Biological Inorganic Chemistry
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Lecture 47
Catalysis by manganese and cobalt ions

Hello, dear students. So, good morning everybody. So, today we just partly will finish the manganese part and then we will move quickly to the cobalt ions, which are very much useful in different biological catalysis reactions.

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So, what are the things we will see today are basically focused on the manganese ions what we have seen so far, particularly for the photosystem II that is very much oxidizing and so that is why we have a oxidizing center for water oxidation. So, today we will take one more example where we have the non-redox functions.

So, how versatile is the manganese ion that is the most important thing to understand that if you just switch off the electron transfer process, what it can show. That we already know that if you can switch off that things that means you substitute some redox active metal ion by a redox inactive metal ions say zinc what we know that what is the corresponding function so that sort of function will get over here.

Then how biologically we produce at some place we know how ammonia we are getting assimilated in our system. Ammonia is the product from a degradation of the amino acids and the proteins and also the NO the nitric oxide. So, what are the most fundamental catalytic action that we try to understand. And then regarding the cobalt, we will see about the cobalt ion in the system which is very much similar to that of your porphyrin system is the corrin system.

So, the cobalt ion corrin based enzymes we will see and what are the different cobalamin cofactors today we just introduced. And in our next class, we will try to consider all these things, how the cobalt centers will be useful for all these cases.

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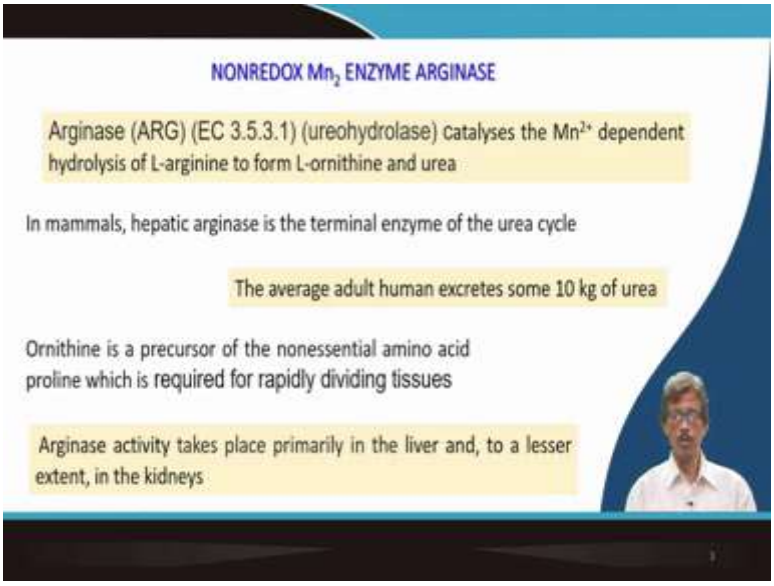

So, we take the magnesium ion and we expect that manganese ions will not show any kind of electron transfer reaction that means the redox activity and then also we have seen earlier that you can have the corresponding superoxide dismutase, the manganese SOD molecules. There we have seen that manganese can subtle between two different oxidation states and it can show the corresponding catalytic activity and which is redox in origin.

Now, we see not the single manganese, but we require the help of double manage and the Mn2 enzyme which is your arginase as we all know now that how it can be considered in terms of its corresponding that nomenclature, the EC number, the enzyme commission number 3.5.3.1 and which is nothing but a ureohydrolase molecule, enzyme. What it can show that it can go for

hydrolysis reaction and the one of the component of that hydrolyzed form is the urea. So, area will be produced.

So, arginase ARG with the number, with the property and is basically a manganese 2 dependent hydrolysis reaction of the substrate L-arginine. So, what is the L-arginine, how it can be converted if you take out that particular part which is responsible for the terminal end where from we can produce the area molecule giving rise to you the L-ornithine molecule.

So, you must be knowing the structure. Until and unless you know the structure nicely then you cannot find out where the manganese center is responsible, how the manual is center is responsible for the cleavage reaction or the cutting of the some part which is producing your urea molecule. So, in our human system, in the hepatic arginase is the terminal enzyme in the urea cycle. We know the urea cycle.

We are producing, our bodies also producing huge amount of urea. And basically we produce throughout the year the average human adult excretes some 10 kg of urea in a year. Throughout the year we basically produce. So, every day we produce the urea in our, from our body. Then how this thing can be related? So, you have the amine acid arginase.

There are proteins or the peptides where we can have the arginase residues that we will also see how we can chemically test for that. Then the product is your ornithine and it is so substrate specific that you can have only go for the L form. L form of arginine can be utilized to give you again that L form of the ornithine. So, is basically a precursor, the product is a non-essential amino acid, proline, so which is required for rapidly dividing tissues and other, the tissue formation and all these things is also a non-essential amino acid, but we require that.

So, if we are not getting through food, what will you do? We try to get it from some other mechanisms, so that is why the arginase is available, the manganese centers of arginine will basically cut one part of the L-arginine such that urea can be removed and you will end up with the L-ornithine. Then this L-ornithine can also be utilized nicely for the production of your non-essential amino acid L-proline. So, you get the all these things and the conversion from one amino acid form to the other amine acid form is very important.

So, where this activity is the highest one or the maximum one and is basically primarily taking place in our liver and to a lesser extent in the kidneys. So, these basically our vital organs, we know liver is our vital organ and is the largest or the biggest organ in our body. So, that can take care of all these things.

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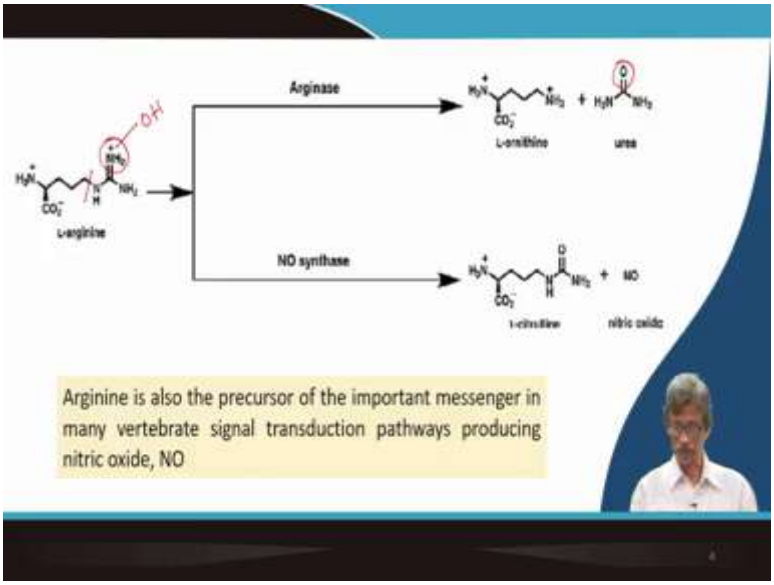

So, you just try to know now the corresponding structured, the chemical structure of L-arginine is definitely amino acid and the zwitterionic form we start from the left where you get this is the form that the zwitterionic form what you get from the left. The zwitterionic form what you get from there is your NH3 plus and CO2 minus. Then we all know that you can have the substitutions from there.

We know what is glycine, but if the substitution is a very big one and with a spacer of 1, 2, 3 carbons centers and then some group which we know that group is well known is the guanidine function. So, at the end basically, so the dangling part or the pendant part you have a long chain of three carbons center, most of the time it is only one carbon center for histidine, cysteine and other amino acid residues.

But if you can have a three carbon spacer to attach the dangling group or the pendant group which is your guanidine, it can have something to do with all these things. It will not be utilized for the metal ion coordination. So, initially what you can think that immediately you cannot have

the very strong coordination for the metal ion, but during the cleavage part, say from the top you apply the arginase, the enzyme arginase is working on this L-arginine.

So, what do you see from the conversion, from the hydrolytic reaction we all know when we are working on amide we know what are the products. We have seen for the, your carboxypeptidase activity also and when you have the ester, what is the hydrolyzed product that we know. So, when you have the arginase, you know that arginine will have the corresponding guanine residue at the end and that guanine residue after hydrolysis will produce the urea molecule.

So, looking at the structure also you try to understand how the urea will be forming. So, you have to cut one CN bond. So, what is that bond basically that you get from there. So, you have to cut this particular bond what do you get, this particular bond you have to cut, and the remaining part you have to take. It is not that particular part also. You have to take out this particular part, group also as oxygen. So, your urea is your oxygen.

So, most of the time, if you look at the system, that will immediately tell you what reaction it can go, follow. So, initially people what identified? People identified the presence of L-arginine in protein molecules or the peptide molecules then the formation of the urea. So, you must know the chemical tests, the useful chemical tests what you can have which can detect the formation of urea in solution also.

The way we know the formation of ammonia in some medium, we can find out if ammonia is forming from any particular reaction, we can detect that particular presence of ammonia. So, what is remaining over there? So, the guanine part is there. So, that guanidine part is there, so not there. So, the remaining amino acid is L-ornithine.

So, try to understand, try to remember and try to compare with the structure of these ornithine with some essential amino acids or naturally occurring amino acids what the changes, because this sort of amino acid is also available to us, where you can have a big spacer unit and then you have the NH2 function which after protonation give you NH3 plus.

Now, the same substrate can also be degraded in a different manner, which is producing nitric oxide. So, if you are instead of cutting it in terms of your urea production, if you are going for your nitric oxide production, so you see now the N as well as the corresponding one if you can

go for some OH function over there and if you are able to cut it from there, that means this entire group NH2OH we all know that if you have the corresponding CO function, if you go for the corresponding NH-OH double bond NH-OH which is your hydroxylamine function.

So, some sort of hydroxylamine function if we are able to produce, if the biosynthetic root can able to produce it that means the formation of new nitrogen oxygen bond. So, that new nitrogen oxygen bond and finally the cleavage of the C double bond N can produce the nitric oxide, but the amino acid which will be producing by that process is the L-citrulline.

So, we will discuss all these in detail also. So, what is that, is the precursor for important messenger in many vertebrate signal transduction pathways producing nitric oxide, NO. So, NO is the basically signaling molecule.

For our many physiological conditions we have to produce the NO, but right now you should try to understand it, try to know it also that NO is a very simple small inorganic molecule. Since carbon is not there, we do not consider it is the organic molecule. And we are able to produce from the, by organic medium, so NO can be there. So, in many cases and NO also we know is a very good ligand in coordination chemistry.

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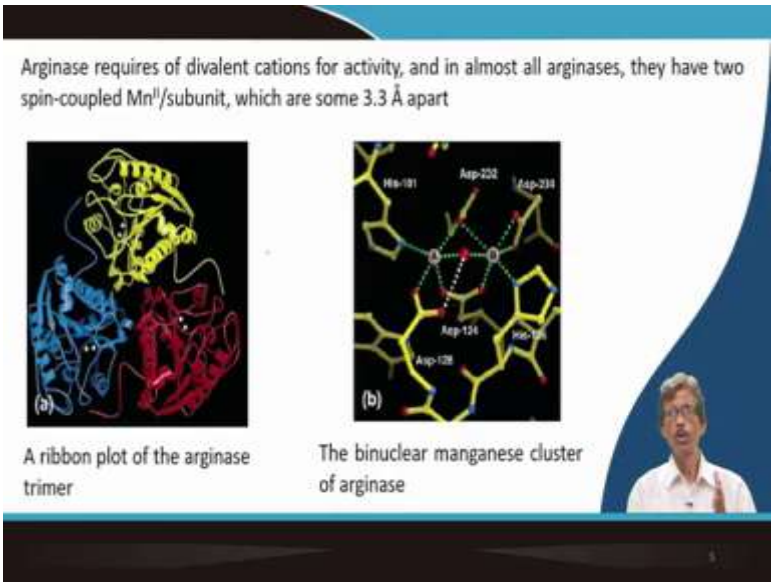

So, what is required then? It requires basically the corresponding divalent cations, not cation, cations, that means more than one manganese center will be requiring for its activity and two of

these manganese 2 centers. So, manganese 2 you should know the corresponding electronic configuration, number of unpaired electrons it will have.

So, if you can consider that whether you will have a paramagnetic center or a diamagnetic center then if these two paramagnetic centers are coupling to each other, we will be considering that these two are spin coupled, because the spin coupling is important. That also tells us that how far these manganese centers are sitting.

If they are close by or near-by say 3.3 angstrom distance then you will have the corresponding interactions between the magnetic orbitals, that means the orbitals which are carrying the unpaired spin density. So, what is that particular structure, the protein structure? And the protein structure always we know that we can present it in the ribbon plot. So, the ribbon plot of arginase which is a trimer, so we are making our life complicated take this challenge in that way that you make your life complicated, then you try to resolve it.

So, is a dimeric unit is one particular part, but the entire thing is the trimer. So, you get this particular one. So, you have the three colored part. So, is basically the cyan, yellow and red part, but everywhere you will find, you will be finding that you have the white sphere, small, small spheres in these three units those are your manganese centers.

So, because this trimeric part is important, we all know now we are master enough in knowing all these things for these corresponding assembly or the self assembly part of the different protein chains like that what we have seen case of hemoglobin. Hemoglobin was a tetramer. So, there is no difficulty in understanding that how the arginase timer will look like. So, three dimensionally you have to place these three assemblies, three part of these proteins such that it can sustain or it can stabilize the binuclear entity based on the manganese center.

So, all these amino acid residues will come. So, if you look at this particular sphere, small spheres of manganese, you will now elaborate it out to the corresponding dinuclear Mn2 system. So, we will try, immediately we will try to see what. You will try to the dotted green line. The dotted green lines are nothing but telling you the coordination of the manganese a, a is the left manganese, and b is the right manganese in this particular projection.

So, the manganese, so in the left you are seeing only 1, 2, 3, 4, 5, the coordination number five, that means you must have a vacancy such that you can have some interaction with the substrate molecule or with a product molecule, but the b molecule Mn b is hexacoordinated. But what is there for the bridging, the red big sphere is nothing but your, this red big sphere is the, can be water molecule or can be hydroxido ion, but they are bridged by the manganese 2 center. So, your water as well as the hydroxido group can be a very good nucleophile for the cutting of the molecule for the hydrolysis reaction of that L-arginine.

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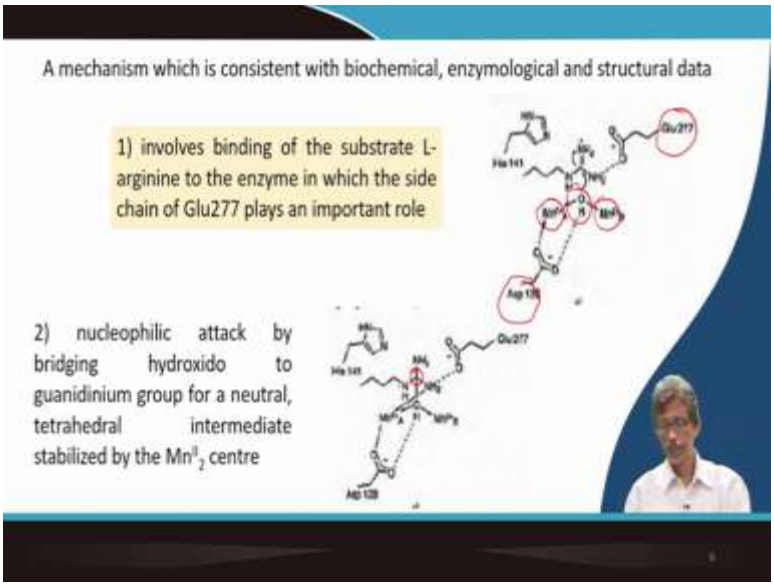

So, what mechanism you can follow basically from here that what sort of mechanism we can understand in terms of all these studies, you can have the biochemical study, you can have the enzymological study as well as the structural data, whatever we can find it out. So, now, we will focus our attention on these two manganese only. So, you have the manganese a and manage b. So, these are your these white spheres.

o, this is your white sphere and you have the bridge by your hydroxide group. Now, if you see the alignment of the corresponding amino acid will come in such a way that your corresponding part, corresponding group which will just simply go away is close to the OH bridging unit. So, that, if that is your nucleophile and that nucleophile therefore can attack.

So, for the mechanistic part is the step number one is nothing but your substrate will come and will sit there, but on the right hand top you have the glutamate 277, but the glutamate 277 with

the CO2 minus end, the CO2 minus and from there. So, this glutamate 277 with that C end basically try to stabilize further that NH2-NH2 end of that particular arginine molecule, not only the interaction close to that of your manganese center, but it should be hydrogen bonded or secondary coordination sphere interactions can take place like that. So, hydrogen bonding network can be there for glutamate 277.

It can also from that this particular amino acid residue aspartate. So, that is basically controlling also, stabilizing the manganese-manganese unit by hydrogen bonding with the OH of the hydroxido function. What is the second step? Second step is that OH can attack basically. Already we have seen that the arrow is there. So, is the guanidinium group will be now attacked and a neutral tetrahedral intermediate. So, if your OH is attacking that carbon which was a planner carbons, C double bonded C, so this particular one.

So, you will have, so where is your tetrahedral intermediate. So, the tetrahedral intermediate is your this carbon. So, you should always try to focus your attention that the hydroxido group which is bridging these two manganese center is now attacking the corresponding guanidinium group and giving you the tetrahedral intermediate and that tetrahedral intermediate is basically still stabilizing the dimanganese entity.

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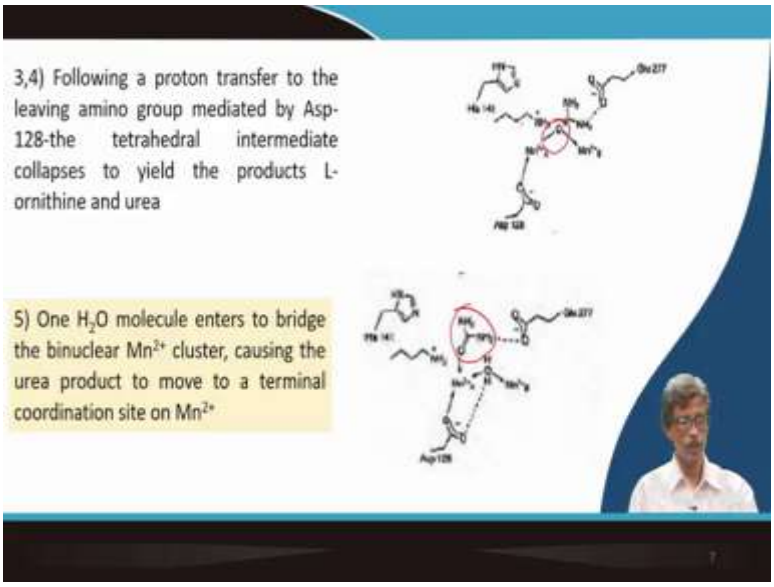

So, then the two follow up steps three and four, you will have the proton transfer, because this is locked now, the substrate is locked. That is why we always call that enzyme substrate activity is

nothing but your lock and key arrangement. So, if your enzyme is locked, substrate is coming as the key, try to open up that lock and is positioning of that particular lock and the perfect lock of that key for that particular lock is important.

And once it is locked you can have now the proton transfer such that you are moving towards the product formation. So, the tetrahedral intermediate you can have. Already I told you that aspartate 128 giving you that and the product L-ornithine will be forming in that way. So, now you have the cleavage. So, once it is cleaved, you have the transfer of these things.

So, that we have the carbon nitrogen bond which were originally present with the guanidinium group is now broken, because now you have the tetrahedral intermediate but that is in the presence of this manganese will be stable, but if we have the proton transfer, it will not be stabilized further. You have the cutting of this carbon nitrogen bond and protonation of the three NH2 end.

So, that basically give you the product as well as the ammonia function. So, once this particular urea, so now you have the formation of the urea from the left, you are producing urea, so it is your urea molecule. So, this urea molecule is not going away. When it is forming immediately it is not going away. Is being trapped inside at that particular active site where you have two manganese centers are there, but already that is not under control of the hydroxido bridge.

So, once that vacancy is created, a new water molecule will come and occupy that particular position, because it is only stabilize, the dimanganese entity can only be stabilized with either a water bridge or a hydroxido bridge.

So, that water molecule will come and that will remove that particular urea molecule, because in the other part that means the previous step, you have the urea is taking care of this particular part that urea is trying to bridge these two manganese center, but when water is coming, so not only proton transfer, but water will also try to post that particular formed urea molecule.

And this particular one is moving and now going to manganese a. What I told you that the manganese a was five coordinate. So, that five coordinate manganese a will have some attraction for the urea molecule and urea we know that as oxygen have lone pair of electrons and those electrons can be transferred to the manganese forming a coordinate interaction.

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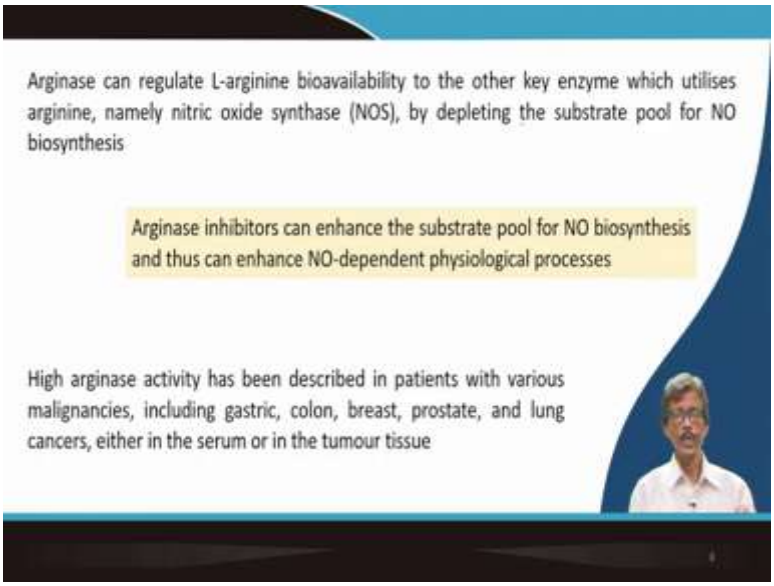

So, this particular case also you can have the corresponding bioavailability of other key enzymes like NOS and which is basically if the substrate is depleting in the pool that NO biosynthesis will be hampered. So, there will be a competition between these arginase as well as the NOS. So, that competition can be dependent on how much your arginase is active or if we can have some additional point of view if you think about the inhibitors.

So, if you have some inhibitors like this that can enhance the substrate pool that means the arginase will not the other path, the arginase hydrolysis path, but it will remain as arginine and that arginine can be utilized for your NOS activity but that we are not going to consider, but it has some repercussion definitely.

So, high arginase activity has been described. So, we do not want, so that is why we go for the inhibitors or say going for the reduced activity of the arginase. So, many types of disease conditions malignancies, gastric, colon, breast, prostate and lung cancers and the serum and the tumor issues from these places. Basically you can have some diseased conditions out of these over activity of your arginase.

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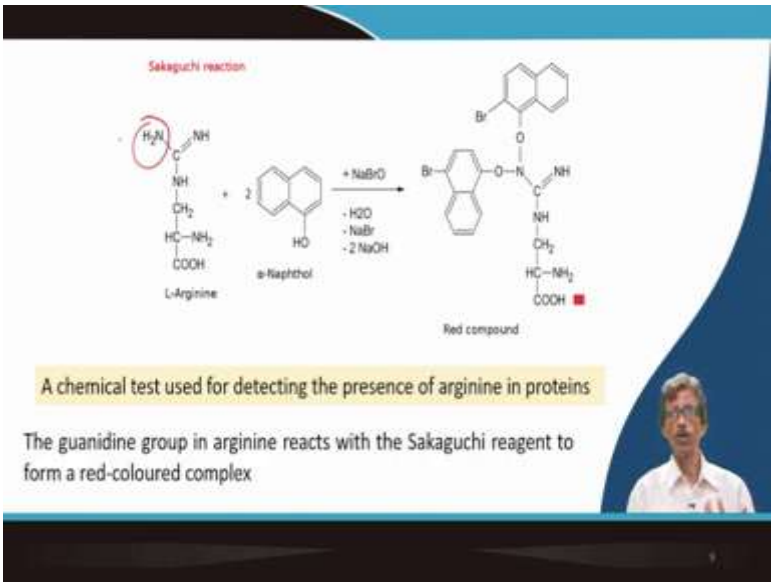

So, what is that particular type very important, chemical test what you can detect which as a chemist we should always know where you can have the arginine and if that arginine is present in the proteins, how we can determine that particular amount of arginine in these proteins. So, arginine, we have arginine as the guanidine group and that guanidine in arginine reacts with Sakaguchi reagent.

So, is a scientist named Japanese, is a food scientist, Japanese food scientists Sakaguchi introduced that thing which is forming a red colored complex, but what is that reagent. Reagent is a very simple reagent is nothing but 1-naphthol or alpha-naphthol as well as sodium hypobromite, NaOBr or NaBrO in presence of a base. So, if you have this L-arginine, what you can have this function basically.

So, if this NH2 function can be derivatized and that derivation can be formed by the beta-naphthol, attachment of the beta-naphthol unit through O minus as well as the bromination of the para position of the beta-naphthol ring giving you a red colored complex, because the visually you can detect that or spectrophotomatically you can quantify it the amount of arginine present in that particular protein molecule.

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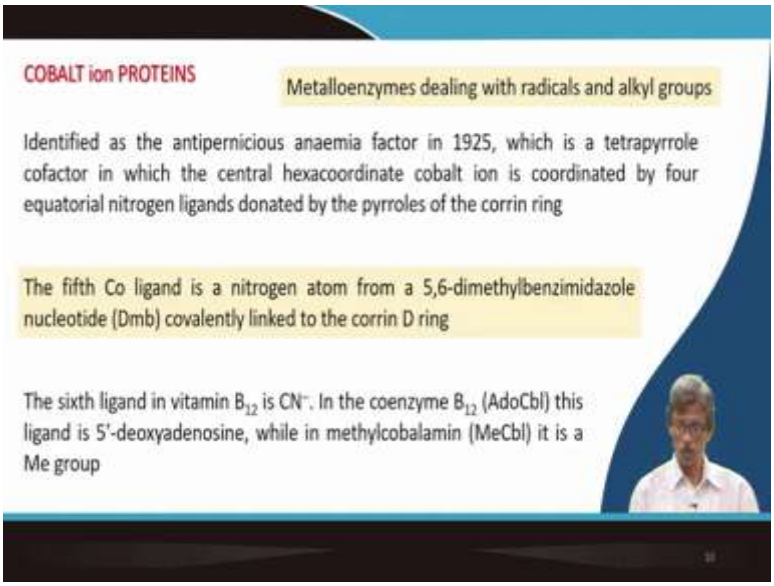

Now, we just moved to see what are there in the proteins, the cobalt system, the cobalt molecules. So, the cobalt based molecules are the enzymes or the cofactors what we can have. So, we will have, we will now see, it will just extend up to my next class also that there will be some metalloenzymes which are having cobalt center. Like manganese, if we have now cobalt center what extra thing we will be able to achieve through that.

But interestingly two very important categories which are not very much common with other metal ions what we have discussed so far, apart from in one case, that of your copper case that is your galactose oxidase that is the formation of the tyrosine residues and all these things, but is not predominantly centered around the metal ion, which is responsible for your metal carbon bond, cleavage of the metal carbon bond and the formation of the radicals.

So, mostly when we talk about the metalloenzymes which are basically dealing the formation of the radicals and the alkyl function or the alkyl radical those are your cobalt proteins. So, long back say almost 96 years back it has been considered as the anti-pernicious anemia, so is a type of anemia is not due to the absence of iron, absence of myoglobin and absence of hemoglobin, but it has some useful role for co-catalytic role or coenzyme factor for the pernicious anemia. But if you want to stop, you must have to take the vitamin B12.

So, that is why it is named also as a vitamin and which is giving us some information that it will have that tetrapyrrole cofactor and that tetrapyrrole cofactor giving you a hexacoordination

around cobalt. So, it is tetrapyrrole like your porphyrin. But it is something modified tetrapyrrole unit. The four bonds will be coming and binding to your cobalt center again in a fashion which is in the plane like that of your myoglobin and hemoglobin, but you can have the two other coordination positions like the coordination number five and the coordination number six.

So, the fifth center around the cobalt is the nitrogen atom. So, the fifth cobalt ligand is derived from 5,6-dimethylbenzimidazole. So, that 5, 6-dimethylbenzimidazole as a nucleotide, so that is why it is dimethylbenzimidazole nucleotide will be covalently linking to the corrin D ring. We know is a tetrapyrrole, so a, b, c, d. So, from top left then right a, b, c and d. So, the D ring will have some a different kind of linking.

Then the sixth ligand will come. If the sixth ligand is typically cyanide ion, because it is basically an artifact when people identify this first time and they use sodium cyanide for this processing that particular process, but during that particular process the cobalt carbon bond is broken and that has been substituted by cyanide ion.

So, it is typically an example of anion metathesis reaction around the cobalt Center. And what we identified initially as your vitamin B12, where the thing is known as your cyanocobalamin. So, now you can have the AdoCbl. AdoCbl is that you can have the coenzyme. Now, the other molecules we will be considering as the coenzyme.

You bring 5 prime-deoxyadenosine that is why it is Ado. So, 5 prime-deoxyadenosine if you bind it to the coenzyme, so it will be, all the series will be the coenzyme B12 so you have the cyanocobalamin, you have ado cobalamin, and finally, you can have the methyl cobalamin. So, what is that methyl? So, it is only we are considering the sixth position.

If you vary the sixth position, so definitely the way we know we define it for the myoglobin and hemoglobin, the sixth coordination site is important. That sixth coordination site when it is holding dioxygen molecule we get the oxymyoglobin, when it is holding oxygen, you get the oxyhemoglobin center, because this all these positions four from the plane and the fifth is booked. You have the position, the variable positions on the sixth position.

So, if you have the corresponding cobalt carbon bond for the sixth positions, what will you find? You will find that it is also a labile part, the reactions are such, goal such a manner that you can cut that particular cobalt carbon bond.

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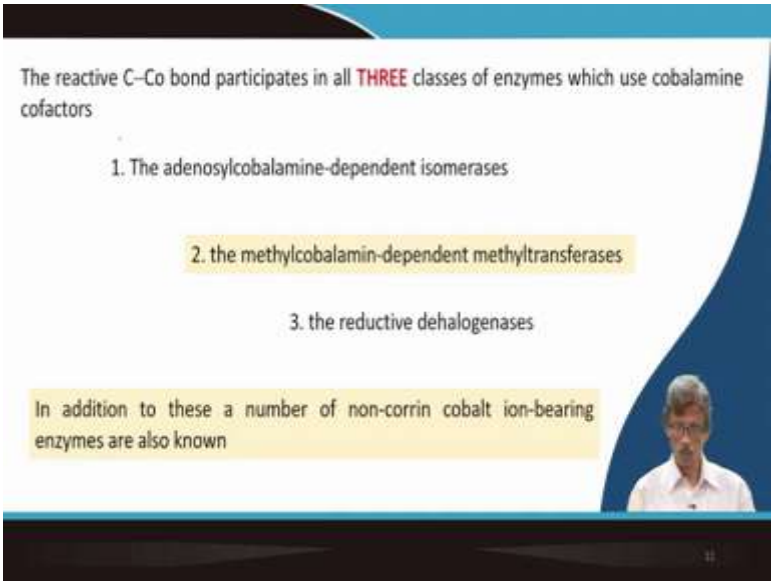

It is the active then and it do participate in three classes of enzymes. In our next class, we will talk about all these classes of enzymes. So, we will definitely talk about these three classes of enzymes using cobalamine cofactors. So, is basically helping the reaction. Then the adenosylcobalamine can go for the isomerase reaction. So, you have the corresponding free radical formation and abstraction of some other group or the bond breaking at other point.

Then methylcobalamin, so AdoCBl and MeCBl give you two different reactions in this particular case, which is very useful that it can go for immediately the methyl transfer reactions. The methyl transfer reaction is very important biology even in the biochemistry as well as in organic chemistry. And third type is your, for your dehalogenase reactions.

Then if we can squeeze the class little bit or if time permits, we can go for something where you can have some species where the corrin ring is not available and cobalt center is only available, but it can show some other enzymatic activity.

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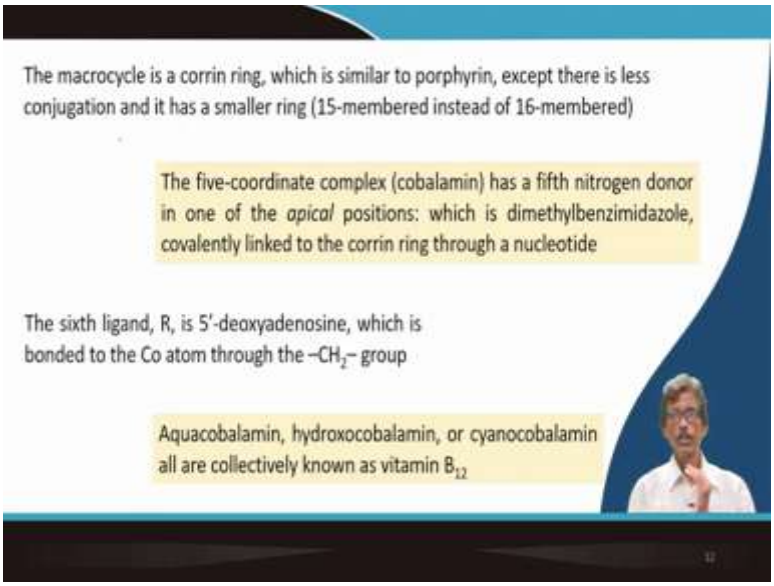

So, the macrocycle is the corrin ring which is similar to your porphyry and there is a less conjugation that means some are saturated and it is 15 membered instead of 16 membered. So, already I told you the fifth coordination site you can have which is a dimethylbenzimidazole. Then further it is covalently linked to the corrin ring through a nucleotide.

So, that is why your structure is little bit complicated. Try to understand, try to compare with that of your structure what we have learned in case of your myoglobin. Then the sixth will come as 5 prime-deoxyadenosine, that is why the ado thing is coming, which is bonded to the cobalt center through a CH2 group. So, you link the carbon bond through a CH2 group.

So, you link the carbon bond through CH2 is giving you the corresponding cobalt carbon bond. Then further like cyanocobalamin, you can have the aquacobalamin, aqua is the neutral water molecule. So, your charge will be different the activity will be different. And also we will see that cobalt can stay in three different oxidation states plus 1 plus 2 as well as plus 3 and your reactivity pattern will change.

So, the aqo, hydroxo and cyanocobalamin all together they will be terming, giving the term as vitamin B12 like your, the special thing is your cyanocobalamin. The first one is your cyanocobalamin.

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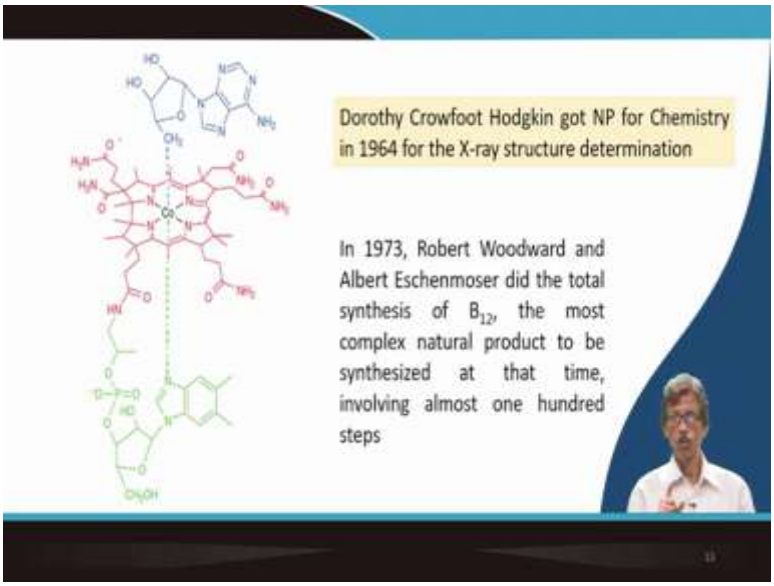

So, it is professor Hodgkin, so Dorothy Crawford Hodgkin he got the Nobel Prize in, she got it in 1964 for the x-ray structure determination. The first time we came to know in 1964 what is the huge structure. Previously we are only have the analytical informations and all these things. So, you now you can have, you know nicely, you practice yourself about the corrin ring and how the corrin is different from the porphyrin ring and all these positions then you have the fifth position.

So, fifth position is similar to that of your globin coordination, but it is connected to the ring now. So, benzimidazole group is now that nitrogen instead of imidazole of the histidine, now it is benzimidazole. So, that benzimidazole nitrogen is coordinate into your cobalt center and finally you have the CH2 carbon bond and that CH2 carbon bond is giving you the entire structure.

But nine years later only is not before that it is Professor Woodward and Eschenmoser did the total synthesis, how we can prepare the whole thing in the laboratory the vitamin B12 synthesis that was a challenge during that time. And you see the synthesis has 100, almost 100 different steps of that particular reaction. So, if you see, you will be amazed to know that how much you should be, you must have the patience to follow a reaction which are having 100 steps.

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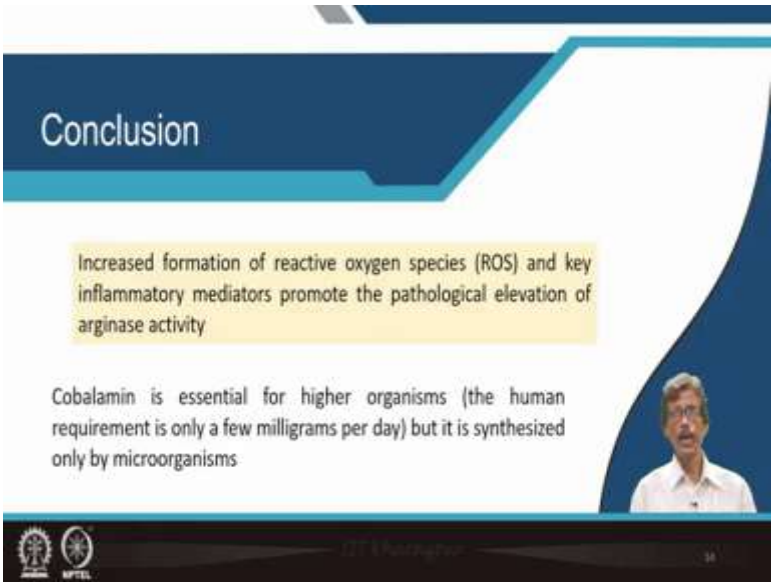

So, what we have seen that we can see for the first part of our lecture that the formation of ROS is also important and that ROS is the key inflammatory mediator promote the pathological elevation of arginase activity. So, we must have something like the ROS activity, the superoxide activity or hydrogen peroxide activity that can have some relationship with the arginase activity.

So, once you read it, you try to learn more out of this thing, the relationship of ROS with that of your arginase activity. And this cobalamin we require for our survival also to stop the pernicious anemia that human requirement is also, but only few milligrams only, but microorganisms all produce for us.

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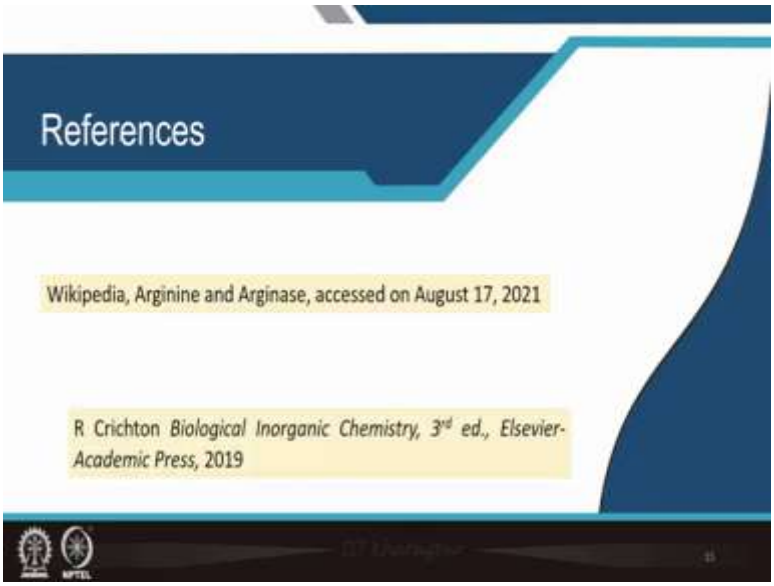

So, you read both the Wikipedia pages for arginine and arginase and also the book of Crichton. So, thank you very much for your kind attention.