

CHEM 153A Week 9

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Glycolysis (Continued)

Importance of Phosphorylated Intermediates

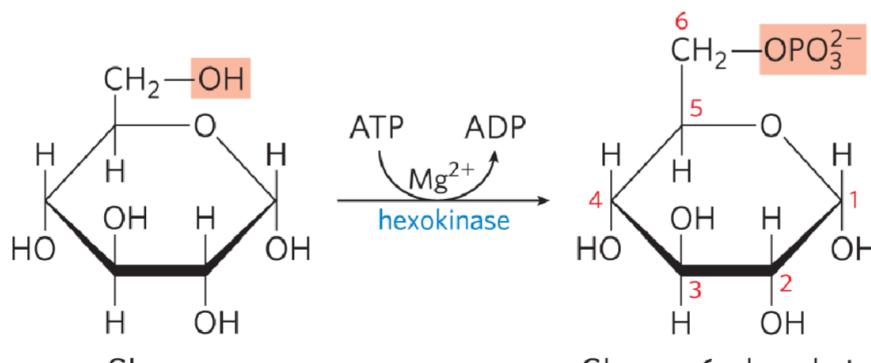
- All nine intermediates are phosphorylated
- Functions of the phosphoryl groups:
 - Prevent glycolytic intermediates from leaving the cell
 - Serve as essential components in the enzymatic conservation of metabolic energy
 - Lower the activation energy and increase the specificity of the enzymatic reactions

The Preparatory Phase of Glycolysis Requires ATP

- In the preparatory phase of glycolysis:
 - Two molecules of ATP are invested to activate **glucose** to **fructose 1,6-bisphosphate**
 - The bond between C-3 and C-4 of fructose 1,6-bisphosphate is then broken to yield two molecules of triose phosphate

(Step 1) Phosphorylation of Glucose

- Hexokinase activates glucose by phosphorylating at C-6 to yield **glucose 6-phosphate**
 - ATP serves as the phosphoryl donor
 - hexokinase requires Mg^{2+} for its activity
 - irreversible under intracellular conditions



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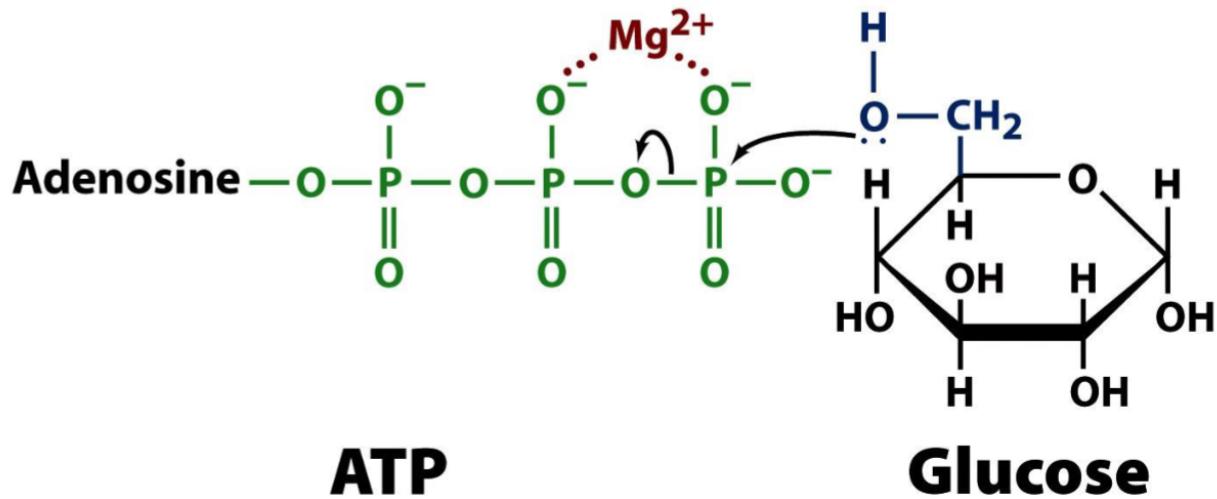
$$\Delta G'^\circ = -16.7 \text{ kJ/mol}$$

Hexokinase commits glucose to the hexose phosphate pool by converting glucose to glucose-6-phosphate (G6P)

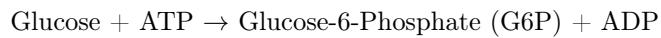
Hexokinase mechanism basics

- Hexokinase relies on magnesium for stabilizing triphosphate
- Shielding the negative phosphate charges allows for nucleophilic attack by hydroxyl
- Example in metal-ion catalysis

partial mechanism:



Hexokinase Reaction:

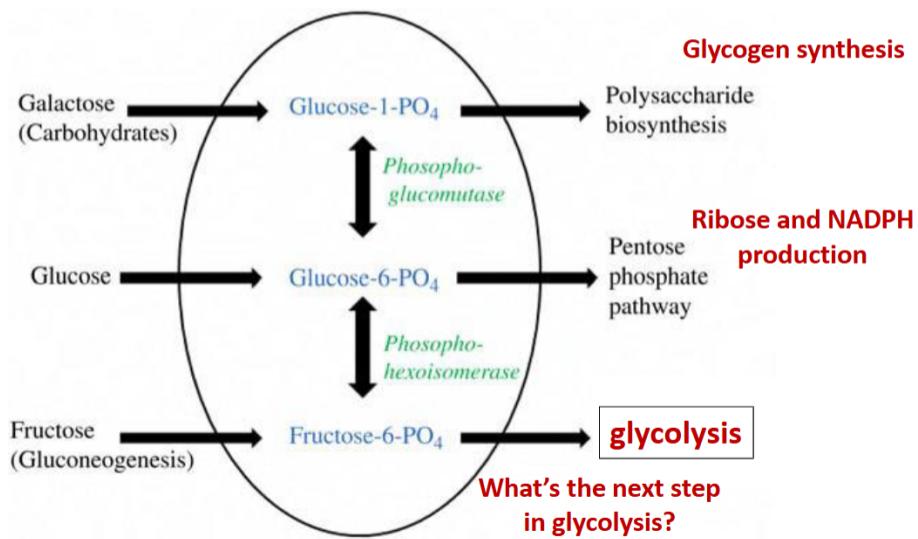


Commitment to Metabolic Pool:

- The hexose phosphate pool
- Significance:
 - Traps glucose inside the cell (G6P cannot cross the cell membrane)
 - Commits glucose to further metabolism within the cell

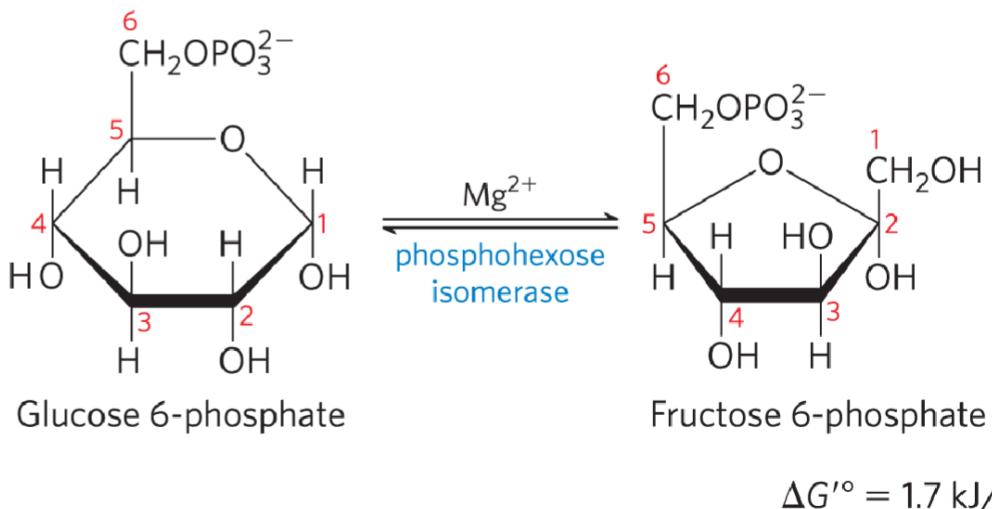
The pool of hexoses

These intermediates aren't just shared by glycolysis and gluconeogenesis...



(Step 2) Conversion of Glucose 6-Phosphate to Fructose 6-Phosphate

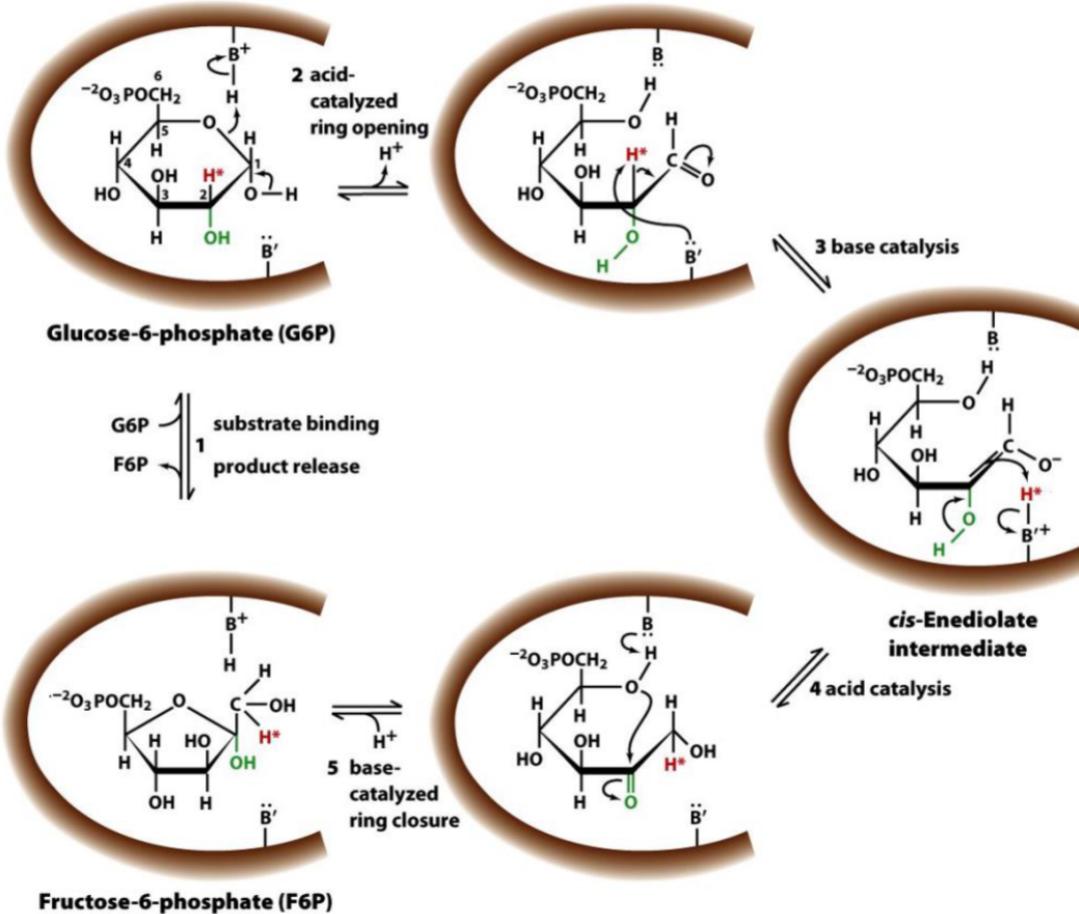
- Phosphohexose isomerase (phosphoglucose isomerase) catalyzes the reversible isomerization of glucose 6-phosphate to fructose 6-phosphate
 - mechanism involves an enediol intermediate
 - reaction readily proceeds in either direction



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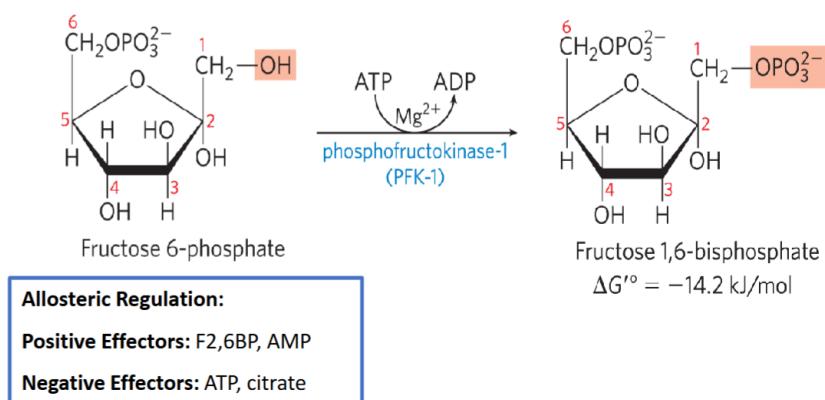
- The rearrangement of G6P to F6P is critical for the efficient progression of glycolysis. It ensures compatibility with downstream enzymes, facilitates the symmetrical cleavage of the sugar, and prepares the molecule for the energy-investment step catalyzed by PFK-1. Without this rearrangement, glycolysis could not proceed in a coordinated or efficient manner.

Phosphohexose isomerase mechanism



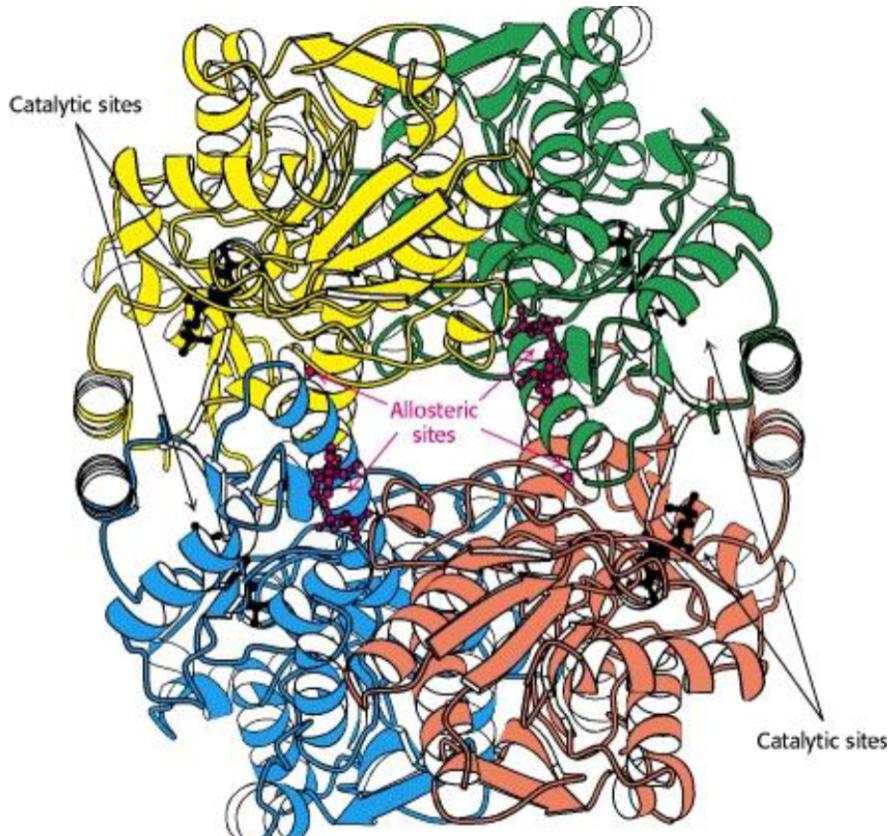
(Step 3) Phosphorylation of Fructose 6-Phosphate to Fructose 1,6-Bisphosphate

- Phosphofructokinase-1 (PFK-1) is a key regulatory enzyme in glycolysis
- Catalyzes the transfer of a phosphoryl group from ATP to fructose 6-phosphate to yield fructose 1,6-bisphosphate
 - Essentially irreversible under cellular conditions
 - The first "committed" step in the glycolytic pathway



Allosteric Regulation of PFK-1

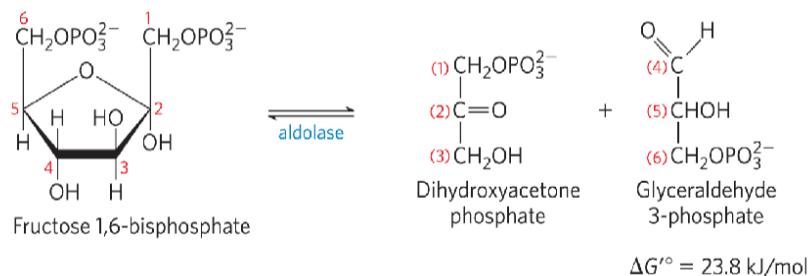
- Activity increases when:
 - ATP supply is depleted
 - ADP and AMP accumulate
- Fructose 2,6-bisphosphate is a potent allosteric activator
- PFK-1 acts as a metabolic "gatekeeper", integrating signals from the cell's energy status and hormonal environment. This regulation allows glycolysis to be precisely tuned to the cell's energy demands, maintaining metabolic balance and energy homeostasis



- Fructose 6-Phosphate (F6P), an intermediate of glycolysis, is phosphorylated by phosphofructokinase-2 (PFK-2) to form Fructose 2,6-bisphosphate (F2, 6BP). F2,6BP is not an intermediate in glycolysis or gluconeogenesis but acts as a potent allosteric regulator of PFK-1, stimulating glycolysis and inhibiting gluconeogenesis.

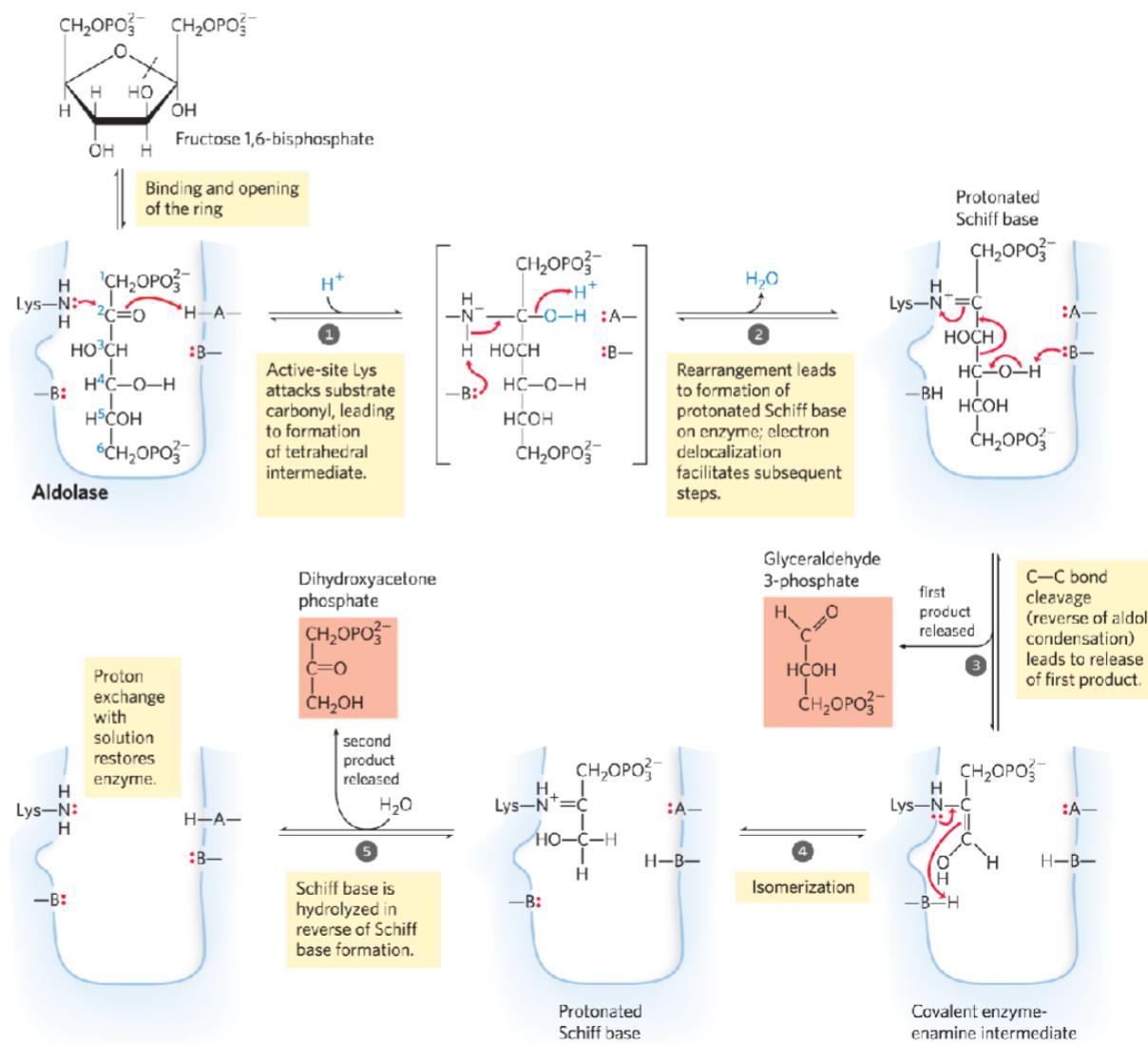
(Step 4) Cleavage of Fructose 1,6-Bisphosphate

- Fructose 1,6-Bisphosphate aldolase (aldolase) catalyzes a reverse aldol condensation and cleaves fructose 1,6-bisphosphate to yield **glyceraldehyde 3-phosphate** and **dihydroxyacetone phosphate**
- Reversible because reactant concentrations are low in the cell.



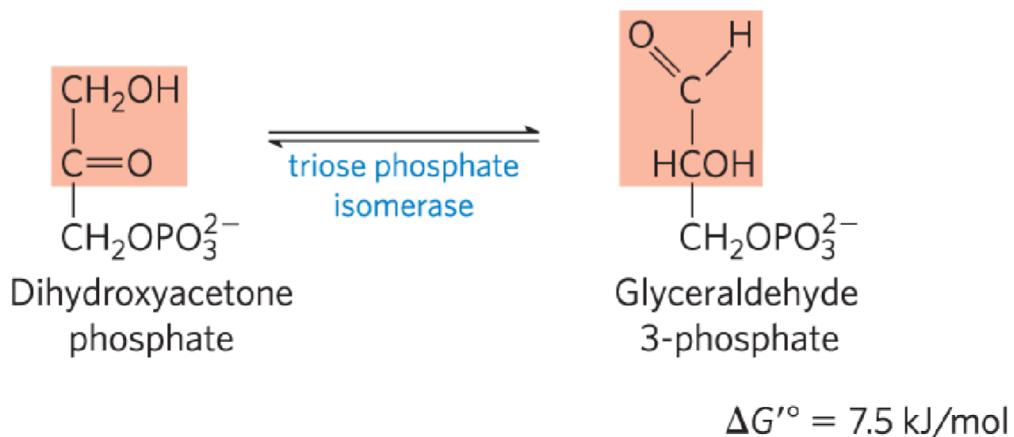
The Class I Aldolase Reaction

- Class I = found in animals and plants
- Class II = found in fungi and bacteria
 - Do not form the Schiff base intermediate



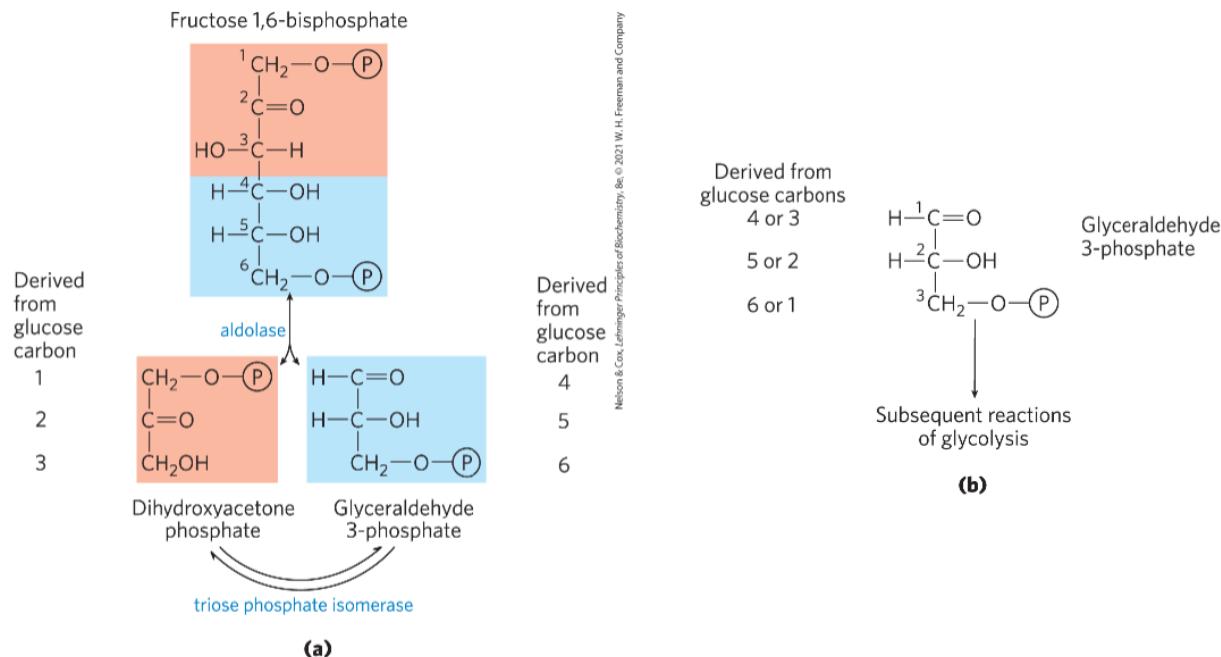
(Step 5) Interconversion of the Triose Phosphates

- Triose phosphate isomerase converts dihydroxyacetone phosphate to glyceraldehyde 3-phosphate
 - reversible
 - final step of the preparatory phase of glycolysis



Fate of the Glucose Carbons in the Formation of Glyceraldehyde 3-Phosphate

- After Step 5 of glycolysis, the carbon atoms derived from C-1, C-2, and C-3 of the starting glucose are chemically indistinguishable from C-6, C-5, and C-4, respectively



The Payoff Phase of Glycolysis Yields ATP and NADH

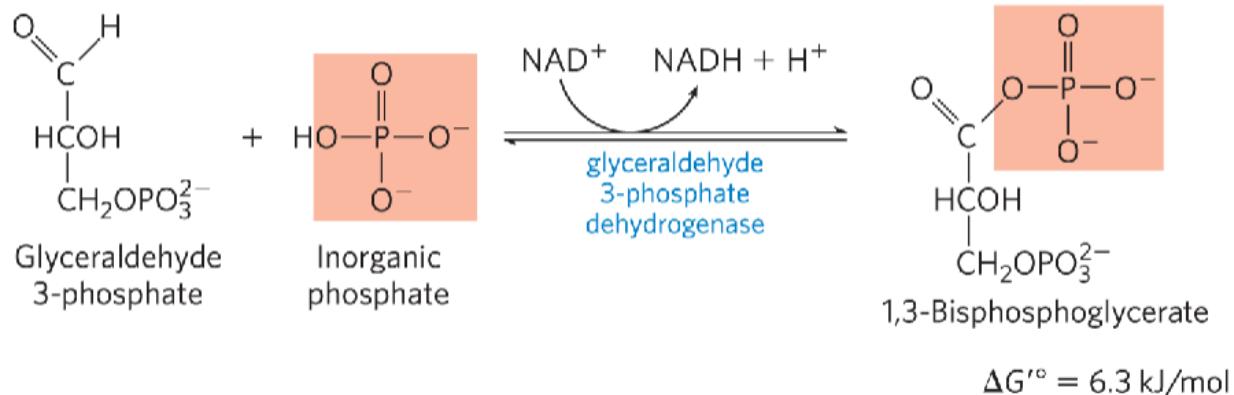
In the payoff phase of glycolysis:

- Each of the two molecules of glyceraldehyde 3-phosphate undergoes **oxidation at C-1**

- Some energy from the oxidation reaction is conserved in the form of one **NADH** and two ATP per triose phosphate oxidized

(Step 6) Oxidation of Glyceraldehyde 3-Phosphate to 1,3-Bisphosphoglycerate

- Glyceraldehyde 3-Phosphate Dehydrogenase** catalyzes the oxidation of glyceraldehyde 3-phosphate to **1,3-bisphosphoglycerate**
- This is an energy-conserving reaction

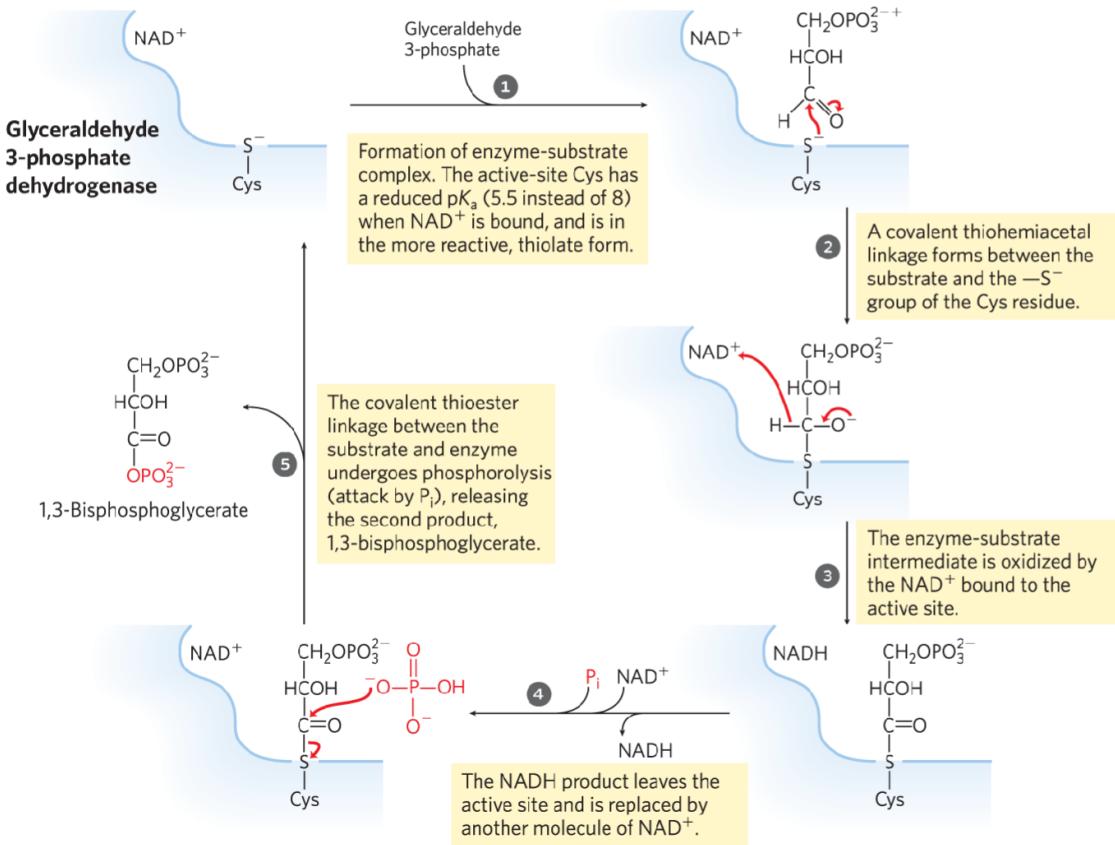


This reduction step stores energy with the formation of the acyl phosphate and in the form of high-energy electrons within NADH

The First Step of the Payoff Phase is an Energy-Conserving Reaction

- Formation of the **acyl phosphate** group at C-1 of 1,3-bisphosphoglycerate conserves the free energy of oxidation
- acyl phosphates have a very high standard free energy of hydrolysis ($\Delta G'^\circ = -49.3 \text{ kJ/mol}$)

The Glyceraldehyde 3-Phosphate Dehydrogenase Reaction



- First, the thiolate ion attacks the carbonyl group of the substrate to form a thiohemiacetal, which is then oxidized to a thioester by transfer of a hydride ion (a hydrogen with two electrons, H^-) to an enzyme-bound NAD^+ , with concurrent release of a proton (H^+). Thus, in effect, two hydrogen atoms are removed from the substrate.
- Once NADH is formed, its affinity for the enzyme decreases, so that a free NAD^+ displaces this NADH . The thioester is an energy-rich intermediate, and by phosphorolysis the high-energy 1,3-bisphosphoglycerate is generated with the release of the free enzyme. Thus, the substrate aldehyde group is oxidized to a carboxylic acid group, with conservation of most of the energy of oxidation in formation of the anhydride bond between carboxylic and phosphoric acids.

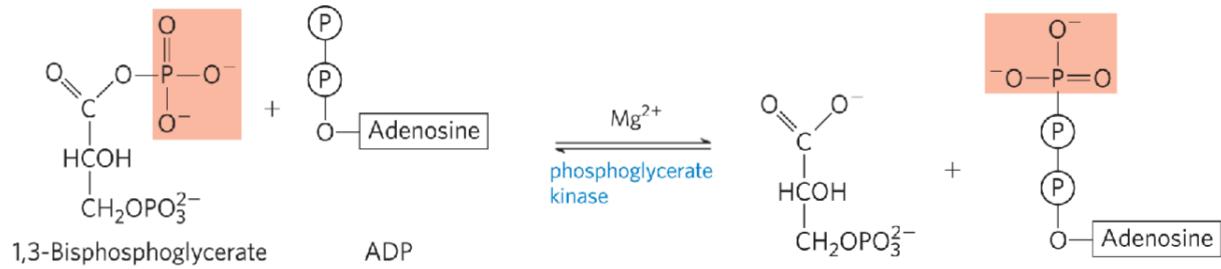
Why This Process Works

- The **thioester intermediate** serves as a critical energy-rich intermediate that conserves the energy released during the oxidation of G3P. This conserved energy is then used to drive the unfavorable phosphorylation step.
- NAD^+ not only acts as an electron acceptor, forming NADH , but also activates the cysteine residue for catalysis.
- The release of NADH ensures that the enzyme is ready to catalyze subsequent reactions efficiently.

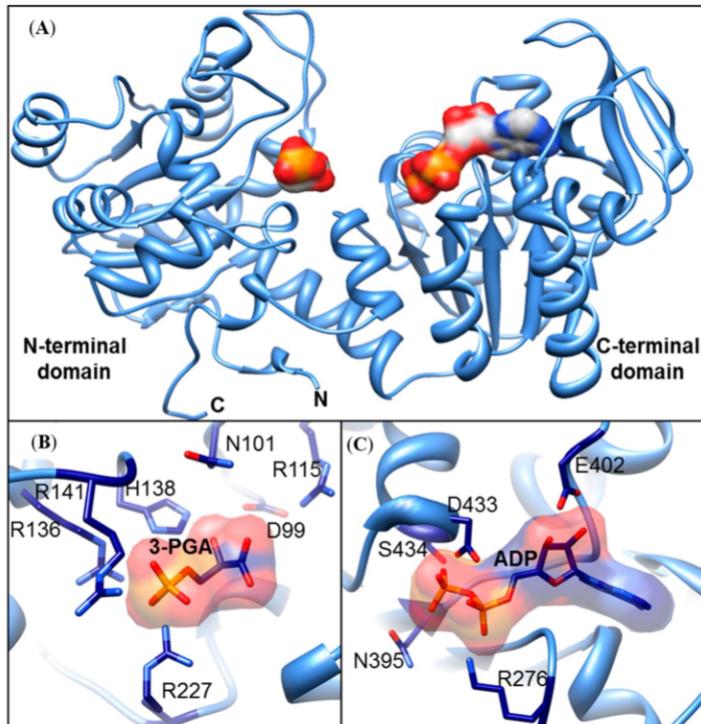
(Step 7) Phosphoryl Transfer from 1,3-Bisphosphoglycerate to ADP

- Phosphoglycerate Kinase transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and **3-phosphoglycerate**.

- substrate-level phosphorylation

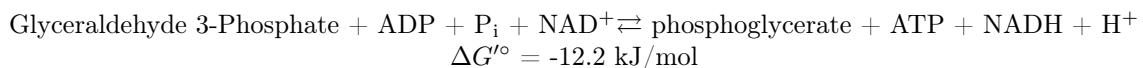


In silico structural model of maize ZmPGK1



Steps 6 and 7 of Glycolysis Consistute an Energy-Coupling Process

- The sum of the two reactions is:



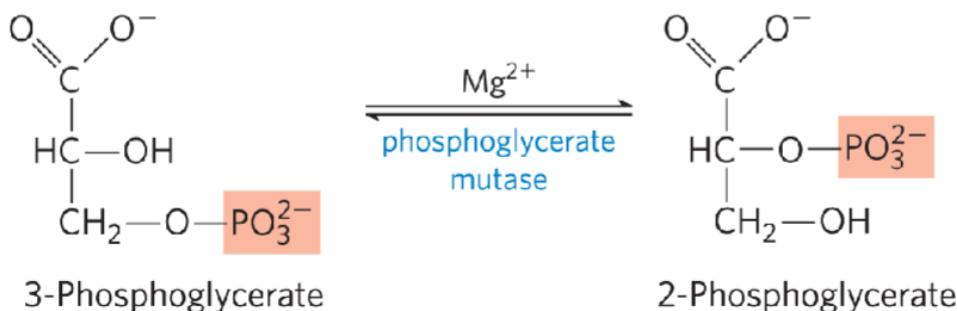
- substrate-level phosphorylation** = the formation of ATP by phosphoryl group transfer from a substrate different from **respiration-linked phosphorylation**
- G3P dehydrogenase is coupled to phosphoglycerate kinase
 - G3P dehydrogenase is forming a high energy phosphate while phosphoglycerate kinase is removing the phosphoryl group and adding it to ADP ($\Delta G < 0$ overall)

		$\Delta G^{\circ'}$
GAPDH	$\text{GAP} + \text{NAD}^+ + \text{P}_i \rightarrow 1,3\text{-BPG} + \text{NADH} + \text{H}^+$	+6.3
first substrate-level phosphorylation	PGK + $1,3\text{-BPG} + \text{ADP} \rightarrow 3\text{-PG} + \text{ATP}$	-18.8
	$\text{GAP} + \text{NAD}^+ + \text{ADP} + \text{P}_i \rightarrow 3\text{-PG} + \text{ATP} + \text{NADH} + \text{H}^+$	-12.5

(Step 8) Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

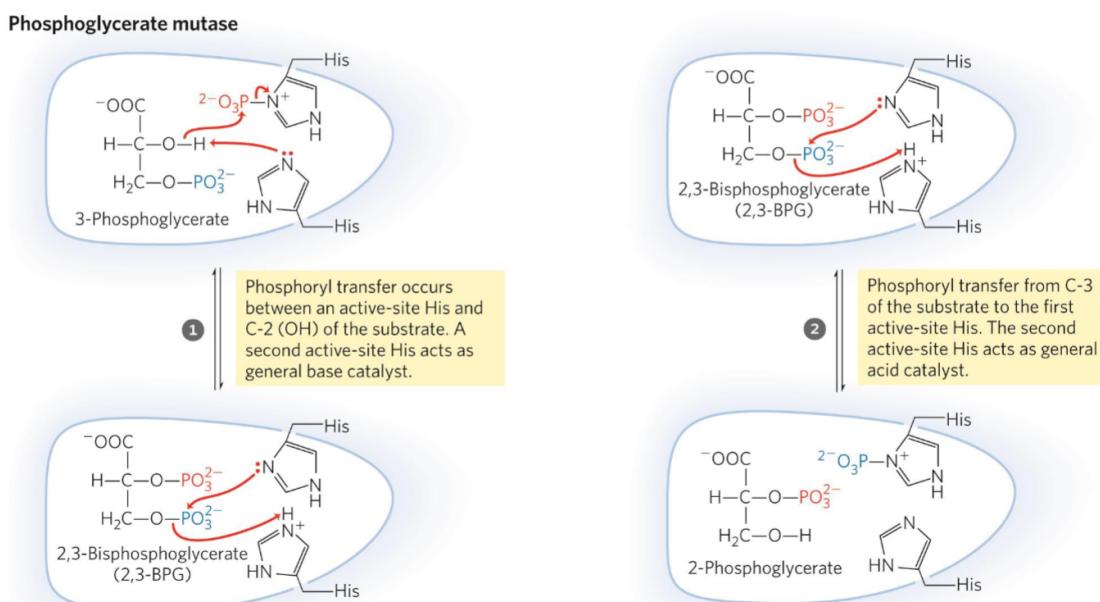
- phosphoglycerate mutase catalyzes a reversible shift of the phosphoryl group between C-2 and C-3 of glycerate

– requires Mg^{2+}



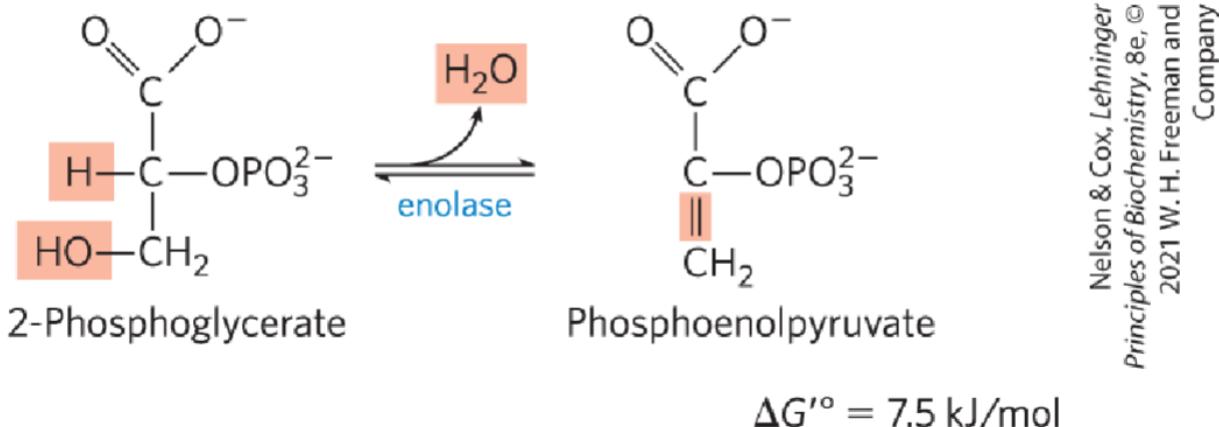
$$\Delta G'^{\circ} = 4.4 \text{ kJ/mol}$$

The Phosphoglycerate Mutase Reaction



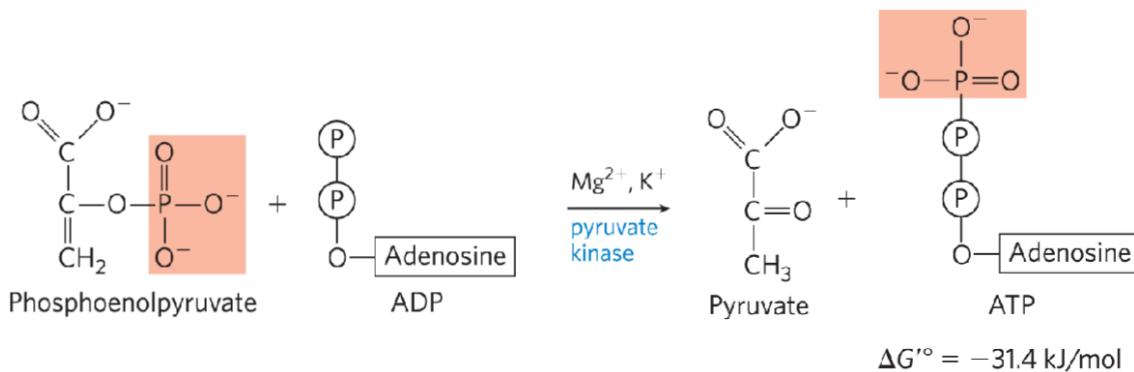
(Step 9) Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

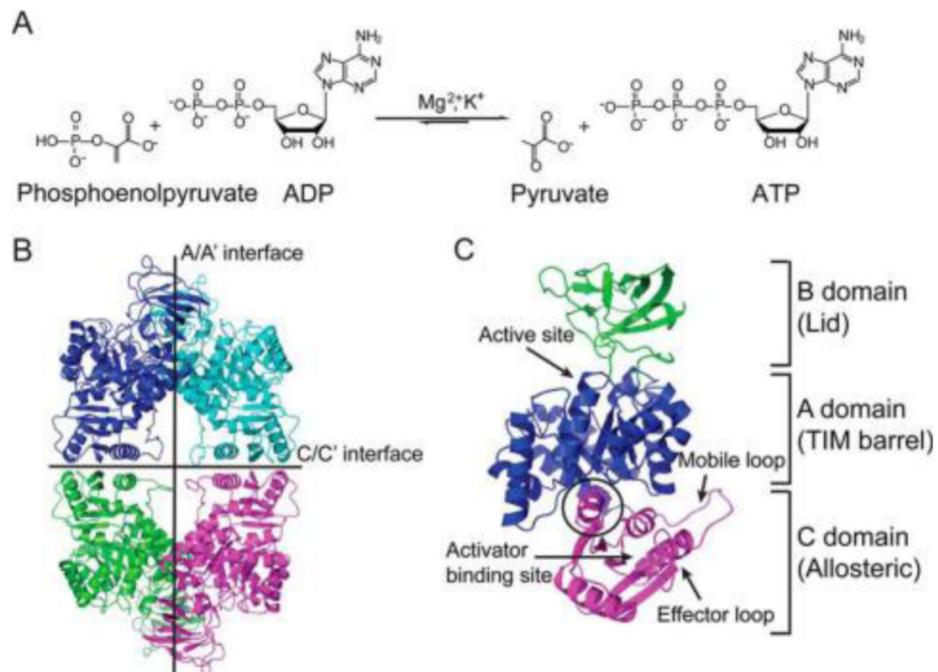
- **enolase** promotes reversible removal of a molecule of water from 2-phosphoglycerate to yield **phosphoenolpyruvate (PEP)**
 - energy-conserving reaction
 - mechanism involves a Mg^{2+} -stabilized enolic intermediate



(Step 10) Transfer of the Phosphoryl Group from Phosphoenolpyruvate to ADP

- **pyruvate kinase** catalyzes the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, yielding **pyruvate**
- Requires K^+ and either Mg^{2+} or Mn^{2+}
- **substrate-level phosphorylation** - the formation of ATP by phosphoryl group transfer from a substrate different from **respiration-linked phosphorylation**

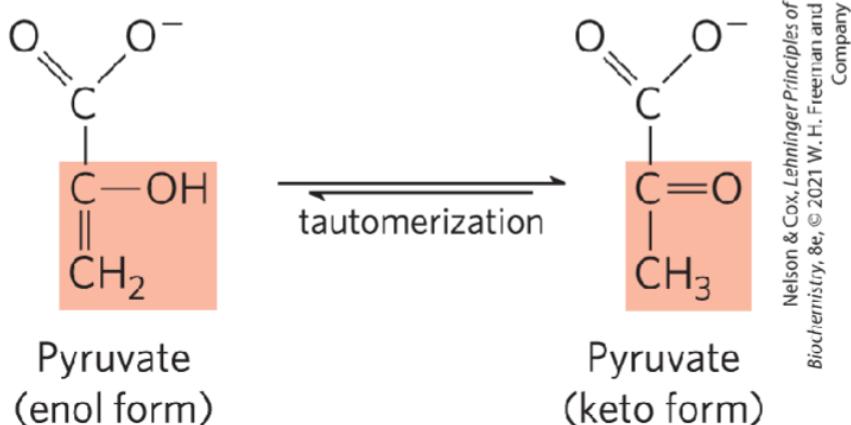




A, the reaction catalyzed by pyruvate kinase. B, structure of *E. coli* pyruvate kinase type 1 tetramer with the tetrameric A/A' and C/C' interfaces labeled. C, pyruvate kinase type 1 monomer showing the active site and allosteric binding site. The monomer is colored by domain, and the helix that connects the allosteric domain with the active site domain is circled

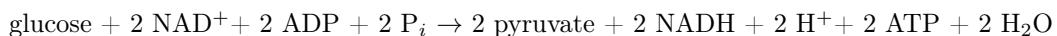
Pyruvate in its Enol Form Spontaneously Tautomerizes to its Keto Form

- **pyruvate kinase** catalyzes the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, yielding **pyruvate**
 - requires K^+ and either Mg^{2+} or Mn^{3+}



The Overall Balance Sheet Shows a Net Gain of Two ATP and Two ADH per Glucose

- Subtracting the two ATP spent in the preparatory phase, the net equation for the overall process is:



Glycolysis Overview

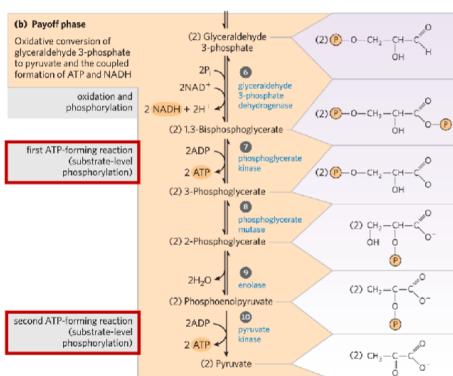


Table 15-1 ΔG°_f and ΔG for the Reactions of Glycolysis in Heart Muscle^a

Reaction	Enzyme	ΔG°_f (kJ · mol ⁻¹)	ΔG (kJ · mol ⁻¹)
1	Hexokinase	-20.9	-27.2
2	PGI	+2.2	-1.4
3	PFK	-17.2	-25.9
4	Aldolase	+22.8	-5.9
5	TIM	+7.9	~0
6 + 7	GAPDH + PGK	-16.7	-1.1
8	PGM	+4.7	-0.6
9	Enolase	-3.2	-2.4
10	PK	-23.0	-13.9

^aCalculated from data in Newsholme, E.A. and Start, C., *Regulation in Metabolism*, p. 97, Wiley (1973).



Energy Remaining in Pyruvate

- Energy stored in pyruvate can be extracted by:
 - **aerobic processes:**
 - * oxidative reactions in the citric acid cycle (TCA cycle)
 - * oxidative phosphorylation
 - **anaerobic processes:**
 - * reduction to lactate
 - * reduction to ethanol
- pyruvate can provide the carbon skeleton for alanine synthesis or fatty acid synthesis