Introduction to Biomolecules
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Lecture – 18
Glycolysis (Part 1/2)

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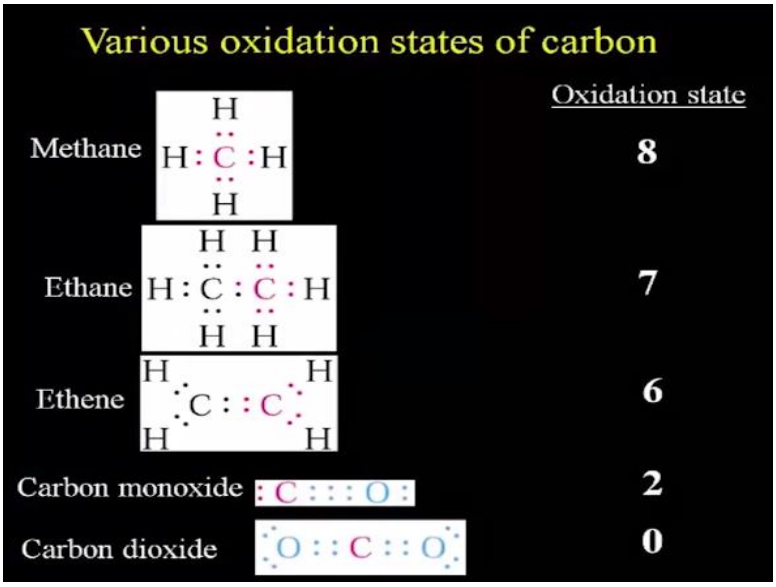

So let us begin. So, someone has a question about carbon's oxidation state in methane. So, see if you look at this slide you see here 4 pairs of electrons shared between carbon and hydrogen, so that is how you get 8. So, each electron here is spending more time with carbon than with the hydrogen. So, carbon is not going to be able to accept any more electrons. Yes, it is a total 8 electrons, not 8 positive charge, it is not 8+. So, we move to the next one.

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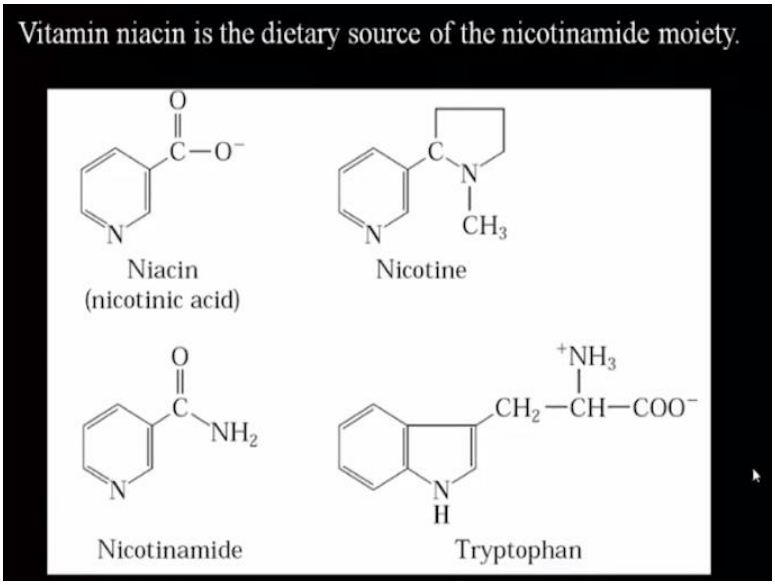

So, there was another question what is protein domain, so alright. So, what is a protein domain? So, protein domain is when the polypeptide chain folds into three-dimensional structure so you have certain regions that form an independent module like structures and we call them as protein domain like yesterday you saw the Rossmann fold that is in NAD binding proteins. So, these are specific three-dimensional structures.

These are a higher order in the hierarchy of structure than the secondary structures, but these are not like the whole protein structure. Sometimes it can be whole protein structure, certain proteins have one single well conserved domain meaning that particular structure is seen in other proteins as well in other organisms, but many proteins particularly larger proteins have multiple independent shapes within them.

And these independent shapes may be conserved and they could function like a module. So you may find like for example if you take a protein that acts as a transcription factor, so you will have a region that enables it to bind DNA and this DNA binding part of that protein will have a certain three-dimensional structure and that could be there in many other DNA binding proteins as well.

And in addition to binding to DNA that protein might have other domains which might help it to interact with a variety of molecules, a transcription factor might have another domain that interacts with RNA polymerase or it might interact with the protein involved in transcription activation or suppression. So, you could have modular structures in a protein and these modules may be mixed and matched.

And such independent three-dimensional structures that are identifiable as specific structure they are the domains. Is that clear? So yesterday we stopped with NAD structure how it accepts electrons in the form of hydride ions that is 2 electrons and 1 proton and so we saw that it is in this nicotinamide domain like this one attached to another nucleotide that is adenine nucleotide. So, the two phosphoric acids connect these two.

So, this is like a benzenoid structure, this is more like a benzene and when it binds these double bonds shift here and that is called quinonoid structure because it resembles quinine. So, then we stopped at this slide so where the nicotinamide comes. So, the nicotinamide normally comes from niacin, this is a B complex vitamin and it is required in the diet, although it can be made from tryptophan.

So, the amount of nicotinamide made from the tryptophan is not sufficient to produce all the NAD that we want and sometimes the protein that we have in our food may not have sufficient amount of tryptophan. For example, if you are eating too much of corn and corn is deficient in tryptophan, so you will not have required tryptophan and that is why its avoidability in the diet as niacin is critical.

Synthetically, originally people made from nicotine and that is why the names are similar, but the nicotine available from tobacco cannot make niacin or nicotinamide. So smoking is not going to provide the raw precursors to make NAD and due to this when your diet lacks niacin so you have vitamin deficiency leading to diseases.

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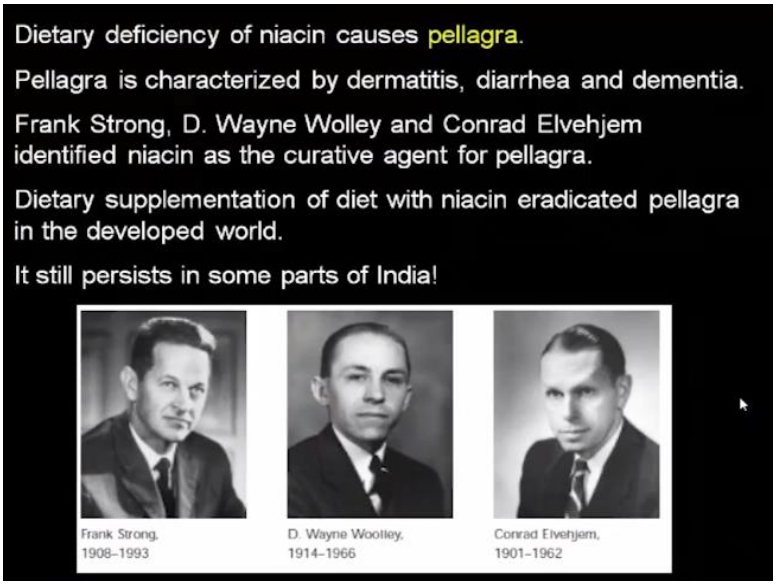

And that disease is called pellagra. So, pellagra is a disease that is characterized by skin infection or abnormalities of the skin, rough skin. The Italian word for that is what is pellagra. Diarrhea and dementia, people forget things to. So, the 3 D's characterize pellagra and that is caused by niacin. So, lot of people used to die due to pellagra earlier particularly in the part of the world where people were dependent on corn.

For example, Southwest United States used to have a problem of deficiency of niacin. Then these 3 scientists identified that the main defect is the absence of niacin in the diet. So, someone else, I forgot the name, identified it is due to the nutritional deficiency and these three people identified the deficient nutrient is actually niacin and niacin is a very inexpensive molecule.

And it can easily be supplemented in one of the many things that you eat in a daily basis and that takes care of pellagra and pellagra is eradicated in the developed world. But I know since our priorities as I told you previously when we were talking about vitamin A for want of a quarter of a carrot a lot of children go blind, similarly we have niacin deficiency, particularly North Central Deccan plateau still pellagra persists.

So many of you should think about such things. When you want to build a career when you think about high phi things you need to realize as a large part of our problems are these kinds of very mundane things, not because we lack big machines or sophisticated equipment or algorithms or defense weapons and so on. So, we still have nutritional deficiencies.

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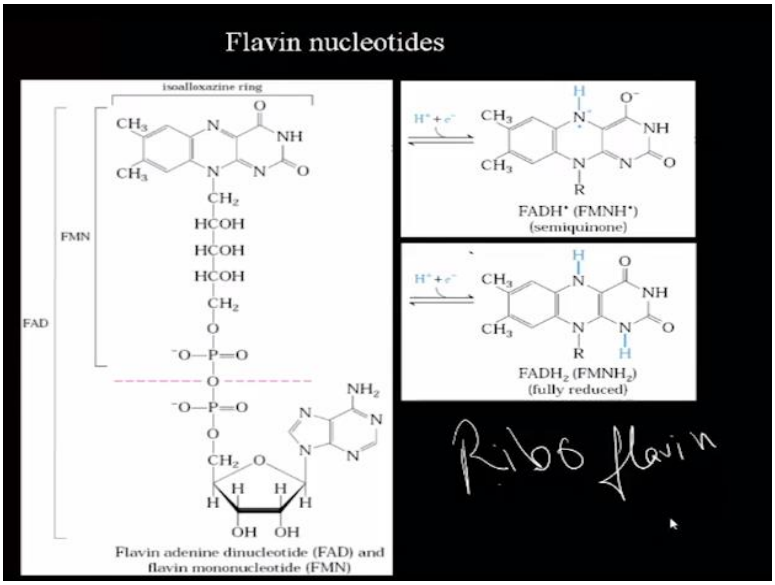

The next electron carrier that we need to familiarize ourselves with is flavin nucleotides. So here enters the simazine the next B complex vitamin. So, this vitamin is riboflavin, let me write the spelling. So this is the vitamin, this is again another B complex vitamin and its main structure is this isoalloxazine ring as you see here. So, this string is called isoalloxazine ring and this is the one that is involved in electron carrying temporarily.

And so this one might exist like above this pink dashed line, so this is flavin mononucleotide. So, this is the structure of the flavin mononucleotide and if it is in dinucleotide linkage with adenine nucleotide just like in NAD, then you call it as FAD flavine adenine dinucleotide. So, it exists in both forms. So, some of the enzymes carry this and some of the enzyme carry FAD.

And an important difference from NAD or NADP is that the proteins that use flavin nucleotides as electron carrier the flavin moiety is tightly bound to the enzyme. Sometimes it is even covalently bound, so therefore this is a good example of a prosthetic group. When we talk about prosthetic group meaning the co-factor that is tightly bound to the enzyme rather than a cofactor that is loosely bound.

So, NAD is loosely bound. It can get reduced by one reaction at that instead of one enzyme then it can freely diffuse out and participate in another uh enzymatic reaction, whereas flavin nucleotides do not do that. So, these temporarily hold electrons in the actin site. When the electrons are taken from a substrate or donated to a substrate so these temporarily hold electrons in this isoalloxazine ring as you see here.

And the type of electron transfer here is single electron and single proton instead of hydride ion as you saw in NAD here it is one proton one electron. So, basically one hydrogen atom and this nitrogen temporarily occurs a positive charge and that is semiquinone. So, then it can accept one more and it gets reduced to FADH 2 or FMNH 2. So, this is fully reduced. So, therefore this flavin nucleotide containing enzymes can participate in reactions where you need to take two electrons from a donor and give one electron at a time to an acceptor which may accept only one electron at a time.

So, we will encounter this kind of reactions when we go into oxidative phosphorylation and photosynthesis. For example, in photosynthesis when you take up electrons by oxidizing water usually it releases a molecular oxygen 2 molecules of water are split. So, you end up getting 4 electrons and 4 protons and there should be some carrier that should be able to accept 4 electrons. And the next step where the electrons are transferred those acceptors takes one electron at a time.

So, you need an electron carrier that can handle numbers within that, should be able to take one to four readily and should be able to give one to four like it may need to give one at a time, four times or four in one go or two at a time twice and so on. So it should be able to handle in the range of one to four. So here FADH 2 and FMNH can handle in terms of accepting one at a time twice and donating two in one go or one at a time twice.

So that is what these can do and due to these features these flavin nucleotides participate in a higher diversity of reactions than what NAD does. But NAD using enzymes are large numbers overall, but in terms of the diversity the flavin proteins, flavoproteins you know the proteins that contain flaming nucleotides as cofactors they are more diverse. And another interesting feature is if you remember we learnt about the reduction potential, standard reduction potential, etc.

So here the reduction potential of FAD and FMN varies depending on how this ring structure is influenced by the binding to the enzyme active site. So, depending on the tightness with which they are bound their reduction potential may be altered. So, these are characteristics of the flavin nucleotides. So, two primary things that you do not want to forget are it can take

one electron at a time are two electrons in one go and it can donate one electron at a time or two electrons in one go that is one.

Second these are prosthetic groups, these are tightly bound. And their absorption spectra again varies among the different forms like FAD fully oxidized one, partially oxidized one, fully reduced one. So, all of them have characteristic absorption spectrum and that is very useful in assaying the oxidation state.

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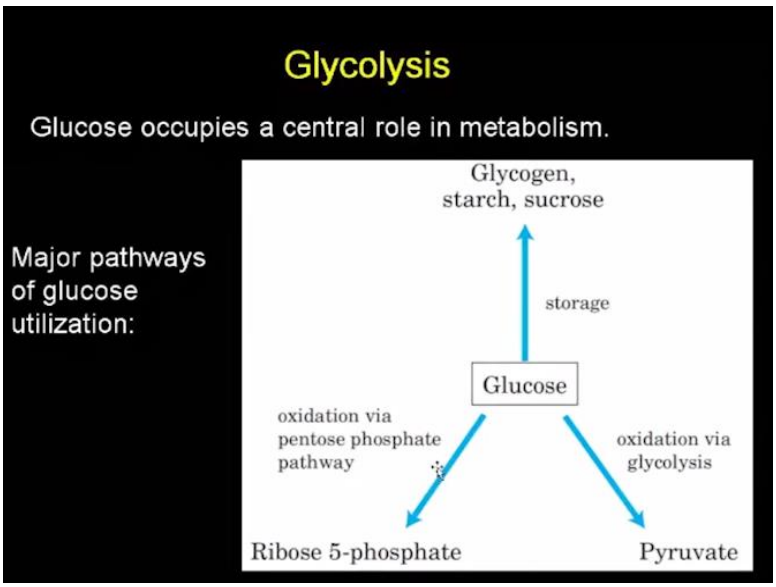

So, with this we have equipped ourselves with the knowledge required to get into metabolism. So the one of the primary things we learnt is we are talking about molecular conversions where energy is involved, either energy is required for the reaction or due to the reaction energy is liberated and where is this energy coming from we learned that it is to do with the kind of atoms and the kind of bonds that are there.

And that is what makes some molecules like the person standing on the 6 feet tall diving board or someone in the water. So that is one main thing. So that is how the chemical energy difference we understand. And the second thing is in biology this difference is primarily in terms of electron affinity. So, therefore we learnt about the electrochemical relationship. So, we learned about how the difference in delta E is connected to delta G.

So, we learned about this transducer all those things. So, we have gotten the basic bioenergetic foundations required and now we also saw the energy release is in incremental form. It is not that you explode a bomb to get a little bit energy that you need to charge you

mobile phone. So, you get it in increments and that is where we have this intermediate electron carriers coupled to transducers.

So, we have not gone into the transducer concept very seriously, so we will get it to it at some point. So now we have learnt about this intermediate steps. So, having equipped with all this fundamental knowledge and we also learned about the type of biochemical reactions, remember oxidation-reduction reaction, carbon-carbon bond formation and breakage. There the importance of carbonyl carbons, partial positive charge on the carbon.

Partial negative charge on the oxygen and how that would influence the charge on an adjacent carbon becoming carbanion. Then we saw the internal rearrangement isomerization and so on. So, with all these knowledges, now we will get into an actual biochemical pathway. So that biochemical pathway, if you want to learn one biochemical pathway in your life and that biochemical pathway is glycolysis.

So, the word tells you it is lysing a carbohydrate molecule, right. Glyco we know is a common generic thing and gluco means it refers to one monosaccharide that is glucose. If you want to name a general carbohydrate thing you use the word glyco. So here lysis means breaking down. So it is splitting some carbohydrate that is what the name is. So here actually we split glucose, it is actually gluco lysis that is what happens.

So, this is the best understood of all biochemical pathways. So, when we talk about best understood we do not mean just the sequence of the reactions or the structures of the intermediates, in addition we also mean the nature of the enzymes, the mechanism of the catalysis, reversibility or irreversibility of the reaction thermodynamic aspects of that reaction, regulation of this reaction and how the intermediates are connected to other biochemical pathways.

In all that aspects, in its entirety glycolysis is the best understood biochemical pathway. And second this is a central pathway in the metabolism in all organisms. So, it is highly conserved in that sense. And also the mechanisms involved in the interconversions that happen here they are also conserved all the way from bacteria to human. So, in summary this occupies the central thing in terms of evolutionary conservation of the mechanisms.

Importance to the organism and that importance being similarly important in all organisms, in all those respects this is a central pathway. So now briefly let us look at the major things that can happen to glucose. So, glucose may be joined with the glucose-glucose multiple of them and make starch, glycogen or convert isomers into fructose and you have glucose fructose disaccharide that is sucrose.

This is what sugarcane does and stores in the sugarcane sap in its stem, starch in all the grains, glycogen in our liver. So, this is one thing that can happen to glucose that comes in the diet are produced by photosynthesis. So, photosynthesis end result is producing glucose. And what else can happen to glucose? It can be oxidized to pyruvic acid via glycolysis and this pyruvic acid is partially oxidized product from glucose.

It can be fully oxidized to carbon dioxide through another cycle that we will learn after glycolysis. And the third thing it can provide the five carbon sugar the ribose required for nucleic acid biosynthesis because nucleic acid backbone is ribose. So, all these things come from glucose. So it can be storage of energy, primary source of energy and it can also provide the raw material or precursors for other important macromolecules in the cell. So this is the metabolic fate of glucose.

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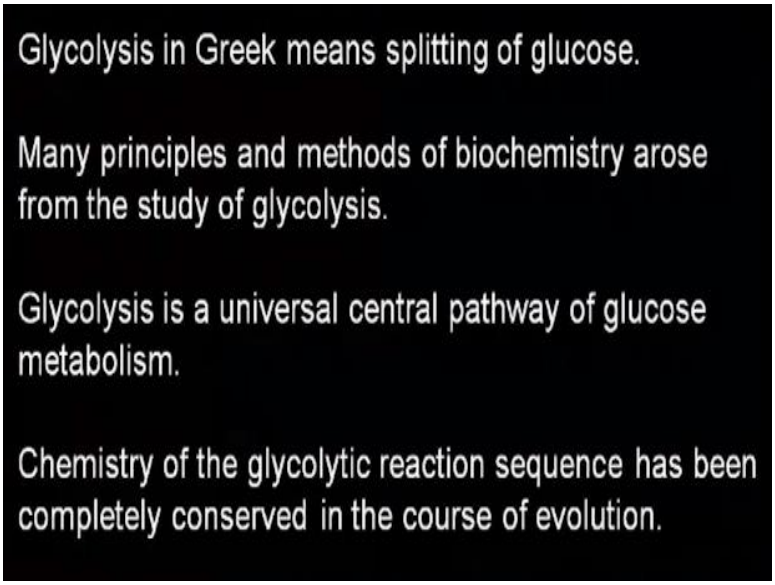

So, this I just told you glycolysis means splitting off glucose. So, this again I told the major principles and methods of biochemistry arose from the study of glycolysis. Through the process of trying to understand glycolysis when people discovered a lot about enzymes,

enzyme kinetics, properties of enzymes, their active site and how substrate binds to enzyme, reaction mechanism, all those.

Many principles of biochemistry came from research on glycolysis. And this again I told you it is a universal central pathway of glucose and this is true in multiple organisms and that is why we call it as universal. And the mechanisms of this conversion, the chemistry of glycolysis is again completely conserved. The way glucose becomes glucose 6-phosphate, the first reaction in E. coli is the way it happens in our body too. So, it has been totally conserved for about 3 billion years.

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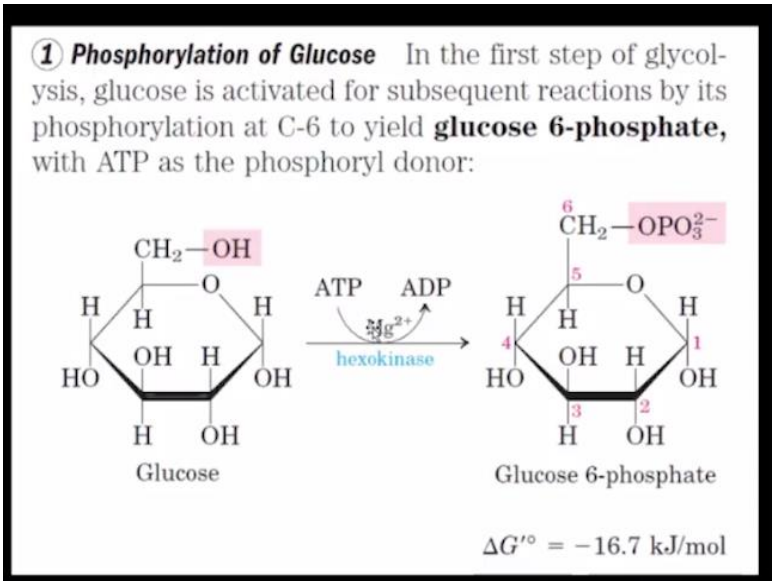

Alright so with that introduction to its importance, let us start looking at the individual steps. So, the first step is energizing or activating glucose for further reactions. So that is getting rid of this poor leaving group and adding this good leaving group this is phosphoryl group transfer. Remember one of the third variety of the reactions that we learned is group transfer and in that we learnt about phosphoryl group transfer.

And there we did have this very example like carbohydrates get phosphorylated and they are the energized version that participates in reactions. For example to make glycogen or starch you do not add glucose-glucose that thing actually comes from a phosphorylated version of glucose and this is catalyzed by hexokinase, a highly conserved enzyme and there are the enzyme family, there is another one called glucokinase.

So, we do not get into those details, if we understand that enzyme that phosphorylates a hexose like glucose, we call it as hexokinase and the phosphoryl group transfer is from ATP. So, if you remember how ATP is used it is not ATP is hydrolyzed to ADP plus phosphate instead the phosphate group may be temporarily transferred to the enzyme and then ADP is released and from the enzyme the phosphate group may be transferred to glucose in this case.

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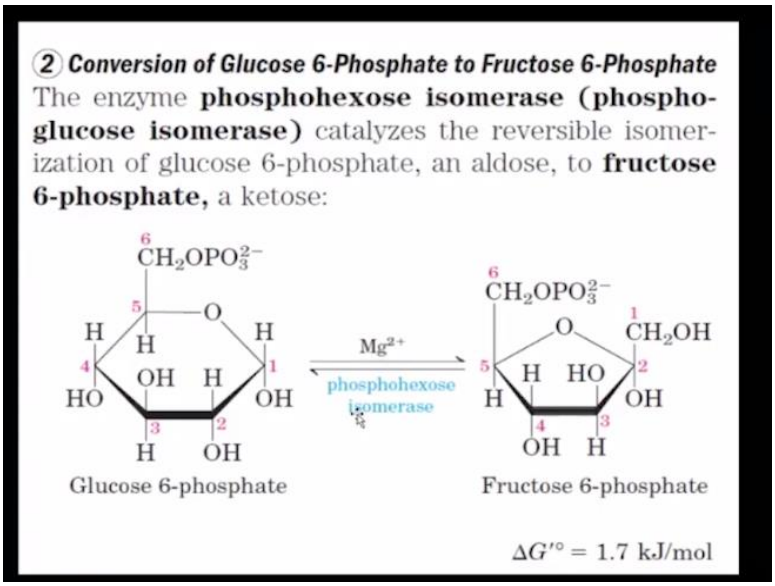

And one important thing we want to consider here is this ATP and magnesium, so they usually exist together as a coordinated structure shown here. So, this divalent cation takes care of charges of two acid groups of adjacent phosphates. So, this is actually magnesium ATP 2– charges, so this and this or whichever like it could be coordinated between these two and these two may be free or this may be here.

So, this is what is magnesium ATP. So, ATP when it is actually existing in our cell it exists in this form and this true with magnesium ADP as well and this is how they are participating in this reaction. So, this is the first step glucose 6-phosphate formation from glucose and the next step is something that we have learnt about its importance that is the importance of carbonyl carbon.

So that is the context in which we learned this, why glucose 6-phosphate has to become fructose 6-phosphate. So, this is an isomerization because glucose and fructose are isomers so that isomerization. So, this is internal rearrangement of bonds primarily switching a double bond from first carbon to the second requirement so that is what it is happening. So, this in linearized form as we will see in the next picture.

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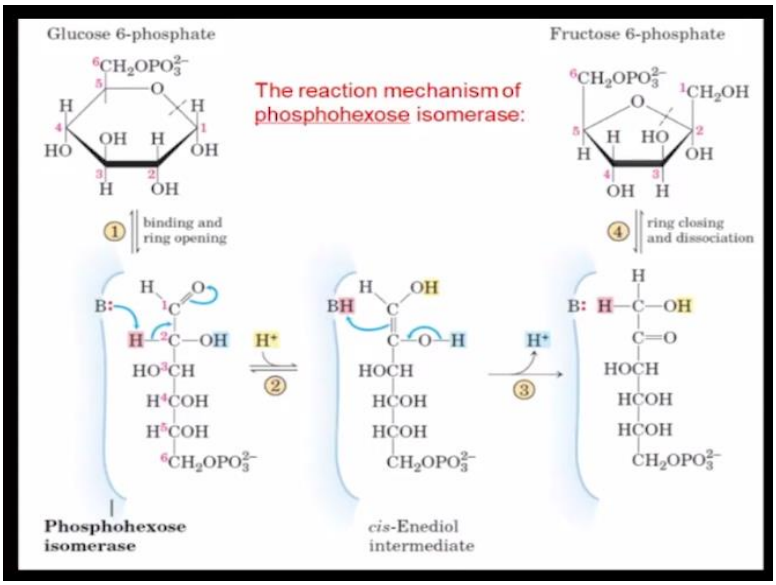

So, this is the linearized version of glucose. So, when it binds to the active site of the enzyme phosphohexose isomerase, so this shaded blue is the enzymes active site. So this is like the active site, this is like the other parts of the enzyme. So, in the active site first the ring structure binds and it readily opens because ring structure is always in equilibrium with this linear structure and this double bond from carbon 1 switches to carbon 2.

So, it is internal rearrangement of double bond that is what happens in this reaction. So now let us look at how this reaction happens in the active site. So, remember we have learnt in enzyme catalysis in terms of the mechanisms of enzyme catalysis of course the general thing applicable to all enzymes we learnt is the binding energy the multiple non-coherent interactions that happen between enzyme active site and substrate provides significant part of the energy required to reduce the activation energy, so that is one thing.

Second, we learned that in the enzyme active site we may have proton donors and proton acceptors and that kind of a thing we call as general acid-base catalysis and sometimes that will stabilize and charged intermediate by providing proton or accepting proton. So that example we see here that reaction mechanism example. So, there we just learnt as one of the possible reaction mechanisms, here we see that as an example.

That time also we had a part of these molecules and this we have already sort of done there in that example, so here we see the actual reaction. So here you have a base that can temporarily accept this proton and that allows a double bond switching to here and therefore this will

become hydroxyl. So that would be enediol intermediate. See this is an ene, alkene and you have two alcohol groups therefore it is an enediol intermediates.

It is cis because both are on the same side of this double bond, cis-trans isomerism coming here. Once that enediol intermediate forms, so the required proton comes from the solvent so mostly it is all aqueous medium, so proton is not an issue. And then this transfer of protons and back this accepting, sorry donating the proton and accepting electron like the reverse of what happened here ends up making the double bond here and this becomes single.

So essentially the double bond in the first carbon now has become the second one. So why this rearrangement? Now you see this carbon adjacent to this carbonyl carbon will be a carbanion. It will have a tendency readily to lose hydrogen attached to it and that enables the cleavage here into two trioses three carbon carbons that is what is going to happen when we actually have that lysis to justify the name of this pathway.

The pathway name is glycolysis and the lysis actually happen between the third and fourth carbon and that is facilitated by the formation of this double bond here. This is the main reason why glucose becomes fructose. The actual thing version of glucose is glucose 6-phosphate ends up becoming fructose 6-phosphate and now ring closure and dissociation from the active site. So, this is the reaction mechanism of phosphohexose isomerase.

So, we will go through at least four different reaction mechanisms here and you need to read, understand and remember these reaction mechanisms, one of them will come in the exam. So, therefore glycolysis reaction mechanism you cannot say you will not be able to study. I know you are capable of learning lot more than this because you have already done that and there is evidence that you can really do this.

So, the primary objective is going from carbonyl group from carbon 1 to carbon 2 that enables cleavage between three and four, so that is the main thing that you need to remember here.

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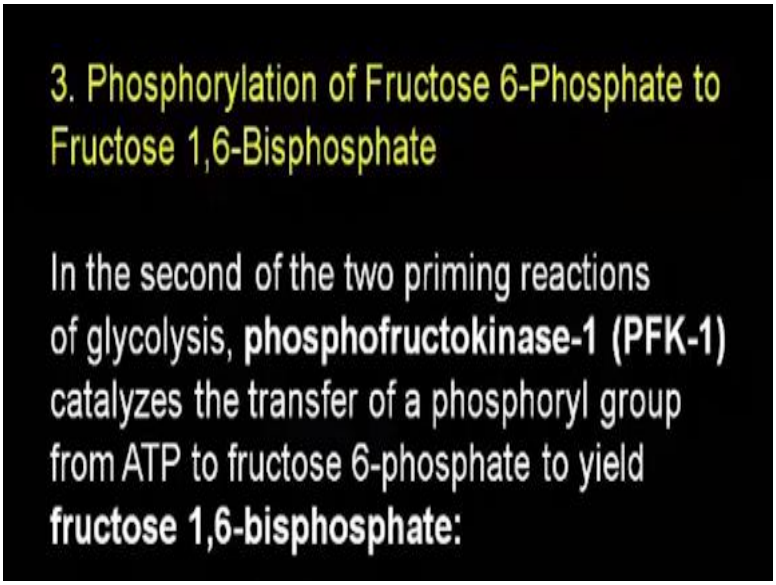

So third one is another priming state, on more phosphate gets added that is fructose 6-phosphate becomes fructose 1, 6-bisphosphate meaning the second phosphate is not added to the first phosphate, instead it is added to another hydroxyl group on a different carbon of fructose and this is catalyzed by phosphofructokinase, kinase means something that transfers phosphoryl group and enzyme the transfers phosphoryl group.

Once again, the donor is ATP. So here actually we are consuming energy, twice we have taken ATP. We have not yet started realizing the benefit of oxidation here. This is like adding you need to release energy from the firewood but to light up the firewood you need to provide light, like your matchbox and that is what we are doing here.

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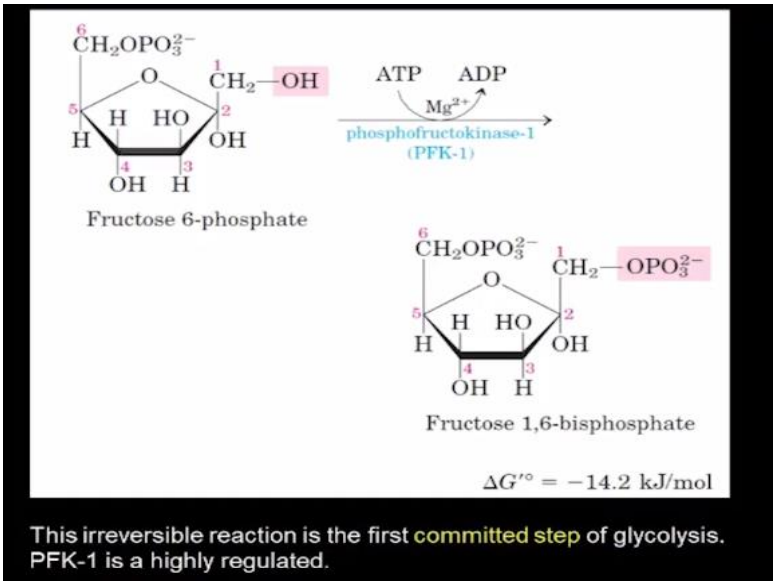

So, phosphofructokinase phosphorylates the first carbon and you get this fructose one six 1, 6-bisphosphate. So, this reaction is irreversible, it goes only in one direction. Of course, when it participates ATP your magnesium is always involved. So now you understand the importance of metal ions in food. These are important for reactions in the body and the product the fructose 1, 6-bisphosphate can only participate in glycolysis.

It does not get into any other metabolic pathway. So therefore, there is no point in producing this molecule if it is not for the continued progression through glycolysis. If glycolysis is not required, then this enzyme is subject to serious regulation, it will be inhibited so that you do not make this molecule because this can only participate in glycolysis, rather this phosphorylation commits fructose 6-phosphate to glycolysis.

Otherwise, this can go into many other things. By undergoing this phosphorylation, it is committing to glycolysis that is why it is called a committed step. So, this committed step is not synonymous with the rate limiting step. Rate limiting step meaning in a series of reactions the individual reaction whose rate is slowest compared to the other individual reactions. So, therefore the overall pathways speed depends on that one particular reaction and they are the rate limiting steps.

Sometimes, the committed step may be the rate limiting step as well, but these need not be always connected. They are independent and due to this commitment, this enzyme subject to serious regulation. So, we will learn about the enzyme regulation after learning glycolysis.

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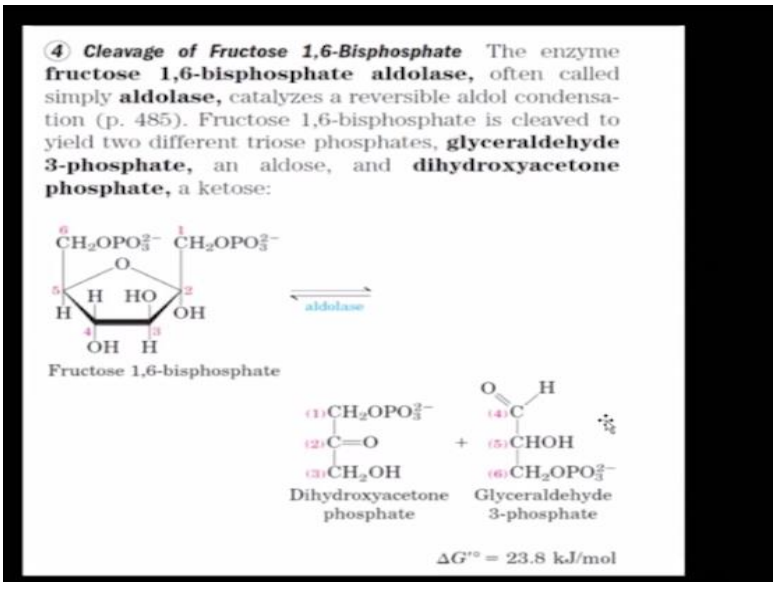

So next the central reaction of glycolysis which is splitting the hexose on the fructose 1, 6-bisphosphate now gets cleaved into that is between the third carbon and fourth carbon a cleave and in its linear structure you have ketone if we go back, so you have the ketone and therefore it is going to become dihydroxyacetone phosphate. Due to the presence of ketone, it is going to be an acetone.

Because this is an acetone structure in which you have two hydroxyl groups and one hydroxyl group is in ester linkage with phosphate. So, therefore it is called dihydroxyacetone phosphate or very affectionately DHAP that is what is formed. And the other one the bottom part of the molecule 4, 5, 6 will be aldehyde. So, this is catalyzed by aldolase.

So here remember the second type of generalized reactions that we learnt is carbon-carbon bond formation or break of the carbon-carbon bond. And here you see an example of carbon-carbon bond and we learned that carbonyl groups help in this bond formation or bond breakage. So that is where we learned aldol condensation as well as glycine condensation, where it is in an ester form it is glycine, otherwise they both are similar.

So, this is a reverse of an aldol condensation. These two joining together to make fructose is the aldol condensation and that is why this enzyme is called aldolase. And this reaction mechanism obviously important and we need to learn and that is described in the next slide.

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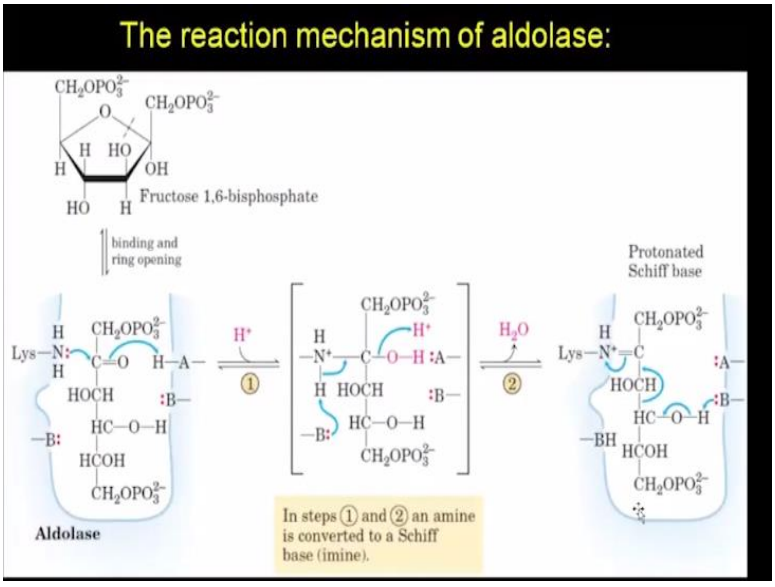

So again, ring binding and ring opening and the central thing here in this reaction mechanism, in the previous one we saw enediol intermediate formation and its stabilization based on base

catalysis how it is helping that. Here it is Schiff base formation between the amino group of lysine side chain, you know lysine side chain is CH 2 CH 2 CH 2 CH 2 NH 2 and that amino group participates in this.

So, this is a nucleophile and this is positively charged one and this nucleophilic attack leads to the Schiff base formation. So, this is an imine version of the Schiff base and once this forms this charge is taken care by a base catalysis here. So, this you know readily accepts an electron or you can say this proton is abstracted and therefore it is justifying base catalysis and that leads to a double bond here, a proper Schiff base.

Then you look this side, here you have an acid as well as base groups available for acid and base catalysis with this direction of electron flow. Again, an abstraction of proton leading to a double bond here and that leads to a cleavage because here if it is going to be double bond then carbon cannot have this and that is how this cleavage is enabled. And this will therefore end up being CHO. So that is glyceraldehyde phosphate and that is going to be released in the next one.

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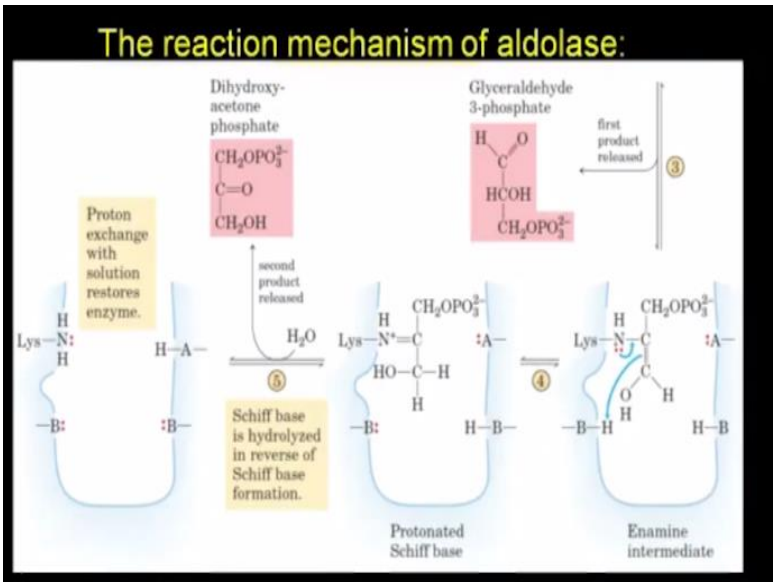

The actual bond cleavage and the first product release will be the step here. So the glyceraldehyde phosphate is released and this is temporarily an enamine intermediate is attached to the active site. Now the donation of this proton the base group has now acts as an acid and that leads to this hydroxyl group and switching the double bond now to this. Actually, the reverse of what we saw of the reaction 1 and 2.

And then you have the hydrolysis of that leading to release of the dihydroxyacetone phosphate and the enzyme gets restored through solvent-based proton exchange to the original shape. So, this is how the fructose 1, 6-phosphate gets cleaved into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. This is a very important reaction mechanism. So you are not going to miss this.

Take the required amount of time, go through it multiple times, if needed take an introductory organic chemistry textbook, learn the mechanisms of electron abstraction, proton abstraction, nucleophile attack, electrophile attack, Schiff base all that that are required learn thoroughly and patiently and be clear with the reaction mechanism of aldolase because this is **very** very important in our understanding of glycolysis.

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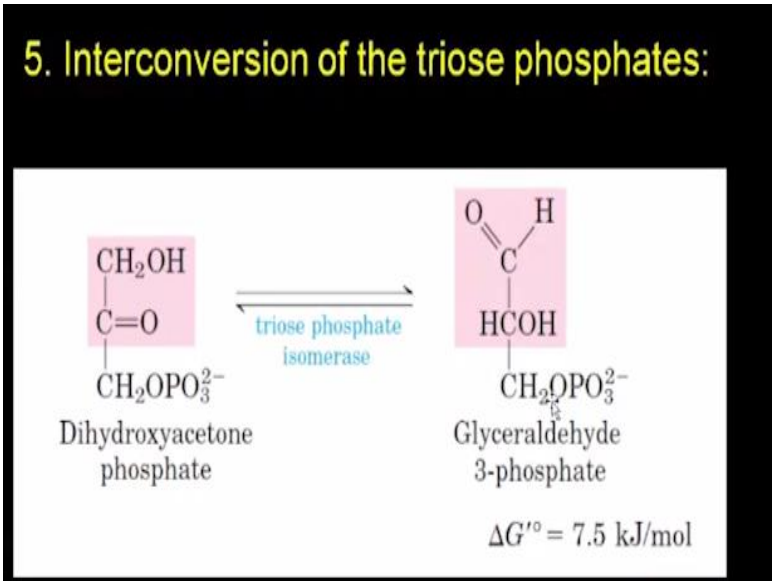

And the next one is essentially this glyceraldehyde 3-phosphate is what is going to participate in the subsequent reactions. So as a result, these three carbon moieties need to be isomerized, the bonds have to be rearranged here, again a double bond switch just like what we saw on in two steps ago. The same thing here the double bond rearrangement carbonyl group from here to this and you get glyceraldehyde 3-phosphate.

So, this is catalyzed by triose phosphate isomerase because both are triose, sorry this is triose and this is keto sugar, you cannot directly call it as triose. So, triose phosphate isomerase catalyzes this interconversion and since the subsequent reactions will siphon out glyceraldehyde 3-phosphate, this dihydroxyacetone phosphate eventually gets converted into glyceraldehyde 3-phosphate. So, this is the fifth step.

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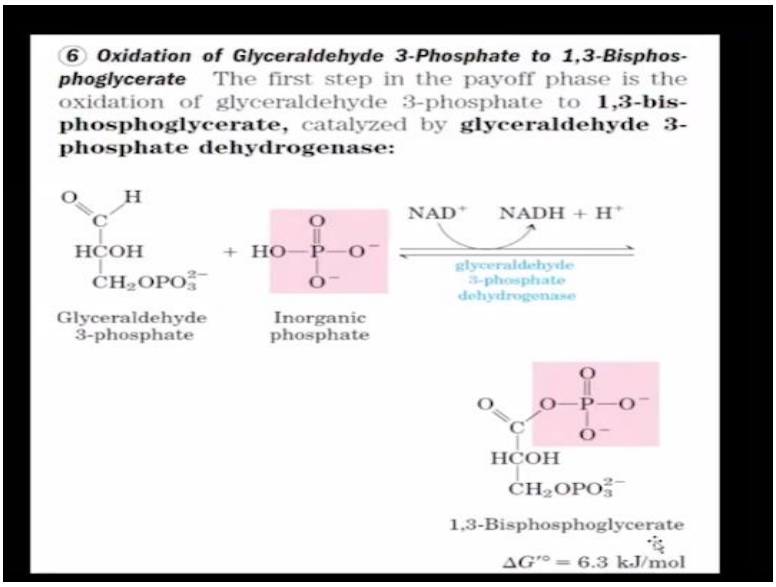

Now you have the energy yielding phase starting. So, till now we have invested two ATP molecules per glucose per hexose, now that is cleaved into two trioses. So now when we talk about glyceraldehyde 3-phosphate per glucose molecule that entered here it should be 2. So therefore, from now onwards everything should be considered 2 molecules as if they are going through.

So for one molecule of glucose this reaction is actually twice that means the means for one molecule of glucose you have two NAD oxidase NAD getting reduced to NADH. So, the electrons are stored in this temporarily. So, the energy is conserved in making this. So roughly one reduced NAD equals 3 ATP. So, you actually have six ATP forming power from a single glucose molecule.

So, if you subtract the 2 ATP invested a initially, so you have got 4 net ATP gain at this dehydrogenation step. Remember oxidation-reduction reactions in biology is often dehydrogenations, meaning hydrogen atoms are lost and usually it is 2 hydrogen atoms and that is exactly what you see in this case. And this aldehyde now gets oxidized to an acid carboxylic acid which is in m anhydride linkage, mixed anhydride linkage with the phosphoric acid that is 1, 3-bisphosphoglycerate.