VIVEKANANDA COLLEGE
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NAAC ACCREDITED 'A' GRADE

Topic:	RESPIRATION
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Name of the Department:	Botany (Morning)

Embden-Meyerhof Pathway or Glycolysis:

This catabolic pathway or EMP Pathway leads to splitting of glucose to two molecules of pyruvic acid i.e. a six- carbon compound is broken down to two molecules of three-carbon compounds. This transformation occurs in several steps and, in the process two molecules of ATP are generated by substrate-level phosphorylation.

- Glycolysis **takes place in the extra mitochondrial part of the cell** (or **the soluble cytoplasm**). Altogether 10 different enzymes are involved which are present in soluble state in the cytosol as free entities.

- In the sequential reactions of glycolysis , three types of chemical transformations are noted. These are:
  - **Degradation of glucose to yield pyruvate,**
  - **Phosphorylation of ADP to ATP by high energy phosphate compounds formed during glycolysis,**
  - **Transfer of a hydride ion to NAD^+ , by forming NADH.**

- During glycolysis, the 6-carbon glucose is broken down into two molecules of 3-carbon pyruvate via 10 enzyme catalysed sequential reactions. These reactions are grouped under two phases, phase I (**preparatory phase**) and phase II (**pay off phase**).

The steps of glycolysis and the names of the enzymes are given below in Figure. -----

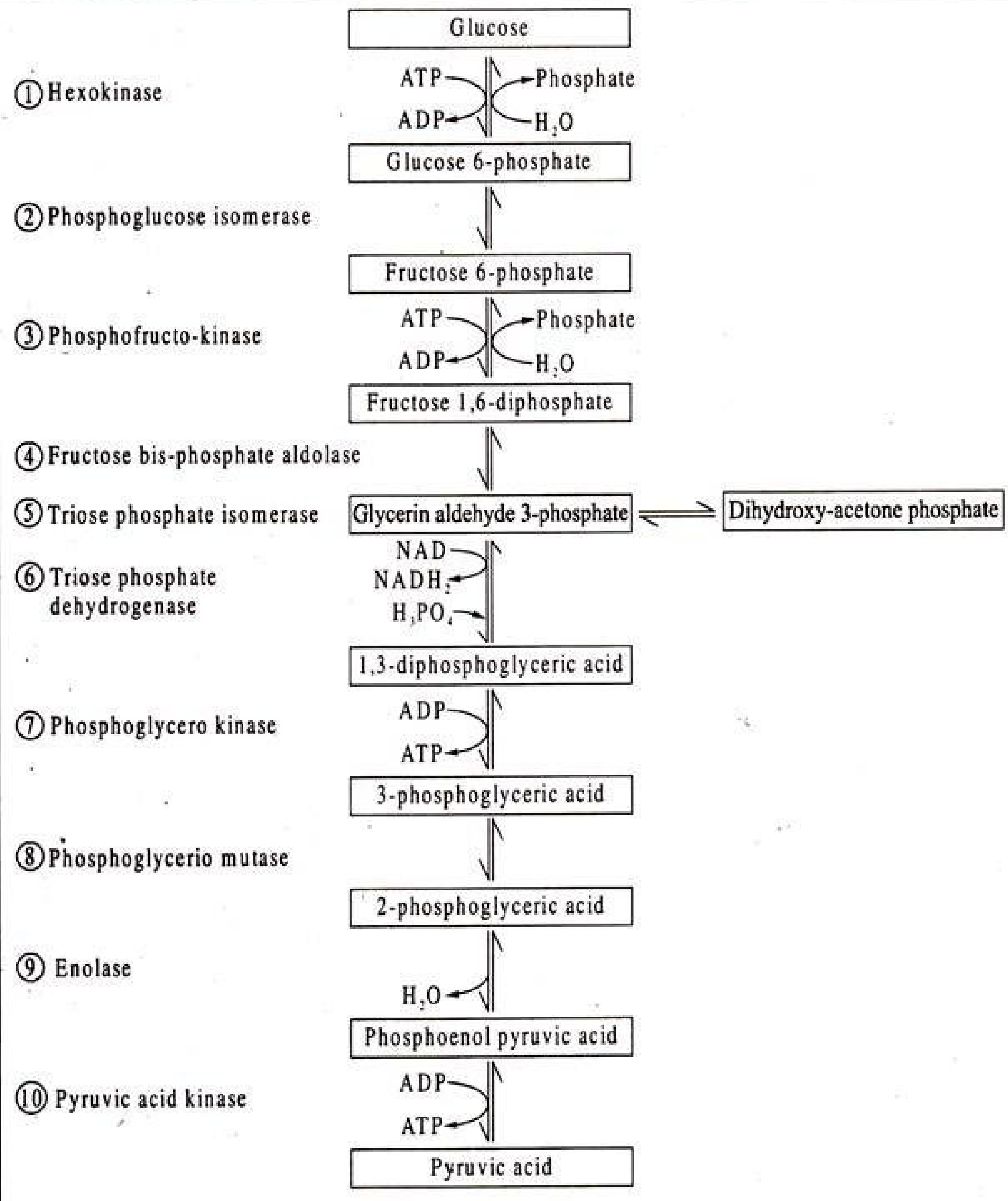


Fig. 8.43 : The glycolytic pathway

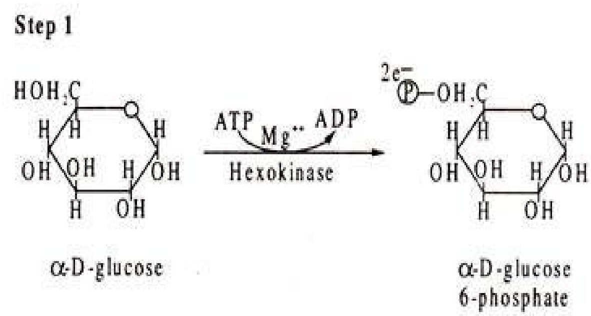
- **Phase 1 or Preparatory phase:** It consists of the **first 5 steps**.This phase requires an investment of 2 molecules of ATP to activate the glucose molecule and prepare it for its cleavage into two 3-carbon pieces.

The details of the individual steps of the glycolytic pathway are discussed:

Step 1: Phosphorylation of Glucose

In the first step a neutral glucose molecule is activated to negatively charge glucose-6-phosphate by the **enzyme hexokinase** using **ATP** as the **phosphate donor and Mg++ as cofactor**. The hexokinase reaction is strongly exergonic and is not freely reversible. The reverse reaction regenerating glucose from glucose 6-phosphate is catalysed by a different enzyme glucose 6-phosphatase which liberates inorganic phosphate.

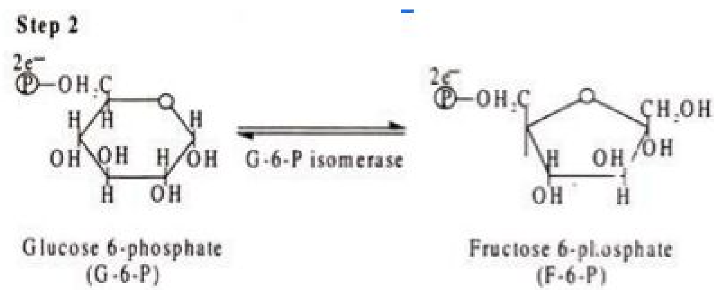
Glucose→Glucose 6-phosphate



Step 2: Isomerization of Glucose 6-phosphate

The second step of glycolysis involves isomerization of aldohexose phosphate(G-6-P) to ketohexose phosphate(F-6-P) by an **isomerase** . the reaction is freely reversible.

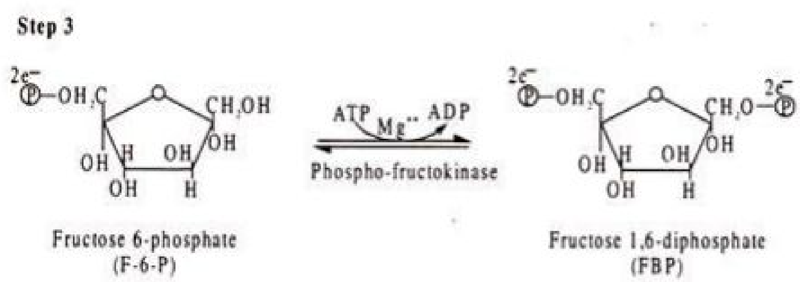
Glucose 6-phosphate↔Fructose 6-phosphate



Step 3: Phosphorylation of Fructose 6-phosphate

In the third step, fructose 6-phosphate is further phosphorylated to fructose bisphosphate. The reaction is catalysed by fructose 6-phosphate kinase also known as **phosphofructokinase**. It **requires ATP and Mg++** and drives the reaction unidirectionally. The reverse reaction (FBP —> F 6-P) is catalysed by di-phospho-fructose phosphatase.

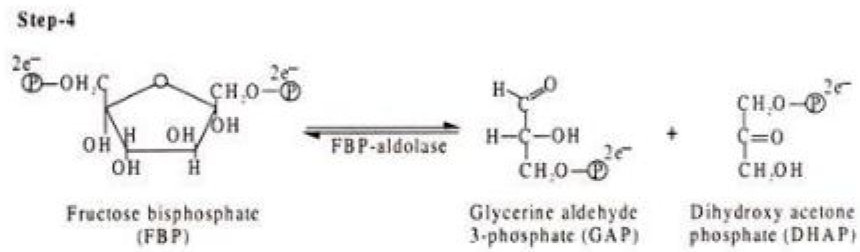
Fructose 6-phosphate→Fructose 1,6-bisphosphate



Step 4: Cleavage of Fructose 1,6-bisphosphate

The fourth step consists of cleavage of FBP into two triose phosphates, GAP and DHAP, catalysed by a lyase, called **FBP aldolase.** The aldolase reaction is reversible and the reverse reaction consists of an aldol condensation. Hence the enzyme is known as aldolase. The cleavage reaction distributes carbon atoms 1, 2 and 3 of FBP to GAP and carbon atoms 4, 5 and 6 to DHAP.

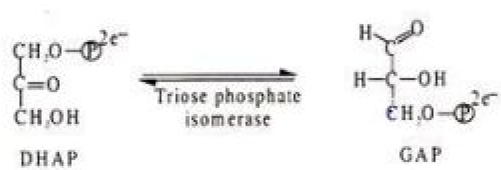
Fructose 1,6-bisphosphate↔Dihydroxyacetone phosphate + Glyceraldehyde 3-phosphate



Step 5: Isomerization of Dihydroxyacetone phosphate

The **two triose phosphates** are **freely inter-convertible** through the action of **triose phosphate isomerase**, but the equilibrium is strongly in favour of DHAP ,so that, at any given time, about 90% of the triose phosphates is in the form of DHAP. Further breakdown takes place only from GAP. As more and more of GAP is consumed in the subsequent steps of glycolysis, DHAP is converted to GAP to maintain the supply of GAP.

Dihydroxyacetone phosphate↔Glyceraldehyde 3-phosphate

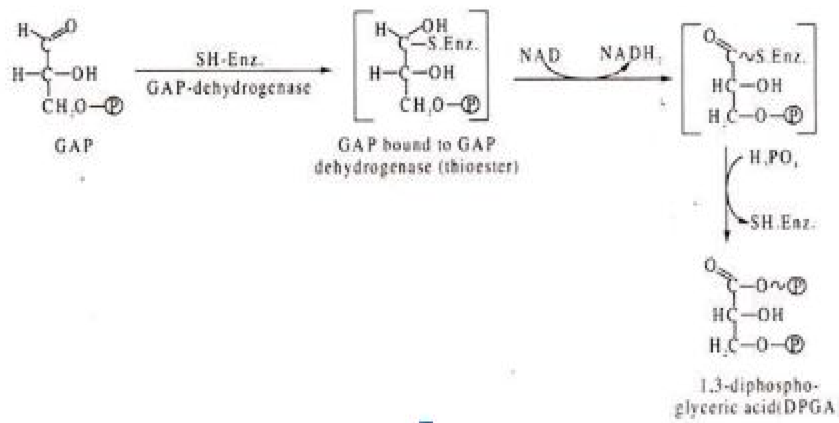


- **Phase II or Payoff Phase**:- The **last 5 reactions of glycolysis** constitute this phase. This phase represents the payoff of glycolysis, in which the energy liberated during conversion of 3 molecules of glyceraldehyde 3-phosphate to 2 molecules of pyruvate is converted by the coupled phosphorylation of 4 molecules of ADP to ATP. Although 4 per molecule of glucose is oxidised, since 2 molecules of ATP are invested in phase I. the phase II is thus energy conserving. Energy is also conserved in the payoff phase in the formation of two molecules of NADH per molecule of glucose.

Step 6: Oxidative phosphorylation of Glyceraldehyde 3-phosphate

The sixth step involves a complex reaction in which GAP is oxidized to di-phosphoglyceric acid, **NAD (coenzyme)** acting as the H-acceptor to form $NADH_2$. The reaction is catalysed by the enzyme **glyceraldehyde 3-phosphate dehydrogenase.**The product is 1, 3-di-phosphoglyceric acid (DPGA).

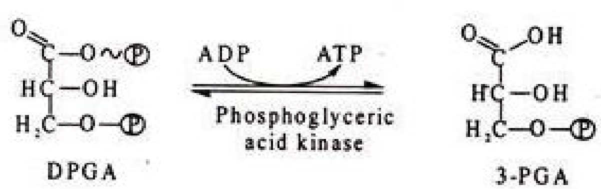
Glyceraldehyde 3-phosphate↔1,3-bisphosphoglycerate



Step 7:Transfer of phosphate from 1,3-bisphosphoglycerate to ADP

The 7th step consists of transfer of the energy-rich phosphate group of DPGA to DPGA to ADP forming ATP and 3-Phosphoglyceric acid (3-PGA). The reaction is catalysed by the enzyme, **phosphoglyceric acid kinase**. The mode of ATP formation involving transfer of an energy-rich phosphate group to ADP is known as substrate-level phosphorylation.

1,3-bisphosphoglycerate↔3-phosphoglycerate



Step 8: Isomerization of 3-phosphoglycerate

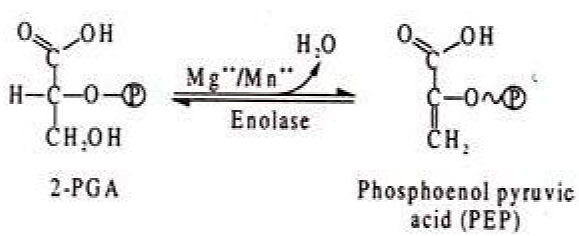
In the 8th step, 3-PGA is transformed to 2-phosphoglyceric acid (2-PGA) by transfer of the phosphate group to the middle carbon atom. The reaction is catalysed by the enzyme, **phosphoglyceromutase**. The reaction is freely reversible and requires **Mg++**.

3-phosphoglycerate↔2-phosphoglycerate

Step 9: Dehydration of 2-phosphoglycerate

The 9th step, catalysed by the enzyme, **enolase** consists of dehydration of 2-PGA to form phosphoenol pyruvic acid (PEP). As a result of removal of H_2O from 2-PGA, the phosphate bond becomes energy-rich. The enzyme requires **Mg++ or Mn++ as cofactor** and is strongly inhibited by fluoride which binds the co-factors and prevents enolase function.

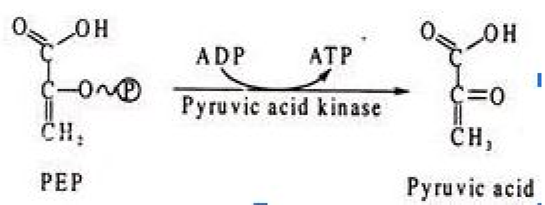
2-phosphoglycerate↔phosphoenol pyruvate



Step 10:Transfer of Phosphate from PEP to ADP

The last step of the glycolytic pathway is catalysed by the enzyme **pyruvic acid kinase** which transfers the energy-rich phosphate group of PEP to ADP forming ATP, thereby affecting the second substrate-level phosphorylation. The reaction is strongly exergonic and is irreversible.

Phosphoenol pyruvate→pyruvate



It may be noted that except three reactions of the glycolytic pathway, viz.

- The first step catalysed by hexokinase,
- the third step catalysed by phosphofructokinase and
- the last step catalysed by pyruvic acid kinase, **the other reactions are reversible**. In **all these three irreversible steps, ATP is one of the reactants.**

Summing up, the glycolytic pathway results in the cleavage of a glucose molecule first into two trioses which are inter-convertible. The triose is oxidized to glyceric acid and subsequently converted to pyruvic acid. Thus, from each half of glucose, one pyruvic acid is formed i.e. **two molecules of pyruvic acids from one molecule of glucose are produced.**

There is only one oxidation-reduction step catalysed by glyceraldehyde phosphate dehydrogenase in which NADH2 is formed i.e. **two NADH2 are produced per glucose molecule,** one from each half. Furthermore, two steps are ATP consuming, viz. conversion of glucose to glucose

6-phosphate and fructose 6-phosphate to fructose bisphosphate catalysed by the respective kinases; and there are two ATP producing steps for each half of glucose-viz. conversion of di-phosphoglyceric acid to phosphoglyceric acid (Step 7) and conversion of phosphoenol pyruvic acid to pyruvic acid (Step 10).

In making a balance-sheet of the inputs and outputs of the glycolytic pathway, it is observed that **for every molecule of glucose degraded to two molecules of pyruvic acid, two molecules of ATP are consumed and four molecules of ATP are produced, and two molecules of NAD are reduced to NADH2.**

The whole of glycolysis can be represented by the following simple equation:

Glucose + 2ADP + 2Pi + $2NAD^+$ = 2 pyruvate + 2ATP + 2NADH + $2H^+$