CARBOHYDRATES METABOLISM

Glycolysis process

Glycolysis is a series of reactions that takes place in the cytoplasm of all prokaryotes and eukaryotes. Glycolysis converts one molecule of glucose into two molecules of pyruvate [which are then converted to acetyl coenzyme A (CoA) ready for entry into the citric acid cycle]. Two ATP molecules are needed for early reactions in the glycolytic pathway but four ATPs are generated later, giving a net yield of two ATPs per molecule of glucose degraded. Overall, glycolysis has a dual role. The first is to generate ATP. Although only two ATPs per glucose are made directly from the reactions of the glycolytic pathway, it also feeds substrates into the citric acid cycle and oxidative phosphorylation, where most ATP is made. The second role is to produce intermediates that act as precursors for a number of biosynthetic pathways.Thus acetyl CoA, for example, is the precursor for fatty acid synthesis.

The process of glycolysis are divided into two phases as preparatory phase and pay off phase.

A. Preparatory phase: in this phase, one mol of glucose is cleaved into two triose phosphates by the invested of two mol of ATP. The energy is invested in this process of glycolysis, thus first five reactions constitute the preparatory phase.

2.pay off phase: In pay off phase, some of the chemical energy of glucose ic conserved in the form of ATP and NADH. In this phase, two molecules of glyceraldehyde 3- phosphate is converted into two molecules of pyruvate and yeild ultimately 2 ATP molecules directly and 6ATP via NADH. so, this phase is energy yeilding phase. That's why remaining five reactions of glycolysis constitutes pay off phase.

The individual steps in glycolysis are described below.

1. Glucose is phosphorylated by ATP to form glucose 6-phosphate and ADP.The reaction is catalyzed by the enzyme hexokinase.

2. Glucose 6-phosphate is converted to fructose 6-phosphate by phosphoglucoisomerase.This isomerization involves the conversion of an aldose to a ketose, a conversion that is better seen by viewing the open chain representations of these molecules.

3. Fructose 6-phosphate is phosphorylated by ATP to form fructose 1,6- bisphosphate and ADP. The enzyme catalyzing this step is phosphofructokinase (PFK).

4. Aldolase splits fructose 1,6-bisphosphate (a six-carbon molecule) into two three-carbon molecules, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

5. Glyceraldehyde 3-phosphate is the only molecule that can be used for the rest of glycolysis. However, the dihydroxyacetone phosphate formed in the previous step can rapidly be converted to glyceraldehyde 3-phosphate by triose phosphate isomerase. This is an equilibrium reaction;

as the glyceraldehyde 3-phosphate is used by the rest of glycolysis, more dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate as replacement.Thus effectively, for each molecule of fructose 1,6-bisphosphate that is cleaved in step 4, two molecules of glyceraldehyde 3-phosphate continue down the pathway.

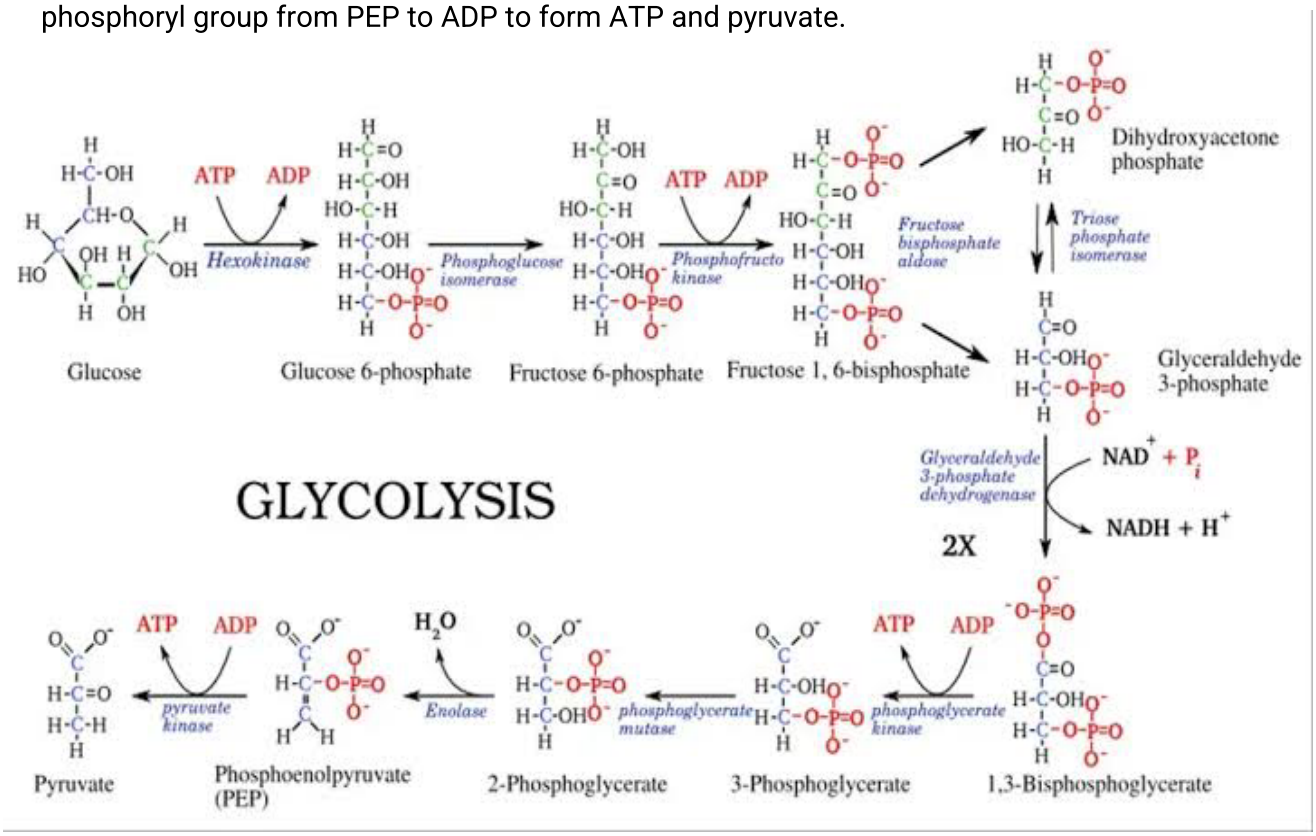
6. Glyceraldehyde 3-phosphate is converted to 1,3-bisphosphoglycerate. The reaction is catalyzed by glyceraldehyde 3-phosphate dehydrogenase and uses inorganic phosphate and NAD . The other product is NADH. The energy for creating this new high-energy phosphate bond comes from the oxidation of the aldehyde group of the glyceraldehyde 3-phosphate.

7. The newly created high-energy phosphate bond of 1,3-bisphosphoglycerate is now used to synthesize ATP. Phosphoglycerate kinase catalyzes the transfer of the phosphoryl group from the 1,3-bisphosphoglycerate to ADP, generating ATP and 3-phosphoglycerate.

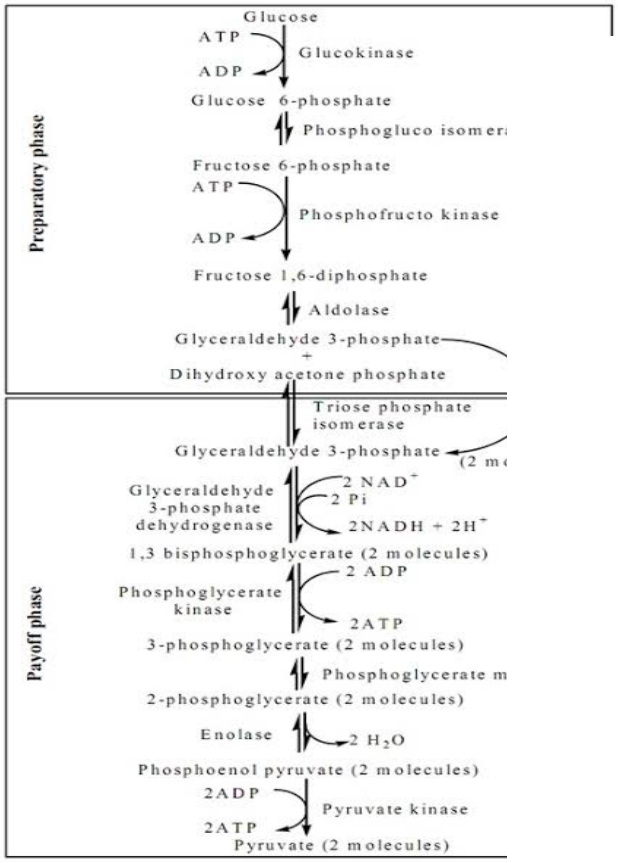
8. 3-Phosphoglycerate is converted to 2-phosphoglycerate by phosphoglycerate mutase. Thus the reaction is a movement of the phosphate group to a different carbon atom within the same molecule.

9. Enolase catalyzes the dehydration of 2-phosphoglycerate to form phosphoenolpyruvate (PEP). This reaction converts the low-energy phosphate ester bond of 2-phosphoglycerate into the high -energy phosphate bond of PEP.

10. In the last reaction, pyruvate kinase catalyzes the physiologically irreversible transfer of the phosphoryl group from PEP to ADP to form ATP and pyruvate.



Substrate-level phosphorylation: There are two distinct methods by which cells synthesize ATP. In oxidative phosphorylation, involving the electron transport chain, the generation of ATP is linked to the oxidation of NADH and FADH2 to NAD and FAD respectively and occurs via the generation of a proton gradient across the inner mitochondrial membrane. In contrast, the two ATP synthetic reactions in glycolysis (catalyzed by phosphoglycerate kinase and pyruvate kinase) involve the direct transfer of a phosphate from a sugar–phosphate intermediate to ADP; these reactions are examples of substrate-level phosphorylation. A third example of substrate-level phosphorylation is the synthesis of GTP by succinate dehydrogenase in the citric acid cycle . The GTP can be used to phosphorylate ADP to form ATP.



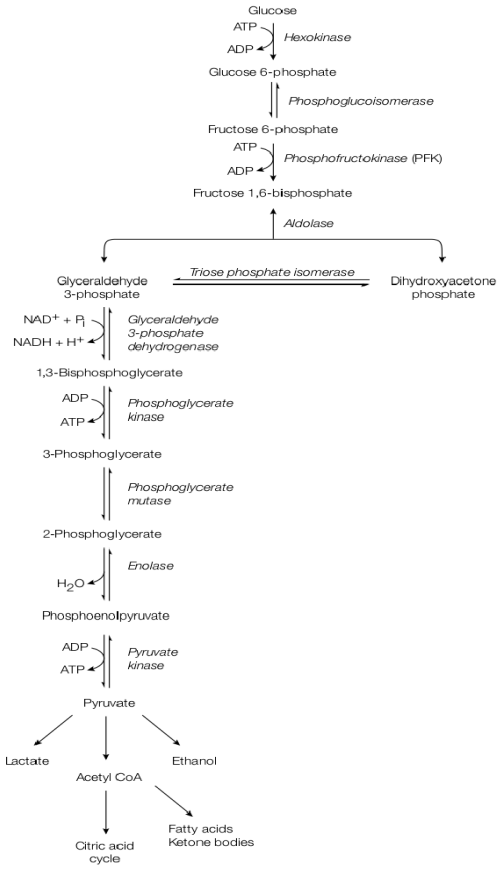


Fig. 1. The reactions of glycolysis (glucose to pyruvate) plus fates of pyruvate.

Fates of pyruvate

● Entry into the citric acid cycle. Glycolysis releases relatively little of the energy present in a glucose molecule; much more is released by the subsequent operation of the citric acid cycle and oxidative phosphorylation.Following this route under aerobic conditions, pyruvate is converted to acetyl CoA by the enzyme pyruvate dehydrogenase and the acetyl CoA then enters the citric acid cycle. The pyruvate dehydrogenase reaction is an oxidative decarboxylation.

Pyruvate dehydrogenase

pyruvate + NAD + CoA ⟶ acetyl CoA + CO2 + NADH

● Conversion to fatty acid or ketone bodies. When the cellular energy level is high (ATP in excess), the rate of the citric acid cycle decreases and acetyl CoA begins to accumulate. Under these conditions, acetyl CoA can be used for fatty acid synthesis or the synthesis of ketone bodies .

● Conversion to lactate. The NAD used during glycolysis (in the formation of 1,3 bisphosphoglycerate by glyceraldehyde 3-phosphate dehydrogenase;must be regenerated if glycolysis is to continue. Under aerobic conditions, NAD is regenerated by the reoxidation of NADH via the electron transport chain (see Topic L2). When oxygen is limiting, as in muscle during vigorous contraction, the reoxidation of NADH to NAD by the electron transport chain becomes insufficient to maintain glycolysis. Under these conditions, NAD is regenerated instea by conversion of the pyruvate to lactate by lactate dehydrogenase:

Lactate dehydrogenase

pyruvate + NADH+ H + ---------- lactate + NAD+

When sufficient oxygen becomes available once more, NAD levels rise through operation of the electron transport chain. The lactate dehydrogenase reaction then reverses to regenerate pyruvate that is converted by pyruvate dehydrogenase to acetyl CoA which can enter the citric acid cycle ,Thus the operation of lactate dehydrogenase in mammals is a mechanism for the reoxidation of NADH to NAD hence allowing glycolysis to continue, and ATP to be made, under anaerobic conditions. The process is even more sophisticated in the case of vigorously contracting skeletal muscle.Here the lactate produced is transported in the bloodstream to the liver where it is converted back to glucose and can return once again via the bloodstream to the skeletal muscle to be metabolized to yield energy. This is the Cori cycle and is described in Topic J4. Finally, in some microorganisms, lactate is the normal product from pyruvate.

● Conversion to ethanol. In yeast and some other microorganisms under anaerobic conditions, the NAD required for the continuation of glycolysis is regenerated by a process called alcoholic fermentation. The pyruvate is converted to acetaldehyde (by pyruvate decarboxylase) and then to ethanol (by alcohol dehydrogenase), the latter reaction reoxidizing the NADH to NAD :

Pyruvate decarboxylase

pyruvate + H ⟶ . acetaldehyde + CO2

Alcohol dehydrogenase

acetaldehyde + NADH + H+ ethanol + NAD+

Energy yield : Early in glycolysis, two ATPs are required for the conversion of glucose to glucose 6-phosphate by hexokinase and for the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate by PFK. However, fructose 1,6-bisphosphate then gives rise to two three-carbon units, each of which generates twoATPs in subsequent steps (catalyzed by phosphoglycerate kinase and pyruvate kinase) giving a net yield of two ATPs per original glucose molecule . The overall reaction is:

$$\text{Glucose.} + 2Pi + 2ADP + 2NAD \rightarrow 2 \text{ pyruvate} + 2ATP + 2NADH + 2H + 2H2O$$

Note that, under aerobic conditions, the two NADH molecules that are synthesized are re-oxidized via the electron transport chain generating ATP.Given the cytoplasmic location of these NADH molecules, each is re-oxidized via the glycerol 3-phosphate shuttle and produces approximately two ATPs during oxidative phosphorylation or via the malate–aspartate shuttle and produces approximately three ATPs during oxidative phosphorylation.

REGULATION OF GLYCOLYSIS :

1. Phosphofructokinase(PFK) : The most important control step of glycolysis is the irreversible reaction catalyzed by phosphofructokinase (PFK). The enzyme is regulated in several ways:

● ATP/AMP. PFK is allosterically inhibited by ATP but this inhibition is reversed by AMP. This allows glycolysis to be responsive to the energy needs of the cell, speeding up when ATP is in short supply (and AMP is plentiful) so that more ATP can be made, and slowing down when sufficient ATP is already available.

● Citrate. PFK is also inhibited by citrate, the first product of the citric acid cycle proper . A high level of citrate signals that there is a plentiful supply of citric acid cycle intermediates already and hence no additional breakdown of glucose via glycolysis is needed.

● Fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate (F-2,6-BP) is synthesized from fructose 6-phosphate by an enzyme called phosphofructokinase 2 (PFK2), a different enzyme from PFK. F-2,6-BP is hydrolyzed back to fructose 6-phosphate by fructose bisphosphatase 2 (FBPase2). Amazingly, both PFK2 and FBPase2 are activities catalyzed by the same polypeptide; hence this is a bi-functional enzyme. Fructose 6-phosphate stimulates the synthesis of F-2,6-BP and inhibits its hydrolysis .F-2,6-BP in turn strongly activates PFK and hence stimulates glycolysis. Themoverall effect is that when fructose 6-phosphate levels are high, PFK (and hence glycolysis) is stimulated. PFK2 and FBPase2 are also controlled by covalent modification (see Topic C5). When blood glucose levels fall, the hormone glucagon is released into the bloodstream and triggers a cAMP cascade that leads to phosphorylation of the PFK2/FBPase2 polypeptide at a single serine residue. This activates FBPase2 and inhibitsPFK2, lowering the level of F-2,6-BP and hence decreasing the rate of glycolysis. The reverse is true as glucose levels rise; the phosphate group is removed from the PFK2/FBPase2 polypeptide by a phosphatase, thus

inhibiting FBPase2 and activating PFK2, raising the level of F-2,6-BP and hence increasing the rate of glycolysis. F-2,6-BP is also important in preventing glycolysis (glucose degradation) and gluconeogenesis (glucose synthesis) operating simultaneously. This is called reciprocal regulation.

● H ions. PFK is inhibited by H ions and hence the rate of glycolysis decreases when the pH falls significantly. This prevents the excessive formation of lactate (i.e. lactic acid) under anaerobic conditions (see above) and hence prevents the medical condition known as acidosis (a deleterious drop in blood pH).

2.**Hexokinase:** Hexokinase, which catalyzes the first irreversible step of glycolysis, is inhibited by glucose 6-phosphate. Thus when PFK is inhibited, fructose 6-phosphate builds up and so does glucose 6-phosphate since these two metabolites are in equilibrium via phosphoglucoisomerase (see Fig. 1). The hexokinase inhibition then reinforces the inhibition at the PFK step. At first sight this seems unusual since it is usually the first irreversible step of a pathway (the committed step) that is the main control step. On this basis, it may appear that hexokinase should be the main control enzyme, not PFK. However, glucose 6-phosphate, the product of the hexokinase reaction, can also feed into glycogen synthesis or the pentose phosphate pathway (see Topic J5). Thus the first irreversible step that is unique to glycolysis is that catalyzed by PFK and hence this is the main control step.

3.Pyruvate kinase: Pyruvate kinase catalyzes the third irreversible step in glycolysis. It is activated by fructose 1,6-bisphosphate. ATP and the amino acid alanine allosterically inhibit the enzyme so that glycolysis slows when supplies of ATP and biosynthetic precursors (indicated by the levels of Ala) are already sufficiently high. In addition, in a control similar to that for PFK , when the blood glucose concentration is low, glucagon is released and stimulates phosphorylation of the enzyme via a cAMP cascade (see Topic J7). This covalent modification inhibits the enzyme so that glycolysis slows down in times of low blood glucose levels.

- FRUCTOSE METABOLISM:Fructose is an abundant sugar in the human diet; sucrose (table sugar) is a disaccharide which when hydrolyzed yields fructose and glucose and fructose is also a major sugar in fruits and honey. There are two pathways for the metabolism of fructose; one occurs in muscle and adipose tissue, the other in liver.

  1. In muscle and adipose tissue, fructose can be phosphorylated by hexokinase (which is capable of phosphorylating both glucose and fructose) to form fructose 6-phosphate which then enters glycolysis.

  2. In liver, the cells contain mainly glucokinase instead of hexokinase and this enzyme phosphorylates only glucose. Thus in liver, fructose is metabolizedinstead by the fructose 1-phosphate pathway.Fructose is converted to fructose 1-phosphate by fructokinase.Fructose 1-phosphate is then split into glyceraldehyde and dihydroxyacetone phosphate by fructose 1-phosphate aldolase. The dihydroxyacetone

feeds into glycolysis at the triose phosphate isomerase step.The glyceraldehyde is phosphorylated by triose kinase to glyceraldehyde 3-phosphate and so also enters glycolysis.