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Subject - Zoology

B.SC -1st year Semester – II

Paper – II: Cell Biology

UNIT- 2

TOPIC - 2.2- Oxidative phosphorylation – Glycolysis and Kreb’s cycle.

Presented By

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INTRODUCTION

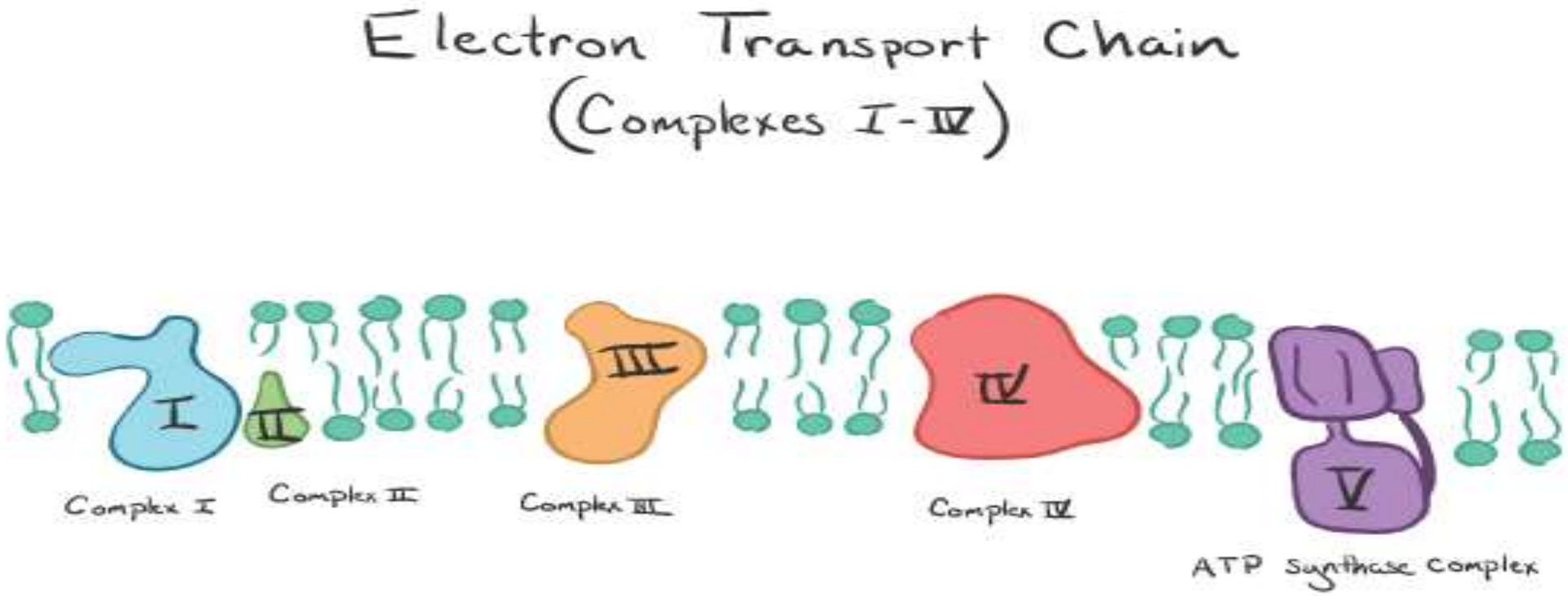
- There are a lot of different ways organisms acquire food. Just think about how sharks, bees, plants, and bacteria eat.
- Almost all aerobic organisms (organisms that require oxygen to live) use oxidative phosphorylation, in one way or another, to produce the basic energy currency of the cell needs to function:
- ATP (adenosine triphosphate).
- Oxidative phosphorylation is the fourth step of cellular respiration, and produces the most of the energy in cellular respiration.

Where does oxidative phosphorylation fit into cellular respiration?

- Glycolysis, where the simple sugar glucose is broken down, occurs in the cytosol.
- Pyruvate, the product from glycolysis, is transformed into acetyl CoA in the mitochondria for the next step.
- The citric acid cycle, where acetyl CoA is modified in the mitochondria to produce energy precursors in preparation for the next step.
- **Oxidative phosphorylation**, the process where electron transport from the energy precursors from the citric acid cycle (step 3) leads to the phosphorylation of ADP, producing ATP. This also occurs in the mitochondria.

What is oxidative phosphorylation?

Oxidative phosphorylation is the process where energy is harnessed through a series of protein complexes embedded in the inner-membrane of mitochondria (called the electron transport chain and ATP synthase) to create ATP. Oxidative phosphorylation can be broken down into two parts: 1) Oxidation of NADH and $FADH_2$, and 2) Phosphorylation.



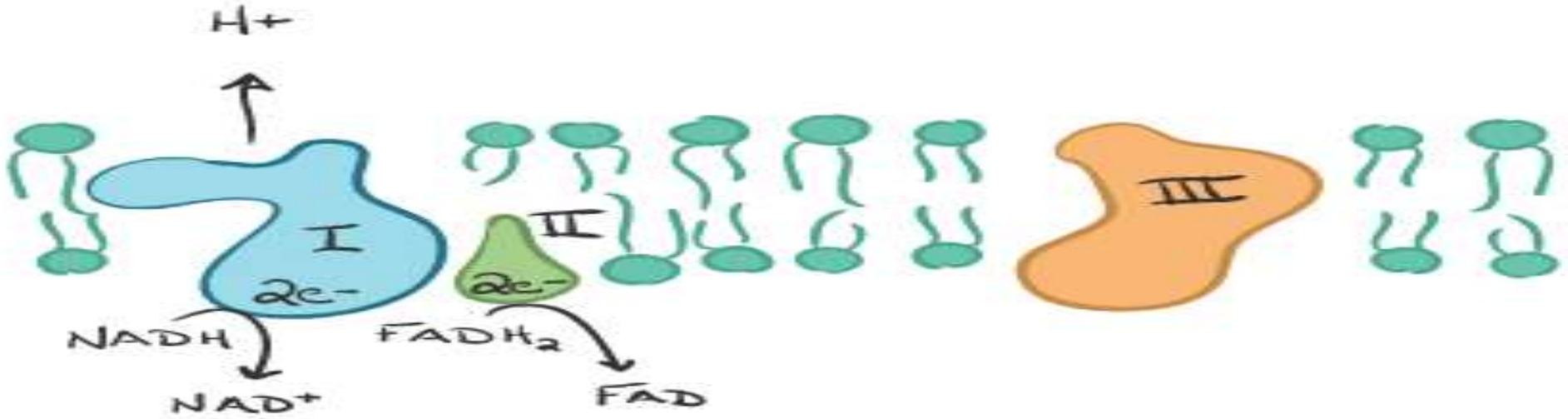
1. Oxidation of NADH and $FADH_2$ - losing electrons via high energy molecules

Step 1

Oxidative phosphorylation starts with the arrival of 3 NADH and 1 $FADH_2$ from the citric acid cycle, which shuttle high energy molecules to the electron transport chain. NADH transfers its high energy molecules to protein complex 1, while $FADH_2$ transfers its high energy molecules to protein complex 2. Shuttling high energy molecules causes a loss of electrons from NADH and $FADH_2$, called *oxidation* (other molecules are also capable of being oxidized).

The opposite of oxidation is *reduction*, where a molecule *gains electrons* (which is seen in the citric acid cycle). Here's an easy way to remember which process gains or loses electrons:

"***LEO** the lion says **GER**"*
Lose Electrons Oxidation (LEO)
Gain Electrons Reduction (GER)

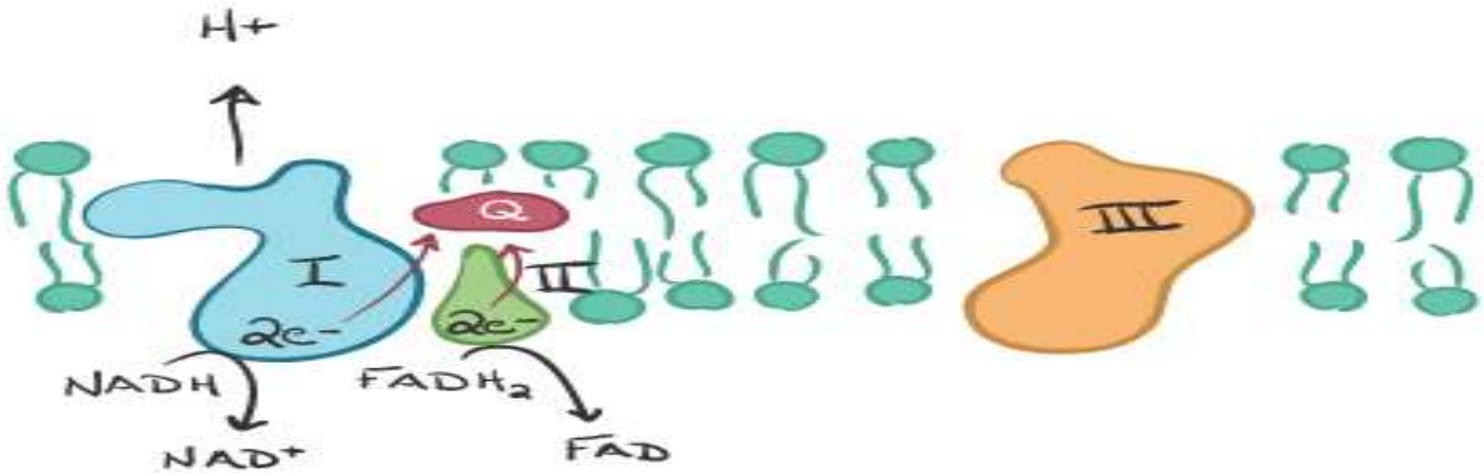


Step 2 - Hitting the gym to pump some serious hydrogens

The process of NADH oxidation leads to the pumping of protons (single positively-charged hydrogen atoms denoted as H^+) through protein complex 1 from the matrix to the intermembrane space. The electrons that were received by protein complex 1 are given to another membrane-bound electron carrier called *ubiquinone* or Q.

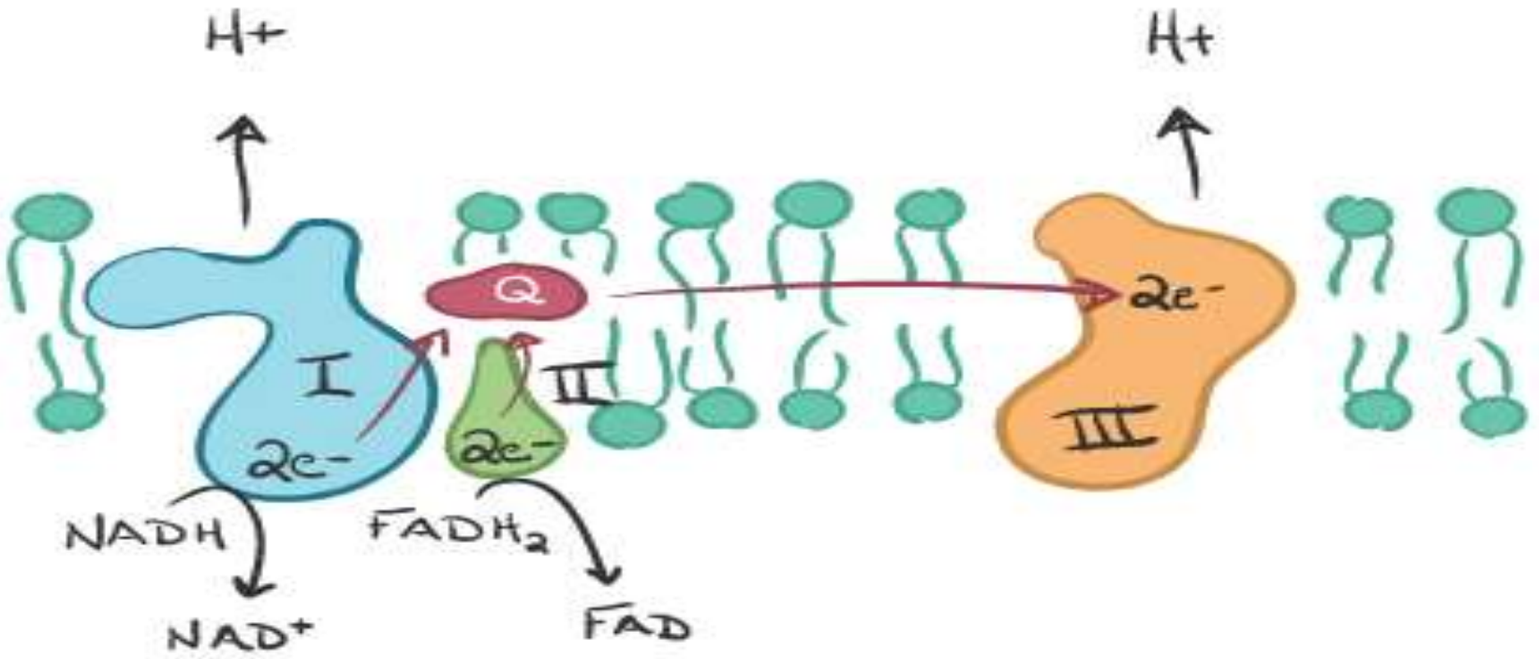
This process of transferring electrons drives the pumping of protons, which is seen as unfavorable. Electron transfer driving proton pumping is repeated in complexes 3 and 4 (which we will discuss in steps 2 - 5). As this action is repeated, protons will accumulate in the intermembrane space. This accumulation of protons is how the cell temporarily stores transformed energy.

Note - $FADH_2$ has a slightly different route than NADH. After its arrival at protein complex 2, its high energy electrons are directly transferred to Q, to form reduced Q, or QH_2. There is no hydrogen pumping for the exchange of the $FADH_2$ electrons here.



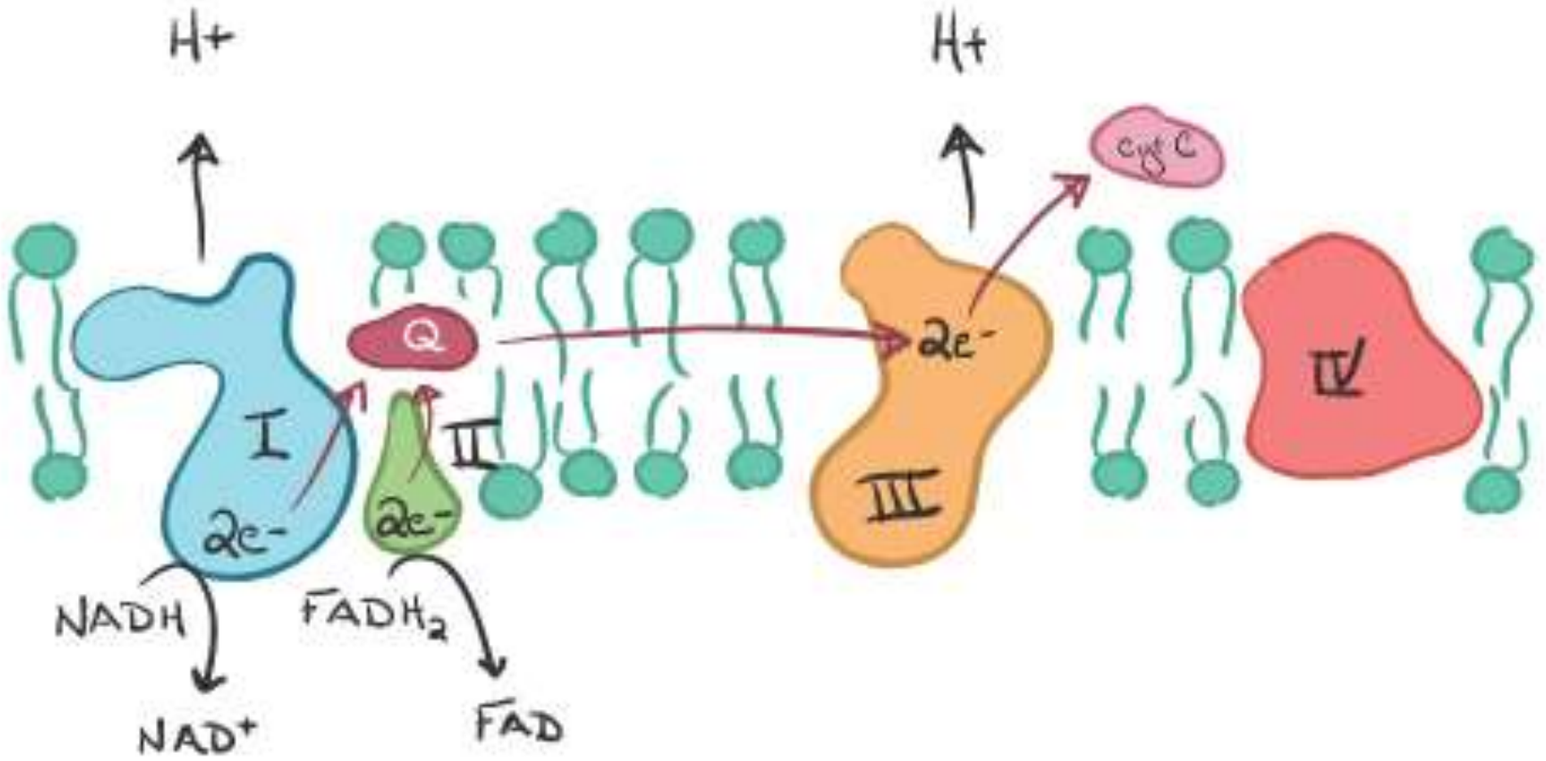
Step 3

The rest of the steps are now the same for the high energy molecules from NADH and $FADH_2$ in earlier steps. Inside the nonpolar region of the phospholipid bilayer, UQH_2 (which is also a nonpolar compound) transports the electrons to *protein complex 3*. UQH_2 also carries protons. When UQH_2 delivers electrons to protein complex 3, it also donates its protons to be pumped.



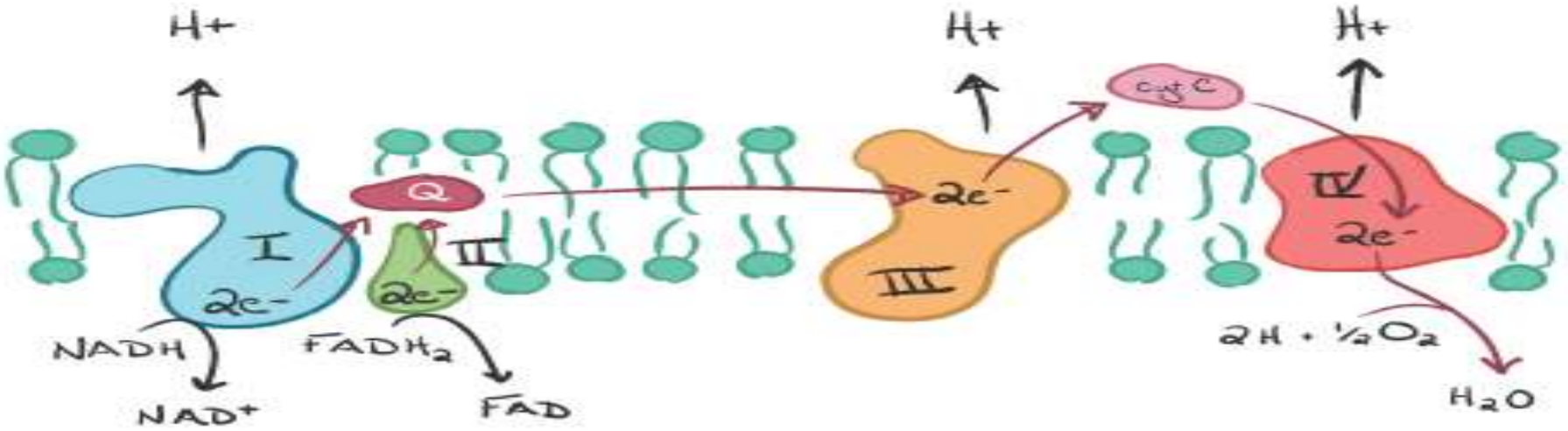
Step 4

The electrons that arrived at protein complex 3 are picked up by *cytochrome C* (or "*cyt C*"), the last electron carrier. This action also causes protons to be pumped into the intermembrane space.



Step 5

Cytochrome C carries the electrons to the final protein complex, *protein complex 4*. Once again, energy released via electron shuttling allows for another proton to be pumped into the intermembrane space. The electrons are then drawn to oxygen, which is the final *electron acceptor*. Its important to note that oxygen must be present for oxidative phosphorylation to occur. Water is formed as oxygen receives the electrons from protein complex 4, and combines with protons on the inside of the cell.



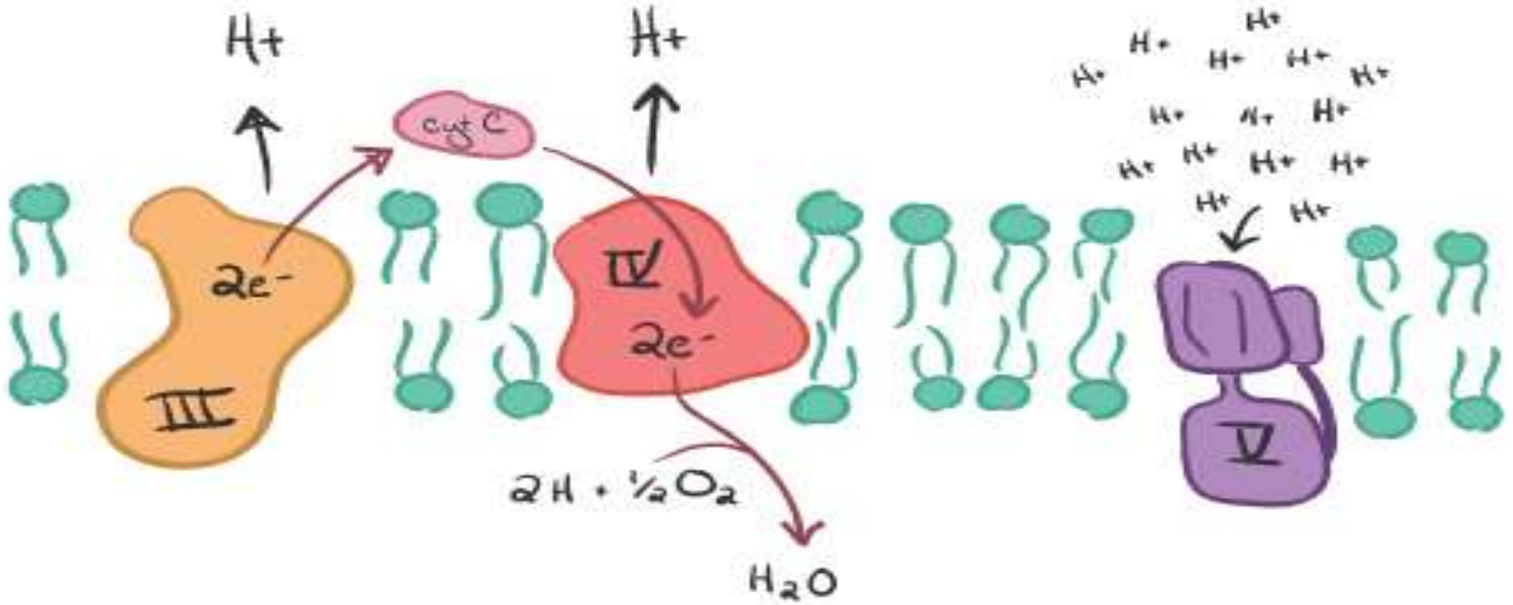
In summary

- +3 NADH
- +1 $FADH_2$
- +3 Hydrogen protons (H^+)
- -2 Hydrogen protons (H^+)
- -½ O_2
- +1 H_2O

2. Phosphorylation - the production of ATP

Step 6

As a result of part 1 (Oxidation of NADH and $FADH_2$), an electrochemical gradient is created, meaning there is a difference in electrical charge between the two sides of the inner mitochondrial membrane. The outside, or exterior, of the mitochondrial membrane is positive because of the accumulation of the protons (H^+), and the inside is negative due to the loss of the protons. A chemical concentration gradient has also developed on either side of the membrane. The electrochemical gradient is how the cell transfers the stored energy from the reduced NADH and $FADH_2$.

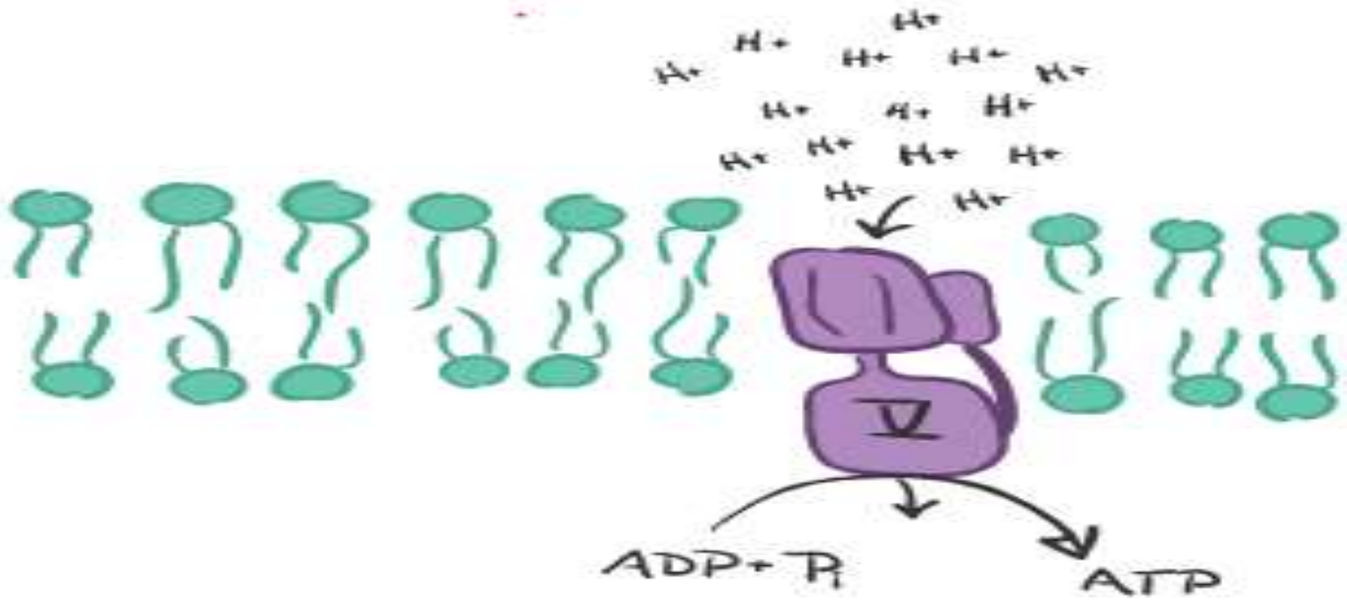


Step 7

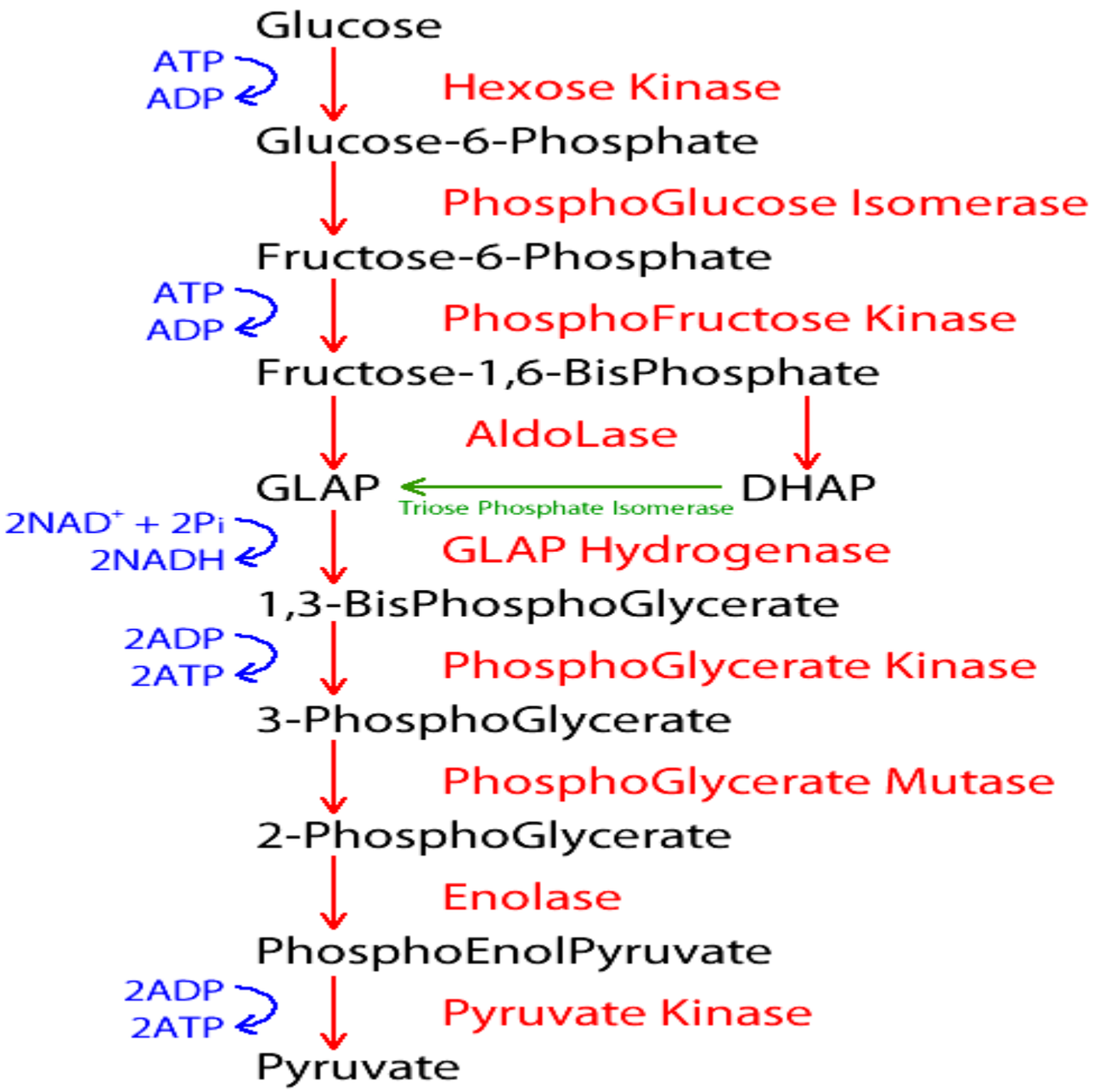
When there is a high concentration of protons on the outside of the mitochondrial membrane, protons are pushed through *ATP synthase*. This movement of protons causes ATP synthase to spin, and bind ADP and Pi, producing ATP. Finally, ATP is made!

In summary

- -ADP
- -Pi
- +ATP



Glycolysis


Glucose
ATP
ADP
Hexose Kinase
Glucose-6-Phosphate
PhosphoGlucose Isomerase
Fructose-6-Phosphate
ATP
ADP
PhosphoFructose Kinase
Fructose-1,6-BisPhosphate
AldoLase
GLAP
Triose Phosphate Isomerase
DHAP
2NAD+ + 2Pi
2NADH
GLAP Hydrogenase
1,3-BisPhosphoGlycerate
2ADP
2ATP
PhosphoGlycerate Kinase
3-PhosphoGlycerate
PhosphoGlycerate Mutase
2-PhosphoGlycerate
Enolase
PhosphoEnolPyruvate
2ADP
2ATP
Pyruvate Kinase
Pyruvate

Glycolysis

Glucose is the major form in which carbohydrate absorbed from the intestinal tract is presented to cells of the body. Glycolysis is the metabolic pathway that converts glucose $C_6H_{12}O_6$, into pyruvate, CH_3COCOO^- + H^+ in the presence of oxygen.The free energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate) and NADH .Pyruvate can be further processed anaerobically to lactate. This pathway is called Embden Meyerhof pathway.

Reactions of Glycolysis

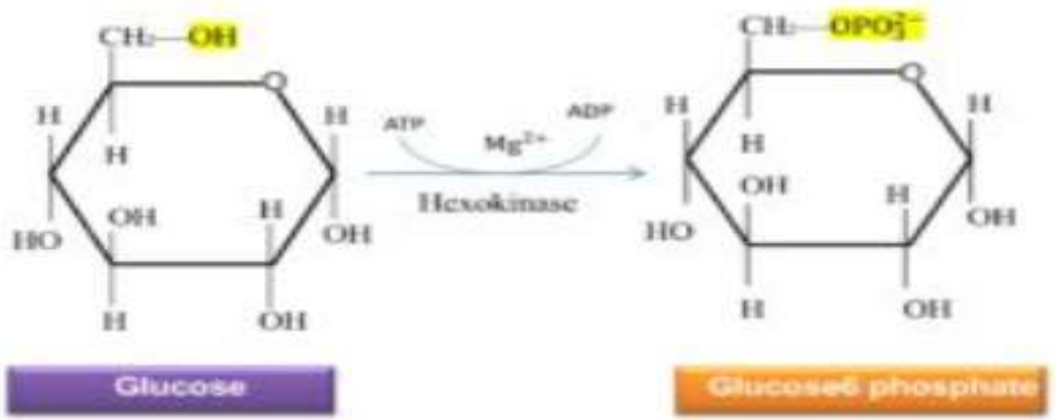
The breakdown of glucose (6-carbon compound) to two molecules of pyruvate (3-carbon compound) is brought about by sequential action of 10 enzymes which can be divided into two phases :

First Phase/Primary Phase/Preparatory Phase

The first five steps are regarded as the preparatory (or investment) phase. Since they consume energy to convert the glucose into two three-carbon sugar phosphates. In this phase two molecules of ATP are invested.

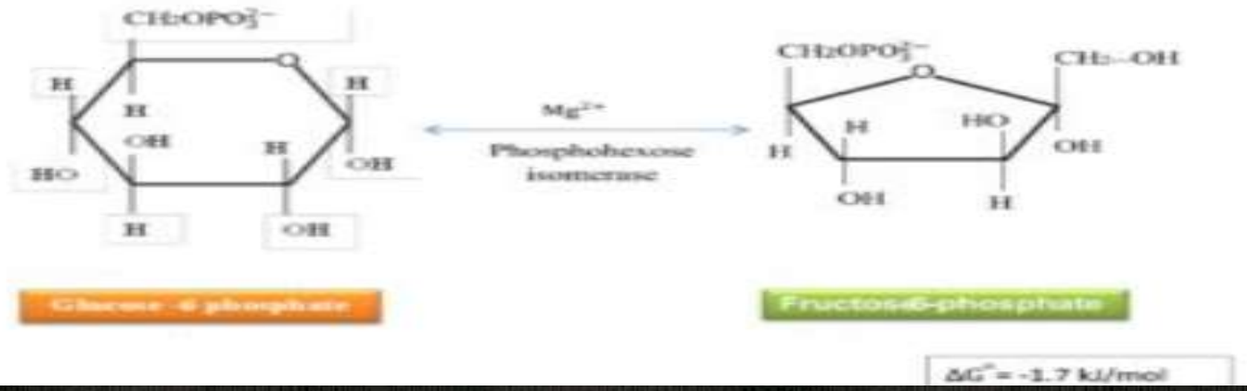
1.First Step

In the 1st step glucose is phosphorylated at C-6 by hexokinase to yield glucose 6-phosphate, with ATP as the phosphate donor. Hexokinase requires for activity. This reaction is irreversible under intracellular conditions.



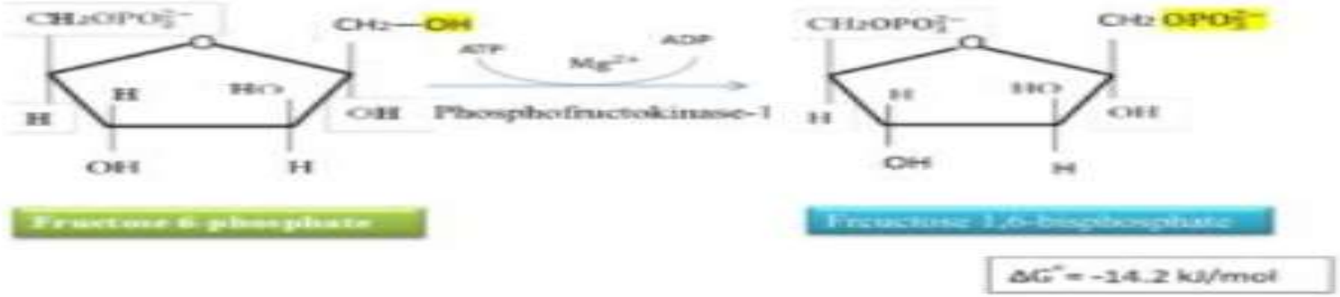
2. Second Step

Glucose 6- Phosphate is then rearranged into fructose 6-phosphate (F6P) by phosphohexose isomerase. This reaction is freely reversible under normal cell conditions.



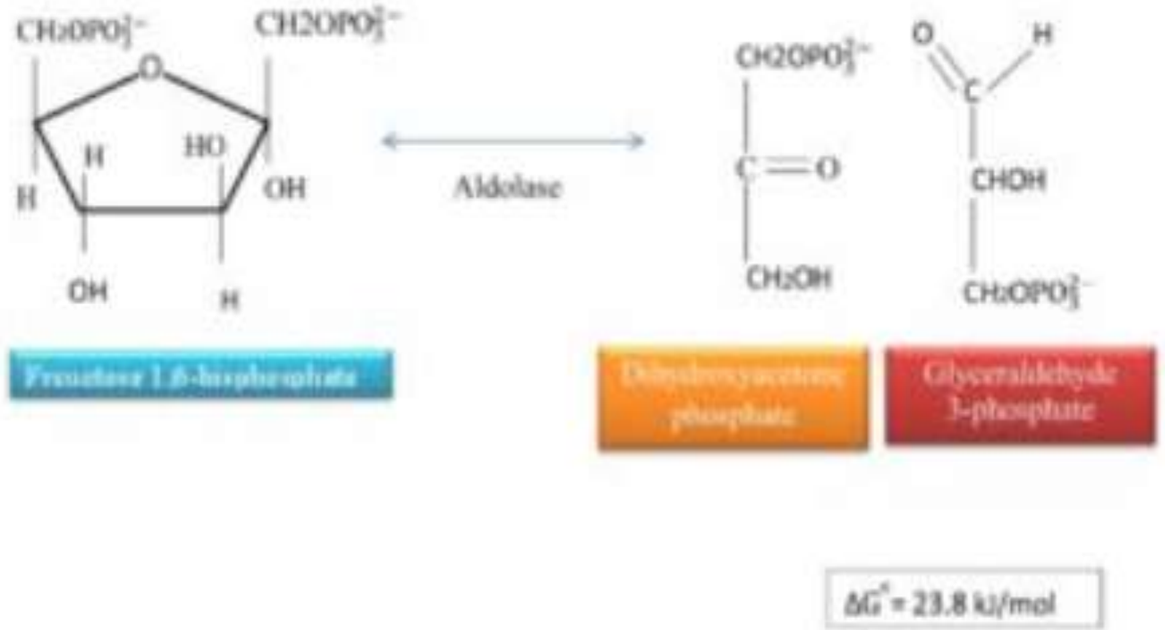
3. Third Step

Phosphofructokinase-1 catalyzes this step at ATP dependent phosphorylation of fructose 6-phosphate to give fructose 1,6- bisphosphate. The reaction is irreversible under intracellular conditions.



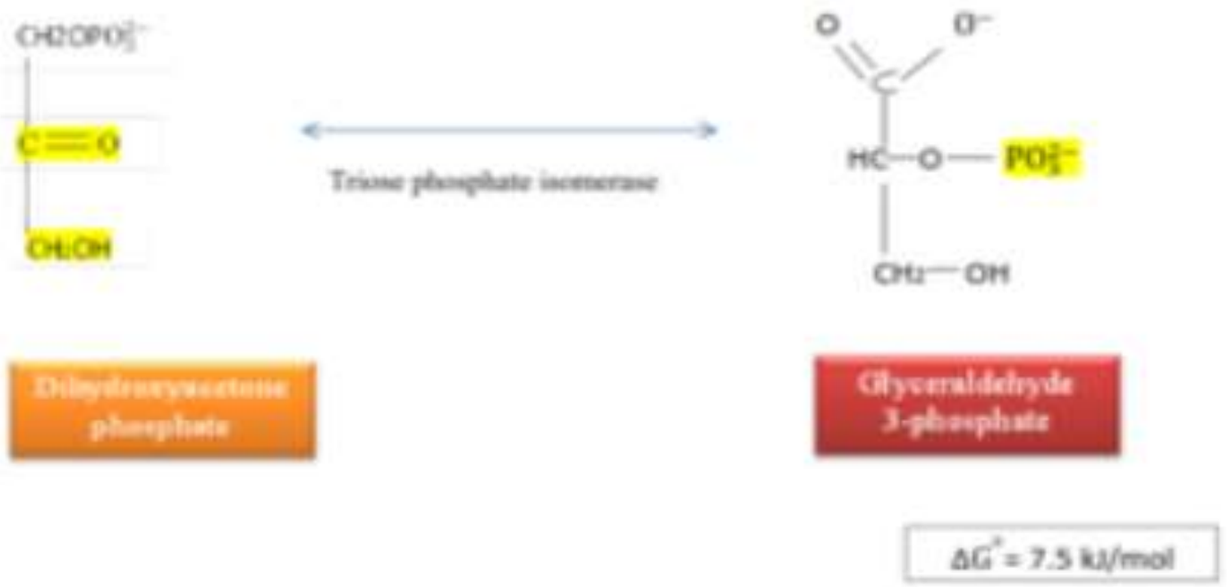
4. Fourth Step

Fructose 1,6 bisphosphate is cleaved by the enzyme aldolase to yield two different triose phosphates, gluceraldehyde 3-phosphate, an aldose and dihydroxyacetone phosphate, a ketose.



5. Fifth Step

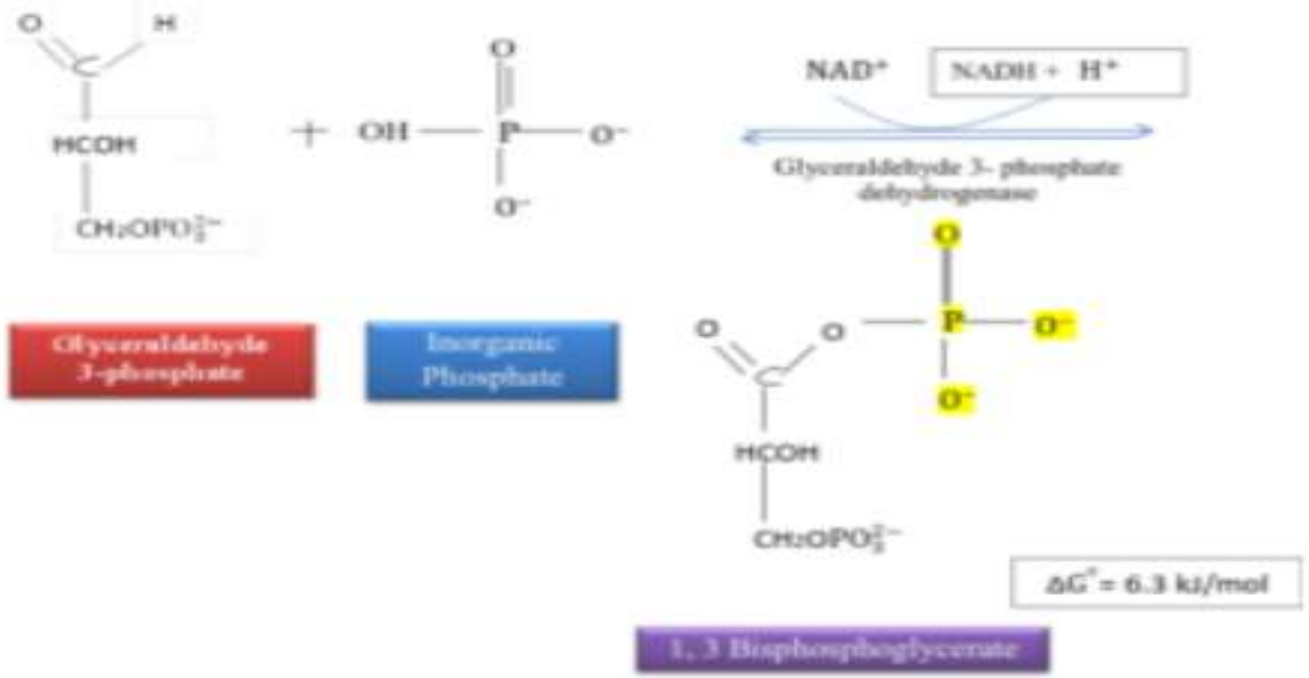
In this step Dihydroxyacetone phosphate is isomerized to glyceraldehyde-3-phosphate by the enzyme phosphotriose isomerase .This reaction completes the preparatory phase of glycolysis.



Second Phase/Secondary Phase/Pay –off Phase

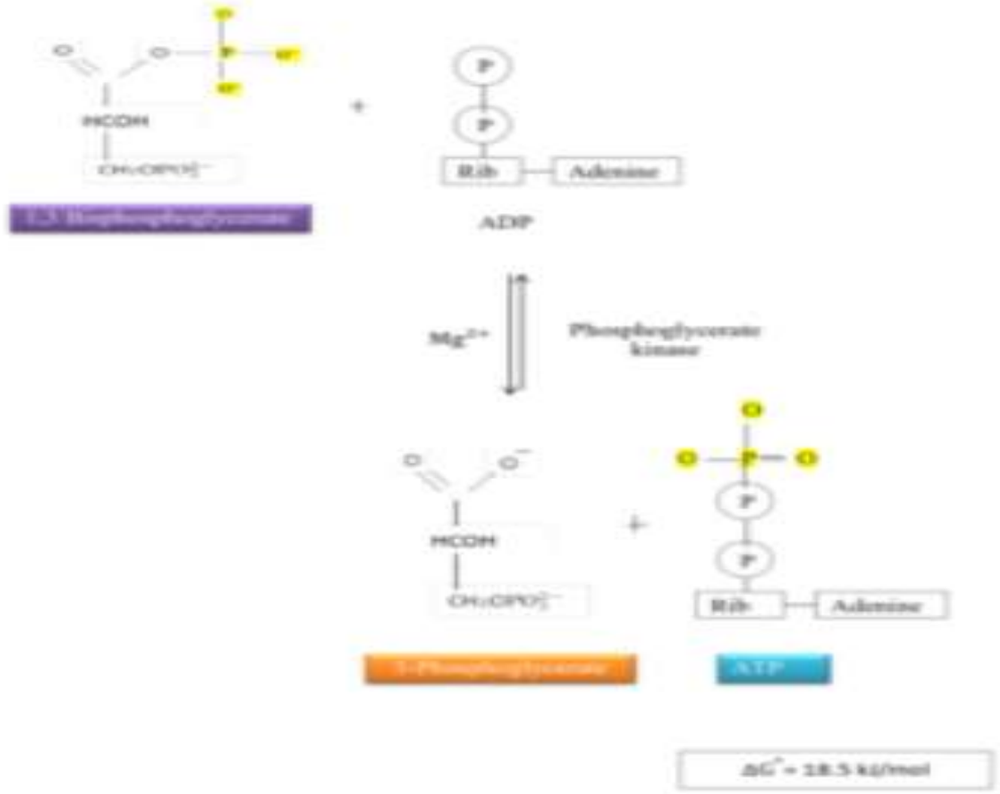
6. Sixth Step

The first step in the pay off phase is the oxidation of glyceraldehyde 3- phosphate to 1, 3 bisphosphoglycerate, catalyzed by glyceraldehyde 3- phosphate dehydrogenase.



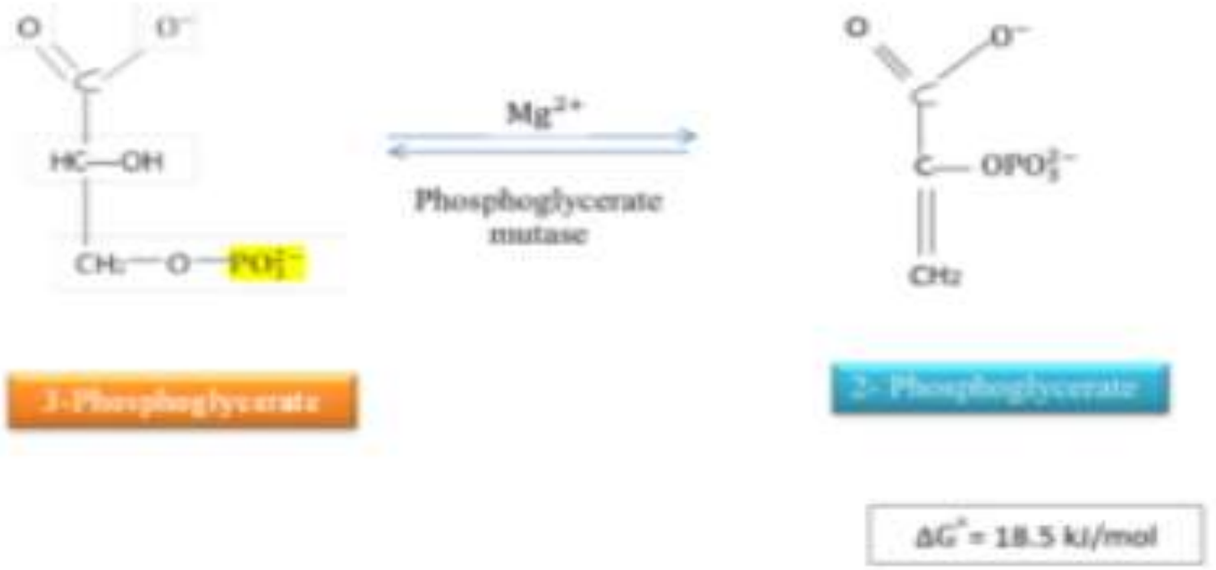
7. Seventh Step

The enzyme phosphoglycerate kinase transfers the high energy phosphoryl group from the carboxyl group of 1,3 bisphosphoglycerate to ADP, forming ATP and 3-phosphoglycerate.



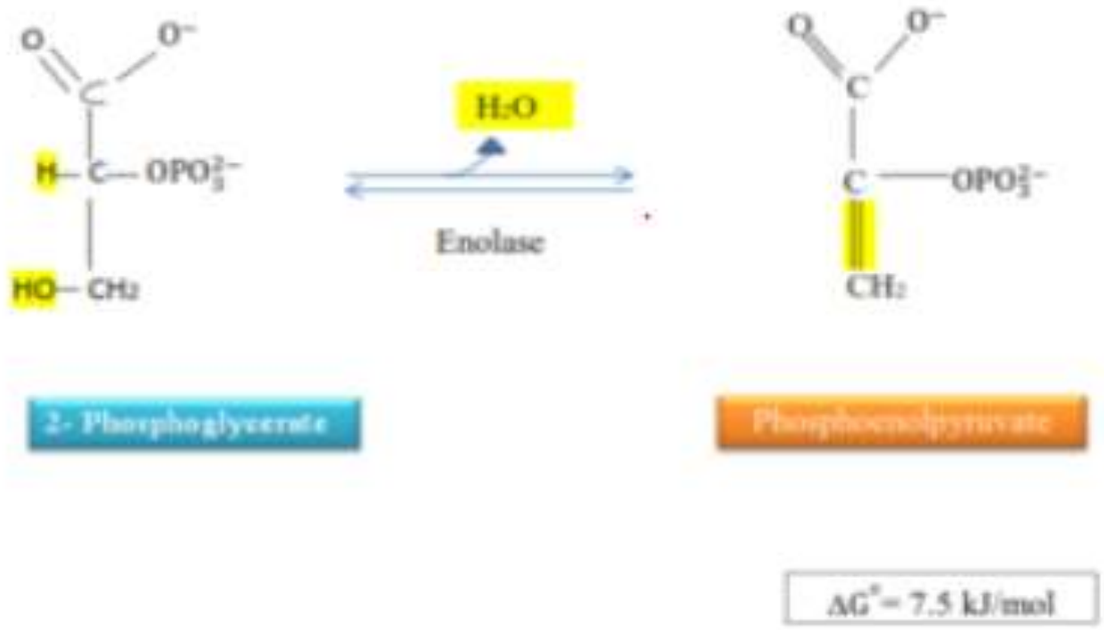
8. Eight Step

In the eighth step the enzyme phosphoglycerate mutase isomerizes 3-Phosphoglycerate to 2-Phosphoglycerate.



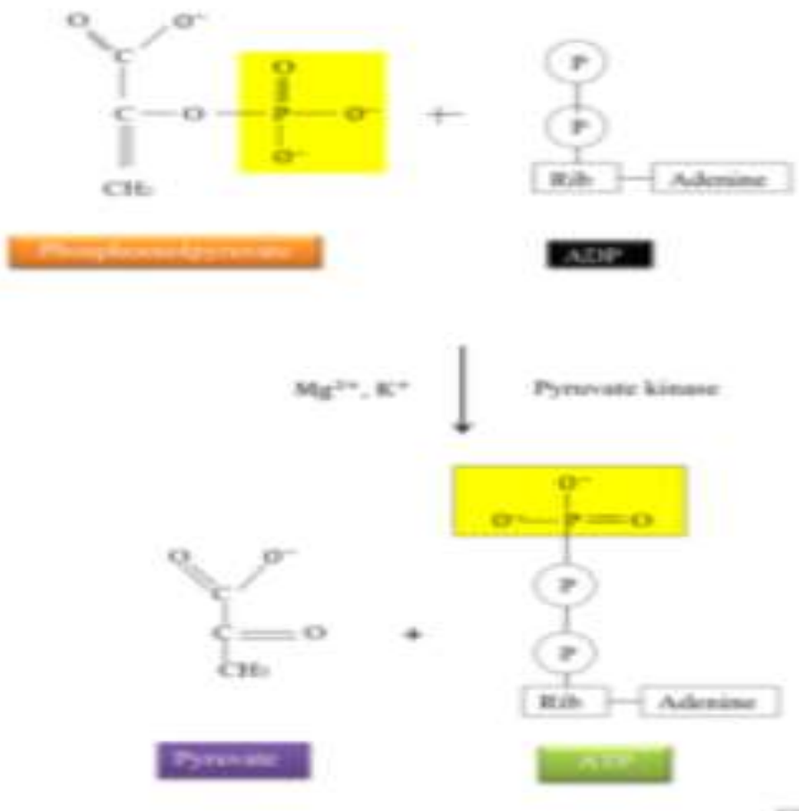
9. Ninth Step

In this step the enzyme enolase promotes reversible removal of a molecule of water from 2-phosphoglycerate to yield phosphoenolpyruvate.



10. Tenth Step

The high energy phosphate of phosphoenol pyruvate is transferred to ADP by the enzyme pyruvate kinase to generate 2 molecules of ATP per molecule of glucose oxidized. Enol pyruvate formed in this reaction is converted spontaneously to the keto form of pyruvate.



Glycolysis: Energy balance sheet

- Hexokinase: - 1 ATP
- Phosphofructokinase: -1 ATP
- GAPDH: +2 NADH
- Phsophoglycerate kinase: +2 ATP
- Pyruvate kinase: +2 ATP

Total/ molecule of glucose: +2 ATP, +2 NADH

Fate of Pyruvate

- NADH is formed from NAD^+ during glycolysis.
- The redox balance of the cell has to be maintained for further cycles of glycolysis to continue.
- NAD^+ can be regenerated by one of the following reactions /pathways:
- Pyruvate is converted to lactate
- Pyruvate is converted to ethanol
- In the presence of O2, NAD^+ is regenerated by ETC. Pyruvate is converted to acetyl CoA which enters TCA cycle and gets completely oxidized to CO_2.

Entry of other sugars into glycolysis

- **Fructose** is phosphorylated by fructokinase (liver) or hexokinase (adipose) on the 1 or 6 positions resp.
- Fructose-6-phosphate is an intermediate of glycolysis.
- Fructose-1-phosphate is acted upon by an aldolase-like enz that gives DHAP and glyceraldehyde.
- DHAP is a glycolysis intermediate and glyceraldehyde can be phosphorylated to glyceraldehyde-3-P.
- **Glycerol** is phosphorylated to G-3-P which is then converted to glyceraldehyde 3 phosphate.
- **Galactose** has a slightly complicated multi-step pathway for conversion to glucose-1-phosphate.
- gal → gal-1-P → UDP-gal → UDP-glc → glc-1-P.
- If this pathway is disrupted because of defect in one or more enz involved in the conversion of gal to glc-1-P, then galactose accumulates in the blood and the subject suffers from galactosemia which is a genetic disorder, an inborn error of metabolism.

Regulation of Glycolysis

Enzyme	Activator
Hexokinase	AMP/ADP
Phosphofructokinase	AMP/ADP, Fructose-2,6-bisphosphate
Pyruvte kinase	AMP/ADP Fructose-1,6-bisphosphate

Enzyme	Inhibitor
Hexokinase	Glucose-6-phosphate
Phosphofructokinase	ATP, Citrate
Pyruvate kinase	ATP, Acetyl CoA, Alanine

Cori Cycle

- Lactate is formed in the active muscle to regenerate NAD^+ from NADH so that glycolysis can continue.
- The muscle cannot spare NAD^+ for re-conversion of lactate back to pyruvate.
- Thus, lactate is transported to the liver, where, in the presence of oxygen, it undergoes gluconeogenesis to form glucose.
- The glucose is supplied by the liver to various tissues including muscle.
- This inter-organ cooperation during high muscular activity is called as the Cori cycle.