Organic Chemistry In Biology And Drug Development
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Lecture – 40
Chemistry of Cofactors /Coenzymes (Contd.)

Welcome back. Now, we will describe the chemistry of remaining Vitamin B complex cofactors and will study the reactions in which they participate along with the enzyme.

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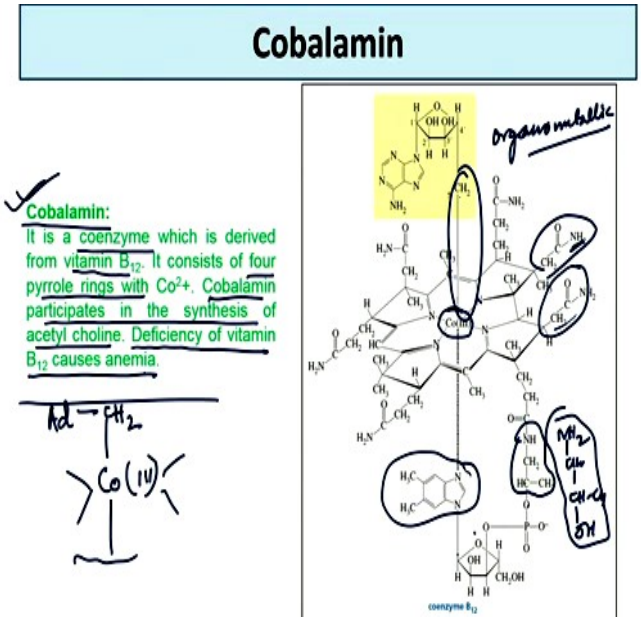

So, what is left now? It is Cobalamin. Cobalamin is the coenzyme of vitamin B_{12}. Now, the structure of this coenzyme cobalamin is shown. 4 Nitrogen atoms of tetra pyrrole type of ring is coordinated to the Cobalt (III) in a square planer fashion. However, cobalt is hexa coordinated.

There is an adenine moiety where the OH of CH_2OH is replaced and attached tothe metal cobalt. This is an organometallic system. There is an organometallic bond between the CH_2 that is the 5 prime CH_2 in adenine. This is attached to the adenine and to the sugar. So, this is adenosine, the CH_2 is attached to the cobalt by a covalent bond.

What is the other side of the hexa coordination side? The 4 sides are occupied by these nitrogens. One is the CH_2 of the adenosine and other is this acetic acid moiety or forming

the amide. There is a methyl here and then other is propionamide. This is your lactate CH_3, CH, OH, no, this is lactate this is your CH 2.

This is NH, CH_2, CH, CH_3 and OH.. This is an amino alcohol. So, the amino alcohol is attached. Here is the amide bond. Here it is attached to the phosphate and then phosphate is interestingly attached to this 3 prime. So, the 2,1 is a N-glycoside, but this is a different type of base not encountered earlier.

This has got an imidazole and the imidazole nitrogen is directly coordinating to the cobalt. So, that is a very complicated type of structure. You have these pyrrole moieties and then occupying this these 4 sides here. Then you have the methylene attached to the cobalt of adenosine and this is a benztriazole, the triazole nitrogen is coordinating to the cobalt. So, that is the structure. But we can just simplify it that Co (III) and adenosine CH_2 is attached to cobalt and we are not concerned about whatever the other things are. Because this will be sufficient in order to explain the reactions.

Now, vitamin B_{12} consists of 4 pyrrole rings it says, they are not really perfect pyrroles because they are substituted. Pyrroles and they are in different oxidation level. Then cobalamin participates in the synthesis of acetylcholine that is very important. Acetylcholine is another neurotransmitter. We talked about some neurotransmitters in some of the sessions of PLP dependent enzymes. Deficiency of vitamin B_{12} causes anemia.

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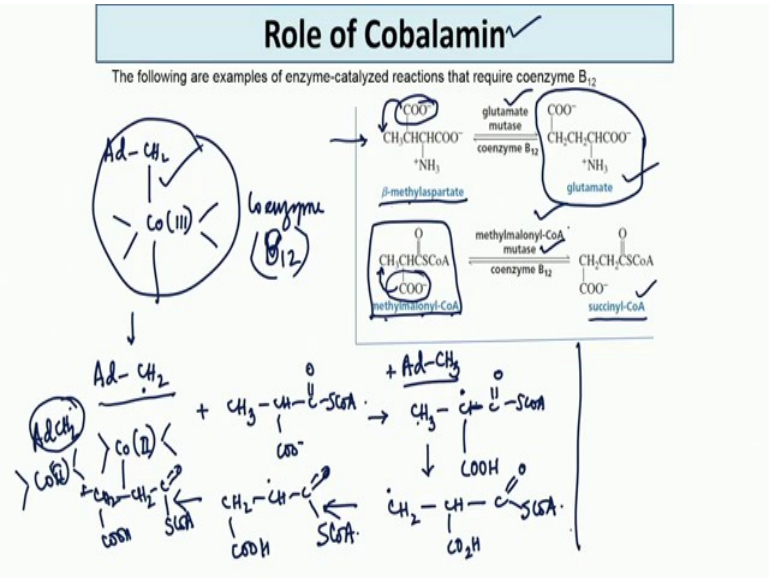

Let us see what type of reaction it catalyzes. I just pointed out two reactions here, one is methylmalonyl-CoA what we have encountered just in the previous session. So, this methylmalonyl-CoA can undergo rearrangement. This participates in reactions which are isomerization reactions.

This carboxy can migrate to the terminal carbon. This has migrated to the terminal carbon and one of the hydrogen migrates here resulting in succinyl CoA. So, you have started methylmalonyl-CoA and this is also mutation reaction because this is change of the constitution of the molecule. So, this reaction is carried out by the enzyme mutase. They are basically isomers. So, through this isomerization you get succinyl CoA because their molecular formula remains the same and only their constitution remains different.

You can have glutamate mutase. What happens here? This is CO_2, this is beta methyl aspartate, you have a methyl here and this is beta methyl aspartate. See if the carboxy is migrating to this carbon and the hydrogen goes to this carbon, then what happens? You have this glutamate. So, beta methyl aspartate can be converted into glutamate. The name of the enzymes are glutamate mutase and this is methylmalonyl-CoA mutase.

Of course, this can be glutamate mutase. You can call it actually beta methanol aspartate mutants because we are doing mutation of these. It is beta methyl aspartate mutase which gives you glutamate. This is methylmalonyl-CoA mutase which gives you succinyl CoA. But the thing is that these reactions are reversible. So for the reverse reactions you can call this as glutamate mutants and you can call this as succinyl CoA mutase. Now, let us see how these mutations are done. What happens here? I told you this is adenine. The simplest way of writing the vitamin B_{12} chemistry is that this is the cobalt and there are other ligands here. This bond will be very reactive and unstable. It can undergo homolytic fission forming the adenine CH_2 radical and obviously the cobalt will now get back its electron that it has donated here. So, cobalt will become cobalt (II). Now, you have CH_3 CHCOO minus and then COSCoA, *i.e.* methylmalonyl-CoA. And then what happens? These first hydrogen should be picked up because that gives a stabilized radical.

Then if you migrate one hydrogen from here you get dot here and then CH. This is CO_2H and you have COSCoA, then the CO_2H moves here and you get CH dot COSCoA. So, this one will become Ad. After hydrogen abstraction it becomes CH dot.

Then this will become CH_2CO_2H and what is called succinyl coenzyme. Adenine becomes Ad CH_2 dot and then it can add to the cobalt which was waiting to be combined again. It forms the vitamin the coenzyme form of vitamin B_{12}.

In summary, this is a very reactive carbon metal bond. It can homolytically dissociates resulting in a radical at the adenosyl part and the cobalt goes to cobalt 2 and then these abstracts hydrogen first here. So it gave a dot and then the hydrogen migrates from here and goes to there. So, CO_2H then migrates resulting in a radical producing here. The CO_2H migrates here again, producing the radical at this position. Then this abstracts the hydrogen from the adenosine $CH_3$and that can combine with the cobalt (III) complex to make the coenzyme or vitamin B_{12}. So, that is the mechanism.

In case of the other possible mechanism, this initially abstracts hydrogen from here, CO_2H migrates there and it forms the radical here and then that abstracts the hydrogen from the Ad CH_3.

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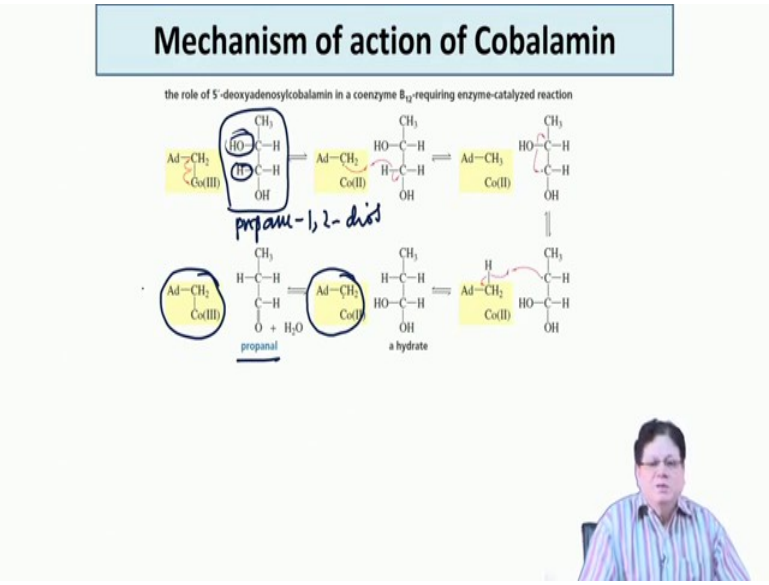

Here other reactions mechanisms are shown here. One reaction is that if you take this propane-1,2- diol, it has been found the ultimate product is propionaldehyde. So, propane-1,2-diolgoes to propanal. That means, to have propanal you have to move this OH to this carbon, so that this carbon bears two Hs and then one water molecule will be lost resulting in the formation of the aldehyde.

So, basically what happens here? It abstracts the hydrogen from here forming a radical at this position, then OH migrates from here to there forming a radical here. Then this abstracts the hydrogen from the methyl of the adenosyl part and then you can again form the coenzyme form of vitamin B_{12} complex. So, this is a unique mechanism.

Up till now we have not encountered any radical type of reaction. This is the cobalamin chemistry or the vitamin B_{12} chemistry. The reactions are done via radical intermediates. People have used this vitamin B_{12} coenzyme form for generating radicals and doing radical reactions.

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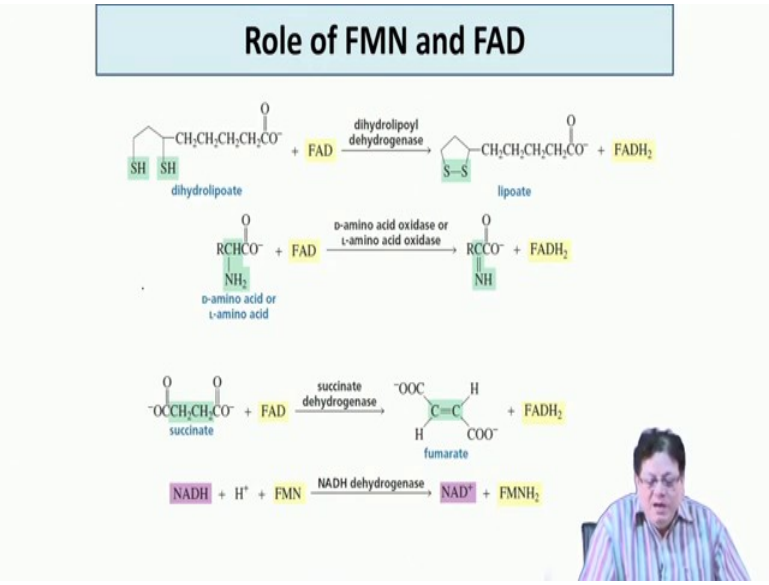

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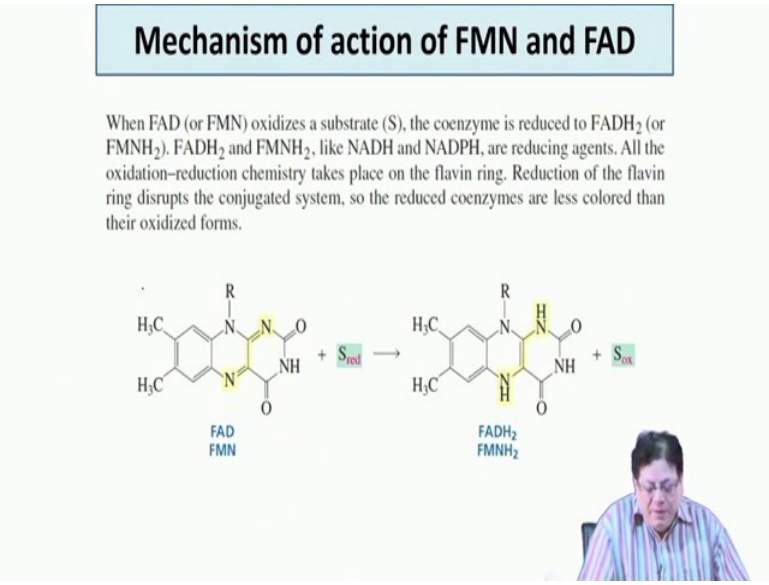

We have already covered FAD. FMN is very similar to what FAD does. FAD is the oxidized form and then FAD goes to always $FADH_2$. Then $FADH_2$ can be converted back to FAD by NAD plus or NADP plus.

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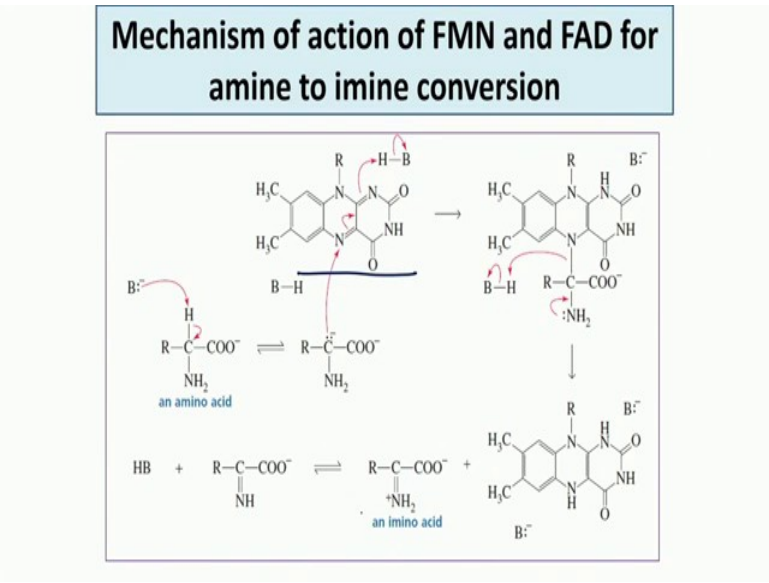

Let us quickly go to the NAD plus and NADP plus.

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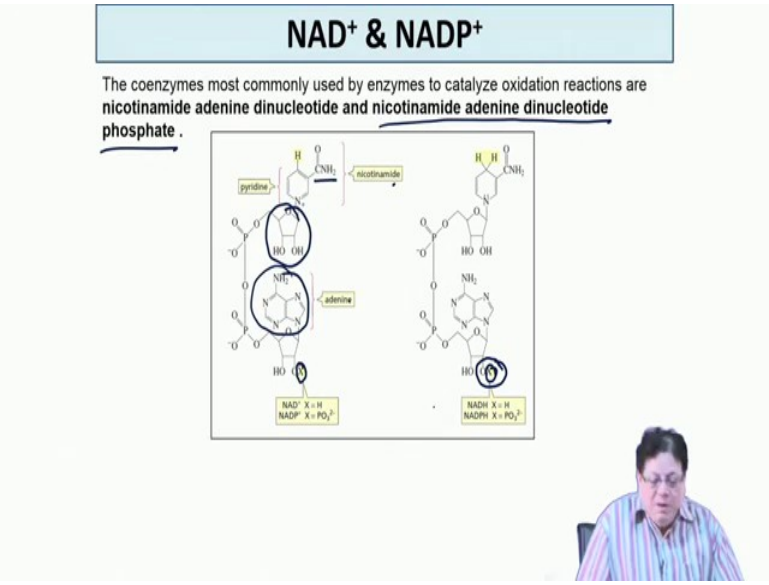

So, what are these? Structure is quite complicated, but the vitamin form is actually nicotinic acid. Nicotinic acid is pyridine-3-carboxylic acid. This is the coenzyme form. So, nicotinic acid is converted to nicotinamide and then this is the ribose, then you have the pyrophosphate and then again you have ribose and then that is attached to adenine. So, this is called Nicotinamide Adenine Dinucleotide, NAD.

Sometimes this x may be a phosphate group and that gives you nicotinamide adenine dinucleotide phosphate. The phosphate is basically including the oxygen. If this is fully the phosphate group then that is called the NADP plus. Some reactions are catalyzed by NAD plus some are NADP plus depending on the enzyme.

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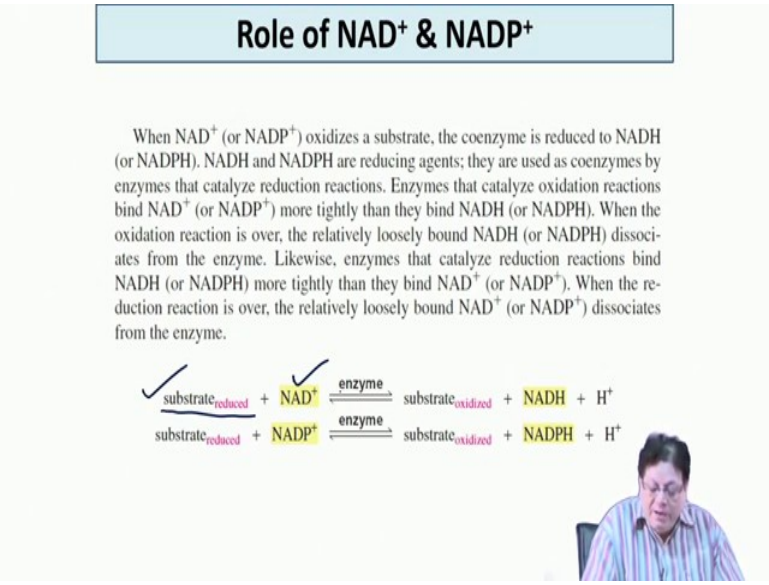

Now, what is the role of NAD plus? If a substrate is reduced like $FADH_2$, then the substrate is oxidized and NAD plus is reduced to NADH. It could be an NADP plus on the substrate. In presence of the enzyme, you get the oxidized substrate and NADPH and proton.

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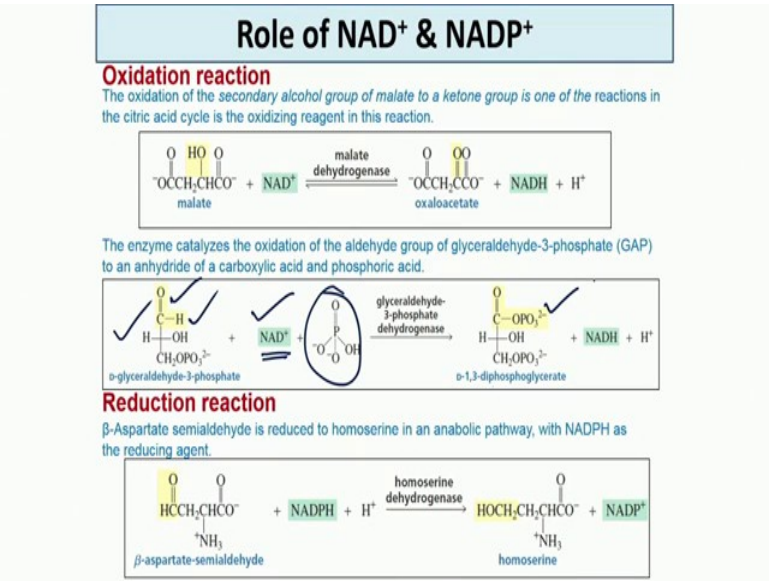

So, there are many reactions that are shown here. There is one important reaction in glycolytic cycle where D-glyceraldehyde-3-phosphate is converted to D-1,3-diphosphoglycerate in presence of NAD plus and a phosphate. So, the aldehyde is

oxidized to the carboxylic acid and that is converted into the phosphate. So, basically you have to oxidize this and oxidizing agent is this NAD plus. An enzyme is required for this. In a test tube, if you take this and add NAD plus and phosphate you can get this, but that will be extremely slow. It is the enzyme which brings all these 3 components together and does the reaction. There are many such examples which are there. The question is how NAD plus works.

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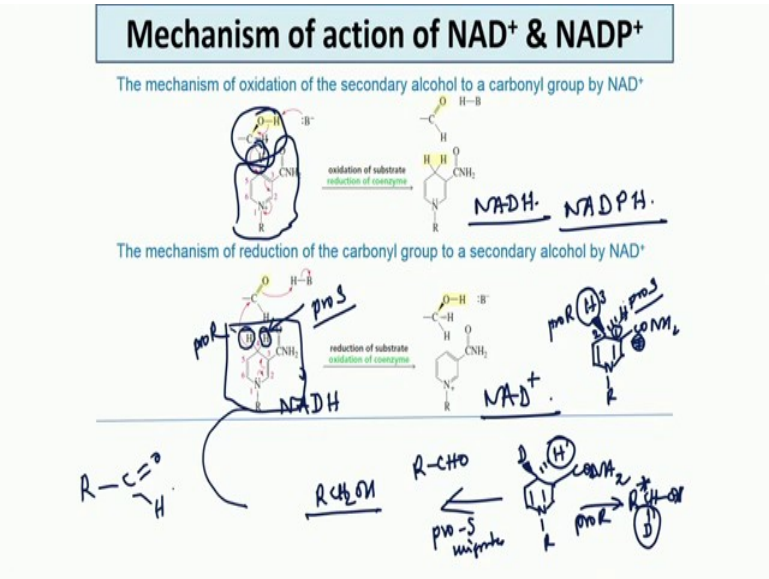

Now, NAD plus is again a complicated structure, but this nicotinamide is the functional part. So, we can write NAD plus always in the abbreviated form R and the nitrogen is positively charged. As nitrogen is positively charged like pyridoxal phosphate it becomes an electron sink and this 4th carbon is very vulnerable because of electron pulling by the nitrogen. So, what happens? If you take an alcohol then the hydrogen can migrate as hydride. It attacks this carbon as a nucleophile and so there are two hydrogens here and in the process this alcohol becomes aldehyde. It is example of alcohol dehydrogenase.

Alcohol dehydrogenase means you get aldehyde from alcohol in presence of NAD plus or NADP plus. Where is the hydrogen gone? This hydrogen from the alcohol part goes to NADH or NADPH. For the reverse reaction, see NAD plus NADH, this is oxidizing agent and other is reducing agent. If you want to oxidize the hydride is added to NAD plus. Reversible reactions are also possible. If you want to reduce aldehyde to an alcohol it is done by another enzyme. Then you take this NADH and then transfer the hydride

from the NADH to the aldehyde and resulting in the formation of the alcohol, and NADH goes to NAD plus.

Now, all these migrations have been demonstrated, that this is the hydrogen from NADH that migrates and attacks the carbinol carbon. If you make a deuterium here and that is transferred to this aromatic ring. These two hydrogens are enantiotopic.

If you have a chiral group here in the form of adenosine these will be diastereotopic. You can assign them as pro R and pro S. So, in one case it is the beta hydrogen and this is the alpha hydrogen.

You give preference in the priority order to this hydrogen over the alpha hydrogen. This is pro R and that is your pro S. If one is pro R the other will be pro S.

So, either pro R hydrogen or pro S hydrogen is going to attack the carbonyl carbon. So, only one hydrogen will be transferred. I have a deuterium here and hydrogen here and I do this NAD, NADH mediated reaction and I have an aldehyde. What is the product if pro S is migrating? The product will be RCH_2OH because this one is the pro S hydrogen. So, this hydrogen will be taken up and you will get RCH_2.

If this is pro S you will get RCHDOH. But remember now, you are making a chiral center so that the deuterium will be delivered in a particular way either from the Re face or from the Si face. There are stereo chemical issues involved in NAD mediated reactions. It is basically the hydrogen that is involved here.

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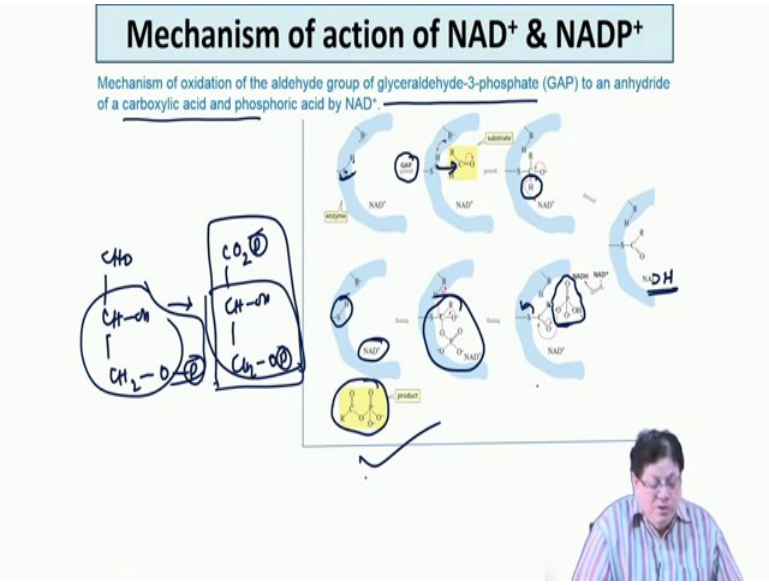

Glyceraldehyde-3-phosphate goes to 1,3-CO_2 phosphate and this is CHOH and this is CH_2OP.

So, what is the possible mechanism? If you can write this as R it will be RCHO initially. There is a cysteine moiety that delivers the hydride. The base takes the hydrogen and this attacks the carbonyl and this becomes O minus. This is the tetrahedral intermediate that comes back. It is very similar to Cannizzaro reaction. This O minus comes back and hydride now is going to NAD plus. So, NAD plus becomes NADH. So, this becomes SCOR, so thioester and this will be NADH.

This is mono phosphate. The mono phosphate now attacks this one and basically it releases. It again breaks the carbon sulfur bond and it becomes the cysteine. The sulfur of cysteine takes the hydrogen from the original base. This is the R CO and phosphate. This is a very important reaction in the glycolytic cycle.

In summary, this is the enzyme which does this oxidative phosphorylation. The glyceraldehydes-3-phosphate binds, then the cysteine attacks as a nucleophile, the base abstracts the hydrogen, the cysteine S attacks the carbonyl forming a tetrahedral intermediate. Then the O minus comes back and in a very similar to Cannizzaro reaction the hydride is transferred to NADH. The phosphate now attacks the carbonyl and breaks the carbon sulphur bond.

So, initially this one will be a NADH, but later it will be oxidized to the NAD plus. After the expansion of the carbon sulfur bond you get 1,3-phosphoglycerate.

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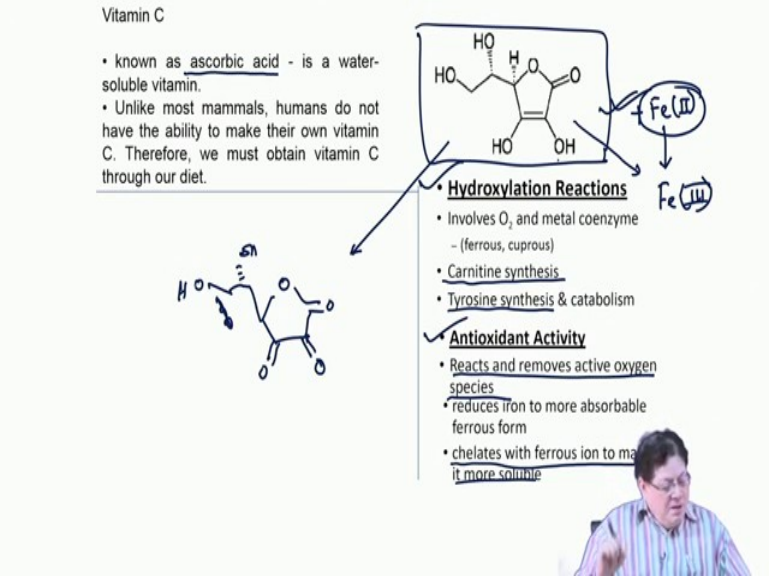

Now, the last one is vitamin C. This is the last coenzymes that we want to discuss. The vitamin B group is now complete. We have discussed vitamin B_{12}, the cyano cobalamin, NAD^+, NADH, $NADP^+$, NADPH and other vitamins like the vitamin B_6, coenzyme like PLP, folic acid, biotin, and thiamine. We have covered most of it. Now, only vitamin C is remaining. Vitamin C is also a very important coenzyme and that is called ascorbic acid.

The structure of ascorbic acid is given here. This is the reduced form of ascorbic acid. Ascorbic acid is a very good antioxidant. Antioxidant scavenges out harmful radicals that are produced inside the body. It all starts with the oxygen going into the peroxo and then into the superoxide.

First it takes one electron and goes to the superoxide, then to the peroxide. That can go to the hydroxyl radical. So, all these radicals are extremely damaging. Vitamin C can take care of these radicals. Vitamin E is also an antioxidant, vitamin A is also an antioxidant, but vitamin C is extremely powerful antioxidant.

However, we are discussing coenzyme activity. So, we will see that it is involved in what type of reactions. It helps reactions which does hydroxylation and it also has antioxidant

activity. What are these hydroxylation reactions? The muscle building compound called carnitine and also tyrosine is formed from phenylalanine because you need one hydroxyl group at the para position of the aromatic ring. Another example of hydroxylation reaction is formation of neurotransmitters like epinephrine.

We will talk about that epinephrine, noradrenaline adrenaline all those things. Antioxidant reacts and removes the reactive oxygen species like peroxide, superoxide and hydroxide radical.

Another important thing is that it can chelate to ferrous ion to make it more soluble. We need iron to produce our red blood cells and it helps to chelate with the ferrous and then form the ferrous more soluble in the aqueous medium.

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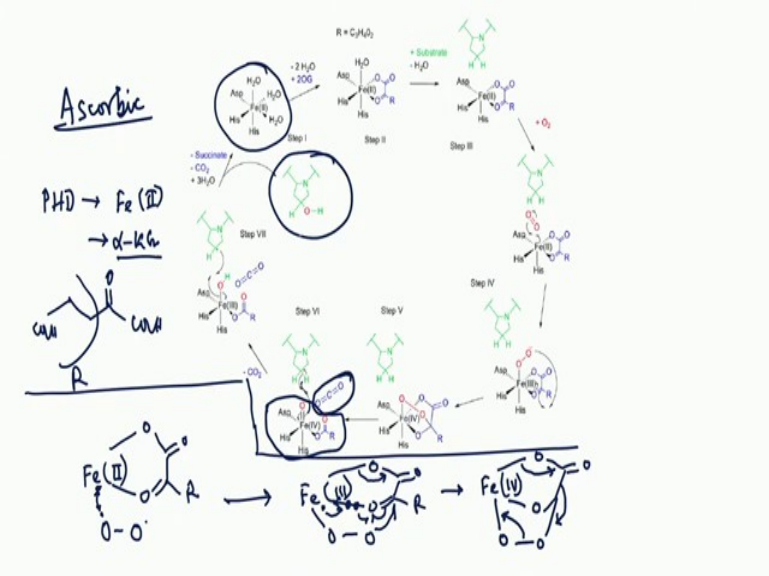

Now, we are going to talk about this reaction. Our muscles are made up of proteins which are called collagens and collagens are present as triple helix. So, that triple helix means it gives lot of strength.

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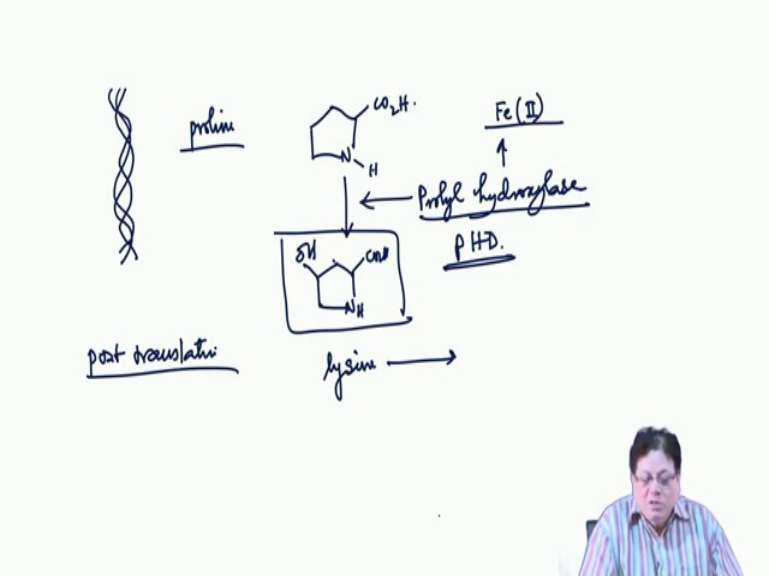

Now, we know that the helix are basically formed due to hydrogen bond between the backbone of carbonyl and the nitrogen NH of the amide. They form hydrogen bonds and give stability to the helix. So, in order to form the triple helix it has been found that the proline is the only secondary amino acid that has to be converted into 4-hydroxyproline. So, there should be a hydroxy group. So, this hydroxylation requires an enzyme called proline hydroxylase. This is also abbreviated as PHD.

This is very important hydroxylation because this 4-hydroxyproline is present in collagen. This hydroxyl groups gives extra stability by forming other type of hydrogen bond. That gives us the strength.

Similarly, there is the lysine moiety that also can undergo hydroxylation. But it will be a lysine hydroxylase. Now for the first time we will introduce a metal ion to catalyze a reaction, i.e the metal ion as a cofactor. We know that different metal ions like zinc, iron, cobalt are very important in case some of the enzymatic reactions. Now, we are encountering the iron, the ferrous as a cofactor. What is the role of ascorbic acid here?

Prolyl hydroxylase does post translational modification. Post translational modification means when the polypeptide is made it will contain all this protein amino acids, here the number is 20. So, it will initially contain only proline. Once the peptide comes out from the ribosome then this enzyme prolyl hydroxylase does post translational modification by which the proline moieties are converted into hydroxyproline. This hydroxyproline give

you extra stability. This hydroxylation is very important because that helps the polypeptide to form triple helix.

What is the mechanism? Prolyl hydroxylase requires the ferrous oxidation state. I told you that Vitamin C is the reduced form of ascorbic acid. Now, ferrous ion has a very strong tendency to go into the ferric in presence of oxygen and this hydroxylation is carried out by oxygen.

Both of these are the carbonyl. So, that is the oxidized form of ascorbic acid. So, ascorbic acid's duty is to keep the iron in the +2 oxidation state.

We are discussing the mechanism of this hydroxylation of proline to form 4-hydroxyproline. The ascorbic acid is acting as an antioxidant. Its role is to keep the iron in the +2 state. Now, the question is how this hydroxylation is carried out by this prolyl hydroxylase or PHD. In addition to ferrous, this PHD enzyme requires another co-substrate which is alpha ketoglutarate or 2-oxoglutarate. What is 2-oxoglutarate? In presence of the oxygen, it helps the iron to form a very super oxidant which is called a ferryl oxo species where the iron is in the +4 state. Now, how it is formed? This is the resting state of the enzyme and the cofactor iron. So, it has got 3 water molecules, an aspartate, 2 histidine as ligand.

It is alpha ketoglutarate which is basically CH_2CO_2H. This part you can write as R. This becomes RCO_2H. So, this acts as a bidentate ligand to expel the two water molecules and itself chelates to the iron. But there is no change in oxidation level of iron here because both the bonds are formed by electrons coming from this oxygen. So, this is the intermediate.

Now, oxygen comes and iron reacts with the oxygen. This is the textbook mechanism, but there are still certain drawbacks because the oxygen will be in the triplet state that is the natural oxygen. So, what will happen? Iron was in the +2 oxidation level and you have this oxygen that is CO which is coordinating to the iron. So, this is the situation. Oxygen is in the triplet state. So, what will happen? Now, iron will donate one electron here and form a covalent bond between this and that. This forms a covalent bond and resulting the iron will be in the +3 oxidation level and this becomes a peroxo radical. You have iron that chelation is still there. This is the bidentate ligand, so double bond OR and this was connected to the iron.

Now, what will happen? This attacks here and one of the electron comes here. Remember this bond was formed from two electrons which are coming from the oxygen. One of the electron will come back here. It will be a lone pair and this bond intact to the iron will donate one electron to form the covalent bond here.

So that means, you get Fe in the +4 oxidation state and earlier it was +2. Then one electron was given to form this iron oxygen bond and one electron is given to make up for the loss of one electron in this coordinate bond. So, ferryl is the Fe^{4+}, then you have oxygen and that is attached to the iron. Now, there will be release of carbon dioxide. So, this ferryl-oxo is formed resulting in the formation of this ferryl-oxo. What comes out is carbon dioxide.

Now, the super oxidant ferryl-oxo abstracts a hydrogen from here and these becomes a radical, and this radical then adds the oxygen and one of the electron comes back to the iron. So, you get 4-hydroxyl-proline and the iron is brought back to the original state. This reaction is oxygen dependent and it is also dependent on alpha ketoglutarate. But in presence of oxygen, the iron has a great tendency to be converted to the Fe (III). If there is formation of any Fe (III) that will immediately be brought to the Fe (II) level by ascorbic acid and to maintain this catalytic cycle, ascorbic acid itself will be converted into the dehydroascorbic acid.

These metal mediated reactions are also very important in the medicinal chemistry aspect. Many drugs actually coordinate to the metal iron that is absolutely required as a cofactor in many enzymatic reactions which are target for drug discovery program. So, that takes care of our first part of this course ‘the organic chemistry and biology’.

So, we have discussed the amino acids- the building blocks of life, carbohydrates, lipids, nucleic protein enzymes, protein, nucleic acids, the modern the recombinant technology, the molecular biology tools. Finally, we have discussed the coenzyme chemistry which is an application of organic chemistry in understanding the reaction mechanism.

So, from next session onwards we will switch on to the medicinal aspects. Now, you have a very good platform on which the medicinal chemistry can be taught because that requires organic chemistry background as well as the biochemistry that are involved and that are required to be known in order to venture into any medicinal chemistry or drug development topic.