Bioinorganic Chemistry
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Lecture - 31
Tungsten Enzymes – III

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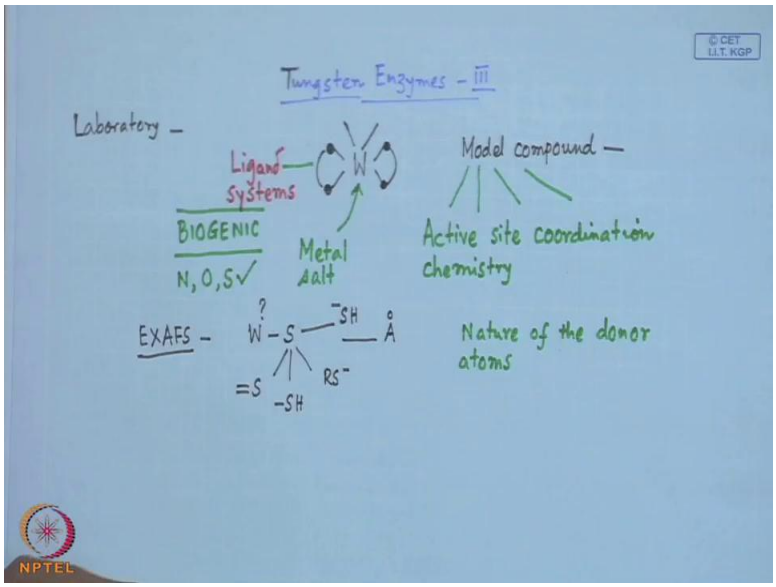

Hello, good morning everybody. So, today we are at the end of the part on tungsten enzymes which we were just discussing. So, part three we are just reached and today we will be taking about something which is closely related to any synthetic inorganic chemist. So, what we do in the laboratory basically. So, what we basically mimic some of these centers where we know that tungsten is there, and which is some environment and if we can model that environment in the laboratory for certain modern systems such that we can explore the two of these sites. If we just consider that these two are blocked by the ligand, so how? Because the tungsten has tremendous role to play based on the metal ion character on the basis of the available oxidation states, and if these two sites we can produce in the laboratory in a model compound.

So, we will have in our hand the model compound, and that model compound can be assessed starting from all other spectroscopic technique including the X-ray structure determination to know that how these model compounds can be similar to that of the active site; that means the site what is present in the enzyme. So, basically what we will be looking for that is the active site coordination chemistry based on the environment

around the tungsten. So, there are several points to know apart from the salt what we can use that what metal salt we can use to get the corresponding model compound and what are the ligand system. So, in direct information's what we can have in our hand about the nature of the ligand system that these two atoms which are directly bound to the tungsten site are sulfur sites.

So, they are of biological origin. So, we simply talk to them as they are biogenic in nature. So these biogenic ligands, they can be mostly supplying the donor atoms like nitrogen, oxygen and sulfur. And in this particular case, the sulfur has been identified very nicely not only from the protein excess structure determination but also from others spectroscopic evidences such as x-ray absorptions fine structure which is a very useful technique extended x-ray absorption fine structure. So, this extended x-ray absorption fine structure can tell us about the separation between the active site; that means the metal site and the immediate coordinating bond which is given by the sulfur atom.

So, that also tells us if we know roughly not exactly like that of our X ray structure determination, but this particular spectroscopic technique can give us something which is similar to that of our determination of bond lengths by x-ray structure determination is the tungsten and sulfur separation. So, this tungsten sulfur separation if it matches with the corresponding oxidation states of tungsten like tetravalent, pentavalent and hexavalent and the corresponding sulfur environment; that means we can have several sulfur groups we know that it can be double-bounded sulfur, it can be SH coordination, it can be RS minus thioether sulfur or it can be SH minus. So these basically the nature, all these information can give some idea about the nature of the donor atoms. So, nature of the donor atoms around the tungsten environment can be nicely modeled.

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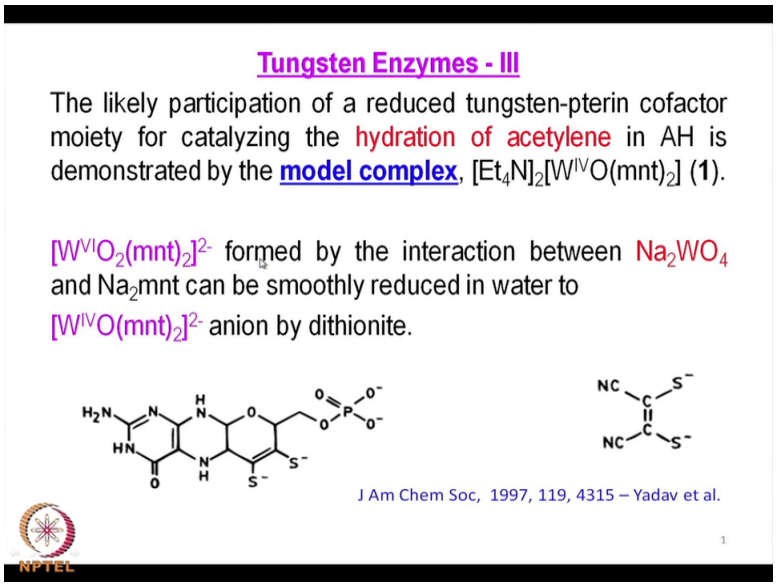

So, this modeling experiment. So, whatever scientific discovering we can have in our hand, we always try to have some model experiment. So, what basically this tungsten-pterin cofactor is giving us around the tungsten site as the coordinating ligand which we can find for the typical reaction which is a modeling of the reaction which is the hydration of acetylene which is a very simple reaction; one molecule of water goes to attach to acetylene molecule and acetylene we all know has a carbon-carbon triple bound. So, addition of water molecule which is been activated by the tungsten centre and that hydration of acetylene provides us the corresponding transform product as acetaldehyde.

So, in AH; that means acetylene hydrates, we can have a corresponding model compound which is oxo tungsten compound having two ligands; type ligands are abbreviated as mnt, mnt is nothing but maleonitriledithiol. So, maleonitriledithiol is providing two sulfur groups each from this particular unit. So, this is the basic backbone of this mnt 2 minus liagand. So, the ligand has a corresponding charge of two negative. So, basically it is a ligand L 2 minus. So, this L 2 minus ligand when attaching to the tungsten side and tungsten is there as a corresponding oxo group.

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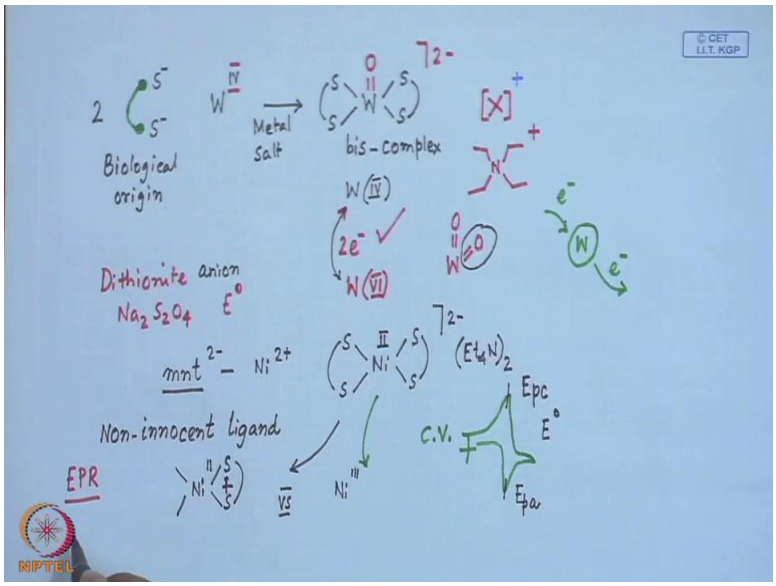

So, if we have this ligand. So, whatever we just abbreviating as the two donor atoms; so these donor groups on that particular maleonitriledithiol is nothing but S minus and this S minus. So, if two of these ligands are coming around a tungsten site which is tungsten four. So if two of these are coming, what we basically get? We get the corresponding bis complex. We call it as a bis complex because the metal ligand stoichiometry is 1 is to 2; one metal centre is attaching to two ligands. So, it's giving rise to the corresponding bis complex. So, we have this S unit and depending upon the metal salt what we are using because sometimes we have seen that some of these compounds which are very stable in their corresponding oxo form. So, metal salt can itself be some oxo salt such that when immediately this compound is forming we have the oxo tungsten compound. So, here two negative charge and two, two, four.

So, altogether six negative charges are accumulating on the tungsten when if the tungsten is in plus 4 oxidation state. So, we will have a negative charge of 2 minus on the complex species which can be balanced by some big cation. So, big cation is there some organic cation. So, some organic cation is there. We basically go for some useful cation. So, this is not X; this is some tetraethyl salt type of thing. So, basically is this particular one. So, it is a big cation, so tetraethyl ammonium cation. So, tetraethyl ammonium cation if it is there. So, that tetraethyl ammonium cation can go and attach to that particular anionic species. So, this is the cationic species and there should be some correspondence between the size of the cation and size of anion such that in the crystal

lattice good packing can take place and we can isolate the corresponding salt as tetraethyl ammonium salt of this particular compound.

So, very easily even in any small laboratory we can make this compound and this particular ligand what is coming and what has been identified from the biological origin. So, this S minus S minus has some biological origin because we know that these are the tungsten corresponding tungsten-pterine factor and this is the complex unit. So, this two are the corresponding tungsten-pterine unit. So, where we have the S minus S minus donor atoms on a complex base unit and you have the carbon-carbon double bond unit. So, backbone is there. So, interestingly the choice of this particular unit for biological tungsten centre in all these different tungsten enzymes one such is that of our acetylene hydratase. So, acetylene hydratase when we try to model it we must have the similarity of that particular part; that means we must have the S minus S minus donor groups on these two and not only that, but also we can have a C-C double bound in the backbone such that we can have a planar unit related to this particular coordination.

So, when this mnt 2 minus is giving us this particular salt. So, we basically get the model compound. So, very simply we can have the model compound related to the acetylene hydratase enzyme of tungsten. So, this particular compound when we get, so this is one form and also this particular one when O 2 and O 2 minus is this is another form; that means when we have this O 2 minus form this is the tungsten in the plus 4 oxidation state. If we go for the corresponding compound in tungsten 6 plus state, we must have another oxo unit to the same basic unit. So, this is the compound where we have tungsten in plus 4 oxidation state and we all know the catalytic system what we can have in the biological system also that this can settle between tungsten four and tungsten six. So, how we get this compound is very simple. So, it is a two electron oxidation or reduction step for this.

So, if this is oxidation and reduction reversible we have two electron transfer and this tungsten site also we can have a mono-oxo species and a dioxo species. So, when we have in the plus four oxidation state we have the mono-oxo species and that immediately activates by water or by dioxygen whatever it is to the corresponding dioxo form and this dioxo form can give rise to the corresponding model compound in plus six oxidation state. So, we have both the two complexes. Now we just want to explore that which particular bound is the catalytic species whether the plus four oxidation state; that means

the tetravalent tungsten centre is the catalytic site or the hexavalent tungsten site and immediately we can make this compound because in laboratory this ligand is very easy to synthesize and this particular ligand and when its reacts with sodium tungstate where Na 2 WO 4; sodium tungsten has tungsten in plus 6 oxidation state.

So, this is the salt what we can have with corresponding tungsten in plus 6 oxidation state. So, immediate reaction of sodium tungstate with that of our mnt ligand mnt 2 minus ligand or the corresponding species sodium salt of mnt sodium salt of maleonitriledithiol when reacts with Na 2 WO 4, it immediately gives the corresponding dioxo compound; that means we are not able to change the corresponding oxidation state what is present for the starting metal salt, reacting metal salt which is there as plus 6 oxidation state and that immediately goes through the incorporation of the two ligand units. So, this immediately reacts with Na 2 mnt and can be smoothly reduced in water to corresponding tungsten four by dithionite S 2 O 4 2 minus. So, dithionite is a very good reducing agent which is sodium dithionite is Na 2 S 2 O 4.

So, it has a corresponding strong reduction potential. So, here in this particular case if we want to go back from the starting compound; that means the tungsten prepared in plus six oxidation state using the maleonitriledithiol ligand, we use the dithionite anion as its corresponding sodium salt because this is a solid compound which is commercially available and this particular sodium dithionite can reduce immediately the corresponding tungsten in plus 6 oxidation state to tungsten in plus 4 oxidation state and the dioxo compound which was present there. So, dioxo compound will be losing one of this oxygen; that means the tungsten double oxygen bound. So, will be losing one oxygen to the reaction with sodium dithionite because sodium dithionite can react with this oxygen atom from this particular species to form the corresponding thiosulfate and sulfite anion and its corresponding reduction is shown.

And we are able to reduce the corresponding compound in that plus four oxidation state where the mono-oxo tungsten compound can be stabilized. So, this is a very smooth reduction reaction in water. We need the solvent basically in water and this reduction basically gives us the corresponding compound in plus 4 oxidation state and the reducing agent is dithionite because the corresponding redox potential between the tungsten in plus 6 and tungsten in plus 4 must match with the corresponding E 0 value of the

reducing agents. So we have the ligand, we have the metal salt and we get two such model compound for further study.

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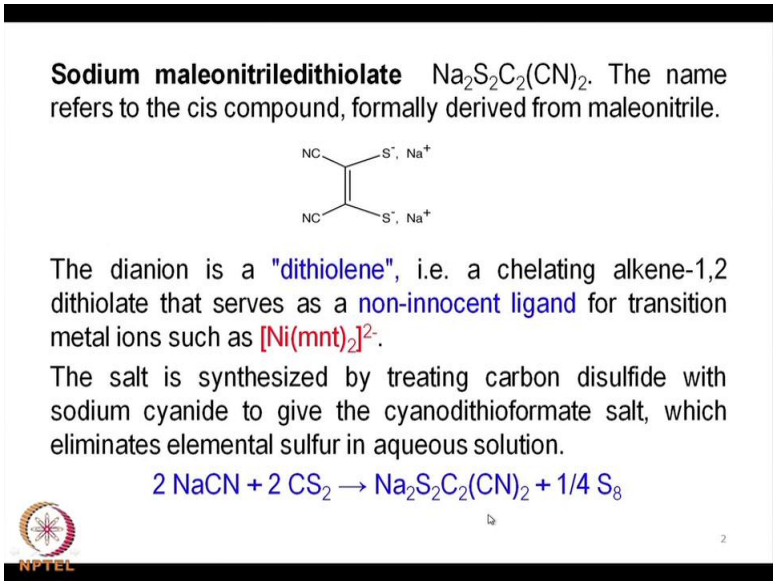

So, this particular ligand is at its corresponding sodium salt. So, sodium mnt sodium maleonitriledithiolate is a compound where we attach the dithiol unit on maleonitrile and the dithiol is a dithiolene unit because we have the C-C double bound and two sulfur groups as S minus S minus. So, alkene-1, 2 dithiolate and it serves as a very good non-innocent ligand for transition metals such as nickel mnt 2 minus. So, when we have these mnt ligand mnt 2 minus, not only its reactivity with that of our tungsten metal ion, but it is well known as well as well established for the typical coordination and chemistry research that it can bind very nicely with the corresponding nickel salt as well. But while binding the nickel salt, we all know that nickel will have no preference for the corresponding oxo formation or any other species formation immediately. So, around nickel it basically behaves as a simple bidentate ligand.

So, S S type bidentate ligand is there and two such ligands are providing altogether four negative charges and nickel is in the common oxidation state; that means the plus 2 oxidation state. So, overall charge on the complex would be 2 minus and like that of our tungsten compound what we have seen just now that the tungsten compound can be crystallized, can be isolated as precipitate from the solution as its corresponding

tetraethylammonium, tetrabutyl or any other bulky cation salt. Similarly this can also be isolated as the corresponding tetraethyl ammonium salt.

So, we require two such tetraethylammonium cation to isolate the corresponding bis compound having a formulation of Ni L 2 in the solid state, but this ligand has some interesting property to know these are also known as redox non-innocent ligand. So, these are redox non-innocent ligand which we all should know and they respect to these ligands because whenever we are taking some electron transfer because all these systems starting from what we know in our some pervious classes as the electron transfer proteins and those electron transfer proteins are there and they show some reactions where those reactions are mostly metal ion centered; that means the metal can be oxidized or it can be reduced back. What is happing here also for these different tungsten enzymes that we are looking our attention we are just focusing our attention on the metal centre; that means the tungsten center and this can accept electron or it can donate electron to some other system.

So, it has some stability for two other oxidation states where the centre can go up for one electron oxidation and go down by one electron reduction. So, in the similar situation these compounds can be synthesized in the laboratory again. And if we just go for the corresponding oxidation and reduction reaction and one typical techniques laboratory techniques is the corresponding cyclic voltammetric measurements. So, if we can have the corresponding cyclic voltammogram and the electron transfer can be identified by the corresponding redox wave which is as known as the corresponding cyclic voltammogram. So, if this cyclic voltammogram is available from there and if we can notice that at one particular potential it is getting oxidized and another particular potential it is reduced. So, if the oxidation is our E p a, the anodic peak potential, the reduction is the E p c the cathodic peak potential. And average of these two basically is represented as is corresponding E 0 or E half value.

But interesting thing is that if both the ligand centre as well as the metal centre because the delocalization through this ligand is such that it is very difficult to go for some selective oxidation from the metal orbital or from the ligand orbital because they are very much overlapped and covalency of the system is such that we have the corresponding metal centre orbital's the molecular orbital's if we talk in terms of the corresponding molecular orbital's, the orbital's which are available on nickel and what are available on

sulfur when they are forming the corresponding molecular orbital's, they are having the nature which is closely related to the nickel orbital as well as the sulfur orbital

So, during the oxidation whether these oxidation this particular oxidation; that means the value of this E p a whether we are going for some oxidation on the metal centre. If the metal is getting oxidized, we should get a corresponding compound of nickel in plus three oxidation state and another alternative for that is if the ligand is getting oxidized. So, nickel will stay as nickel in plus two oxidation state and the ligand what is there. So, ligand will be reduced as its corresponding anion radical. So, these two situations that means versus this, this versus this. So, these two situations can be very easily identified if we just look at the corresponding EPR spectrum. So, if we the corresponding electron paramagnetic resonance is there in our hand and we get the corresponding EPR spectrum for the oxidized form, whatever oxidation we can do we can go for the electrochemical coulometric oxidation as well as we can go for the corresponding chemical oxidation by using some oxidizing agent good oxidizing agent.

So, this particular trivalent nickel if the oxidation is metal centered; that means the oxidation is taking place on the nickel and within the species what is formed in the solution because this particular thing is basically a solution technique and we are talking all in terms of the corresponding solution because whatever reactions whatever reactivity patterns we are looking for all these metalloenzymes those are basically in solution. So, this particular trivalent nickel has one character as sticky peer spectrum which is different compared to that of the spectrum what is present if we go for the corresponding radical species on the organic part; that means organic ligand part which is providing the corresponding anionic radical or the cationic radical if we just go for the corresponding oxidation. So, if this is oxidation we go for the corresponding cationic radical on the ligand and if we go for the reduction we get the corresponding anionic radical.

So, this can be cationic radical or the anionic radical. So, the radical can be radical versus the corresponding typical trivalent nickel can be distinguished by measuring the corresponding EPR spectrum. So, that basically tells us what is known as the corresponding redox non-innocent ligand; that means during oxidation whether it is an electrochemical oxidation or a chemical oxidation, the ligand can be oxidized or reduced back from one oxidation state level higher or lower. So, use of this maleonitriledithiolate salt of sodium, we can prepare very nicely by using carbon disulfide with sodium

cyanide to give the cyanodithioformate salt which eliminates elemental sulfur in aqueous solution giving the corresponding sodium slat of maleonitriledithiol.

So, we have the CS 2 backbone and this CS 2 backbone basically after coupling giving rise to the corresponding C-C bond and this C-C bond formation is taking place during the preparation of this sodium dithiolate ligand and the cyano groups are attached to this particular carbon which is already present to that particular system and we have this carbon and sulfur from the carbon disulfide and CN is also attached over there. So, we get the corresponding species with the elimination of one of this sulfur which is coming out as elemental sulfur.

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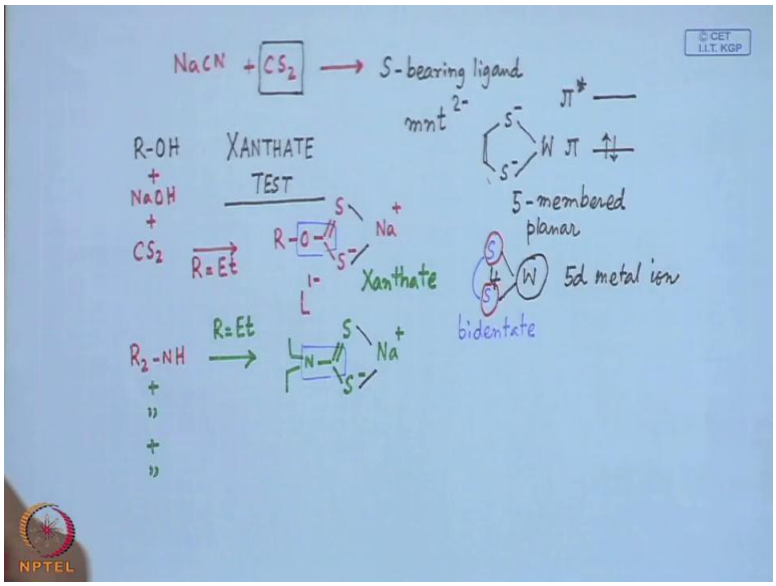

So, this particular technique by using a typical salt inorganic salt sodium cyanide and carbon disulfide; so the reaction of these carbon disulfides are very useful and we can have several such ligand systems; that means we will get something where we can have some sulfur bearing ligand. So, we basically get a sulfur bearing ligand. So, in this particular case we get maleonitriledithiol dinegative anion which is S S bidentate ligand. So, having in backbone which is little bit robust and due to the presence of this double bond, it is a redox non-innocent ligand because which can be very easily oxidized or reduce because we have this pie electron density. So, whenever we have this pie level present in the ligand system, we must have the corresponding pie star level also available

and due to this pie level we have the electron unit and this pie level we can have the electron.

So, we can push one electron in the pie star level or we can take off one electron from the pie level such that we can go for the corresponding oxidation state of the maleonitriledithiolate ligand. So, two other interesting reactions based on carbon disulfide is also useful to know at this context that the reaction of this carbon disulfide how easily and how nicely give some sulfur bearing ligands which we all know from our school days; when we first tested the presence of alcohol, the alcohol is R OH if it is methanol or if it is ethanol and the presence of this alcohol can be justified in a test-tube or in laboratory by doing the reaction and that reaction is well known as the corresponding XANTHATE test and that XANTHATE test is nothing but we do the reaction in basically by the use of corresponding sodium hydroxide or potassium hydroxide; that means the base and the same reagent; that means carbon disulfide.

So, what we get from that reaction is a yellow product if we go for the corresponding reaction on ethyl alcohol; that means R is equal to ethyl group. We get the corresponding system as ethyl xanthate which is again a corresponding salt and if when we use sodium as the corresponding base sodium hydroxide from the base. So, we basically get the sodium ethyl sodium salt of ethyl xanthate. But here the incorporation of two such sulfur atoms are different compared to the incorporation of sulfur atoms in maleonitriledithiol that these two sulfur groups what are already present in carbon disulfide unit is still present in this and this particular one can function also as a very good ligand, because it has L 1 minus charge. Similarly another reaction on the amine; that mean, if we have R 2 NH instead of R OH; that means some secondary amine which has a very good reaction yield for the same reaction; that means the same base is given as carbon disulfide.

In this particular case whatever thing we get, we get as a corresponding xanthate; that is why the reaction is also know as a corresponding xanthate test. So, xanthate salt is prepared and here what we get; that means if R is equal to ethyl, you get this one as its corresponding sodium salt. So, these two has some similarity here we have only that corresponding bond differences, differences in the bond backbone. Here in this particular one, the carbon disulfide is forming a bond. So, we have a C-O backbone and here we have a C-N backbone. So, this is the only difference; otherwise these two ligand systems are same, but the electronic structure should be definitely different because the nature of

these two bonds are completely different for the carbon oxygen bound and the carbon nitrogen bound but like that of our maleonitriledithiol these has also two sulfur donor groups.

So, this can also function as a very good bidentate ligand. So, this is also a bidentate ligand, but the nature of this bidentate ligand is completely different compared to that of our bidented ligand what we get from maleonitriledithiol because when these two sulfur atoms, just now what we have seen, is forming coordinate bound to the tungsten centre to get the corresponding model tungstoenzyme system. So, this is one, so but this is you have a corresponding C-C double bound in the backbone and we have a 5-membered chelate ring. So, this is basically giving us a corresponding 5-membered chelate ring and we have a typical planar arrangement is not a 6-membered one but 6-membered if we get that is also distorted from the planarity and it is also a procured one, but this is much more stable and which is highly planar. So, 5-membered planar ring is also assured due to the backbone with bearing the corresponding C-C double bound.

So, 5-membered planar backbone which is important but in this particular case if these two sulfur atoms from xanthate and two sulfur atoms from the corresponding diethyl carbonate is involved in binding, say, with that of our tungsten or any other metal ion, we get a different type of bonding where we have a 4-membered ring having no double bond backbone. So, it is not necessarily that it will be highly planar to that of our 5-membered ring, but the 4-membered ring is not all the time stabilized by some groups. But in this case if we just go for the corresponding tungsten compound because the tungsten is a 5d metal ion. So, due to its nature; that means is a 5d metal ion, its ionic size in plus 4 or plus 6 oxidation state is bigger and this can show interaction with the bigger donor atom. The sizes of the sulfurs are comparatively bigger than that of all oxygen and nitrogen.

So, you have bigger sulfur atoms and these two bigger sulfur atoms can go and bind to the tungsten centre, but the corresponding compound the type of the compounds are completely different. So we should think of that, that though we have the same sulfur-sulfur coordination around tungsten like that of our mnt coordination, but we can have some different reactivity pattern of these compounds. So, nature of this ligand basically, so the ligand basically dictate the type of compound what we can have whether that particular compound you can so ask the corresponding enzymatic reactivity or not, that can be very easily charged if we have the corresponding ligand system.

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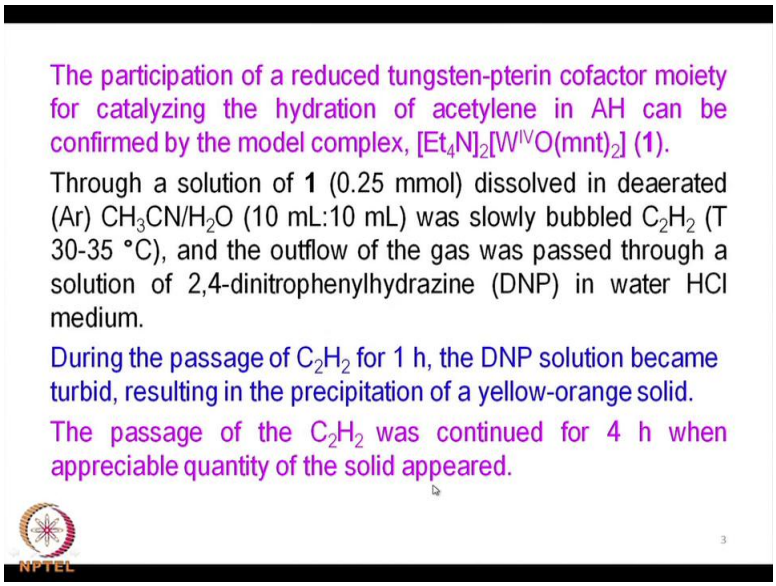

So, this maleonitriledithiol when we make that and we want to see the corresponding reaction; that means what reaction we have the model compound in our hand and we can go for the corresponding model reaction.

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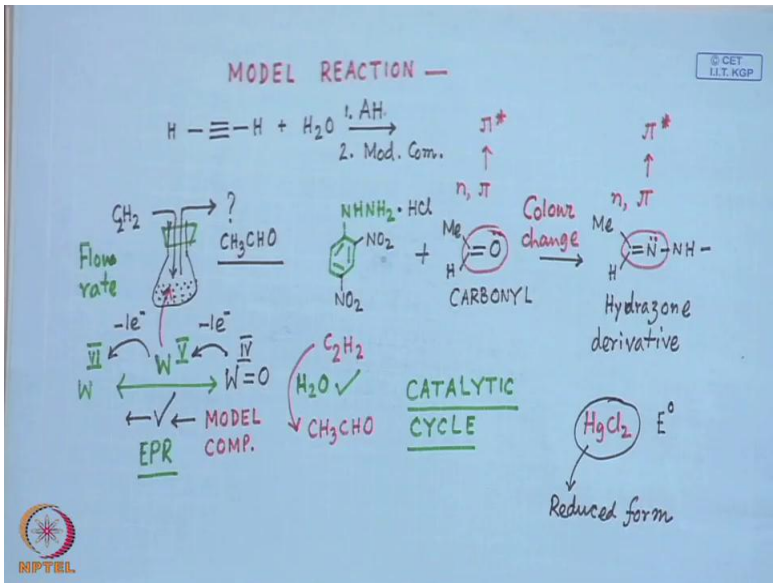

So, what is that model reaction? It is the model reaction is targeted for some specific enzymatic reaction. So, in this particular case the model reaction is the corresponding hydration reaction on acetylene. So, we must have to have some mechanism where this water molecule can be activated by the corresponding tungsten enzyme. So, tungsten

enzyme is our acetylene hydratase which is responsible for the hydration of the acetylene group by the water molecule. So, the ligand system what is present for the enzyme is the tungsten-pterin unit and which is catalyzing the H, so the model compound if we can take and in that particular oxidation state; that means the plus four oxidation state if we can go for the corresponding reaction of this with acetylene in presence of water.

So, we have the three things; we have this instead of this acetylene hydrates, we just take because the reaction is known for acetylene hydratase and we go for the model compound which is the maleonitriledithiolate compound of tungsten. So, this is the compound one which we level here. So, what we do that is a typical experimental setup for this reaction, what we just go for this we get from this particular reference is a journal of a American Chemical Society reference and in that particular paper, the people who had showing this particular acetylene hydratase reaction, they took the solution of this one this compound is a anionic compound because the complex part is anionic which is stabilized by the cationic ammonium salt and which is taken as in a one is to one acetronitrile water mixture which is deaerated; that means oxygen is not there by argon.

So, argon gas is passed through the solution and through purging this argon gas through the solution we are excluding the presence of dioxygen for the change in oxidation state of the tungsten or the change in a corresponding oxidation state of the non-innocent ligand and also the corresponding activation of the dioxygen molecule for insertion into the acetylene backbone. So, through deaerated system; that means in inert atmosphere what we can see because this is also deaerated because we are using water but it should be dioxygen free because that can immediately establish the corresponding role of the water molecule for this hydration reaction. So, this hydration reaction is typically dependent on the water molecule present in the reaction medium.

So, what we do within the solution where this compound is present in solution aqueous acetronitrile is the medium and we slowly bubble acetylene gas because this acetylene is a gas and in-house; that means in the laboratory, we can generate the bubbles of acetylene by reacting calcium carbide with water and this acetylene gas at a temperature of 30 to 35 degree not very much higher than that of our room temperature because the room temperature in most of the cases it ranges between 25 to 28. So, it is slightly higher than that of the common laboratory room temperature at 30 to 35 degree and what is happing there, just we have bubble of this acetylene gas and at the outlet the gas is

coming out from the system. So, if we have a corresponding reaction vessel for the typical one is the corresponding gas inlet and another is the corresponding gas outlet.

So, acetylene as the inlet and this we can have the compound in aqueous acetronitrile water. So, what is coming out that has to be tested. So, testing of this particular gas because this may not be in the gaseous phase also because this particular acetylene if we cannot control the corresponding flow rate the flow rate of purging this acetylene gas is important because this particular acetylene gas can then be the typical carrier gas for the product also. So, in the outlet, so outlet what is coming out that the gas after the reaction is coming out from this. So, we can just sometime we can just cock the system for some time and then we can take out the gas by purging more acetylene into the reaction vessel. So with the carrier gas, carrier gas is our acetylene. Some of the product gases in the gaseous form is also coming out. So, that outlet is basically then passed through a solution of 2, 4-dinitrophenylhydrazine; that means 2, 4-DNP in water HCl medium; that means 2, 4-DNP hydrochloride because this is a well known reagent for everybody from our school days.

We know this particular reagent which is nothing but a hydrogen derivative which is phenyl hydrogen we all know. So, this phenyl hydrogen when have substitutions at position two by nitro group and position four we get 2, 4-DNP. So, this 2, 4-DNP and due to its poor solubility in water medium, we can put some drops of hydrochloric acid or the whole system; that means 2, 4-DNP is available as its corresponding hydrochloride salt which is highly soluble in water. So, this particular reagent is well known for going some adhoc reaction with some ketone or aldehyde; that means if we have a carbonyl function in water medium; that means in some solvent as water. So, the carbonyl function if it is present and this one is also in solubilized form of water in the hydrochloride form, this immediately goes to react with this particular carbonyl function to give some color reaction.

So, if we are able to produce this acetylene to some carbonyl compound; that means if we are able to transform; that means mimic the reaction of the corresponding acetylene hydratase which is shown by the tungsten enzyme in the laboratory by a model tungsten compound of maleonitriledithiol, we see that acetaldehyde what is coming out from acetylene. So, this acetaldehyde immediately reacts with the 2, 4-dinitrohydrazine reagent. In this particular case this is H and this therefore M e is again a carbonyl

compound and which is forming N NH; that means corresponding hydrazone derivative. So, we basically get the corresponding hydrazone derivative from the system and this is insoluble in water all say very less soluble in water. So, it immediately separate out from the reaction medium and it basically indicated that whether the carbonyl function has been generated from this reaction is the corresponding color change due to this reaction.

Because whatever change are taking place there is that we have the carbonyl function C double bound O which is getting changed to some C double bound N in function. And this carbonyl function has a typical pie to pie star transition at one particular wave length, but in most of the cases they are in the ultraviolet region; that is why this carbonyl function is colorless in nature but this particular having a small energy gap; that means we can go towards the visible range for this pie-pie star or sometime the n pie star transition is also available. So, if we have the non-bonding level; that means we have the lone pair of electrons on these nitrogen's on these oxygen's. So, if lone pair of electrons is also involved for transition, we get the corresponding n pie star transition and we see the corresponding color change for this reaction. So, any color change or any separation of the solid material from this particular reaction can be identified that the activity; that means acetylene hydratase activity has been established through the model reaction.

So during the passage, when we pass the acetylene gas for one hour because this conversation rate for this transformation from acetylene to acetaldehyde is not very fast. The D N P solution slowly becomes turbid and the turbidity is due to the formation of the corresponding 2, 4-dinitrophenylhydrazone derivative which is caused due to the precipitation of a yellow-orange solid. So, this 2, 4-dinitrophenylhydrazine as well as the acetylene giving us the acetaldehyde they are all colorless. But due that hydrazone derivative or due to the formation of the corresponding C double bound N; that means the amine bond on the molecule, we get slight color change from the ultraviolet region to the visible region which is yellow-orange in color and that immediately confirms that we get the corresponding acetaldehyde.

So, not only one hour the passage is continued for this bubbling of acetylene gas for four hour when appreciable amount of solid appeared which was earlier has a turbidity; that means turbidity means very small particles are formed initially and which are highly distributed throughout the entire solution. But when more and more hydrazone derivatives are formed, they go for agglomeration and bigger particles are formed and

those bigger particles are basically ultimately heavier than the solvent medium, heavier than the solution medium and the solid particles are generated from there and those solid particles are precipitating out from the medium.

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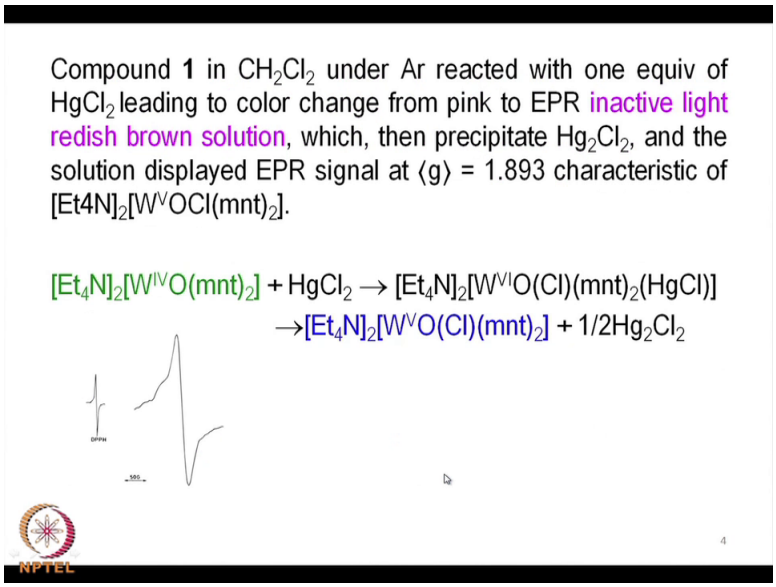

So, when we get this particular reaction it is our need that we have used certain compound of tungsten which is in plus four oxidation state and definitely this is a mono-oxo compound. So, we just took the corresponding mono-oxo derivative of the model compound. So, this model compound what we can have in our hand is the mono-oxo derivate and neatly we just go for it. So, if this is the involvement of this mono-oxo compound for the transformation of acetylene to acetaldehyde, we should be able to talk little bit about the corresponding catalytic cycle. So, what is that catalytic cycle for this particular transformation? So, to know that catalytic cycle we see that definitely the water molecule is attached. So, it is showing the corresponding acetylene hydratase reaction, but how this particular species is changing; that means how the activation is taking place through a mono-oxo species to a dioxo species; that means how this particular water molecule is getting activated.

So, activation of this water molecule by the tungsten species is important to know and whether this particular oxidation state is shuttling between this tetravalent to the hexavalent state with the involvement of one more oxo form; that means one over oxo atom attached to the tungsten. And the involvement of other intermediate oxidation state;

that means if it is going from tungsten four to tungsten six and as an intermediate step whether the tungsten five is involved there or not. Why we want to know or charge the involvement of tungsten 5 is very simple, that if we just go for this 4 to 5; that means it is a one electron oxidation step and 5 to 6 is also one electron oxidation step. But this entire reaction whatever reaction we get from here is also a 2 electron transfer case and if this 2 electron transfer is not taking place in a single step; that means we are not jumping from a tungsten in plus 4 oxidation state to a tungsten in plus 6 oxidation state, definitely we get these in a stepwise manner; that means transfer from one step and transfer from other step.

So, we definitely should be able generate the intermediate oxidation state. So, this intermediate oxidation state whether this can go for making this particular pentavalent state of tungsten as the most catalytically active species. So, if the catalytically active species which is not very much stable has a very transient lifetime, the lifetime of tungsten five is also not because at the intermediate state is confirmed to a tungsten five but which immediately collapse to tungsten 6 with the removal of the product molecule. So, that how we can understand? With this we can again understand by knowing the corresponding E P R signature. So, if the tungsten pentavalent state has a characteristic EPR spectrum, we can identify in the solution state while bubbling these all these things; that means whether we can generate the tungsten 5 in the solution, whether this tungsten 5 is being generated within the solution.

So, we take out because we are going for the reaction in a slow phase; that means sometime we go for one hour studying and then it can continue for a 4 hours time for appreciable amount of precipitation of the corresponding hydrazone derivative. So, for that purpose if we take out some adequate amount of this particular intermediate species from this and we can run the corresponding EPR spectrum, then only we can try to identify the presence of tungsten in the pentavalent state. So, that signature is very important to identify from the reaction medium. So, when this is reacted with this particular one. So, not that we are going for the electrochemical reduction or we can go for some chemical reaction., so any other oxidizing agent like that of mercury chloride can be used to oxidize this particular species through some intermediate formation of this tungsten 5 oxidation state.

So, that we will just discuss next day in detail that how we can use this particular reagent because this mercuric chloride is well known for its corresponding oxidizing ability. It has a typical E 0 value for oxidation and when this as a reagent is added to the tungsten corresponding tungsten four species, we get some intermediate species where the tungsten is interacting with that of our mercury centre. And next step this particular oxidizing agent is being released as its reduced form and we generate some species as tungsten in the plus 5 oxidation state and that plus 5 oxidation state we should be able to detect by EPR techniques. So, what should be the nature of the corresponding EPR spectrum and how we can identify that EPR from the solution by using the corresponding EPR spectrometer; that we will see in our next class.

Thank you.