Glycolysis

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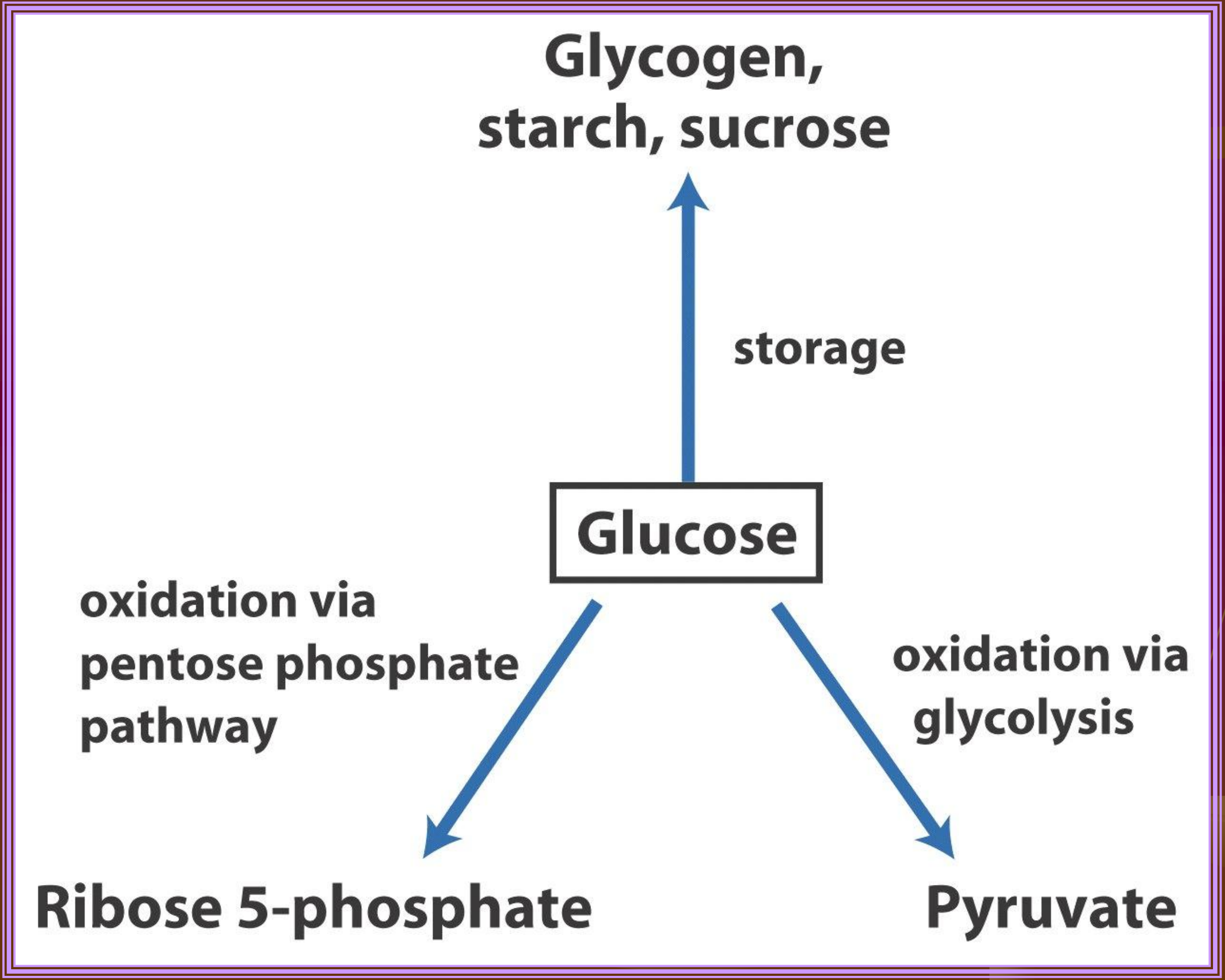
Glycolysis

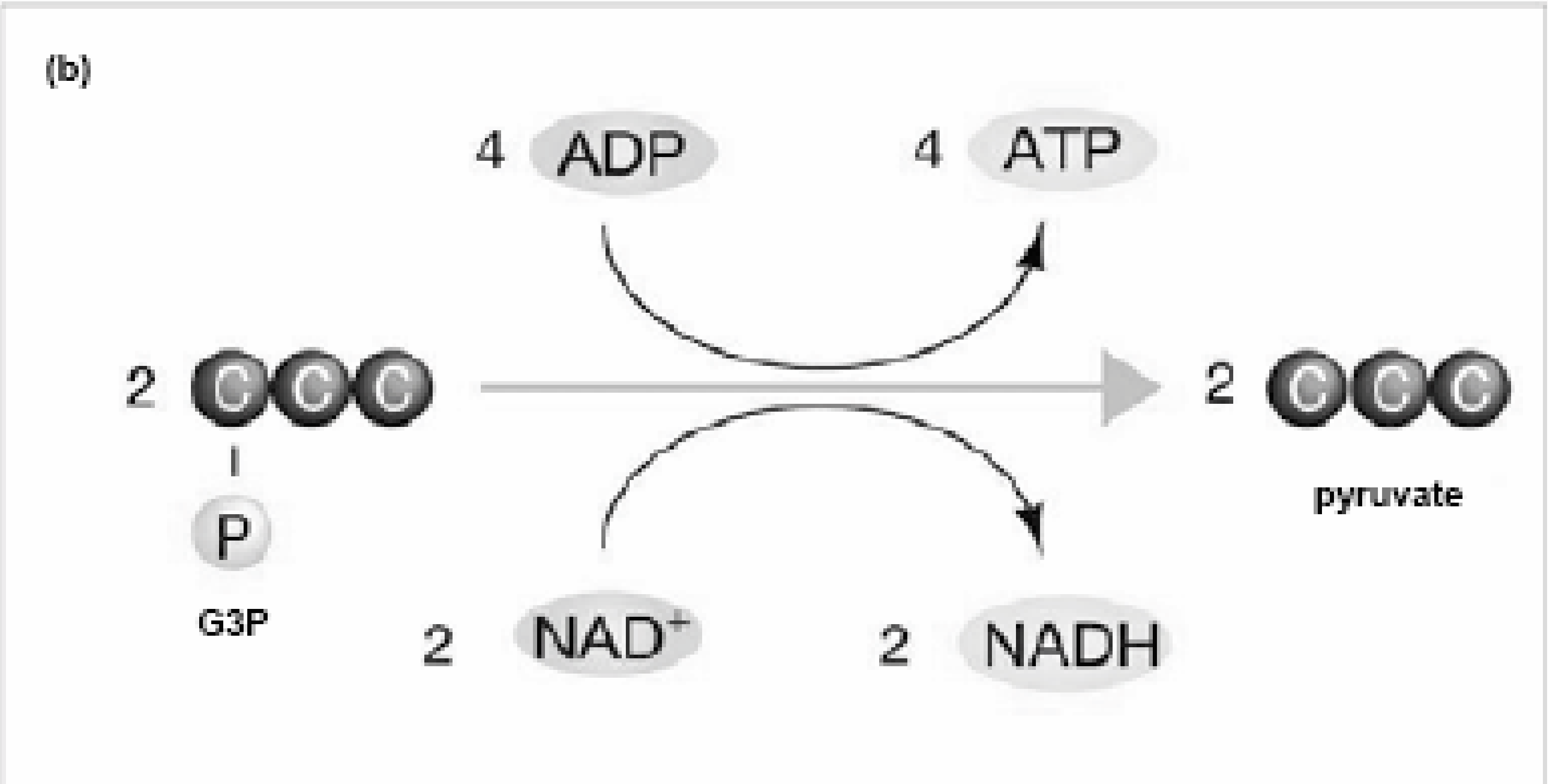
- **Universal energy-harvesting process of life, very primitive**
  - **Evolved early in life's history**
  - **Means "sugar-splitting"**
    - 6-C sugar glucose is split in half to make two 3-C compounds

Glycolysis

1. Occurs in the cytoplasm
2. Anaerobic, not really a part of aerobic respiration
3. Glucose molecule → 2 pyruvic acid molecules (3 carbons)
4. 2 ATP used up, 4 ATP formed
5. 2 NADH formed
6. Pyruvic acid and NADH enter mitochondria

Major Pathways For Glucose





Energy harvest

- Glycolysis converts D-glucose to two molecules of pyruvate.
- This process gives the net synthesis of two ATP and two NADH molecules.
- Glycolysis is only the first step of aerobic metabolism.
- D-glucose is the major fuel of most organisms. In glycolysis, glucose is degraded in a series of steps to form two molecules of the three-carbon molecule pyruvate.
- Some of the free energy released in this process is conserved by forming ATP and NADH.
- This breakdown of glucose to smaller carbon molecules is likely to be the oldest form of enzyme-catalyzed catabolism, and even now it occurs in almost every cell of every organism.

Fermentation and respiration

- Heterotrophs obtain energy from oxidation/reduction reactions.
- Aerobes obtain most energy from respiration – the oxidation of organic fuels by molecular oxygen.
- Here glycolysis is only the beginning.
- The products are further broken down and oxidized. Anaerobes obtain energy from fermentation – passing electrons from one organic intermediate to another. They only do the glycolysis part and stop there.

Cellular Respiration

- Cellular respiration is the name given to metabolic pathways in which cells harvest the energy from the metabolism of food molecules

- Glucose breakdown is one of the main pathways of cellular respiration and occurs in 3 stages

  - Glycolysis

  - Krebs cycle

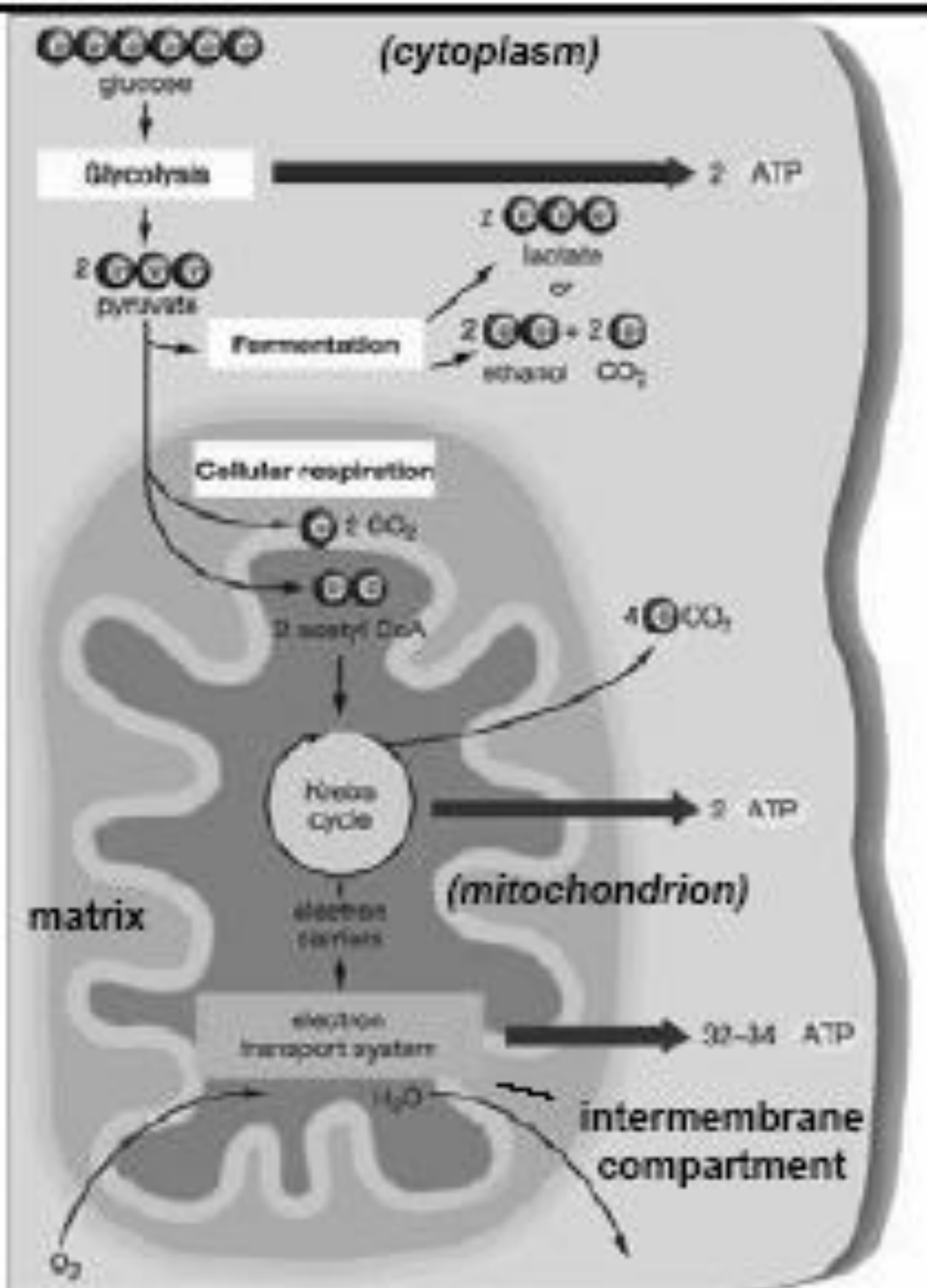
  - Electron transport system

Harvesting energy from glucose

- A. Glycolysis occurs in the cytoplasm and splits glucose in half, producing 2 ATP

- B. In the mitochondria, an additional 34-36 ATP are produced

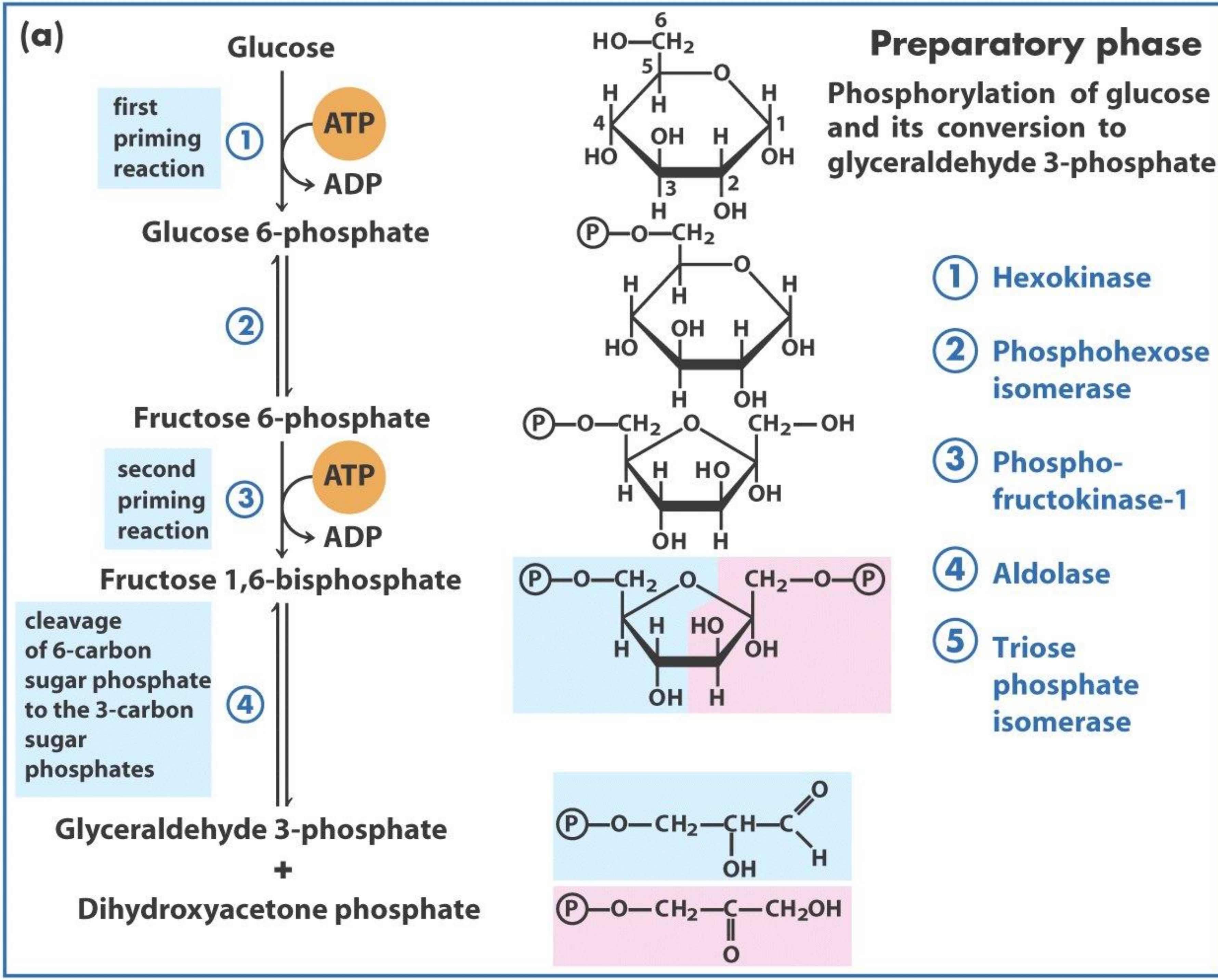
- C. In the absence of oxygen, fermentation occurs, producing only 2 total ATP per glucose


(cytoplasm)
glucose
Glycolysis
2 ATP
2 pyruvate
2 lactate
or
Fermentation
2 ethanol + 2 CO_2
Cellular respiration
2 CO_2
2 acetyl CoA
4 CO_2
Krebs cycle
2 ATP
(mitochondrion)
matrix
electron carriers
electron transport system
32–34 ATP
H_2O
intermembrane compartment
O_2

Two stages of glycolysis

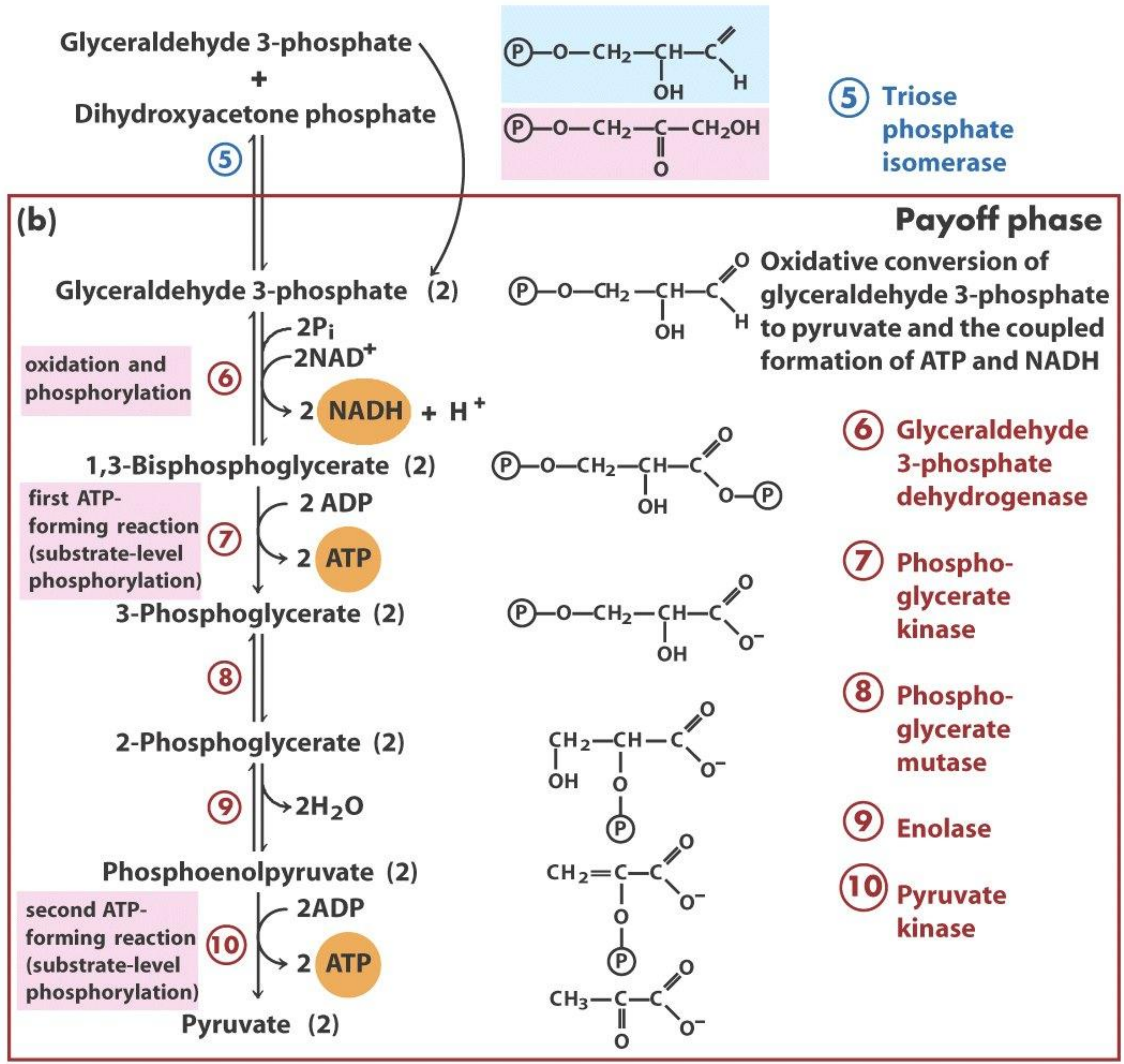
1) **preparatory phase.**

- Starting from D-glucose, there are two phosphorylations (at the expense of ATP).
- The sugar is split to two molecules, ultimately giving two molecules of glyceraldehyde-3-phosphate.


(a)
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to the 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase

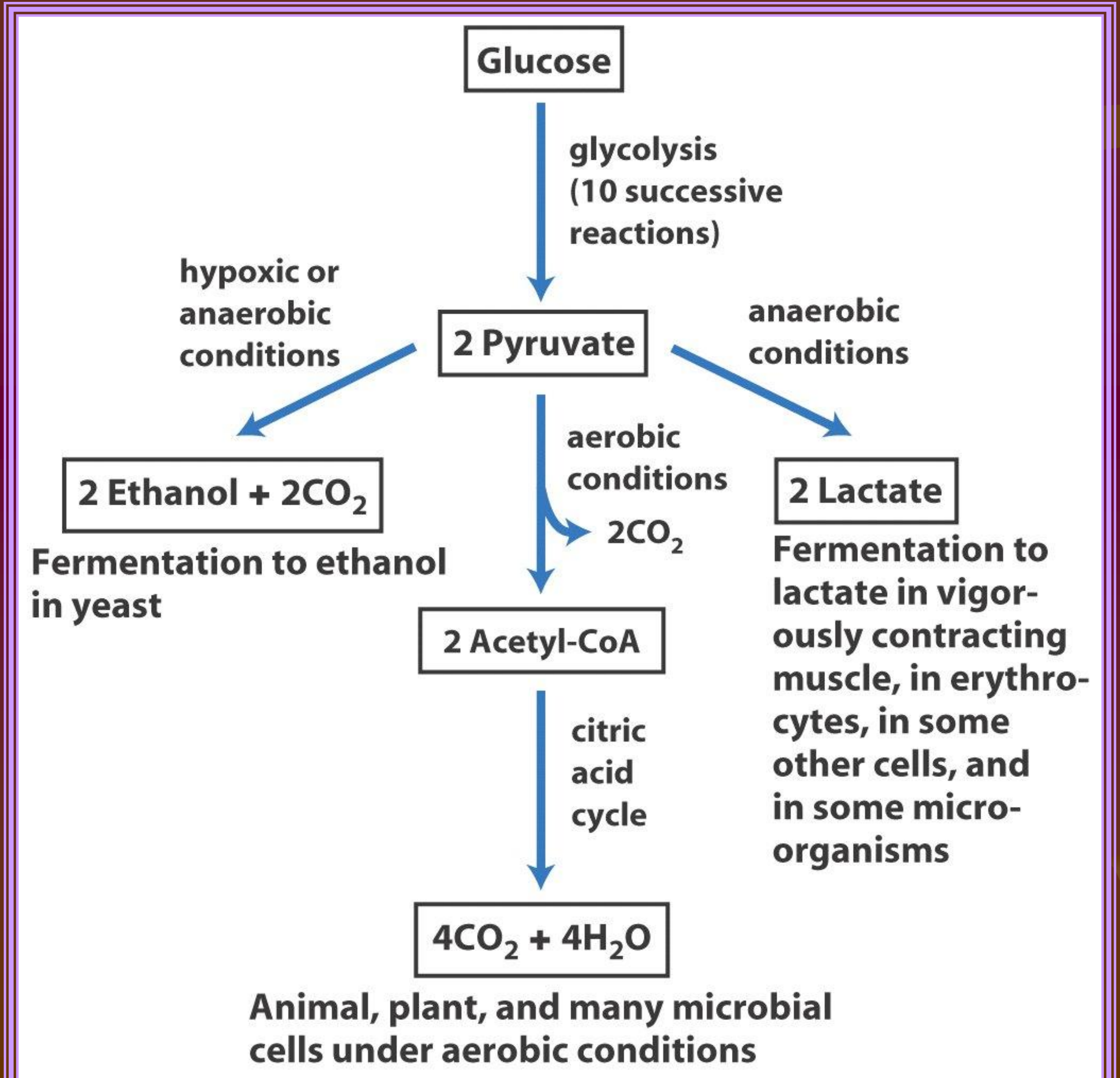
2) **Then comes the payoff phase.**

- Each molecule of glyceraldehyde-3-phosphate is converted in several steps to pyruvate, giving synthesis of 2 molecules of ATP and one molecule of NADH.
- The net yield from one glucose is 2 molecules of ATP and 2 molecules of NADH (remember that there are two molecules of glyceraldehyde-3-phosphate for each molecule of glucose).



Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
5 Triose phosphate isomerase
(b)
Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
Glyceraldehyde 3-phosphate (2)
2Pi
2NAD+
oxidation and phosphorylation
6
2 NADH + H+
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2 ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H2O
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phosphoglycerate kinase
8 Phosphoglycerate mutase
9 Enolase
10 Pyruvate kinase

Further Metabolism of Pyruvate



- Pyruvate is then further metabolized via one of three routes .
- It can be oxidized to give the acetyl group of acetyl-coenzyme A, which is then oxidized completely to give CO2.
- It can be reduced to lactate (a type of fermentation).
- It can be converted to ethanol and CO2 (another type of fermentation)

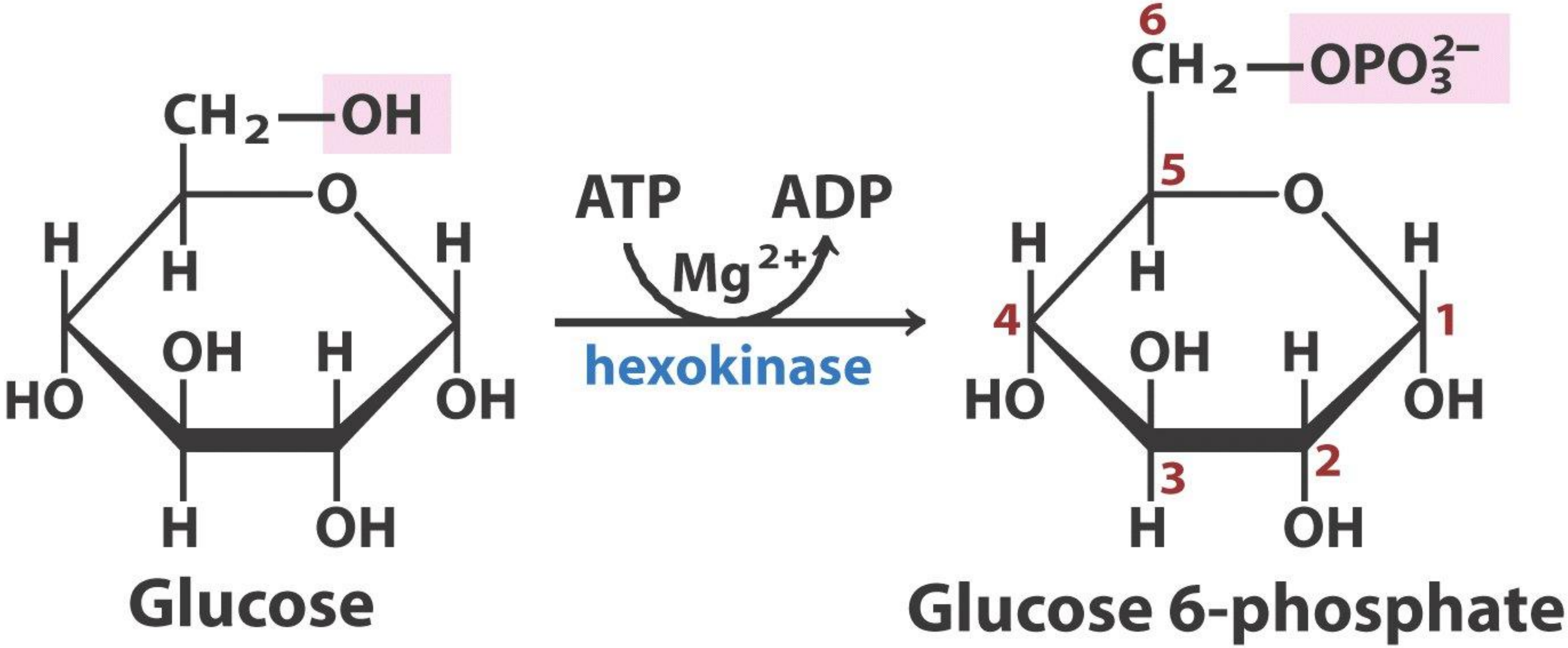
Importance of phosphorylated intermediates

- All intermediates in glycolysis are phosphorylated. These phosphoryl groups appear to have three functions.
- Negative charge prevents the intermediates from leaving the cell
- Phosphates are donated to ADP to form ATP
- Binding energy from phosphates allows glycolytic enzymes to increase in efficiency.

The preparatory phase of glycolysis

- In the first stage of glycolysis, two molecules of ATP are ‘invested’, phosphorylating the hexose sugar to give fructose 1,6-bisphosphate.
- This is then cleaved, giving two molecules of glyceraldehyde 3-phosphate at the end of the preparatory phase.
- The name glycolysis means ‘sugar splitting’ to describe this cleavage reaction.

Rxn 1: Phosphorylation of Glucose



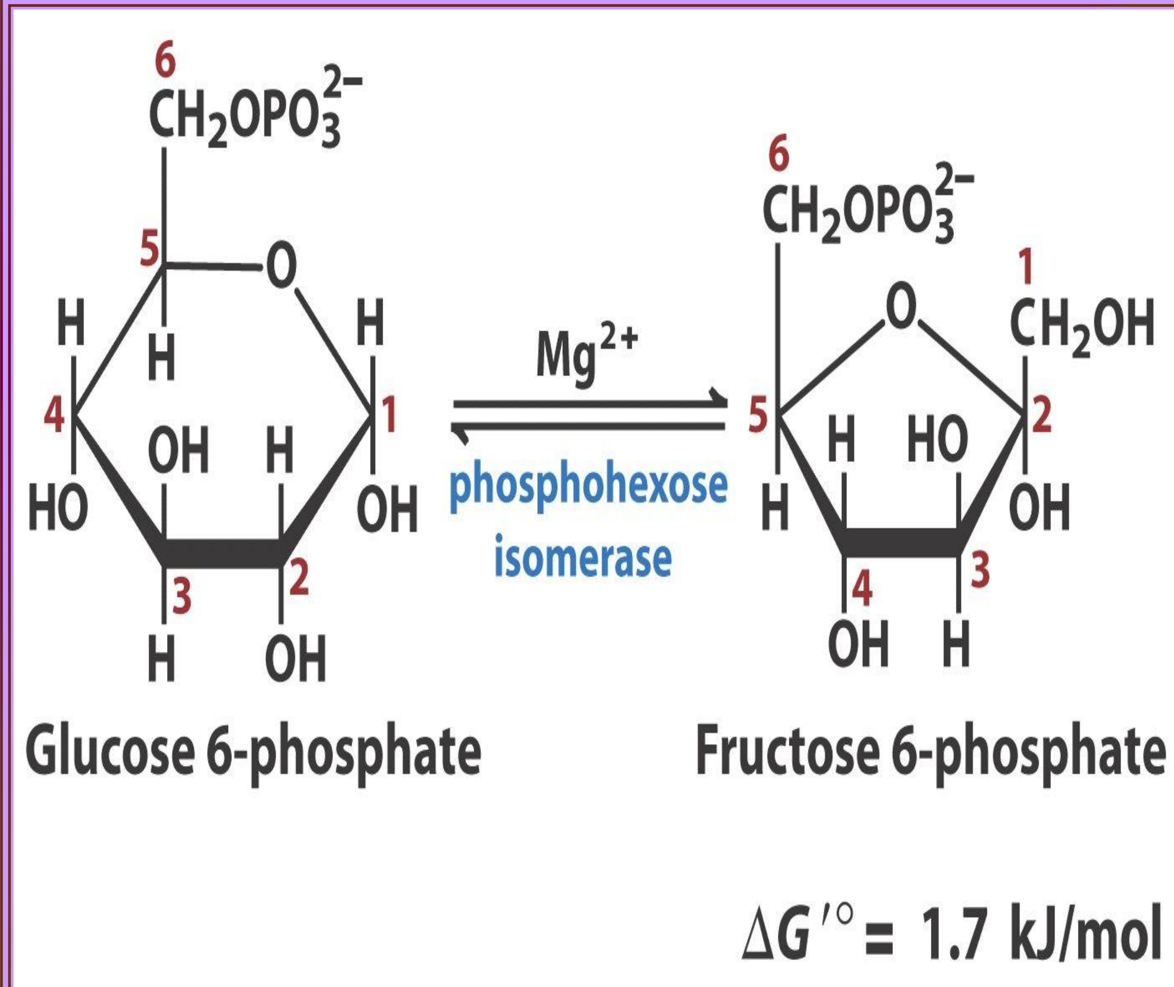
$\Delta G'^{\circ} = -16.7\ \mathrm{kJ/mol}$

1. Phosphorylation of glucose:

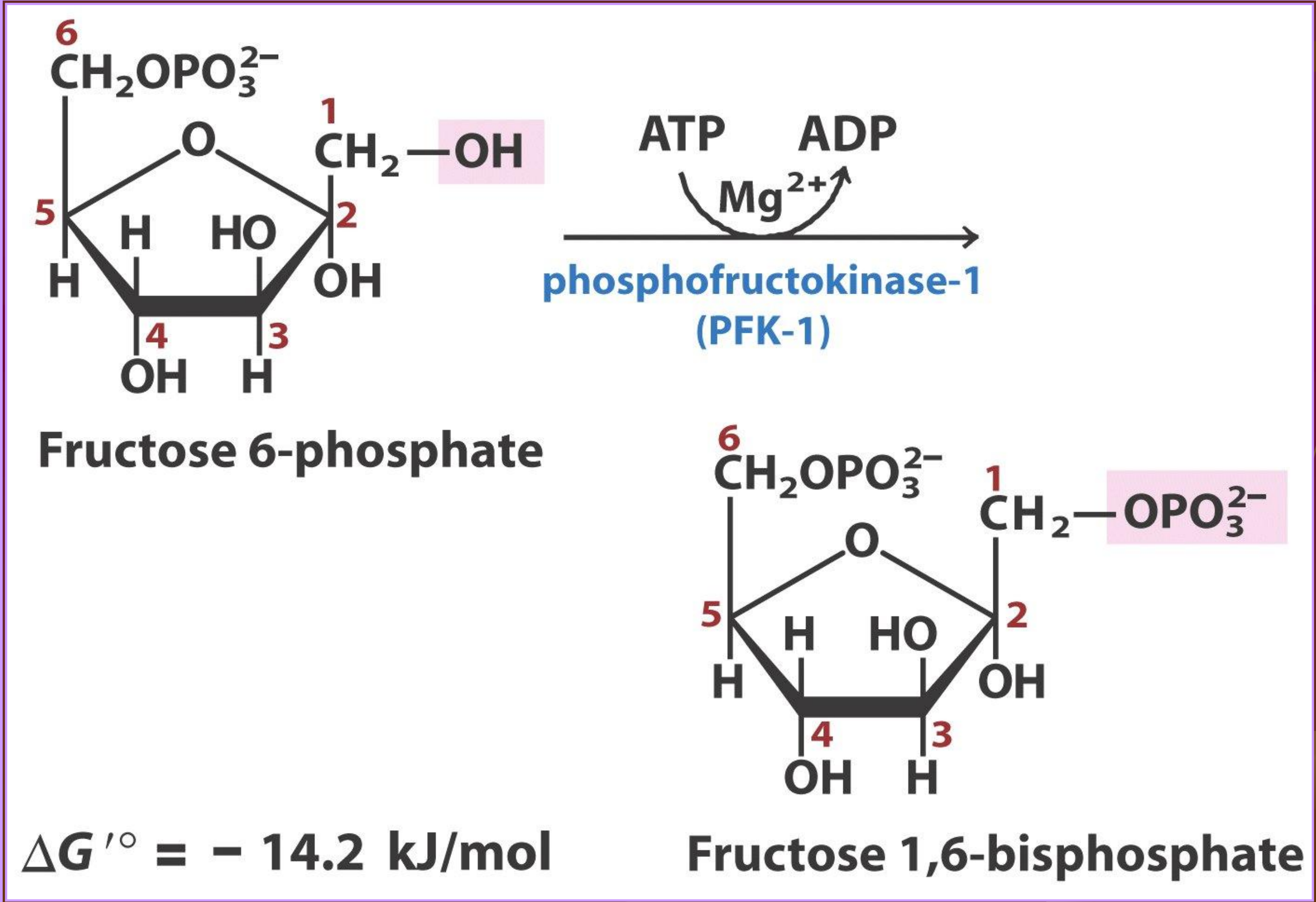
- Reaction is at C6 to give glucose-6-phosphate
- Catalyzed by the enzyme **hexokinase** (remember from the enzyme section?), with ATP as the phosphoryl donor
- a-D-glucose + ATP → a-D-glucose-6-phosphate + ADP
- Reaction is essentially irreversible under cellular conditions
- This reaction and essentially all that involve ATP also require Mg2+. This is because ATP is always complexed with Mg2+ in the cell, so the substrate is better thought of as MgATP2– instead of ATP4–.

Rxn 2: Conversion to Fructose 6-phosphate

- Catalyzed by phophohexose isomerase
- Reaction is readily reversible – DG'° = 1.7 kJ/mol

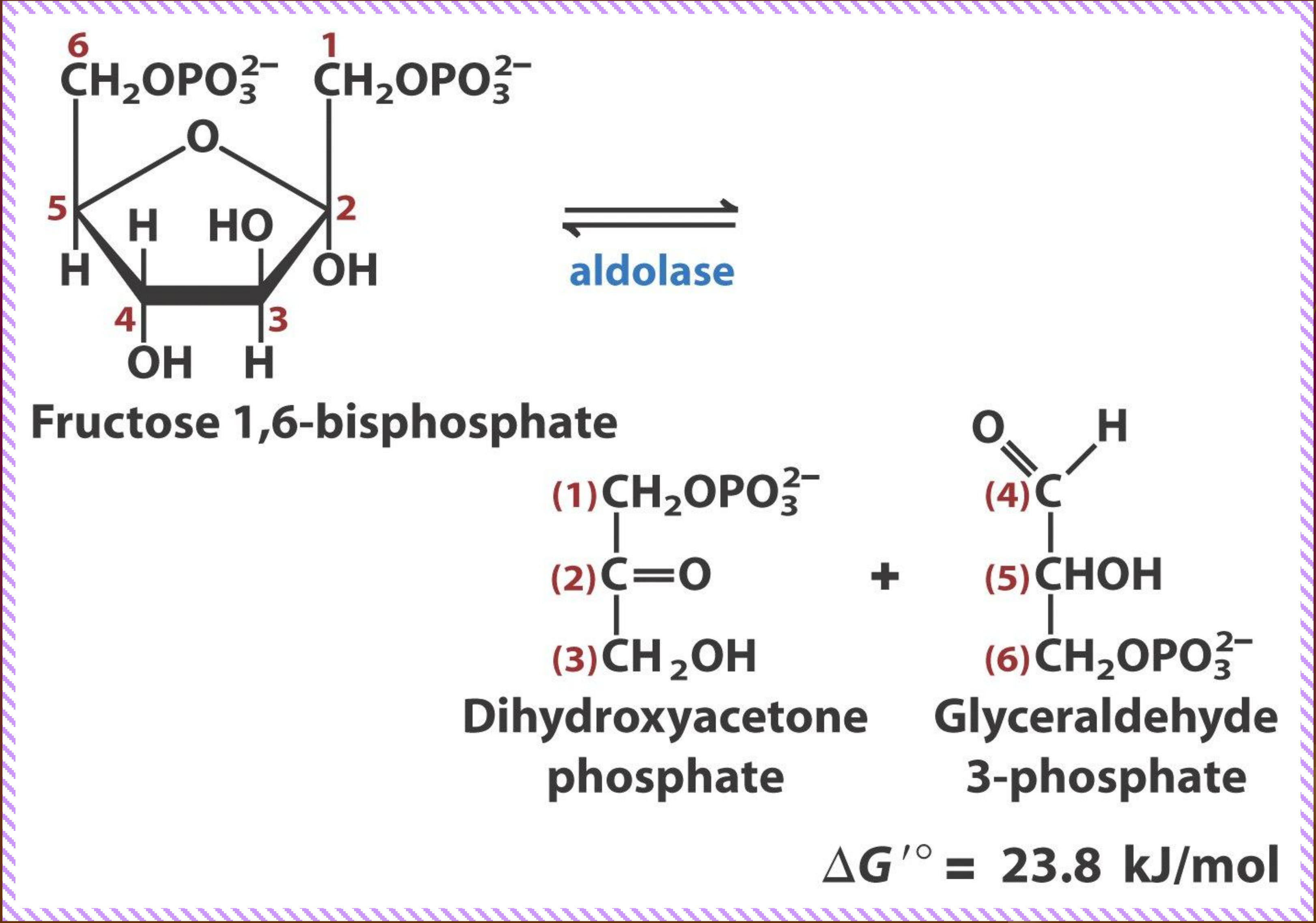


Rxn 3: Phosphorylation to Fructose 1,6-bisphosphate



- This is the second of the two ‘priming’ phosphorylation reactions.
- Catalyzed by **phosphofructokinase (PFK-1)**.
- The reaction is essentially irreversible
- This reaction is a major point of regulation in glycolysis. Activity is increased when supply of ATP is low or when ADP and AMP are at high concentration. Activity is inhibited when ATP is high and is well-supplied with other fuel sources – *i.e.* fatty acids.

Rxn 4: Cleavage to DHAP and G 3-P



Cleavage of fructose 1,6-bisphosphate

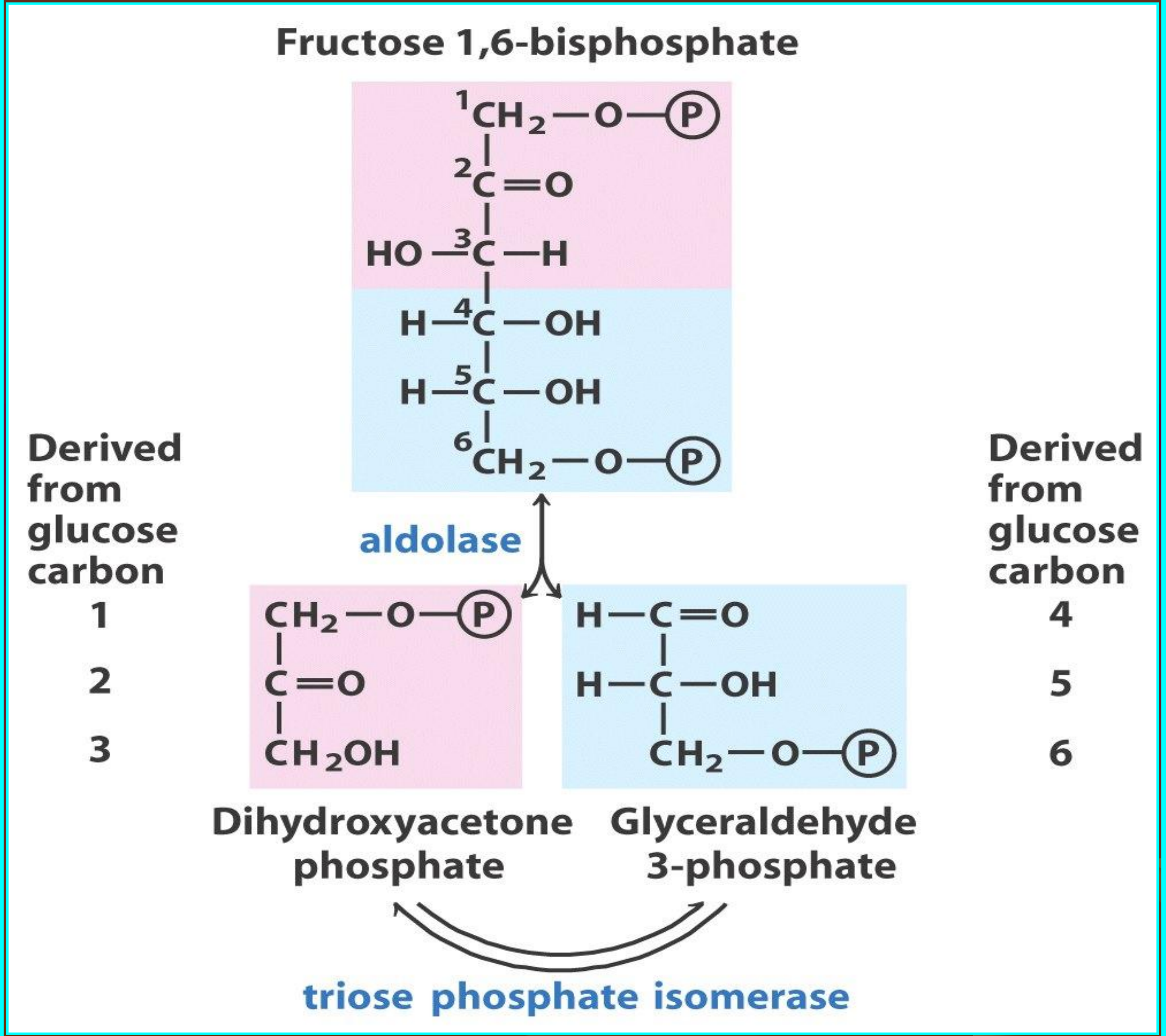
- Fructose 1,6-bisphosphate → dihydroxyacetone phosphate + glyceraldehyde 3-phosphate
- This reaction gives two triose phosphates from one molecule of the 6-carbon fructose 1,6-bisphosphate
- Catalyzed by fructose 1,6-bisphosphate aldolase, or just **aldolase**
- Although this reaction has a positive standard-state DG (23.8 kJ/mol), it proceeds in glycolysis because the triose phosphate products undergo further reaction rapidly, keeping them at low concentration and making the reaction favorable.

Rxn 5: Conversion of DHAP to Glyceraldehyde 3-phosphate

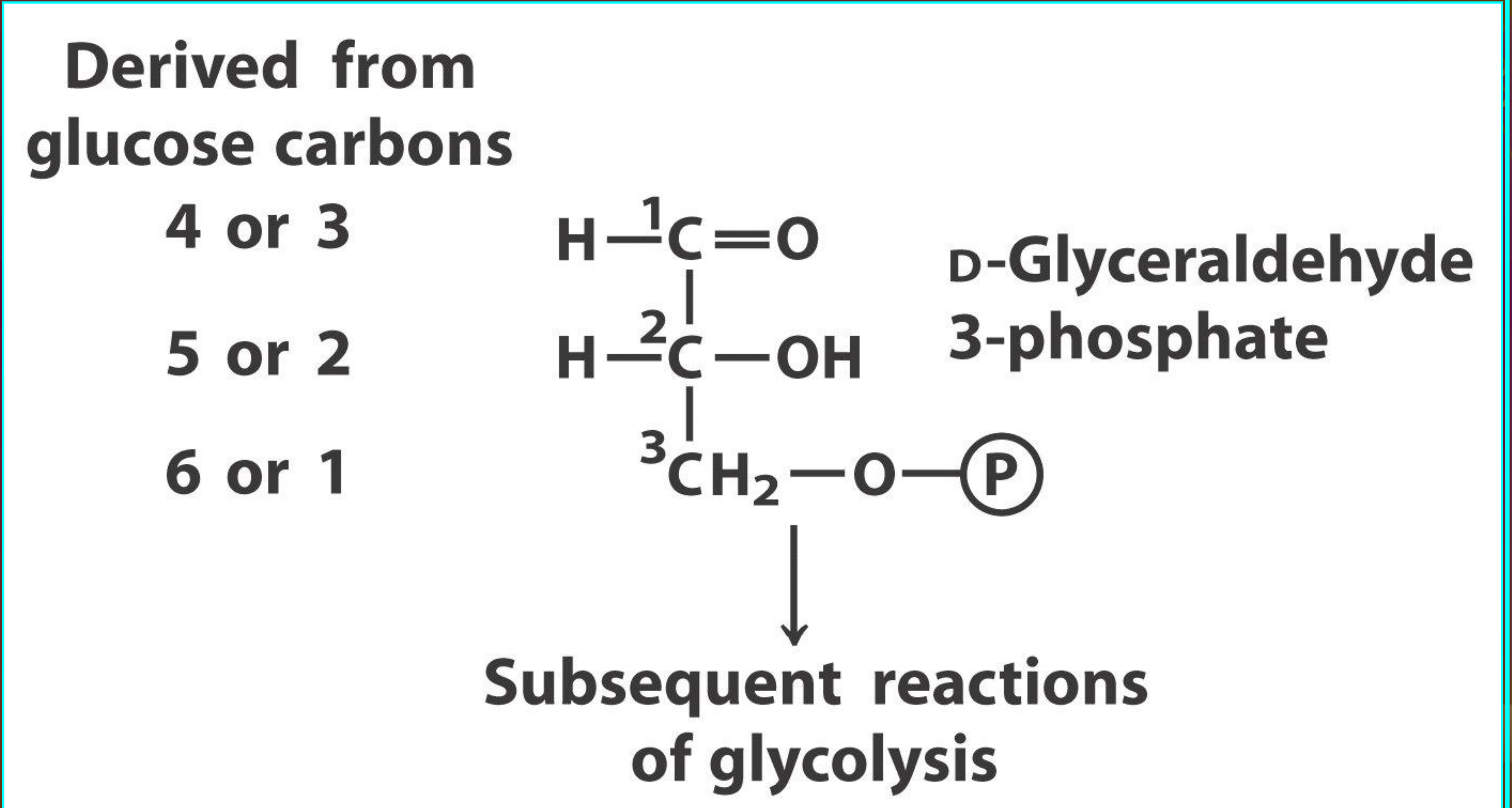
- Dihydroxyacetone phosphate → glyceraldehyde 3-phosphate
- Reaction catalyzed by **triose phosphate isomerase**

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

Fate of Carbon Atoms From Glucose

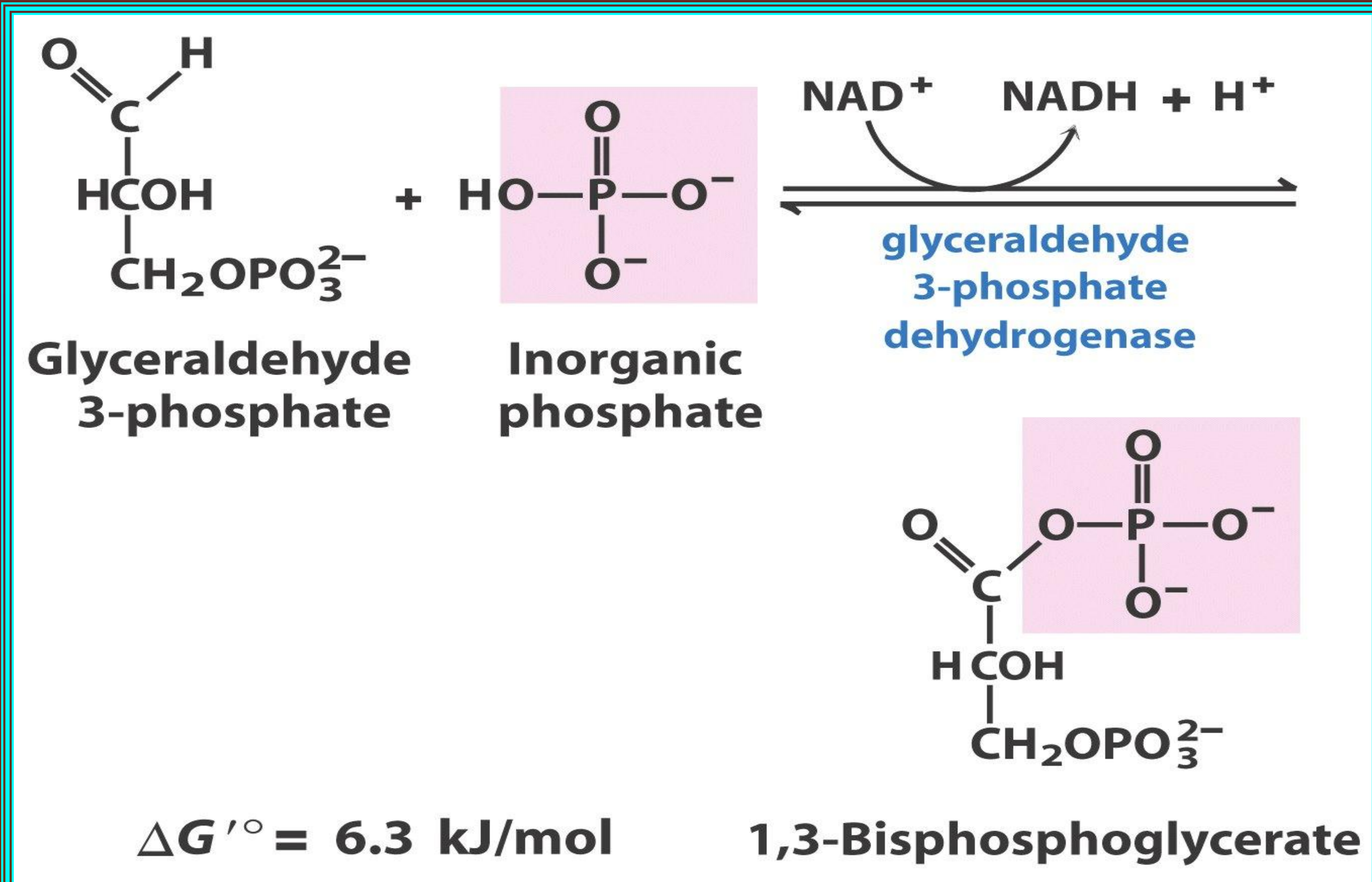


G 3-P Numbering and Origin of Carbon Atoms

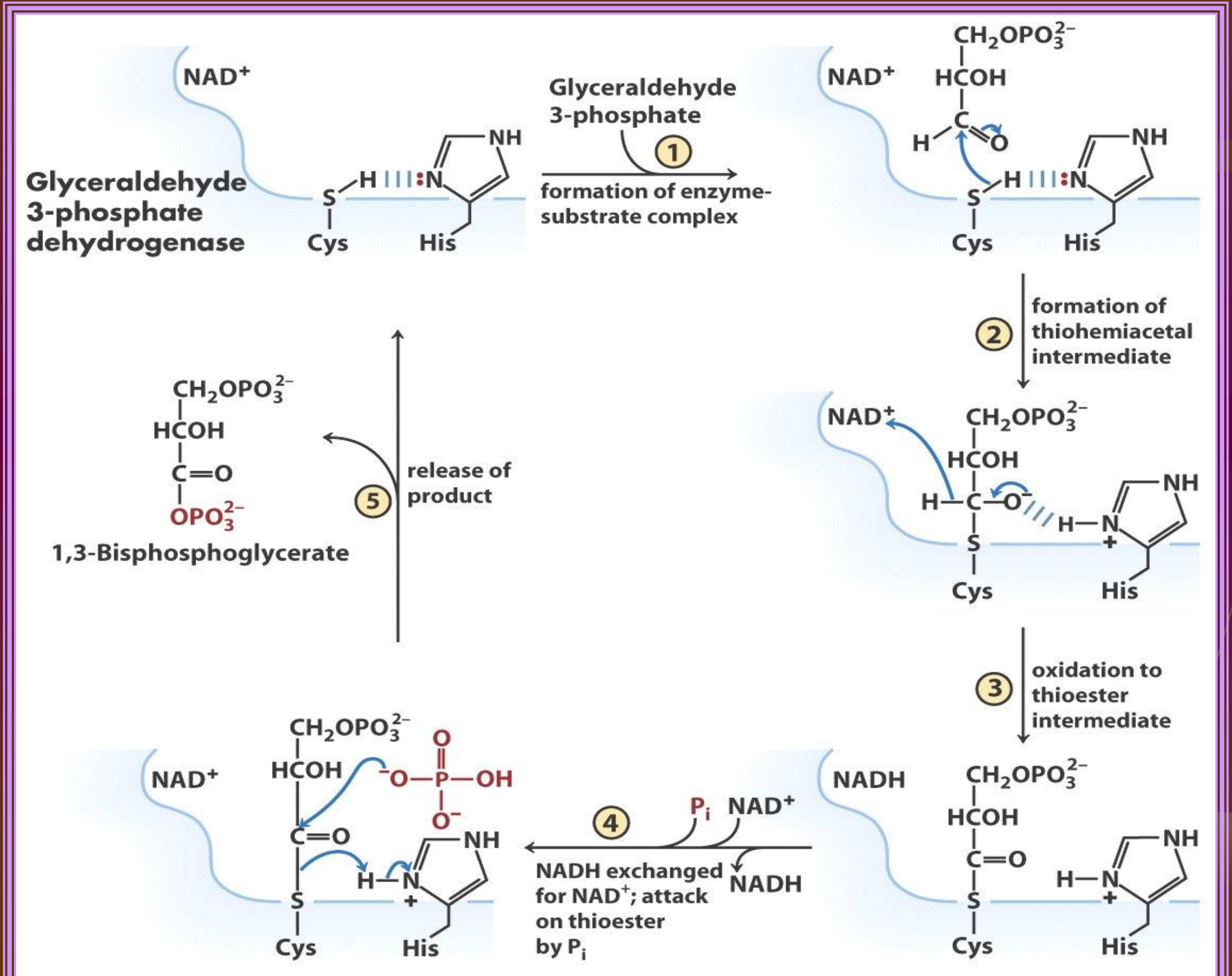


- *C. The payoff phase of glycolysis*
- Both molecules of glyceraldehyde 3-phosphate that are derived from one molecule of glucose now follow the same pathway in the payoff phase.
- Here the further reactions are coupled to the generation of ATP from ADP and the reduction of NAD+ to NADH.

Rxn 6: Oxidation to 1,3-bisphophoglycerate



Rxn 6: Glyceraldehyde 3-phosphate dehydrogenase

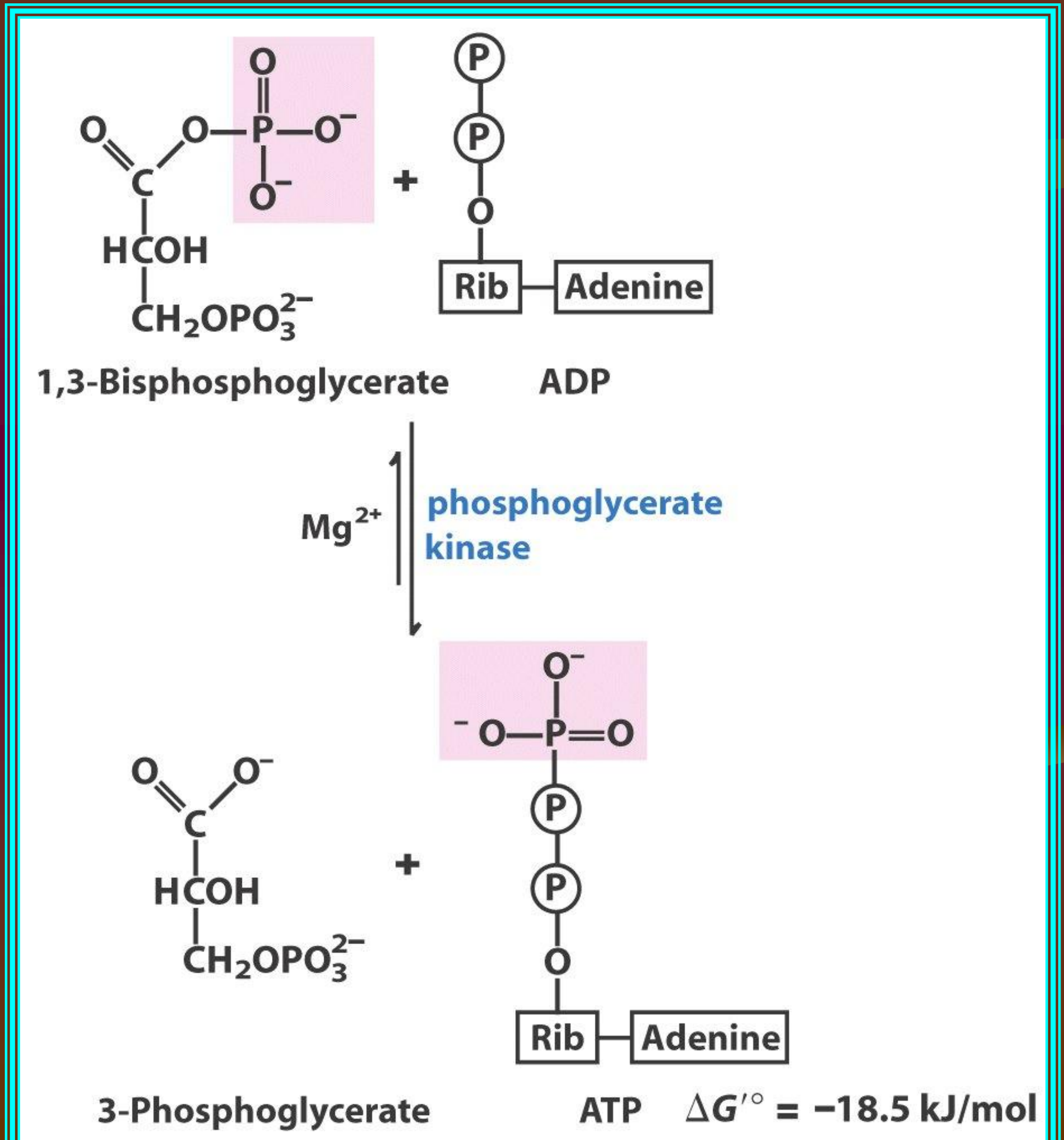


6. Oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate

- glyceraldehyde 3-phosphate + Pi + NAD+ → 1,3-bisphosphoglycerate + NADH + H+
- Reaction is catalyzed by **glyceraldehyde 3-phosphate dehydrogenase .**
- This is an oxidation reaction, but because the phosporyl group is added to the adehydic carbon to give an **acyl phosphate**, much of the energy is conserved (and will be used later to generate ATP).

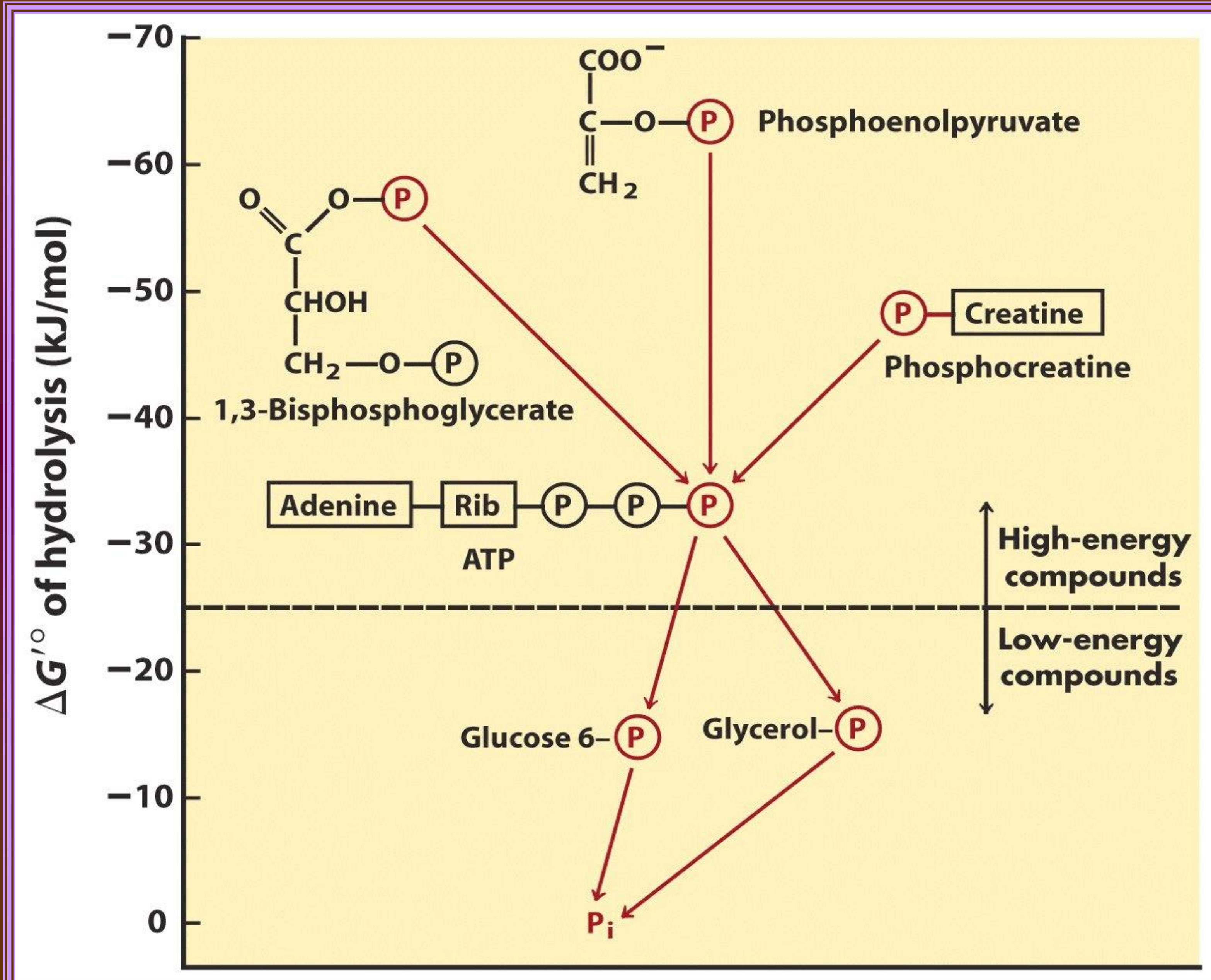
- Energy is conserved because this phosphoryl group is now highly activated to be transferred to ADP.
- The acceptor of hydrogen is NAD^+ to give NADH. The reduction of NAD^+ proceeds by enzymatic transfer of a hydride ion (:H–).
- The other hydrogen atom of the substrate molecule is released to solution as H^+.

Rxn 7: Phosphoryl Transfer to Form 3-Phosphoglycerate and ATP

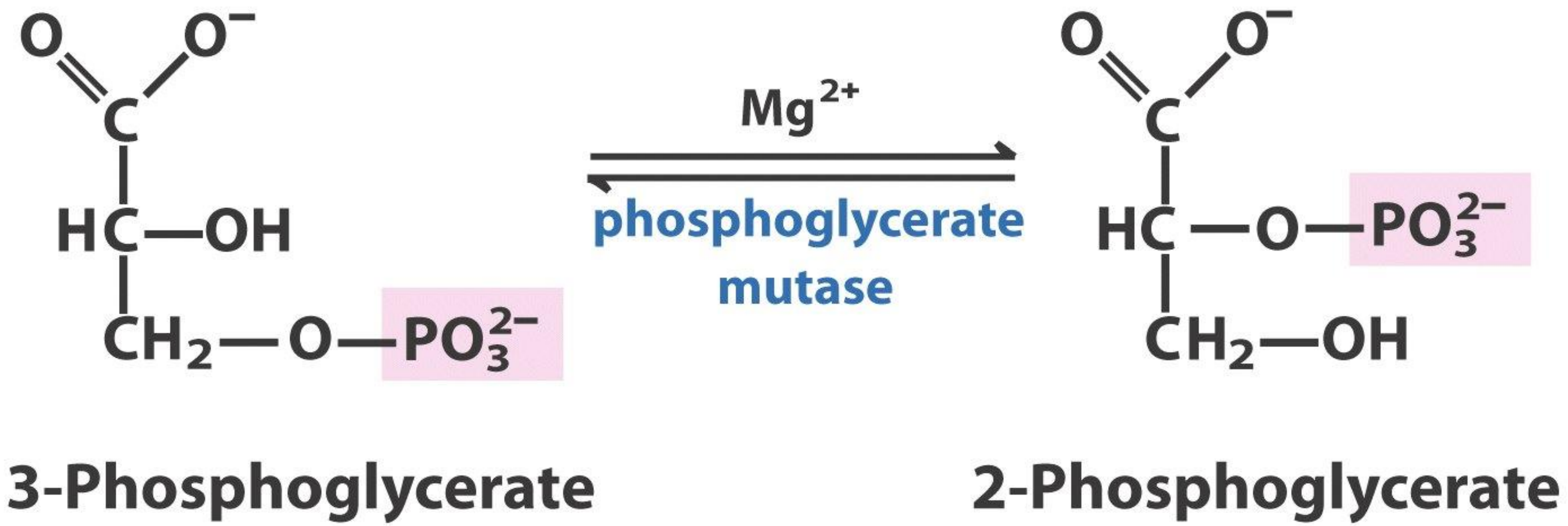


- 1,3-bisphosphoglycerate + ADP → 3-phosphoglycerate + ATP (DG'° = –18.5 kJ/mol)
- Reaction is catalyzed by the enzyme **phosphoglycerate kinase**. (Note that the enzyme is named for the reverse reaction).
- the formation of ATP here is referred to as **substrate-level phosphorylation** to distinguish it from **respiration-linked** phosphorylation (which we will cover later).
- In substrate-level phosphorylation, the phosphoryl group is transferred directly from a substrate, whereas respiration-linked phosphorylation involves the H+ gradient across a membrane, which is coupled to ATP generation.

Phosphoryl Group Transfer Potential

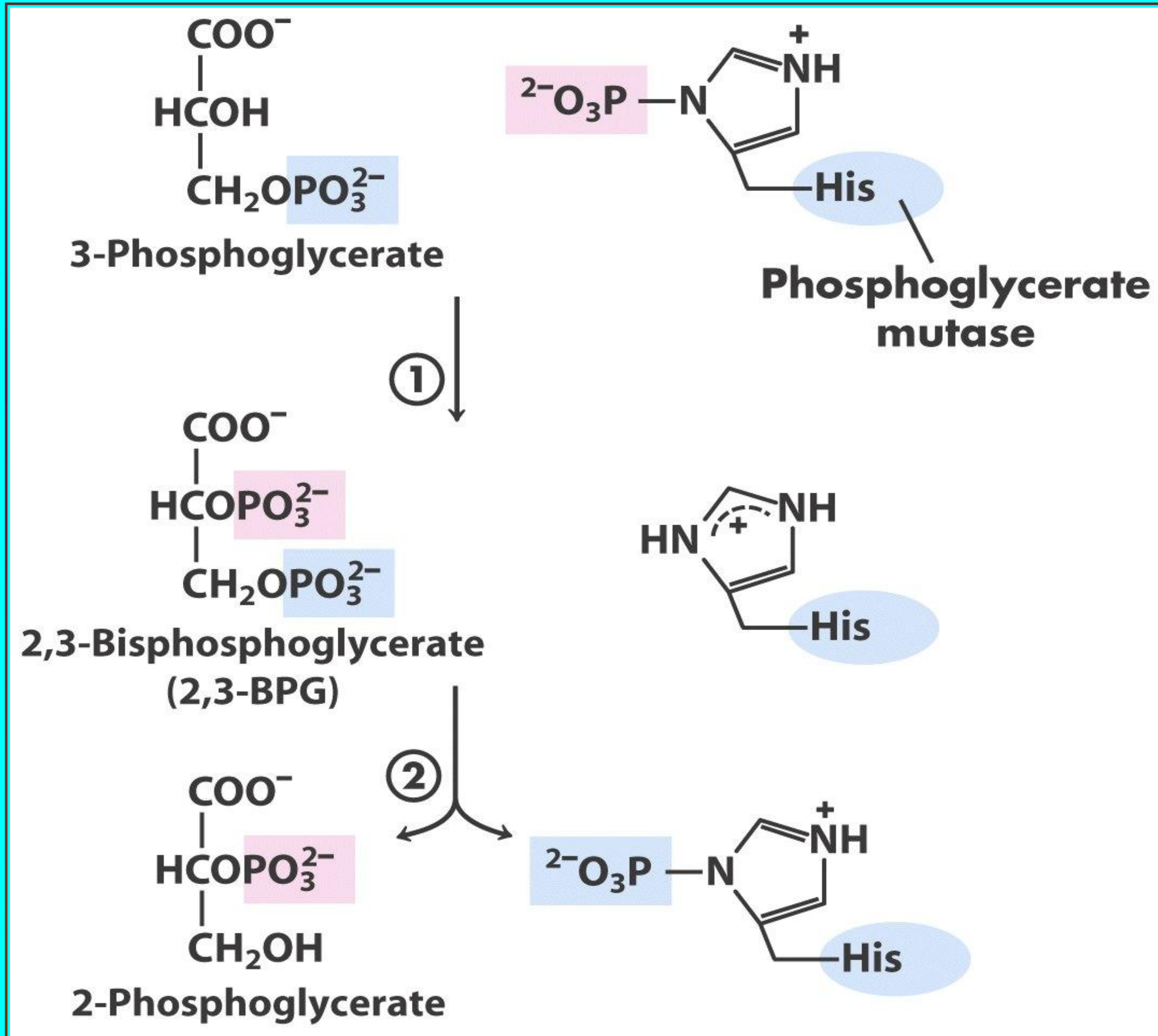


Rxn 8: Conversion to 2-Phosphoglycerate



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

Rxn 8: Mechanism of phosphoglycerate mutase

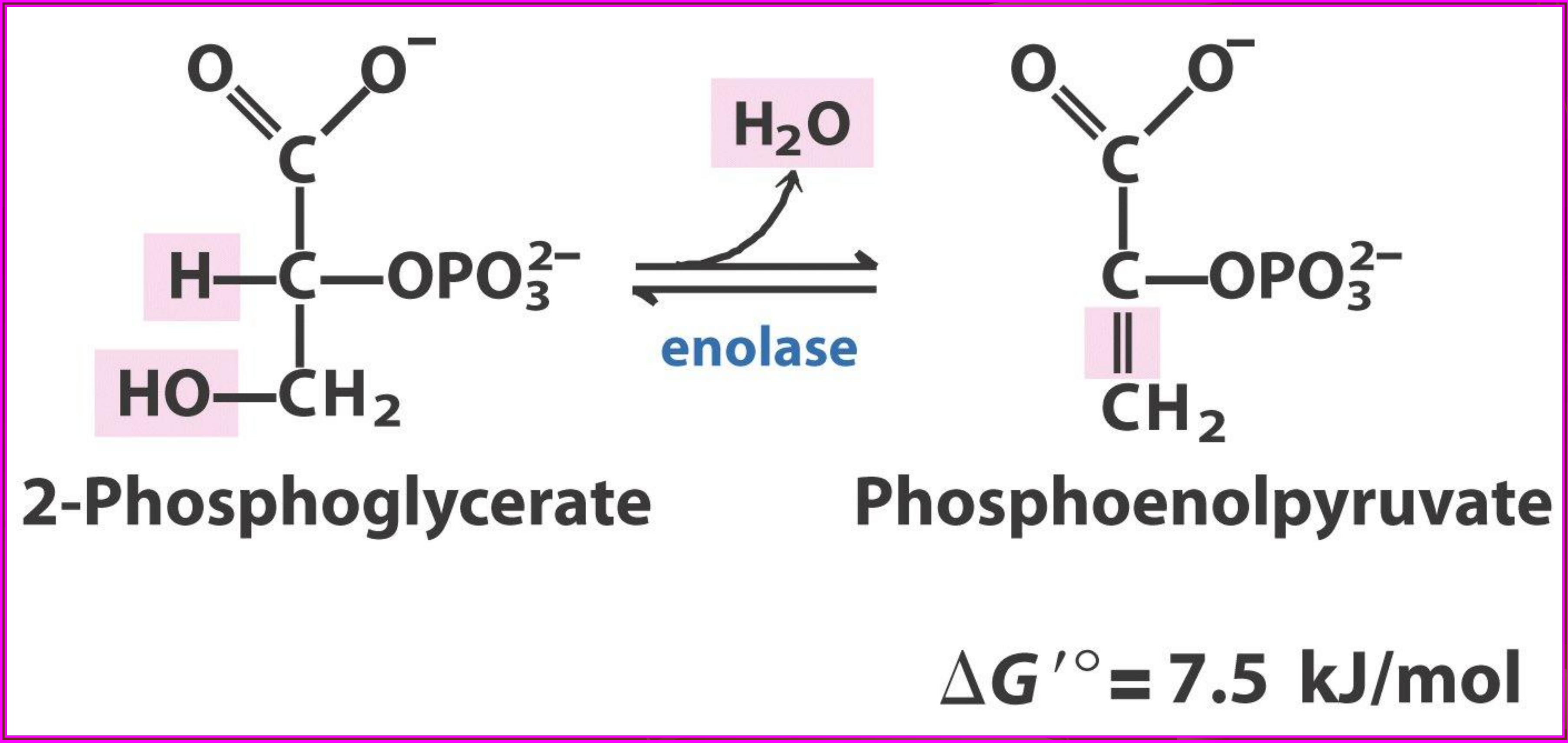


- Catalyzed by **phosphoglycerate mutase**
- Two steps :

--- a phosphoryl group is first transferred from the enzyme to the substrate to give an intermediate, 2,3-bisphosphoglycerate (BPG, remember this from regulation of hemoglobin's affinity for oxygen?).

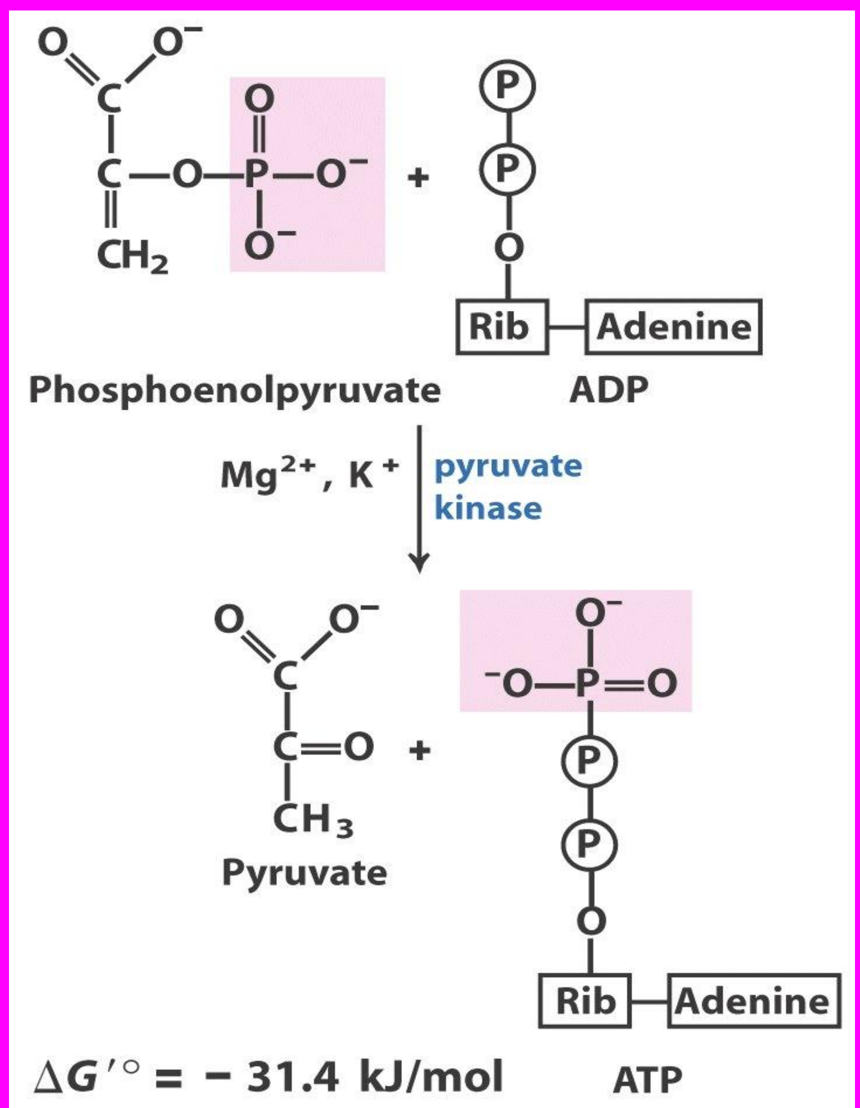
---In the second step the phosphoryl group at the 3 position is transferred back to the enzyme to give the product, 2-phosphoglycerate, and regenerate the phosphorylated enzyme.

Rxn 9: Dehydration to Phosphoenolpyruvate



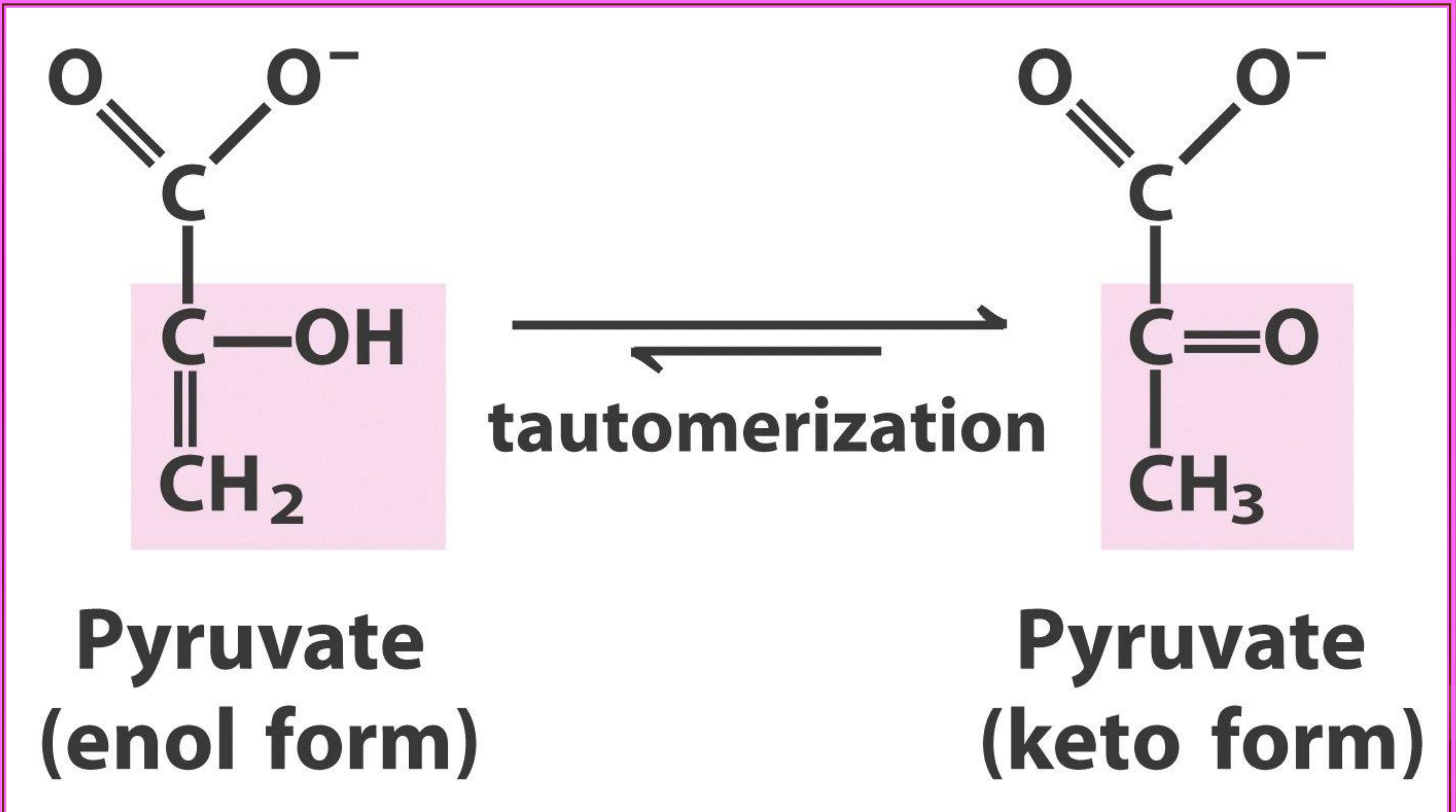
- 2-phosphoglycerate → phosphoenolpyruvate + H_2O
- Catalyzed by **enolase**
- This is the second reaction that generates a compound with high phosphoryl group transfer potential.
- The transfer potential of phosphoenolpyruvate is used in the next step.

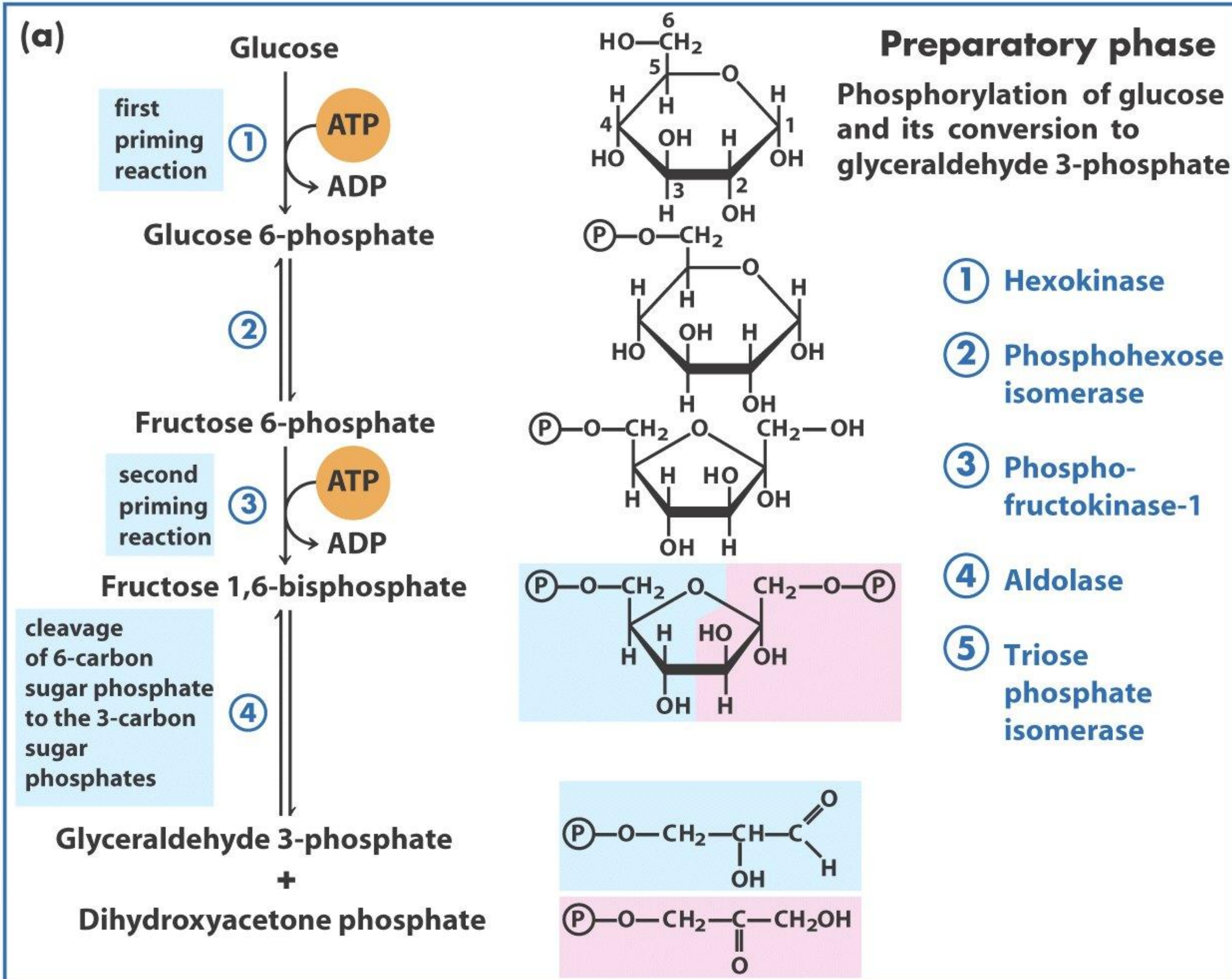
Rxn 10: Phosphoryl Transfer to Generate Pyruvate and ATP

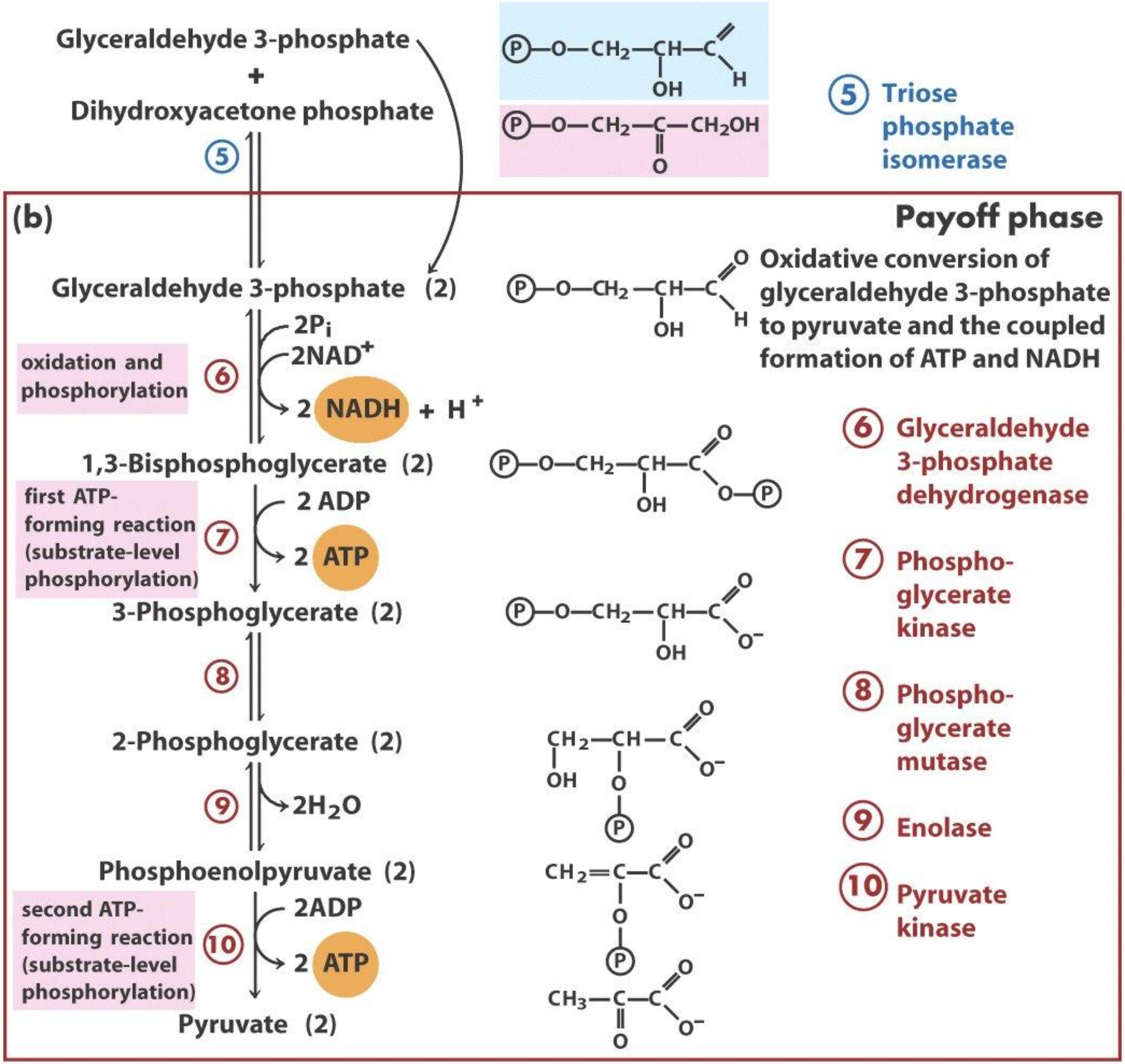


- Phosphoenolpyruvate + ADP → pyruvate + ATP
- Catalyzed by **pyruvate kinase**
- Reaction has a large negative free energy (DG'° = –31.4 kJ/mol)
- This reaction is also an important point in regulation, as we will see later.

Tautomerization of Pyruvate




(a)
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to the 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase



Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
5 Triose phosphate isomerase
(b)
Payoff phase
Glyceraldehyde 3-phosphate (2)
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
$2P_i$
$2NAD^+$
oxidation and phosphorylation
6
2 NADH + H^+
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2 ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
$2H_2O$
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phosphoglycerate kinase
8 Phosphoglycerate mutase
9 Enolase
10 Pyruvate kinase

The Balance Sheet From Glycolysis

Glucose + **2 ATP** + **2 NAD$^+$** + **4 ADP** + 2 P_i $\rightarrow$
2 pyruvate + **2 ADP** + **2 NADH** + 2 H^+ + **4 ATP** + 2 H_2O

After canceling…

Glucose + 2 NAD$^+$ + 2 ADP + 2 P_i -->
2 pyruvate + 2 NADH + 2 H^+ + 2 ATP + 2 H_2O

Key Points

1. In glycolysis, D-glucose is converted to two molecules of pyruvate.

2. Glycolysis gives the net synthesis of two molecules of ATP and two molecules of NADH.

3. Glycolysis is the end of the line for the energy-producing steps of anaerobic metabolism but only the first step for aerobic metabolism.