GLYCOLYSIS

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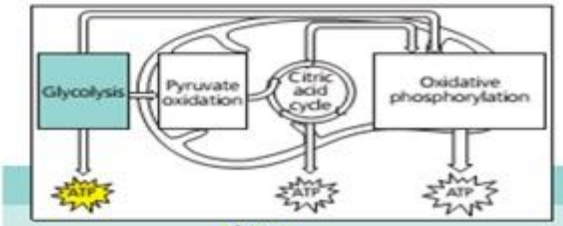
1. The word *glycolysis* means “sugar splitting,”

2. Glucose, a six-carbon sugar, is split into two three-carbon sugars. These smaller sugars are then oxidized and their remaining atoms rearranged to form two molecules of pyruvate *with the concomitant net production of two molecules of ATP*

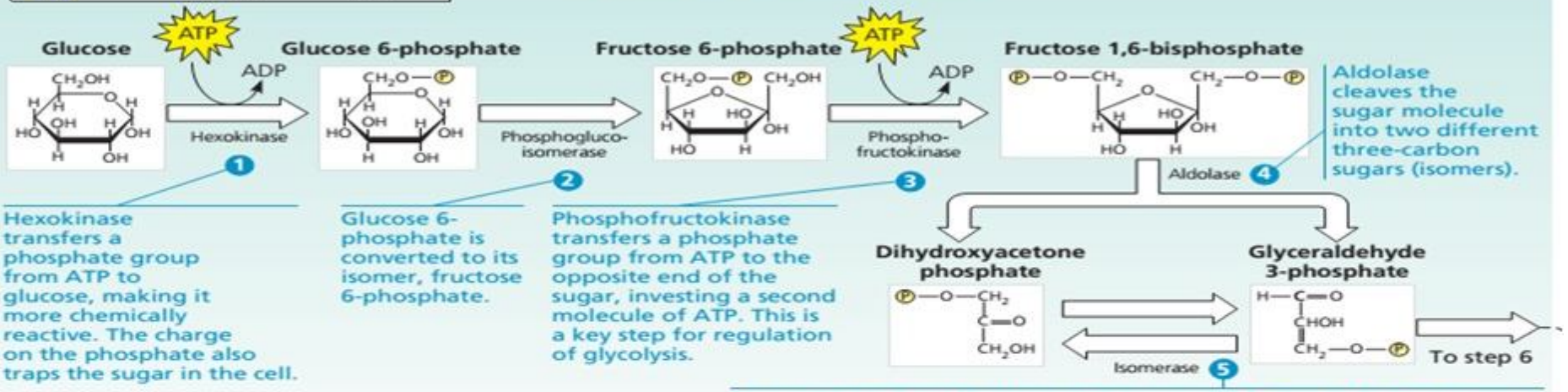
3. This process is anaerobic (i.e., it does not require O2) because it evolved before substantial amounts of oxygen accumulated in the atmosphere.



A closer look at glycolysis. The orientation diagram on the left relates glycolysis to the entire process of respiration. Note that glycolysis is a source of ATP and NADH.

WHAT IF? *What would happen if you removed the dihydroxyacetone phosphate generated in step 4 as fast as it was produced?*

Glycolysis: Energy Investment Phase



Hexokinase transfers a phosphate group from ATP to glucose, making it more chemically reactive. The charge on the phosphate also traps the sugar in the cell.

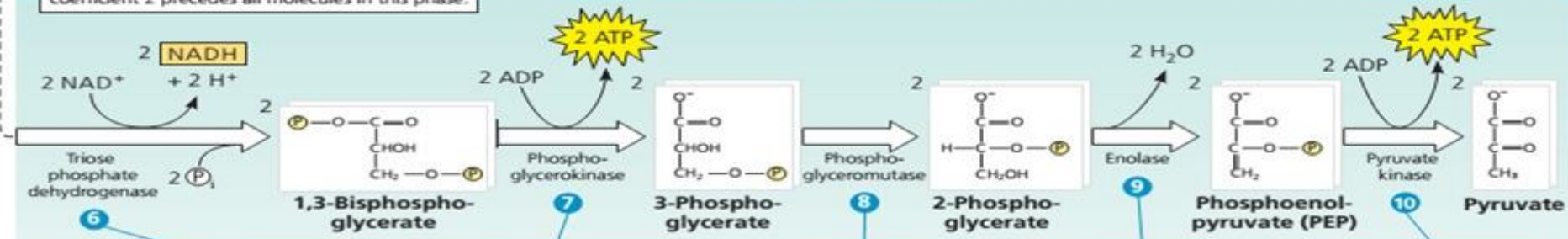
Glucose 6-phosphate is converted to its isomer, fructose 6-phosphate.

Phosphofructokinase transfers a phosphate group from ATP to the opposite end of the sugar, investing a second molecule of ATP. This is a key step for regulation of glycolysis.

Isomerase catalyzes the reversible conversion between the two isomers. This reaction never reaches equilibrium: Glyceraldehyde 3-phosphate is used as the substrate of the next reaction (step 6) as fast as it forms.

The energy payoff phase occurs after glucose is split into two three-carbon sugars. Thus, the coefficient 2 precedes all molecules in this phase.

Glycolysis: Energy Payoff Phase



This enzyme catalyzes two sequential reactions. First, the sugar is oxidized by the transfer of electrons to NAD^+, forming NADH. Second, the energy released from this exergonic redox reaction is used to attach a phosphate group to the oxidized substrate, making a product of very high potential energy.

The phosphate group added in the previous step is transferred to ADP (substrate-level phosphorylation) in an exergonic reaction. The carbonyl group of a sugar has been oxidized to the carboxyl group ($—COO^-$) of an organic acid (3-phosphoglycerate).

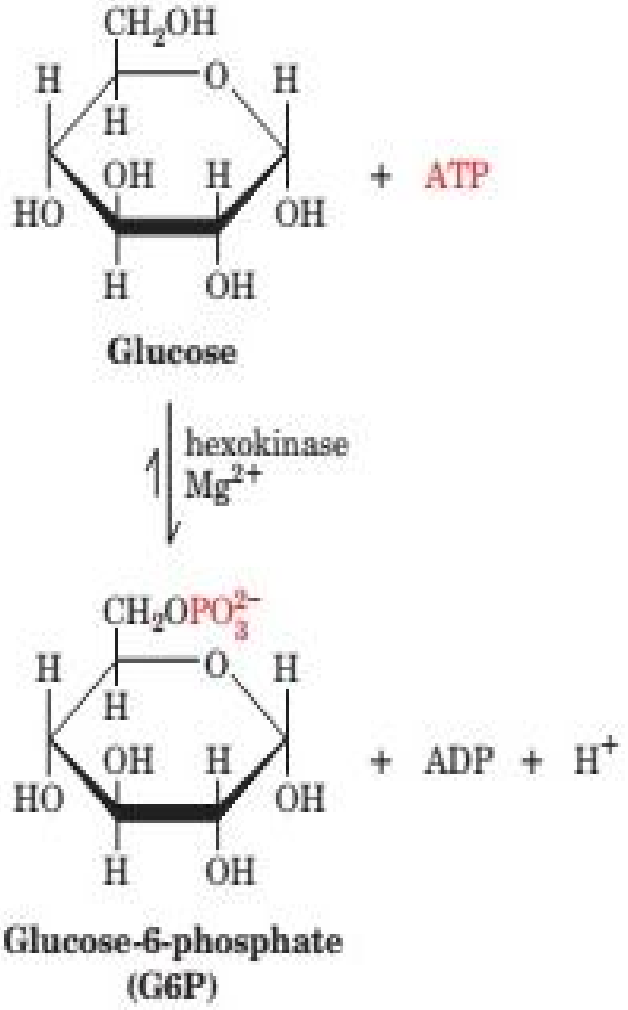
This enzyme relocates the remaining phosphate group.

Enolase causes a double bond to form in the substrate by extracting a water molecule, yielding phosphoenolpyruvate (PEP), a compound with a very high potential energy.

The phosphate group is transferred from PEP to ADP (a second example of substrate-level phosphorylation), forming pyruvate.

Hexokinase: First ATP Utilization

- Reaction 1 of glycolysis is the transfer of a phosphoryl group from ATP to **glucose** to form **glucose-6-phosphate (G6P)** in a reaction catalyzed by **hexokinase (HK)**
- HK is a relatively nonspecific enzyme contained in all cells that catalyzes the phosphorylation of hexoses such as D-glucose, D-mannose, and D-fructose.
- *Kinases* are enzymes that catalyze the transfer of a phosphoryl group from ATP to an acceptor. Hexokinase, then, catalyzes the transfer of a phosphoryl group from ATP to a variety of six-carbon sugars (hexoses), such as glucose and mannose. *Hexokinase, like adenylate kinase and all other kinases, requires Mg^{2+} (or another divalent metal ion such as Mn^{2+}) for activity.* The divalent metal ion forms a complex with ATP.



Kinetics and Mechanism of the Hexokinase Reaction

- Liver cells also contain **glucokinase,** which catalyzes the same reaction but which is primarily involved in the maintenance of blood glucose levels.
- The second substrate for HK, as with other kinases, is an Mg^{2+}–ATP complex. In fact, uncomplexed ATP is a potent competitive inhibitor of HK.
- Mg^{2+} is essential for kinase enzymatic activity (other divalent metal ions such as Mn^{2+} often satisfy the metal ion requirements of kinases *in vitro*, but Mg^{2+} is the normal physiological species).
- Hexokinase forms a ternary complex with glucose and Mg^{2+}–ATP before the reaction occurs.
- The Mg2+, by complexing with the phosphate oxygen atoms, is thought to shield their negative charges, making the phosphorus atom more accessible for the nucleophilic attack of the C6-OH group of glucose.

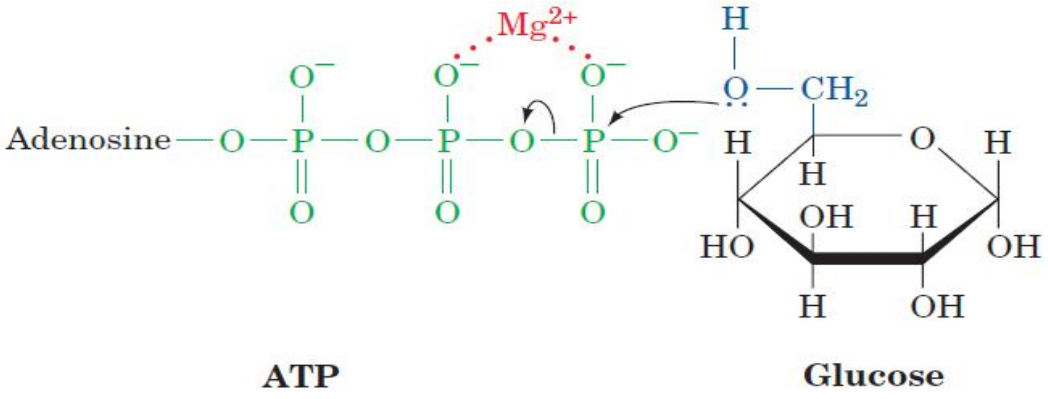


Figure 17-4 **The nucleophilic attack of the C6—OH group of glucose on the γ phosphate of an Mg^{2+}–ATP complex.** The position of the Mg^{2+} ion is shown as an example; its actual position(s) has not been conclusively established. In any case, the Mg^{2+} functions to shield the negatively charged groups of ATP and thereby facilitates the nucleophilic attack.

An important mechanistic question is why does HK catalyze the transfer of a phosphoryl group from ATP to glucose to yield G6P, but not to water to yield ADP + P_i (ATP hydrolysis)?

Water is certainly small enough to fit into the phosphoryl acceptor group's enzymatic binding site. Furthermore, phosphoryl transfer from ATP to water is more exergonic than it is to glucose particularly since $[H_2O] = 55.5M$ and [glucose] = 5 to 10 mM *in vivo*. Yet HK catalyzes phosphoryl transfer to glucose 40,000 times faster than it does to water.

- Comparison of the X-ray crystallographic structures of HK and the glucose–HK complex revealed that the binding of glucose induces a large conformational change in the HK.

- Hexokinase consists of two lobes, which move toward each other when glucose is bound. The two lobes that form its active site cleft swing together by up to 11.5 Å so as to engulf the glucose in a manner that suggests the closing of jaws.

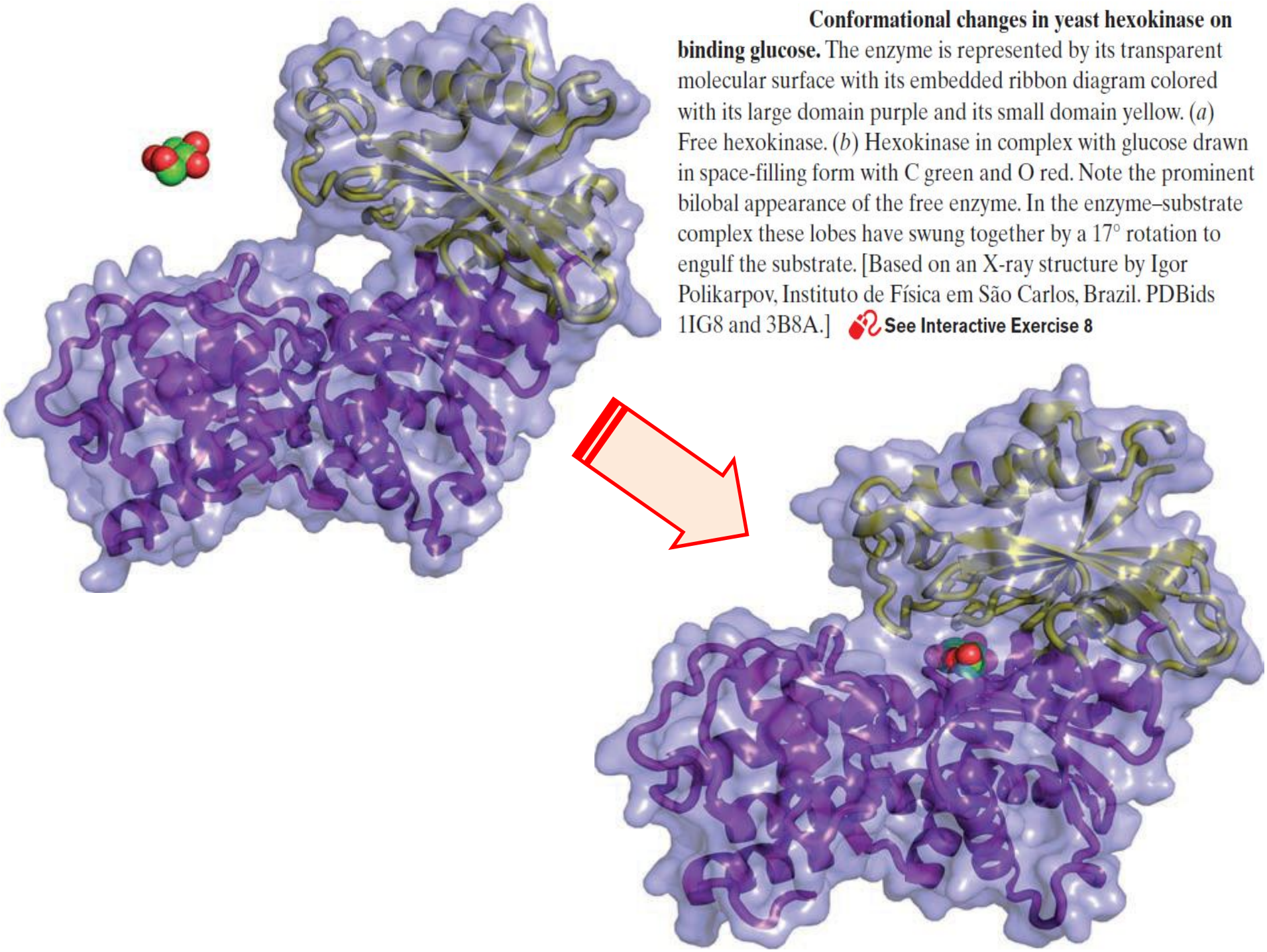
- *This movement places the ATP in close proximity to the group of glucose and excludes water from the active site.*

- If the *catalytic* and *reacting groups* were in the proper position for reaction while the enzyme was in the open position, *ATP hydrolysis would almost certainly be the dominant* reaction.

- On glucose binding, one lobe rotates 12 degrees with respect to the other, resulting in movements of the polypeptide backbone of as much as 8 Å.

- The cleft between the lobes closes, and the bound glucose becomes surrounded by protein, except for the hydroxyl group of carbon 6, which will accept the phosphoryl group from ATP.

- The closing of the cleft in hexokinase is a striking example of the role *of induced fit* in enzyme action.



Conformational changes in yeast hexokinase on binding glucose. The enzyme is represented by its transparent molecular surface with its embedded ribbon diagram colored with its large domain purple and its small domain yellow. (*a*) Free hexokinase. (*b*) Hexokinase in complex with glucose drawn in space-filling form with C green and O red. Note the prominent bilobal appearance of the free enzyme. In the enzyme–substrate complex these lobes have swung together by a 17° rotation to engulf the substrate. [Based on an X-ray structure by Igor Polikarpov, Instituto de Física em São Carlos, Brazil. PDBids 1IG8 and 3B8A.] **See Interactive Exercise 8**

The glucose-induced structural changes are significant in two respects.

- First, the environment around the glucose becomes more nonpolar, which favors reaction between the hydrophilic hydroxyl group of glucose and the terminal phosphoryl group of ATP.

- Second, the conformational changes enable the kinase to discriminate against H_2O as a substrate. The closing of the cleft keeps water molecules away from the active site. If hexokinase were rigid, a molecule of H_2O occupying the binding site for the $-CH_2OH$ of glucose could attack the γ phosphoryl group of ATP, forming ADP and Pi.

- In other words, a rigid kinase would likely also be an ATPase. It is interesting to note that other kinases taking part in glycolysis—phosphofructokinase, phosphoglycerate kinase, and pyruvate kinase—also contain clefts between lobes that close when substrate is bound, although the structures of these enzymes are different in other regards. *Substrate-induced cleft* closing is a general feature of kinases.

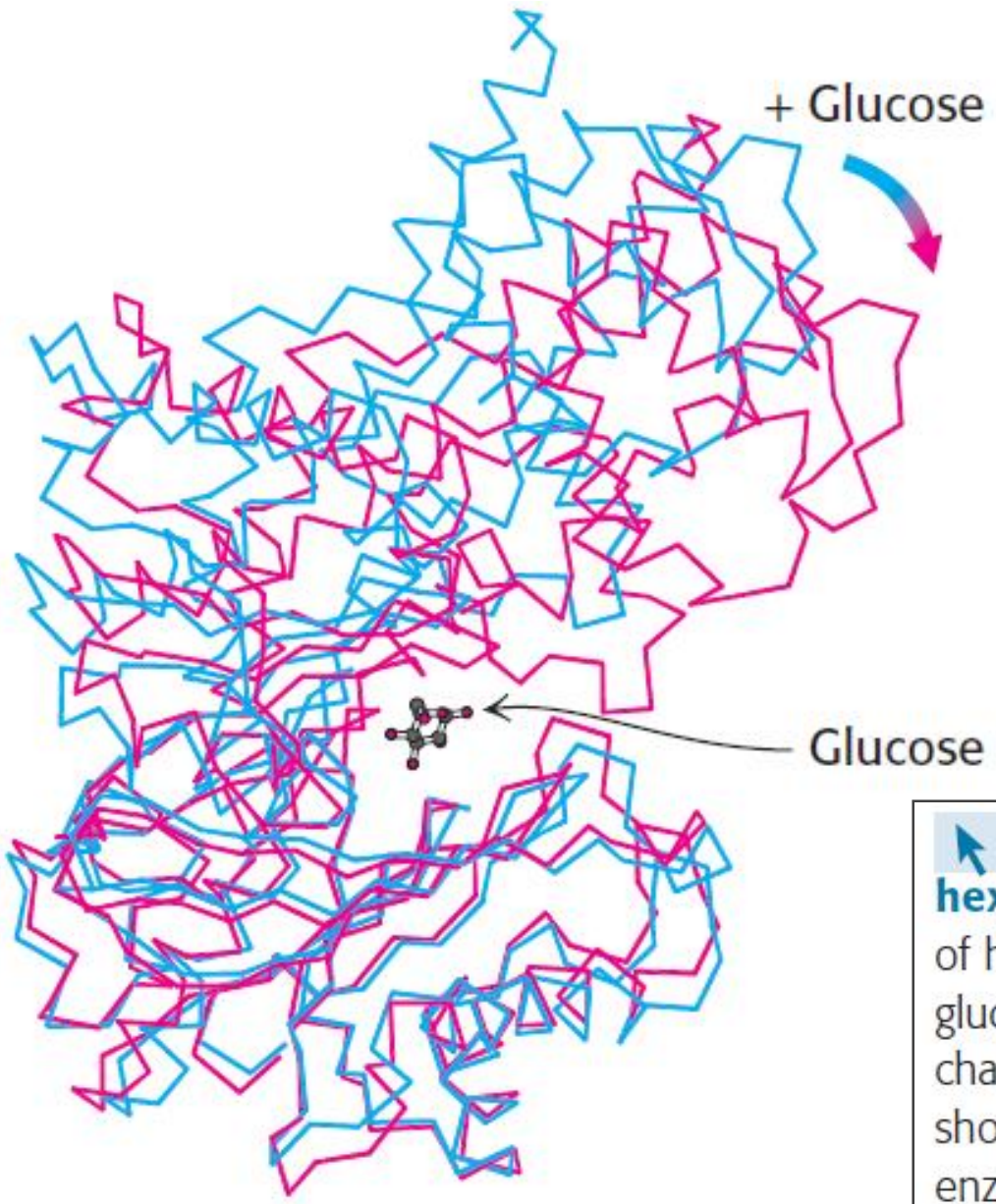


Figure 16.3 Induced fit in hexokinase. As shown in blue, the two lobes of hexokinase are separated in the absence of glucose. The conformation of hexokinase changes markedly on binding glucose, as shown in red. *Notice* that two lobes of the enzyme come together and surround the substrate, creating the necessary environment for catalysis. [Courtesy of Dr. Thomas Steitz.]

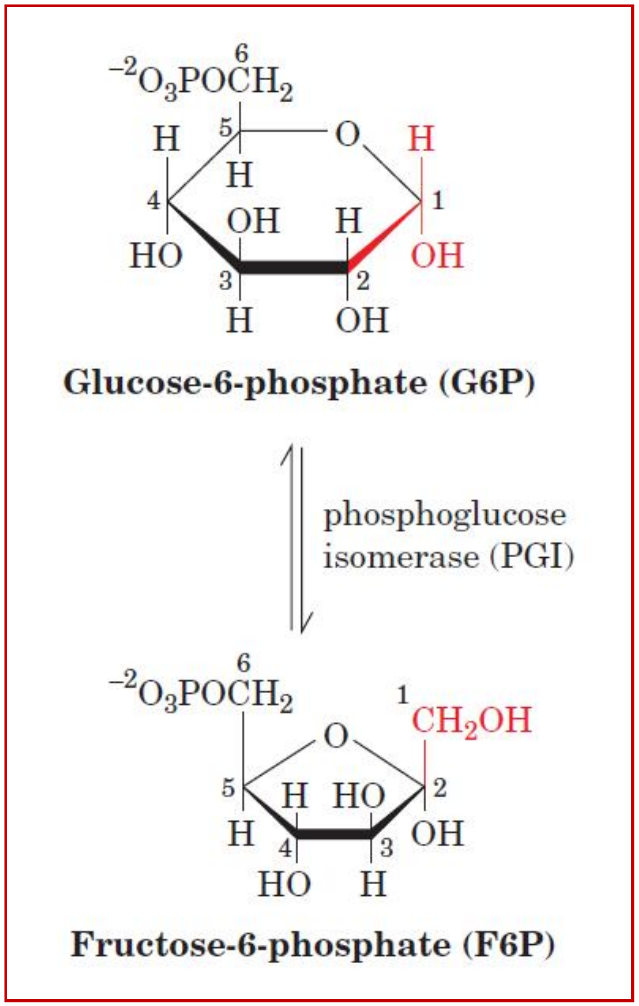
Phosphoglucose Isomerase

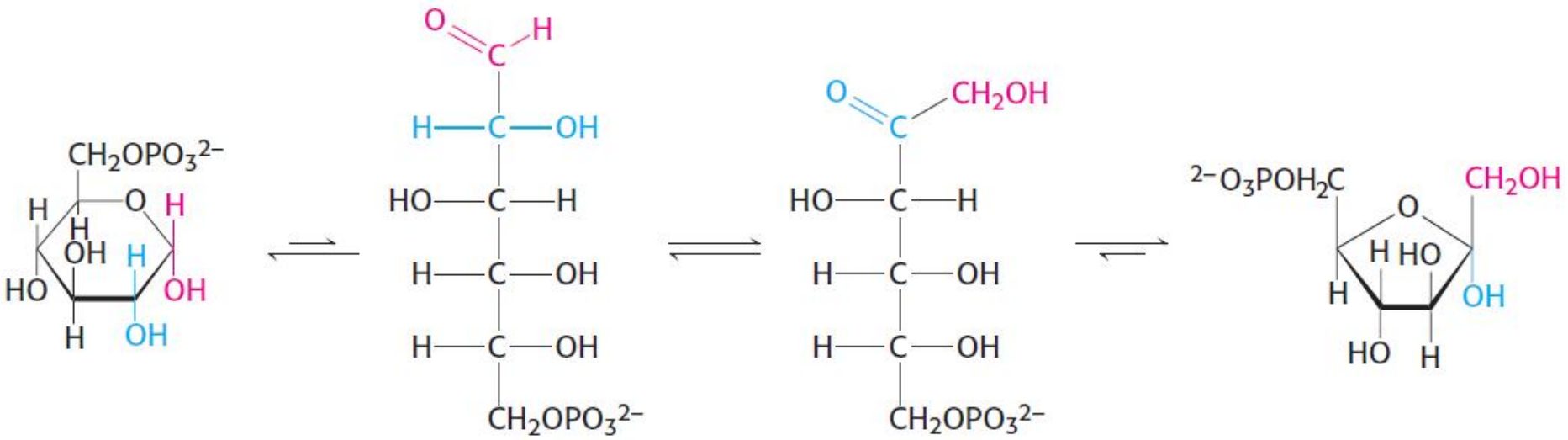
Reaction 2 of glycolysis is the conversion of G6P to **fructose-6-phosphate (F6P)** by **phosphoglucose isomerase (PGI; also called glucose-6-phosphate isomerase).** This is the isomerization of an aldose to a ketose:

Since G6P and F6P both exist predominantly in their cyclic forms, the reaction requires ring opening, followed by isomerization, and subsequent ring closure.

The amino acid side chain of the His and lys enzyme participate in the catalytic mechanism. X-ray structure of PGI reveals that Glu 216 and His 388 form a hydrogen bonded catalytic dyad, which facilitates the action of His 388 as

an acid–base catalyst.





Glucose 6-phosphate (G-6P)

Glucose 6-phosphate (open-chain form)

Fructose 6-phosphate (open-chain form)

Fructose 6-phosphate (F-6P)

A proposed reaction mechanism for the PGI reaction involves general acid–base catalysis by the enzyme:

Step 1 Substrate binding.

Step 2 An acid, presumably the Lys ε-amino group, catalyzes ring opening.

Step 3 A base, presumably the imidazole portion of the His–Glu dyad, abstracts the acidic proton from C2 to form a *cis-enediolate intermediate* (this proton is acidic because it is α to a carbonyl group).

Step 4 The proton is replaced on C1 in an overall proton transfer. Protons abstracted by bases are labile and exchange rapidly with solvent protons. Nevertheless, Irwin Rose confirmed this step by demonstrating that [2-^{3}H]G6P is occasionally converted to [1-^{3}H]F6P by intramolecular proton transfer before the ^{3}H has had a chance to exchange with the medium.

Step 5 Ring closure to form the product, which is subsequently released to yield free enzyme, thereby completing the catalytic cycle.

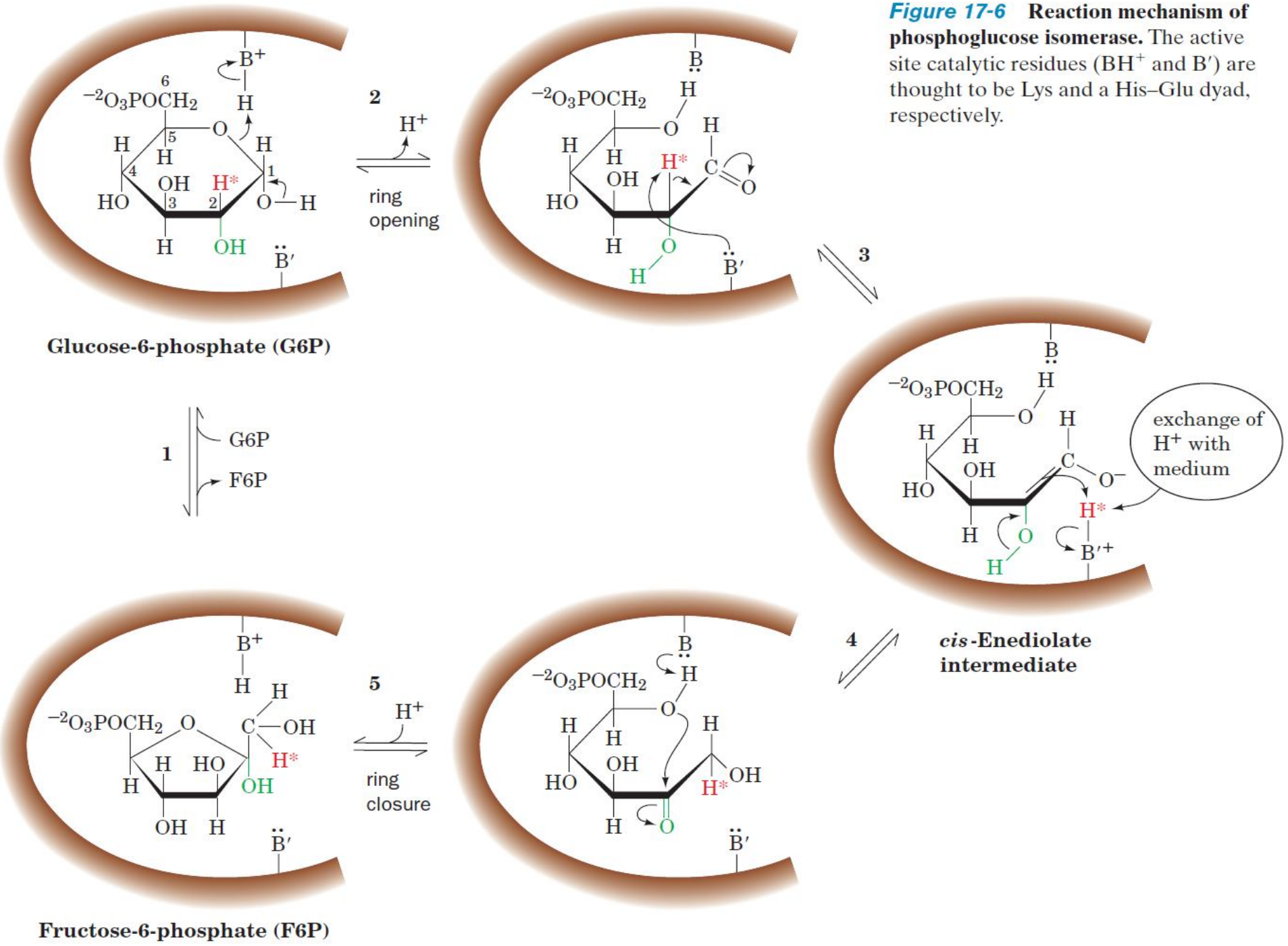
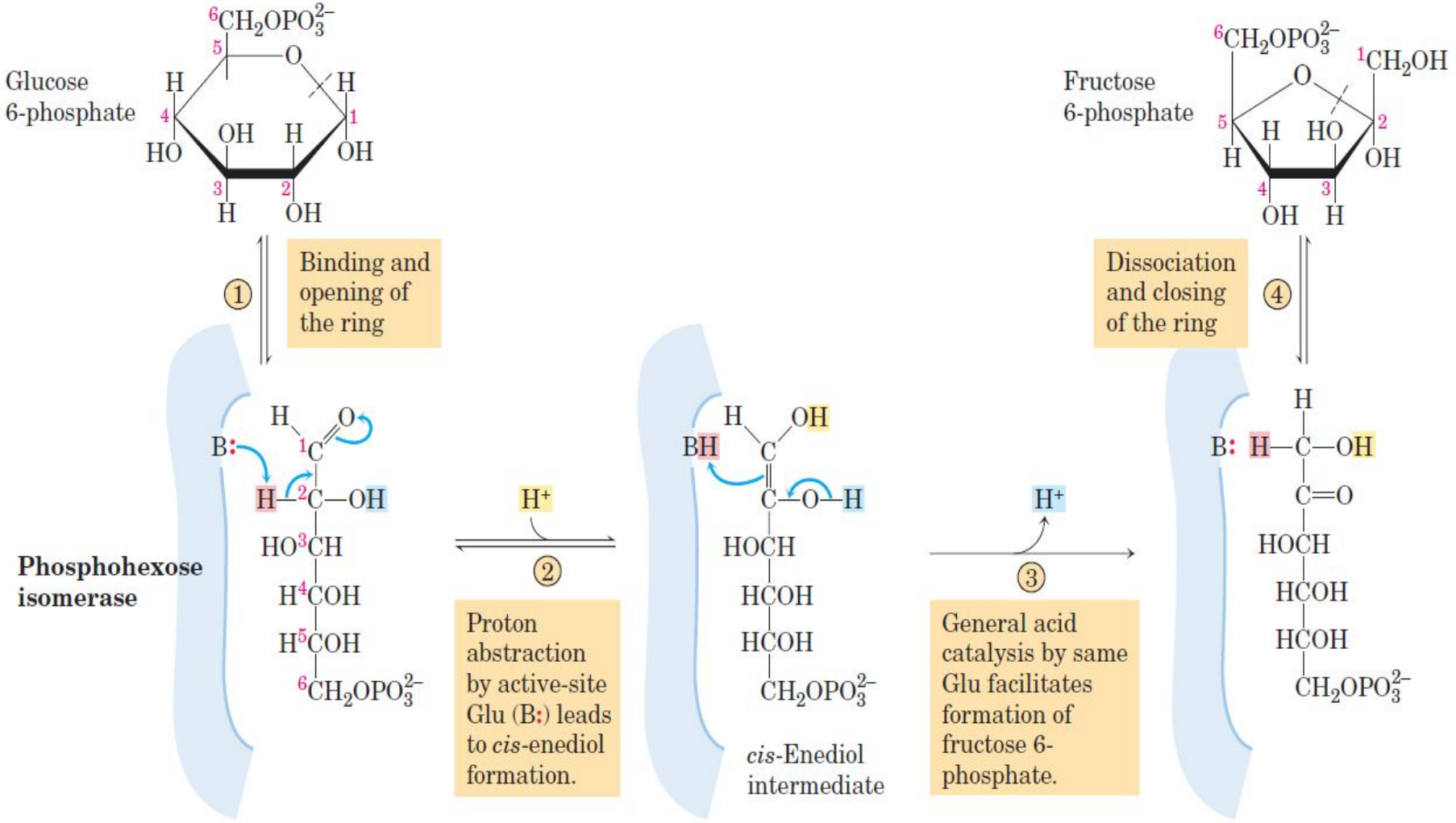


Figure 17-6 **Reaction mechanism of phosphoglucose isomerase.** The active site catalytic residues (BH^+ and B′) are thought to be Lys and a His–Glu dyad, respectively.



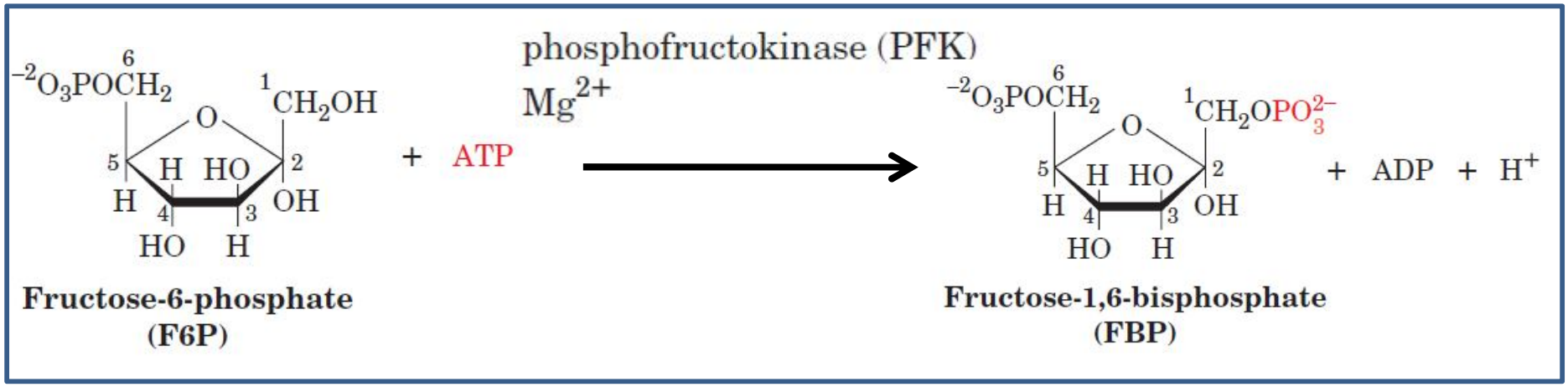
MECHANISM FIGURE 14–4 **The phosphohexose isomerase reaction.** The ring opening and closing reactions (steps ① and ④) are catalyzed by an active-site His residue, by mechanisms omitted here for simplicity. The proton (pink) initially at C-2 is made more easily abstractable by electron withdrawal by the adjacent carbonyl and nearby hydroxyl group. After its transfer from C-2 to the active-site Glu residue (a weak acid), the proton is freely exchanged with the surrounding solution; that is, the proton abstracted from C-2 in step ② is not necessarily the same one that is added to C-1 in step ③.

Phosphohexose Isomerase Mechanism

Phosphofructokinase: Second ATP Utilization

In Reaction 3 of glycolysis, **phosphofructokinase (PFK)** phosphorylates F6P to yield fructose-1,6-bisphosphate **[FBP or F1,6P;** previously known as **fructose-1,6- diphosphate (FDP)]:**

- This reaction is similar to the hexokinase reaction
- PFK catalyzes the nucleophilic attack by the C1-OH group of F6P on the electrophilic γ-phosphorus atom of the Mg^{2+}–ATP complex.

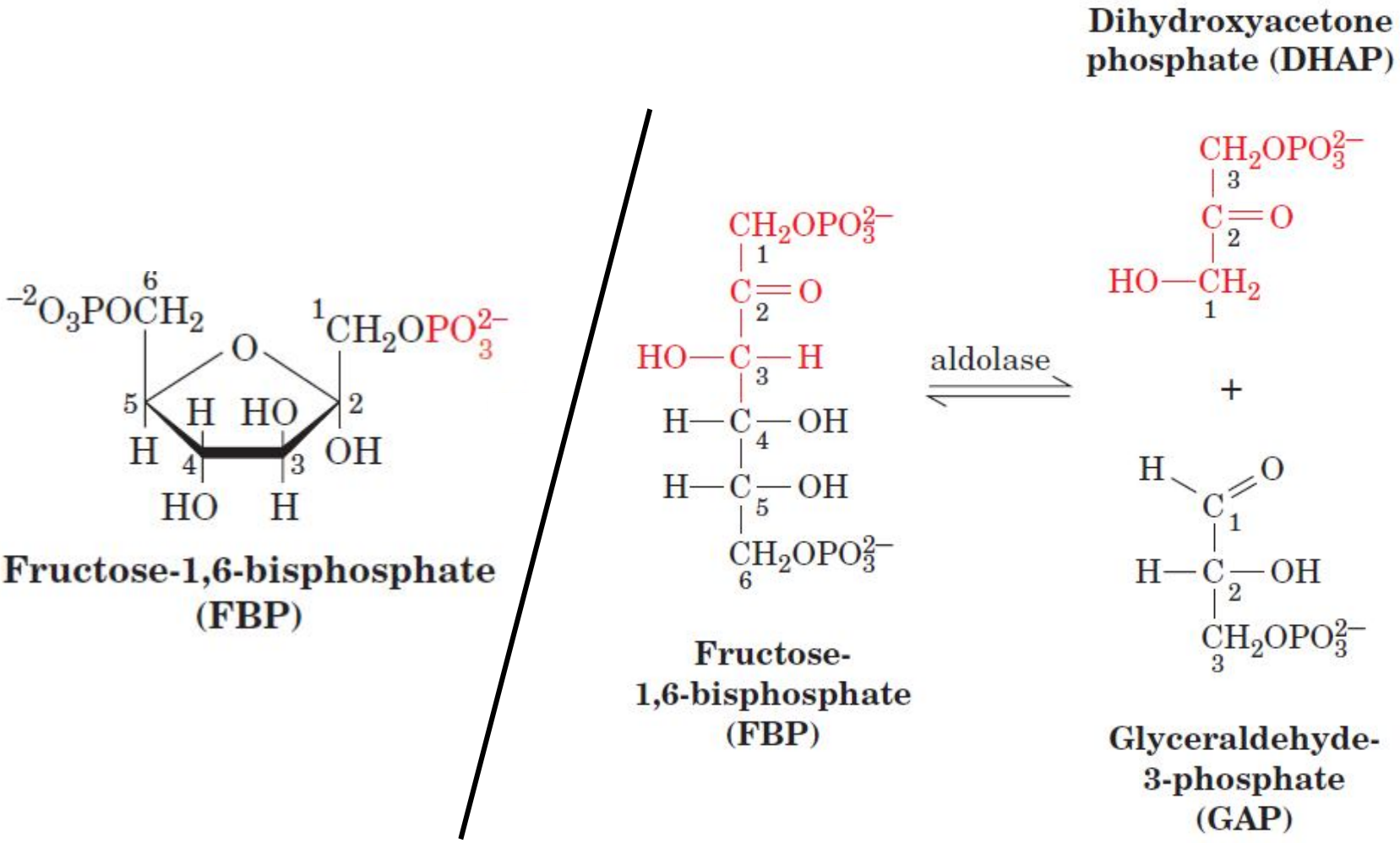


PFK plays a central role in the control of glycolysis because it catalyzes one of the pathway's rate-determining reactions.

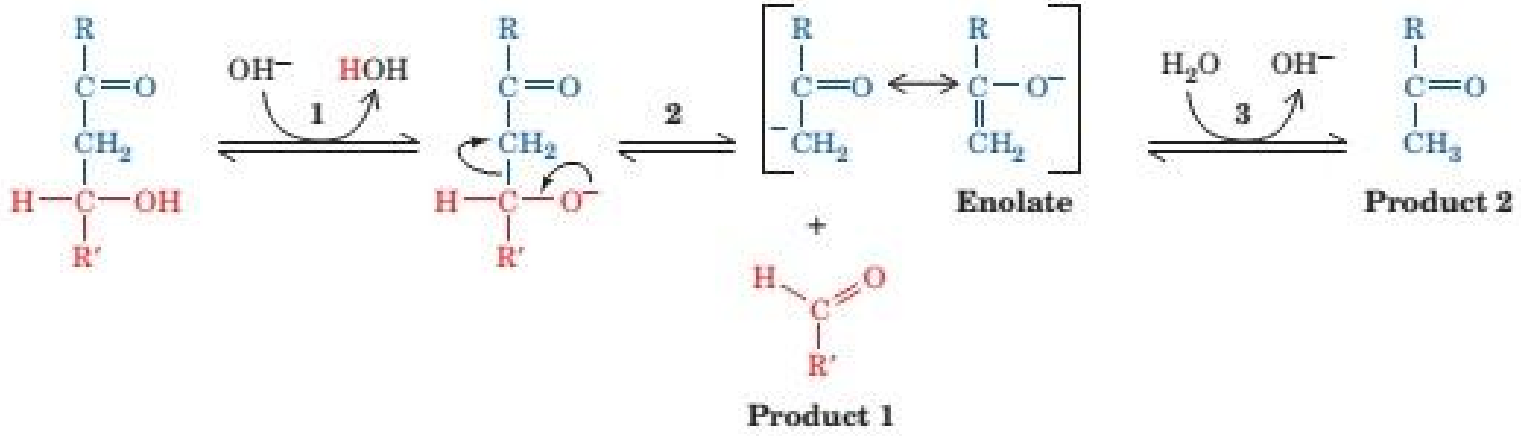
In many organisms the activity of PFK is enhanced allosterically by several substances, including AMP, and inhibited allosterically by several other substances, including ATP and citrate.

The six-carbon sugar is cleaved into two three-carbon fragments: 4th Reaction; Aldolase

Aldolase catalyzes Reaction 4 of glycolysis, the cleavage of **FBP** to form the two trioses **glyceraldehyde-3-phosphate (GAP)** and **dihydroxyacetone phosphate (DHAP):**



- This reaction is an **aldol cleavage (retro aldol condensation)** whose nonenzymatic base-catalyzed mechanism is shown in Fig.



- Note that aldol cleavage between C3 and C4 of FBP requires a carbonyl at C2 and a hydroxyl at C4. Hence, the “logic” of Reaction 2 in the glycolytic pathway, the isomerization of G6P to F6P, is clear.
- Aldol cleavage of G6P would have resulted in products of unequal carbon chain length, while aldol cleavage of FBP results in two interconvertible C3 compounds that can therefore enter a common degradative pathway.

There Are Two Mechanistic Classes of Aldolases

Aldol cleavage is catalyzed by stabilizing its enolate intermediate through increased electron delocalization.
There are two types of aldolases that are classified according to the chemistry they employ to stabilize the enolate.

In Class I aldolases, which occur in animals and plants, the reaction occurs as follows

Step 1 Substrate binding.

Step 2 Reaction of the FBP carbonyl group with the ε-amino group of the active site Lys 229 to form an iminium cation, that is, a protonated Schiff base.

Step 3 C3-O4 bond cleavage resulting in enamine formation and the release of GAP. The iminium ion is a better electron-withdrawing group than is the oxygen atom of the precursor carbonyl group. Thus, catalysis occurs because the enamine intermediate is more stable than the corresponding enolate intermediate of the base-catalyzed aldol cleavage reaction.

Step 4 Protonation of the enamine to an iminium cation.

Step 5 Hydrolysis of this iminium cation to release DHAP, with regeneration of the free enzyme.

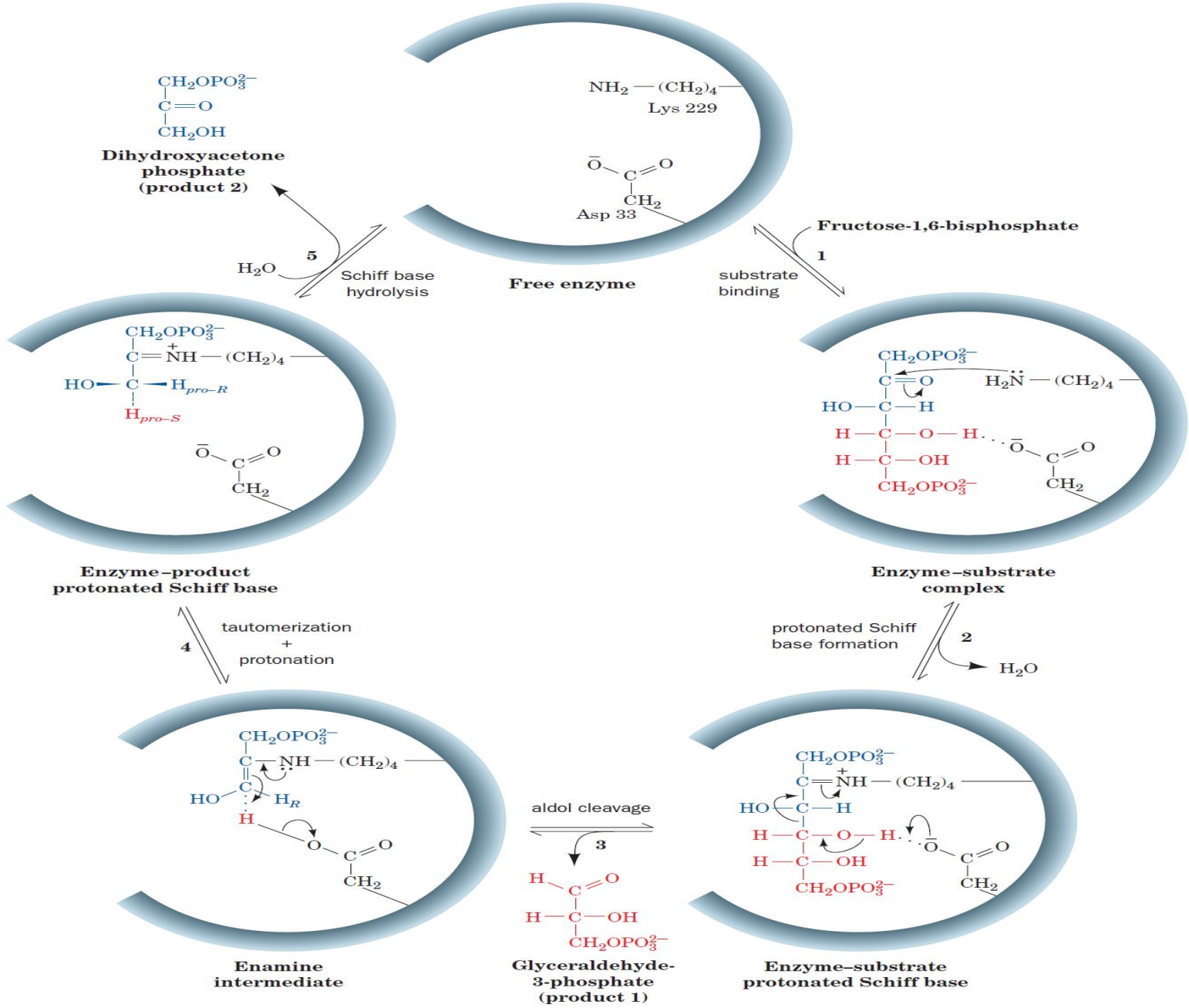
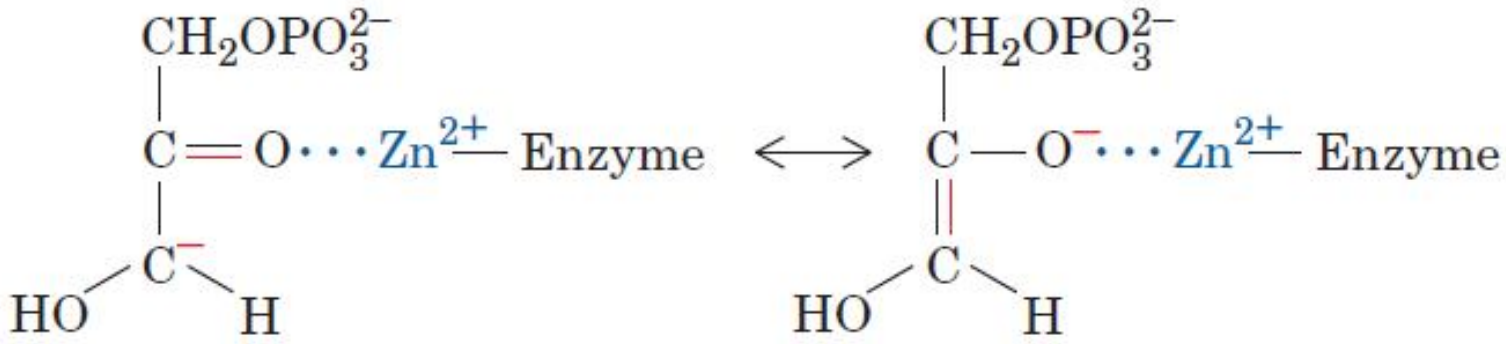


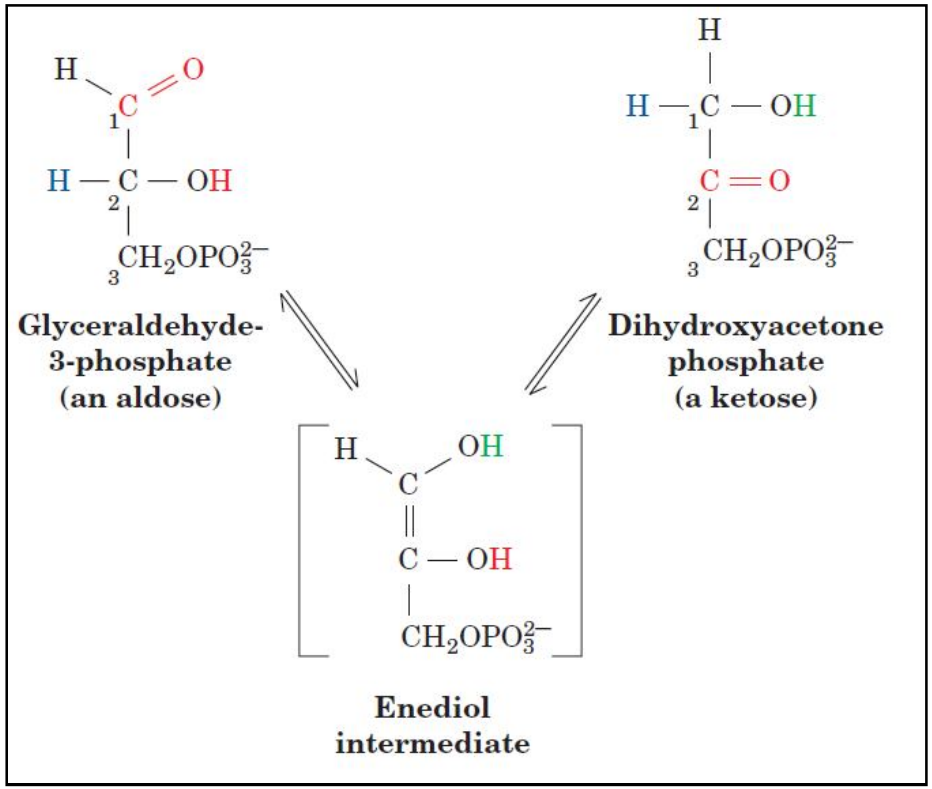
Figure 17-9 **Enzymatic mechanism of Class I aldolase.** The reaction involves **(1)** substrate binding; **(2)** Schiff base formation between the enzyme's active site Lys residue and FBP; **(3)** aldol cleavage to form an enamine intermediate of the enzyme and DHAP with release of GAP (shown with its *re* face up); **(4)** tautomerization and protonation to the iminium form of the Schiff base; and **(5)** hydrolysis of the Schiff base with release of DHAP. **See the Animated Figures.**

Class II aldolases, which occur in fungi, algae, and some bacteria, do not form a Schiff base with the substrate. Rather, a divalent cation, usually Zn^{2+} or Fe^{2+}, polarizes the carbonyl oxygen of the substrate to stabilize the enolate intermediate of the reaction



Interconversion of the Triose Phosphates: Triose Phosphate Isomerase

- Only one of the products of the aldol cleavage reaction, GAP, continues along the glycolytic pathway.
- However, DHAP and GAP are ketose–aldose isomers just as are F6P and G6P.
- Dihydroxyacetone phosphate, is rapidly and reversibly converted to glyceraldehyde 3-phosphate by the fifth enzyme of the glycolytic sequence, **triose phosphate isomerase (TPI**, sometimes abbreviated **TIM).** The reaction mechanism is similar to the reaction promoted by phosphohexose isomerase in step 2 of glycolysis.



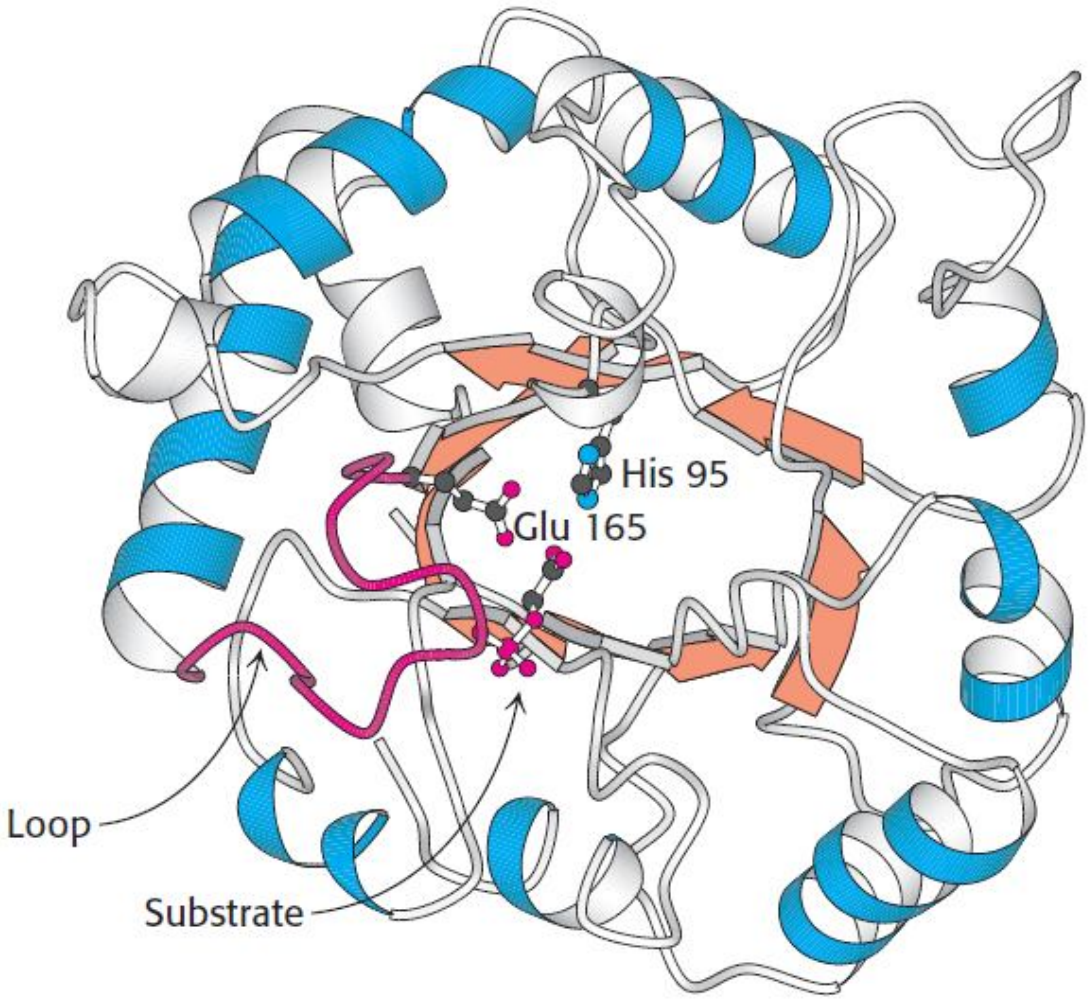


Figure 16.4 Structure of triose phosphate isomerase. This enzyme consists of a central core of eight parallel β strands (orange) surrounded by eight α helices (blue). This structural motif, called an αβ barrel, is also found in the glycolytic enzymes aldolase, enolase, and pyruvate kinase. *Notice* that 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. [Drawn from 2YPI.pdb.]

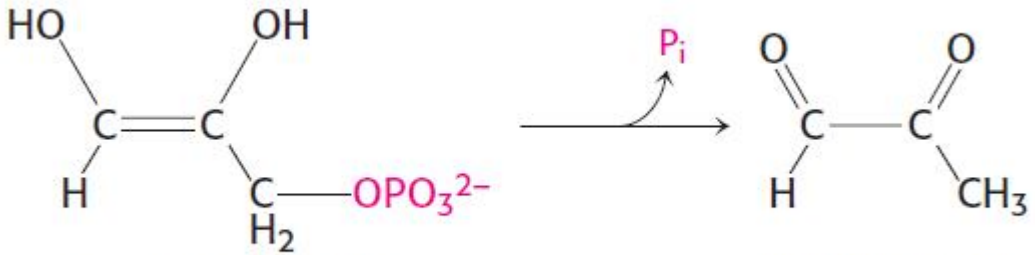
Mechanism: Triose phosphate isomerase salvages a three-carbon fragment

- ✓ TPI catalyzes the transfer of a hydrogen atom from carbon 1 to carbon 2, an intramolecular oxidation–reduction. This isomerization of a ketose into an aldose proceeds through an *enediol intermediate.*
- ✓ X-ray crystallographic and other studies showed that glutamate 165 plays the role of a general acid–base catalyst: it abstracts a proton (H1) from carbon 1 and then donates it to carbon 2. However, the carboxylate group of glutamate 165 by itself is not basic enough to pull a proton away from a carbon atom adjacent to a carbonyl group.
- ✓ Histidine 95 assists catalysis by donating a proton to stabilize the negative charge that develops on the C-2 carbonyl group.

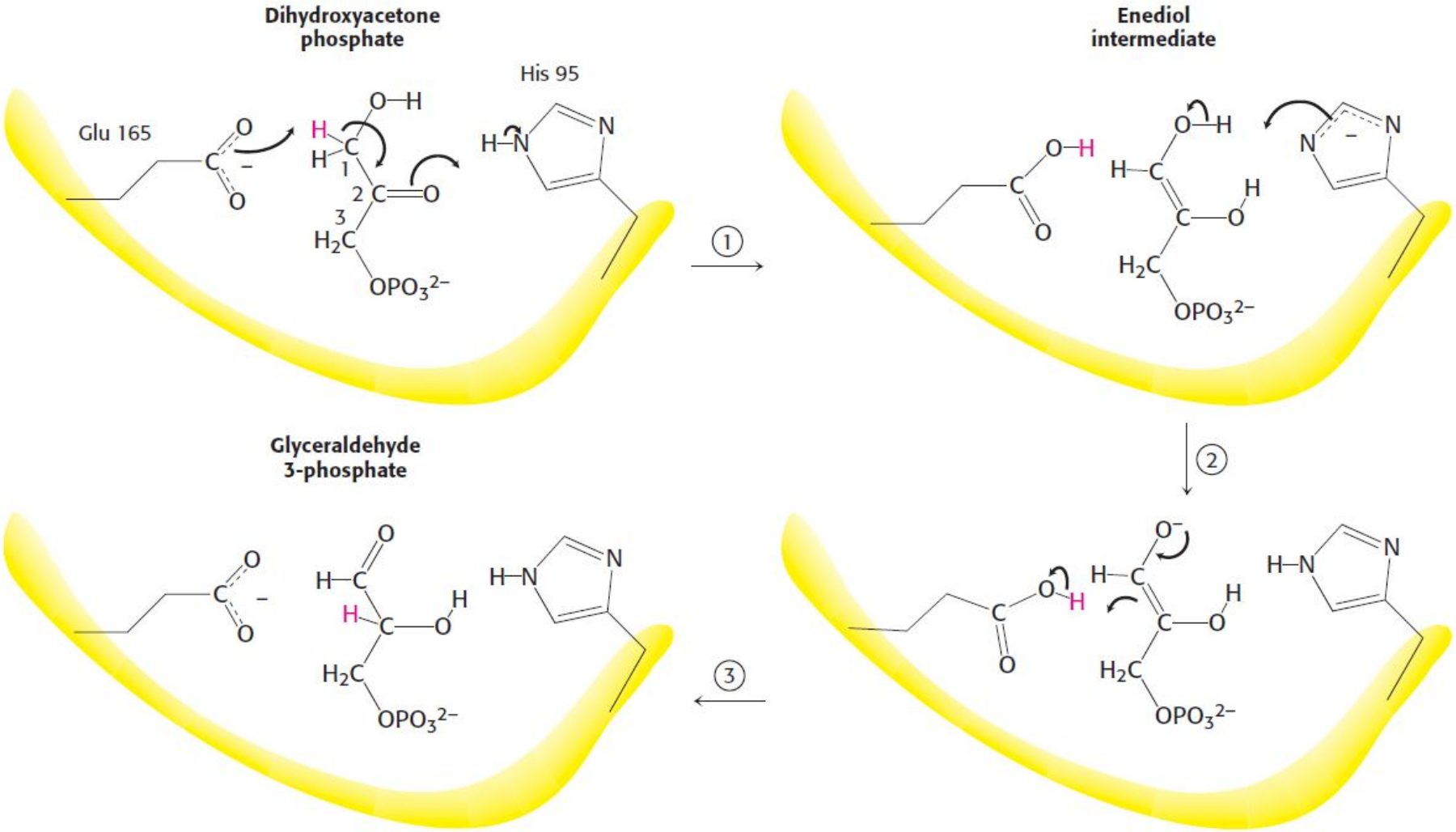
Two features of this enzyme are noteworthy.

1. First, TPI displays great catalytic prowess. It accelerates isomerization by a factor of 10^{10} compared with the rate obtained with a simple base catalyst such as acetate ion.
2. Second, TPI suppresses an undesired side reaction, the decomposition of the enediol intermediate into methyl glyoxal and orthophosphate.

- ✓ In solution, this physiologically useless reaction (the decomposition of the enediol intermediate into methyl glyoxal and orthophosphate) is 100 times as fast as isomerization.
- ✓ Moreover, methyl glyoxal is a highly reactive compound that can modify the structure and function of a variety of biomolecules, including proteins and DNA. The reaction of methyl glyoxal with a biomolecule is an example of deleterious reactions called advance **glycation end products**.
- ✓ Hence, TPI must prevent the enediol from leaving the enzyme. This labile intermediate is trapped in the active site by the movement of a loop of 10 residues. This loop serves as a lid on the active site, shutting it when the enediol is present and reopening it when isomerization is completed.
- ✓ *We see here a striking example of one means of preventing an undesirable alternative reaction: the active site is kept closed until the desirable reaction takes place.*



Enediol intermediate **Methyl glyoxal**



Catalytic mechanism of triose phosphate isomerase.

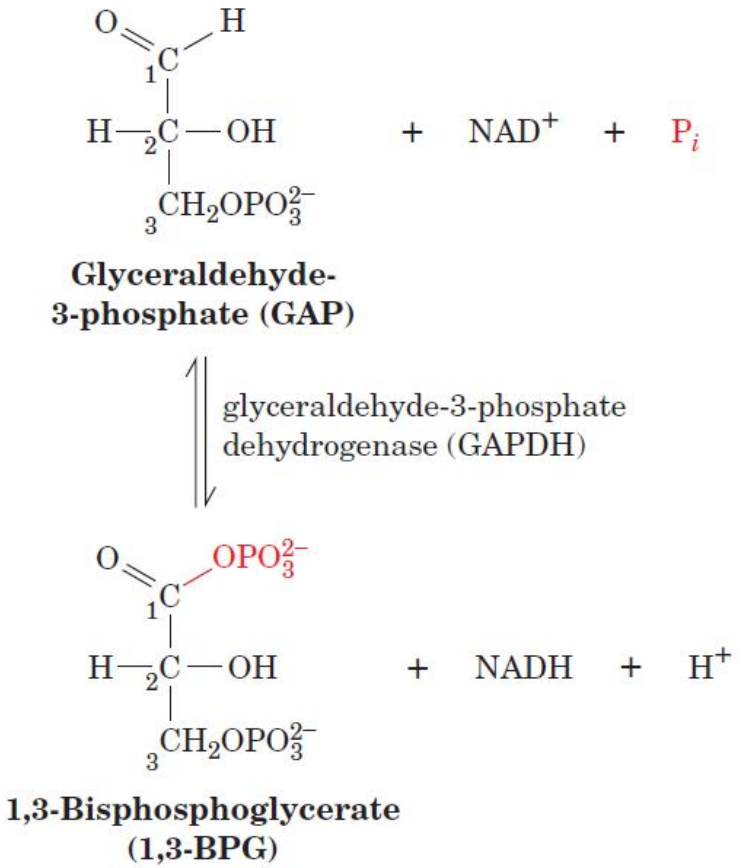
(1) Glutamate 165 acts as a general base by abstracting a proton (H+) from carbon 1. Histidine 95, acting as a general acid, donates a proton to the oxygen atom bonded to carbon 2, forming the enediol intermediate. (2) Glutamic acid, now acting as a general acid, donates a proton to C-2 while histidine removes a proton from the OH group of C-l. (3) The product is formed, and glutamate and histidine are returned to their ionized and neutral forms, respectively.

The Payoff Phase of Glycolysis Yields ATP and NADH

Glyceraldehyde-3-Phosphate Dehydrogenase: First "High-Energy" Intermediate Formation

Reaction 6 of glycolysis involves the oxidation and phosphorylation of GAP by NAD and P*i as catalyzed by* **glyceraldehyde-3-phosphate dehydrogenase (GAPDH)**.

In this reaction, aldehyde oxidation, an exergonic reaction, drives the synthesis of the acyl phosphate ***1,3-bisphosphoglycerate (1,3-BPG; previously called 1,3- diphosphoglycerate).***



Step 1 GAP binds to the enzyme.

Step 2 The essential sulfhydryl group, acting as a nucleophile, attacks the aldehyde to form a **thiohemiacetal.**

Step 3 The thiohemiacetal undergoes oxidation to an acyl thioester by direct transfer of a hydride to NAD^+. This intermediate, which has been isolated, has a high grouptransfer potential. *The energy of aldehyde oxidation has not been dissipated but has been conserved through the synthesis of the thioester and the reduction of* NAD^+ *to NADH.*

Step 4 Another molecule of NAD^+ replaces NADH.

Step 5 The thioester intermediate undergoes nucleophilic attack by P*i to regenerate free enzyme and form* 1,3-BPG. This "high-energy" mixed anhydride generates ATP from ADP in the next reaction of glycolysis.

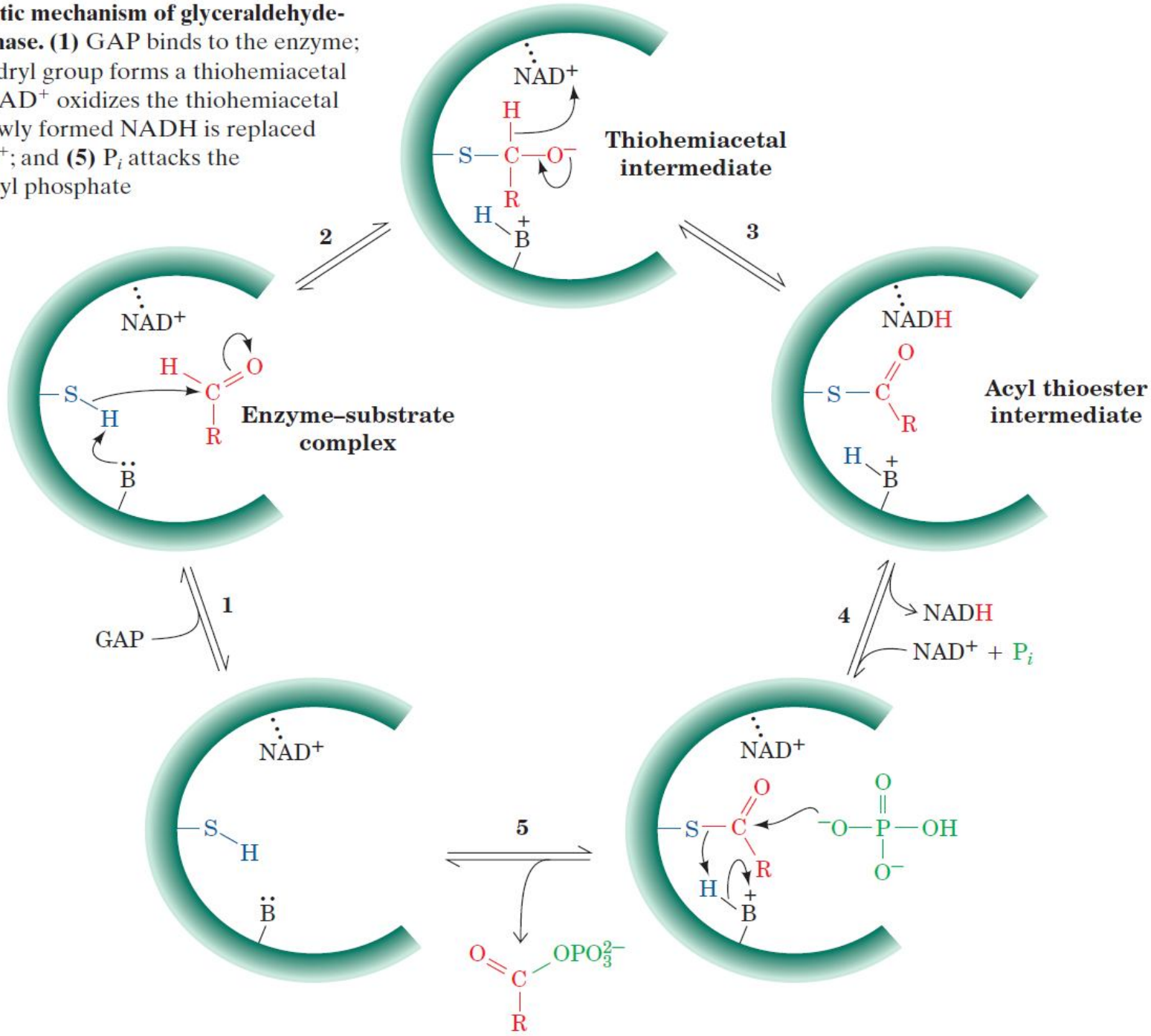


Figure 17-14 **Enzymatic mechanism of glyceraldehyde-3-phosphate dehydrogenase. (1)** GAP binds to the enzyme; **(2)** the active site sulfhydryl group forms a thiohemiacetal with the substrate; **(3)** NAD^+ oxidizes the thiohemiacetal to a thioester; **(4)** the newly formed NADH is replaced on the enzyme by NAD^+; and **(5)** P_i attacks the thioester, forming the acyl phosphate product, 1,3-BPG, and regenerating the active enzyme.

See the Animated Figures

Mechanism: Phosphorylation is coupled to the oxidation of glyceraldehyde 3-phosphate by a thioester intermediate

The active site of glyceraldehyde 3-phosphate dehydrogenase includes a reactive cysteine residue, as well as NAD^+ and a crucial histidine (Figure 16.7). Let us consider in detail how these components cooperate in the reaction mechanism (Figure 16.8). In step 1, the aldehyde substrate reacts with the sulfhydryl group of cysteine 149 on the enzyme to form a hemithioacetal. Step 2 is the *transfer of a hydride ion to a molecule of* NAD^+ *that is tightly bound to the enzyme and is adjacent to the cysteine residue.* This reaction is favored by the deprotonation of the hemithioacetal by histidine 176. The products of this reaction are the reduced coenzyme NADH and a thioester intermediate. *This thioester intermediate has a free energy close to that of the reactants* (see Figure 16.6). In step 3, the NADH formed from the aldehyde oxidation leaves the enzyme and is replaced by a second molecule of NAD^+. This step is important because the positive charge on NAD^+ polarizes the thioester intermediate to facilitate the attack by orthophosphate. In step 4, orthophosphate attacks the thioester to form 1,3-BPG and free the cysteine residue. This example illustrates the essence of energy transformations and of metabolism itself: energy released by carbon oxidation is converted into high phosphoryl-transfer potential.

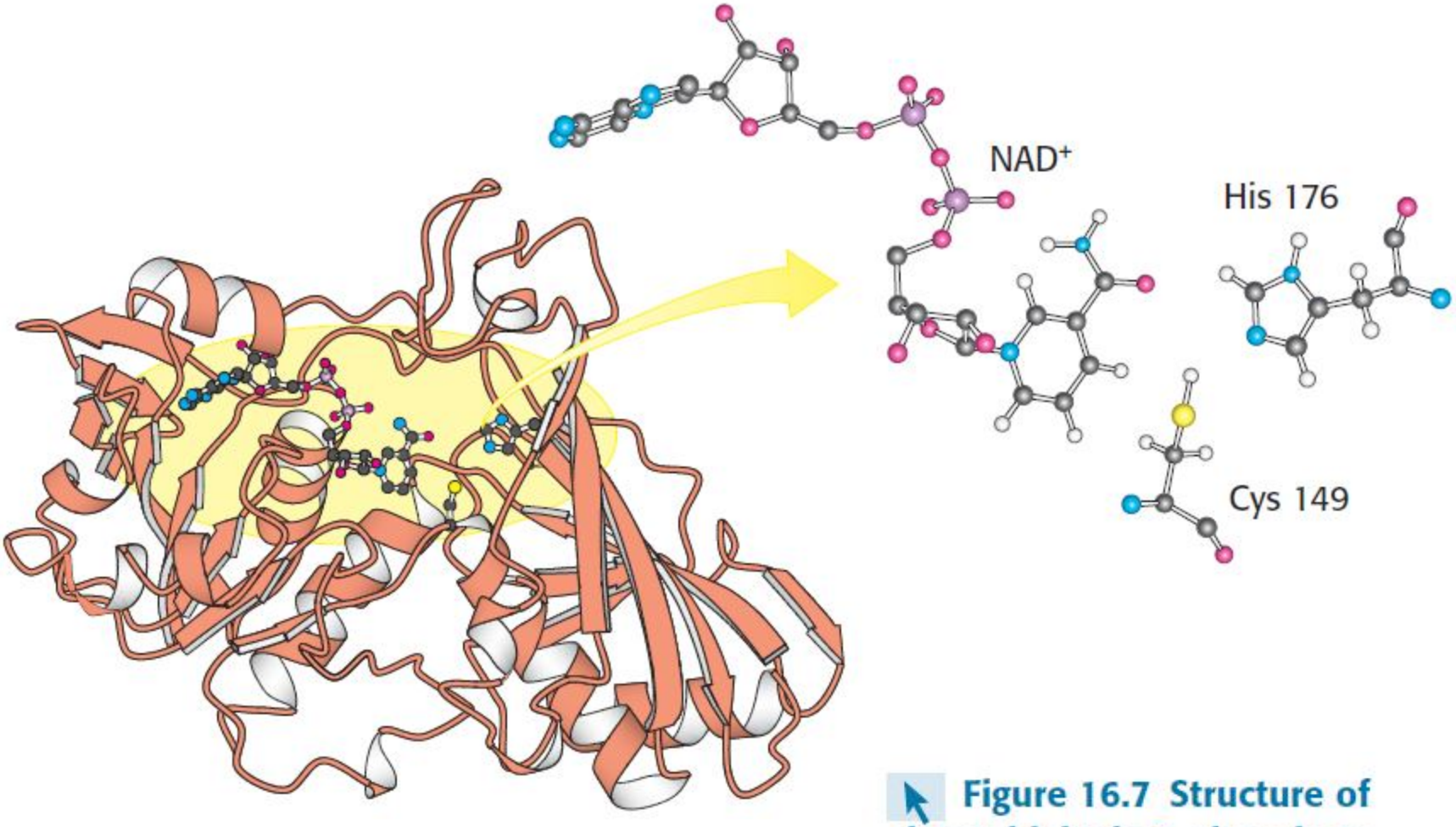


Figure 16.7 Structure of glyceraldehyde 3-phosphate dehydrogenase. *Notice* that the active site includes a cysteine residue and a histidine residue adjacent to a bound NAD^+ molecule. The sulfur atom of cysteine will link with the substrate to form a transitory thioester intermediate. [Drawn from 1GAD.pdb.]

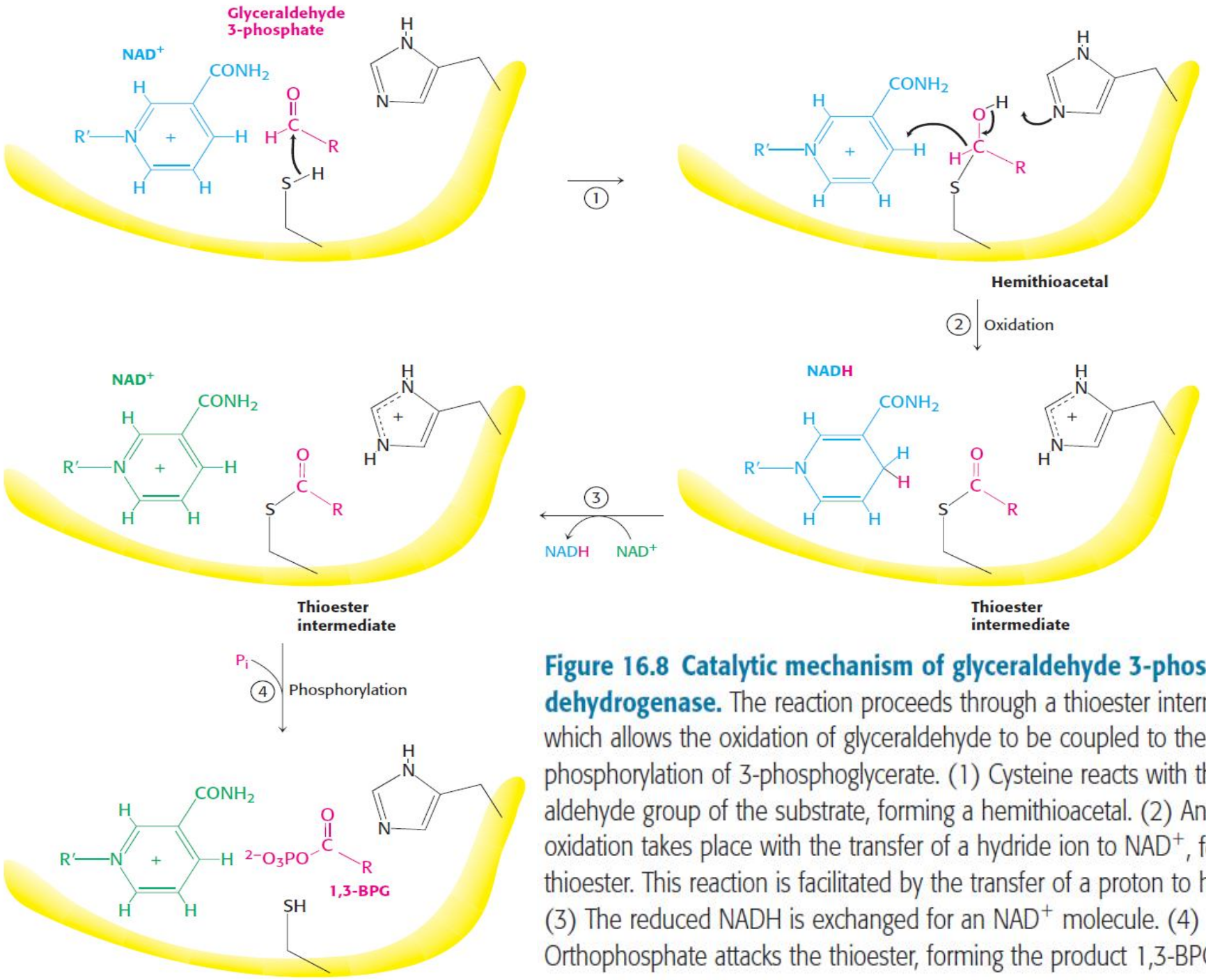
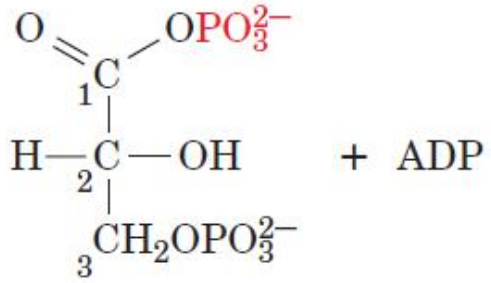


Figure 16.8 Catalytic mechanism of glyceraldehyde 3-phosphate dehydrogenase. The reaction proceeds through a thioester intermediate, which allows the oxidation of glyceraldehyde to be coupled to the phosphorylation of 3-phosphoglycerate. (1) Cysteine reacts with the aldehyde group of the substrate, forming a hemithioacetal. (2) An oxidation takes place with the transfer of a hydride ion to NAD^+, forming a thioester. This reaction is facilitated by the transfer of a proton to histidine. (3) The reduced NADH is exchanged for an NAD^+ molecule. (4) Orthophosphate attacks the thioester, forming the product 1,3-BPG.

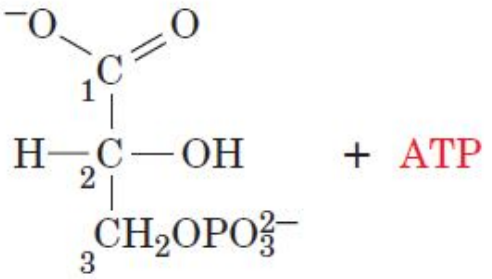
Phosphoglycerate Kinase: First ATP Generation

Reaction 7 of the glycolytic pathway results in the first formation of ATP together with **3-phosphoglycerate (3PG) in** a reaction catalyzed by **phosphoglycerate kinase (PGK):**



1,3-Bisphosphoglycerate (1,3-BPG)

Mg^{2+} phosphoglycerate kinase (PGK)



3-Phosphoglycerate (3PG)

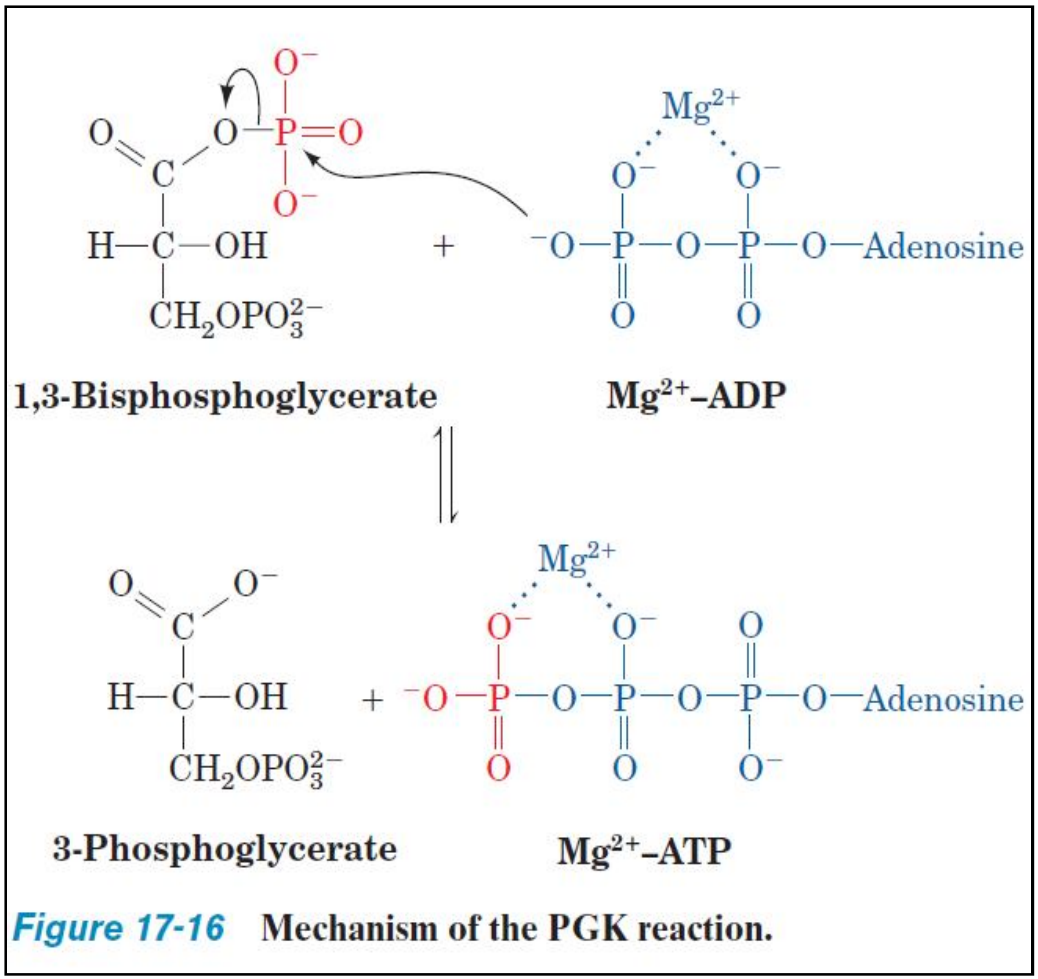


Figure 17-16 **Mechanism of the PGK reaction.**

- 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

$$\text{GAP} + \text{P}_i + \text{NAD}^+ \longrightarrow \text{1,3-BPG} + \text{NADH} \qquad \Delta G^{\circ\prime} = +6.7\ \text{kJ} \cdot \text{mol}^{-1}$$

$$\text{1,3-BPG} + \text{ADP} \longrightarrow \text{3PG} + \text{ATP} \qquad \Delta G^{\circ\prime} = -18.8\ \text{kJ} \cdot \text{mol}^{-1}$$

$$\text{GAP} + \text{P}_i + \text{NAD}^+ + \text{ADP} \longrightarrow \text{3PG} + \text{NADH} + \text{ATP} \qquad \Delta G^{\circ\prime} = -12.1\ \text{kJ} \cdot \text{mol}^{-1}$$

- Thus the overall reaction is exergonic. the actual free-energy change, Δ*G*, is determined by the standard free-energy change, Δ*G*, and the mass-action ratio, *Q*, which is the ratio [products]/[reactants]

$$\Delta G = \Delta G'^{\circ} + RT \ln Q$$

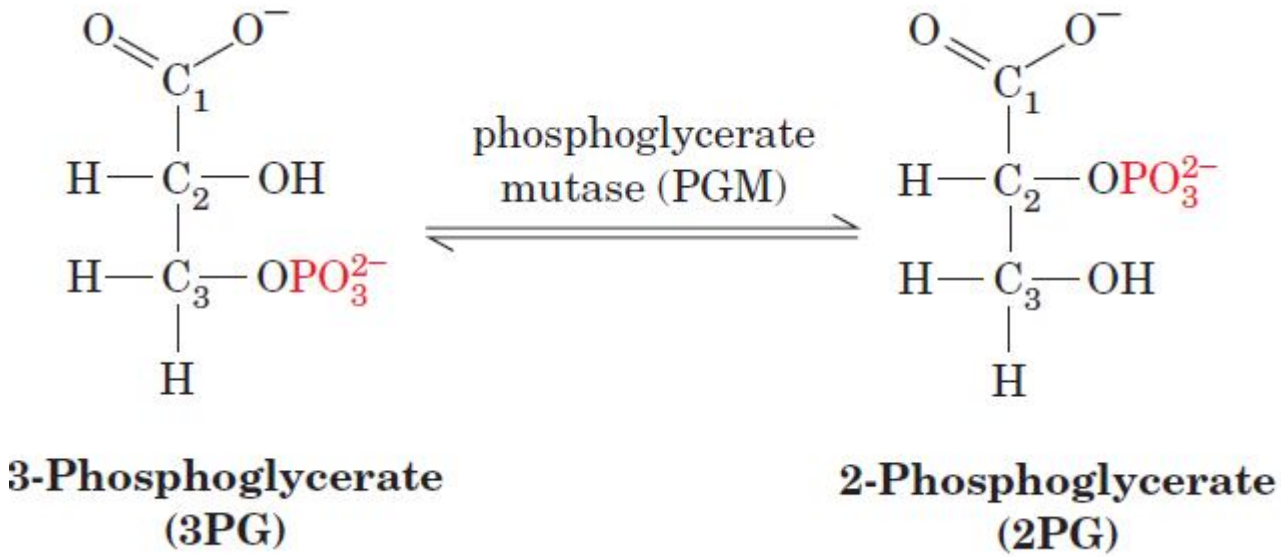
$$= \Delta G'^{\circ} + RT \ln \frac{[\text{1,3-bisphosphoglycerate}][\text{NADH}]}{[\text{glyceraldehyde 3-phosphate}][\text{P}_i][\text{NAD}^+]}$$

- **Notice that** [H+] is not included in *Q.* In biochemical calculations, [H+] is assumed to be a constant (10^{-7} M), and this constant is included in the definition of ΔG^{o}

Conversion of 3-Phosphoglycerate to 2- Phosphoglycerate Phosphoglycerate Mutase

In Reaction 8 of glycolysis, 3PG is converted to **2-phosphoglycerate (2PG)** by **phosphoglycerate mutase (PGM):**

A **mutase** catalyzes the transfer of a functional group from one position to another on a molecule. This reaction is a necessary preparation for the next reaction in glycolysis, which generates a “high-energy” phosphoryl compound for use in ATP synthesis.



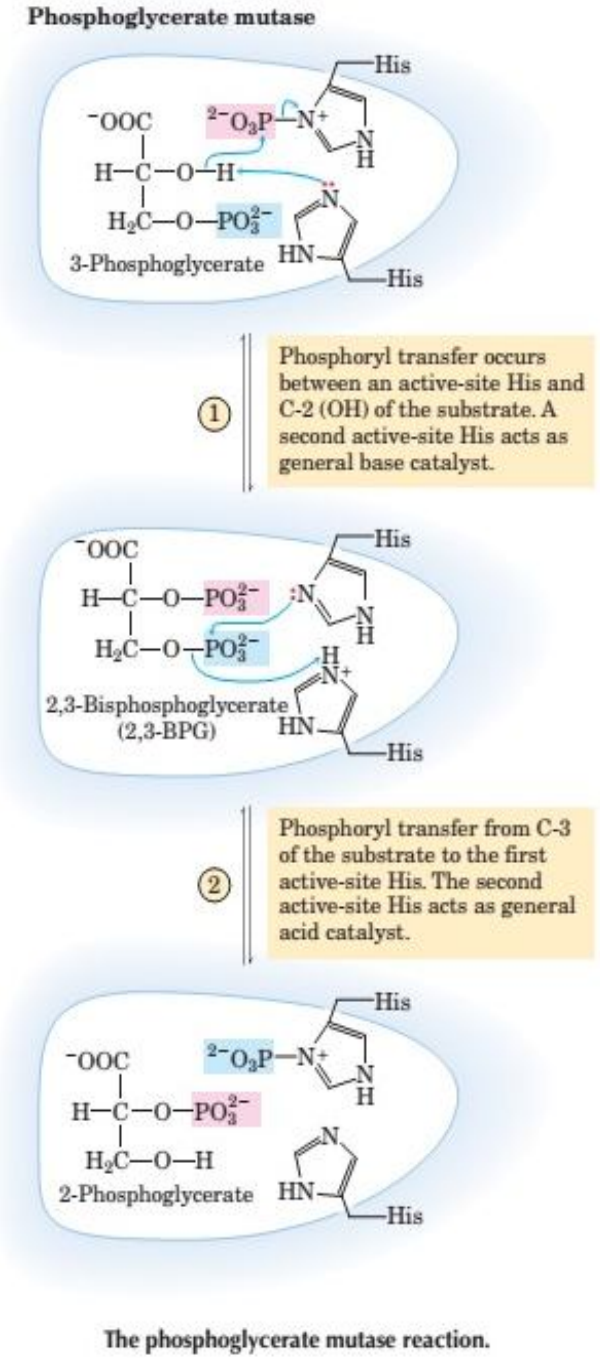
Reaction Mechanism of PGM :

The reaction occurs in two steps:

A phosphoryl group initially attached to a His residue of the mutase is transferred to the hydroxyl group at C-2 of 3-phosphoglycerate, forming 2,3-bisphosphoglycerate (2,3-BPG).

The phosphoryl group at C-3 of 2,3-BPG is then transferred to the same His residue, producing 2-phosphoglycerate and regenerating the phosphorylated enzyme.

Phosphoglycerate mutase is initially phosphorylated by phosphoryl transfer from 2,3-BPG, which is required in small quantities to initiate the catalytic cycle and is continuously regenerated by that cycle.

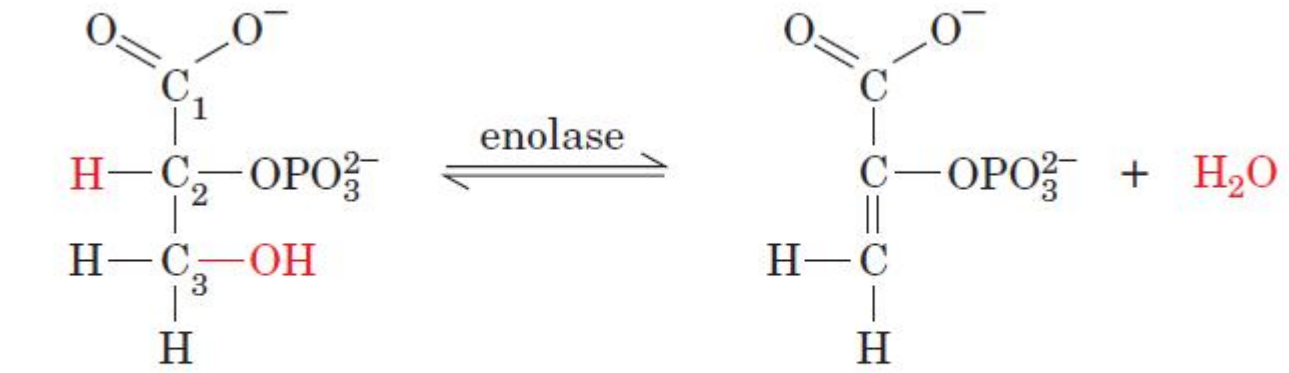


The phosphoglycerate mutase reaction.

Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate Enolase: Second "High-Energy" Intermediate Formation

In Reaction 9 of glycolysis, 2PG is dehydrated to **phosphoenolpyruvate (PEP)** in a reaction catalyzed by **enolase:**

- ✓ The enzyme forms a complex with a divalent cation such as Mg2+ before the substrate is bound. A second divalent metal ion then binds to the enzyme.
- ✓ Fluoride ion inhibits glycolysis, resulting in the accumulation of 2PG and 3PG. It does so by strongly inhibiting enolase in the presence of P*i*. *F^- and Pi form a* tightly bound complex with the Mg^{2+} at the enzyme's active site, blocking substrate binding and thereby inactivating the enzyme. Enolase's substrate, 2PG, therefore builds up and, as it does so, is equilibrated with 3PG by PGM.



2-Phosphoglycerate (2PG) **Phosphoenolpyruvate (PEP)**

a. Catalytic Mechanism of Enolase

The dehydration (elimination of H_2O) catalyzed by enolase might occur in one of three ways (Fig. 16-9*a*): (1) The —OH group at C3 can leave first, generating a carbocation at C3; (2) the C2 proton can leave first, generating a carbanion at C2; or (3) the reaction can be concerted. Isotope exchange studies by Paul Boyer demonstrated that the C2 proton of 2PG exchanges with solvent 12 times faster than the rate of PEP formation. However, the C3 oxygen exchanges with solvent at a rate roughly equivalent with the overall reaction rate. This suggests the following mechanism (Fig. 17-21):

Step 1 Rapid carbanion formation at C2 facilitated by a general base on the enzyme. The abstracted proton can readily exchange with the solvent, accounting for its observed rapid exchange rate.

Step 2 Rate-limiting elimination of the —OH group at C3. This is consistent with the slow rate of exchange of this hydroxyl group with solvent.

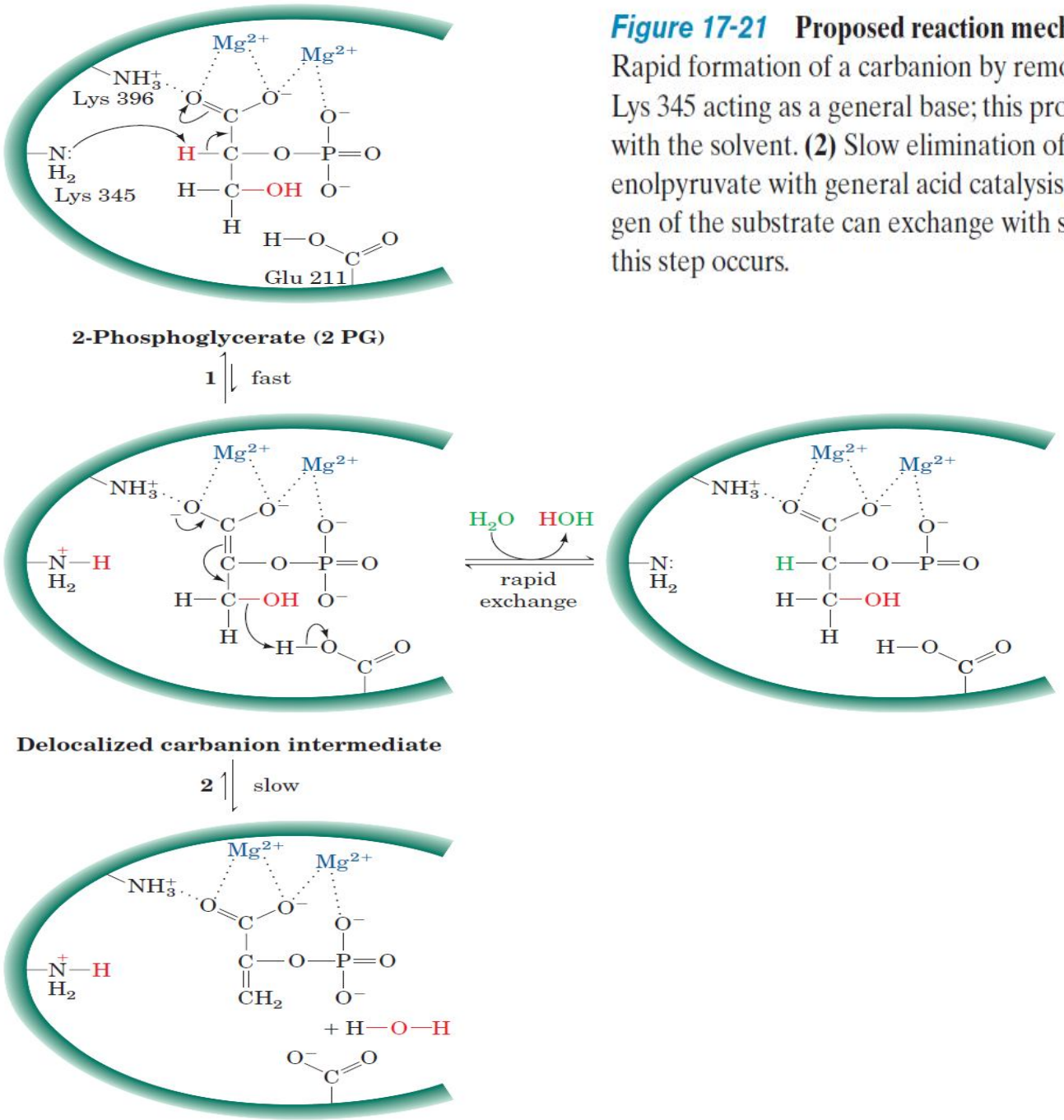
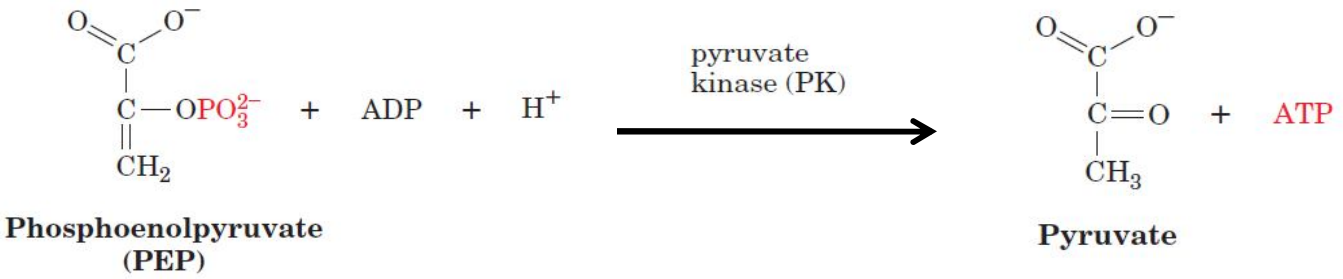


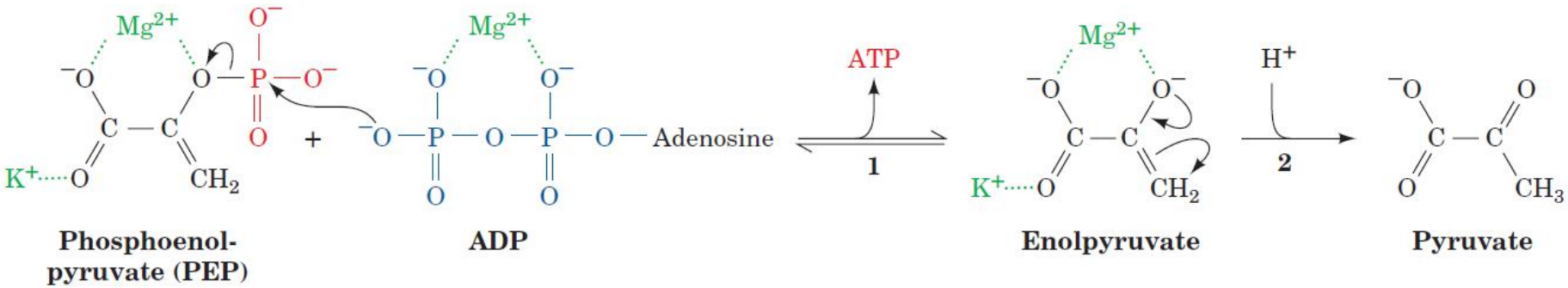
Figure 17-21 **Proposed reaction mechanism of enolase. (1)** Rapid formation of a carbanion by removal of a proton at C2 by Lys 345 acting as a general base; this proton can rapidly exchange with the solvent. **(2)** Slow elimination of H_2O to form phosphoenolpyruvate with general acid catalysis by Glu 211; the C3 oxygen of the substrate can exchange with solvent only as rapidly as this step occurs.

Pyruvate Kinase: Second ATP Generation

In Reaction 10 of glycolysis, its final reaction, **pyruvate kinase (PK)** couples the free energy of PEP hydrolysis to the synthesis of ATP to form pyruvate:



Catalytic Mechanism of PK



$\Delta G^{\circ\prime} = +14.6\ \text{kJ}\cdot\text{mol}^{-1}$ $\Delta G^{\circ\prime} = -46\ \text{kJ}\cdot\text{mol}^{-1}$

Overall $\Delta G^{\circ\prime} = -31.4\ \text{kJ}\cdot\text{mol}^{-1}$

Figure 17-22 **Mechanism of the reaction catalyzed by pyruvate kinase. (1)** Nucleophilic attack of an ADP β-phosphoryl oxygen atom on the phosphorus atom of PEP to form ATP and enolpyruvate; and **(2)** tautomerization of enolpyruvate to pyruvate.

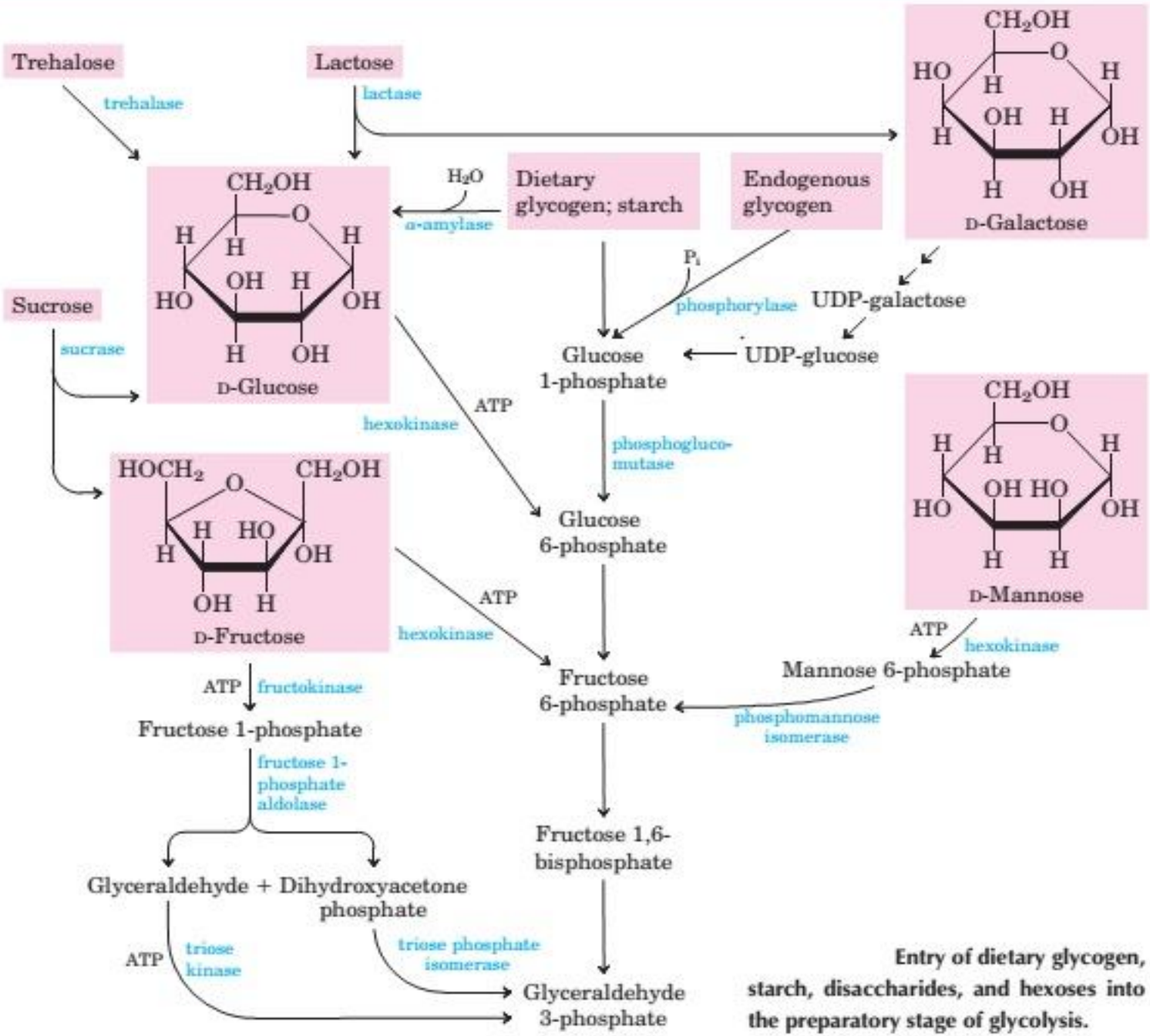
a. Catalytic Mechanism of PK

The PK reaction, which requires the participation of both monovalent (K^+) and divalent (Mg^{2+}) cations, occurs as follows (Fig. 17-22):

Step 1 A β-phosphoryl oxygen of ADP nucleophilically attacks the PEP phosphorus atom, thereby displacing enolpyruvate and forming ATP. This reaction conserves the free energy of PEP hydrolysis.

Step 2 Enolpyruvate converts to pyruvate. This enol–keto tautomerization is sufficiently exergonic to drive the coupled endergonic synthesis of ATP (Section 16-4Ba).

We can now see the "logic" of the enolase reaction. The standard free energy of hydrolysis of 2PG ($\Delta G^{\circ\prime}$) is only $-17.6\ kJ \cdot mol^{-1}$, which is insufficient to drive ATP synthesis ($\Delta G^{\circ\prime} = 30.5\ kJ \cdot mol^{-1}$ for ATP synthesis from ADP and P_i). The dehydration of 2PG results in the formation of a "high-energy" compound capable of such synthesis [the standard free energy of hydrolysis of PEP is $-61.9\ kJ \cdot mol^{-1}$ (Fig. 16-25)]. In other words, PEP is a "high-energy" compound, 2PG is not.



Entry of dietary glycogen, starch, disaccharides, and hexoses into the preparatory stage of glycolysis.