METABOLIC BIOCHEMISTRY

GLYCOLYSIS

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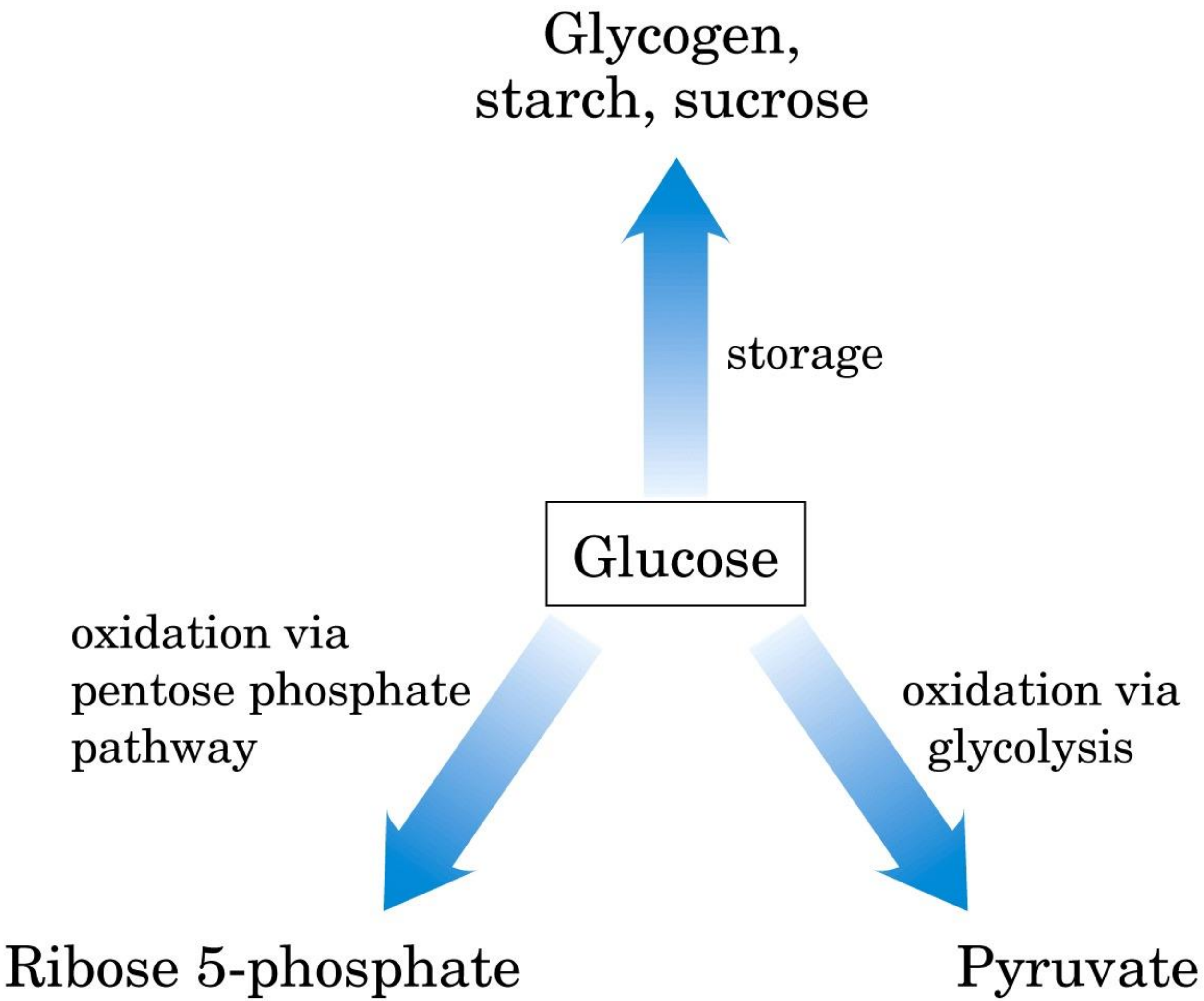
Major pathways of glucose utilization



Glycolysis

What is glycolysis?

- Ten step metabolic pathway to convert glucose into two molecules of pyruvate and two molecules each of *NADH* and *ATP.*

- All carbohydrates to be catabolized must enter the glycolytic pathway.

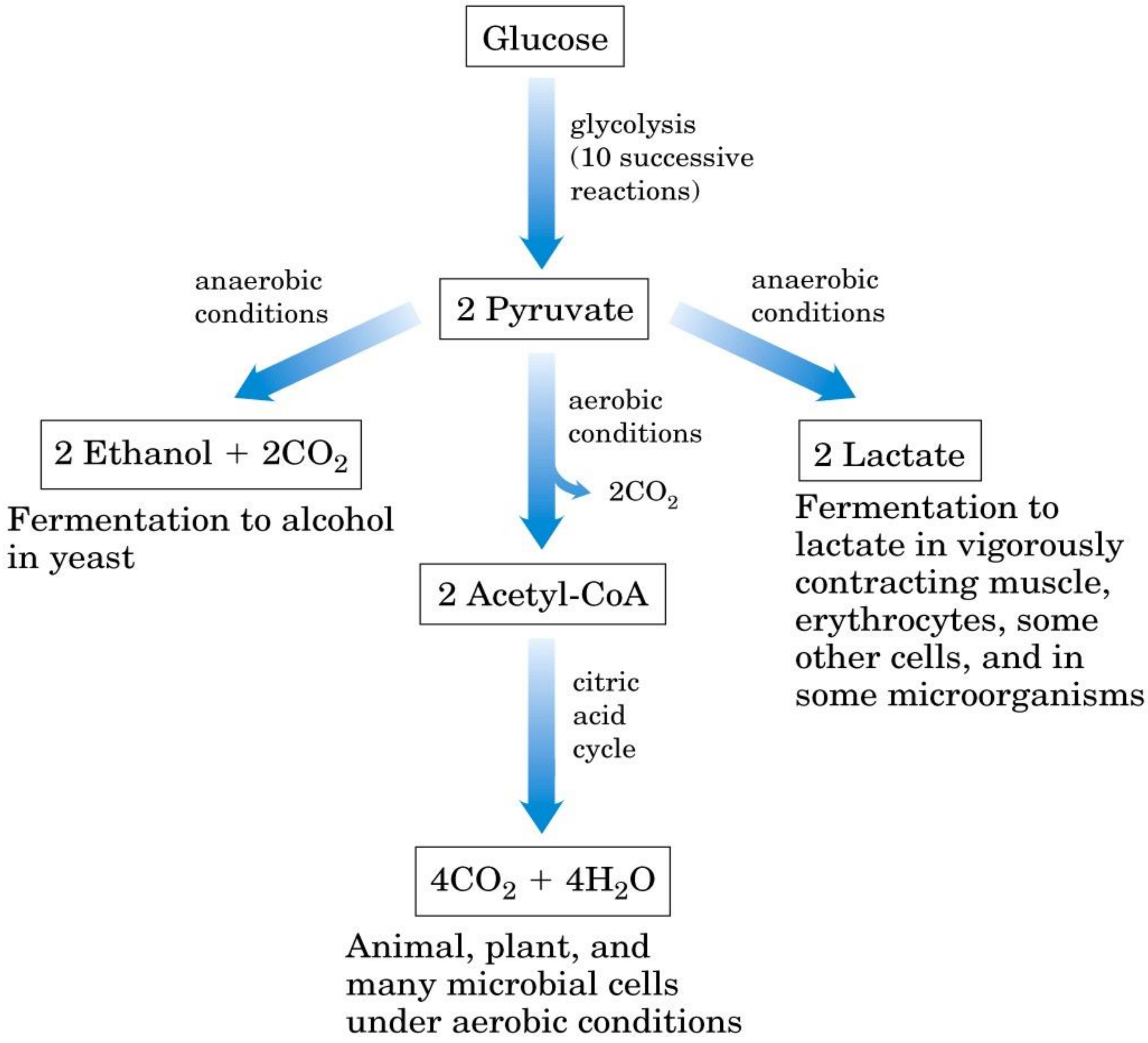
- The glycolytic breakdown of glucose is the sole source of metabolic energy in some mammalian tissues and cell types (erythrocytes, renal medulla, brain, and sperm)

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

(1) degradation of the carbon skeleton of glucose to yield pyruvate,

(2) phosphorylation of ADP to ATP by high-energy phosphate compounds formed during glycolysis, and

(3) transfer of a hydride ion (anion of hydrogen, H^-) to NAD, forming NADH.


Glucose
glycolysis (10 successive reactions)
anaerobic conditions
2 Pyruvate
anaerobic conditions
2 Ethanol + $2CO_2$
Fermentation to alcohol in yeast
aerobic conditions
$2CO_2$
2 Lactate
Fermentation to lactate in vigorously contracting muscle, erythrocytes, some other cells, and in some microorganisms
2 Acetyl-CoA
citric acid cycle
$4CO_2 + 4H_2O$
Animal, plant, and many microbial cells under aerobic conditions

Energy Remaining in Pyruvate

- Glycolysis releases only a small fraction of the total available energy of the glucose molecule;

- The two molecules of pyruvate formed by glycolysis still contain most of the chemical potential energy of glucose, energy that can be extracted by oxidative reactions in the citric acid cycle and oxidative phosphorylation.

Importance of Phosphorylated Intermediates

Each of the nine glycolytic intermediates between glucose and pyruvate is phosphorylated. The phosphoryl groups appear to have three functions.

1. The phosphorylated glycolytic intermediates cannot leave the cell.

2. High-energy phosphate compounds formed in glycolysis (1,3-bisphosphoglycerate and phosphoenolpyruvate) donate phosphoryl groups to ADP to form ATP.

3. Binding energy resulting from the binding of phosphate groups to the active sites of enzymes lowers the activation energy and increases the specificity of the enzymatic reactions.

Glycolysis has two stages

A. An ***energy investment*** phase.
Reactions, 1-5. Glucose to two glyceraldehyde 3-phosphate molecules. Two ATPs are invested.

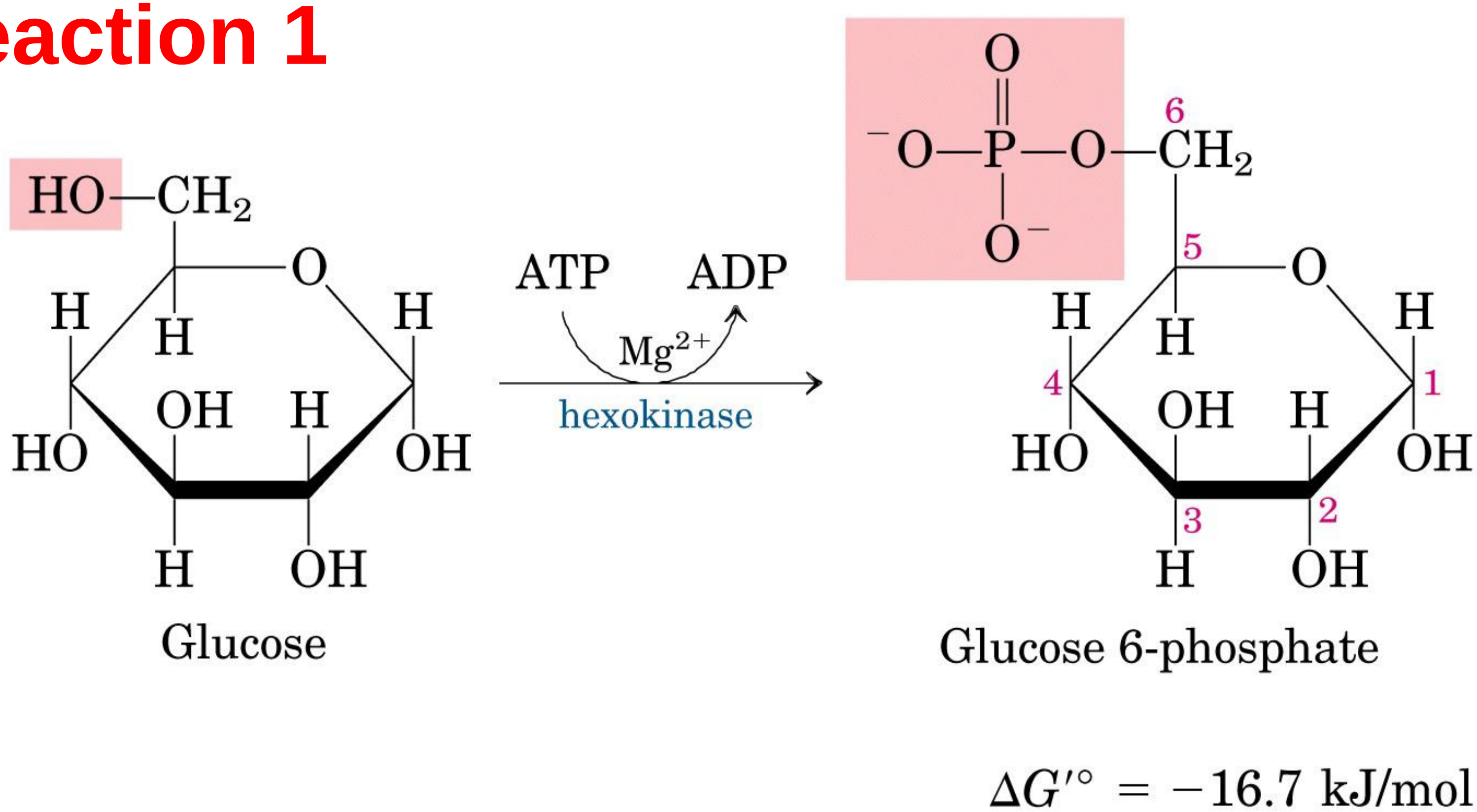
B. An ***energy payoff*** phase.
Reactions 6-10. Two glyceraldehyde 3-phosphate molecules to two pyruvate plus four ATP molecules.

A **net of two ATP** molecules overall plus **two NADH**.

Detailed Reactions of glycolysis

Preparatory Phase

Reaction 1

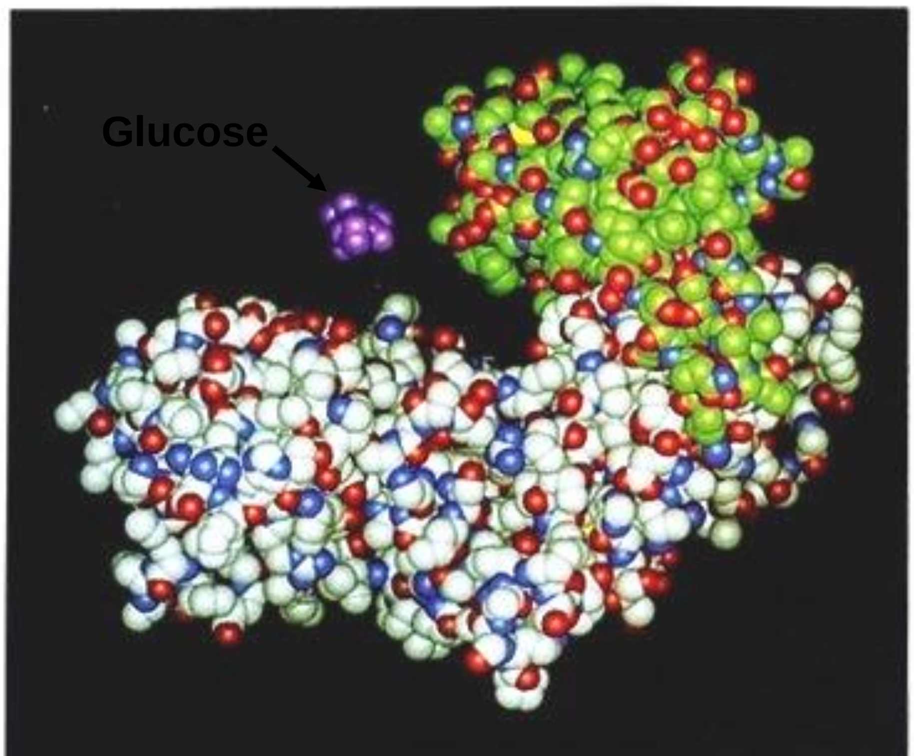


This reaction, which is irreversible under intracellular conditions, is catalyzed by **hexokinase**

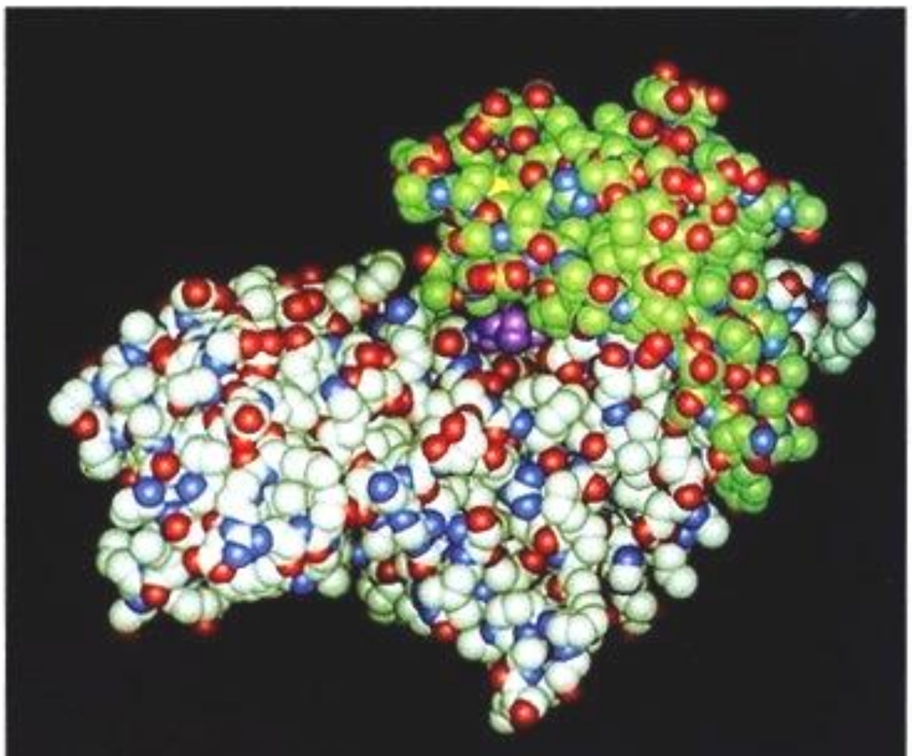
First ATP Utilization

Hexokinase and its complex with glucose (induced fit).

In the enzyme-substrate complex the two lobes swing together to engulf the substrate. This excludes H_2O from the active site which prevents ATP hydrolysis.



(a)



(b)

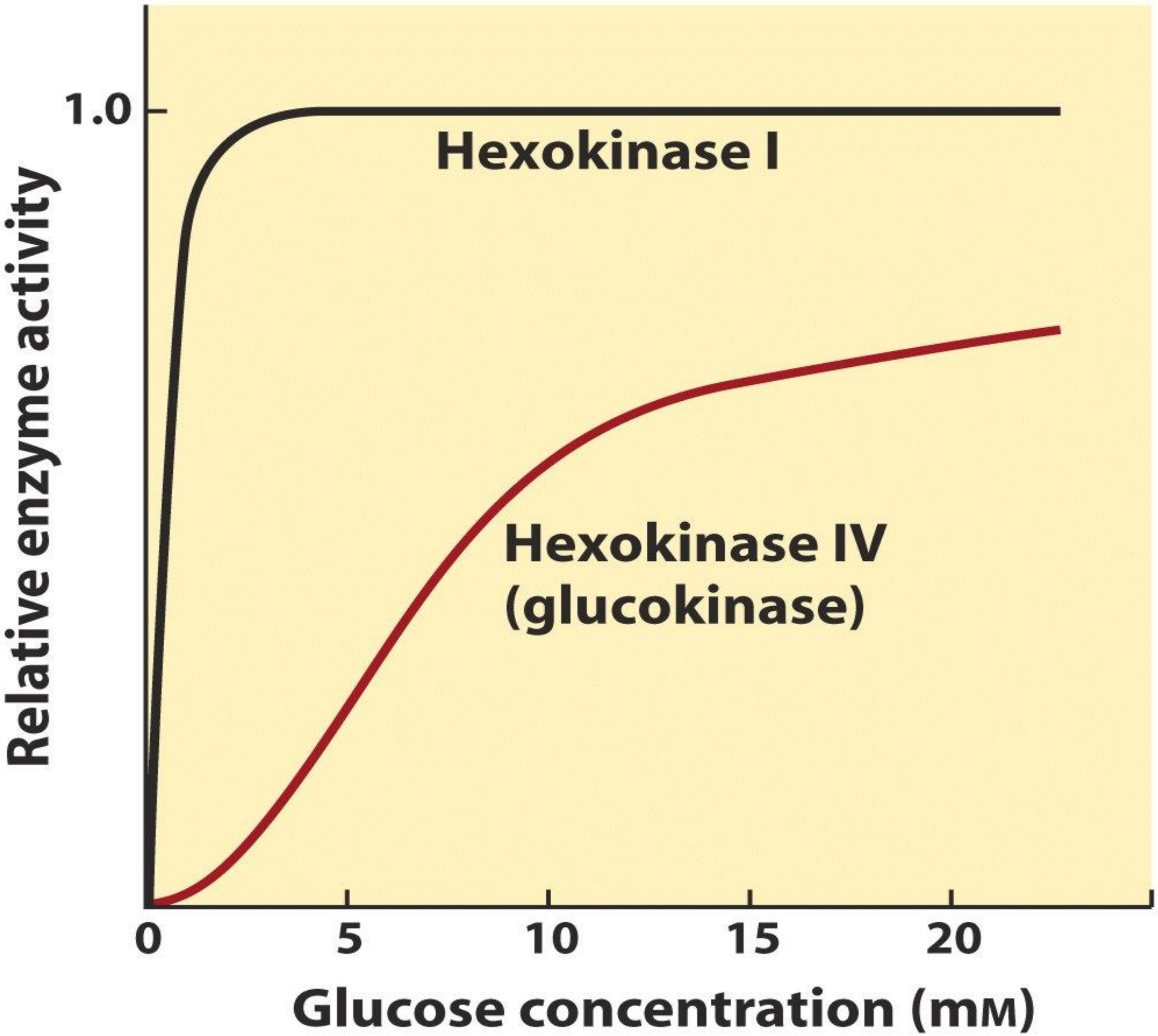
Hexokinase vs. Glucokinase

Hexokinase

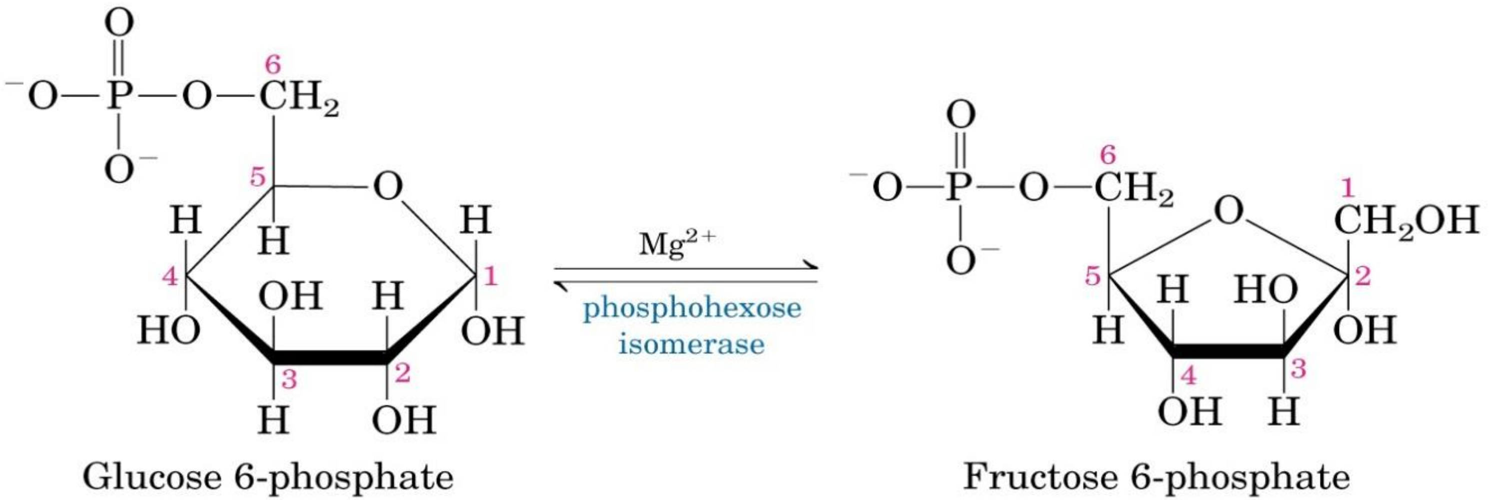
– Found in the cytosol of most tissues
– Low specificity
– Low Km: (Km = 0.1 mM) high affinity for glucose
– Inhibited by it's product Glucose-6-phosphate

Glucokinase: Found in the Liver and pancreatic β cells

- Also a 'hexokinase'= hexokinase IV
- High Km (Km ~10mM) High specificity for glucose
- Inhibited by fructose-6-phosphate (not glu-6-p)

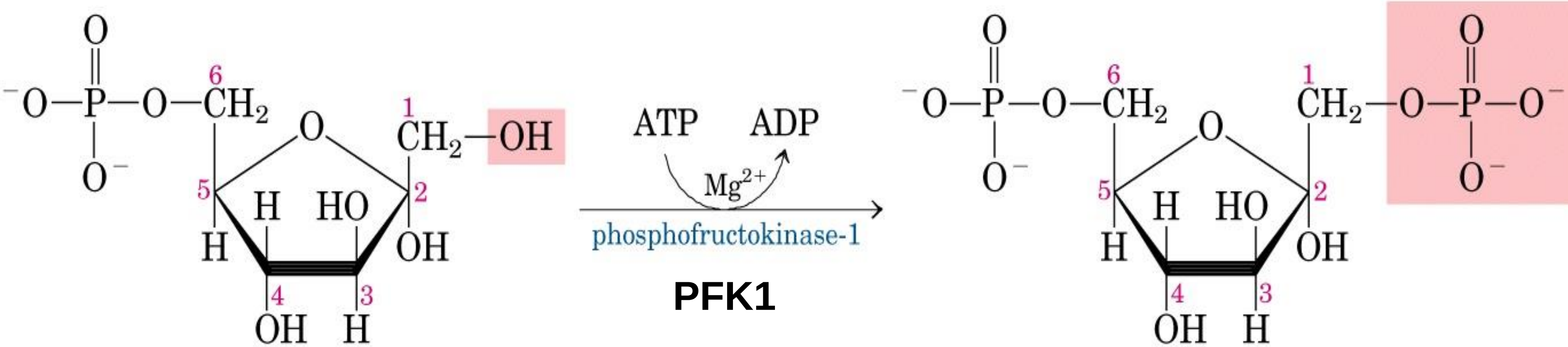

1.0
Hexokinase I
Hexokinase IV
(glucokinase)
Relative enzyme activity
0
5
10
15
20
Glucose concentration (mM)

Reaction 2 : The enzyme **phosphohexose isomerase** catalyzes the reversible isomerization of glucose 6-phosphate, an aldose, to **fructose 6-phosphate,** a ketose:



$$\Delta G'^{\circ} = 1.7 \text{ kJ/mol}$$

Reaction 3: The PFK-1 reaction is essentially irreversible under cellular conditions, and it is the first “committed” step in the glycolytic pathway fructose 1,6-bisphosphate is targeted for glycolysis.



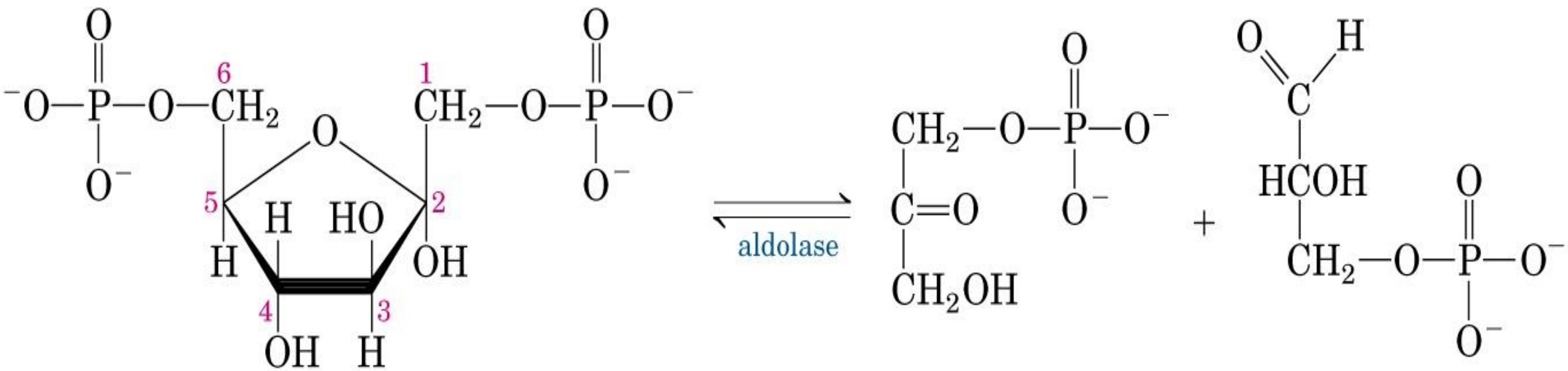
Fructose 6-phosphate

Fructose 1,6-bisphosphate

$$\Delta G'^{\circ} = -14.2 \text{ kJ/mol}$$

Second ATP Utilization

Reaction 4: **by** the enzyme **aldolase,**Fructose 1,6-bisphosphate is cleaved to yield two different triose phosphates, **glyceraldehyde 3-phosphate,** an aldose, and **dihydroxyacetone phosphate,** a ketose:



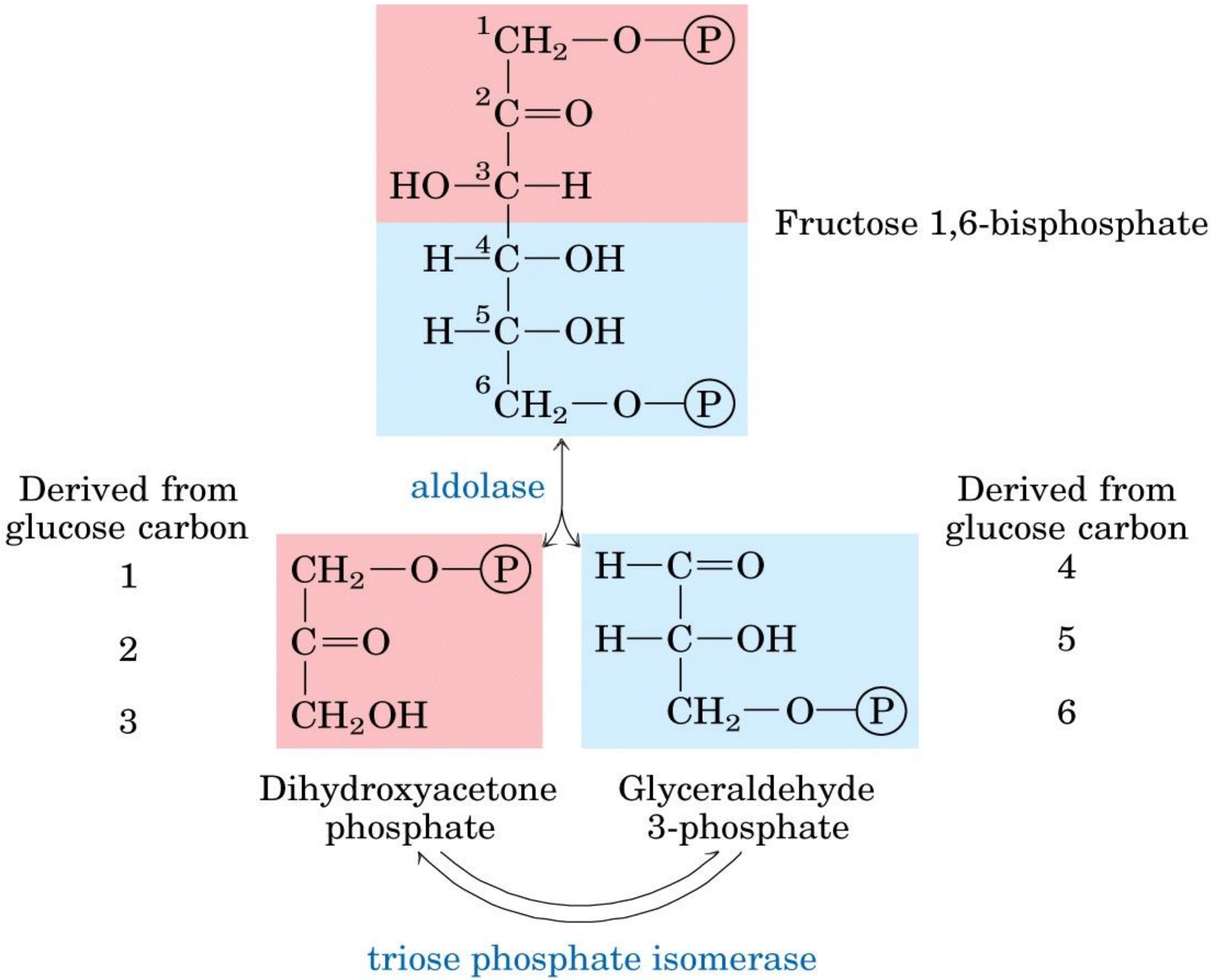
Fructose 1,6-bisphosphate

Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

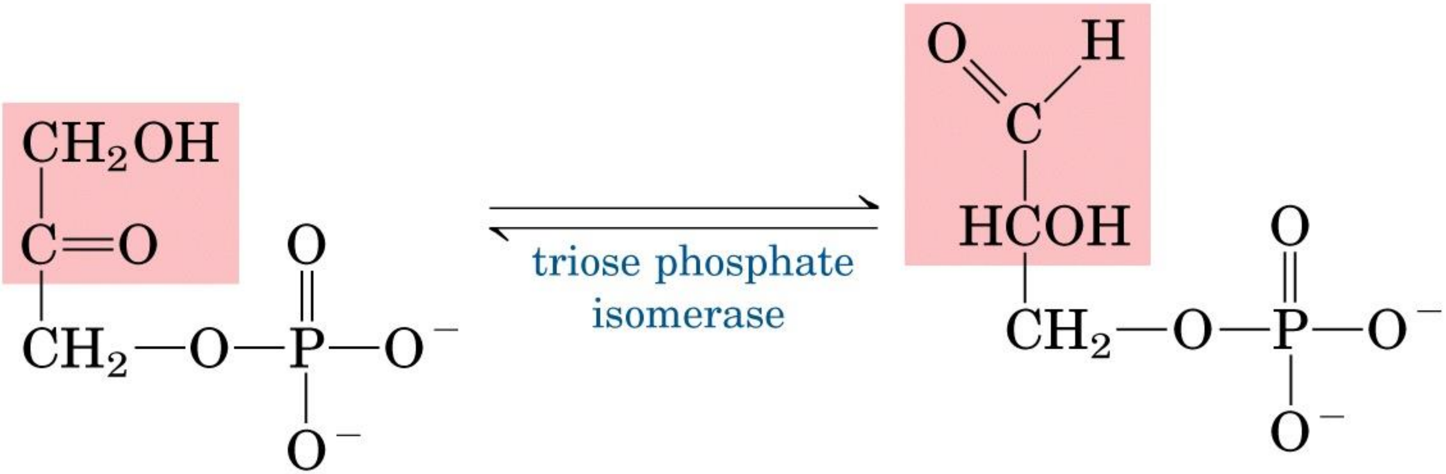
$\Delta G'^{\circ} = 23.8\ \text{kJ/mol}$

Aldolase acts in the reverse direction during the process of **gluconeogenesis**


Fructose 1,6-bisphosphate
aldolase
Derived from glucose carbon
1
2
3
Derived from glucose carbon
4
5
6
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
triose phosphate isomerase

(a)

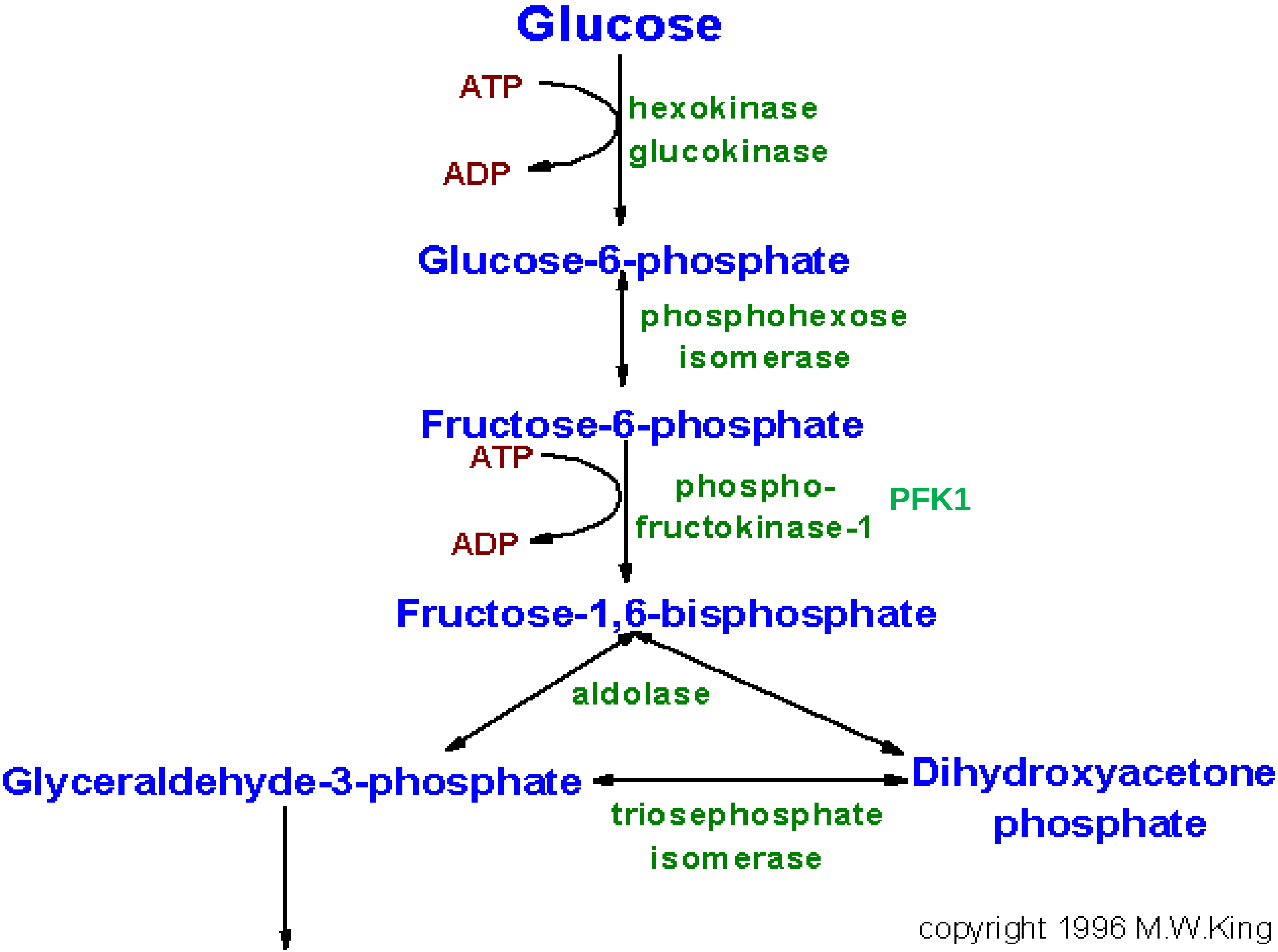
Reaction 5



Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

$\Delta G'^{\circ} = 7.5\ \text{kJ/mol}$


Glucose
ATP
ADP
hexokinase
glucokinase
Glucose-6-phosphate
phosphohexose
isomerase
Fructose-6-phosphate
ATP
ADP
phospho-
fructokinase-1
PFK1
Fructose-1,6-bisphosphate
aldolase
Glyceraldehyde-3-phosphate
Dihydroxyacetone
phosphate
triosephosphate
isomerase

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End of First Phase:

- Production of two glyceraldehyde 3-phosphate molecules from one glucose molecule with the expenditure of two ATPs.
- Therefore: the energy yields of the following steps are multiplied by two.

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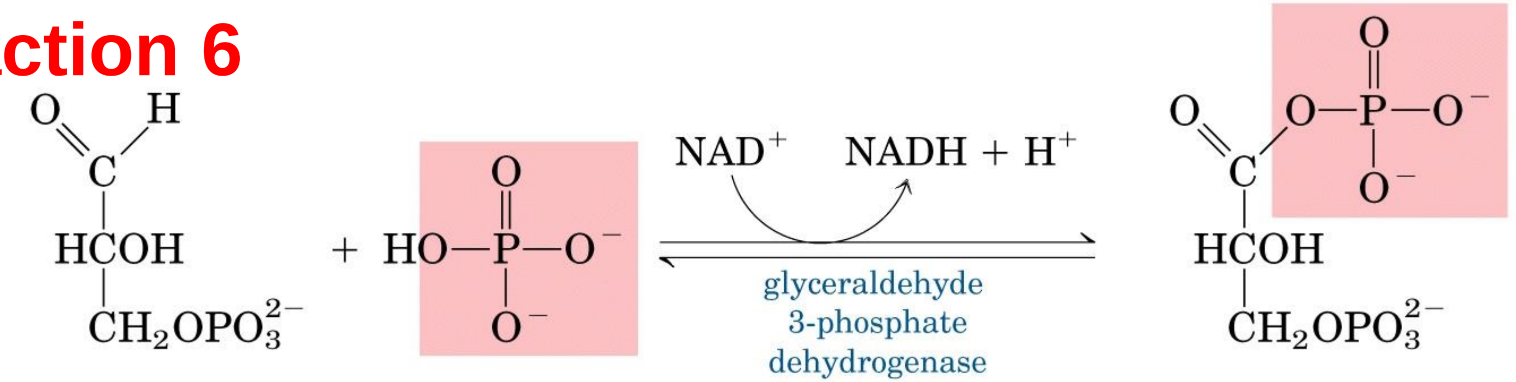
Second Phase:

Reaction 6



Glyceraldehyde 3-phosphate + Inorganic phosphate ⇌ 1,3-Bisphosphoglycerate

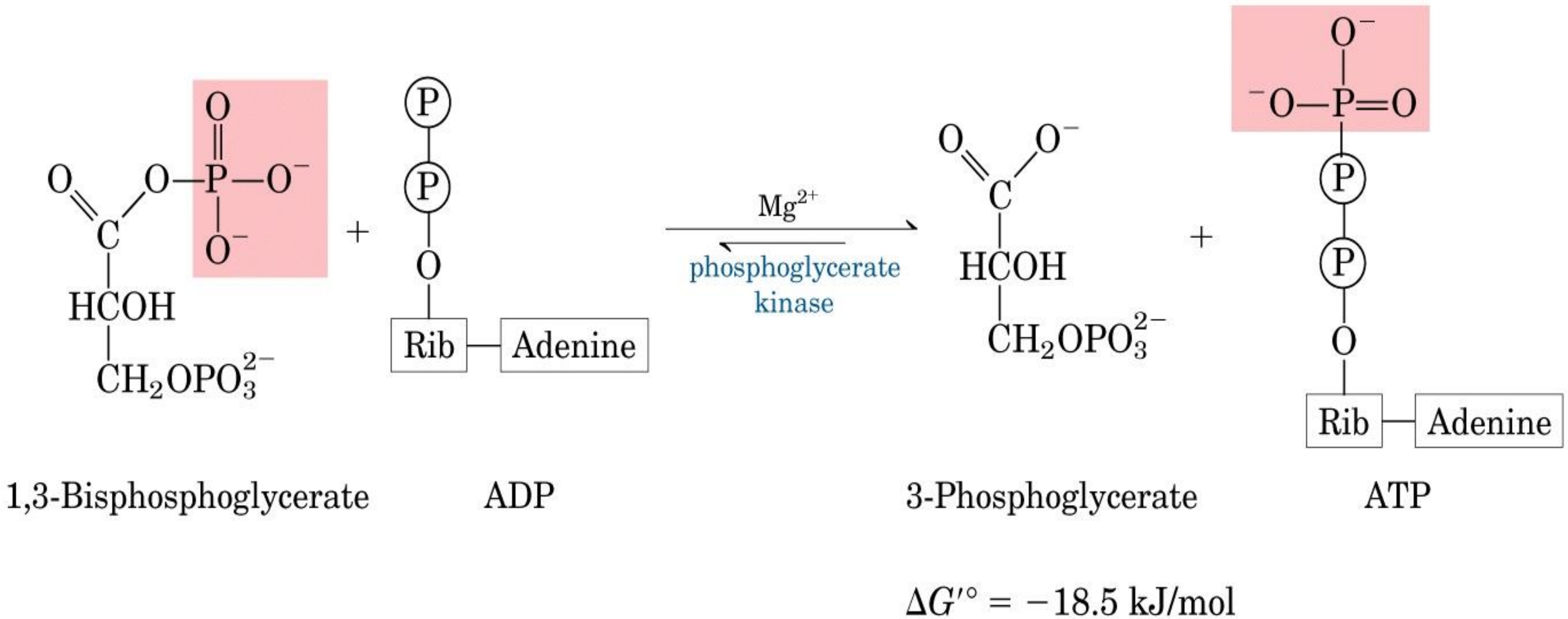
$\Delta G'^{\circ} = 6.3 \text{ kJ/mol}$

- The aldehyde group of glyceraldehyde 3-phosphate is oxidized, not to a free carboxyl group but to a carboxylic acid anhydride with phosphoric acid. This type of anhydride, called an **acyl phosphate, has a very high standard** free energy of hydrolysis that is conserved by formation of the acyl phosphate group at C-1.

1ST energy-conserving reaction of glycolysis that eventually lead to the formation of ATP.

Reaction 7

ATP formation, substrate-level phosphorylation



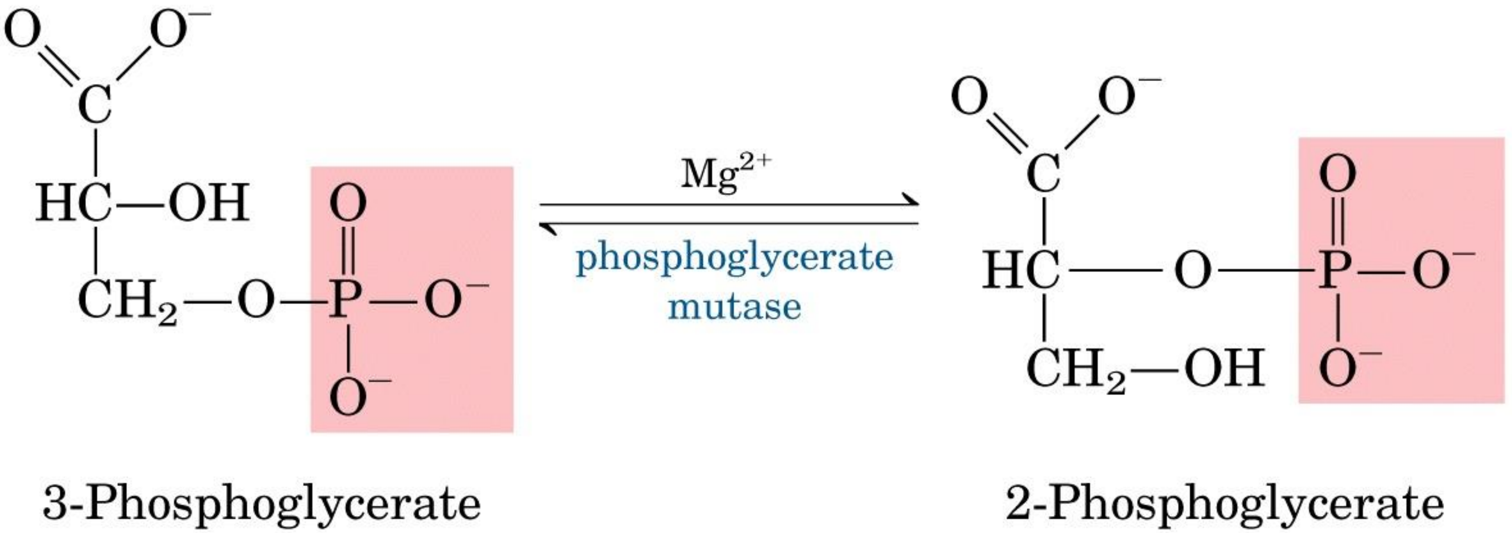
Notice that phosphoglycerate kinase is named for the reverse reaction. it catalyzes the reaction in both directions. This enzyme acts in the direction suggested by its name during gluconeogenesis.

- The formation of ATP by phosphoryl group transfer from a substrate such as 1,3-bisphosphoglycerate is referred to as a **substrate-level phosphorylation, to distinguish this mechanism from respiration-linked phosphorylation.**

- **Substrate-level phosphorylations** involve soluble enzymes and chemical intermediates (1,3-bisphosphoglycerate in this case).

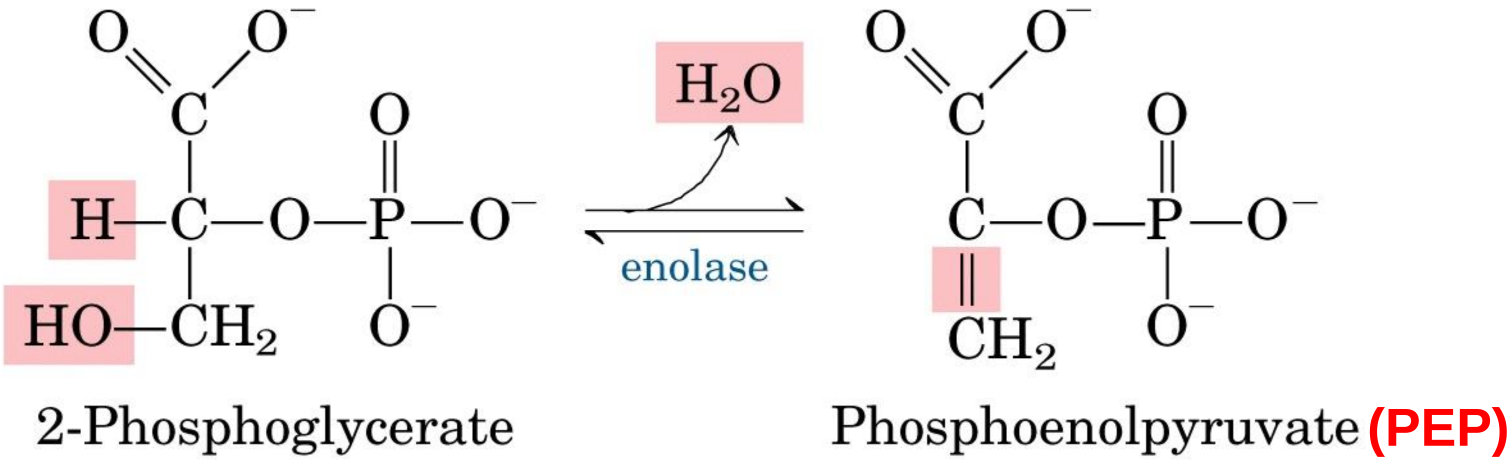
- **Respiration-linked phosphorylations**, on the other hand, involve membrane-bound enzymes and transmembrane gradients of protons

Reaction 8



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

Reaction 9

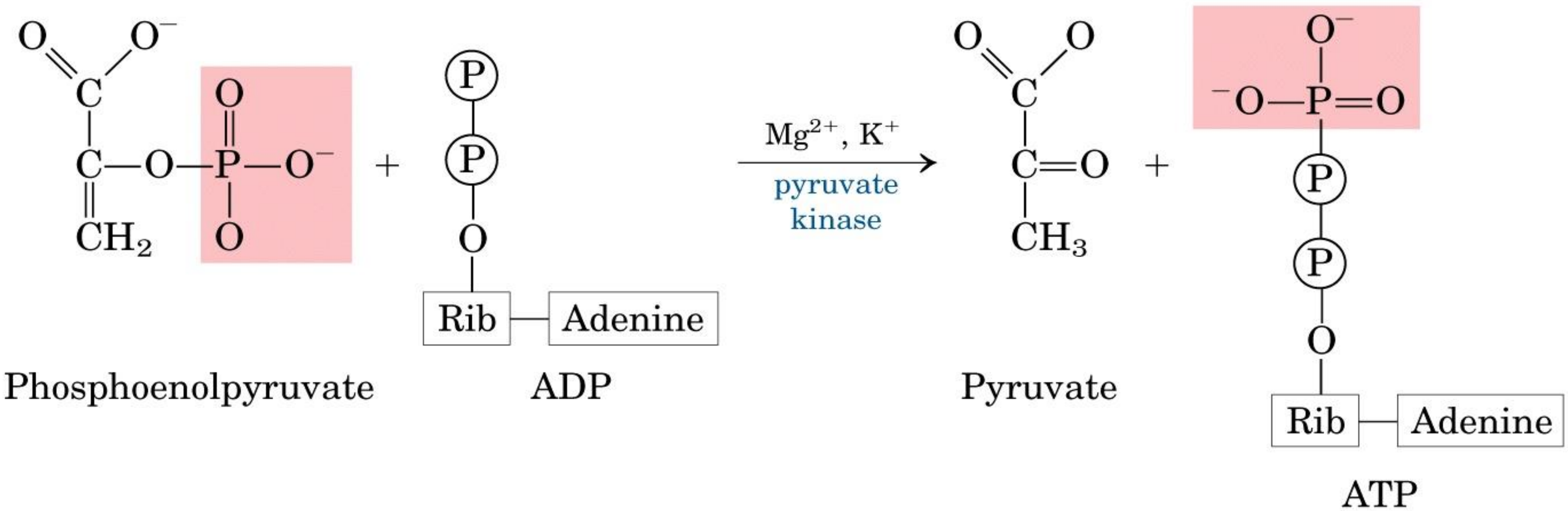


(PEP)

$\Delta G'^{\circ} = 7.5$ kJ/mol

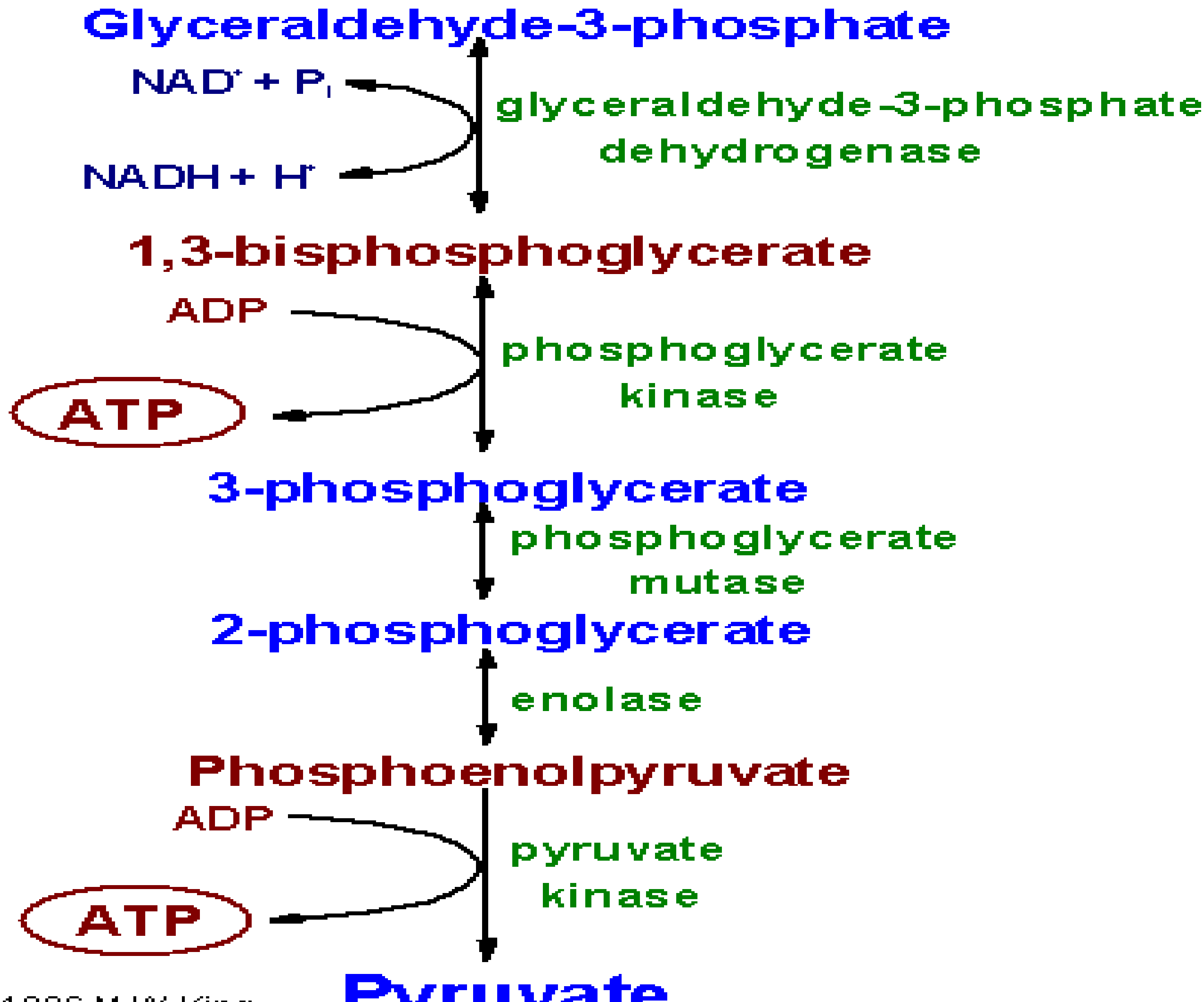
2nd energy-conserving reaction of glycolysis that eventually lead to the formation of ATP.

Reaction 10:
ATP formation, substrate-level phosphorylation



$\Delta G'^{\circ} = -31.4$ kJ/mol

The pyruvate kinase reaction is essentially irreversible under intracellular conditions and is an important site of regulation.



Glyceraldehyde-3-phosphate
NAD+ + Pi
NADH + H+
glyceraldehyde-3-phosphate dehydrogenase
1,3-bisphosphoglycerate
ADP
ATP
phosphoglycerate kinase
3-phosphoglycerate
phosphoglycerate mutase
2-phosphoglycerate
enolase
Phosphoenolpyruvate
ADP
ATP
pyruvate kinase
Pyruvate

The Overall Balance Sheet Shows a Net Gain of ATP

Glucose + 2ATP + 2NAD + 4ADP + 2Pi $\longrightarrow$

2 pyruvate + 2ADP + 2NADH + 2H + 4ATP + 2H2O

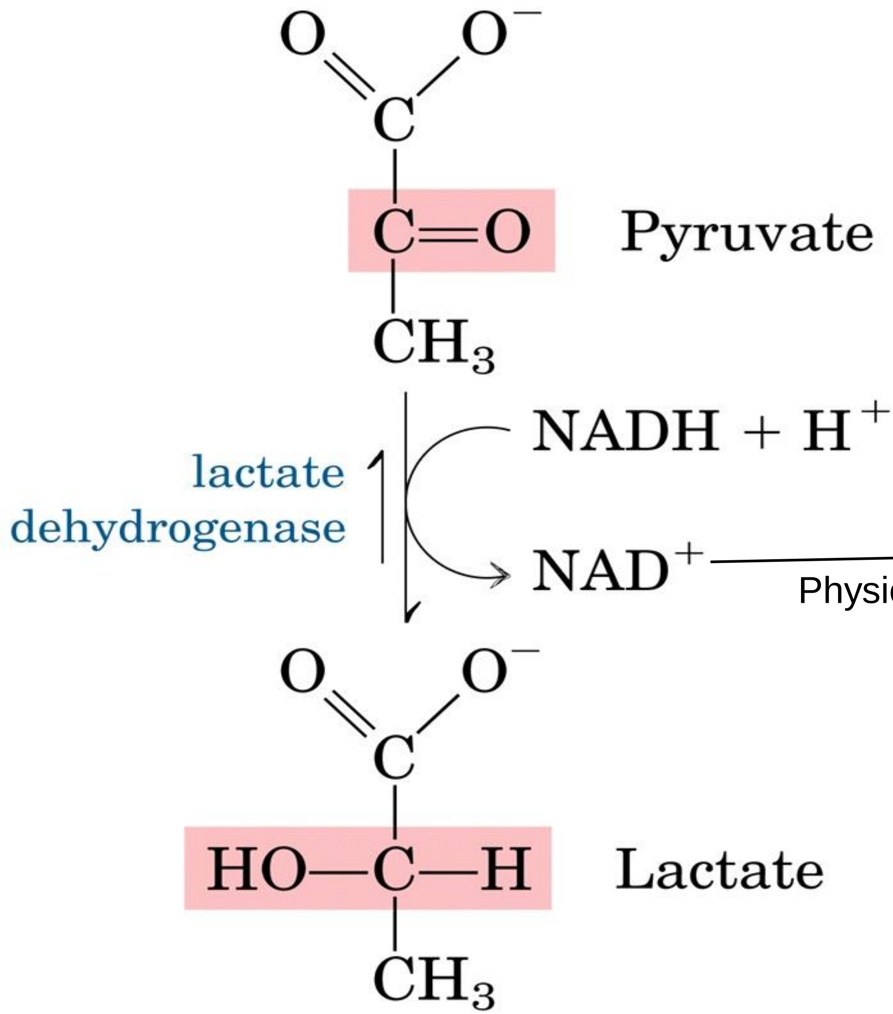
Canceling out common terms on both sides of the equation gives the overall equation for glycolysis under aerobic conditions:

Glucose + 2NAD + 2ADP + 2Pi $\longrightarrow$

2 pyruvate + 2NADH + 2H + 2ATP + 2H2O

The two molecules of NADH provides the energy for synthesis of ATP by respiration linked phosphorylation (oxidative phosphorylation)

Fermentation

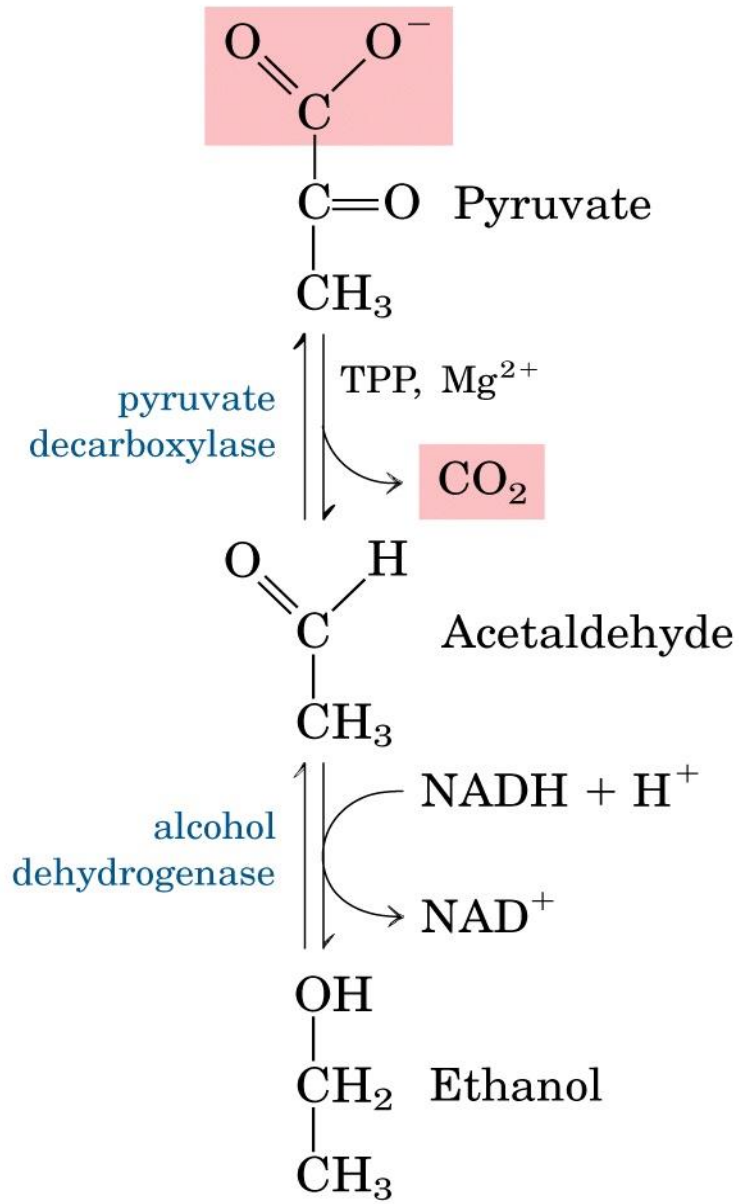


Physical requirement of glycolysis

Rx 6

$$\Delta G'^{\circ} = -25.1 \text{ kJ/mol}$$

Some cells ferment pyruvic acid to lactic acid



Other cells ferment pyruvic acid to alcohol