Overview and Integration of Cellular Metabolism

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Lecture 04: Glycolysis, alcohol and lactic acid fermentation

Hello everyone, in today's session of Overview and Integration of Cellular Metabolism, we will discuss Glycolysis, Alcohol and Lactic Acid Fermentation. Now, in the previous class we already have discussed the digestion and absorption of carbohydrate. In this class we will discuss different concepts of glycolysis like definition of glycolysis that is what is glycolysis, then the sites of glycolysis, different phases of glycolysis, basic biochemical reactions including reversible, irreversible and rate limiting steps of glycolysis and also different inhibitors, then clinical and applied aspects related to this specific metabolic pathway ok. So, in the previous class you have already known that carbohydrate are absorbed in the simpler form and enters circulation. In the circulation the major metabolite of carbohydrate is definitely glucose. Now, glucose enters inside the cell through different glucose transporters, see these are the glucose transporters.

Now, after entering into the cell glucose can undergo different biochemical reaction. It is not only the source of energy, it is also used as precursor for different intermediate for different other biochemical reaction. Now, here we will discuss how glucose is entering glycolysis. Definitely it forms glucose 6 phosphate.

Now, glucose 6 phosphate can once again undergo glycolysis as well as glycogenesis that is glycogen formation and also pentose phosphate pathway. Here we will discuss glycolysis specifically. Now, what is glycolysis? Basically glycolysis this term is derived from Greek words one is glykes which means sweet or sugar and the other one is lysis, splitting. So, this is basically the splitting of glucose glycolysis. Now it is also known with the name of the discoverers Embden, Meyerhof and Parnas.

So, it is also known as EMP pathway. Now, in glycolysis what is actually happening? It is that metabolic pathway where one molecule of glucose is degraded by sequential enzyme catalyzed reactions to yield pyruvate a 3 carbon compound pyruvate is generated by splitting glucose. Now, during this sequential reaction some free energy are released and those free energies are stored in the form of ATP and NADH. So, basically

glycolysis is splitting of glucose to form pyruvate as well as production of ATP and NADH. Now where glycolysis occurs? Glycolysis is basically an universal central pathway of glucose metabolism present almost in every cell not only that this is the largest flux of carbon.

Now there are multiple cells or tissues like erythrocyte, RBC, renal medulla, brain, sperm cells. These cells these tissues are solely dependent on glycolysis for the production for the supply of energy and the enzymes which are required for glycolysis are present in cytosol. So, basically glycolysis is a cytosolic metabolic pathway. Now there are 2 phases of glycolysis one is preparatory phase another is payoff phase. Now let us see what happens in these phases.

Now in preparatory phase these are the reactions what happens? First the glucose is converted to glucose 6 phosphate. So, basically we call this step as activation of glucose. Now just like other monosaccharides like fructose, galactose glucose also needs to be activated to enter the metabolic pathway and how these activation occurs by phosphorylation. Phosphorylation with the help of hexokinase sometimes glucokinase as well. Now these 2 enzymes either of the one actually converts glucose to glucose 6 phosphate.

This is the first priming reaction and here the phosphate donor is definitely ATP. Now glucose 6 phosphate next is converted to fructose 6 phosphate this is an isomerization reaction. So, the enzyme here is phosphohexose isomerase. Next, fructose 6 phosphate once again undergo another priming reaction by phosphorylation with the help of the enzyme phosphofluctokinase 1. Now this enzyme is very important this metabolic reaction is very important.

Now you can see that those 2 phosphorylation reaction like glucose to glucose 6 phosphate as well as fructose to fructose to fructose 1 6 bisphosphate these 2 reactions are irreversible reaction whereas, isomerization is a reversible one. Now formation of fructose bis 1 6 bisphosphate is the committed pathway for glycolysis. Why? See these intermediates from glucose like glucose 6 phosphate, fructose 6 phosphate they can enter or undergo various other biochemical reaction they can enter in different other metabolic pathway other than glycolysis. But formation of fructose bis 1 6 bisphosphate is the targeted step these molecule can only enter glycolysis. So, this is the second priming reaction as well as the committed steps for glycolis step for glycolysis and also the rate limiting step of glycolysis ok.

So, there is formation of fructose 1 6 bisphosphate once again the phosphate phosphate group donor is ATP. Next fructose 1 6 bisphosphate is split to 2 different 3 carbon

molecule one is glyceraldehyde 3 phosphate another is dihydroxyacetone phosphate. So, this is the step based on which glycolysis is name lysis is the splitting here the splitting is splitting has happened. So, this splitting occurs with the in with the help of the enzyme aldolase this is basically aldolase A. Now these 2 3 carbon molecule are inter convertible, but in normal intracellular condition mostly dihydroxyacetone phosphate is converted to glyceraldehyde 3 phosphate the enzyme is triose phosphate isomerase.

So, what we can see at the end of the preparatory phase there are 2 molecules of ATP there are 2 molecules of ATP which are utilized one is here another is here and those energy free in those energies are stored as free energy in the intermediates and also there is formation of a common product glyceraldehyde 3 phosphate. So, basically there are 2 glyceraldehyde 3 phosphate form at the end of preparatory phase of glycolysis. Now payoff phase is how these stored energy in the intermediate those free energy how they are helping to form other molecules which finally, can be utilized in energy formation in electron transport chain as well as substrate level phosphorylation let us see. So, in payoff phase we are having 2 molecules of glyceraldehyde 3 phosphate. Now both the molecules undergo same type of processing.

Now what happens first the glyceraldehyde 3 phosphate undergo oxidation and phosphorylation. Now remember in these type of phosphorylation the ATP is not utilized ATP is not the phosphate donor rather inorganic phosphate is used here and also because there is oxidation. So, basically there is dehydrogenation and NAD is converted to NADH here and the enzyme definitely here is a dehydrogenase glyceraldehyde 3 phosphate dehydrogenase. So, there is formation of 1 3 bisphosphoglycerate 2 molecules remember there are 2 molecules every time. Now from this 1 3 bisphosphoglycerate a substrate level phosphorylation occurs.

So, what is substrate level phosphorylation here the phosphate donor 2 ADP is not ATP rather it is a substrate which is giving of the phosphate group. So, here 1 3 bisphosphoglycerate 1 of the phosphate group is donated to ADP to form ATP and there is formation of 3 phosphoglycerate and the enzyme here is kinase phosphoglycerate kinase. Next there is phosphate group shifting from 3 to 2 position of carbon forming 2 phosphoglycerate with the help of the enzyme phosphoglycerate mutase. Now this 2 phosphoglycerate undergoes dehydration or hydrolysis actually with formation of phosphanol pyruvate with the help of the enzyme enolase. Now this enzyme enolase can be inhibited by fluoride, fluoride inhibit the enzyme enolase what is the importance we will discuss it later.

Now phosphanol pyruvate then undergo another substrate level phosphorylation and forms pyruvate again because there is substrate level phosphorylation there is formation

of ATP from ADP and the enzyme is another kinase which is pyruvate kinase. Now this is the payoff phase where we can see there is formation of NADH here then there is formation of ATP at substrate level phosphorylation here and here. So, this is the payoff phase where the stored energy in the intermediate are finally, giving finally, producing NADH as well as ATP's. Now what is the fate of this pyruvate finally, now pyruvate can be processed aerobically as well as anaerobically. In most of the cells most of the aerobic cells pyruvate enters TCA cycle or citric acid cycle it is also known as citric acid cycle where different reducing equivalent in the form of NADH or FADH2O form and they enter electron transport chain and finally, they are oxidized by oxidative phosphorylation forming ATP and also regenerating NAD that is the oxidized form.

So, NAD is form at the end of the electron transport chain once again it is regenerated, but and that regenerated NAD can once again be utilized in glycolysis, but in case of anaerobic glycolysis where oxygen supply is low in those tissues or in those organism who undergo anaerobic metabolism their regeneration of NAD cannot be done because there is no electron transport chain. So, there is no oxidative phosphorylation there is no regeneration of NAD then how these NAD can be replenished. So, in those organisms or in those tissues where there is anaerobic metabolism is occurring their pyruvate is converted to lactate with the help of the enzyme lactate dehydrogenase and their NADH is converted to NAD and these NAD can be reutilized in glycolysis. So, basically the flux of NAD is maintained in anaerobic glycolysis by forming lactate. Now, lactate specifically does not have any metabolic role in case of glycolysis rather accumulation of excess lactate can cause muscle fatigue as well as acidosis lactic acid accumulation.

So, there can be acidosis low pH. So, excess lactic acid accumulation can cause lactic acid with all these symptoms. So, basically anaerobic glycolysis there is formation of lactate and aerobic glycolysis there is formation of pyruvate and that pyruvate enters TCA cycle and is utilized in electron transport chain for formation of ATP. Now as we have discussed that in aerobic condition pyruvate forming acetyl coenzyme A can enter TCA cycle or citric acid cycle, but in case of anaerobic condition or hypoxic condition it can form lactate in different organisms. It can also form alcohol that is alcohol fermentation in hypoxic or anaerobic condition different organisms pyruvate can be converted to ethanol and releases carbon dioxide in different organism and those are utilized metabolically.

So, that is alcohol fermentation process. So, this is the overall balance sheet of glycolysis here each molecule of glucose gives rise to two molecules of glyceraldehyde 3 phosphate two molecules of glyceraldehyde 3 phosphate and they and the whole process undergo 10 specific reaction where glucose forms two molecules of pyruvate 4 ATP investing 2 ATP investing 2 ATP one inorganic phosphate is there to actually 2 for 2

molecules of pyruvate and then there is formation of NADH as well as ADP. Now if you simplify the reaction we can see there is investment of glucose which finally, forms 2 molecules of pyruvate from NAD there is formation of NADH from inorganic phosphate and ADP finally, there is formation of ATP and further water formation water molecule formation. So, this is the final balance sheet of glycolysis. Now let us see what is the net ATP production at the end of glycolysis.

So, this is the step here in payoff phase of glycolysis this is the payoff phase of glycolysis here you can see for formation of 1 3 bisphosphoglycerate there is 2 molecules of NADH because there are 2 molecules of glyceraldehyde 3 phosphate from this 2 molecules of NADH when this NADH is basically oxidized there is energy production which is equivalent to 3 molecules of ATP. As well as there are substrate level phosphorylation from where we are getting 2 molecules of ATP and also another level of substrate level phosphorylation there are 2 molecules of ATP. Now 3 ATP is produced from 1 molecule of NADH. So, basically there is 6 molecules of ATP because you remember there are 2 glyceraldehyde 3 phosphate from there 2 NADH is produced each NADH is contributing in formation of 3 molecules of ATP.

So, there are 6 ATPs. So, 6 ATP, but remember in preparatory phase there is investment of 2 ATP molecule 2 ATP molecule. So, 2 ATP are utilized. So, at the end of glycolysis what we are getting 6 plus 2 plus 2 minus 2. So, there are 8 ATP molecules are formed at the end of glycolysis. Now once again it has been seen that the energy is not specifically 2 molecule sorry 3 molecules of ATP on oxidation of NADH it is actually 2.

5 the energy is equivalent to 2.5 molecules of ATP. So, if we recalculate it we will see that there are 5 molecules of ATP from NADH then 4 molecules from substrate level phosphorylation of ATP and 2 molecules of ATP are invested in preparatory phase. So, basically there are 7 molecules of ATP at the end of glycolysis. So, remember this is the old calculation this is the newer one where at the end of glycolysis there is formation of 7 molecules of ATP. Now what are the applied importance and clinical aspects of glycolysis? Now remember as we have discussed that the enzyme enolase is inhibited by fluoride.

Now what is the clinical utilization of this phenomena? Now when we collect blood for estimation of blood glucose in blood there are multiple molecules molecules of RBCs. Now RBC if you do remember is solely dependent on glycolysis for the provision of metabolic energy. Now because there is only very low amount of ATP form in glycolysis. So, there is excessive utilization of glucose for provision of ATP. Now what happens in the stored blood in the collected blood which is which have been collected for estimation of blood glucose if that blood is kept for long time what happens the RBCs present in the

blood they utilize glucose for their energy requirement.

Now what will happen the content of glucose will be reduced in blood sample now to stop that what we will do we add fluoride in the vials with the anticoagulants we add sodium fluoride. Now that sodium fluoride actually inhibit enolase. So, basically enolase is in an enolase is inhibited by sodium fluoride which helps to keep the blood glucose content in the amount as it is. So, this is one clinical aspect. Next pyruvate kinase deficiency causes hemolytic anemia.

Now remember once again that is that this concept is once again with regard to RBCs. Now in RBCs if there is pyruvate kinase deficiency what will happen definitely ATP production will not be there. Now RBC has sodium potassium ATP sperm. Now this sodium potassium ATP sperm is actually maintaining the shape as well as the as well as the life span of RBC. So, if this sodium potassium ATP sperm is not acting properly due to the absence or deficiency of ATP what will happen RBC will swells up and will burst that will cause hemolytic anemia.

Then another concept that is lactic acidosis is precipitated by anoxia or hypoxia as we have already discussed there is formation of lactate from pyruvate in case of anaerobic glycolysis. Now if there is continuous or severe hypoxia there will be formation of excessive amount of lactate and that will cause lactic acidosis. So, these are the different clinical aspects or applied aspects of glycolysis. So, at the end of this session these are the key points that glycolysis is the formation of pyruvate by splitting glucose this is the 6 carbon compound which has been split off to form pyruvate with simultaneous production of ATP as well as NADH and this NADH is finally, utilized to form ATP after entering into the electron transport chain. And then glycolysis can be aerobic by formation of pyruvate which enters once again TCA cycle to electron transport chain or it can be anaerobic which forms lactate for regeneration of NAD at the end of the aerobic glycolysis phase there is production of 8 ATP rather 7 ATP this is the recent concept at the end of glycolysis there is production of 7 ATP molecules. So, these are all for today. Thank you.