Embden Meyerhof Pathway

Class – M.Sc. Microbiology
Code- MMB-201 (Biochemistry)

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THE BREAKDOWN OF GLUCOSE TO PYRUVATE

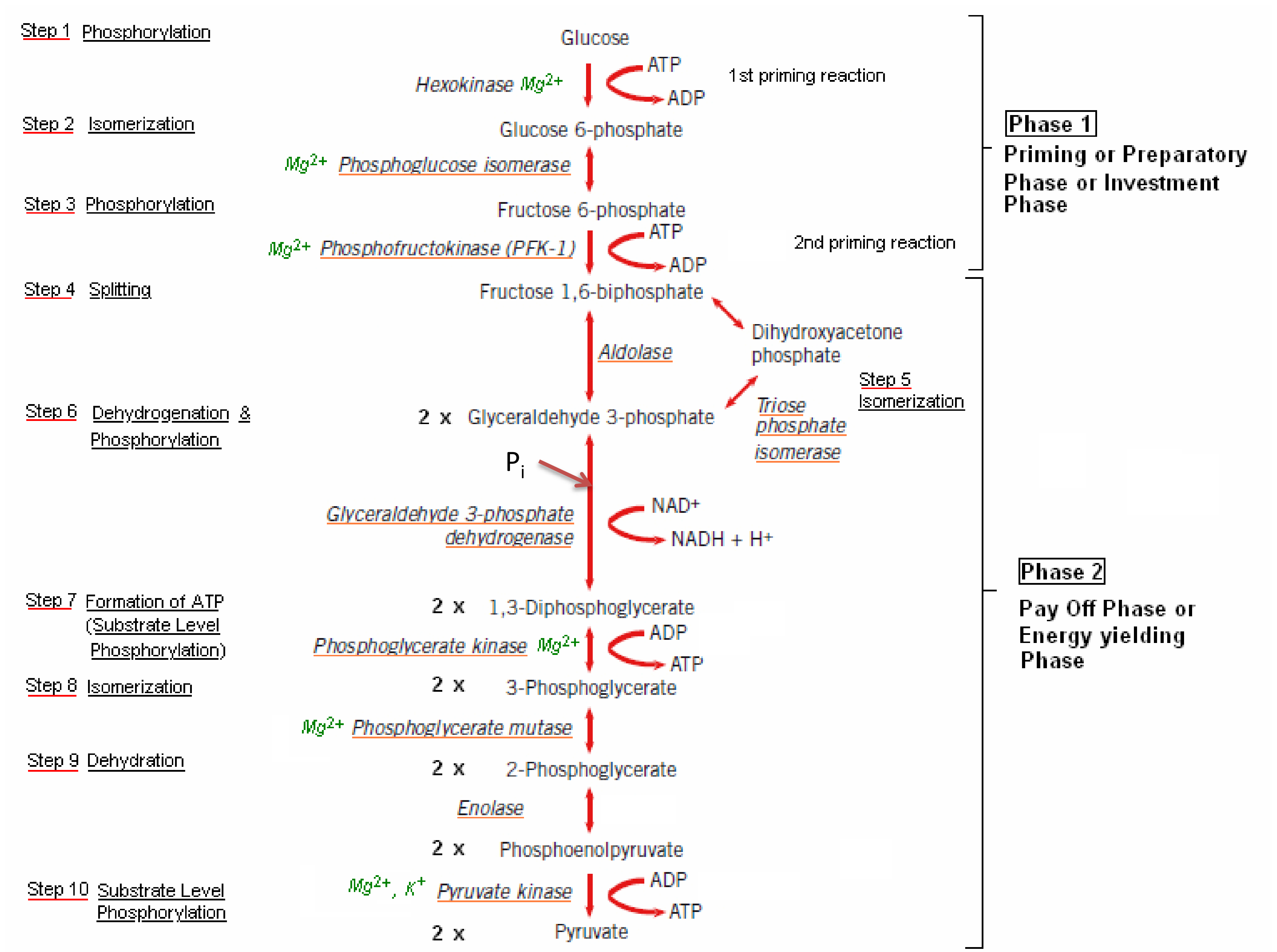
Three routes: (1) the Embden-Meyerhof pathway (Glycolysis), (2) the pentose phosphate pathway, and (3) the Entner-Doudoroff pathway.

The Embden-Meyerhof Pathway

- Most common pathway for glucose degradation to pyruvate in aerobic respiration.
- It is found in all major groups of microorganisms and functions in the presence or absence of O_2.
- Occurs in the cytoplasmic matrix of procaryotic and eucaryotic microbes.

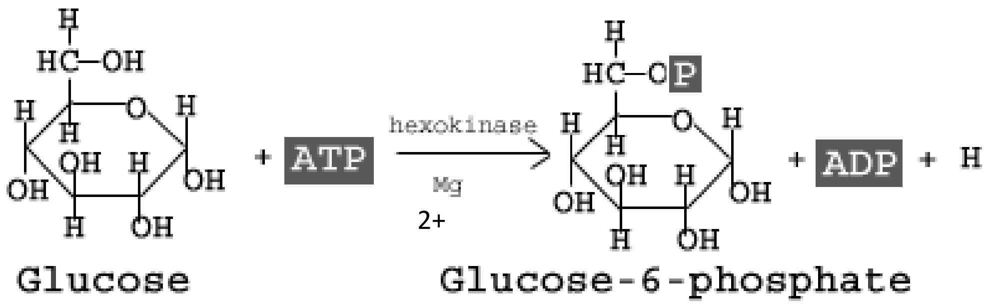
- Also known as glycolysis.
- Greek: *glycos*: sugar, *lysis*: breakdown
- Scheme of glycolysis was given by Gustav Embden, Otto Meyerhof, and J. Parnas, and is often referred to as the **EMP** pathway
- Glycolysis is common step in both aerobic & anaerobic respiration.
- Glucose is oxidized partially into 2 molecules of puruvate in 10 enzyme catalyzed steps.
- The entire glycolysis pathway can be separated into two phases:

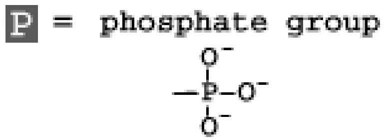
1) The ***Preparatory Phase*** - in which ATP is consumed and is hence also known as the ***investment phase*.**
2) The ***Pay Off Phase*** - in which ATP is produced by ***substrate level phosphorylation***.



Step 1 Phosphorylation
Glucose
ATP
Hexokinase Mg2+
ADP
1st priming reaction
Step 2 Isomerization
Glucose 6-phosphate
Mg2+ Phosphoglucose isomerase
Step 3 Phosphorylation
Fructose 6-phosphate
ATP
Mg2+ Phosphofructokinase (PFK-1)
ADP
2nd priming reaction
Phase 1
Priming or Preparatory Phase or Investment Phase
Step 4 Splitting
Fructose 1,6-biphosphate
Aldolase
Dihydroxyacetone phosphate
Step 5 Isomerization
Triose phosphate isomerase
Step 6 Dehydrogenation & Phosphorylation
2 x Glyceraldehyde 3-phosphate
Pi
Glyceraldehyde 3-phosphate dehydrogenase
NAD+
NADH + H+
Phase 2
Pay Off Phase or Energy yielding Phase
Step 7 Formation of ATP (Substrate Level Phosphorylation)
2 x 1,3-Diphosphoglycerate
Phosphoglycerate kinase Mg2+
ADP
ATP
Step 8 Isomerization
2 x 3-Phosphoglycerate
Mg2+ Phosphoglycerate mutase
Step 9 Dehydration
2 x 2-Phosphoglycerate
Enolase
2 x Phosphoenolpyruvate
Step 10 Substrate Level Phosphorylation
Mg2+, K+ Pyruvate kinase
ADP
ATP
2 x Pyruvate

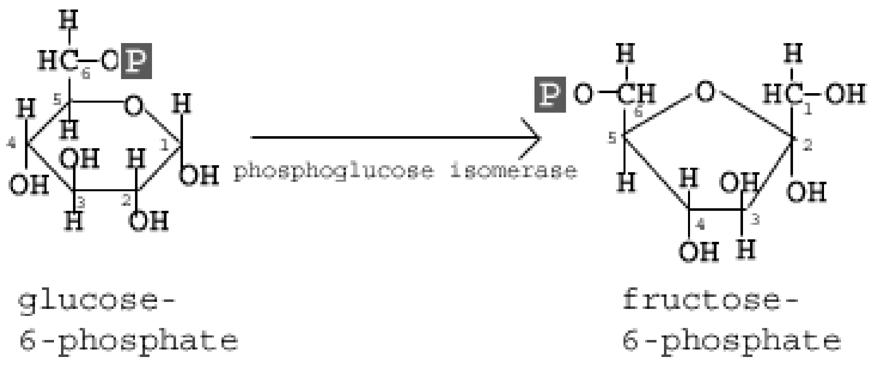
Step 1:



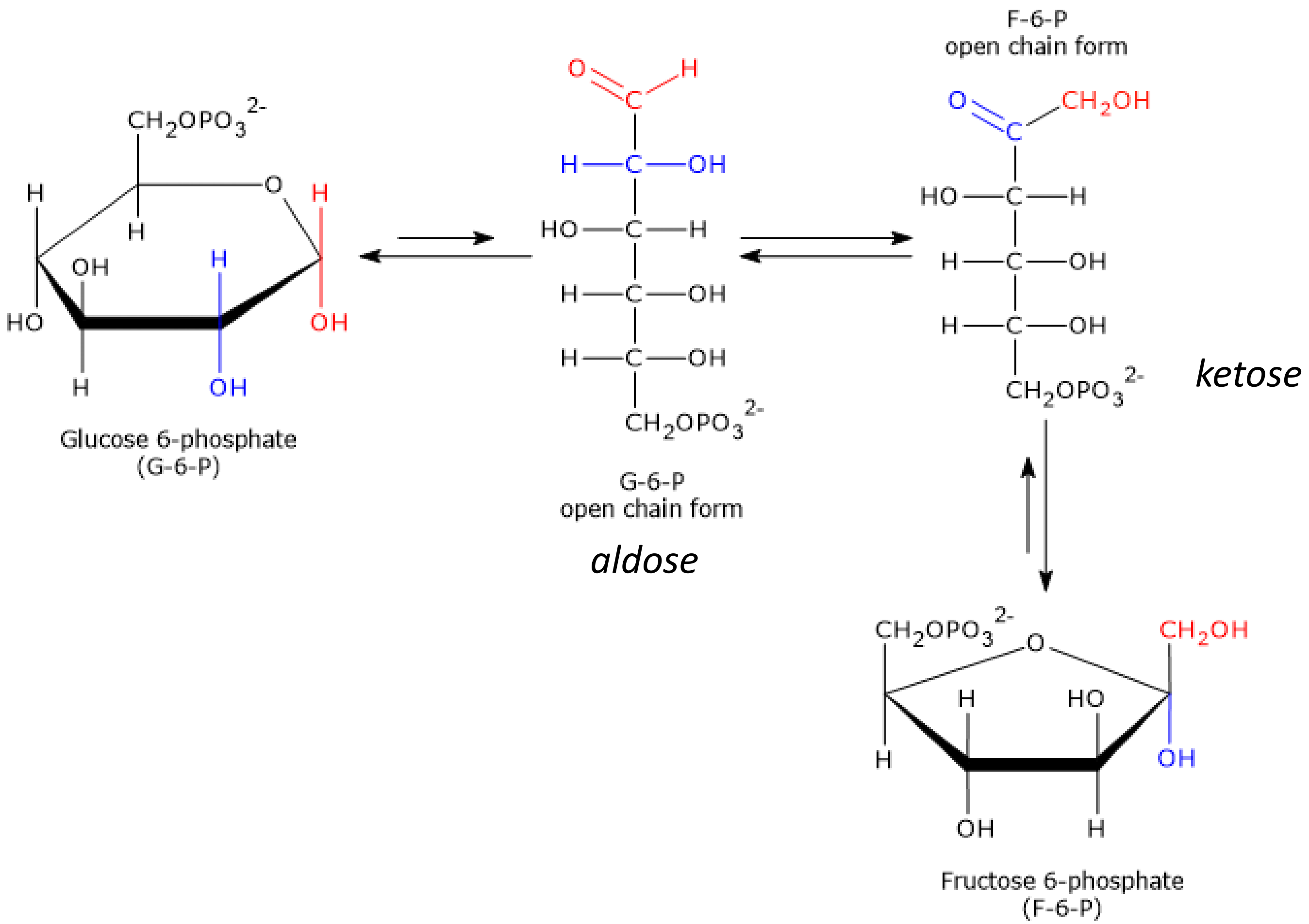


- The glucose ring is phosphorylated and it occurs with the help of the enzyme hexokinase.
- Magnesium (Mg^{2+}) plays a crucial role in this step. It acts as a cofactor by binding to the ATP molecule, helping to stabilize it.
- Specifically, Mg^{2+} shields the negative charges on the phosphate groups of ATP, which reduces the repulsion between them and makes the transfer of the phosphate group to glucose more favorable.
- This stabilization is essential for the proper functioning of the enzyme and the progression of the glycolysis pathway.
- This step is important for trapping glucose (as glucose-6-phosphate) inside the cell and preparing it for further breakdown.

Step 2:

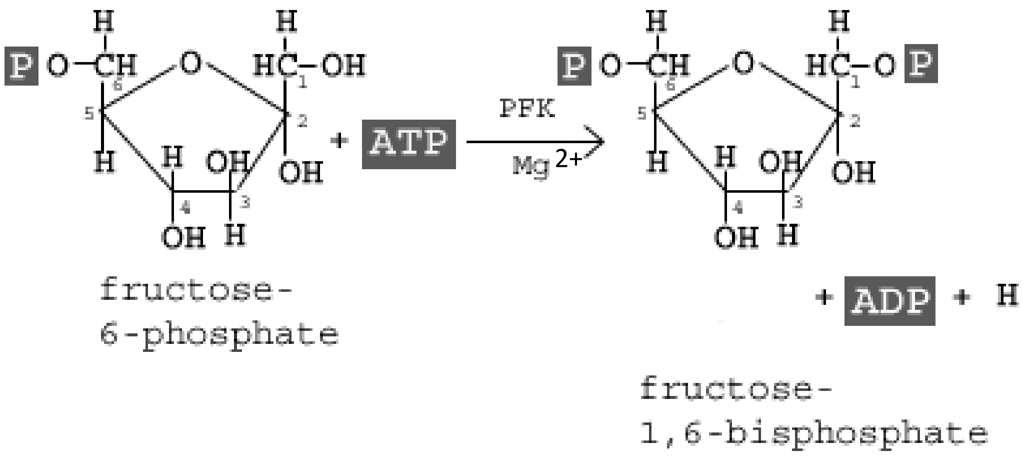


- The second step of glycolysis involves the conversion of glucose-6-phosphate (aldose) to fructose-6-phosphate (F6P) (ketose).
- This reaction occurs with the help of the enzyme phosphoglucose isomerase (PI).
- As the name of the enzyme suggests, this reaction involves an isomerization reaction.
- The reaction involves the rearrangement of the carbon-oxygen bond to transform the six-membered ring into a five-membered ring.
- The rearrangement takes place when the *six-membered ring opens* and *then closes* in such a way that the *first carbon becomes now external to the ring*.



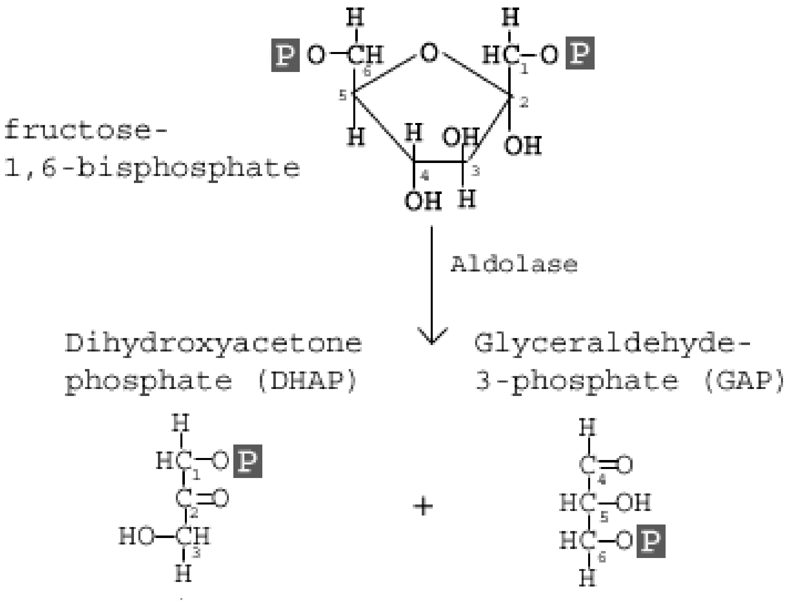
- Magnesium (Mg^{2+}) does not play a direct catalytic role in this specific step of glycolysis.
- However, magnesium ions can still be indirectly involved by maintaining the overall cellular environment necessary for enzyme activity and stability.

Step3:



- In the third step of glycolysis, fructose-6-phosphate is converted to fructose- 1,6-*bis*phosphate (FBP).
- Similar to the reaction that occurs in step 1 of glycolysis, a second molecule of ATP provides the phosphate group that is added on to the F6P molecule.
- The enzyme that catalyzes this reaction is phosphofructokinase (PFK).
- As in step 1, a magnesium ions are involved to help shield negative charges.

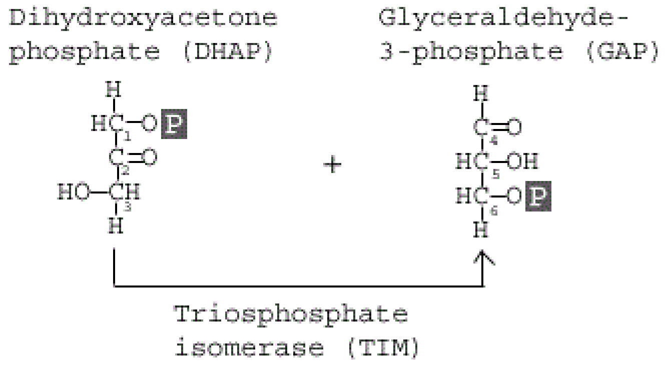
Step 4:



- The enzyme Aldolase splits fructose 1, 6-bisphosphate into two sugars, i.e., dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP), which are isomers of each other.

- The enzyme that catalyzes this reaction is aldolase.

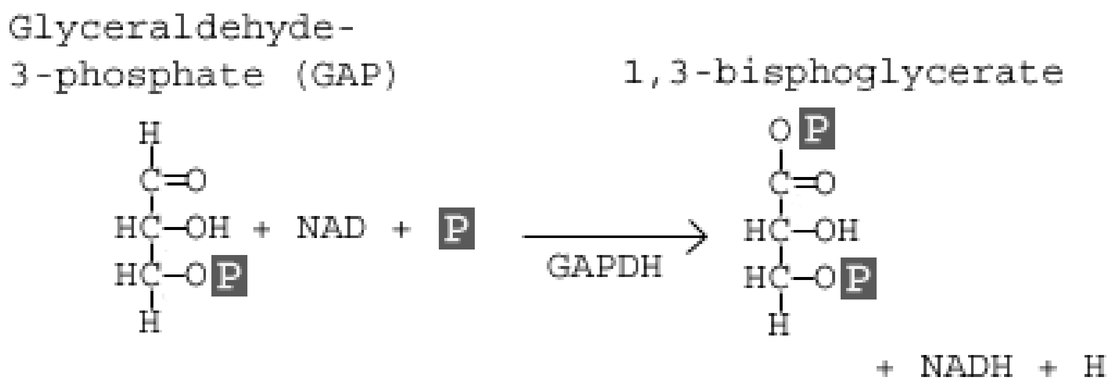
Step 5:



- The enzyme triosephosphate isomerase rapidly inter-converts the molecules dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP).

- GAP is the only molecule that continues in the glycolytic pathway. As a result, all of the DHAP molecules produced are further acted on by the enzyme triosephosphate isomerase, which reorganizes the DHAP into GAP so it can continue in glycolysis.

Step 6:



- In this step, two main events take place:

1) glyceraldehyde-3-phosphate is oxidized by the coenzyme nicotinamide adenine dinucleotide (NAD);

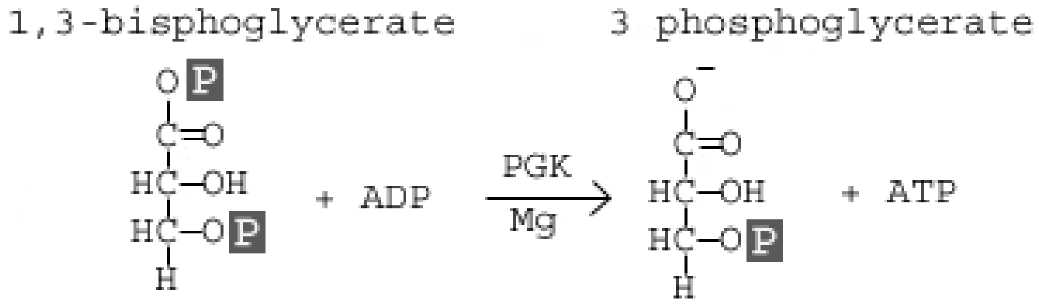
2) the molecule is phosphorylated by the addition of a free phosphate group.

- The enzyme that catalyzes this reaction is glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

- The enzyme GAPDH contains appropriate structures and holds the molecule in a conformation such that it allows the NAD molecule to pull a hydrogen off the GAP, converting the NAD to NADH.

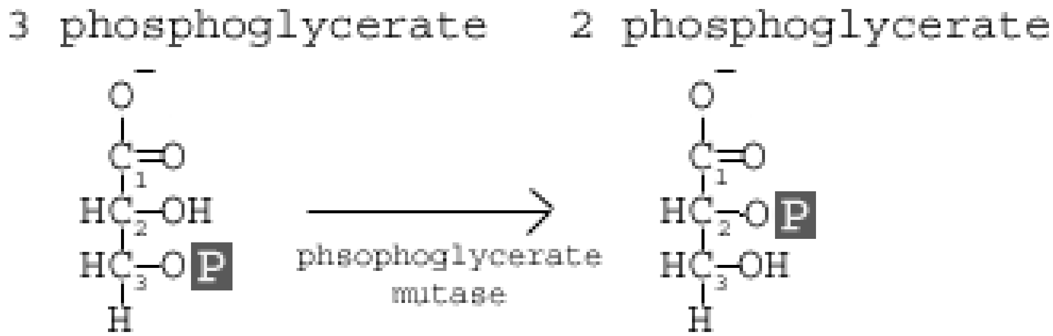
- The phosphate group then attacks the GAP molecule and releases it from the enzyme to yield 1,3 bisphoglycerate, NADH, and a hydrogen atom.

Step 7:



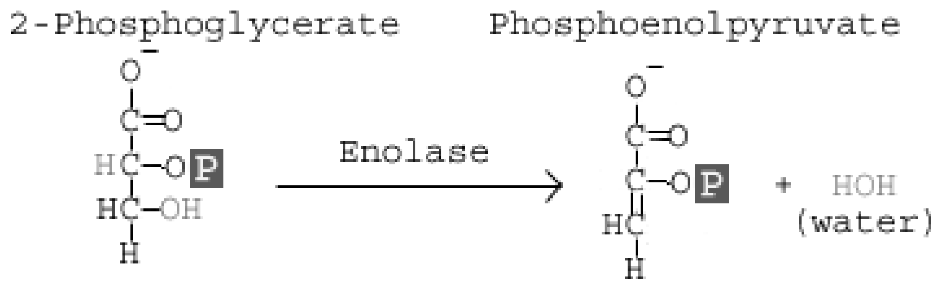
- In this step, 1,3 bisphoglycerate is converted to 3-phosphoglycerate by the enzyme phosphoglycerate kinase (PGK).
- This reaction involves the loss of a phosphate group from the starting material. The phosphate is transferred to a molecule of ADP that yields our first molecule of ATP.
- Since we actually have two molecules of 1,3 bisphoglycerate (because there were two 3-carbon products from stage 1 of glycolysis), we actually synthesize two molecules of ATP at this step.
- With this synthesis of ATP, we have cancelled the first two molecules of ATP that we used, leaving us with a net of 0 ATP molecules up to this stage of glycolysis.
- Again, we see that an atom of magnesium is involved to shield the negative charges on the phosphate groups of the ATP molecule.

Step 8:



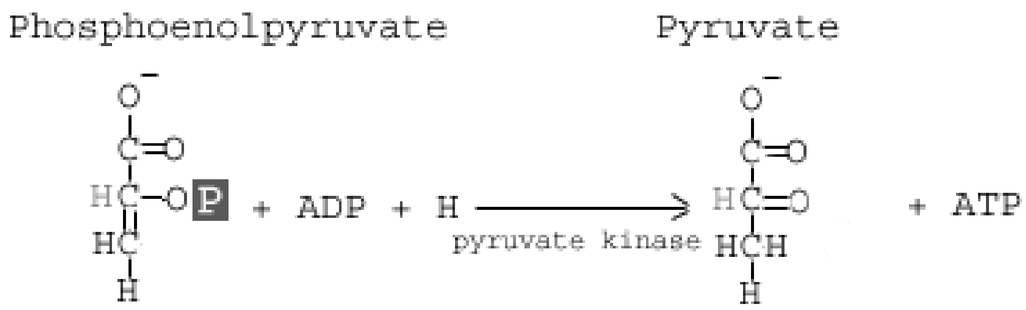
- This step involves a simple rearrangement of the position of the phosphate group on the 3 phosphoglycerate molecule, making it 2 phosphoglycerate.
- The molecule responsible for catalyzing this reaction is called phosphoglycerate mutase (PGM). A *mutase* is an enzyme that catalyzes the transfer of a functional group from one position on a molecule to another.
- The reaction mechanism proceeds by first adding an additional phosphate group to the 2′ position of the 3 phosphoglycerate.
- The enzyme then removes the phosphate from the 3′ position leaving just the 2′ phosphate, and thus yielding 2 phsophoglycerate.
- In this way, the enzyme is also restored to its original, phosphorylated state.

Step 9:



- This step involves the conversion of 2 phosphoglycerate to phosphoenolpyruvate (PEP).
- The reaction is catalyzed by the enzyme enolase.
- Enolase works by removing a water group, or *dehydrating* the 2 phosphoglycerate.
- The specificity of the enzyme pocket allows for the reaction to occur through a series of complicated steps.

Step 10:



- The final step of glycolysis converts phosphoenolpyruvate into pyruvate with the help of the enzyme pyruvate kinase.
- As the enzyme's name suggests, this reaction involves the transfer of a phosphate group.
- The phosphate group attached to the 2′ carbon of the PEP is transferred to a molecule of ADP, yielding ATP.
- Again, since there are two molecules of PEP, here we actually generate 2 ATP molecules.

Steps 1 and 3 =	**– 2ATP**
Steps 7 and 10 =	**+ 4 ATP**
Net ATP produced =	**+ 2.**

- Magnesium (Mg^{2+}) and potassium (K^{+}) are essential cofactors in this step.

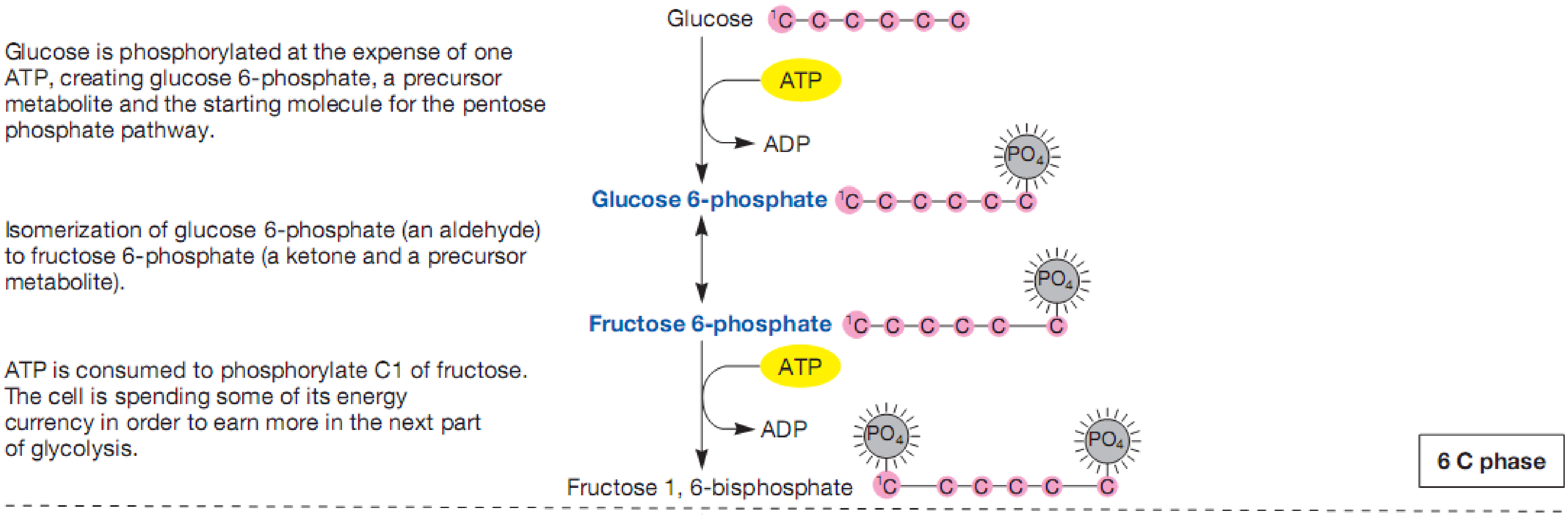
- Mg^{2+} binds to ATP and stabilizes the negative charges on its phosphate groups, similar to its role in other ATP-dependent reactions.

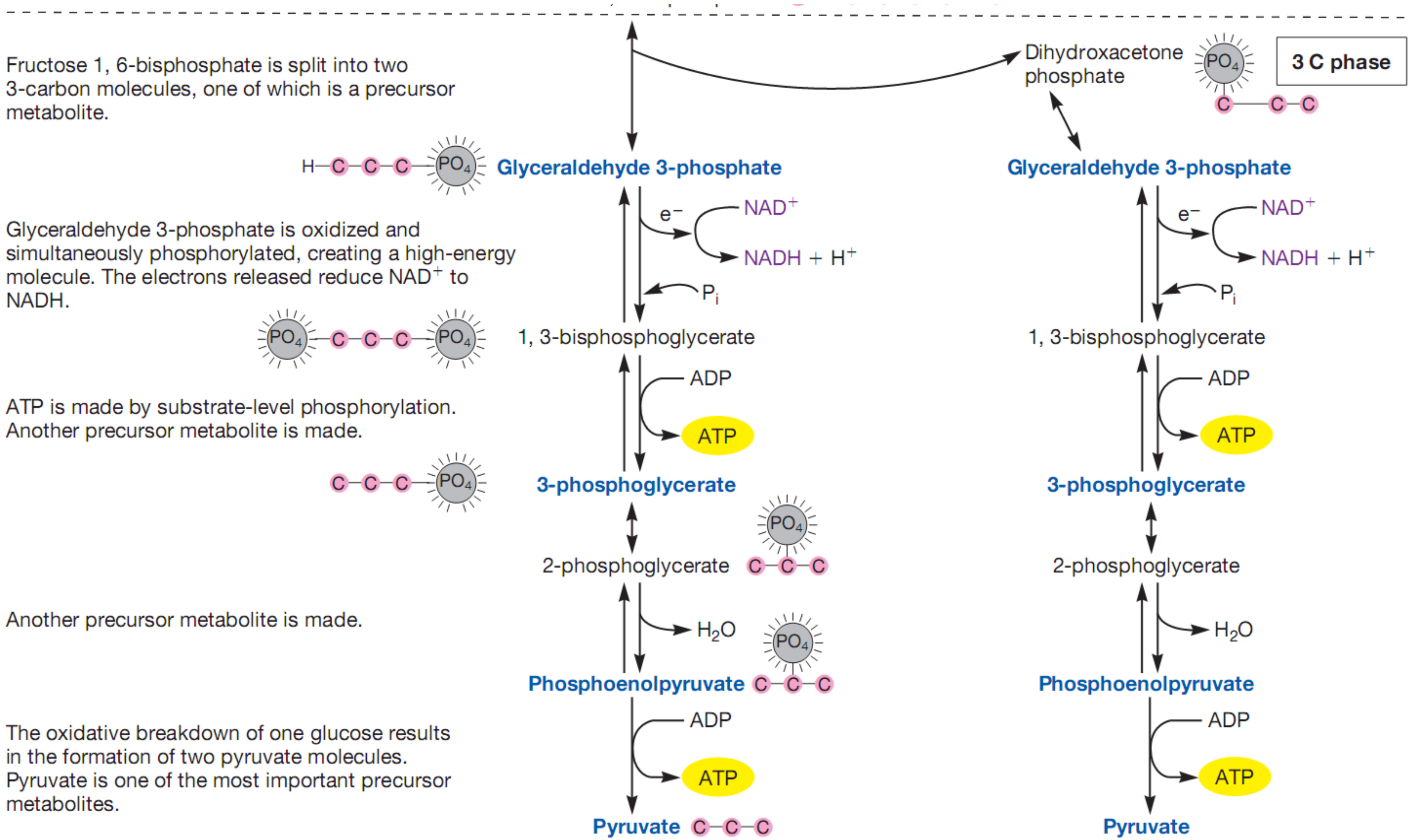
- Mg^{2+} is also directly involved in the catalytic activity of pyruvate kinase. It helps in the proper positioning of the substrate and the enzyme, facilitating the transfer of the phosphate group from PEP to ADP, forming ATP and pyruvate.

- Potassium ions (K^+) help maintain the structural conformation of pyruvate kinase, which is necessary for its enzymatic activity. K^+ ensures the enzyme is in the correct shape to catalyze the reaction efficiently.

- K^+ enhances the catalytic efficiency of pyruvate kinase by stabilizing the enzyme-substrate complex. This stabilization is crucial for the effective transfer of the phosphate group from PEP to ADP.

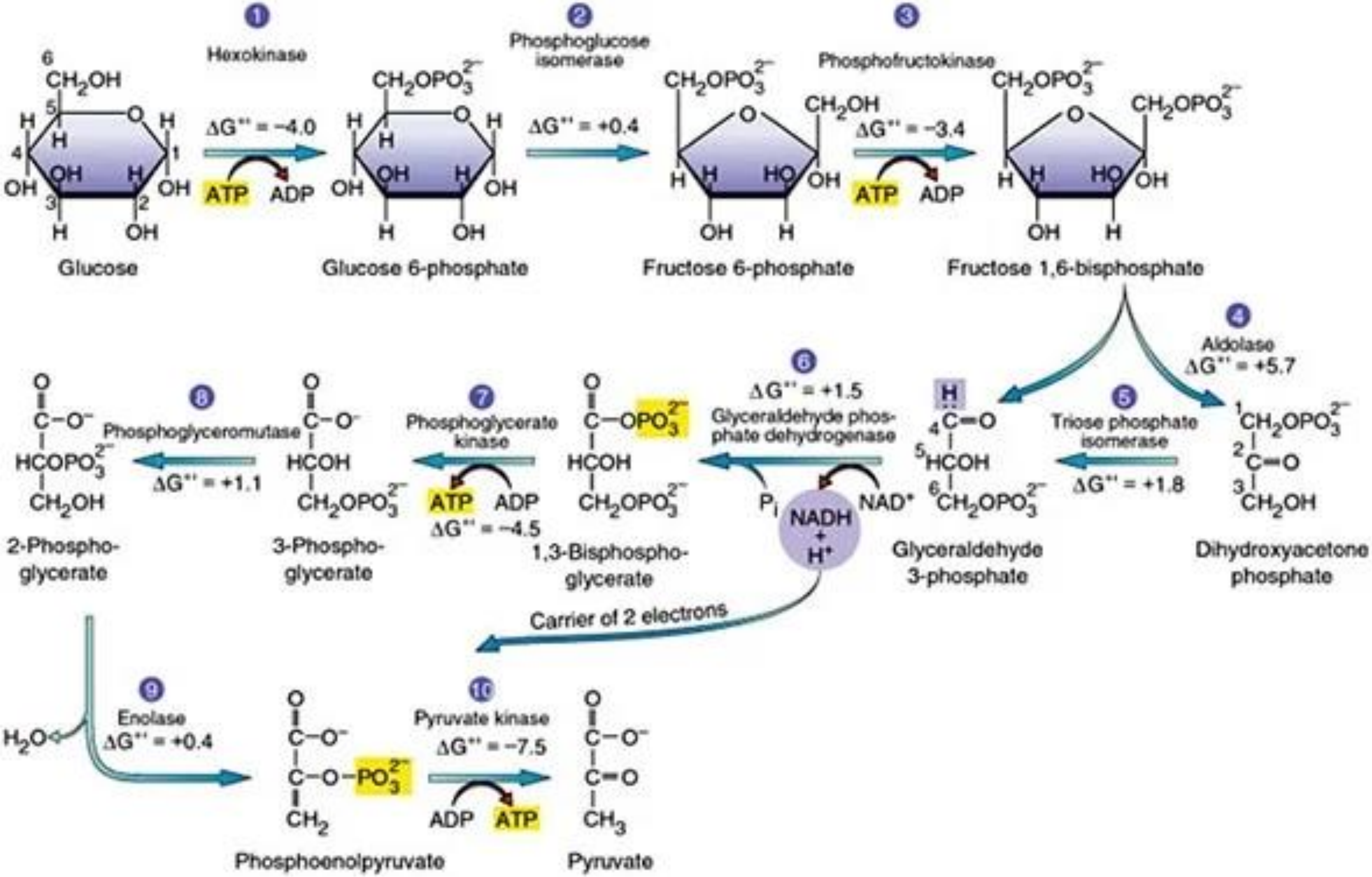
The Embden-Meyerhof pathway is also an important amphibolic pathway, as it generates several precursor metabolites (shown in blue).





$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i + 2\text{NAD}^+ \longrightarrow$$

$$2 \text{ pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+$$


1
Hexokinase
ΔG°' = −4.0
ATP
ADP
Glucose
2
Phosphoglucose isomerase
ΔG°' = +0.4
Glucose 6-phosphate
3
Phosphofructokinase
ΔG°' = −3.4
ATP
ADP
Fructose 6-phosphate
Fructose 1,6-bisphosphate
4
Aldolase
ΔG°' = +5.7
5
Triose phosphate isomerase
ΔG°' = +1.8
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
6
ΔG°' = +1.5
Glyceraldehyde phosphate dehydrogenase
Pi
NAD+
NADH + H+
1,3-Bisphosphoglycerate
7
Phosphoglycerate kinase
ATP
ADP
ΔG°' = −4.5
3-Phosphoglycerate
8
Phosphoglyceromutase
ΔG°' = +1.1
2-Phosphoglycerate
Carrier of 2 electrons
9
Enolase
ΔG°' = +0.4
H2O
Phosphoenolpyruvate
10
Pyruvate kinase
ΔG°' = −7.5
ADP
ATP
Pyruvate