Faculty Science

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M.Sc. III Sem Paper- H3002 (Phytochemistry and Metabolism)

Topic- Glycolysis and its regulation

Introduction- Metabolism, the sum of all the chemical transformations taking place in a cell or organism, occurs through a series of enzyme-catalyzed reactions that constitute metabolic pathways. In a pathway, the product of one reaction serves as the substrate of the subsequent reaction. Different pathways can also intersect, forming an integrated and purposeful network of chemical reactions. Most pathways are classified into catabolic (degrade complex molecules such as carbohydrates, fats and proteins to a few simple products such as lactic acid, CO_2, NH_3 and H_2O) and anabolic (synthesize complex end products from simple precursors). Catabolic pathways release energy in the form of ATP. Anabolic pathways require energy, which is generally provided by the breakdown of ATP.

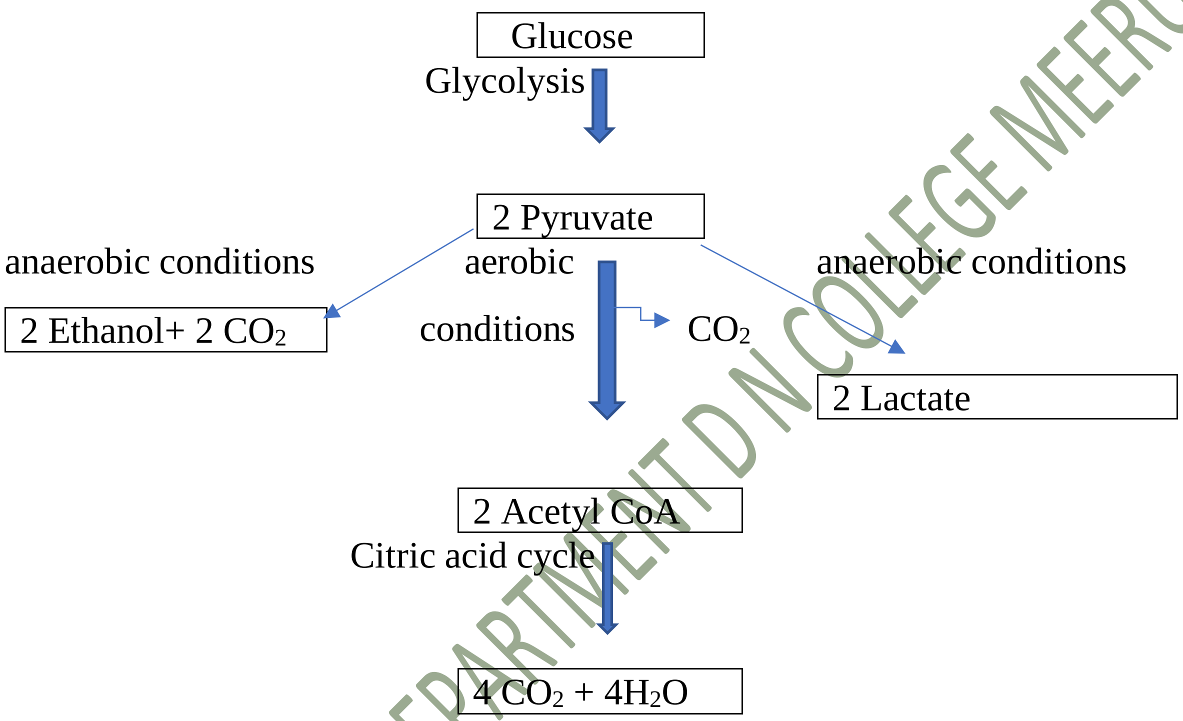
The role of few enzymes

Kinase, Phosphatase and Phosphorylase: **Kinase** is the enzyme that catalyzes transfer of a phosphoryl group from ATP or GTP to an acceptor molecule- a sugar (e.g. **Hexokinase**), a protein (e.g. **Glycogen phosphorylase kinase**). In other word kinases catalyze the phosphorylation of the molecules. **Phosphatase** is the enzyme that catalyzes the removal of phosphoryl group from a phosphate ester with water as the attacking molecule (e.g. **Glucose-6-phosphatase**). Phosphatases catalyze the dephosphorylation of the molecules. **Phosphorylase** is the enzyme that catalyzes the addition of a phosphoryl group from inorganic phosphate to an acceptor (e.g**. Glycogen phosphorylase**).

Cellular respiration is a process in which all living organisms obtained energy from the breakdown of glucose. It is of two types:

1. Aerobic cellular respiration- It involves glycolysis, citric acid cycle or Krebs cycle and electron transport chain with oxidative phosphorylation. Glycolysis occurs in the cytoplasm and involves the breakdown of glucose into pyruvate. Two molecules of ATP and two molecules of the high energy NADH are also produced in glycolysis. Pyruvate is oxidized with the loss of its carboxyl group as CO_2, to yield the acetyl group of acetyl Co A, the acetyl group is then oxidized completely to CO_2 by citric acid cycle. The electrons from these oxidations are passed to oxygen through a chain of carriers in mitochondria forming water. The energy from the electron transfer reactions drive the synthesis of ATP in mitochondria.

2. Anaerobic cellular respiration- Pyruvate is reduced to lactate by **lactic acid fermentation**. When vigorously contracting skeletal muscle must function under low oxygen conditions, NADH cannot be re-oxidized to NAD^+ and NAD^+ is required as electron acceptor for further oxidation of pyruvate. Under this condition pyruvate is reduced to lactate, accepting electrons from NADH and thereby regenerating NAD^+ necessary for glycolysis to continue. Lactate is also the product of glycolysis under anaerobic condition in some microorganisms. In some plants and microorganisms such as yeast, pyruvate is converted under anaerobic conditions into ethanol and carbon dioxide. This process is known as **alcohol fermentation**.



Three catabolic fates of the Pyruvate

Glycolysis- Glycolysis is a set of reactions that takes place in the cytoplasm of prokaryotes and eukaryotes. The roles of glycolysis are to produce energy and intermediates for biosynthetic pathways. The conversion of glucose to pyruvate occurs in two stages. The first five reactions of glycolysis correspond to an energy investment phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP. The subsequent reactions of glycolysis constitute an energy generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation and two molecules of NADH per glucose molecule metabolized.

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

2. Isomerization of glucose-6-phosphate- Glucose-6-phosphate is converted to fructose-6-phosphate by phosphoglucose isomerase.

3.Phosphorylation of fructose-6-phosphate- Fructose-6-phosphate is phosphorylated by ATP to form fructose 1,6-bisphosphate and ADP. This reaction is catalyzed by the enzyme phosphofructokinase.

4. Cleavage of fructose 1,6-bisphosphate- The enzyme aldolase splits fructose 1,6-bisphosphate into 2 three carbon molecules, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

5. Isomerization of dihydroxyacetone phosphate- Dihydroxyacetone phosphate is reversibly converted to glyceraldehyde 3-phosphate by the enzyme triose phosphate isomerase.

6. Oxidation of glyceraldehyde 3-phosphate- 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.

7. Synthesis of 3-phosphoglycerate- 3-Phosphoglycerate kinase catalyzes the transfer of the phosphoryl group from 1,3-bisphosphoglycerate to ADP, generating ATP and 3-phosphoglycerate. The reaction is catalyzed by phosphoglycerate kinase, which, unlike most other kinases, is physiologically reversible.

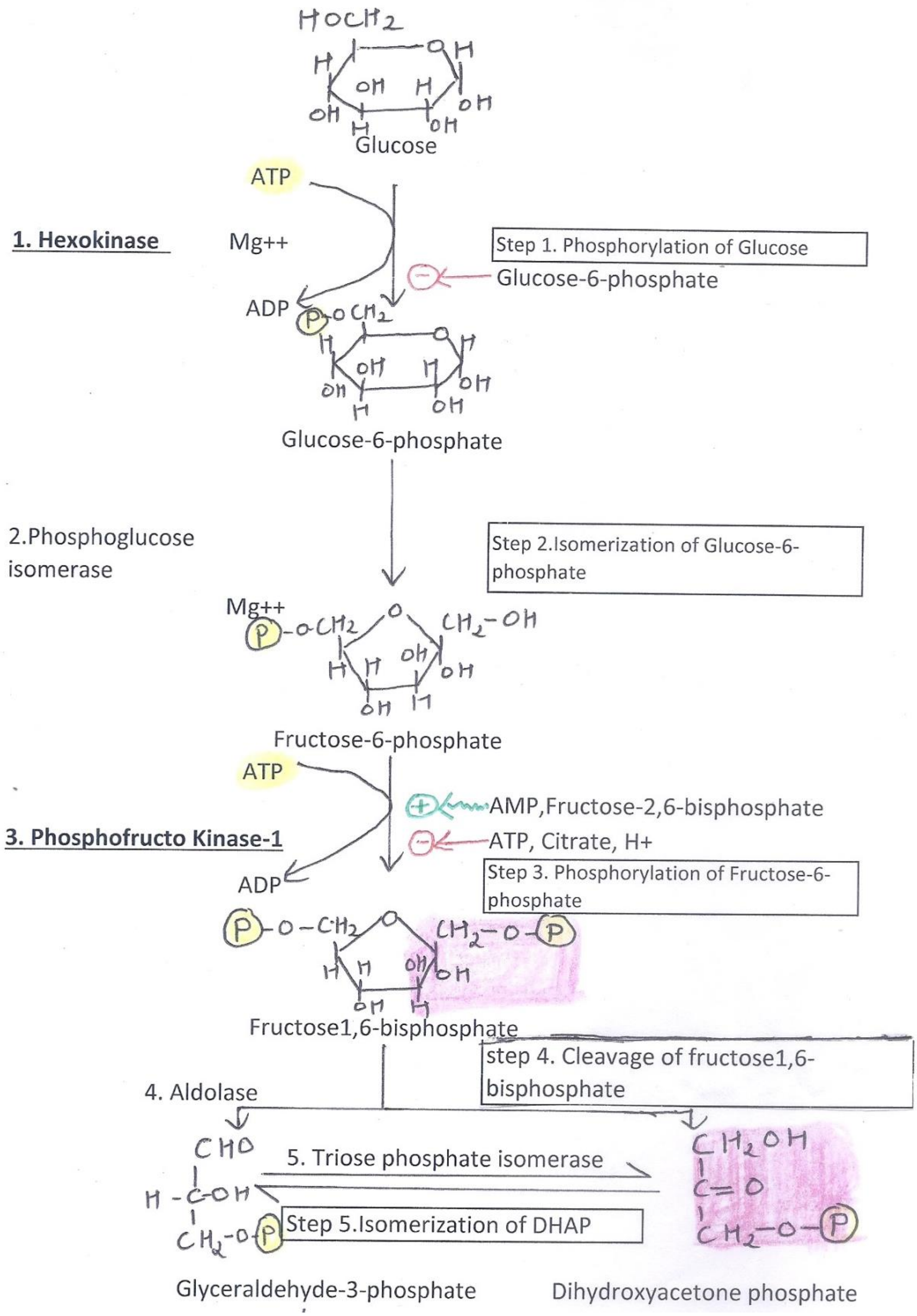
8. Shifting of phosphate group from C3 to C2- 3-Phosphoglycerate is converted to 2-phosphoglycerate by the enzyme phosphoglycerate mutase.

9. Dehydration of 2-phosphoglycerate- Enolase catalyzes the dehydration of 2-phosphoglycerate to form Phosphoenolpyruvate.

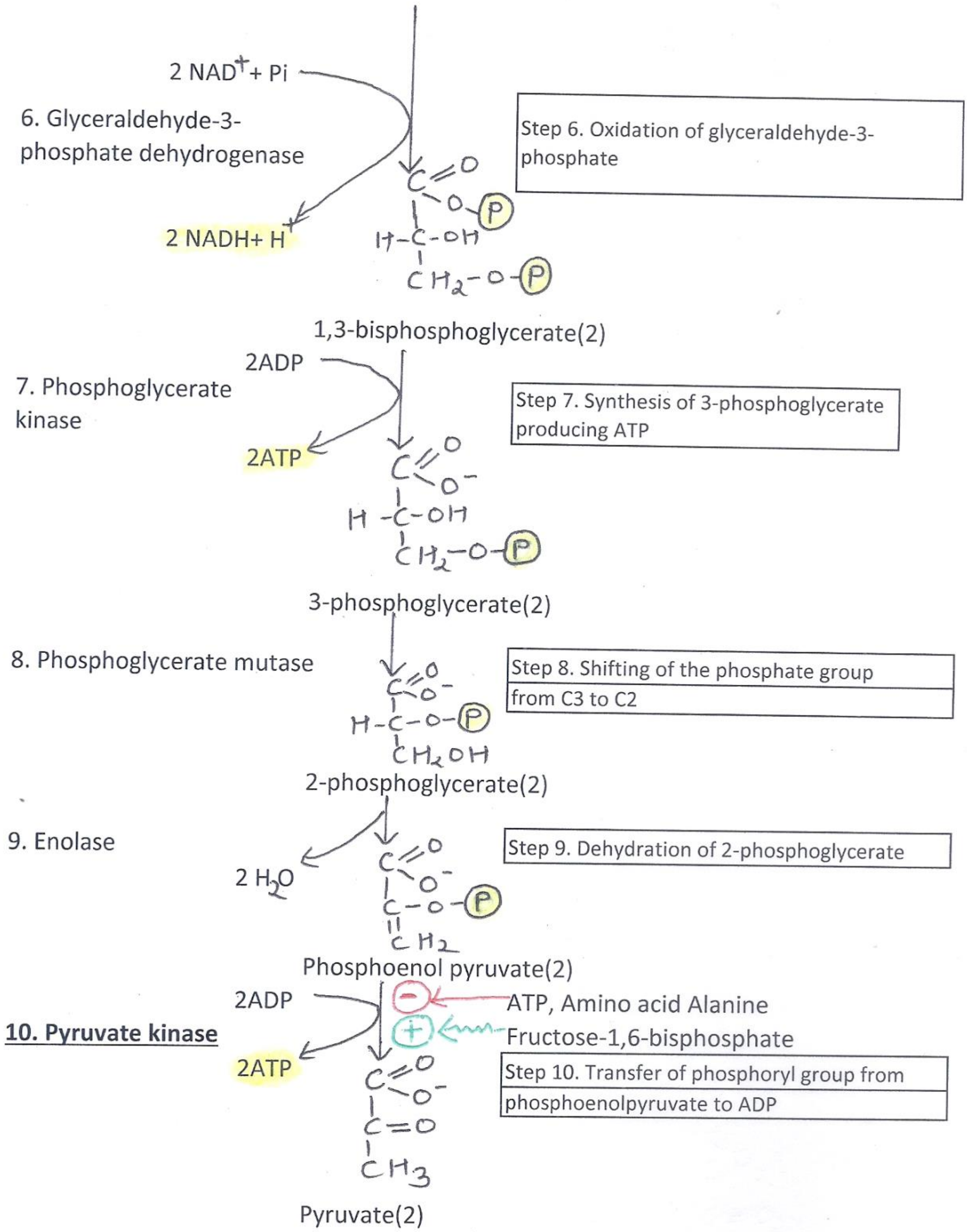
10. Transfer of phosphoryl group from phosphoenolpyruvate to ADP- Pyruvate kinase catalyzes the irreversible transfer of phosphoryl group from phosphoenolpyruvate to ADP to form ATP and pyruvate. Pyruvate kinase was inappropriately named before it was recognized that it did not directly catalyze phosphorylation of pyruvate, which does not occur under physiological conditions.

The overall equation for glycolysis is

$$\text{Glucose} + 2\,NAD^+ + 2ADP + P_i \longrightarrow 2\,\text{Pyruvate} + 2NADH + H^+ + 2ATP + 2H_2O$$



Glucose
ATP
1. Hexokinase
Mg++
Step 1. Phosphorylation of Glucose
Glucose-6-phosphate
ADP
Glucose-6-phosphate
2.Phosphoglucose isomerase
Step 2.Isomerization of Glucose-6-phosphate
Mg++
Fructose-6-phosphate
ATP
AMP,Fructose-2,6-bisphosphate
3. Phosphofructo Kinase-1
ATP, Citrate, H+
Step 3. Phosphorylation of Fructose-6-phosphate
ADP
Fructose1,6-bisphosphate
step 4. Cleavage of fructose1,6-bisphosphate
4. Aldolase
5. Triose phosphate isomerase
Step 5.Isomerization of DHAP
Glyceraldehyde-3-phosphate
Dihydroxyacetone phosphate



Reactions of Glycolysis

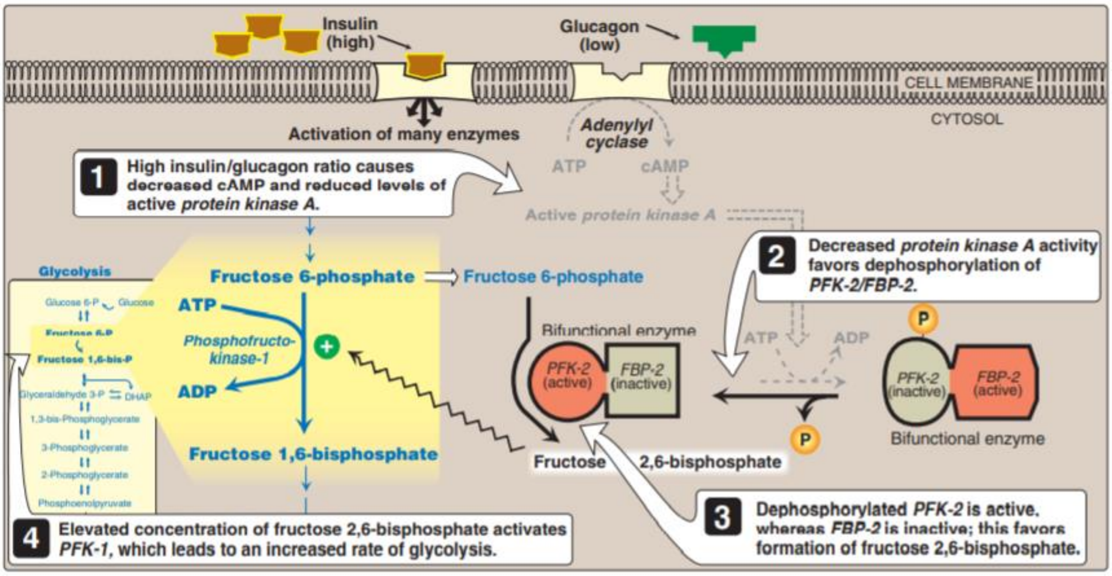
Inhibitors (-) and activators (+) of the enzymes of the pathway

Regulation of glycolysis

There are three points of regulation of glycolysis and these points are enzymes that catalyse Irreversible steps into glycolytic pathway; phosphofructokinase, hexokinase and pyruvate kinase.

Phosphofructokinase1 (PFK1) is the most important control step of glycolysis. This enzyme is regulated in several ways:

i) ATP/AMP- PFK1 is allosterically inhibited by elevated level of ATP and also by elevated level of citrate. PFK1 is activated allosterically by high concentration of AMP, which signal that the cell's energy stores are depleted.



ii) Fructose 2, 6-bisphosphate -Fructose 2,6-bisphosphate is synthesized from Fructose 6-phosphate by an enzyme phosphofructokinase 2 a different enzyme from PFK 1. Fructose 2, 6-bisphosphate is hydrolysed back to Fructose 6-phosphate by Fructose bisphosphatase2. Both PFK2 and FBPase2 are activities catalysed by the same polypeptide, this is a bifunctional enzyme. Fructose 6 phosphate stimulates the synthesis of fructose 2, 6-bisphosphate and inhibits its hydrolysis. Fructose 2, 6-bisphosphate in turn strongly activates PFK1 and hence stimulates glycolysis. When Fructose 6 phosphate levels are high, PFK is stimulated. PFK2 and FBPase2 are also controlled by phosphorylation and dephosphorylation. When blood glucose levels fall, the hormone glucagon is released into bloodstream and triggers is cAMP cascade that leads to the phosphorylation of PFK2/ FBPase2. This activates FBPase2 and inhibits PFK2, lowering the level of fructose2, 6-bisphosphate and hence decreasing the rate of glycolysis. The reverse is true as glucose levels rise, phosphate group is removed from the PFK2/ FBPase2 by a phosphatase, and hence increasing the rate of glycolysis.

Fructose2, 6-bisphosphate is also important in preventing glycolysis and gluconeogenesis operating simultaneously. This is called reciprocal regulation **iii) Hydrogen ion-** PFK is inhibited by hydrogen ions and hence the rate of glycolysis decreases, when the pH falls. This prevents excessive formation of lactate under anaerobic condition and hence prevents the medical condition known as acidosis.

Hexokinase- Hexokinase is inhibited by glucose 6 phosphate. Thus, when the product of hexokinase reaction accumulates, its rate of production is lowered. Glucose 6 phosphate can also feed into glycogen synthesis or the pentose phosphate pathway.

Pyruvate kinase- Pyruvate kinase is activated by fructose 1,6-bisphosphate. ATP and the amino acid alanine allosterically inhibit the enzyme. When the blood glucose concentration is low, glucagon is released and stimulates phosphorylation of enzyme via cAMP cascade. This covalent modification inhibits the enzyme so that glycolysis slows down.

References:

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