

GLYCOLYSIS Biochemistry - ②

Glycolysis is a process that results in the conversion of a molecule of glucose to two molecules of pyruvic acid.

It is also named as the "^(EMP)Embden-Meyerhof-Parnas" pathway after the investigation which made major contributions to its discovery in the 1930s.

Important characteristics of glycolysis

- i) Of various type of glycolysis EMP-pathway is most common, other pathways are HMP pathway and ED pathway.
 - ii) It is basically an anaerobic pathway and does not require oxygen.
 - iii) It is an enzymatic 10 steps reductive process in which glucose/fructose/sucrose/respiratory substrate is reduced to pyruvic acid with the formation of ATP and NADH.
 - iv) All the reactions of glycolysis are reversible except those catalysed by hexokinase (Step 1), phosphofructokinase (Step 3) and pyruvate kinase (Step 10).
 - v) Enzymes concerned with glycolysis are mostly tetramers or dimers.
 - vi) Glycolysis proceeds more rapidly under anaerobic conditions than under aerobic conditions. This is known as "Pasteur effect".
 - vii) When glucose is added, oxygen consumption is inhibited and this is known as "Crabtree effect".
 - viii) Nearly all the glycolysis enzymes require Mg^{+2} as cofactor.
- Mechanism of Glycolysis

Glycolysis is not a simple process. The glycolytic pathway involves a sequence of 10 enzyme-catalyzed reactions, where a molecule of glucose yields two molecules of pyruvic acid. The enzymes involved in glycolysis are found in the cytoplasm, hence glycolysis occurs in cytoplasm of cells outside the mitochondria.

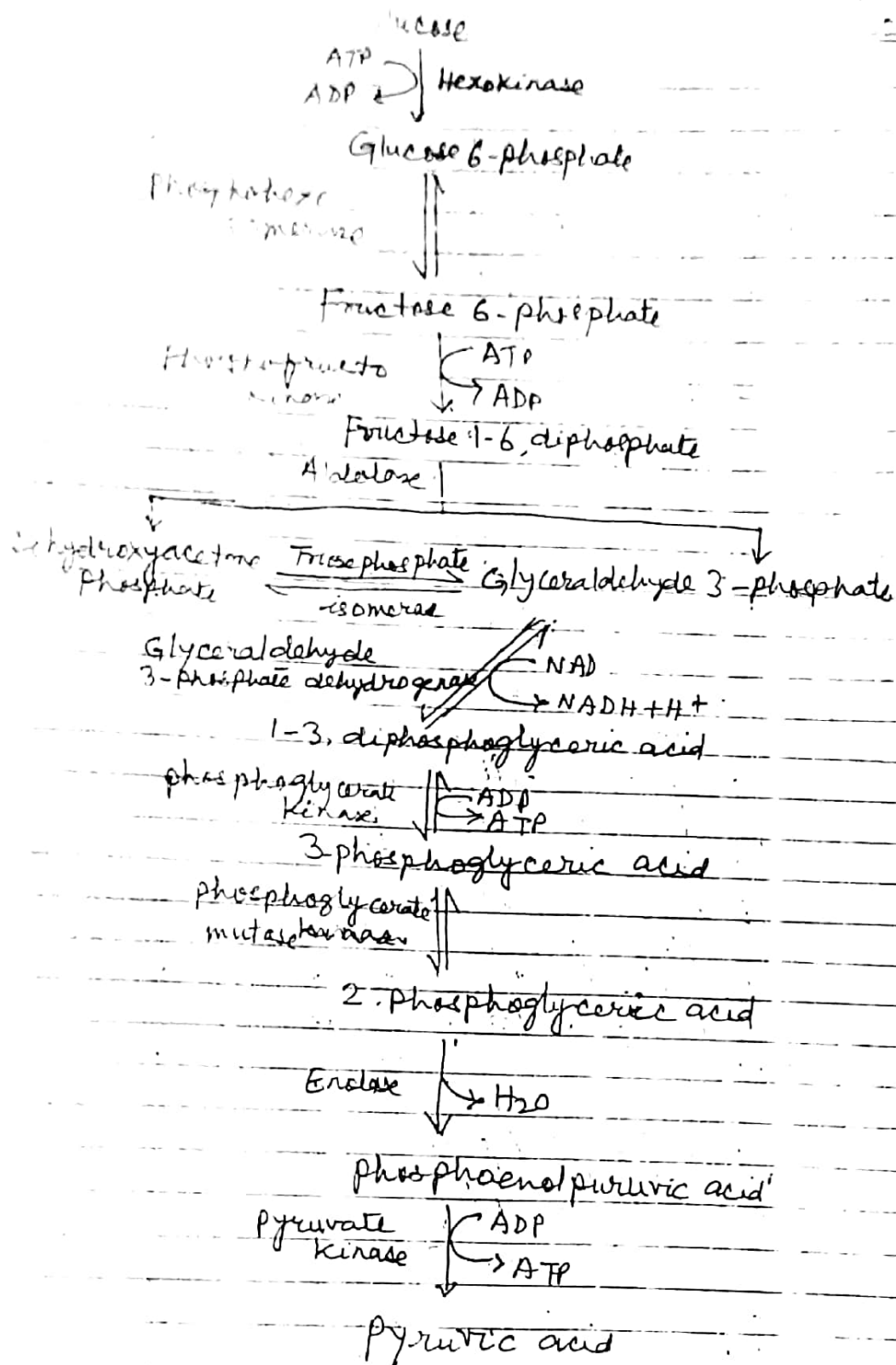
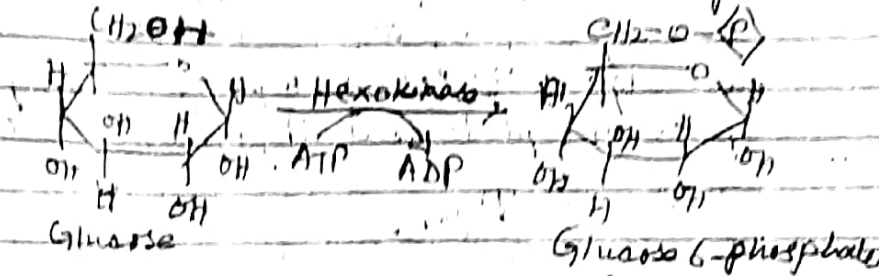
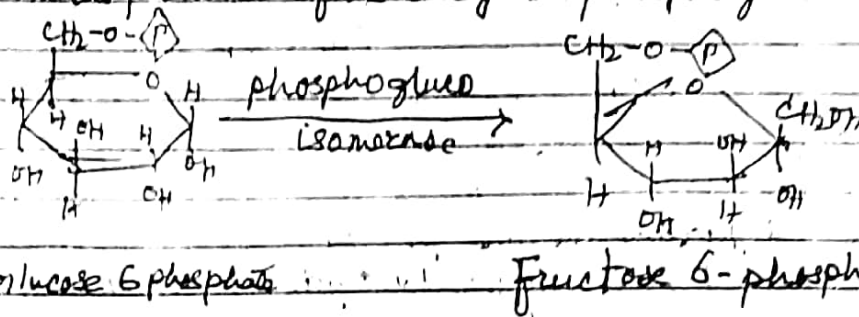


Fig. The glycolytic pathway.

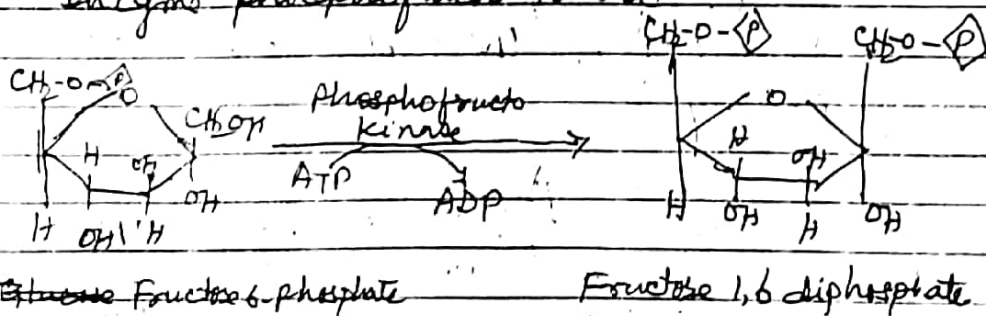
Step - 1 : Phosphorylation : Glucose is converted into Glucose 6-phosphate via hexokinase. This is an irreversible reaction which requires ATP.



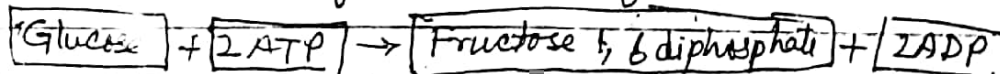
Step 2 : Isomerization : Glucose 6-phosphate is converted to its isomeric form fructose 6-phosphate in the presence of the enzyme phosphoglucose isomerase.



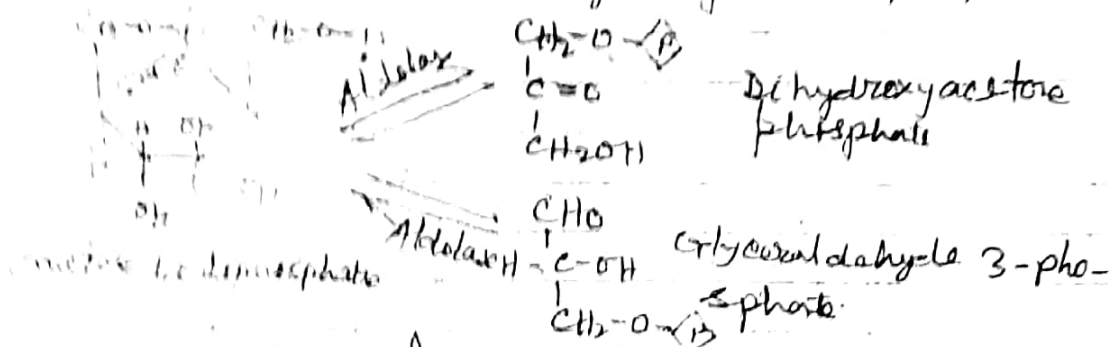
Step 3 : Phosphorylation : A second phosphorylation reaction follows the isomerization. Fructose 6-phosphate is phosphorylated by ATP to fructose 1,6-diphosphate and the reaction is catalyzed by the enzyme phosphofructo kinase.



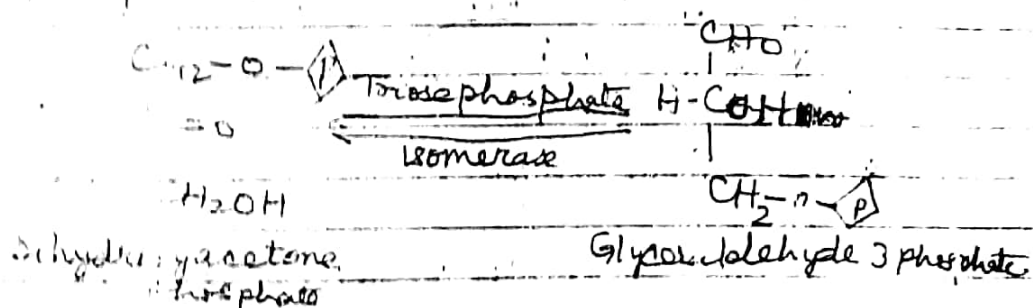
The overall effect of the above three reactions can be represented as follows, which shows the expenditure of 2 molecules of ATP.



the cleavage of fructose 1,6 diphosphate into two molecules involves the splitting of (P₁₆) 6 carbon fructose 1,6-diphosphate molecules between carbon atom 3 and 4 by the enzyme aldolase into two molecules of 3 carbon phosphate, namely glyceraldehyde phosphate and dihydroxyacetone phosphate.



Step 5: Isomerization : Glyceraldehyde 3-phosphate is ~~not~~ the direct pathway of glycolysis but dihydroxyacetone phosphate is not. However dihydroxyacetone phosphate can be readily converted into glyceraldehyde 3-phosphate as both are isomers. Glyceraldehyde 3-phosphate is an aldose and dihydroxyacetone phosphate is a ketose. This isomerization is catalyzed by the enzyme triosephosphate isomerase and this reaction is reversible and thus two molecules of glyceraldehyde 3-phosphate is formed from one molecule of fructose 1,6-diphosphate.

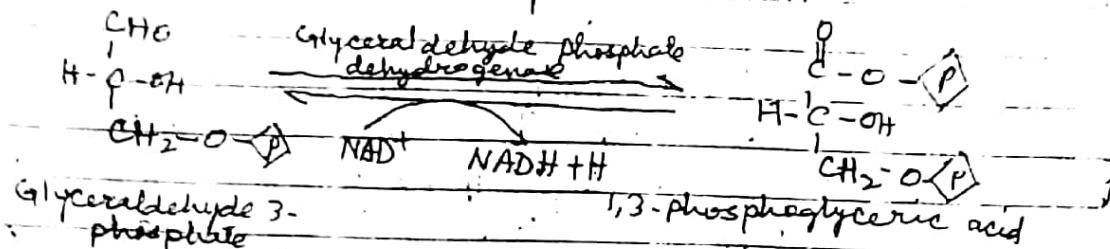


Step 6: Oxidation and phosphorylation. Glyceraldehyde 3-phosphate is oxidized and phosphorylated simultaneously under the influence

of the enzyme glyceraldehyde 3-phosphate dehydrogenase in the presence of NAD and inorganic phosphate to form 1,3-diphosphoglyceric acid.

The co-enzyme in the oxidized form and has a net +ve charge and written as NAD^+ or

When glyceraldehyde 3-phosphate is oxidized, NAD^+ is reduced to NADH and one proton (H^+) is released into the aqueous medium.



Glyceral phosphate shuttle:

The NADH produced during glycolysis can not directly enter the respiratory chain, as mitochondria is impermeable to both NADH and NAD^+ . Hence instead of NADH , the electron from the NADH are carried into the mitochondria a system that operate between the mitochondria and cytoplasm known as Glyceral phosphate shuttle by which each NADH molecule produces only 2 molecules of ATP instead of 3 molecules of ATP.

The first step in the shuttle is the transfer of electron from NADH to Dihydroxyacetone phosphate, thereby resulting in the formation of glyceraldehyde 3-phosphate. This reaction is catalyzed by glyceraldehyde 3-phosphate dehydrogenase that occurs in the cytoplasm.

Glyceral 3-phosphate, acting as a carrier enters the mitochondria where it is re-oxidized to dihydroxyacetone phosphate by the transfer of electrons to FAD . As a result FAD reduced to FADH_2 and dihydroxyacetone phosphate diffuses out from mitochondria into cytoplasm to complete the shuttle.

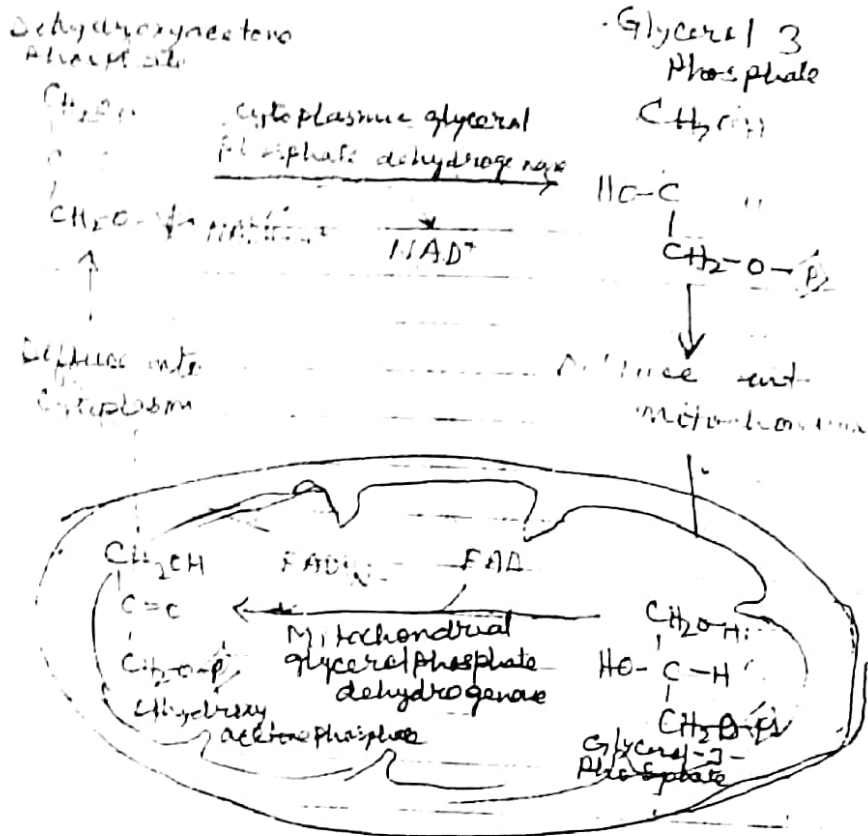


Fig. Glycerol phosphate shuttle.

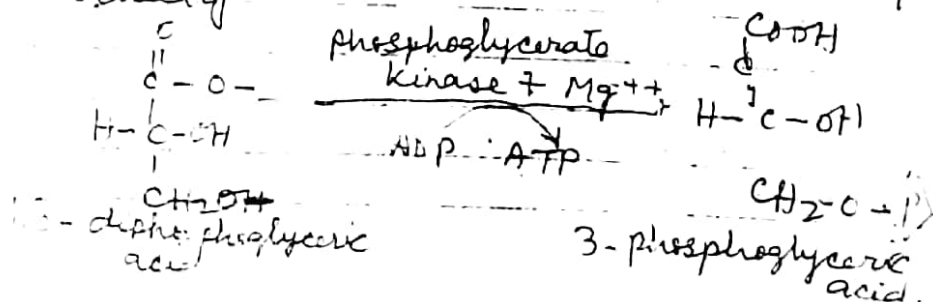
The FADH₂ transfer the electron to the respiratory chain at the level of CVO (the second site of ATP formation), so 2 molecules of ATP rather than 3 molecules are synthesized.

Step 7: Substrate level phosphorylation.

The high energy phosphate group in 1-3-diphosphoglycerate is transfer to ADP resulting in the formation of 3-phosphoglyceric acid and ATP.

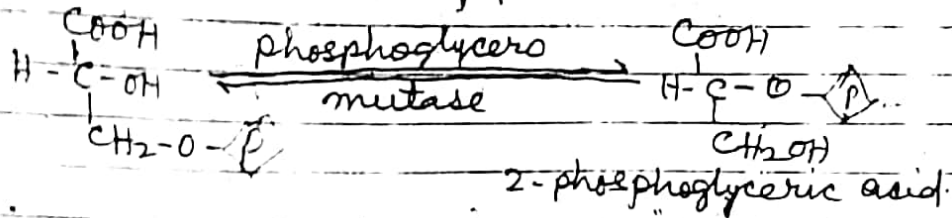
The enzyme that catalyze the reaction is phosphoglycerate kinase in the presence of Mg^{++} .

The phosphorylation which occurs in this step is substrate level phosphorylation, where a high energy phosphate bond is formed directly.

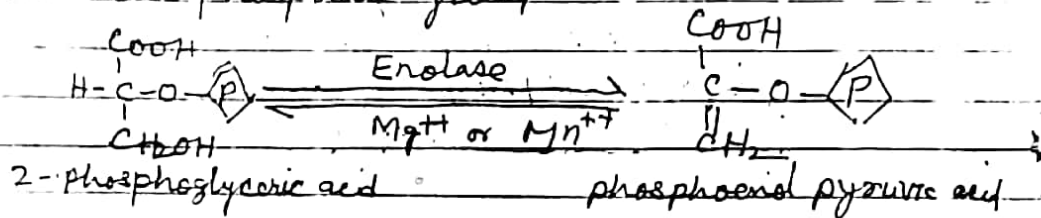


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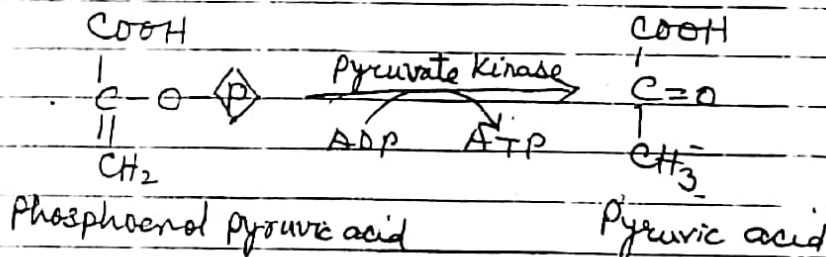
Step-8: Isomerization: The 3-phosphoglyceric acid undergoes internal rearrangement to form 2-phosphoglyceric acid. The reaction is catalyzed by the enzyme phosphoglyceromutase.



Step-9: Dehydration: The 2-phosphoglycerate is dehydrated by the action of enzyme enolase in the presence of Mg^{++} or Mn^{++} to yield phosphoenolpyruvic acid which has a high energy enolic phosphate group.

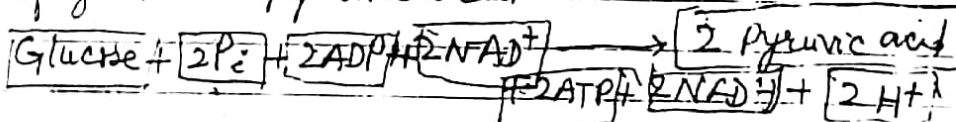


Step-10: Substrate level phosphorylation
In the final step, there is transfer of phosphate from phosphoenolpyruvic acid to ADP. Thus a molecule of ATP is directly synthesized and pyruvic acid is also formed. This reaction is catalyzed by the enzyme pyruvate kinase.



Energy Yield in Glycolysis:

The net reduction in the transformation of glucose to pyruvic acid



Thus two molecules of ATP generated in the conversion of glucose to pyruvic acid. The reduced NAD ($\text{NADH} + \text{H}^+$) enters the electron transport chain, where the reoxidation of NADH to NAD occurs with the liberation of some 2-molecules of ATP (by glycerol phosphate shuttle).

Reactant	
Glucose $\rightarrow$ Glucose 6-phosphate	+1
Fructose 6 phosphate $\rightarrow$ Fructose 1,6-diphosphate	+1
2 1,3-phosphoglyceric acid $\rightarrow$ 2 3-phosphoglyceric acid	+1.2
2 Phosphoglyceric acid $\rightarrow$ 2 pyruvic acid	+2
2 NADH + 2 H^+ $\rightarrow$ 2 pairs of electron through electron transport chain (2x2) by glycerol phosphate shuttle	+4
Net gain	+6