PLANT BIOCHEMISTRY BLOCK-2UNIT-1 BIOSYNTHESIS OF CARBOHYDRATE

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Glycolysis

- This is the first metabolic pathway . Glycolysis is also called Embden-Meyerhoff pathway.
- The complete set of reactions occurs in the cytoplasm of virtually every animal cell.
- The entire process occurs without molecular oxygen. Glycolysis consumes 2 ATP and generates 4ATP. Thus, the process results in the generation of 2 net ATP.
- The process also generates 2 NADH. What happens to pyruvate depends upon the presence or absence of mitochondria in the cell or upon the availability of oxygen in mitochondria – containing cells.
- For the glycolytic pathway to continue, NAD+ has to be regenerated. In erythrocytes (no mitochondria) and in mitochondria – possessing cells under anaerobic conditions, NAD+ is regenerated from NADH during the conversion of pyruvate to lactate.
- It must be controlled. Enzymatic control can be exercised by three different common methods.
- a. Allosteric effectors. The transient binding of molecules to the enzyme to change the conformation. Effect is observed in milliseconds.
- b. Covalent modification. Generally phosphorylation. Effect in seconds. a. Transcription of enzyme. Effect observed in hours. There are only three major points of control of glycolysis. Three points of control Hexokinase and glucokinase:
- Reaction # 1 Hexokinase is found in most tissues. 1. It has a low Km for glucose .

- In mitochondria – possessing cells under aerobic conditions, NAD+ is regenerated by either malate – aspartate shuttle or α-glycerophosphate shuttle, which transfer the reducing equivalents from NADH into mitochondria for electron transport chain, thus regenerating NAD+ in the cytoplasm.
- 2 Reactions Glycolysis consists of two phases. In the first phase, glucose is broken down to two molecules of glyceraldehyde-3-phosphate in a series of five reactions. In the second phase, another series of five reactions convert these two molecules of glyceraldehyde-3-phosphate into two molecules of pyruvate.
- Phase I consumes 2 ATP and Phase II generates 4 ATP. The net ATP production in the entire process is 2. 3 Individual Reactions Reaction
- 1: Hexokinase/Glucokinase a. This is the first intracellular reaction of glycolysis (remember all reactions are in the cytoplasm).
- b. Requires an ATP (Mg). This is one of the investment reactions.
- c. The phosphorylation of glucose traps the glucose inside the cell.
- d. The reaction is considered irreversible.
- e. Hexokinase has a Km for glucose of less than 0.1 mM (high affinity).
- It is also inhibited by the product glucose-6-phosphate
- f. Liver hepatocytes and pancreatic β cells contain another enzyme Glucokinase. lower than normal, thus shifting the curve to the left.
- 11 Control of Glycolysis It is obvious that glycolysis

- #2: Phosphoglucose isomerase. a. This reaction is readily reversible (not a controlling step) and functions in both glycolysis and gluconeogenesis. b. Conversion of an aldose to a ketose. 5 Reaction #3: 6-Phosphofructo-1-kinase (PFK-1) or Phosphofructokinase-1. a. Reaction is the rate-limiting step of glycolysis. b. It is irreversible, and the committed step. It is an allosteric enzyme and also a major regulatory enzyme. c. We have invested our second ATP molecule. Reaction #4: Aldolase. a. We now have two phosphorylated trioses. b. Only glyceraldehyde-3-phosphate is used in glycolysis. Therefore, dihydroxyacetone phosphate has to be converted into glyceraldehyde-3-phosphate. This occurs in the next step.
- 6 Reaction #5: Triose phosphate isomerase. a. Catalyzes the interconversion of dihydroxyacetone phosphate and glyceraldehyde3-phosphate. b. Because of the interconversion, one glucose molecule can be converted to two glyceraldehyde-3-phosphate molecules. Reaction # 6: Glyceraldehyde-3-phosphate dehydrogenase a. The enzyme oxidizes the number one carbon aldehyde and then adds a phosphate group. We have an acid anhydride in the product 1,3-bisphophoglycerate. Remember from bioenergetics that acid anhydrides are high-energy bonds. b. The phosphate on the number 3 carbon is not an high-energy bond 7 c. We have used an NAD+ for the oxidation reaction. The cell has limited amounts of NAD+ , so somewhere along the line we have to regenerate it or glycolysis will stop. d. This reaction is a target for Arsenate (AsO4 3-).

- The arsenate resembles inorganic phosphate (Pi). In the presence of arsenate, the product of the reaction is 1-arseno3-phosphoglycerate. This product is unstable and decomposes into arsenate and 3- phosphoglycerate with no ATP formation. After this step, glycolysis continues. e. The enzyme contains an essential thiol (cysteine-SH) group at the active site. Iodoacetic acid (ICH2COOH) is also an inhibitor of this reaction. It reacts with the active site SH group and inhibits the enzyme. Reaction #
- 7: Phosphoglycerate kinase a. This is the first step of energy production. b. This is referred to as substrate-level phosphorylation as opposed to oxidative phosphorylation that occurs in mitochondrial ATP production. d. We have recovered both ATP that were invested. Remember that each glucose gives 2 phosphoglycerate molecules. 8 Reaction

- # 8: Phosphoglycerate mutase. Reaction#9: Enolase a. Catalyzes the dehydration of 2-phosphoglycerate to form phosphoenolpyruvate (PEP). c. Recall from the bioenergetics lecture that PEP contains an high-energy bond. d. This reaction is inhibited by Fluoride. Reaction
- # 10: Pyruvate Kinase a. Catalyzes the transfer of the phosphate from PEP to ADP to generate ATP and 9 pyruvate. b. Again, this is substrate-level phosphorylation. c. This reaction completes that part of glycolysis that is common to both anaerobic and aerobic metabolism. d. Under aerobic conditions and the presence of mitochondria, pyruvate can enter the citric acid cycle. NAD+ is regenerated by malate/aspartate shuttle or by αglycerophosphate shuttle e. Under anaerobic conditions or in the absence of mitochondria, pyruvate is reduced to lactate via the following reaction and regenerate NAD+ .
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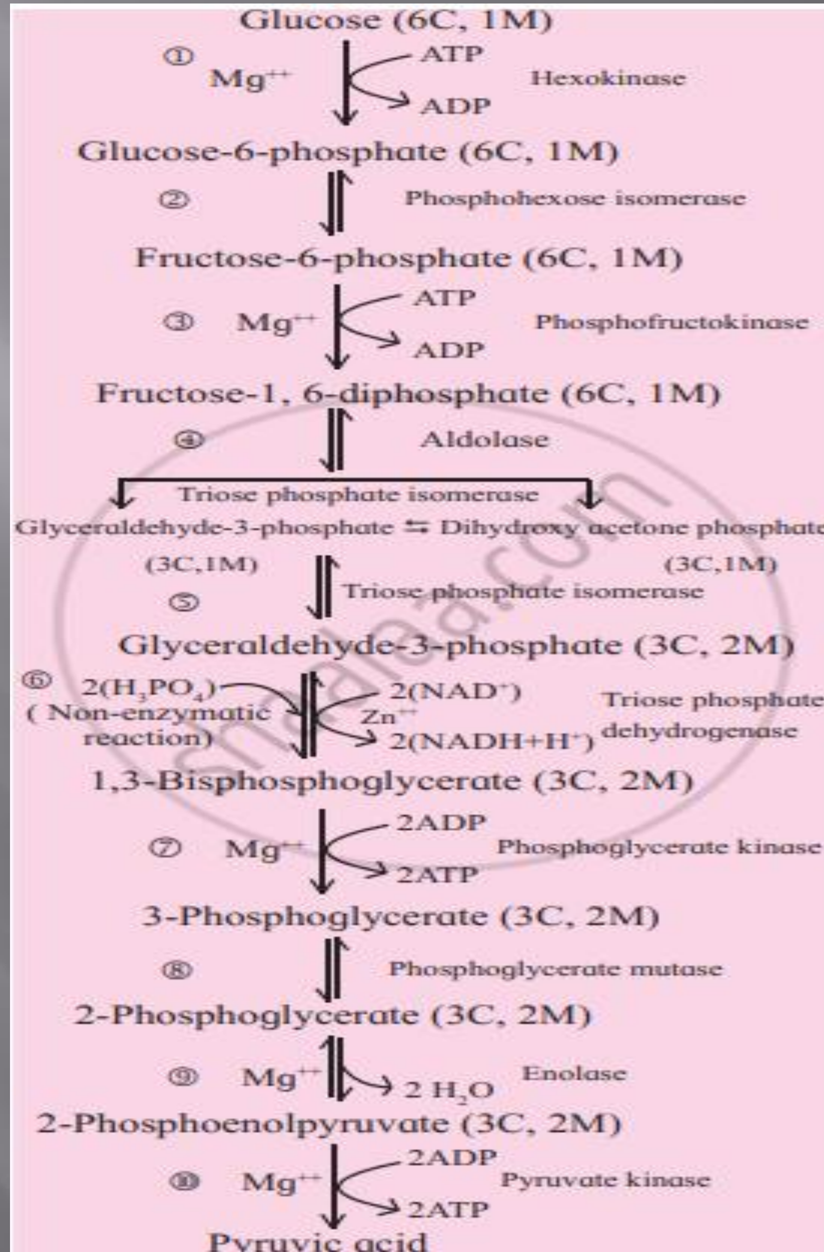
- If the entire glycolytic pathway in RBC occurs via the formation of 2,3-BPG, glycolysis will yield no net ATP because the 2 ATP produced by pyruvate kinase will be equal to 2 ATP consumed in Phase I of glycolysis. e. 2, 3-BPG levels in erythrocytes increase in high altitude where partial pressure of oxygen is low.
- f. Loss-of-function mutations in pyruvate kinase increase the levels of 2,3-BPG in RBC, thus shifting the sigmoidal curve of oxygen binding to hemoglobin to the right.
- If loss-of-function mutations occurs in any of the enzymes upstream of the step



Glucose (6C, 1M)
① Mg++
ATP
ADP
Hexokinase
Glucose-6-phosphate (6C, 1M)
②
Phosphohexose isomerase
Fructose-6-phosphate (6C, 1M)
③ Mg++
ATP
ADP
Phosphofructokinase
Fructose-1, 6-diphosphate (6C, 1M)
④
Aldolase
Triose phosphate isomerase
Glyceraldehyde-3-phosphate ⇆ Dihydroxy acetone phosphate
(3C,1M)
(3C,1M)
⑤
Triose phosphate isomerase
Glyceraldehyde-3-phosphate (3C, 2M)
⑥ 2(H3PO4)
(Non-enzymatic reaction)
2(NAD+)
Zn++
2(NADH+H+)
Triose phosphate dehydrogenase
1,3-Bisphosphoglycerate (3C, 2M)
⑦ Mg++
2ADP
2ATP
Phosphoglycerate kinase
3-Phosphoglycerate (3C, 2M)
⑧
Phosphoglycerate mutase
2-Phosphoglycerate (3C, 2M)
⑨ Mg++
2 H2O
Enolase
2-Phosphoenolpyruvate (3C, 2M)
⑩ Mg++
2ADP
2ATP
Pyruvate kinase
Pyruvic acid

THANKS