Welcome to BCII lesson 3

Chimie Biologique II

Biological Chemistry II

BIO-213

Teacher

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Glycolysis

D-Glucose is the major nutrient for a wide range of organisms. It can be stored by cells in the form of polymers and used upon need to generate ATP.

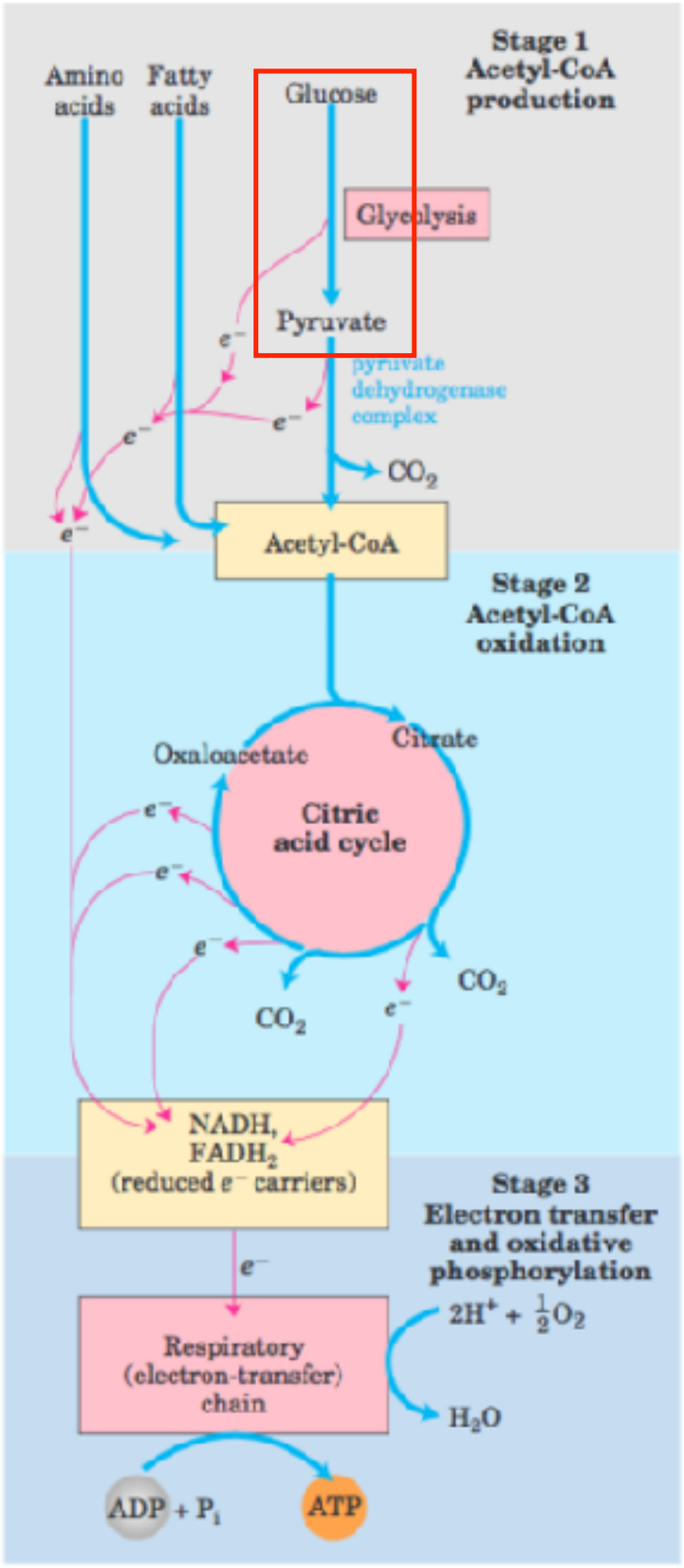
The most ancient and conserved pathway to use glucose is glycolysis that emerged in an anaerobic environment.

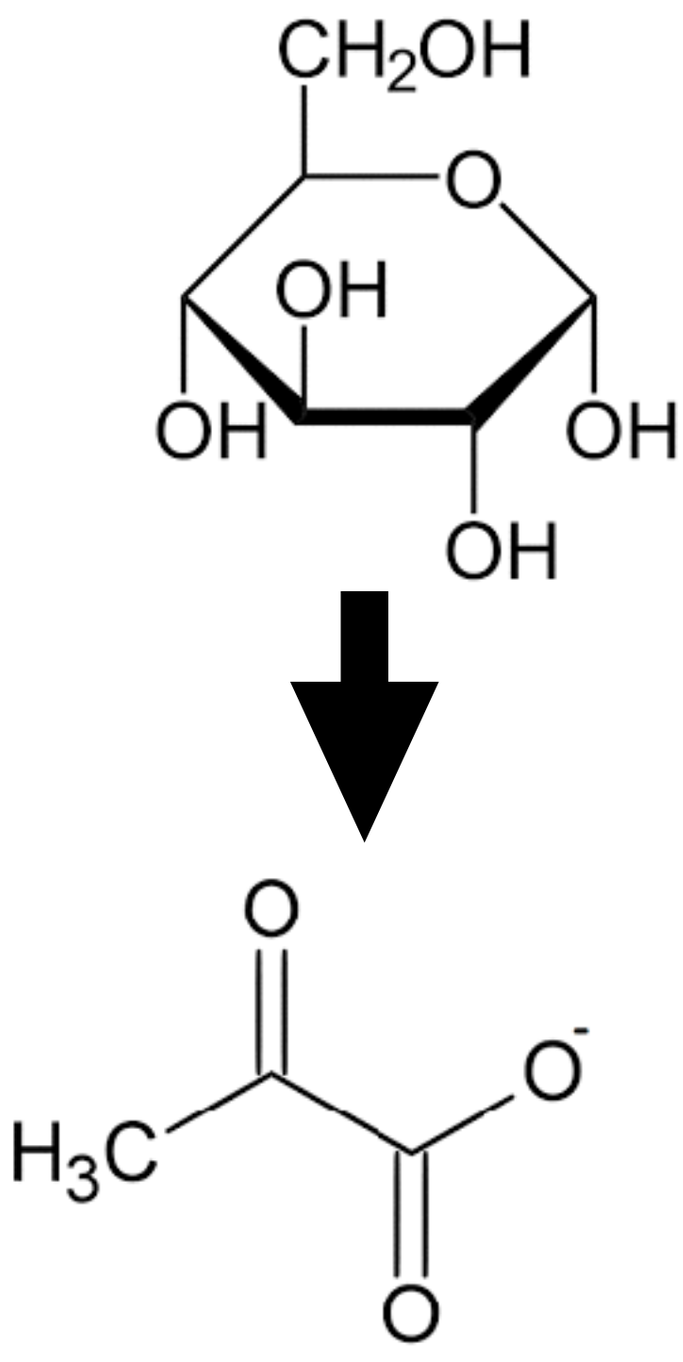
Glycolysis can be divided in two stages (stage I [Preparatory phase], stage II [Payoff phase])

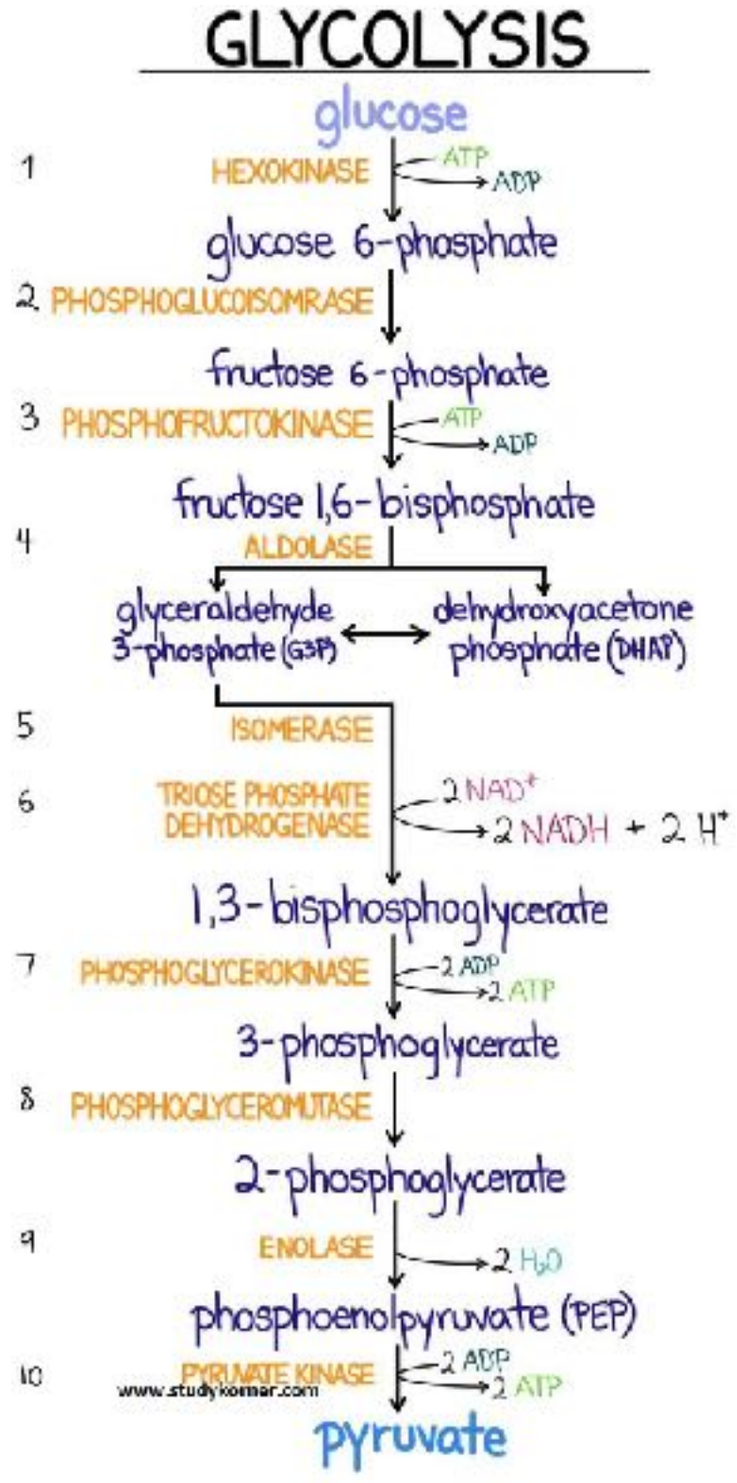
- Stage I traps glucose inside the cell, ‘activates' it, and breaks it down into smaller components

- Stage II oxidises these components to produce ATP, pyruvate and NADH

Glycolysis


Stage 1
Acetyl-CoA production
Amino acids
Fatty acids
Glucose
Glycolysis
Pyruvate
pyruvate dehydrogenase complex
CO_2
Acetyl-CoA
Stage 2
Acetyl-CoA oxidation
Oxaloacetate
Citrate
Citric acid cycle
CO_2
CO_2
$NADH$, $FADH_2$ (reduced e^- carriers)
Stage 3
Electron transfer and oxidative phosphorylation
$2H^+ + \frac{1}{2}O_2$
H_2O
Respiratory (electron-transfer) chain
ADP + P_i
ATP


CH_2OH
O
OH
OH
OH
OH
O
H_3C
O
O^-
O


GLYCOLYSIS
glucose
1 HEXOKINASE
ATP
ADP
glucose 6-phosphate
2 PHOSPHOGLUCOISOMRASE
fructose 6-phosphate
3 PHOSPHOFRUCTOKINASE
ATP
ADP
fructose 1,6-bisphosphate
4 ALDOLASE
glyceraldehyde 3-phosphate (G3P)
dehydroxyacetone phosphate (DHAP)
5 ISOMERASE
6 TRIOSE PHOSPHATE DEHYDROGENASE
2 NAD+
2 NADH + 2 H+
1,3-bisphosphoglycerate
7 PHOSPHOGLYCEROKINASE
2 ADP
2 ATP
3-phosphoglycerate
8 PHOSPHOGLYCEROMUTASE
2-phosphoglycerate
9 ENOLASE
2 H2O
phosphoenolpyruvate (PEP)
10 PYRUVATE KINASE
2 ADP
2 ATP
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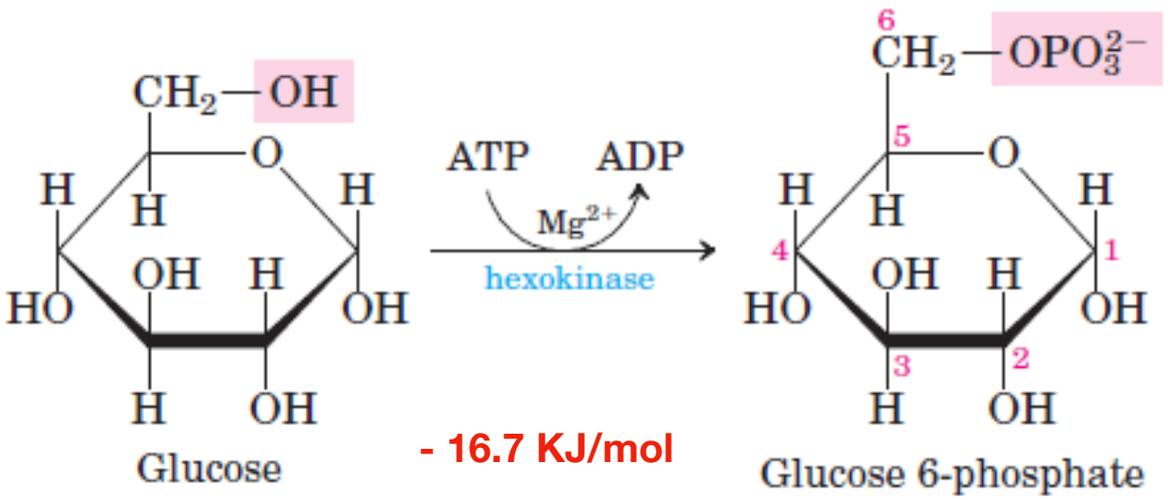
pyruvate

Stage1

Step1: phosphorylation of Glucose



D-Glucose movies into the cell with the help of a membrane transporter. Once in the cytoplasm it undergoes phosphorylation by an enzyme called **hexokinase** to produce Glucose 6-phosphate

This has two consequences:

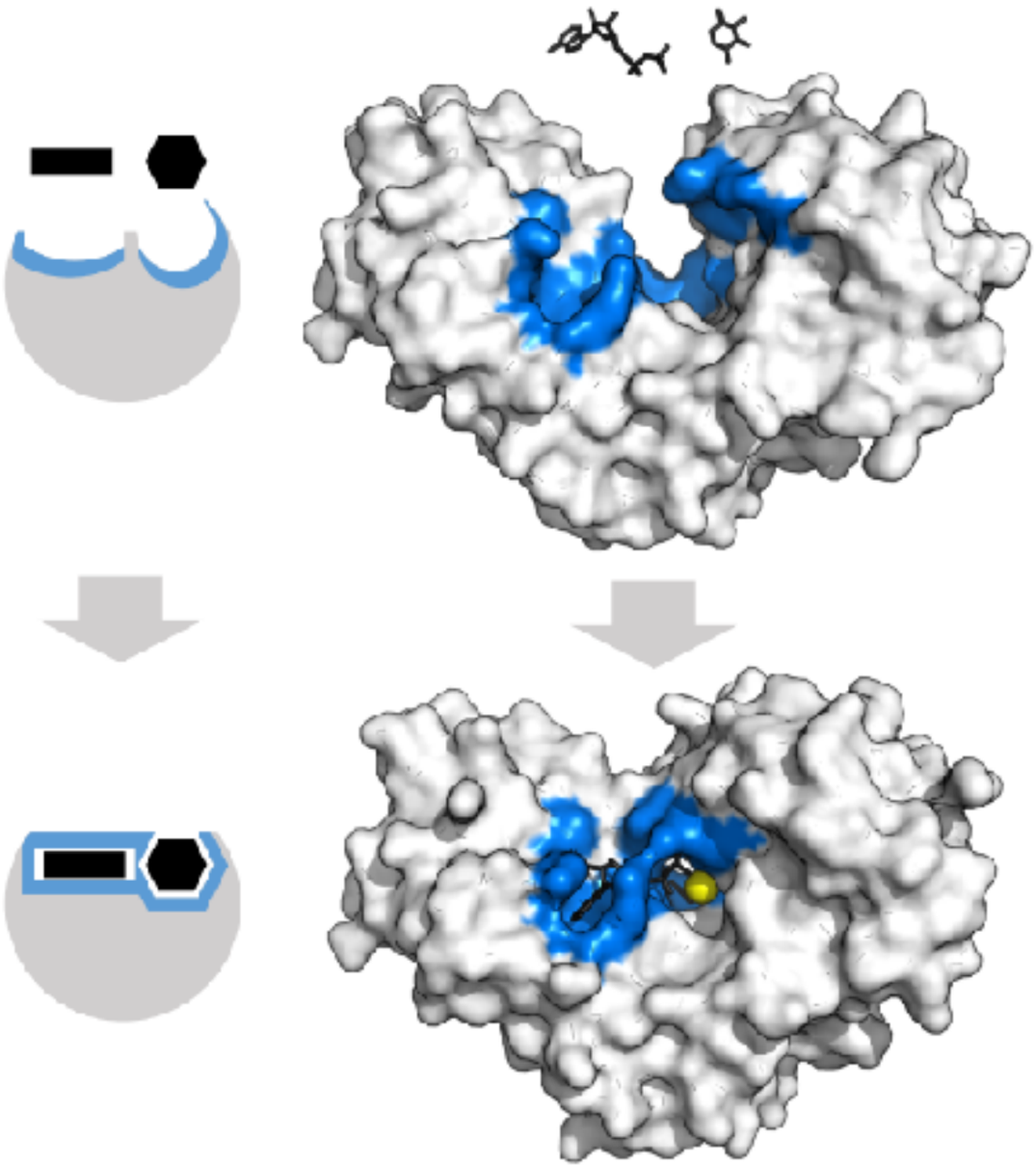
1. Glucose 6-phosphate is structurally different from Glucose and thus it cannot be transported back out from the cell by the the same membrane transporter that allowed it in. Thus Glucose 6-phosphate gets ‘trapped' in the cytoplasm

3. Addition of negative charges (2 here) to the Glucose molecule makes Glucose 6-phosphate more reactive and less stable (higher in energy) if compared with Glucose.

Note: to obtain this we pay an energy price by consuming 1 ATP molecule

Stage1

Step1: phosphorylation of Glucose - HexoKinase



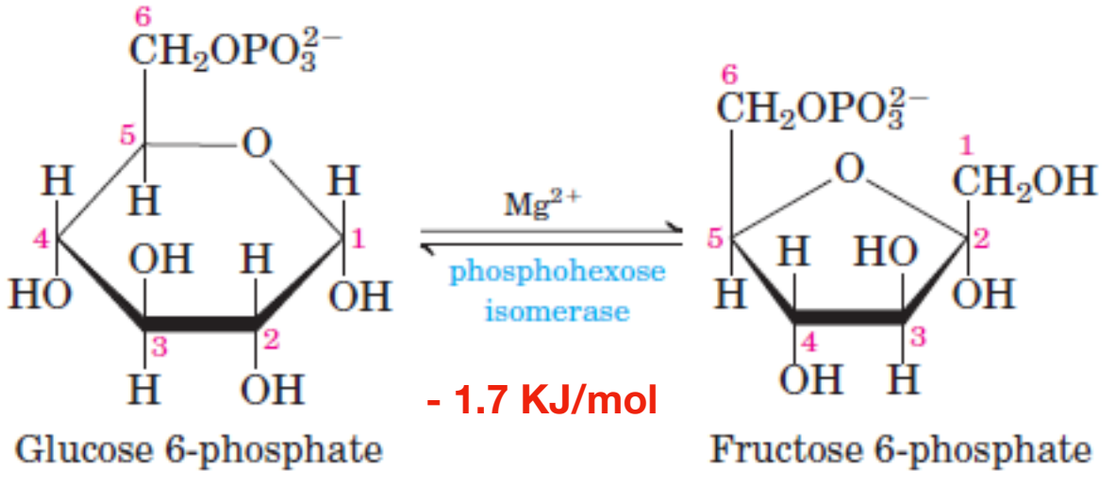
The hexokinase is an enzyme that phosphorylates hexoses (like glucose) using ATP.

Hexokinase (and in general all protein kinases) requires the presence of a divalent cation (Mg2+, or Mn2+) as a cofactor which is actually complexed with ATP.

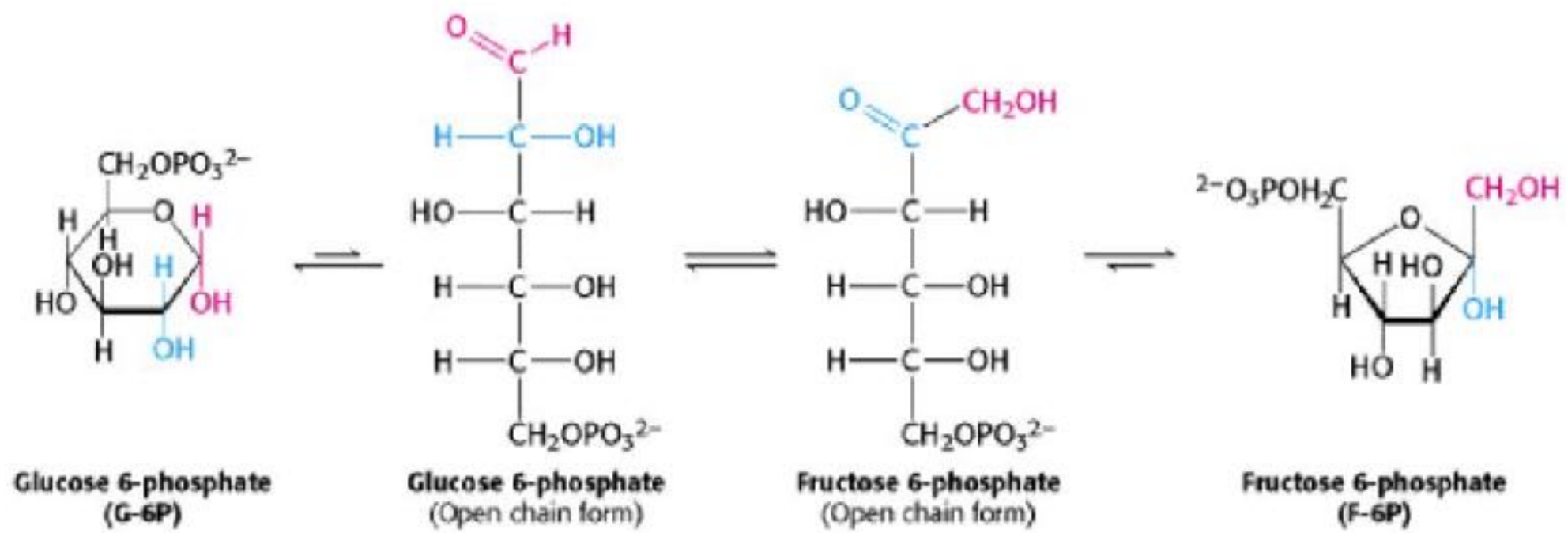
The movement of Glucose into the Hexokinase (HK) active site causes a conformational change whereby the two HK lobes rotate by 12 degrees (10 Å) creating an **induced fit**. This seals off the glucose, orients the carbon 6 towards ATP, and squeezes out water molecules form the active site (desolvation).

Stage1

Step2: Isomerization

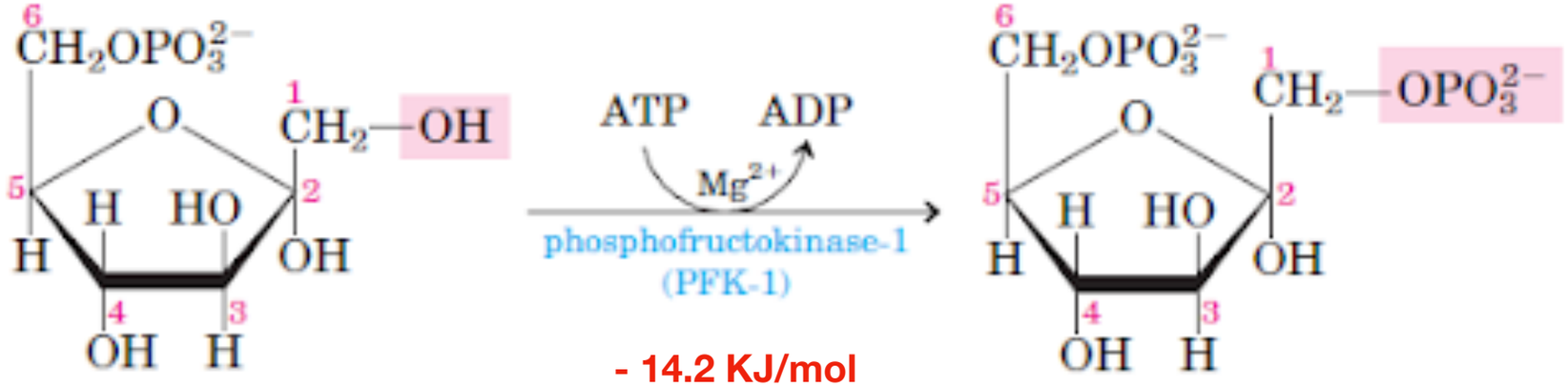


In the second step, the enzyme **phospho-glucose isomerase** transforms an aldose (Glucose 6-phosphate) into a ketose (Fructose 6-phosphate).



Stage1

Step3: Second phosphorylation



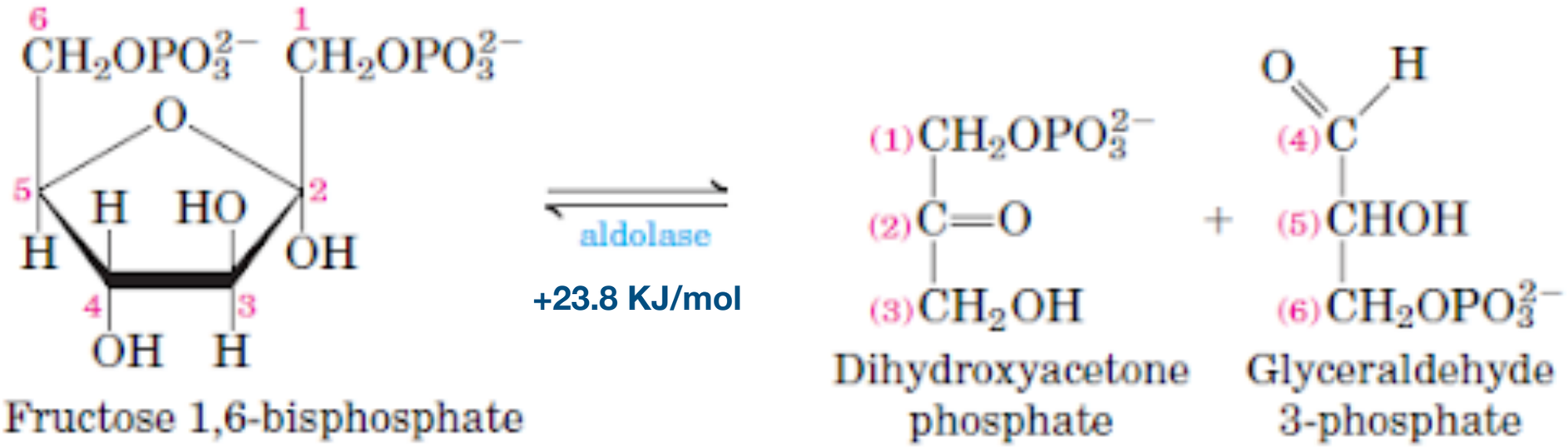
In the third step, the enzyme **phospho-fructo kinase-1** (PFK-1) adds a second phosphate group onto Fructose 6-phosphate to produce Fructose 1,6-bisphosphate.

This step commits the sugar to glycolysis.

PFK-1 is a highly regulated enzyme where its activity is modified according to the cellular concentrations of ATP, ADP, AMP (ATP inhibits - AMP stimulates PFK-1)

Stage1

Step 4: Breakdown of Fructose 1,6-bisphosphate

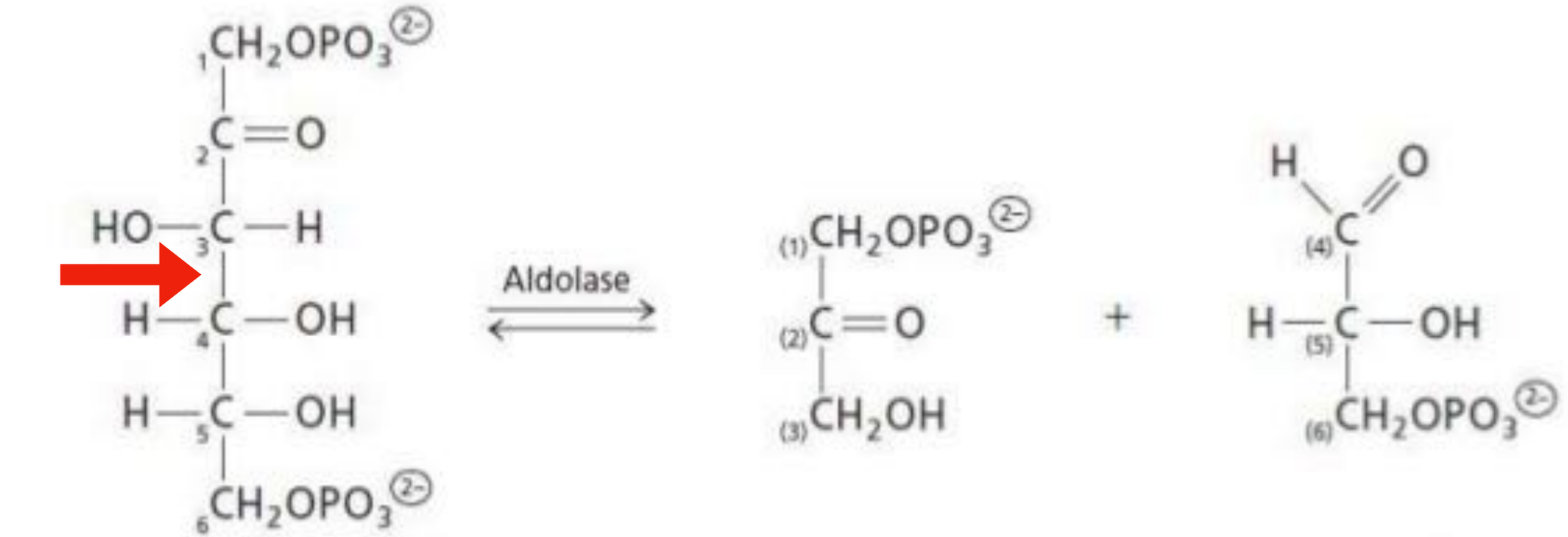


An enzyme called **aldolase** catalyses the breakdown of Fructose 1,6,-bisphosphate into two different three-carbon molecules, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (DHAP)

The glyceraldehyde 3-phosphate feeds directly in the glycolytic pathway without any further change while DHAP needs to be first transformed into glyceraldehyde 3-phosphate to proceed in the pathway.

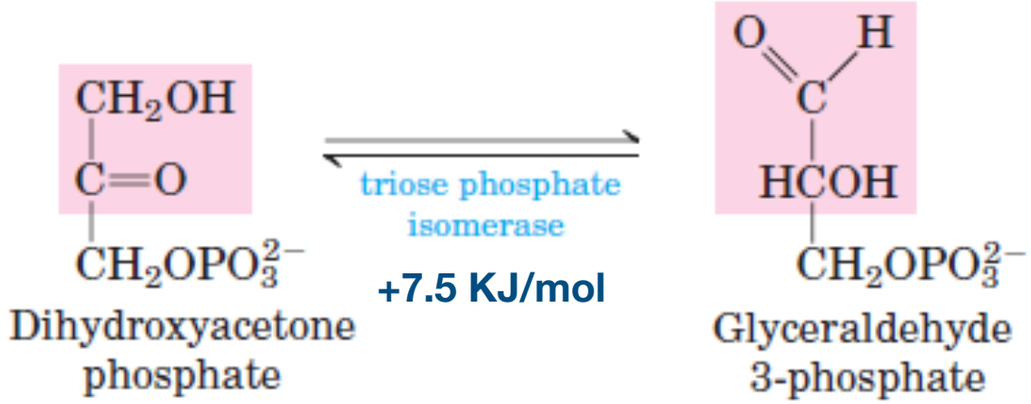
Stage1

Step 4: Breakdown of Fructose 1,6-bisphosphate

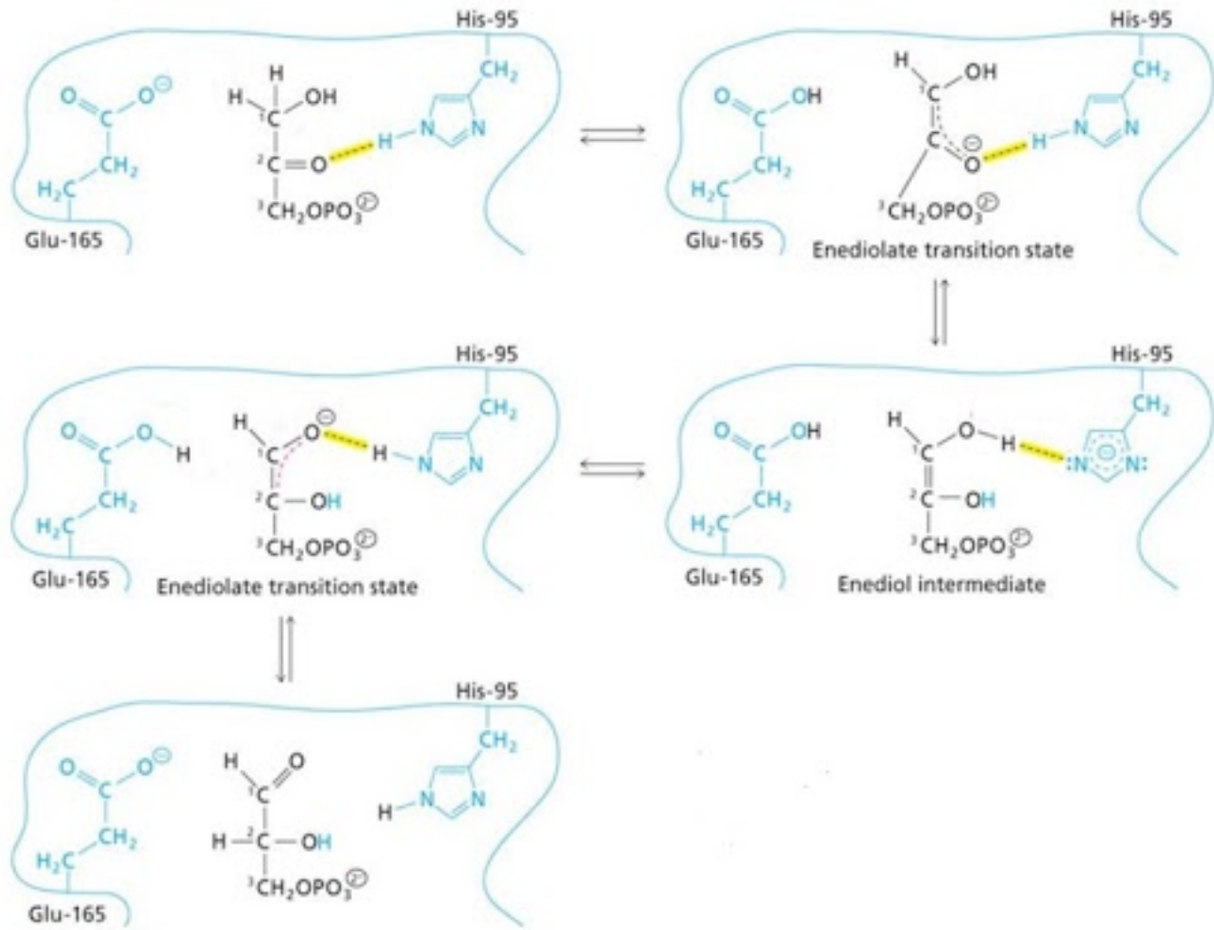


Stage1

Step 5: Isomerisation of DHAP to GA3P



DHAP (96%) GA3P (4%)



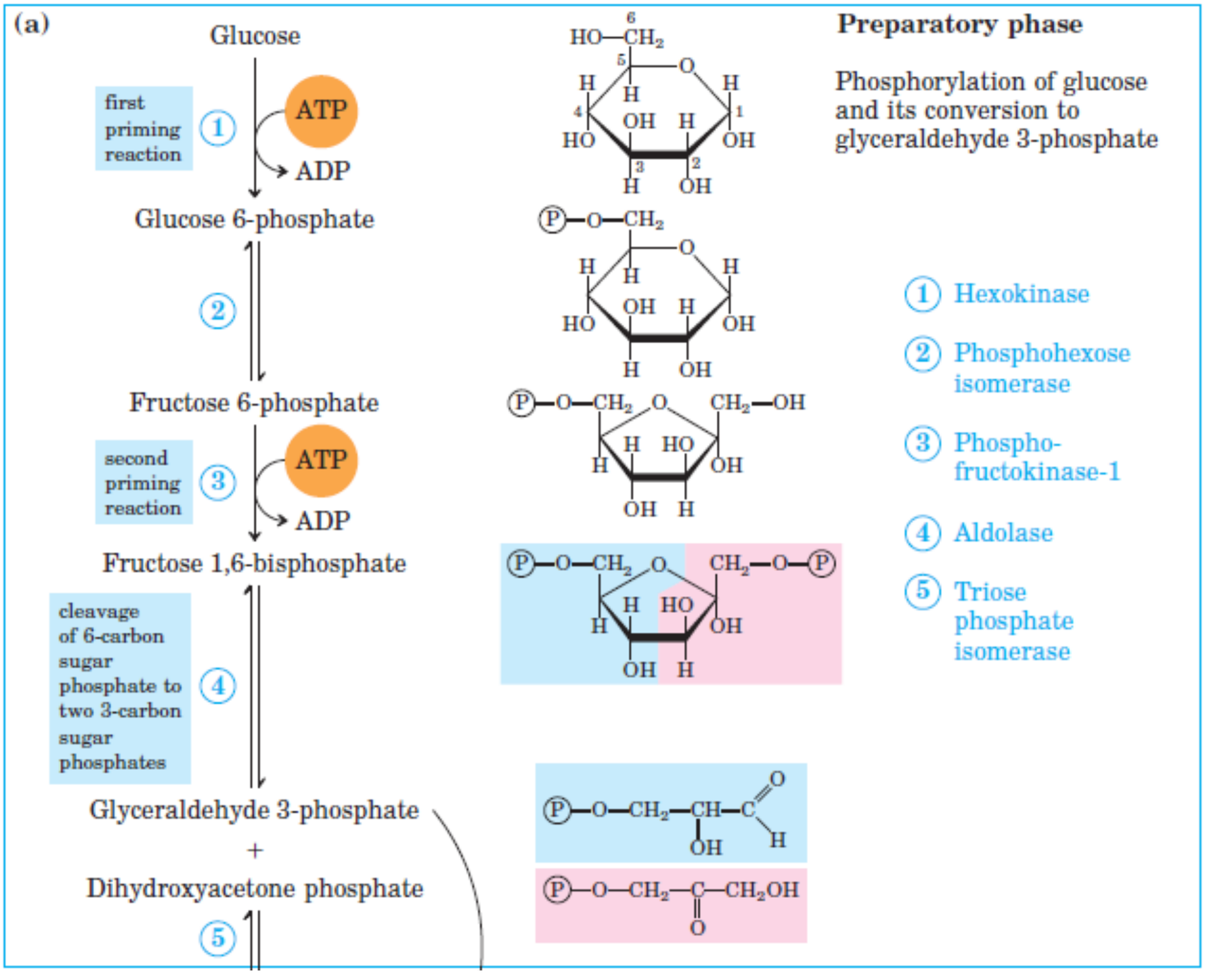
An enzyme called **triose phosphate isomerase** (TPI or TIM) catalyses the rapid and reversible conversion of DHAP to GA3P.

TPI catalyses the conversion of the ketone DHAP to the aldehyde (GA3P) via an intramolecular redox reaction in which an hydrogen is transferred from C1 to C2

TPI increases the rate of this reaction 10 billion folds.

TPI impedes competing reactions to happen (DHAP dephosphorylation)

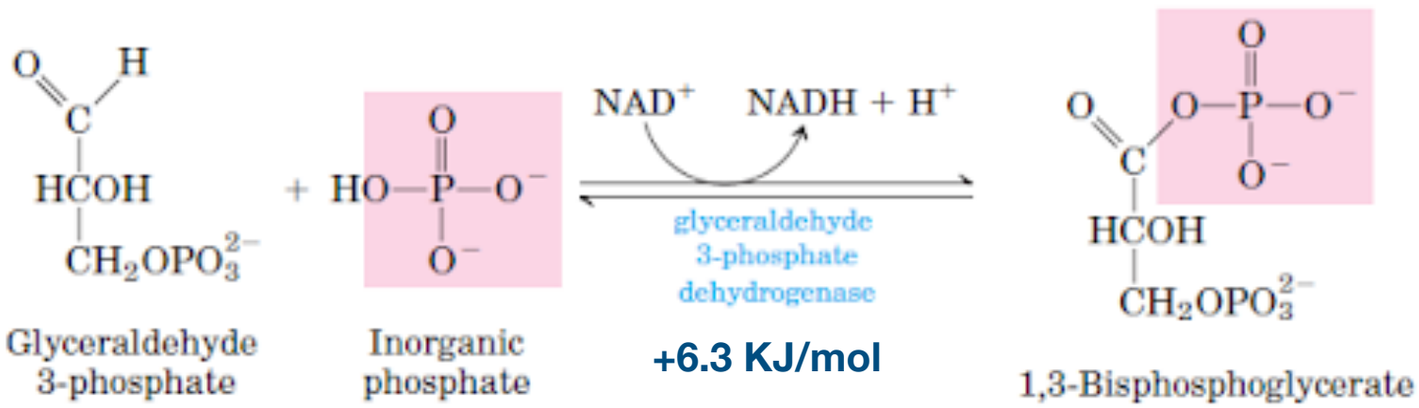
Stage1

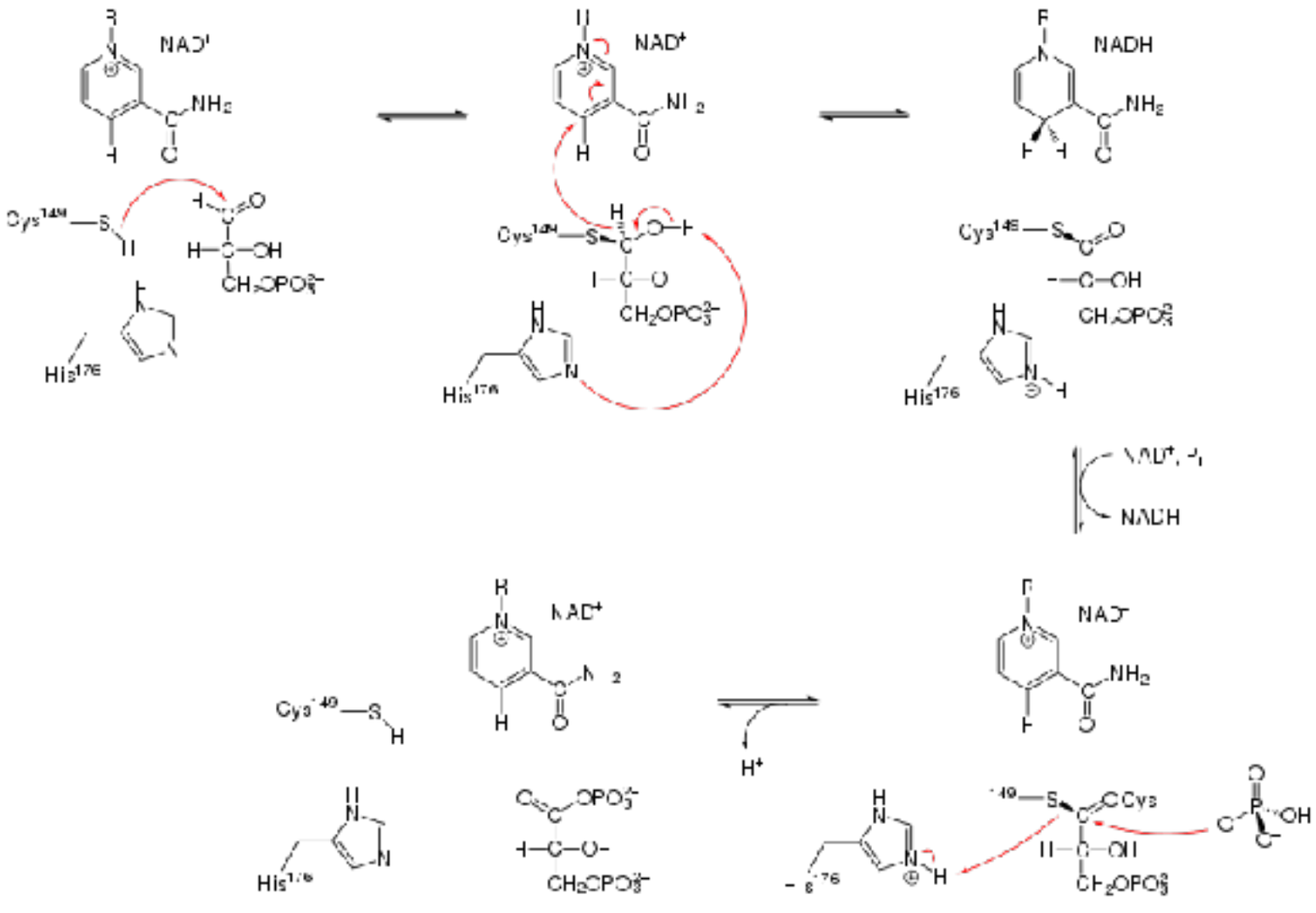

(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Preparatory phase
Phosphorylation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

10’ Break

Stage2

Step1: Conversion of GA3P to 1,3-BPG



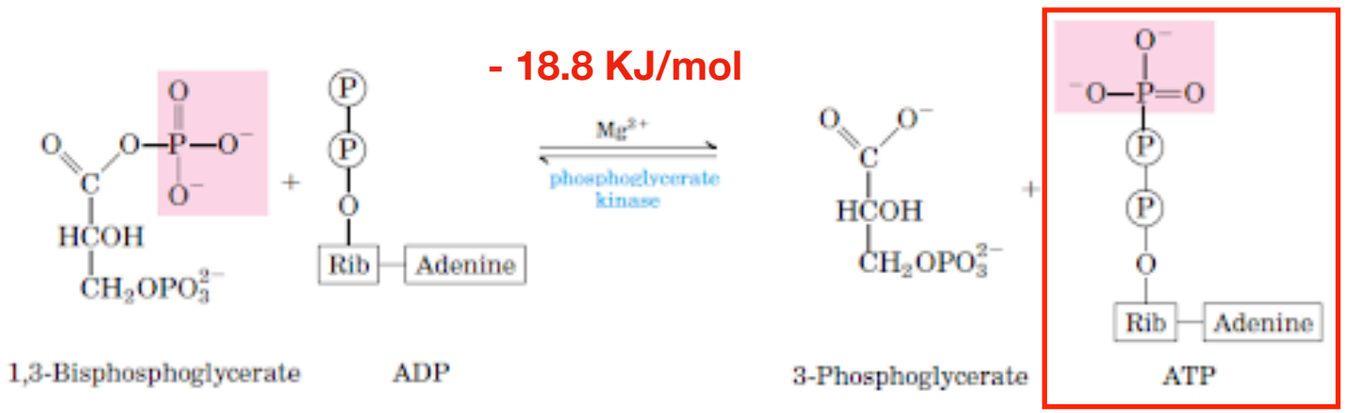


In the first step of stage 2 GA3P is converted into 1,3-bisphosphoglycerate (1,3-BPG) in a reaction catalysed by the enzyme **glyceraldehyde 3-phoshate dehydrogenase** (GAPDH).

1. the SH in Cys149 attacks nucleophilically C1
2. His176 acts as a base and grab an H. At the same time an hydrate group is transferred to NAD+ to form NADH
3. NADH is exchanged for NAD+ and Pi
4. The phosphate attacks the thioester intermediate to form 1,3-BPG

Stage2

Step2: Phosphotransfer from 1,3-BPG to ADP



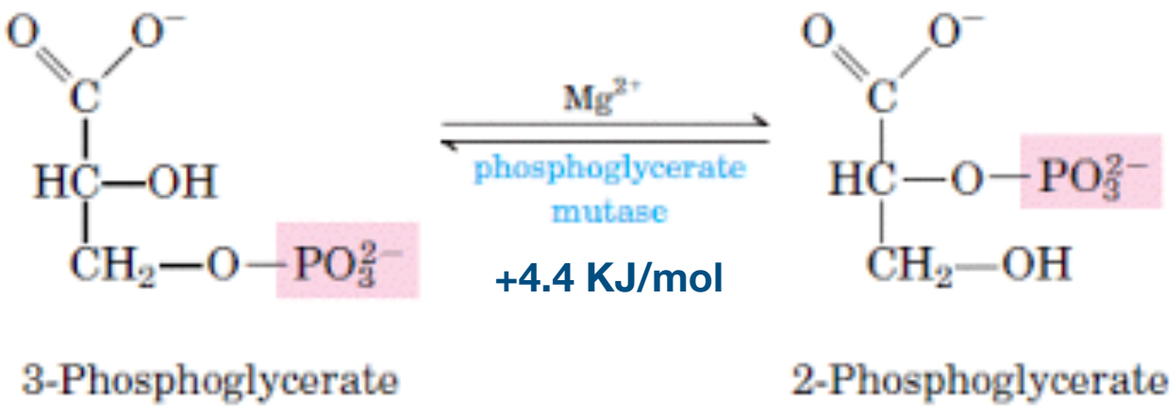
Phosphoenolpyruvate	−61.9
1,3-bisphosphoglycerate (→ 3-phosphoglycerate + P_i)	−49.3
Phosphocreatine	−43.0
ADP (→ AMP + P_i)	−32.8
ATP (→ ADP + P_i)	−30.5
ATP (→ AMP + PP_i)	−45.6
AMP (→ adenosine + P_i)	−14.2
PP_i (→ $2P_i$)	−19
Glucose 1-phosphate	−20.9
Fructose 6-phosphate	−15.9
Glucose 6-phosphate	−13.8
Glycerol 1-phosphate	−9.2
Acetyl-CoA	−31.4

In the second step of stage 2 1,3-BPG is used as a phosphate donor to ADP in a reaction catalysed by the enzyme **glycerophosphate kinase** to produce 3-Phosphoglycerate and **ATP**

As from one molecule of glucose we obtain 2 molecules od GA3P (and as a consequence 2 molecules of 1,3-BPG) at this point we have consumed two ATP molecules (in the preparatory phase) and produced 2 ATP and 2 NADH molecules

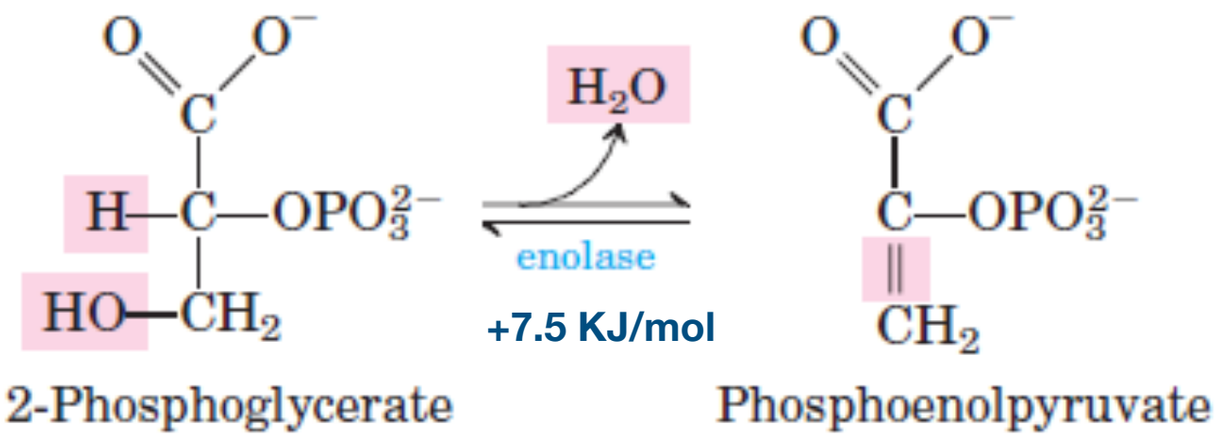
Stage2

Step3: Conversion to 2-Phosphoglycerate



In this step, an enzyme called **phosphoglycrate mutase** catalyses the transfer of a phosphate group from C3 of 3-phosphoglycerate to C2 to form 2-phosphoglycearate.

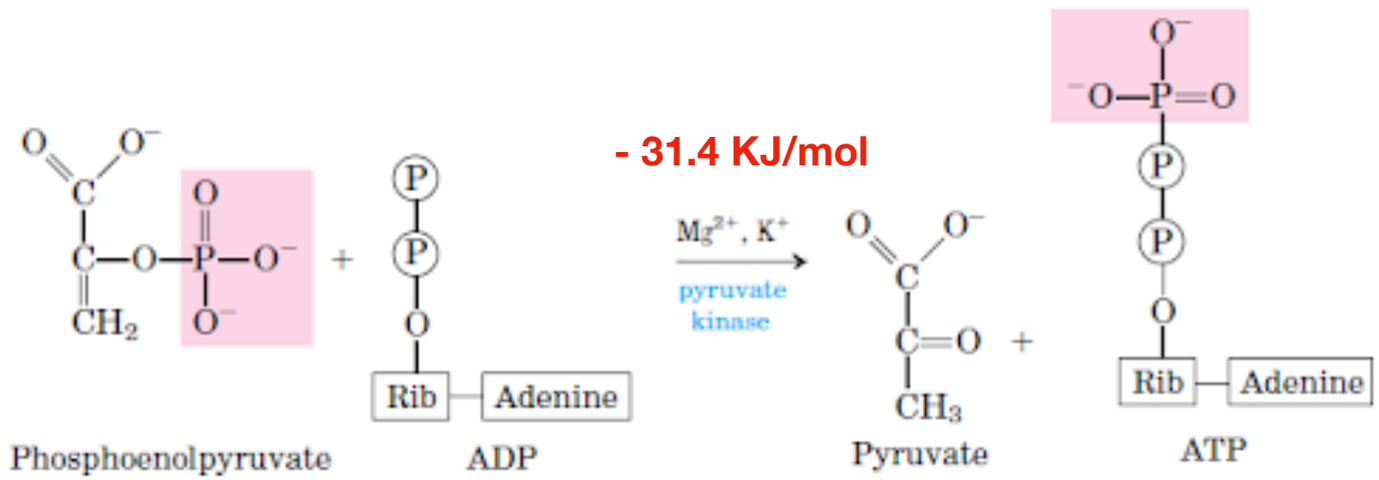
Step4: Conversion to Phosphoenolpyruvate



In step 4, an enzyme called **enolase** converts 2-phosphoglycearate into Phosphoenolpyruvate (PEP). This dehydration reaction increases the phosphoryltrasfer potential of the molecule.

Stage2

Step5: Conversion to pyruvate



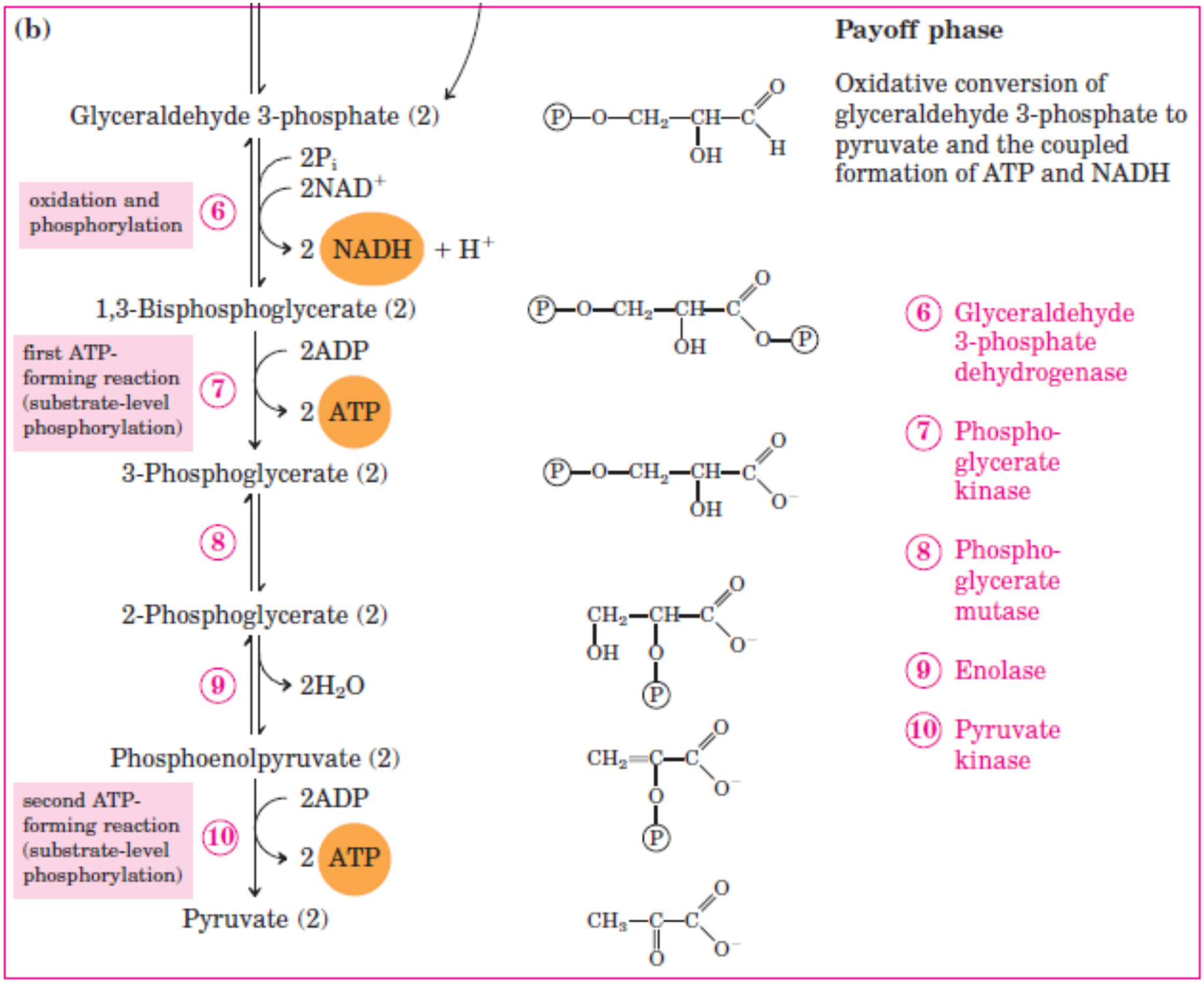
Phosphoenolpyruvate ADP Pyruvate ATP

In the last step **pyruvate kinase** catalyses the phosphoric transfer from PEP to ADP to form pyruvate and ATP.
Enolpyruvate transforms into ketopyruvate spontaneously.

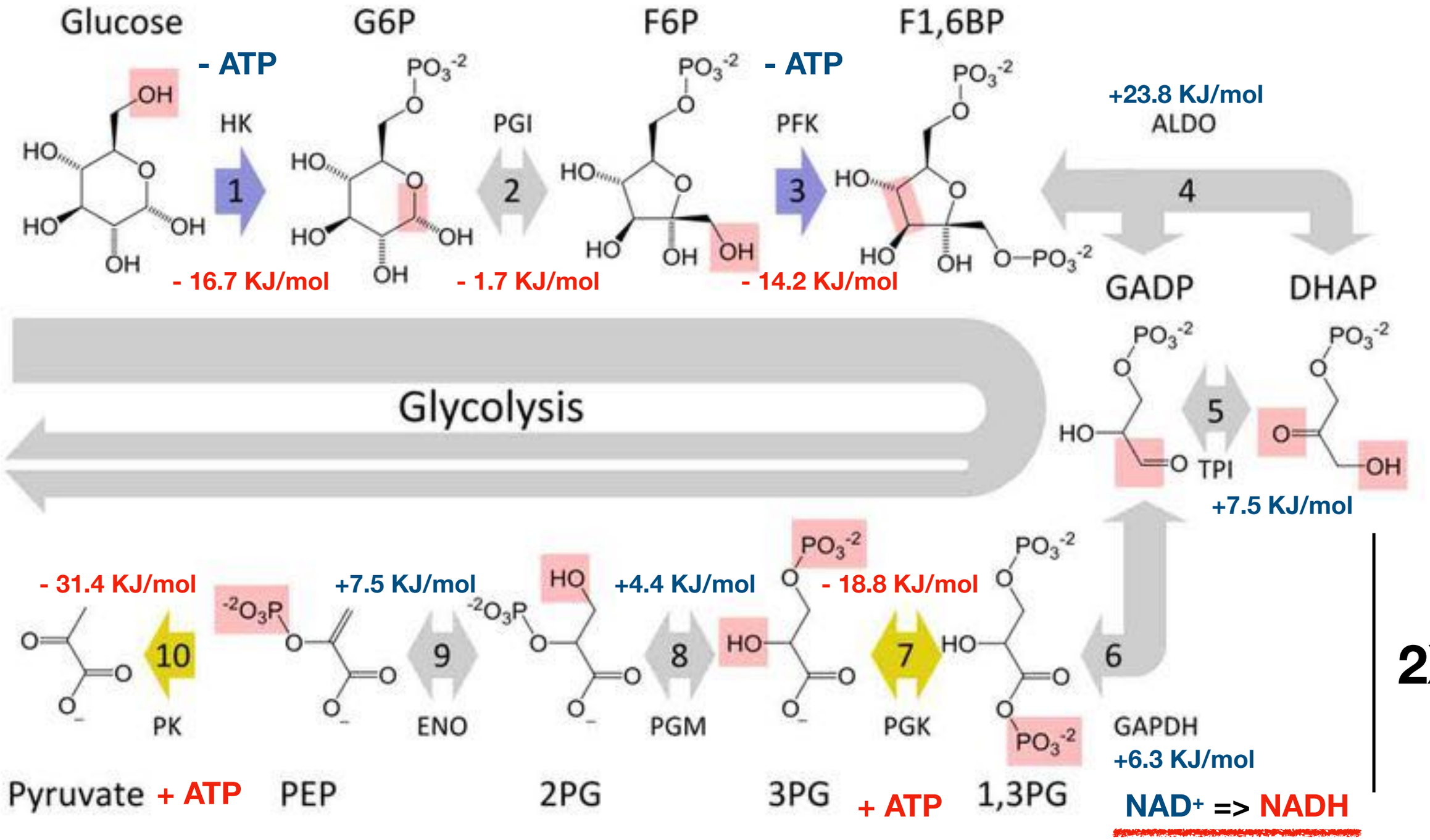
Phosphoenolpyruvate	−61.9
1,3-bisphosphoglycerate (→ 3-phosphoglycerate + P_i)	−49.3
Phosphocreatine	−43.0
ADP (→ AMP + P_i)	−32.8
ATP (→ ADP + P_i)	−30.5
ATP (→ AMP + PP_i)	−45.6
AMP (→ adenosine + P_i)	−14.2
PP_i (→ $2P_i$)	−19
Glucose 1-phosphate	−20.9
Fructose 6-phosphate	−15.9
Glucose 6-phosphate	−13.8
Glycerol 1-phosphate	−9.2
Acetyl-CoA	−31.4

At this point we have consumed 2 ATP molecules in the preparatory phase and produced 4 in the pay off phase (net balance 2 ATP molecules). We have also produced 2 NADH, 2 H2O and 2 H+ .

Stage2

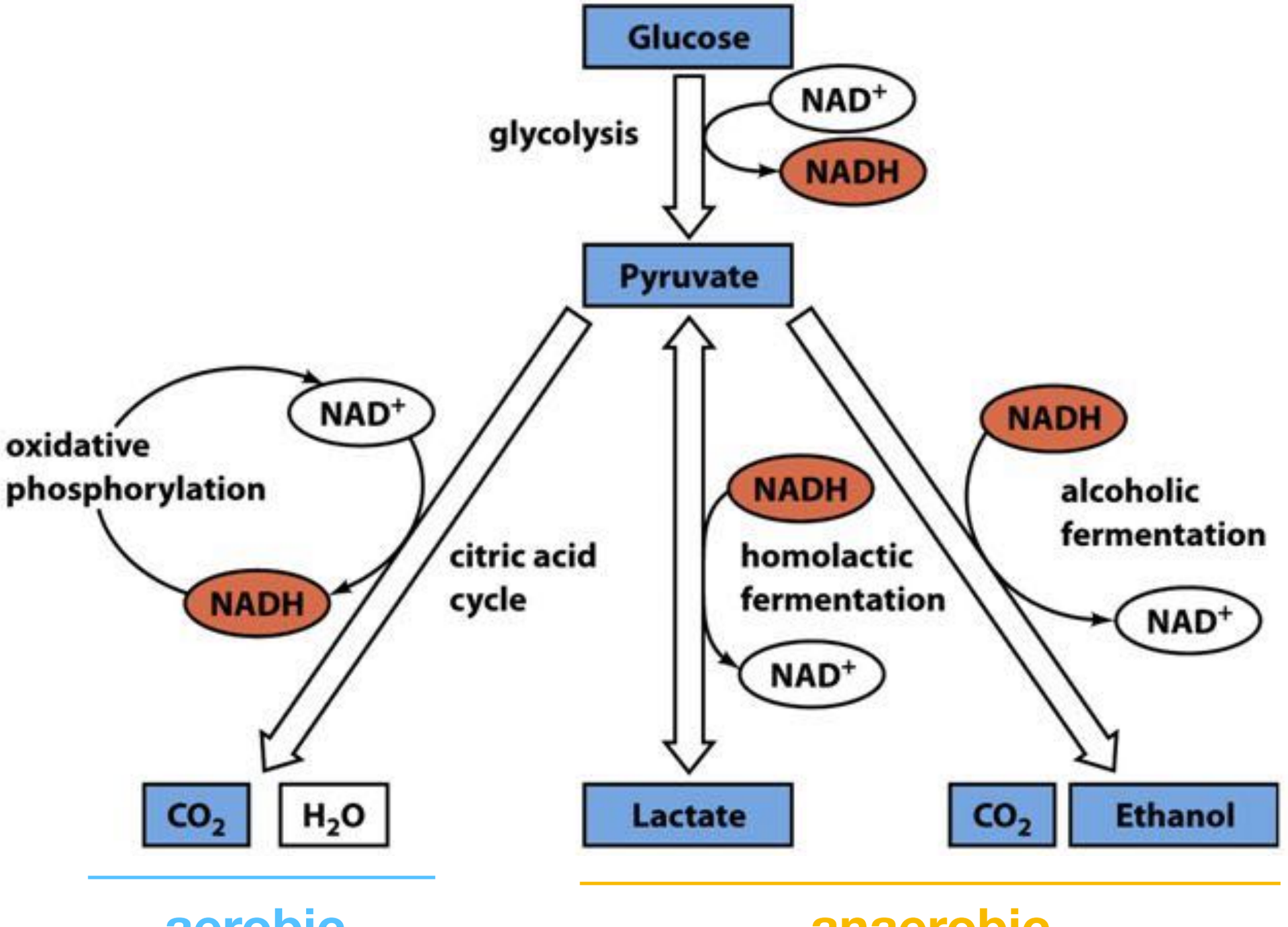

(b)
Glyceraldehyde 3-phosphate (2)
2P_i
2NAD^+
oxidation and phosphorylation
6
2 NADH + H^+
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H_2O
Phosphoenolpyruvate (2)
second ATP-forming reaction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

Glycolysis

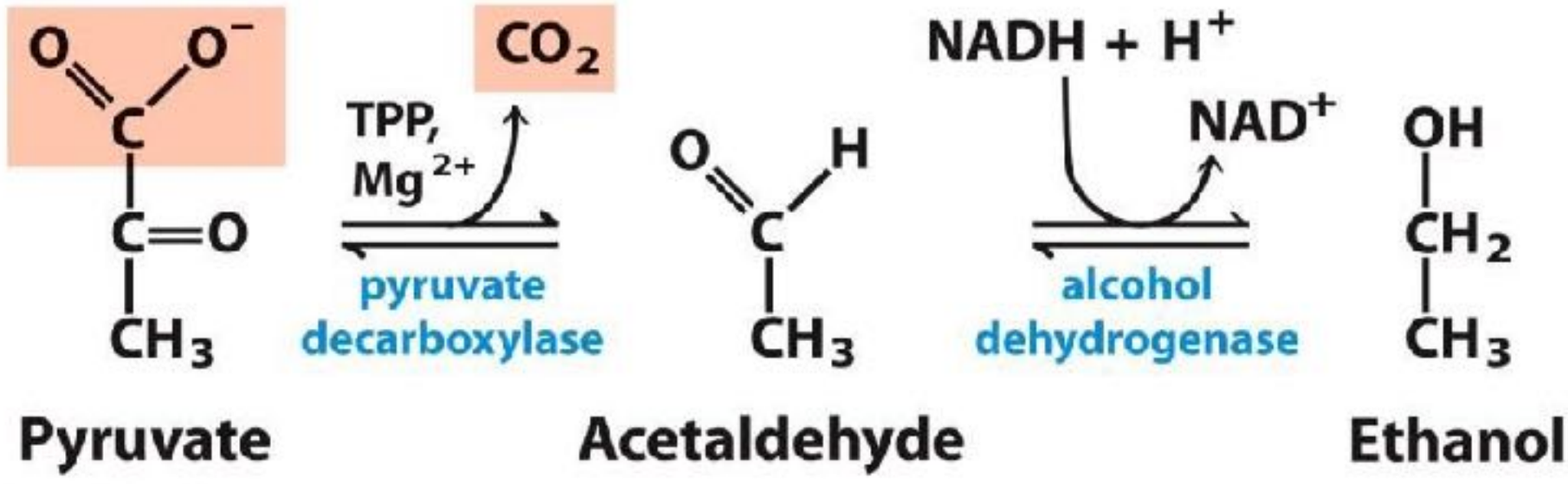


Glucose + 2ADP + 2NAD⁺ + 2Pi => 2 Pyruvate + 2ATP = 2NADH + 2H⁺ + 2 H_2O

The fates of Pyruvate



Ethanol Fermentation



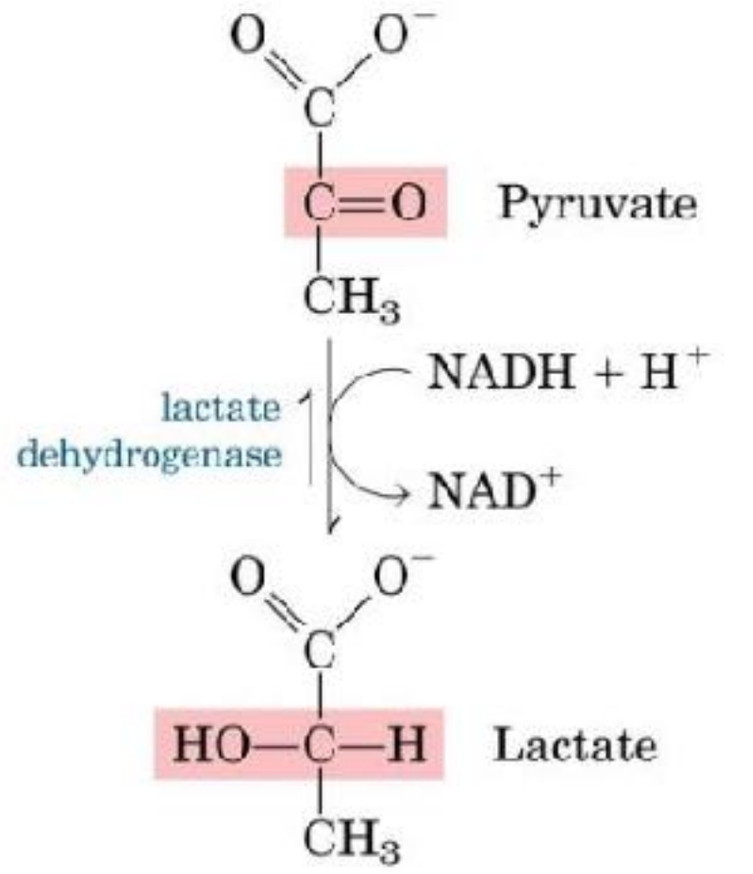
Yeast and several bacteria utilise ethanol (alcoholic) fermentation to regenerate NAD+ and to transform pyruvate into ethanol and carbon dioxide. this process occurs in two steps.

In the first step a decarboxylation reaction is catalysed by the enzyme **pyruvate decarboxylase**. The coenzyme thiamine pyrophosphate (TPP; a Vitamin B1 derivative) assists this process that transforms a pyruvate into acetaldehyde and CO2

The second step is catalysed by **alcohol dehydrogenase**. Alcohol dehydrogenase contains a zinc ion in its active site to help polarise the carbonyl double bond that promotes hydride transfer from NADH

Glucose + 2ADP + 2Pi => 2 Ethanol + 2ATP + $2CO_2$ + $2H_2O$

Lactic Fermentation



Many prokaryotic and eukaryotic organisms can use lactic fermentation. In fact, when the oxygen runs low in our cells, they use lactic fermentation to regenerate NAD+. Lactic fermentation is catalysed by the enzyme **lactate dehydrogenase** (LDH) that promotes the transfer of an hydride group from NADH to Pyruvate to form Lactate and NAD+.

During strenuous exercise, our skeletal muscle cells may not receive an adequate supply of oxygen. Such cells will produce Lactate. Lactate and H+ can activate nociceptive receptors in our muscle cells that cause the sensations of fatigue and pain.

Some pathogenic bacteria such as *clostridium tetani*, *clostridium botulinum* and *clostridium perfrigens* are obligate anaerobes and can only use Lactic fermentation as an exit point from glycolysis.

Take Home Messages

- Cell metabolism can be divided in Catabolic and Anabolic phases that involve converging/ cyclic and diverging pathways respectively
- ATP is the energy currency of the cell
- ATP can be produced by oxidation of nutrients
- Oxidative reactions require electron carriers such as NAD+, NADP+, FMN, and FAD
- Glycolysis is an universal metabolic pathway that produces pyruvate and ATP from Glucose
- Glycolysis can be divided in a preparatory and a pay off phase each consisting of 5 reactions
- Pyruvate (the product of glycolysis) can be used by aerobic and anaerobic pathways (alcoholic and lactic fermentations among others) to reconstitute the cellular NAD+ pool

Questions?