relatively low energy character of glucose 6-phosphate and the lower stability of Mg^{2+}.ADP compared to Mg^{2+}.ATP. Glucose 6-phosphate is an important compound, being at the junction of many metabolic pathways such as glycolysis, glycogenolysis, gluconeogenesis and the hexose monophosphate shunt.

(*a*)   (*b*)

Fig. 21–4.   A space-filling model of a subunit of yeast hexokinase in the "open" (a) and "closed" (b) conformations

The glucose, with which the enzyme complexes, is shown in purple. Note the prominant bilobal appearance of the free enzyme (the C atoms in the small lobe are shaded green, whereas those in the large lobe are light grey; the N and O atoms are blue and red). In the enzyme-substrate complex, these 2 lobes have swung together so as to engulf the substrate. Hexokinase also has been crystallized with a bound analogue of ATP. In the absence of glucose, the enzyme with the bound ATP analogue remains in the open conformation. The structural change caused by glucose results in the formation of additional contacts between the enzyme and ATP. This can explain why the binding of glucose enhances the binding of ATP.

(*Courtesy of Dr. Thomas A. Steiz, Yale University*)

$$\underset{\alpha\text{-}\textsc{d}\text{-glucose}}{\text{(structure)}} + ATP^{4-} \xrightarrow[\substack{Mg^{2+}}]{\substack{\text{Hexokinase,} \\ \text{Glucokinase}}} \underset{\alpha\text{-}\textsc{d}\text{-glucose 6-phosphate}}{\text{(structure)}} + ADP^{3-} + H^+$$

Hexokinase (Fig. 21–4) is found in all tissues and exists in 3 isoenzyme forms, types I, II and III. Each form is composed of a single subunit (MW = 100,000). Brain contains chiefly type I and skeletal muscle, the type II. *All the 3 types are present in human liver and fat*. Type I is found in the cytosol or bound to the mitochondria, whereas type II exists primarily in the cytosol. Hexokinase, like all other kinases, requires Mg^{2+} (or other divalent metal ions such as Mn^{2+}, Ca^{2+} etc) for activity. The 2 lobes of hexokinase remain separate in the absence of its substrate molecule, *i.e.*, glucose. However, the conformation changes markedly on binding with glucose and the 2 lobes of the enzyme come together and surround the substrate. This induced fit is shown in Fig, 21–5. Hexokinase not only acts on glucose but also on some other common hexoses such as fructose and mannose. The activity of hexokinase is inhibited by the product of the raction (*i.e.*, glucose 6-phosphate) which binds the enzyme at an allosteric site. Hexokinase has a high affinity (*i.e.*, low Km value of about 1.0 mM) for its substrate, glucose. The reverse reaction (Glucose 6-phosphate → Glucose) requires a different enzyme, glucose 6-phosphatase with Mg^{2+} as cofactor. This reaction occurs in liver but not in muscle which lacks glucose 6-phosphatase. Yeast hexokinase, however, differs somewhat from the mammalian forms. It is a dimer of identical subunits (MW = 50,000). Also, its activity is not affected by glucose 6-phosphate.

Glucokinase (often designated **hexokinase IV**) is a monomeric inducible enzyme (MW = 48,000) and is found almost exclusively in the liver. Glucokinase differs from mammalian hexokinase in 3 respects :

Fig. 21–5. Computer graphics of the induced fit in hexokinase

As shown in blue, the two lobes of hexokinase are separated in the absence of glucose. The conformation of hexokinase changes markedly on binding glucose, as shown in red. The two lobes of the enzyme come together and surround the substrate. Except around the binding site, the remaining portion of the enzyme in both the conditions (free and bound) is almost superimposed as seen in the figure.

(*a*) It is specific for glucose and does not act on other hexoses.

(*b*) It is not inhibited by glucose 6-phosphate.

(*c*) It has a low affinity (*i.e.*, a much higher Km value of about 10 *m*M) for glucose than hexokinase.

The function of glucokinase is to remove glucose from the blood following a meal and to trap it in the liver cells, thereby allowing storage of glucose as glycogen or, after further metabolism, as fatty acids.

Step 2 : *Isomerization of Glucose 6-phosphate*

Glucose 6-phosphate is reversibly isomerized to frucose 6-phosphate by *phospho-glucoisomerase*. Thus, the 6-membered pyranose ring of glucose 6-phosphate is converted into the 5-membered furanose ring of fructose 6-phosphate. This reaction involves a shift in the carbonyl
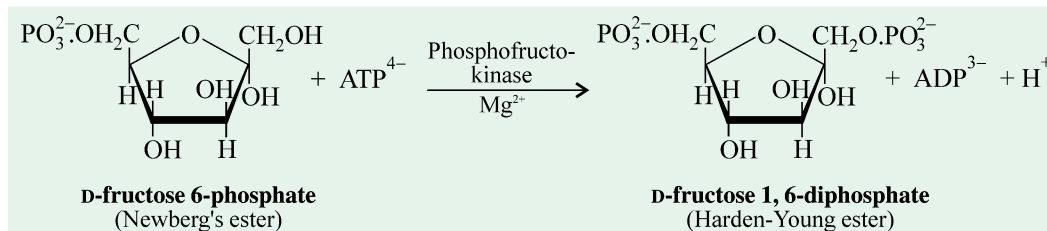
oxygen from C_1 to C_2, thus converting an aldose into a ketose. At equilibrium, the ratio of aldose to ketose is 7 : 3, *i.e.*, glucose 6-phosphate predominates, having concentration over twice that of fructose 6-phosphate. The reaction proceeds readily in either direction because of relatively small standard free energy change. Fructose 6-phosphate has metabolic fates other than glycolysis.

α-D-**glucose 6-phosphate**
(Robinson's ester)

Phosphogluco-
isomerase

α-D-**fructose 6-phosphate**
(Newberg's ester)
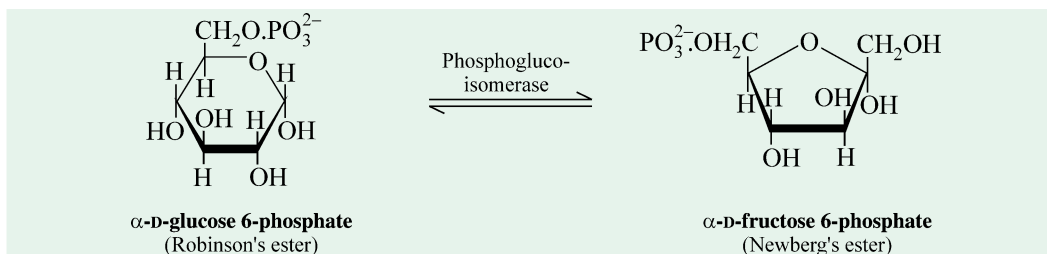
Human phosphoglucoisomerase (MW = 134,000) is a dimer of identical subunits and requires Mg^{2+} for activity. It is specific for glucose 6-phosphate and fructose 6-phosphate. An interesting sidelight of this enzyme is that it binds the α- pyranose form of glucose 6-phosphate, but the open chain form of fructose 6-phosphate. The α- and β-pyranose forms are interconvertible, so all of the glucose 6-phosphate is available to the enzyme.

Step 3 : *Phosphorylation of Fructose 6-phosphate*

This is the second of the two priming or activating reactions of glycolysis (the first one being Step 1). Fructose 6-phosphate is phosphorylated by ATP to produce fructose 1, 6-diphosphate in the presence of another inducible allosteric enzyme, *phosphofructokinase* (abbreviated as PFK). The enzyme catalyzes the transfer of a phosphate group from ATP to fructose 6-phosphate at C1 to yield fructose 1, 6-diphosphate. Since the reaction proceeds with $\Delta G^\circ = -3.4$ kcal/mol, it is essentially irreversible. *It is considered to be the committed step in glycolysis* since the PFK action 'commits' the cell to metabolizing glucose rather than storing or converting it to some other hexose. In addition to being a key step, it is an important control point of glycolysis.

D-**fructose 6-phosphate**
(Newberg's ester)

$+ \text{ATP}^{4-}$

Phosphofructo-
kinase
Mg^{2+}

D-**fructose 1, 6-diphosphate**
(Harden-Young ester)

$+ \text{ADP}^{3-} + \text{H}^+$

The muscle **phosphofructokinase** (MW = 320,000) *is one of the most complex known enzymes*. It is a tetramer of 4 identical subunits (Fig. 21–6)but dissociates into inactive dimers in the presence of citrate. Fructose 6-phosphate, however, promotes their reunion to form the tetramer. It is a major regulatory enzyme in muscle glycolysis because of its allosteric nature. The activity of phosphofructokinase is accelerated whenever the cell is deficient in ATP or there is an excess of ATP breakdown products (*i.e.*, ADP and AMP). The activity is, however, inhibited whenever the cell has plentiful ATP (and other fuels such as citrate or fatty acids) which lowers the affinity of the enzyme for fructose 6-phosphate. For the same reason, PFK is rightly regarded as the '*pace maker*' of glycolysis.

Step 4 : *Cleavage of Fructose 1,6-diphosphate*

This is a unique C–C bond scission reaction. Since fructose 1, 6-diphosphate is a molecule with phosphate group on both ends, it splits in the middle into two different triose phosphates, glyceraldehyde 3-phoshpate (an aldose) and dihydroxyacetone phosphate (a ketose). This reaction
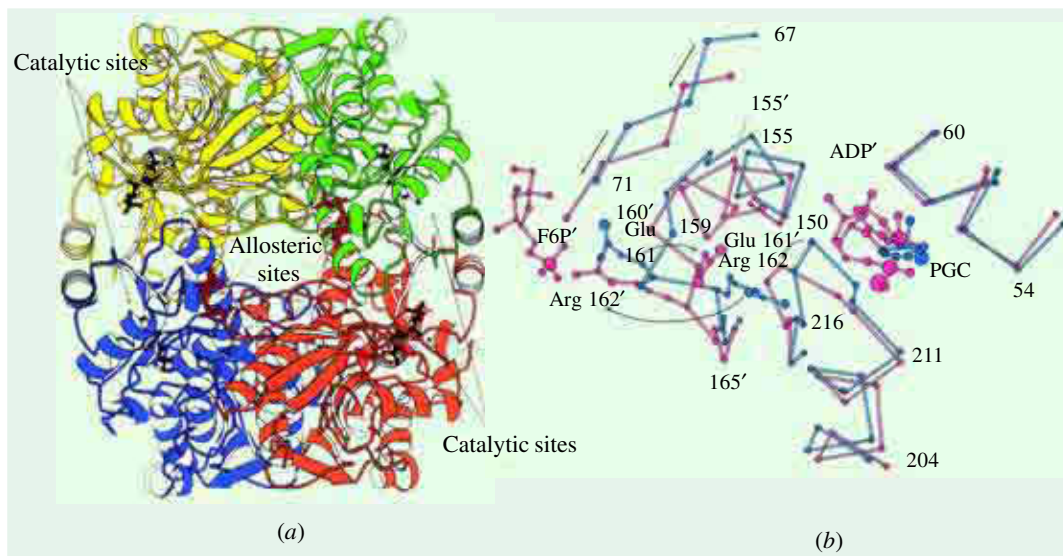
Fig. 21–6. The x-ray structure of phosphofructokinase (PFK)

(*a*) **Ribbon model.** Phosphofructokinase in the liver is a tetramer of four identical subunits. The positions of the catalytic and allosteric sites are indicated.

(*b*) **A superposition of those segments of the T state (*blue*) and R-state (*red*) enzymes that undergo a large conformational rearrangement upon the T→ R allosteric transition** (*indicated by the arrows*). Residues of the R state structure are marked by a prime. Also shown are bound ligands : the nonphysiological inhibitor 2-phosphoglycolate (PGC; a PEP analogue) for the T state, and the cooperative substrate F6P and the activator ADP for the R state.

(*Courtesy : (b) After Schirmer T and Evans PR, 1990*)

is catalyzed by the enzyme *fructose diphosphate aldolase* (often simply called *aldolase*) which cleaves the fructose 1, 6-diphosphate molecule between C_3 and C_4. Carbon atoms 4, 5 and 6 appear in glyceraldehyde 3-phosphate and 1, 2 and 3 in dihydroxyacetone phosphate. Although the aldolase reaction has a highly positive standard free energy change, it can readily proceed in either direction under the *p*H and concentration conditions existing in cells. Thus, this is a reversible aldol condensation type of reaction. The remaining steps in glycolysis involve 3 carbon units, rather than 6 carbon units.
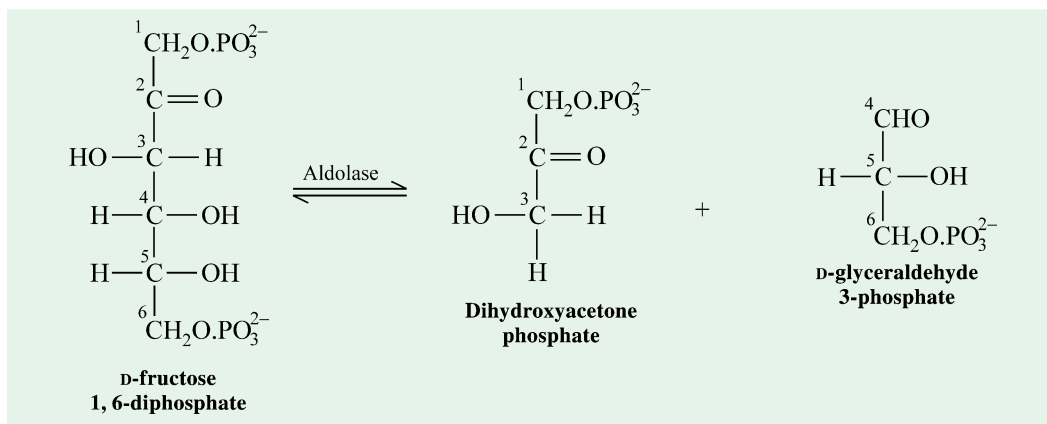

The **aldolase** (name derived from the nature of the reaction it catalyzes) from all animal tissues is a tetramer (MW = 160,000) but various tissues contain primarily one of the 3 different forms, characteristic of muscle, liver and brain respectively. All forms catalyze the above reaction

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but at different rates. The aldolase of animal tissues does not require Mg^{2+} but in many microbes (yeast), aldolase needs Zn^{2+} for its activity.

Step 5: *Isomerization of Dihydroxyacetone phosphate*

Glyceraldehyde 3-phosphate (an aldose) can be directly degraded in the subsequent reaction steps of glycolysis but dihydroxyacetone phosphate (a ketose) cannot be. However, dihydroxyacetone phosphate can be readily and reversibly converted into glyceraldehyde 3-phosphate by the enzyme *triose phosphate isomerase* (also called *phosphotriose isomerase*) in the same way that glucose and fructose phosphates are interconverted by phosphoglucoisomerase. This is an isomerization reaction and occurs very rapidly. At equilibrium, about 95% of the triose phosphate is dihydroxyacetone phosphate. However, the reaction proceeds towards glyceraldehyde 3-phosphate formation because of the efficient removal of this product. It may be noted that by this reaction, carbon atoms 1, 2 and 3 of the starting glucose now become indistinguishable from carbon atoms 6, 5 and 4 respectively. This reaction completes the first phase of glycolysis.
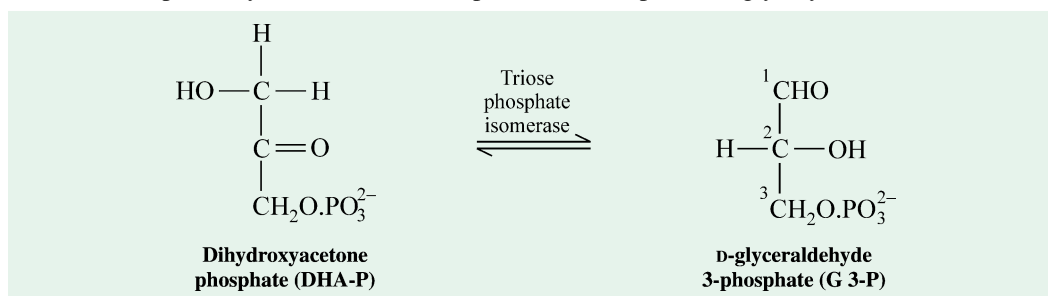
Triose phosphate isomerase (MW = 56,000) is a dimer of two identical subunits (Fig. 21– 7). It is noteworthy that the two enzymes, aldolase and triose phosphate isomerase have
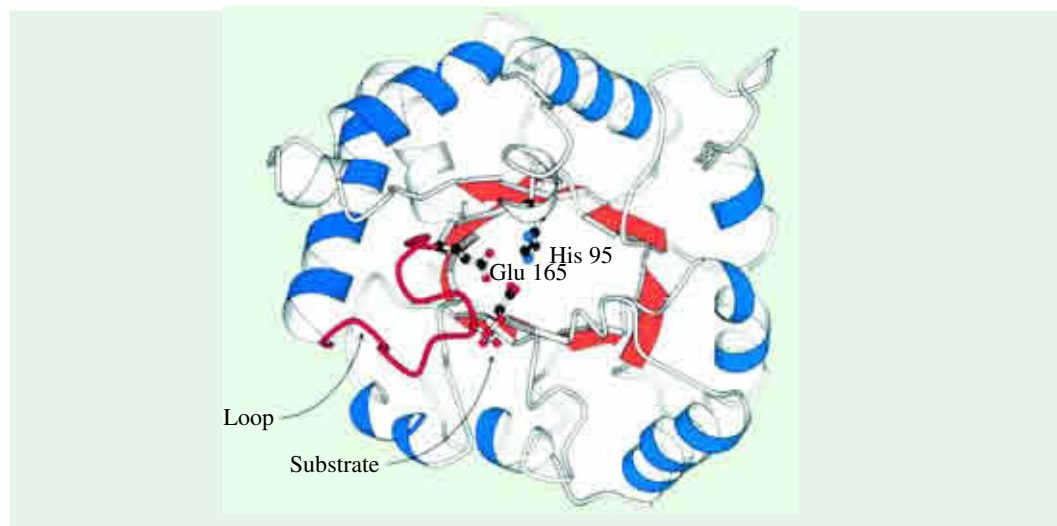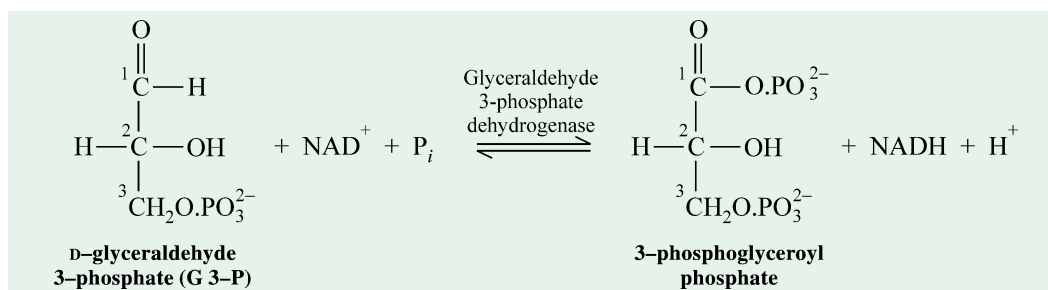

Fig. 21–7. Structure of triose phosphate isomerase

This enzyme consists of a central core of eight parallel β strands (orange) surrounded by eight α helices (blue). This structural motif, called an αβ barrel, is also found in the glycolytic enzymes, aldolase, enolase, and pyruvate kinase. Histidine 95 and glutamate 165, essential components of the active site of triose phosphate isomerase, are located in the barrel. A loop (red) closes off the active site on substrate binding.

a common substrate, dihydroxyacetone phosphate ; the difference being in their mode of action - the former produces fructose 1, 6-diphosphate whereas the latter, glyceraldehyde 3-phosphate.

Step 6 : *Oxidative Phosphorylation of Glyceraldehyde 3-phosphate*

This is first of the two energy-conserving reactions of glycolysis (the second one being Step 9) in which a high energy phosphate compound, 3-phosphoglyceroyl phosphate is formed. Here, glyceraldehyde 3-phosphate is converted into 3-phosphoglyceroyl phosphate (= 1, 3-diphosphoglycerate, 1,3-DPG) by the enzyme *glyceraldehyde* 3-phosphate *dehydrogenase* (= phosphoglyceraldehyde dehydrogenase), which is NAD^+-dependent. In this complex and reversible reaction, the aldehyde group of glyceraldehyde 3-phosphate is dehydrogenated, not to a free carboxyl group, but to a carboxylic anhydride with phosphoric acid, the 3-phosphoglyceroyl phosphate. This type of anhydride called an acyl phosphate has a very high standard free energy of hydrolysis ($\Delta G^{o\prime}$ = – 11.8 kcal/mol) and is thus a super high energy phosphate (refer Table 20–5). The acyl phosphate or 1,3–diphosphoglyceraldehyde conserves much of the free energy liberated during oxidation of the aldehyde group of glyceraldehyde 3-phosphate. The value of $DG^{o\prime}$ for this reaction is + 1.5 kcal/mol.

$$\begin{array}{c}
\overset{O}{\overset{\|}{\underset{1}{C}}}\text{—H} \\
\mid \\
H\text{—}\underset{2}{C}\text{—OH} \\
\mid \\
\underset{3}{CH_2}O.PO_3^{2-}
\end{array}
\quad + \; NAD^+ + P_i
\xrightleftharpoons[]{\substack{\text{Glyceraldehyde}\\\text{3-phosphate}\\\text{dehydrogenase}}}
\begin{array}{c}
\overset{O}{\overset{\|}{\underset{1}{C}}}\text{—O.PO}_3^{2-} \\
\mid \\
H\text{—}\underset{2}{C}\text{—OH} \\
\mid \\
\underset{3}{CH_2}O.PO_3^{2-}
\end{array}
\quad + \; NADH + H^+$$

<center>ᴅ–glyceraldehyde   3–phosphoglyceroyl
3–phosphate (G 3–P)   phosphate</center>

Glyceraldehyde 3-phosphate dehydrogenase (Fig. 21–8) from rabbit skeletal muscles (MW = 146,000) is a tetramer of 4 identical subunits, each consisting of a single polypeptide chain of about 330 amino acid residues. Four thiol (– SH) groups are present on each polypeptide, probably derived from cysteine residues within the polypeptide chain. One of the – SH groups is found at the active site on which an acyl-enzyme complex is formed. Each mole of enzyme also contains 4 moles of NAD^+. The enzyme is, however, inactivated by
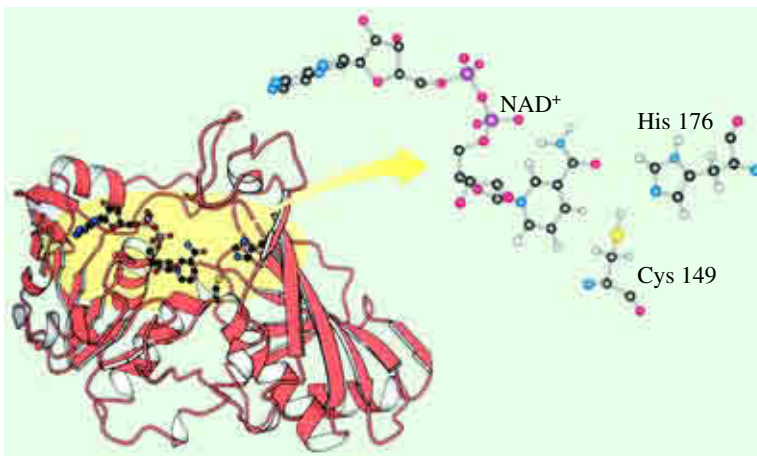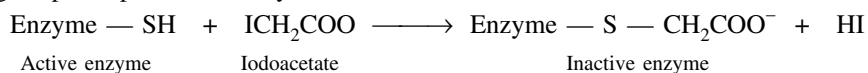

Fig. 21–8. **Structure of glyceraldehyde 3-phosphate dehydrogenase**

The active site includes a cysteine residue and a histidine residue adjacent to a bound NAD^+.

the – SH poison, iodoacetate which combines with the essential – SH group of the enzyme, thus preventing its participation in catalysis.

<center>Enzyme — SH + ICH$_2$COO $\longrightarrow$ Enzyme — S — CH$_2$COO$^-$ + HI</center>

<center>Active enzyme   Iodoacetate   Inactive enzyme</center>

The mechanism of action of glyceraldehyde 3-phosphate dehydrogenase is rather complex and resembles that of a-ketoglutarate dehydrogenase. It involves 3 steps (refer Fig 21–9).
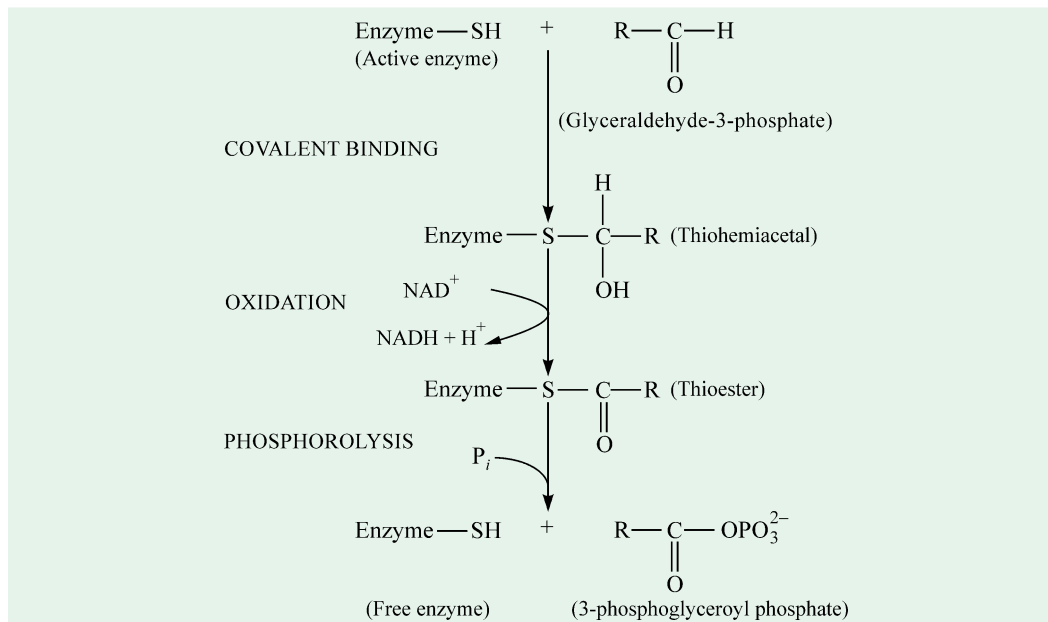
Fig. 21–9. Mechanism of action of glyceraldehyde 3-phosphate dehydrogenase, represented
here as Enzyme—SH

(*a*) *Covalent binding of substrate to —SH group*. The substrate first combines with an —SH
group of an essential cysteine residue present at the active site of the enzyme, forming a
thiohemiacetal.

(*b*) *Oxidation of thiohemiacetal and reduction of NAD$^+$*. Thiohemiacetal (= hemithioacetal)
is then oxidized to produce a high energy covalent acyl-enzyme complex, called thiol ester (=
thioester). The hydrogen removed in this oxidation is transferred to the coenzyme NAD$^+$, also
tightly bound at the active site of the enzyme molecule. The reduction of NAD$^+$ proceeds by the
enzymatic transfer of a hydride ion (:H$^-$) from the aldehyde group of the
substrate, glyceraldehyde 3-phosphate to position 4 of the nicotinamide
ring of NAD$^+$, resulting in its reduction at ring positions 1 and 4 to yield
the reduced coenzyme NADH. The other hydrogen atom of the substrate
molecule appears in the medium as H$^+$. For the same reason, the enzymatic

Hydride ion is a
hydrogen nucleus
and two electrons.

reduction of NAD$^+$ is written to include the hydrogen ion (H$^+$) formed. NADH formed in the
reaction is reoxidized to NAD$^+$ in order to participate in the breakdown of more glucose molecules
to pyruvate.
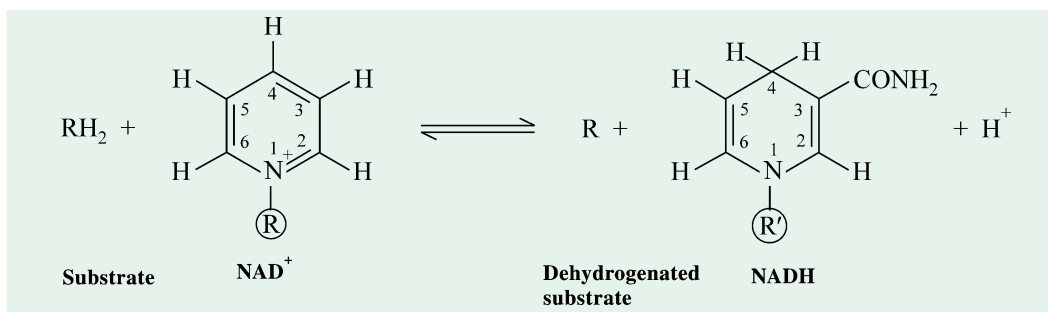
(*c*) *Phosphorolysis of thioester*. Finally, the acyl-enzyme (or thioester) reacts with an inorganic
phosphate (Pi) forming an acyl phosphate called 3-phosphoglyceroyl phosphate and the free enzyme

with a reconstituted —SH group is liberated. A diagrammatic representation of the mechanism of action of glyceraldehyde 3-phosphate dehydrogenase is given in Fig. 21-10.
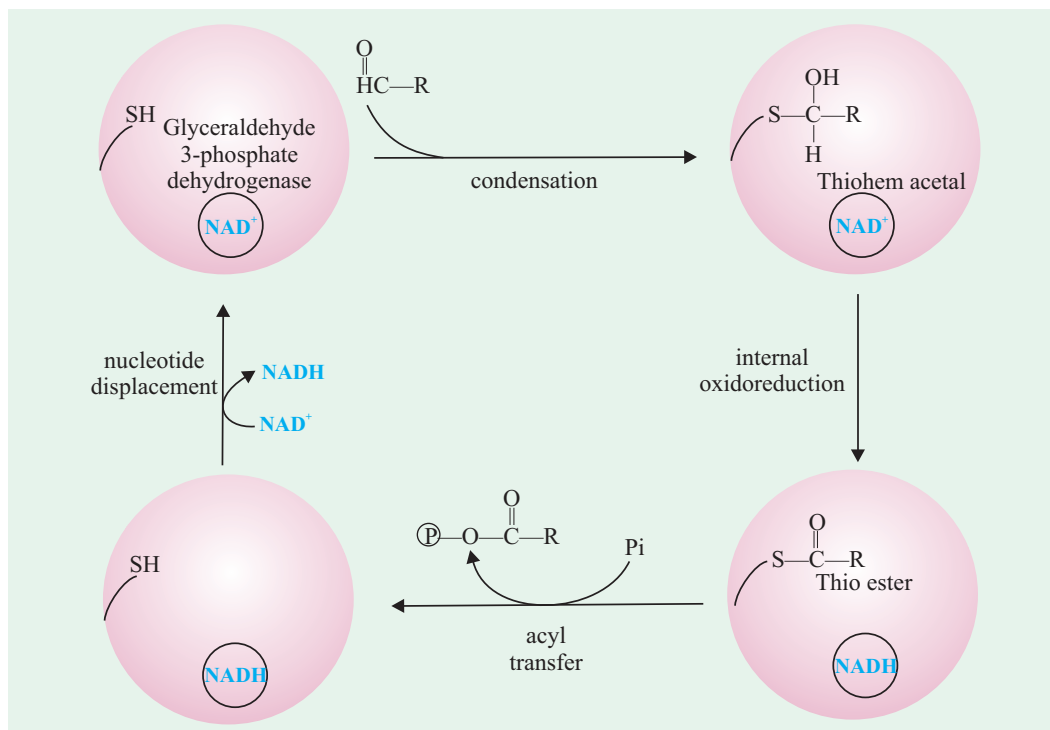

Fig. 21–10. Mechanism of action of glyceraldehyde 3-phosphate dehydrogenase

Large circle represents enzyme, small circle binding site for NAD^+; RCOH, glyceraldehyde 3-phosphate; — SH, the sulfhydryl group of the cysteine residue located at the active site ; and ~P, the high-energy phosphate bond of 1, 3-bisphosphoglycerate.

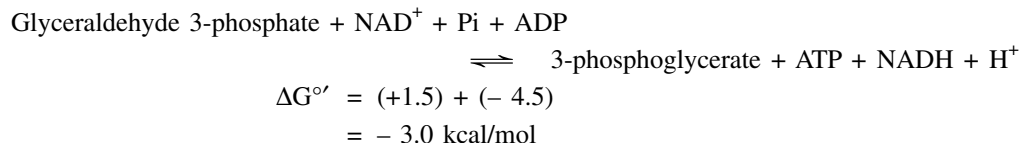**Step 7 :** *Transfer of Phosphate from 1,3-DPG to ADP*

This is the first ATP-generating reaction in glycolysis (the second one being Step 10). It involves the transfer of high-energy phosphate group from the carboxylic group of 3-phosphoglyceroyl phosphate (= 1, 3-diphosphoglycerate or 1,3-DPG) to ADP by the enzyme phosphoglycerate kinase, thus producing ATP and leaving 3-phosphoglycerate. Since 2 moles of triose phosphate are produced per mole of glucose, 2 moles of ATP are generated at this stage per mole of glucose oxidized. The value of $\Delta G°'$ for this essentially reversible reaction is – 4.5 kcal/mol.
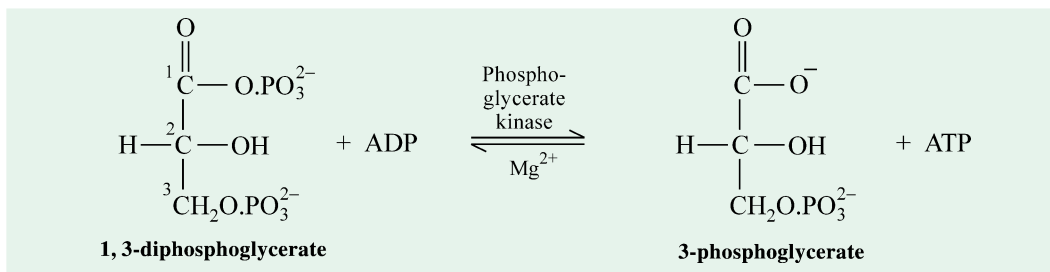
Phosphoglycerate kinase (Fig. 21–11) has a molecular weight of about 45,000. As with other enzymes of this type, there is an absolute requirement for a divalent metal cofactor (Mg^{2+}, Mn^{2+}

or Ca^{2+}). The metal interacts with the ADP or ATP to form the reactive complex. This and the preceding reaction (*i.e.*, Steps 6 and 7) together constitute an energy-coupling process. The sum of these two sequential reactions would give :
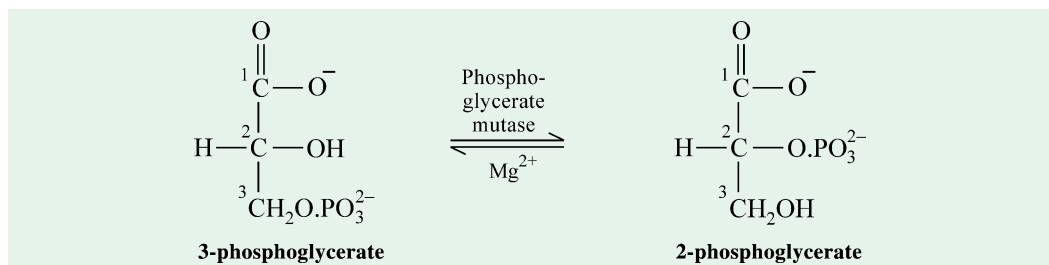
Glyceraldehyde 3-phosphate + NAD^+ + Pi + ADP

$$\rightleftharpoons \text{3-phosphoglycerate + ATP + NADH + } H^+$$

$$\Delta G^{\circ\prime} = (+1.5) + (-4.5)$$

$$= -3.0 \text{ kcal/mol}$$

Thus, the net outcome of Steps 6 and 7 is :

(*a*) Glyceraldehyde 3-phosphate, an aldehyde, is oxidized to 3-phosphoglycerate, a carboxylic acid.

(*b*) NAD+ is reduced to NADH.

(*c*) ATP is produced from ADP and Pi.

In other words, the net result of Steps 6 and 7 is that the energy released on oxidation (or dehydrogenation) of an aldehyde to a carboxylate group is conserved by the coupled formation of ATP from ADP and Pi. Such reactions are called *substrate-level phosphorylations* because the energy required to bring them about arises from the dehydrogenation of an organic substrate molecule (for example, glyceraldehyde 3-phosphate in the present case). This term distinguishes these phosphorylations from *oxidative phosphorylation*, which is the formation of ATP coupled to the oxidation of NADH and $FADH_2$ by oxygen. As the oxidative phosphorylation is coupled to electron transport, it is also called as *respiratory-chain phosphorylation*.

Fig. 21–11. A space-filling model of yeast phosphoglycerate kinase showing its deeply clefted bilobal structure

The substrate-binding site is at the bottom of the cleft as marked by the P atom (*purple*) of 3PG. Compare this structure with that of hexokinase (Fig. 21–4).

(*Courtesy of Herman Watson, University of Bristol, U.K.*)

Step 8 : *Isomerization of 3-phosphoglycerate*

The 3-phosphoglycerate is converted into 2-phosphoglycerate due to the intramolecular shift of phosphoryl group from C_3 to C_2, by the enzyme *phosphoglycerate mutase* (= *phosphoglyceromutase*). This is a reversible reaction with a $\Delta G^{\circ\prime}$ value = + 1.06 kcal/mol.

3-phosphoglycerate   **2-phosphoglycerate**

Phosphoglycerate mutase (MW = 65,000) is a dimer of identical subunits. Mg^{2+} is essential for this reaction. The enzyme requires 2, 3-diphosphoglycerate as cofactor for its action and combines with it to give a phosphoenzyme and either the 2-phosphoglycerate or the 3-phosphoglycerate :
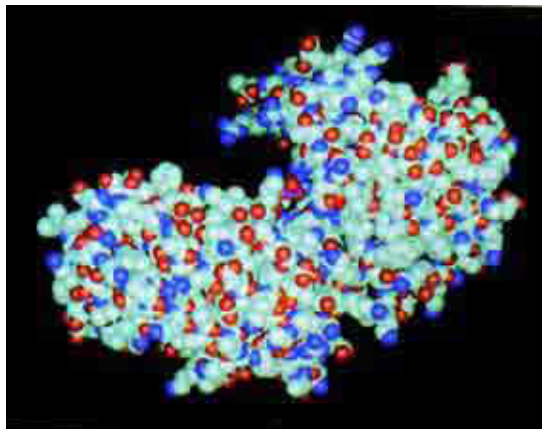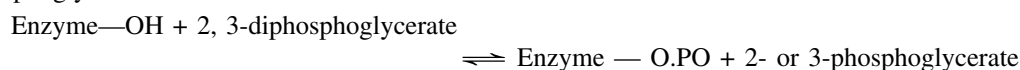
Enzyme—OH + 2, 3-diphosphoglycerate

$$\rightleftharpoons \text{Enzyme — O.PO + 2- or 3-phosphoglycerate}$$

At high concentration of 3-phosphoglycerate, the 3-phosphoglycerate and phosphoenzyme react to produce free enzyme and 2, 3-diphosphoglycerate, which in turn yields the 2-phosphoglycerate. The reaction occurs in reverse direction at high concentration of 2-phosphoglycerate. At equilibrium, the ratio of 2- to the 3-phosphate is 50 : 1. The 2, 3-diphosphoglycerate is produced from 3-phosphoglycerate by the enzyme *2, 3-diphosphoglycerate kinase* as follows :

$$\text{3-phosphoglycerate} + \text{ATP} \longrightarrow \text{2, 3 diphosphoglycerate} + \text{ADP}$$
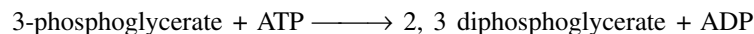
Step 9 : *Dehydration of 2-phosphoglycerate*

This is the second reaction of glycolysis in which is a high-energy phosphate compound (*i.e.*, phosphoenolpyruvate) is formed. The 2-phosphoglycerate is dehydrated by the action of enolase (= *phosphopyruvate hydratase*) to phosphoenolypyruvate (abbreviated as PEP), which is the phosphate ester of enol tautomer of pyruvate. This is a reversible reaction and has a relatively small free energy change value of + 0.44 kcal/mol. At equilibrium, the ratio of 2-phosphoglycerate to phosphoenolpyruvate is 2 : 1. However, phosphoenolpyruvate, and not 2-phosphoglycerate, contains a super high-energy phosphate bond. The loss of water from 2-phosphoglycerate causes a redistribution of energy within the molecule, raising the phosphate on position 2 to the high energy state, thus forming PEP. The reaction is freely reversible since there is little free energy change. The $\Delta G^{\circ\prime}$ value for the hydrolysis of PEP is –14.8 kcal/mol. There is more than sufficient energy to allow synthesis of ATP from PEP in the next step of glycolysis.

2-phosphoglycerate   **Phosphoenolpyruvate**
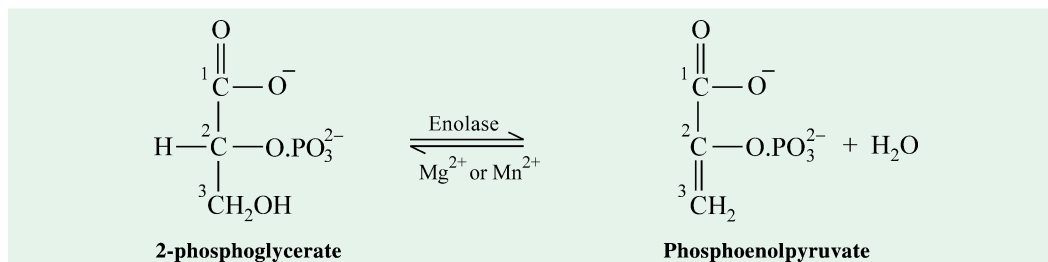
Enolase (MW = 88,000) is a dimer with identical subunits. It requires Mg^{2+}, Mn^{2+}, Zn^{2+} or Cd^{2+} as cofactor in its active site which forms a complex with the enzyme before the substrate is bound. Enolase is inhibited by the simultaneous presence of fluoride and phosphate. In fact, the fluorophosphate ion, which binds with Mg^{2+} forming magnesium fluorophosphate, is the true inhibitor.

Step 10 : *Transfer of Phosphate from PEP to ADP*

This is the second ATP-generating reaction in glycolysis. Here, phosphoenolpyruvate (PEP) is converted into pyruvate in enol form (*i.e.*, enolpyruvate) by the inducible allosteric enzyme *pyruvate kinase* (abbreviated as *PK*). The enzyme catalyzes the transfer of a phosphoryl group from PEP to ADP, thus forming ATP. *This phosphorylation reaction is nonoxidative* in contrast with the one catalyzed by glyceraldehyde 3-phosphate dehydrogenase (*i.e.*, Step 6). This is another physiologically irreversible step in glycolysis (the first one being Step 1) and proceeds with $\Delta G^{\circ\prime}$ = – 7.5 kcal/mol.
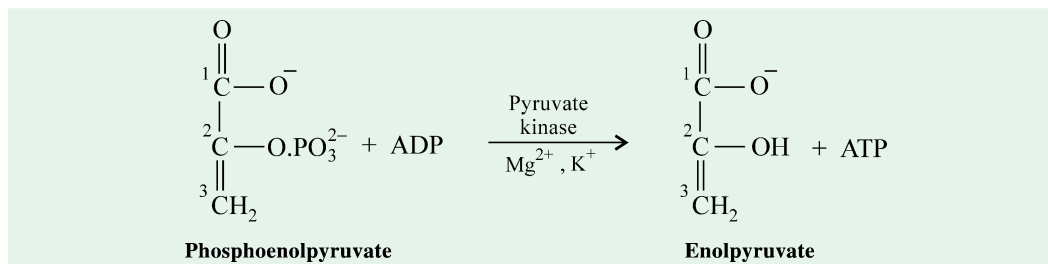
Phosphoenolpyruvate   **Enolpyruvate**

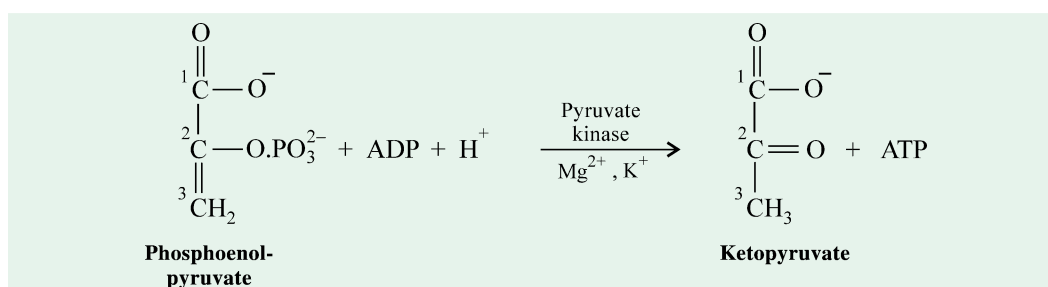The enolpyruvate, however, rearranges rapidly and *nonenzymatically* to yield the keto form of pyurvate (*i.e.*, ketopyruvate). The keto form predominates at *p*H 7.0.

$$
\begin{array}{ccc}
\overset{O}{\underset{\|}{^1C}}-O^- & & \overset{O}{\underset{\|}{^1C}}-O^- \\[4pt]
^2C-OH & \longrightarrow & ^2C=O \\[4pt]
^3CH_2 & & ^3CH_3
\end{array}
$$

Enolpyruvate **Ketopyruvate**
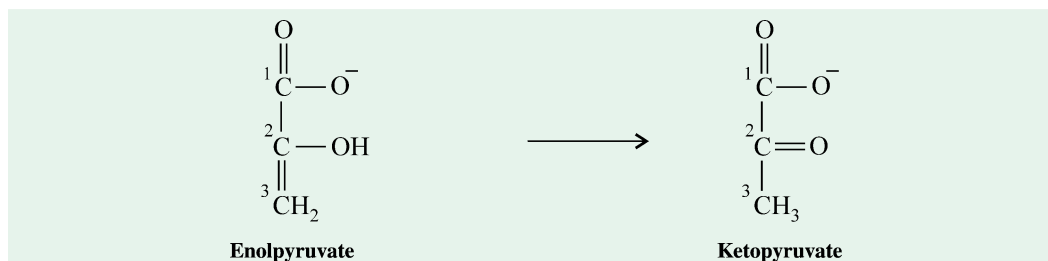
The point of equilibrium of this nonenzymatic reaction is very far to the right. Therefore, it 'drives' the preceding enzymatic reaction to the right by mass action. The two reactions, on addition, give :

$$
\begin{array}{ccc}
\overset{O}{\underset{\|}{^1C}}-O^- & & \overset{O}{\underset{\|}{^1C}}-O^- \\[4pt]
^2C-O.PO_3^{2-} + ADP + H^+ & \xrightarrow[\ Mg^{2+},\,K^+\ ]{\text{Pyruvate kinase}} & ^2C=O + ATP \\[4pt]
^3CH_2 & & ^3CH_3
\end{array}
$$

Phosphoenol-   **Ketopyruvate**
pyruvate

The overall reaction has a very large negative $\Delta G^{\circ\prime}$ value due to the spontaneous conversion of enol form of pyruvate to the keto form. The $\Delta G^{\circ\prime}$ value for hydrolysis of PEP is – 14.8 kcal/mol. About half of this energy is recovered as ATP ($\Delta G^{\circ\prime} = -7.3$ kcal/mol) and the rest (–7.5 kcal/mol) constitutes a large driving force, pushing the reaction far to the right. Since 2 moles of PEP are formed per mole of glucose oxidized, 2 moles of ATP are also produced per mole of glucose. The conversion of phosphoenolpyruvate into pyruvate is the second example of *substrate-level phosphorylation* in glycolysis.

Pyruvate kinase (MW = 190,000 – 250,000) is found in 3 major forms : muscle and brain contain the M type and liver the L type whereas most other tissues contain the A type. Each type controls different catalytic properties in accordance with its differing roles it performs. However, all forms of the enzyme are tetramers of 4 identical subunits. The enzyme is dependent on the concentration of K^+ which increases the affinity of PEP for enzyme. The enzyme also requires Mg^{2+} as it forms a complex with ATP, Mg^{2+}. ATP complex, which is the actual substrate. The participation of both K^+ and Mg^{2+} ions is an unusual, although not unique, instance of complementary requirement for two different cations. Pyruvate kinase is an allosteric enzyme and like phosphofructokinase, its activity is regulated by several means. In general, its activity is high when a net flux of glucose to pyruvate or lactate is required and low during gluconeogenesis (*i.e.*, during formation of glucose or glycogen from noncarbohydrate sources). Pyruvate kinase is also inhibited by acyl-CoA and by long-chain fatty acids, both important fuels for citric acid cycle. Certain amino acids also modulate enzyme activity, *esp.*, in the liver.

In metabolic pathways, the enzymes catalyzing essentially irreversible reactions are the key sites of control. In glycolysis, the steps 1, 3 and 10 which are catalyzed by hexokinase (or glucokinase), phosphofructokinase and pyruvate kinase respectively are virtually irreversible. Hence, they perform regulatory as well as catalytic functions.

STOICHIOMETRY OF GLYCOLYSIS

Overall Balance Sheet

Keeping in mind that each molecule of glucose yields 2 molecules of glyceraldehyde 3-phosphate, the total inputs and the outputs of all the 10 glycolytic reactions may be written as follows :
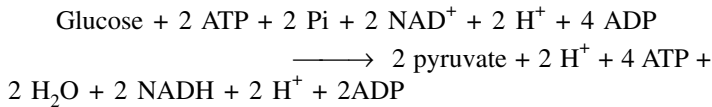
The word stoichiometry is pronounced as **'stoy-ke-om'–etry'.** It combines two Greek words : *stoicheion* = element and *metron* = measure.

Glucose + 2 ATP + 2 Pi + 2 NAD$^+$ + 2 H$^+$ + 4 ADP

$\longrightarrow$ 2 pyruvate + 2 H$^+$ + 4 ATP +

2 H$_2$O + 2 NADH + 2 H$^+$ + 2ADP

On cancelling the common terms, we get the net equation for the transformation of glucose into pyruvate :

Glucose + 2 Pi + 2 ADP + 2 NAD$^+$ $\longrightarrow$ 2 pyruvate + 2 ATP + 2 NADH + 2 H$^+$ + 2 H$_2$O
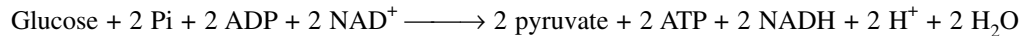
Thus, three things happen simultaneously in glycolysis :

(*a*) Glucose is oxidized to pyruvate.

(*b*) NAD+ is reduced to NADH.

(*c*) ADP is phosphorylated to form ATP.

There can be no EMP pathway without all 3 events which means that NAD$^+$, ADP and Pi, as well as glucose, must be present.

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 21–2.

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 × 2 = 2
10	Phosphoenolpyruvate $\longrightarrow$ Pyruvate		1 × 2 = 2
		2	4
		Net gain of ATP = 4 – 2 = 2	

## MUSCLE (OR ANAEROBIC) GLYCOLYSIS AND HOMOLACTIC FERMENTATION

In plant and animal tissues, under aerobic conditions, the pyruvate is the product of glycolysis and NADH, formed by the dehydrogenation of glyceraldehyde 3-phosphate, is then reoxidized to NAD$^+$ by oxygen. However, under anaerobic conditions, in actively contracting skeletal muscles, the NADH generated in glycolysis cannot be reoxidized by oxygen but must be reoxidized to NAD$^+$ by the pyruvate itself, converting pyruvate into lactate. Such type of glycolytic sequence occurring under anaerobic conditions in the muscle tissues is commonly spoken of as *muscle glycolysis or anaerobic glycolysis*. Besides the skeletal muscles, a large number of microorganisms, the lactic acid bacteria (esp., species of *Lactobacilli, Bacilli, Streptococci and Clostridia*) also follow the same path for the reduction of pyruvate to lactate. Such type of fermentation that yields lactate as the sole product is termed *homolactic fermentation.*

The reduction of pyruvate by NADH to form lactate is catalyzed by lactate dehydrogenase (abbreviated as LDH) which forms the L-isomer of lactate. As the reaction has a large negative value of


| Ketopyruvate | L-lactate |
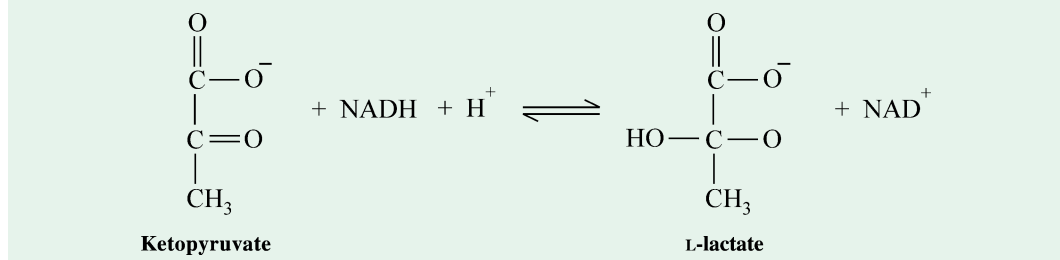
$\Delta G^{\circ\prime}$ (–6.0 kcal/mol), it proceeds far to the right. The reoxidation of NADH via lactate formation allows glycolysis to proceed in the absence of O_2 by regenerating sufficient NAD^+ for the reaction Step 6 of glycolysis, catalyzed by glyceraldehyde 3-phosphate dehydrogenase. Thus, the reduction of pyruvate to lactate is coupled to the oxidation of glyceraldehyde 3-phosphate to 3-phosphoglyceroyl phosphate as shown below :
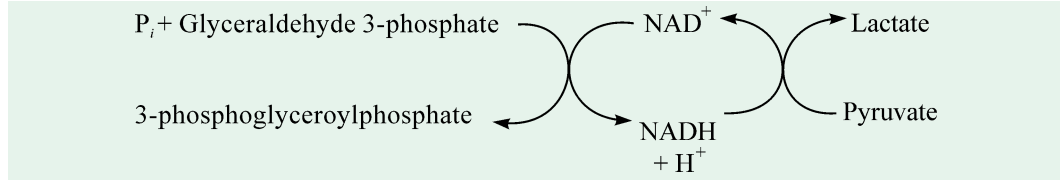
The anaerobic glycolysis, thus, results in the accumulation of 2 moles of lactate per mole of glucose utilized. *Lactate is one of the blind alleys or "dead-end streets" in metabolism and, once formed, it can only be reconverted to pyruvate.* The reconversion is, however, accomplished in the liver cells into which lactate is transported by circulation from muscle cells.

Lactate dehydrogenase (MW = 140,000) is a tetramer consisting of 4 subunits which are of 2 types. These are designated as M (for skeletal muscle) and H (for heart muscle) and differ in sequence. The skeletal muscle contains LDH of M_4 type mainly whereas heart muscle LDH is largely H_4. However, various tissues contain all possible hybrids, M_1H_3, M_2H_2 and M_3H_1. The exact amount of each of these 5 isozyme forms, as they are called, differs with the tissue. The various isozyme forms differ with respect to their K_m value for pyruvate, their turnover numbers or V_{max} and the degree of their allosteric inhibition by pyruvate. The H_4 has a low K_m for pyruvate and is strongly inhibited by pyruvate. It is, thus, better adapted for a more highly aerobic organ which removes lactate and oxidizes it to pyruvate mainly in the mitochondria. In contrast, the M_4 has a higher K_m for pyruvate and is not inhibited by pyruvate. Hence, it is useful in a tissue in which a more anaerobic environment may predominate as in skeletal muscle. *M_4 is catalytically more active than H_4.* Lactate dehydrogenase is characteristically inhibited by oxamate.

The net equation for anaerobic glycolysis in muscles and lactate fermentation in some microbes would then be :

$$\text{Glucose} + 2 \text{ Pi} + 2 \text{ ADP} \longrightarrow 2 \text{ lactate} + 2 \text{ ATP} + 2 \text{ H+} + 2 \text{ H}_2\text{O}$$
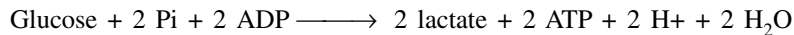
No oxygen is consumed in anaerobic glycolysis. Two steps involve oxidation-reduction, the oxidation of glyceraldehyde 3-phosphate and the reduction of pyruvate to lactate. NAD^+ participates in both reactions. Hence, the two cancel out and there is no net oxidation or reduction.

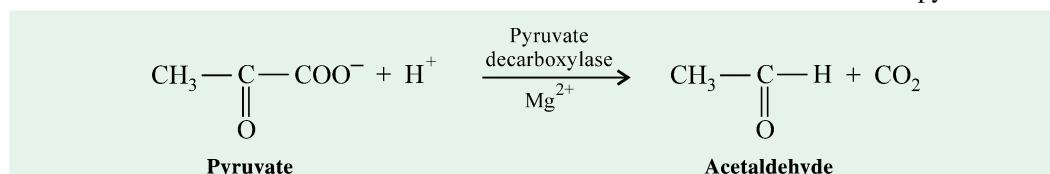In anaerobic glycolysis, however, there occurs no net change in the oxidation state of carbon. This becomes evident by comparing the empirical formula of glucose ($C_6H_{12}O_6$) with that of lactic acid ($C_3H_6O_3$). The ratio of C to H to O is the same (*i.e.*, 1 : 2 : 1) for both, showing that no net oxidation of carbon has occurred. Nevertheless, some of the energy of glucose is extracted by

anaerobic glycolysis, sufficient to give a net gain of 2 ATP per glucose utilized. This is equivalent to an estimated 15 kcal. Since some 56 kcal per mole are produced when glucose is degraded to lactate under standard conditions, the overall efficiency of glycolysis is $15/56 \times 100$ or approximately 25% – a rather high figure !
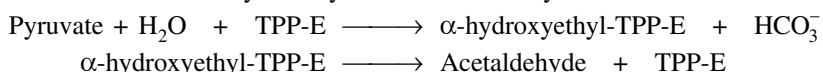
ALCOHOLIC FERMENTATION

In yeast and other microorganisms, the reactions of glycolysis up to pyruvate formation are identical to those described for anaerobic glycolysis and the difference occurs only in its terminal steps. In contrast to animals, which utilize lactate dehydrogenase reaction for the reoxidation of NADH to generate NAD^+, the yeast cells utilize two enzymatic reactions for the purpose, as lactate dehydrogenase is not found in them :
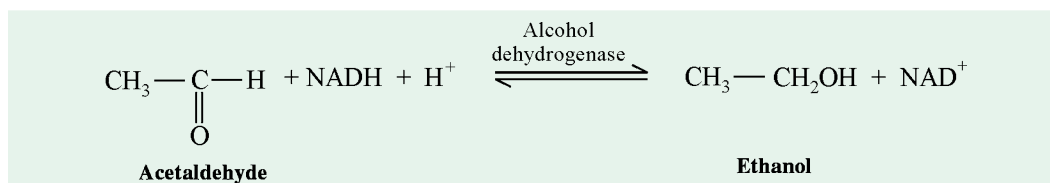
(*a*) In the first step, the pyruvate resulting from glucose breakdown is decarboxylated by the action of *pyruvate decarboxylase* (= *2, oxo-acid carboxylase*) to produce acetaldehyde and carbon dioxide. This is an irreversible reaction and does not involve the net oxidation of pyruvate.

$$CH_3 - \underset{\underset{O}{\|}}{C} - COO^- + H^+ \xrightarrow[\text{Mg}^{2+}]{\text{Pyruvate}\atop\text{decarboxylase}} CH_3 - \underset{\underset{O}{\|}}{C} - H + CO_2$$

Pyruvate   **Acetaldehyde**

Pyruvate decarboxylase (E.C. 4.1.1.1) requires the usual Mg^{2+} for activity although certain trivalent ions such as Al^{3+}, Fe^{3+} may also satisfy the need. It has a tightly bound coenzyme, thiamine pyrophosphate, TPP (= cocarboxylase). TPP functions as a transient carrier of acetaldehyde group. In fact, the carboxyl group of pyruvate is lost as CO_2 and the rest of the molecule (sometimes referred to as *active acetaldehyde*) is simultaneouly transferred to the position 2 of the thiazole ring of TPP, to yield its hydroxyethyl derivative. This is unstable since the hydroxyethyl group quickly dissociates from the coenzyme to yield free acetaldehyde.

$$\text{Pyruvate} + H_2O + \text{TPP-E} \longrightarrow \alpha\text{-hydroxyethyl-TPP-E} + HCO_3^-$$
$$\alpha\text{-hydroxyethyl-TPP-E} \longrightarrow \text{Acetaldehyde} + \text{TPP-E}$$

(*b*) In the second and final step, acetaldehyde is reduced to ethanol by NADH, derived from glyceraldehyde 3-phosphate dehydrogenase reaction, *i.e.*, Step 6 of Glycolysis, through the catalytic action of *alcohol dehydrogenase* (abbreviated as ADH). This is a reversible oxidation-reduction reaction.

$$CH_3 - \underset{\underset{O}{\|}}{C} - H + NADH + H^+ \xrightleftharpoons{\text{Alcohol}\atop\text{dehydrogenase}} CH_3 - CH_2OH + NAD^+$$

Acetaldehyde   **Ethanol**

The yeast **alcohol dehydrogenase** (MW = 151,000) is an NAD-dependent enzyme and contains a zinc ion at its active site.

The conversion of glucose into ethanol is called alcoholic fermentation. Thus, ethanol and CO_2, instead of lactate, are the end products of this process. Alcoholic fermentation is prevented by $NaHSO_3$ which combines with acetaldehyde to give a bisulfite addition compound that is not a substrate for alcohol dehydrogenase.

The net equation for alcoholic fermentation would then be :

$$\text{Glucose} + 2 \text{ Pi} + 2 \text{ ADP} \longrightarrow 2 \text{ Ethanol} + 2 CO_2 + 2 \text{ ATP} + 2 H_2O$$
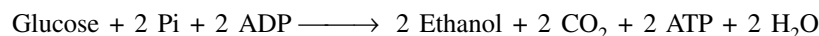
It may be pointed out that NAD^+ and NADH do not appear in this equation because NAD^+ generated during reduction of acetaldehyde is used in the reaction catalyzed by glyceraldehyde 3-

phosphate dehydrogenase, *i.e.*, Step 6 of Glycolysis. Thus, there is no net oxidation-reduction, as in anaerobic glycolysis. Another noteworthy point of alcoholic fermentation is that there is no net change in the ratio of hydrogen to carbon atoms when D-glucose (H/C ratio = 12/6 = 2) is fermented to 2 molecule each of ethanol and CO_2 (H/C ratio = 12/6 = 2). In fact, *in all anaerobic fermentations, the H/C ratio of the reactants and products remains the same*.

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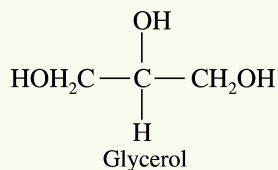
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PROBLEMS

1. Xylose has the same structure as that of glucose except that it has a hydrogen atom at C-6 in place of a hydroxymethyl group. The rate of ATP hydrolysis by hexokinase is markedly enhanced by the addition of xylose. Why ?

2. The intravenous infusion of fructose into healthy volunteers leads to a two- to fivefold increase in the level of lactate in the blood, a far greater increase than that observed after the infusion of the same amount of glucose.

   (*a*) Why is glycolysis more rapid after the infusion of fructose ?

   (*b*) Fructose has been used in place of glucose for intravenous feeding. Why is this use of fructose unwise ?

3. What are the likely consequences of a genetic disorder rendering fructose 1,6-bisphosphatase in liver less sensitive to regulation by fructose 2,6-bisphosphate ?

4. If cells synthesizing glucose from lactate are exposed to CO_2 labeled with ^{14}C, what will be the distribution of label in the newly synthesized glucose ?

5. In the conversion of glucose into two molecules of lactate, the NADH generated earlier in the pathway is oxidized to NAD^+. Why is it not to the cell's advantage to simply make more NAD^+ so that the regeneration would not be necessary ? After all, the cell would save much energy because it would no longer need to synthesize lactic acid dehydrogenase.

6. People with galactosemia display central nervous system abnormalities even if galactose is eliminated from the diet. The precise reason for it is not known. Suggest a plausible explanation.

7. Write a pathway leading from glucose to lactose in mammary gland, and write a balanced equation for the overall pathway.

8. How many high-energy phosphates are generated in (*a*) converting 1 mole of glucose to lactate ? (*b*) converting 2 moles of lactate to glucose ?

9. Write balanced equations for all of the reactions in the catabolism of D-glucose to two molecules of D-glyceraldehyde-3-phosphate (the preparatory phase of glycolysis). For each equation, write the standard free-energy change. Then write the overall or net equation for the preparatory phase of glycolysis, including the net standard free-energy change.

10. In working skeletal muscle under anaerobic conditions, glyceraldehyde-3-phosphate is converted into pyruvate (the payoff phase of glycolysis), and the pyruvate is reduced to lactate. Write balanced equations for all of the reactions in this process, with the standard free-energy change for each. Then write the overall or net equation for the payoff phase of glycolysis (with lactate as the end product), including the net standard free-energy change.

11. The concentration of glucose in human blood plasma is maintained at about 5 mM. The concentration of free glucose inside muscle cells is much lower. Why is the concentration so low in the cell ? What happens to the glucose upon entry into the cell ?

12. Glycerol (see below) obtained from the breakdown of fat is metabolized by being converted into dihydroxyacetone phosphate, an intermediate in glycolysis, in two enzyme-catalyzed reactions. Propose a reaction sequence for the metabolism of glycerol. On which known enzyme-catalyzed reactions is your proposal based ? Write the net equation for the conversion of glycerol to pyruvate based on your scheme.

$$\text{HOH}_2\text{C} - \overset{\overset{\displaystyle \text{OH}}{|}}{\underset{\underset{\displaystyle \text{H}}{|}}{\text{C}}} - \text{CH}_2\text{OH}$$

Glycerol

13. In muscle tissue, the rate of conversion of glycogen to glucose-6-phosphate is determined by the ratio of phosphorylase a (active) to phosphorylase b (less active). Determine what happens to the rate of glycogen breakdown if a muscle preparation containing glycogen phosphorylase is treated with (a) phosphorylase b kinase and ATP ; (b) phosphorylase a phosphatase ; (c) epinephrine.

14. The intracellular use of glucose and glycogen is tightly regulated at four points. In order to compare the regulation of glycolysis when oxygen is plentiful and when it is depleted, consider the utilization of glucose and glycogen by rabbit leg muscle in two physiological settings: a resting rabbit, whose leg-muscle ATP demands are low, and a rabbit who has just sighted its mortal enemy, the coyote, and dashes into its burrow at full speed. For each setting, determine the relative levels (high, intermediate, or low) of AMP, ATP, citrate, and acetyl-CoA and how these levels affect the flow of metabolites through glycolysis by regulating specific enzymes. In periods of stress, rabbit leg muscle produces much of its ATP by anaerobic glycolysis (lactate fermentation) and very little by oxidation of acetyl-CoA derived from fat breakdown.

15. Unlike the rabbit with its short dash, migratory birds require energy for extended periods of time. For example, ducks generally fly several thousand miles during their annual migration. The flight muscles of migratory birds have a high oxidative capacity and obtain the necessary ATP through the oxidation of acetyl-CoA (obtained from fats) *via* the citric acid cycle. Compare the regulation of muscle glycolysis during short-term intense activity, as in the fleeing rabbit, and during extended activity, as in the migrating duck. Why must the regulation in these two settings be different ?

16. If a cell is forced to metabolize glucose anaerobically, how much faster would glycolysis have to proceed to generate the same amount of ATP as it would get if it metabolized glucose aerobically ?