**Biological Inorganic Chemistry**
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**Lecture 35**
**Mononuclear and Dinuclear non-heme enzymes**

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Hello everybody. Welcome back to this class where we are just with iron and in this module this is your last class where we are talking about now some Non-Heme Enzymes. So, we have seen the proteins, the iron-sulfur proteins in your last class now we simply move to the non-heme enzymes because the heme groups we have seen how we can modify that particular environment that means we are trying to extract out the chemistry out of the iron centers.

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So, we will discuss here about the different types of oxidases and oxygenases and particularly their contrasting definitions, monooxygenases only one oxygen atom will be involved; dioxygenases when both the two oxygen atoms are involved. Then mononuclear non-heme centers and finally most important thing for our drug research and all these the beta-lactam biosynthesis we should know about little bit which were dependent on your iron center.

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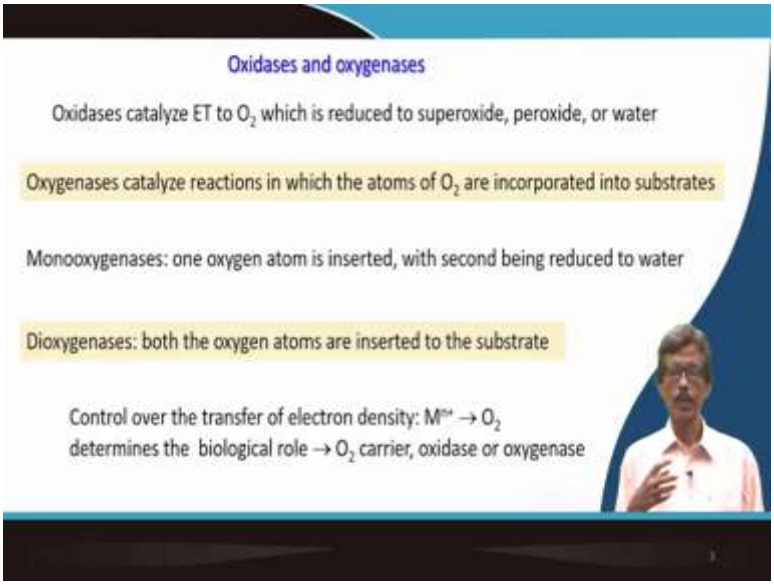


So, two types of these molecules that oxidases and oxygenases where the first category is basically catalyze the electron transfer to O2. So, if we just simply go for electron transfer to O2

how we can reduce it to superoxide we know that if you go for one electron transfer it will be reduced to superoxide, only single electron transfer way we have seen it for your hemoglobin and myoglobin molecules.

Similarly, if you have this also the super oxide molecules in our body also you can produce only when you have a single electron transfer. But in the next case if you go for another electron transfer that means the double electron transfer to the anti-bonding orbitals of your O2 molecule so you have two anti-bonding orbitals the Pi star orbitals you can have so we are having one unpaired electron on the left and another on the right.

So, these two are basically if you provide again two more electrons one will be paired, the second one will be paired also you get the peroxide. And finally, if you are able to put four electron transfer where your oxygen is utilized for our food burning in the case of cytochrome c oxidase what we have seen earlier so is there four electron transfer. So, single electron transfer, double electron transfer and four electron transfer is important for your reactions.

Then the oxygenases catalyze basically reactions in which the atoms of oxygens. If you are directly go for the incorporation of the atom within the substrate ra h; r h or methane, methane we write as CH3 H or CH4. So, if you are able to insert one oxygen atom within the CH bond we go for the CH activation we call and then the insertion of the oxygen atom between carbon and hydrogen bond.

So, your CH bond will be converted to COH that is your hydroxylation the aliphatic hydroxylation. So, that is your oxygenasian reactions we have also seen little bit about your mononuclear heme enzyme like your cytochrome P450. So, oxygenase then mono-oxygenases so one oxygen will take and the second will be reduced to your water molecule. And dioxygenase is both the oxygens we will be able to insert within the substrate.

Then what you can do basically? How we can control; or you have the same iron center it is the important thing that how your ligand can control such that you can have a particular electronic environment which is given by the ligand its geometrical environment its structure and all these can change for your transfer of electron density from m n plus to O2.

The way we know that once you have this it can function as simply the O2 carrier like your myoglobin and hemoglobin which is reversible in nature. Then when we can have the oxidase activity due to that electron transfer only. So, only thing that you have to change the corresponding ligand or the protein environment so studying all these things are very important to us because you want to know the nature we want to disclose the nature how nature is working.

So, if nature uses that sort of ligand so if you go to the laboratory and you are able to make similar type of ligand environment or mimicking of that particular environment then tell us that okay we can have that and that basically gives us that idea that okay we can manipulate the electron transfer from the metal ion center to your O2 because you have to reduce the O2 center. So, it can function as O2 carrier; it can function as oxidase or it can function as your oxygenase.

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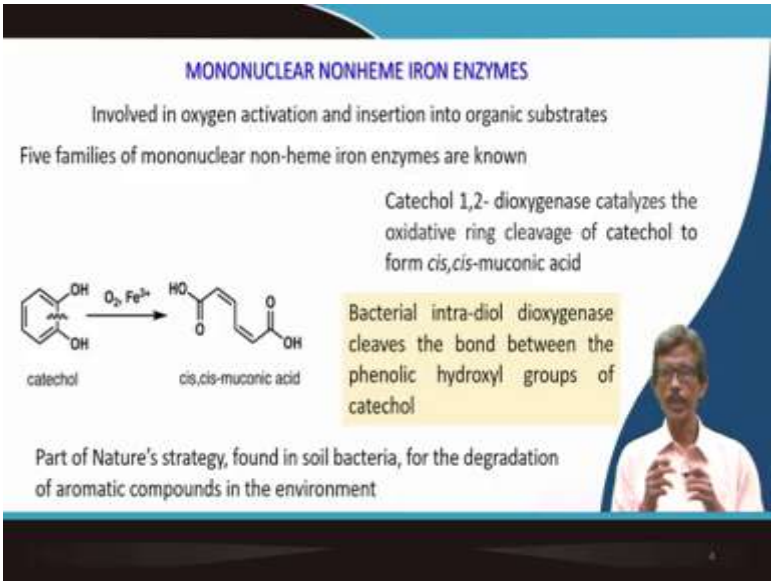


Then we take something where you do not have the porphyrin ring you put the iron that means you have a different type of ligand environment which we can consider as the non-heme iron enzyme and when one iron center is present will be calling as the mononuclear non-heme iron enzyme.

So, these mononuclear non-heme enzymes are many they are plenty in number they are responsible for your oxygen activation and insertion into the organic substrate which is very important. So,metimes we use something in your organic chemistry classes what we use that some activated or very highly reactive corresponding oxygen species even Ko2 the potassium

super oxide which is a powder in powder form you can buy it as a laboratory chemical but that is highly activated oxygen as super oxide O2 is present as a superoxide and it can go for the different types of reaction even single electron transfer that means radical formation.

So, this particular case you have to go for the activation and you have to go for the activation in such a way that you will be able to cut the O-O bond and one of the oxygen you have to take out and put within the substrate like say your methane because our goal is there to convert the methane which is a greenhouse gas to a value added product which is your methanol.

Methanol will be very useful solvent if you are able to convert the methane which is polluting our environment which is also a greenhouse gas to the formation of the corresponding methane that is a one such example once I told you that is the methane monooxygenase.

The bacteria basically can touch that particular system but here the strategy is completely different their two iron centers are there but here we will use only one iron center and the ligand scaffold. There are five different families basically are there for this sort of enzyme because the five are mostly characterized is not that the end of the list any day or any time we can discover more and we can have the new observations and new findings which you can also apply for our chemical knowledge or chemical understanding.

So, you can start from the bacteria basically because the bacterial thing is very important because the assimilation of all these polluting substrates or some species which can go and which can be degraded by bacteria only. So, bacteria what gives you giving it you have a diol and intra-diol dioxygenase activity that means diol-diol you have the in between part, so if you are able to clip the diol-diol in between the particular bond that is your intra diol dioxygenase activity.

So, that cleave basically the bond between the phenolic hydroxyl groups, so you have a phenyl ring and two ortho positions you have the OH-OH that is your diol or the catechol molecule. We know the catechol oxidase we will study in your copper cases, so this diol can be now cleaved basically so we are baking so catechol 1,2- dioxygenase basically can catalyze the oxidative ring cleavage is not only oxidation because we know the catechol can be oxidized to give you the corresponding radical form but you have to break or you have to cut the carbon-carbon bond so which is giving you cis,cis-muconic acid that is the product.

So, you have this catechol your catechol 1,2- dioxygenase will come having a bacterial origin with the help of only ferric ion and O2 molecule is like typical phantoms type of reaction but is little bit complicated reaction and the nature is following that particular strategy because you can have the bacteria which is available in soil and the different aromatic compounds if your aromatic compound is available and the degradation product is one point is the catechol product.

So, catechol is there everywhere from the different vegetables and all that is why we need the catechol oxidase on the skin of the potatoes. So, that if you are able to reach up to the catechol point then this iron mononuclear non heme iron enzymes will come and basically cut the corresponding thing and make a cis,cis-muconic acid and other small or degraded molecules which can be very easily assimilated within the system or within the environment.

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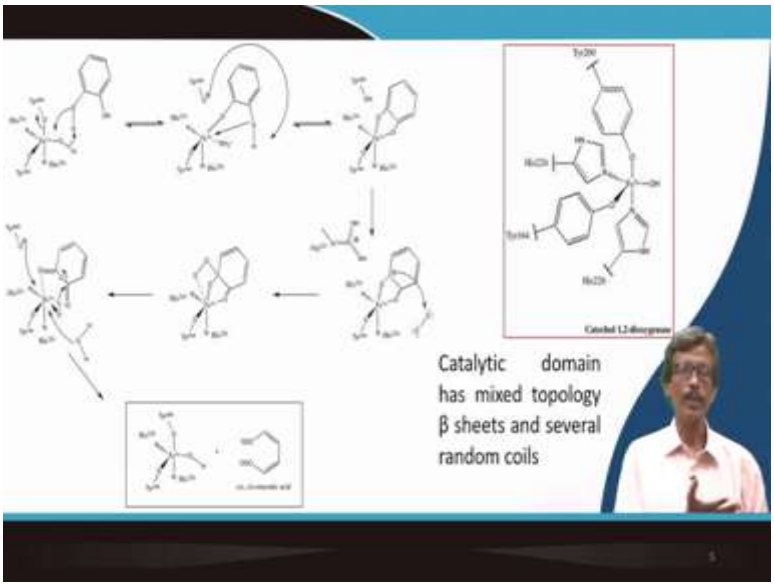


So, you have the protein binding domain and which if you have the corresponding metal ion which is showing the catalytic activity, we will call them as the catalytic domain. So, the catalytic domain can have mixed topology of beta sheets and several random coils. We know that the alpha helix and the beta sheets and in between we have the random coils.

So, the coiling is such that what you can have you have to keep the corresponding mononuclear iron center and this mononuclear iron center is non-heme type that means you do not have any porphyrin ligand available to bind this particular center so when the extra structure is known to us and we find out everything nicely will find that iron center is basically not a octahedral system

because the protein constant is such that you have the histidine residue, tyrosine residue of two tau two tyrosine residues and two histidine residues.

So basically, two plus two you can consider as a NO and another NO, two NO type of ligands. So, the from the protein chain is basically NO-NO ligand is coming and this basically binding your iron if your iron center is like this, so that binding giving you a initially formed mononuclear entity of iron within the protein pocket and then you have the another $5^{th}$ coordination which is can be your water molecule or which can be your hydroxide ion.

So, that particular in the trigonal plane so if you have a trigonal bipyramid geometry within the trigonal plane basically you can have the corresponding OH group is coming. So, if your protein is like this so you see this is the thing so if your protein is like this and if the 2 plus 2 is coordinating from this particular site like your rubredoxin molecules, so rubredoxin is coming like this is a mononuclear one and all the sides are completely occupied no vacant site you have that is why it is only showing you the electron transfer protein or electron transfer reactions.

But if you have some open space so 2 plus 2 is coming like this from one side basically also we will see that one particular face is also sometime fine that a facial coordination from three donor groups but you can have 2 plus 2, 4 donor groups you can bring and from one particular side so that basically you can get a particular thing as the capping from this side basically so one tyrosine, one histidine, another histidine in this, these two are in plane so one at the top, another at the bottom.

So basically, that particular arrangement from one side keeping this iron and you have the water molecule coming from this side. So, this basically tells us that that will be your substrate entry point. So, substrate will also reach from that particular side and approach your iron site for its activity. So, you see do not bother about and do not be perplexed with this thing that is so many structures and so many things is that we have this particular structure now you have to put the substrate.

So, you see at the top basically we have bought that particular substrate and how this substrate will interact with this iron side such that you can have the typical interaction. So, is basically the hydroxide group on the iron center is responsible for your deprotonation first and this

deprotonation is basically allowing that particular O as your O minus or one of the catechol bond unit to coordinate to your iron side.

Then this particular one is basically you can have the corresponding protonation of the tyrosine residues also further so then one proton is going for the protonation of the tyrosine residues of the protein chain so that particular binding is also going.

So, that is the unique activity of the protein envelop such that at some point of time you will be able to protonate the protein chain and that coordination is lost from that particular protein side such that you can have more access around the iron side for your substrate that is why your catechol is coordinating to a bidentate fashion if all the four other positions are occupied it was not so easy to go for a bidented coordination of the catechol unit because in all these cases varying from some stage where you have the peroxide or linkage is one other avenue so two avenues basically have been shown here.

So this is the thing that means the movement basically from top then to left and then to the muconic acid. So, at one particular point under forced condition that iron center can go to a octahedral geometry also. Then you can have the transfer so you are basically cleaving that particular unit so O-O bond cleavage is taking place and O-O bond cleavage is taking place in such a way that within the ring within the benzene ring of the catechol unit your oxygen is getting inserted such that your C-C bond is converted to C-O-C bond.

So, is a seven membered ring seven membered bigger ring you can have such that you can have a corresponding so you are basically cleaving the C-C bond and you will ultimately getting from the both ends if you are able to cleave it you will get the corresponding muconic acid. So, that is the very important and the interesting reactions for the intra diol cleaving agent.

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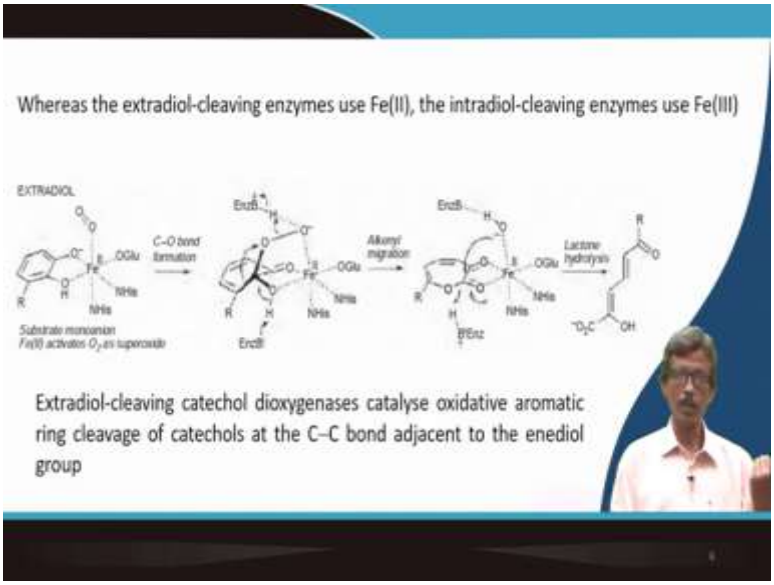


Now, if you go for the extradiol-cleaving system, so extradiol-cleaving system will be of similar type where you can have the iron because iron in all these cases iron will be the low oxidation state because you are allowing to react with the ferric state and your iron center is there and that iron center can give rise to the corresponding transfer of electron to the O2 molecule such that your O2 will be the active species or the reagent and your iron center will be converted to the ferric state.

So, the ferric based thing is there and for the extradiol the if you just simply compare what is the difference between the intra diol thing and the extradiol thing so in the extradiol thing we are now trying to have this again the corresponding carboxylate function now is the the glutamate not the tyrosine residue here you have the corresponding substrate species in the form of a glutamate coordination of oxygen then the histidine of nitrogen and histidium, so you have the three coordination.

So, these three coordination is basically can come through a face of an octahedron type of thing or even for the trigonal bipyramid also because the trichronal bipyramid you have on the top you have three trigonal phases and you have the altogether six trigonal faces in octahedron you have octa but trigonal bipyramid can be considered as a hexahedron.

So, that C-O bond formation can take place from there and what you can have now you have the activated O2 molecule and that activated O2 molecule can attack the carbon of the corresponding

catechol molecule and again the corresponding one can give you the ring expansion for the insertion of the oxygen between the carbon-carbon bond which is getting activated at that particular point.

So, which is outside the O-O part that means O-O is basically they are already bound to your iron center but your C-O-C is forming at some outside of this particular chelation. So, outside the chelation you get the thing and you will get a different product not like that of the previous product. So, this cleavage basically catalyzes basically the oxidative aromatic ring cleavage of catechols where the C-C bond is adjacent to the enediol group.

So, diol function is there then you consider that whether you can have a in function is also there because it is the benzene ring so you can consider as a enediol function so enediol dial function but the bond what is being cleaved is attachment to the iron center but the bond is cutting from the outside of this chelation.

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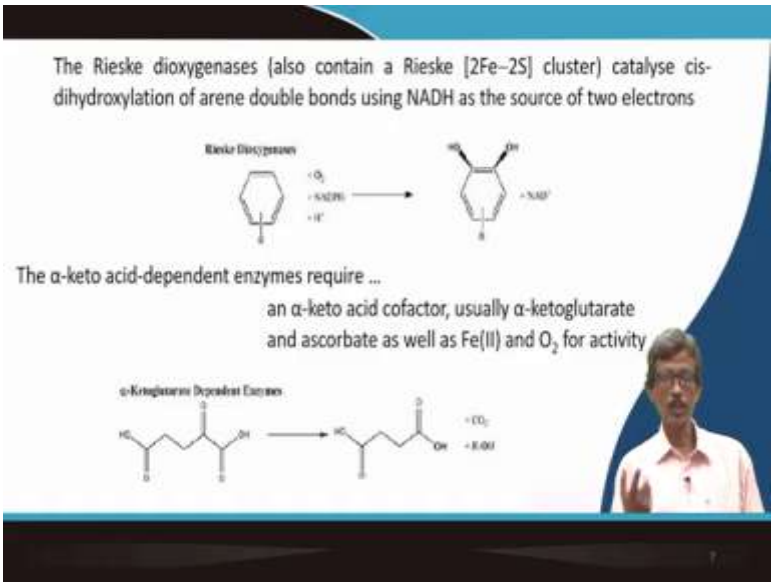


Then we can talk about the Rieske, the Rieske dioxygenase, the dioxygenase now. Also contain a Rieske 2 iron 2 sulfur cluster, so that is why is the mixture of these things what we have learnt in our previous class the Rieske proteins we consider that these two iron two sulfur proteins are there which are responsible for electron transfer.

But if the dioxygen is available showing the catalytic function but is dependent on your escape protein iron sulfur protein so iron sulfur protein that Rieske iron sulfur protein is basically giving the corresponding electron to the catalytic side such that you can see the dioxygenase activity.

So, is basically going for cis-dihydroxylation of iron double bonds, so if you have a arene double bond using NADH and some source of two electrons basically that is why it is dioxygenase reaction because you when you transfer it basically your dioxygen can be converted to your peroxide.

So, is the dioxygenase you have the typical benzene ring or the substituted benzene ring your reagent is O2 your iron center iron sulfur proteins as the Rieske iron sulfur protein then proton and NADPH you will have a double hydroxylation that means two O-H bonds will be inserted within the substrate such that you get a double hydroxylation reaction.

So, another category I told you that you have 5 such categories so basically you should know about the categories only the detailed reactions I have shown you but not for all you can have the corresponding mechanism for all these reactions but the most important that how many types of these particular enzymes have been identified and structurally characterized and studied in detail.

So, another one is alpha keto acid dependent enzymes, so you have a alpha ketoacid now instead of catechol or simple that corresponding benzene molecule or benzene type of molecule is basically required the presence of an ketoacid cofactor which is nothing but a alpha ketoglutarate so a ketoacid which is also a keto acid and ascorbate is a reducing energy is like your NADPH which is a biological available reductant ascorbate is also biologically available reductant RN 2 and O2 again the phantom type of reaction we can go and basically that particular one what is forming there you see the carbon is getting lost the keto function of the ketoacid is destroyed or has been taken out as your carbon dioxide molecule.

So, that has been taken as your carbon dioxide molecule for this particular reaction, so one carbon sort of that particular complicated keto acid is giving you a dicarboxylic acid.

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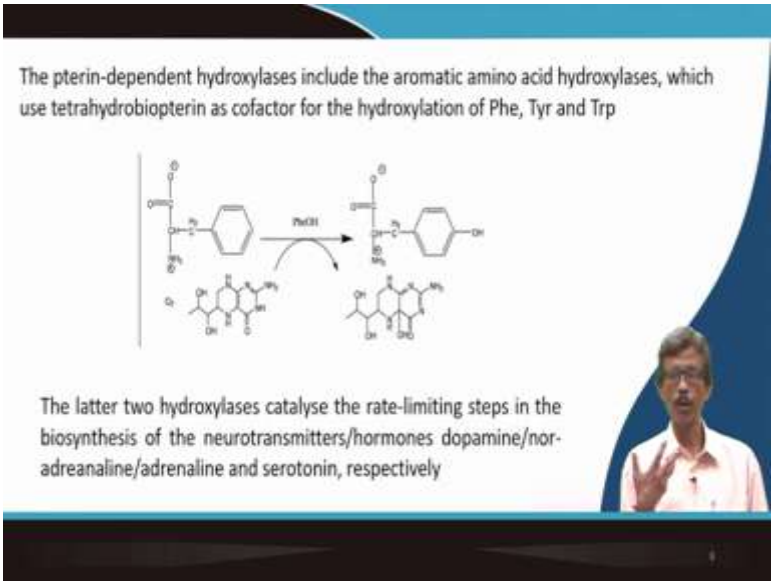


Then another category is your pterin-dependent hydroxylases so you will soon go for hydroxylation reactions of aromatic amino acids, so amino acids we have seen and amino acids can be hydroxylated in the aliphatic part like your methanone oxygenase.

Similarly, the corresponding phenyl part that means the aromatic part can also be hydroxylated and if you can have such not many examples of these amino acids are there which are phenylalanine tyrosine and tryptophan so these are the three so you can have the tetrahydro biopterin, biopterin as your cofactor so that is basically the ligand part which is functioning something for activating of these particular pterin dependent hydroxylases.

So, these hydroxylases are there and you can have the corresponding cofactor the pterin cofactor is there and that pterin cofactor is basically present and you can have the corresponding hydroxylation on that particular pterin cofactor and also you have the conversion of the substrate.

So, these last two types basically why we are studying and why we are trying to learn all these things is that because of their catalytic activity iron dependent catalytic activity we are able to make many useful and physiological important molecules for us also. So, the last two categories of these hydroxylating molecules which are responsible for your catalysis for hydroxylation reactions and also the aromatic hydroxylations that means you have the phenyl ring even if you have the phenol ring like your the corresponding tyrosine residues you will find afterwards also

that if you are having something tyrosinase molecule so tyrosinase they are how you can monitor on this particular reaction.

So, the biosynthesis of very useful molecules such as your neurotransmitters different hormones like dopamine, nor adrenaline, adrenaline and serotonin. So, vitamin synthesis as well as the neurotransmitter synthesis and many useful molecules can be synthesized in our body which are mainly dependent on the catalysis of your iron site or the iron center.

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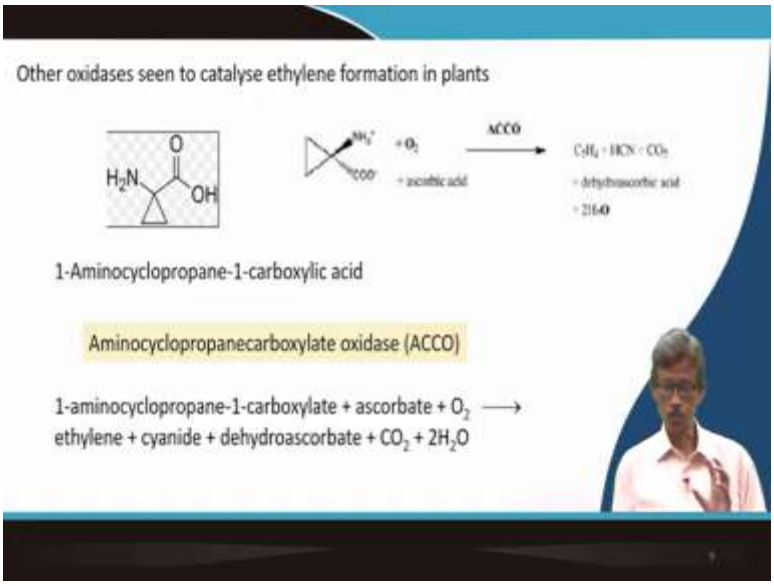


Then we see at the end basically that other oxidases if you can have apart from your all these very useful molecules if we go to the plants, so after human being what we need basically plants also require some hormones because here we have studied that particular hormones and all these things and the neurotransmitters and the other types of hormones also.

So, plant hormones if we simply see and very well characterized well known and studied for many years basically that plant hormone which is nothing but your ethylene molecule C2H4 molecule, we all know the ethylene how we can activate it in the laboratory also how we can activate ethylene to make the polyethylene also in the polymer chemistry taking the help of the polymer chemistry.

But if you have one such substrate which can produce in situ within the plants the ethylene because we all know the ethylene molecule is a required hormone for your plant for fruit

ripening also, the ethylene is required for your fruit ripening, so when you basically cleave it what are the species basically you are able to produce it through the reductant ascorbic acid as well as oxidant O2 and the corresponding enzyme ACCO.

So, what we have we have the substrate as your one amino cyclopropane one carboxylic acid is nothing but a very similar to that of your amino acids what you all know the glycine is NH2CH2COH this on this carbon if you substitute that carbon by a cyclopropane ring so NH2 CH2COH so you have the CH2 it will be CH and then cyclopropane ring which is nothing but your 1-Aminocyclopropane-1- carboxylic acid group.

So if that is available to you so that is your substrate instead of your hydrogen atom you have now a steric bulk of cyclopropane ring and that cyclopropane ring all we know is not a very useful system for any amino acid or this particular type of amino acid also so it is immediately cleaved so that the backbone of this the other part is also not going like this the backbone of this thing basically going as your ethylene molecule but the remainder also is getting clipped into HCN hydrocyanic acid and carbon dioxide

So if you consider that the carboxylate function the CO function the carboxylate function is basically going for you and that carboxylate function is going for your carbon dioxide then the remaining part the CH NH2 function is giving you the corresponding cyanide function so that is one such biologically important reaction where the organic molecule can produce your the inorganic acid as HCN or inorganic anion as the cyanide that we again has been discovered in the same time for your hydrogenases.

So, hydrogenases biologically we produce many cyanide molecules and the cyanides are the very good ligands even in your biological system. So, that acid the amino cyclopropane carboxylate so is the anionic form basically and is oxidase activity so is named as ACCO. So, producing ethylene, producing cyanide as either HCN or CN minus then dehydroascorbate anion because you are using hydro ascorbic acid so that ascorbic acid is getting reduced to dehydroascorbate anion then carbon dioxide and H2O, water molecule is formed there.

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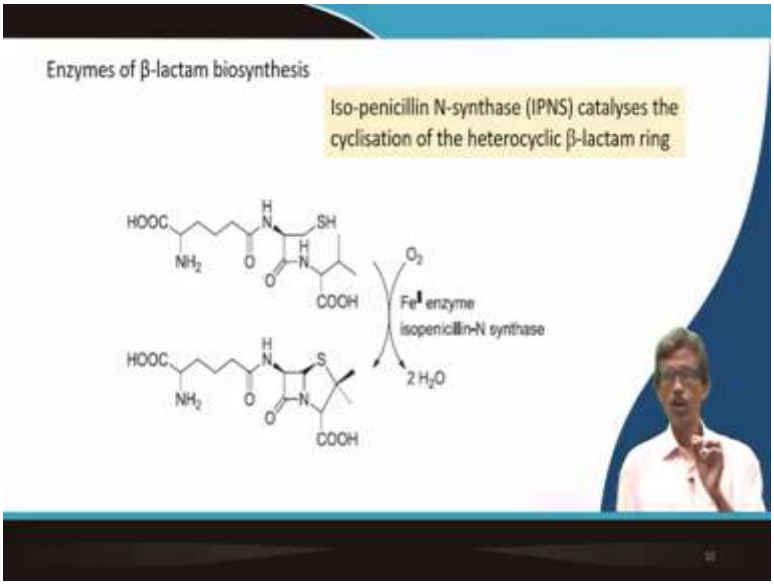


So lastly, we see that the importance in terms of your medicinal point of view that what particular enzyme because people are working in this area extensively to know about these things or know about nicely about the iron as the catalyst whether you can have some cheap catalyst which will be useful for your beta lactam synthesis in the laboratory.

Because the beta electron biosynthesis is known now, so which is nothing but we all know the penicillin is your beta lactam antibiotic so iso-penicillin N-synthase IPNS so which is basically a iron bearing catalyst which is responsible for only one part at the end basically for the ring formation the cyclization.

So just now we have seen that iron based enzymes or the catalysts are required for cutting the ring, cutting the benzene ring, cutting the catechol unit. Now the reverse thing also we can do we can produce the beta lactam ring for some useful molecule like the penicillin amoxicillin and all these things.

So, what we have we have the corresponding one so you know the structure little bit because you have to know the structures as a complicated structure of your penicillin molecule then the last step what is taking place you have the corresponding beta lactam ring. So, beta lactam ring is the corresponding bond formation which is that of your CH2 SH and your NH function. So, you are forming a new carbon nitrogen bond within the beta lactam ring. So, it is not that the NCO bond

but it is the NC bond towards the sulfur side, so sulfur definitely can play some important role with this particular iron two enzyme.

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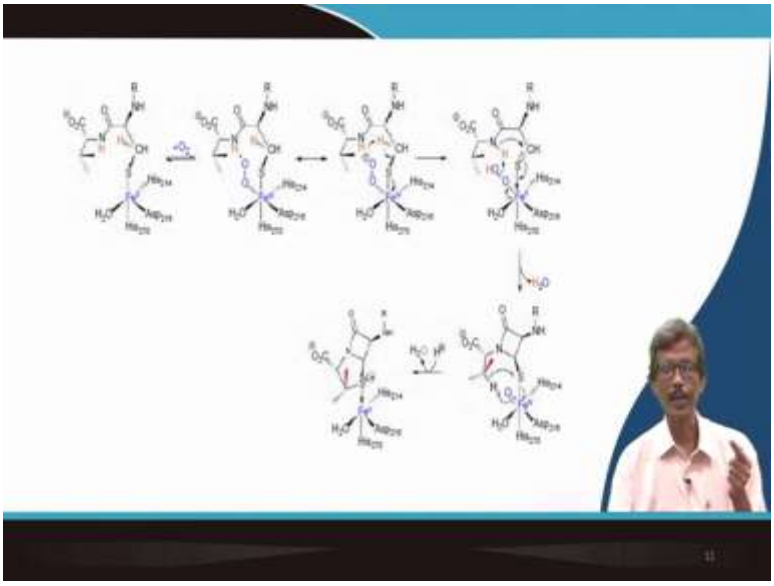

So that basically gives us this particular information that how it basically going for our reactions. So, from the top left also again we start that these iron sides are there now again you have the histidine residues, two histidine residues and one aspartate acid now; aspartic acid now from the minus it is again. N2O so immediately by looking at the corresponding active site you should know that what sort of that corresponding triad or the corresponding facially capping ligand is available to you which is N2O ligand.

So N2O (())(28:48) ligand is there one water is three and then thiol will come because thiol already we have seen like starting from your rubredoxin life that thiolate the cysteine groups are very useful to but your iron side that means the ferrous side. So, once it is binding then the vacant site can take up that particular dioxygen molecule and that dioxygen molecule can be activated to your super oxide.

Then that superoxide condition is very important that can also go for the corresponding activation of the carbon sulfur bond and that carbon sulfur bond towards the right you can have to have the corresponding carbon sulfur bond formation and O-O bond cleavage. So, here basically on the right extreme right of the top that you have this corresponding red marked

hydrogen atoms and when you have the O cleavage that OH group is producing and that OH group is abstracting the hydrogen from the NH function.

And when abstracting that nh function for that hydrogen is basically what you are producing; you are producing the most stable low energy species your water molecule. So, once you produce that water molecule you are getting that particular cyclization of the four membered beta lactam ring.

So once that four member beta lactam ring is formed there and that basically is giving that important information from there once that ring is formed so again you can have the corresponding cyclization from the other part of the ring and you get the corresponding cyclic form. So, is a bicyclic product you will be able to get it with that particular enzyme activity.

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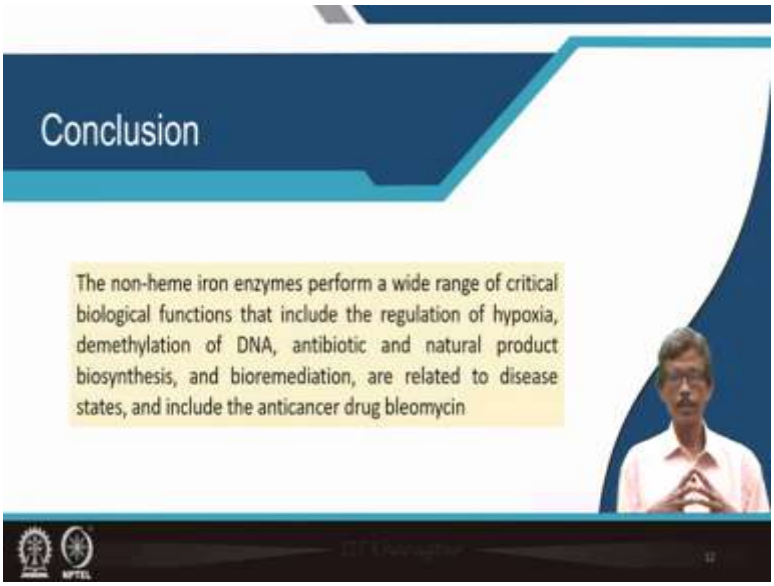


So, at the end basically why we are studying all these things in terms of your non-heme iron enzyme because the non-heme iron enzymes are easy to gather and easy to accept it because you need a very simple ligand system not like that of your porphyrin and it can form many important biological functions and it can have some role in hypoxia condition hypoxic condition where the low oxygen condition in our body the demethylation of the DNA antibiotic production we gave the example for your penicillin molecule.

And then other natural product biosynthesis this is also one sort natural product then the bio remediation that means the removal of some toxic part from our system and related to disease states also and in some anti-cancer drug thing where you have the drug bleomycin which can only be activated in presence of iron as your ferrous ion.

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So, the non-heme oxygenase page you just read it from your Wikipedia and the book of Crichton, okay. So, thank you very much for your kind attention.