Bioinorganic Chemistry
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Lecture - 28
Tungsten Enzymes-I

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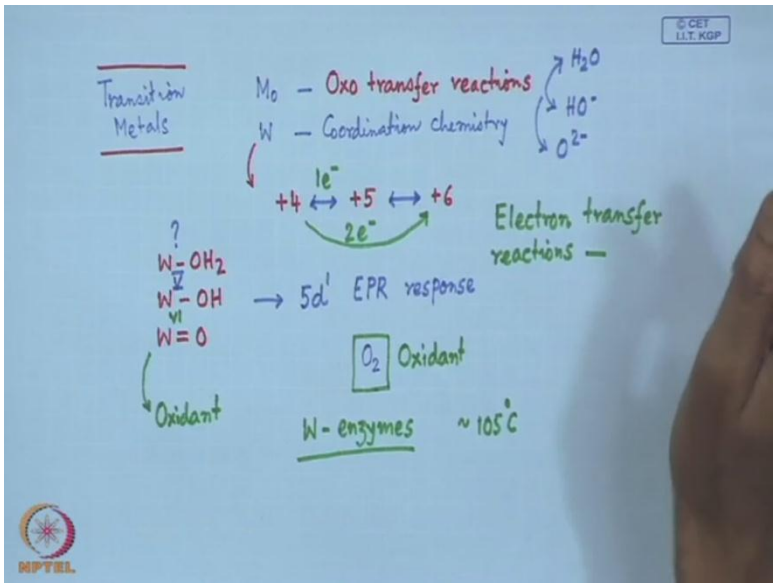

Good morning. So, we will just now we will just continue from the series what we are seeing the transition metals. So, these are we have seen their role in biology the molybdenum then we have seen how both molybdenum and tungsten do participate in biology. And little bit we have discussed about molybdenum and so also with tungsten we will discuss about their coordination chemistry. So, how does this part of the inorganic chemistry can help us to understand the very complex biological reactions, because incase of the other congener of the group that means the molybdenum. We have seen it is mostly dominated by the oxo transfer reaction, where the coordination chemistry is also mostly dominated by the formation of the oxo molybdenum complexes, So, this part of the reactions in biology.

So, these second group and second row and third row metal ions the molybdenum and tungsten when do participate. But they are not much abundant in the biological system only few examples are there, where the tungsten center can play some important role in all these systems. And where these like molybdenum can move from an oxidation state of plus 4 to plus 5, to plus 6 that means when we consider this we can immediately think

of that there is some electron transfer between these two oxidation state and also these two oxidation states. So, what we see that along with this oxo transfer reactions which are mostly dominated by the reactivities with water, hydroxide ion as well as with oxo anion.

So, these groups basically following simple deprotonation they can give rise to the hydroxide group or the oxido group, which can immediately go and bind to that of your tungsten center to give us a tungsten aqua bond. Initially which can further go for deprotonation to give us tungsten hydroxido bond or tungsten oxo bond? And during all these transformations sometime we do not have any control over the oxidation state on the metal ion, whether the tungsten is in plus 4 oxidation state or plus 5 or plus 6 is very difficult to predict. So, we always like that of our molybdenum part we have seen that we rely on some of the useful spectroscopic technique such that if this particular 1 is in plus 5 oxidation state. And if we have 1 unpaired electron on that so, if that is in the 5 d 1 level so, we do expect to have some E P R responses.

So E P R response will immediately tell us even in the solution in a very dilute condition where, the concentration of this tungsten center is very less. Because it is surrounded by huge protein molecule, but this center which is having 1 unpaired electron in 5 d level can show some response for its characteristic E P R signal. So, leaving this inter conversion when we move from aqua complex to an oxo complex we basically go from a plus 4 to plus 5 to plus 6 oxidation state. So, along with oxo transfer reactions what we expect that there is also some electron transfer reactions, electron transfer reactions can also take place with that of the tungsten center. So, during that electron transfer we can have a single electron transfer, or we can straight away a case where we can go for 2 electron transfer. So, we can have the catalytic processes which can be either a single electron transfer case or a double electron transfer case.

So, very simple or very use full electron transfer reactions we can have, which we can get simple for this oxidation by air that means the oxygen, dioxygen present in air can go for some of these transformations. But in the biological system we require that means activating this dioxygen molecule is not so easy in the biological system. So, we there take the help of the water molecule attached to the tungsten center for these electron transfer reactions. Otherwise a normal laboratory environment we expect that some of these transformations are simply catalyzing by the presence of dioxygen molecule.

So that case the dioxygen is the most useful oxidizing agent or oxidant what can be acting on the substrate molecule. But in these cases if this tungsten center is in plus 6 oxidation state that means the hexavalent tungsten center which is attached to some oxo center is now the oxidant. For all these biological transformations instead of the simple dioxygen molecule which is available in the air.

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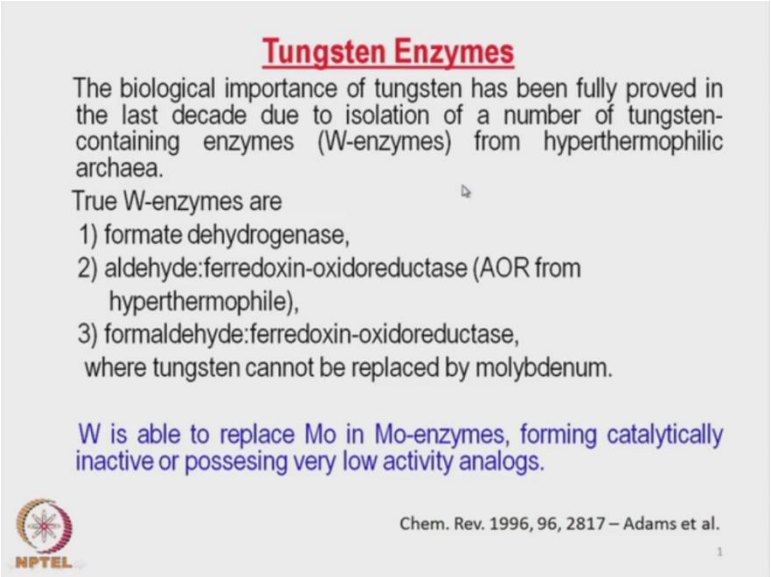

So, in all these tungsten enzymes slowly one after another will discuss, little bit on that because the, we have discussed already the molybdenum enzymes in detail. So, is a very recent phenomenon, So, only during the last decade or last 15 years people could isolate a number of tungsten containing enzymes these we call it as tungsten enzymes in hyperthermophilic archaea. So, hyperthermophilic archaea means that these are very stable towards temperature, So, all these tungsten enzymes are thermally very much stable.

So they do not degrade in the range of say 105 degree centigrade they do not degrade, because otherwise we know that the biological systems what we can have in our body which is only living till we have the corresponding temperature range of our body temperature, which is 37 degree centigrade. But in case of this tungsten enzymes they are very much stable till the temperature is reaching 100 degree centigrade, So, this particular groups they are known as the hyperthermophilic archaea. And these enzymes

the first one is of this category is the formate dehydogenase, when the formate the substrate is the formate anion and which is informed in the redox transfer reactions.

So, formate is sometime getting oxidized to carbon dioxide molecule what we have seen in case of molybdenum centers also that means, the molybdenum enzymes. Then another group of molecules which are very important that means we are talking something which is nothing, but the oxidoreductase family. That means the reversible oxidation and reduction reaction can take place which is mediated by the ferredoxin molecule and which is acting on the substrate aldehyde.

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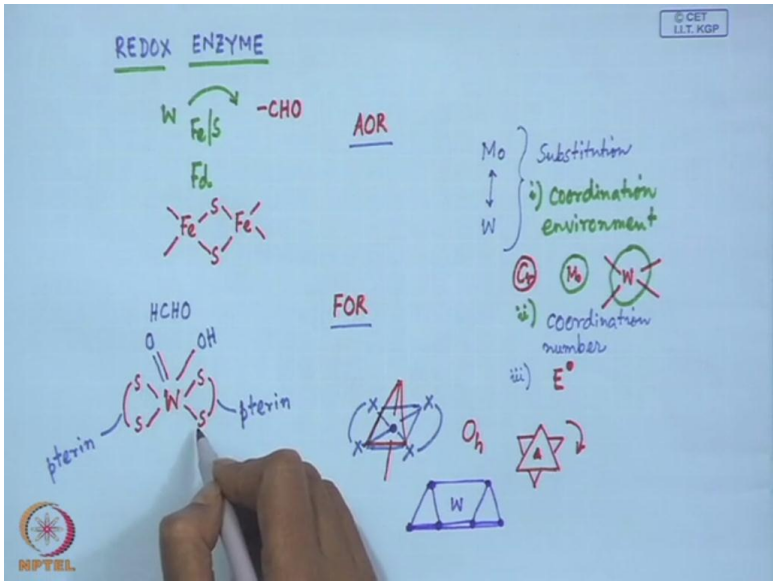

So, is a little bit complex arrangement where we have the redox enzyme, So, what we have in our hand is our redox enzyme. So, as soon as we have some redox enzyme in our hand we obviously can think of that there should be some electron transfer from that enzyme part. But in case of these we have that enzyme which is tungsten centered, and this tungsten centered functioning as a redox enzyme which is then under the category of oxidoreductase family. But which is coupled with some iron sulphur protein molecule which we consider as the ferredoxin molecule that means at least we should have 2 iron centers connected to 2 sulphur groups.

So the basic unit what we can have which is not a rubidoxin unit, but is a ferredoxin unit, so, at least a dimeric unit if not a tetrameric one which is the dimmer of dimmer thing. So, this ferredoxin molecule is basically can give rise to the electron to the system. So,

the electron transfer is mediated through the ferredoxin molecule and we have the aldehyde as our substrate. So, it is working on the aldehyde substrate mediated by the iron sulphur mediator and tungsten is the corresponding catalytic site and another group is that of your formaldehyde ferredoxin oxidoreductase.

So, in 1 group it is acting on aldehyde that means any other aldehyde not that of the formaldehyde. So, it is aldehyde oxidoreductase again from it is a thermophilic bacteria archaea. So, this again belongs to the hyperthermophyl. So, aldehyde oxidoreductase it belongs to AOR family. But the other group which is very much specific on formaldehyde that is why it is labeled as it is very much specific for the substrate which is formaldehyde not any other aldehyde like acetaldehyde or any other group. So this formaldehyde when acting on this particular tungsten enzyme which is belongs to the class of again oxidoreductase and again ferredoxin mediated. So, this formaldehyde 1, So, we have aldehyde oxidoreductase which is 1 family, the AOR family; and when we have the formaldehyde oxidoreductase we get FOR family. So, FOR family is little bit different when we have the substrate as formaldehyde.

So, this FOR family and AOR family the reactivity wise or the source wise sometimes initial isolation was difficult, and once people have isolated that then they went for the corresponding identification of the center as the tungsten 1. So in case of FOR family we cannot replace this particular tungsten center, so tungsten is very much needed for their reactivity. Because the reactivity wise these group of enzymes, the tungsten enzymes are very much similar to that of our molybdenum enzyme. So, if we can isolate some of these native enzymes from the biological sources different biological parts we can take, and those biological sources can be checked whether we can substitute one particular metal center by the other.

If the tungsten enzyme can be replaced by the molybdenum enzyme, because we want to check whether the tungsten is needed for their absolute reactivity for the corresponding transformation or molybdenum can have a different catalytic role or molybdenum can be more reactive. But in this particular one where we truly speaking that they are the true tungsten enzymes and this tungsten cannot be replaced by molybdenum that means tungsten metal ion is very tightly bound to that particular center. And once tungsten is replaced by molybdenum the reactivity for that particular center is also lost.

But in some other cases we can replace this molybdenum centers in different molybdenum enzymes, So, these are the bigger metal ion compared to the molybdenum 1 this is a 4 d element this is a 5 d element. So, 5 d element can go and remove the molybdenum and take that particular position, but during that transfer we get basically a catalytically inactive species which is not active at all and which is not reacting on the substrate if the substrate is our formaldehyde or acetaldehyde. But some other cases if it shows little bit of that reactivity it shows very low activity analogs.

So the substitution of these that means whether we can substitute a molybdenum center by a tungsten center and we just basically check what is the effect for that substitution. So, metal ions substitution is a well-known practice for this bio inorganic aspects of the different enzymes, because sometimes if want to know the immediate coordination environment. And that particular environment, do change because we are changing 1 particular size of the metal ion from molybdenum to a bigger 1 which is tungsten. So, definitely not only the coordination environment, but also the corresponding coordination number might change. So, in that particular case the reactivity pattern is also changing, if we go for substitution of 1 metal center by the other.

So coordination number is also changing, and another important thing which we always should be careful in knowing that thing whenever you go for changing this thing is the corresponding redox electron transfer that means the E 0 values. The E 0 values what we measure for 1 particular center for based on molybdenum to that of our tungsten. So, if that center is on molybdenum and this center is on tungsten, then E 0 values are also different which is well known to us that. If we go down to a group this particular center is weakly oxidizing compared to the other congeners in the 4 d and 3 d c. Because in the 3 d c is we have the chromium on the left, So, whenever we have the chromium which when we stabilize it as in terms of the corresponding oxo group. That means the chromate and dichromate anion which is highly oxidizing compared to the corresponding molybdenum oxo unit then which is again less oxidizing compared to that of our that tungsten center.

So, if you should compare always the E 0 values what we get, because all these reactions are dependent on the corresponding E 0 values and the corresponding thermodynamic stability of the corresponding substrate and the substrate which is giving the corresponding product. So, this low activity analog will basically get when we just

simply change the corresponding center from that of tungsten to molybdenum, or molybdenum to tungsten.

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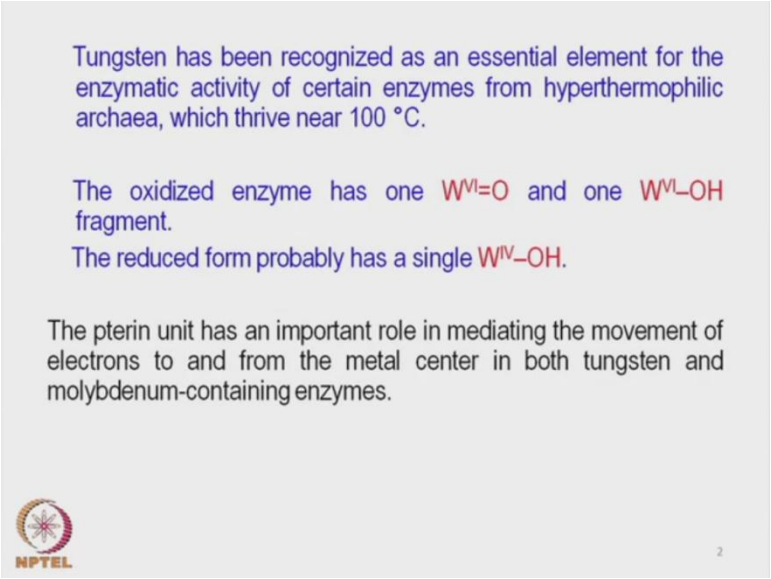

So is essential element in all these enzymatic activity and again we are talking that the hyperthermophilic archaea which thrive near 100 degree centigrade. So, which basically stable and So, can activity till we reach a temperature range of 100 degree centigrade. And what I just now told you that the oxidized enzyme has at least onemolybdenum, tungsten oxo unit having the tungsten center in hexavatent state. And 1 of the is that tungsten in the same oxidation state, but attached to a hydroxide unit. So, if we have the corresponding tungsten center, and if these 2 groups are attached to that corresponding pterin unit, tungsto pterin unit, which are the sulphur groups?

So, these are the sulphur groups so, 4 coordination sites are already attached and the size of these tungsten unit is little bit bigger. So, we have like that we are just covering a particular bigger ball or bigger sphere compared to the smaller 1. So, 4 coordination positions are already taken up and two of these positions are still vacant for the corresponding reactivity to the substrate or the other reagent what is coming out as oxo and hydroxide unit.

So if we see that this is 1 of them is oxo, So, the next 1 can be with the corresponding hydroxide unit. So, hexa coordination can be fulfilled around the tungsten center and depending upon that it basically settle between this water, hydroxide and oxide unit to

show the reactivity. And the pterin unit which is our source for this S S donor groups, the bidentated S S donor units which is involved from the pterin unit. And which has important role in movement of electrons to and from the metal center in both the 2 cases that means in case of tungsten as well as in case of molybdenum. This basically the pterin unit that means the ligand part So, these are our pterin unit if it is for tungsten, we call it as the tungsto pterin unit. If it is for molybdenum we call it as a molybd pterin unit. So, this pterin unit from both the 2 sites can hold basically the tungsten site for its catalytic reactivity.

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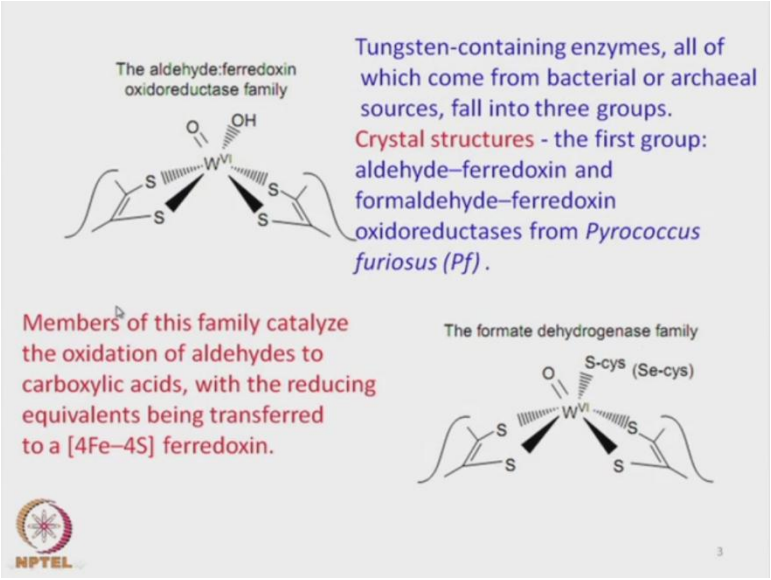

So this is the two, these are the two cases. So, in the first case, which is the corresponding AOR family? The aldehyde ferredoxin, oxidoreductase family; the first one. So, in the first case we have this particular aldehyde ferredoxin oxidoreductase family. So, you have this particular s s pterin unit so, is in diethyl unit. So, this 2 sulphur groups are the theoyl sulfur unit s minus, s minus and the back bone is little bit stabilized in the form of a double bound between these 2 carbon centers.

So is basically a tip little bit of planar unit, So, this entire part what is there is the 2 plus 4 plus 5. This 5 member ring is basically a planar unit with less distortion related to that of our carbon, carbon back bone which is a double bounded 1. Similarly, another group is also present on the right So, which is also from the same pterin is unit the pterin bidentated sulfur, sulfur ligand so, the bidentated S S donor ligand. So, these are

basically covering something because this basically giving some important coordination geometry which we are not getting unlike our first transition series elements.

Because whenever we find in case of iron, nickel, cobalt etcetera all if the metal center is present over there and if we have 1 donor group from here, another donor group from here, another donor group here and another donor group here. So, for the 3 d elements and which is not biologically connected, So, these are non-biological donor groups. So, non biological donor groups they basically prefer for again this if this particular individual unit that means if this particular ring whatever ring it is it can be 5 memberd ring or it can be 6 memberd ring.

But if this part is a planar 1 and depending upon the nature of the metal center we can have the square planar environment or if this particular 5 memberd ring or 6 memberd ring is perpendicular to the other ring we get a tetrahedral geometry. But basically it is settling between either a tetrahedral geometry or a square planar geometry. So, when two of these ligands are attached So, we go for the attachment of the fifth and 6 groups. But in this particular case the attachment of the fifth and sixth which is also true for the iron center which is present in our hemoglobin and the myoglobin molecule. So, with 1 of these groups will come from above the square plane and another will from below the square plane making a corresponding octahedral geometry. So, this octahedral geometry, what we sometimes draw in the form of the corresponding trigonal plane, because this is 1 of such trigonal plane and below we have another.

So, if we just look at the corresponding c 3 axis so being along the c 3 axis so, which is a threefold axis c 3 axis we get some arrangement where we have 1 trigonal plane. So, if we can move these 2 trigonal planes these are the staggered orientation in a typical octahedral geometry, these 2 planes are in staggered orientation. But this particular 1 can move from here to here and you can get a corresponding eclipse orientation, So, between these eclipse orientation we can have a corresponding geometry which is a prism geometry. So, we can have a corresponding hexagonal arrangement where the metals center that means tungsten is sitting over there, and 6 positions 6 coordination sites are available, 6 coordination sites are available for binding.

So, we can have any kind of distortion particularly when we have the bigger metal ions present we can have huge distortion. Because we do not have any control or any ligand

to ligand or donor atom to donor atom interaction between these which is very much true for smaller metal ion like nickel, iron or cobalt where you can have the corresponding interaction between these things and we do not get a huge distortion and the coordination site, but in this particular case. So, we have the 6 coordination site these 2 are fixed bidentated fragments are fixed, but these two are monodentated one.

So we can have a corresponding geometry where you find that these 3 these dotted coordination sites, this is 1 of these 3 fold axis what we have seen just now. This is one 3 fold axis and including O S S; this is another 3 fold axis. So, this 3 fold axis and another 3 fold axis if we consider these two are the two planes, the c 3 planes. So, 1 c 3 plane and the another c 3 plane they can adjust themselves depending upon the corresponding size of the metal center based on the corresponding oxidation state that means whether our tungsten is in plus 4, plus 5 or plus 6 oxidation state we can have these typical arrangements.

So these particular enzymes basically fall into three groups and crystal structures of these 3 groups have been identified So, far and people have identified the corresponding coordination from this pterin unit. The tungsto pterin units to such tungsto pterin units fulfilling this particular site and the other two sites are occupied by oxide and Hydroxide groups. So, these particular one, this AOR family the aldehyde ferredoxin and formaldehyde ferredoxin oxidoreductase. So, AOR family and FOR family these are the 2 families and they are basically identified from pyrococcus furiosus and is abbreviated as p f, pyrococcus furiosus p f.

So it the biological name of the origin for this particular enzyme. So, crystal structure basically tells us that what type of distortions we can have, because this particular geometry is not at all an octahedral geometry. But which is in a different form and between the catalytic turn over basically the adjustment of the coordination center can also take place and when this particular center is acting on aldehyde. So, definitely we expect that this is basically the corresponding oxidation reaction and that oxidation reaction the corresponding aldehydes are getting oxidized.

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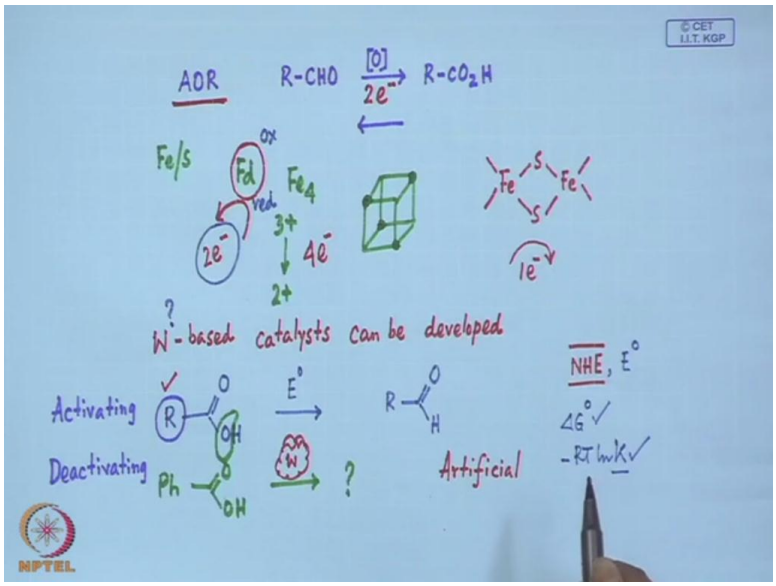

So, these aldehydes what we can have So, in AOR family, the aldehyde oxidoreductase family where, where r is basically the corresponding methyl group we get the acetaldehyde. So, when it is acting on acetaldehyde we get R C O 2 H in the corresponding carboxylic acid formation. So, these we all know from the knowledge of organic chemistry that this is well known oxidation reaction even if you have some benzoldehyde in our hand the bottles of benzoldehyde in the laboratory can be oxidized very easily by the dioxygen present in air.

So dioxygen can be a very good oxidizing agent for all aldehyde like substrate particularly the benzoldehyde and acetaldehyde. And this particular conversion of this acetaldehyde to acetic acid what we have seen in case of the corresponding molybdenum based enzymes, where we can take care of the corresponding oxidation of ethanol to acetaldehyde. So this particular conversion, So, this is an oxidation reaction some electron flow can take place in the reverse direction. So, in the reverse direction electron can go for the corresponding transfer that means once we oxidize the aldehyde to carboxylic acid, the reducing equivalence are being transferred to a 4 iron 4 sulfur ferredoxin.

So far we have seen that this is dependent on iron, sulfur ferredoxin molecules and this ferredoxin is now our F e 4 units. So, 4 sulfur, 4 iron ferredoxin molecule which is we all know is a basically a cube type molecule where all the alternate corners are occupied by

iron and sulphur groups. So, if these are the iron So, other alternative corners are occupied by sulfur groups. So, in this particular case So, this ferredoxin molecule the iron centers are settling between plus three to plus 2 oxidation states that means between the ferrous and ferric oxidation state. So, depending upon the number of electron transfer which is being taken off for this particular oxidation reaction whether we can talking about some electron transfer which is single electron transfer or a double electron transfer.

We should rely on a 2 iron ferredoxin is molecule or a 4 iron ferredoxin molecule because sometimes we find that though you can have 4 iron centers present. And all the 4 iron centers can be reduced to plus 2 or all the 4 iron centers can be oxidized to plus 3. So, you can extract out if it goes from a typically reduced form where all the iron centers are present in plus 2 oxidation state to a system. Where all the iron centers are oxidized to plus 3 oxidation state, we expect to get 4 electron transfer for this particular system. But in most cases we get 1 particular ferredoxin molecule which can provide us 2 electron transfer.

So ferredoxin molecule which is based on 4 iron center which can go for 2 electron transfer, So, either it can be oxidized by 2 electron or it can be reduced by 2 electron. So, which is very much unlike for 2 iron ferredoxin system, because in 2 iron ferredoxin system what we have seen just now that we have 2 iron system and these 2 can settle between the corresponding oxidation state. And here also it can initially go for the single electron transfer that means already the system is in mixed oxidized form that means 1 center is in plus 2 and another center is plus 3.

So it can be reduced for this iron or it can be oxidized for the second iron. So, when this we get for this corresponding oxidation of the aldehyde to acid corresponding acid, this reducing equivalent being transferred to the 4 iron 4 sulfur ferredoxin. So, whatever reducing equivalent we can generate from this particular oxidation reaction that is getting transferred to the ferredoxin molecule. So this reduced form of the ferredoxin molecule then take part in the corresponding catalytic cycle to the next step. And this is 1 particular aldehyde or in the general form it is possible for all other aldehyde molecules starting from the acetaldehyde. But for formate this formate dehydrogenase family little bit different only towards this particular coordination of O H. Because this particular 1 from the cysteine sulfur or selenocysteine selenium it can be attached to this particular

tungsten site, and since these corresponding donors atoms are bigger in size. If it is sulphur or if it is selenium, close to another bigger unit which is our tungsten unit.

So, very easily from the determination of the crystal structure we can identify the nature of these donors groups attached to the tungsten unit. And that also when a bigger unit is attached to the tungsten site though tungsten is in the hexavalent state, which is the smallest possible among the all 3 oxidation states available for the tungsten. But this basically this bigger donor atom can also distort the coordination environment around this tungsten site. So, this coordination center which is attached to the oxygen, and which is attached to the Sulfur or selenium are different. So,that is why the reactivity pattern for formaldehyde hydrogenise is also little bit different compared to that of your normal other general aldehyde.

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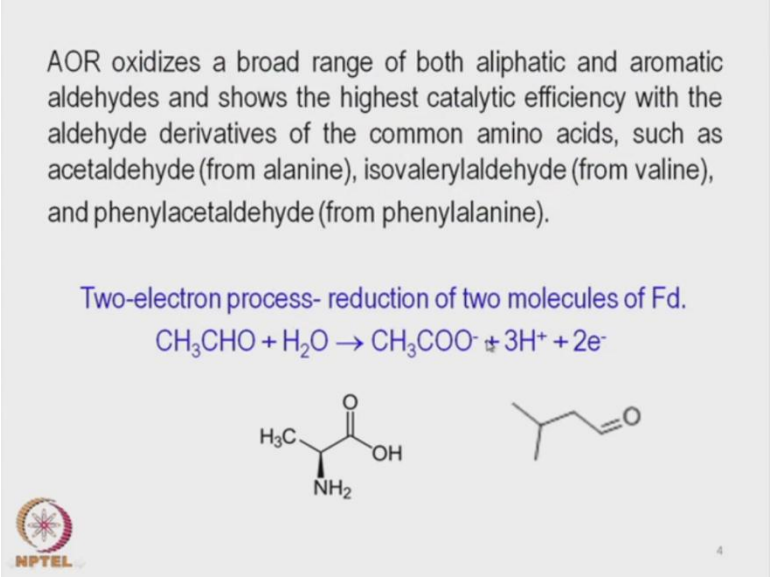

So, this aldehyde oxidoreductase so, they can reduced or oxidized these 2 fragments. So, one is our corresponding aldehyde. So, they can also work on aliphatic and aromatic aldehyde. So, it is very interesting to note that in the biological systems when certain system has been identified and that system when we indentify as it is working on some aliphatic unit. So, this particular aliphatic group is available So, this aliphatic aldehyde is getting oxidized by AOR family of tungsten enzyme.

We expect that whether that particular center can also be operating on the aromatic aldehydes as well, but the biological substrate what is coming out from the biological

system is the aliphatic substrates. So, if people can isolate that particular enzyme, So, AOR family of enzymes. If you can isolate that particular enzyme can be tested for their reactivity on some aromatic aldehyde like benzyl dehydro or any other aldehyde. Because the corresponding enzyme pocket is very important to hold the substrate, because some cavity should always be there which can take up the corresponding aldehyde close to that of our tungsten center which is our catalytic site. And in this particular case if the aliphatic aldehyde is of biological origin and aromatic aldehydes are of synthetic origin they show equal type of catalytic efficiency towards these 2 aldehydes.

So, they can also show good efficiency towards other substrates which are our common amino acids such as, when it can act on acetaldehyde. So, acetaldehyde which we are getting from alanine, alanine is this one. So, it can basically go for the corresponding transformation. So, alanine can transferred to acetaldehyde and that acetaldehyde can be utilized for substrate for AOR family of molecules reacting on it to giving rise to corresponding acid function then isovalerylaldehyde. So, if we have the valine as the corresponding amino acid, So, that valine unit can be converted to its corresponding aldehyde which is isovalerylaldehhyde.

Isovalerylaldehyde is the molecule so, isovalerylaldehyde can also be a good substrate. So, not only acetaldehyde which we identified so, a good range of other aldehyde molecules can be tested for this particular reactivity that means tungsten can be a very good catalytic site. So, that also tempted us to discover some good catalyst which can be based on tungsten center. So, tungsten based catalyst is can be developed with these information. Then also this corresponding other aldehydes where you have the corresponding phenyl acetaldehyde.

So, is not directly a benzoldehyde, because benzoldehyde corresponding aldehyde function is directly attached to the aromatic carbon of the benzene ring. But if we have a C 6 H 5 C H 2 C H O type of acetaldehyde that means the phenyl acetaldehyde which has a biological origin also it is coming from the amino acid phenyl alanine from the deamination reaction. Because all these things can go for, the corresponding deamination reactions like that of our alanine. Because we can go for, the corresponding keto function over here and that when goes for the corresponding oxidation leaving behind us the corresponding decarboxylation for the C O 2 unit.

So, this unit goes as C O 2 and this part is oxidized to acetaldehyde So, that acetaldehyde can be utilized for substrate for this enzymatic activity. So, all these molecules which are directly or in directly supplying some aldehyde base substrates. So, these aldehyde based substrates can be tested for their reactivity which is a very useful 2 electron redox process. That means our acetaldehyde molecule in presence of 1 molecule of water, can be oxidized to acetic acid with the liberation of 3 protons and 2 electrons.

So these liberation of these protons and electrons can take part in basically some of our well known fact of that electro port equilibrium, but whenever we have that electron transfer sidewise. You can have the corresponding proton transfer from the system and these 2 electrons are basically taken up by the oxidized form of the ferredoxin molecule. When we have the 4 iron ferredoxin molecule where the, oxidized form of this is giving us that the iron centers in the plus 3 oxidation state.

And those plus three oxidized state of the iron centers can take up these 2 electron and reduced back to the reduced from of the 4 iron ferredoxin molecule. And these protons are attached to some of the other sites where we have some basic sites can also be available from there and those sites can be attached to these proton liberated from these reactions. So, basically what we find these basically this particular transformation what we are seeing there that is basically a 2 electron transfer reaction.

And this 2 electron transfer reactions already we have seen we have thought of that the individual ferredoxin center can take up those 2 electrons to change from 1 form of the ferredoxin that means ferredoxin in the oxidized form and ferredoxin in the reduced form. So, in the catalytic cycle which direction this particular reaction can go whether from the carboxylic acid to aldehyde or aldehyde to carboxylic that definitely depends on the oxidation state. That means the oxidized form of the tungsten center as well as the state of the ferredoxin that means, whether the available ferredoxin molecules are in the reduced form or the oxidized form.

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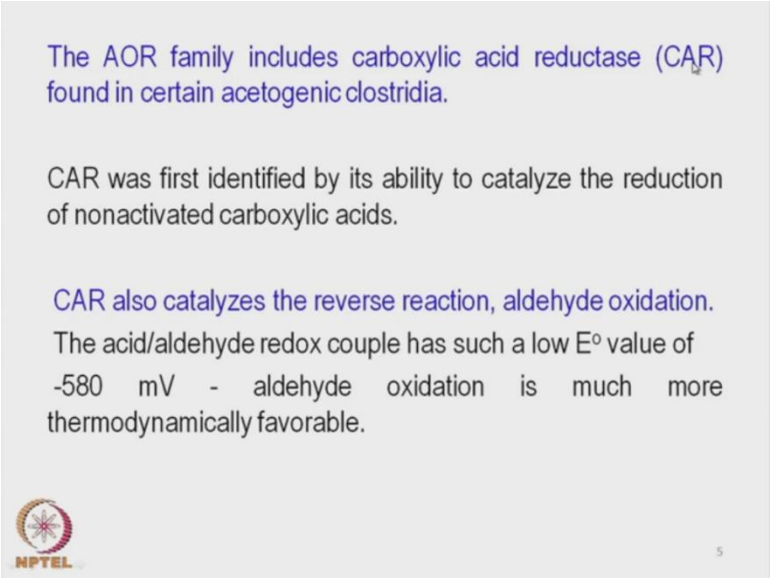

So, knowing this particular type of oxidation based on tungsten, then another group of molecule which we immediately fill that once we are able to oxidize that aldehyde. That means aldehyde oxidoreductase family which is acting on the aldehyde molecules what we are producing is the corresponding carboxylic acid. So, these carboxylic acids if they can be reduced back to the aldehyde we get another group of or another family of corresponding enzymes, those enzymes are known as the corresponding carboxylic acid reductase. So, along with AOR family, we get another family which is C A R family carboxylic acid reductase family and biologically also they can be available from some of the acetogenic clostridia.

So, acetogenic clostridia is the corresponding acetyl function which we can generate that means the acetogenic bacteria or the acetogenic corresponding clostridia can develop to get some of these groups as the corresponding acetyl function, which can be obtained from the corresponding reduction of the carboxylic acid function. This was first identified and its ability to catalyze the reduction of non-activated carboxylic acids. So, if we have some group which is we know that the R group and this R group is attached to the corresponding carboxylic function. And the reactivity for this carboxylic function that means R is there and that is going for the corresponding aldehyde function.

So this particular reactivity that means what we are taking away we are taking away this particular oxygen atom from this particular unit. So, the removal of this particular

oxygen. So, this particular catalytic activity is very much dependent on how we have the corresponding carboxylic function is activated. That means if we just go for R as the phenyl ring R is attached to some benzene ring which is also attached to C double bond O H function. So, what type of reaction based on these enzymes we get and if we get a different reactivity pattern for this reaction, we immediately can see that this phenyl function is basically deactivating the carboxylate function.

So, which is deactivating the carboxylate function for this sort of reaction whereas, the R group if they are activating, and the carboxylate function for the smooth transfer from carboxylic unit to the corresponding aldehyde function. We consider that this particular R function has some role to play for this particular reaction. So, depending upon of all these substrates whether this particular substrate which we I already told you that this particular substrate if it is R is equal to methyl function. And if it is a biological origin So, definitely this has some catalytic function and catalytic rate.

And you can measure the corresponding rate of the catalysis based on that particular substrate which is biologically available for that particular enzyme. But if we, just move for some artificial substrate which we are not getting from the enzymatic system. So, this particular artificial substrate can be tested for the role of these R function for this catalytic action. And whether this particular enzyme whatever tungsten enzyme we are utilizing this for this reaction can be utilized for other biochemical transformations or bio catalytic transformations where the substrate is added or substrate is utilized from outside.

So during this particular conversion what already we discussed that this typical oxidation reaction or the reduction reactions from the corresponding carboxylic acid to aldehyde, is definitely related to some redox couple. Which can be measured very nicely by polaroid graphic measurement or cyclic volt ammetric or any other amperometric measurements for their E 0 values. So, this acetaldehyde acid redox couple has a very low E 0 value which is minus 580 milli volt. So, minus 580 milli volt is a quite negative redox potential compared to other standard reference electrode. If we have a reference electrode like normal hydrogen electrode or saturated calomel electrode or any other standard reference electrode, we just considered these and this as minus 580 milli volt is reported with respect to normal hydrogen electrode.

So, how useful this particular potential is in finding on in identifying the corresponding redox couple for this transformation we can think. So if this potential is very small the corresponding aldehyde oxidation what we have seen that, if it we have in a bottle or if it is in a test tube is very easily oxidized by the oxidation from the dioxygen present in the air. That means no catalyst is required nothing is required only the dioxygen present in air can oxidize nicely these aldehyde to carboxylic acid and the extra oxygen what is there going to attach the aldehyde function is coming from the dioxygen molecule.

And this particular case that this one that means the dioxygen which is functioning as an oxidizing agent. So, E 0 value for, the dioxygen is much more positive compared to the corresponding the reduction potential for the corresponding acetaldehyde conversion. So, once we find the corresponding E 0 values. So, the, if E it is with respect to the N H E, the E0 value we can if we know. And this E 0 value can be correlated with the delta G 0 value that means the free energy change and that free energy change can also be correlated with the corresponding minus R T natural logarithm of K. So, once we find this E 0 value for the any transformation, we can find out the corresponding delta G 0 value and the corresponding K value. So, once we determine the equilibrium constant this K is nothing, but the equilibrium constant.

Once we determine the equilibrium constant for the reaction we immediately know the corresponding reaction whether it is a enzymatic reaction or non-enzymatic reaction, how much fissile the reaction is for transferring 1 site to the other? Therefore, this aldehyde oxidation is much more thermodynamically favorable. So, immediately by determining the corresponding E 0 value from this reaction we can talk about the corresponding driving force. The thermodynamic driving force for this particular reaction in terms of the corresponding comparison with, the equilibrium constant, and we can comment on that this particular reaction. If the K value is very high that means the equilibrium Constant is very high it immediately goes from 1 site to the other due to that particular catalytic transformation. But you can have some kinetic barrier related to this particular reaction and the enzyme environment whatever enzyme environment we can have.

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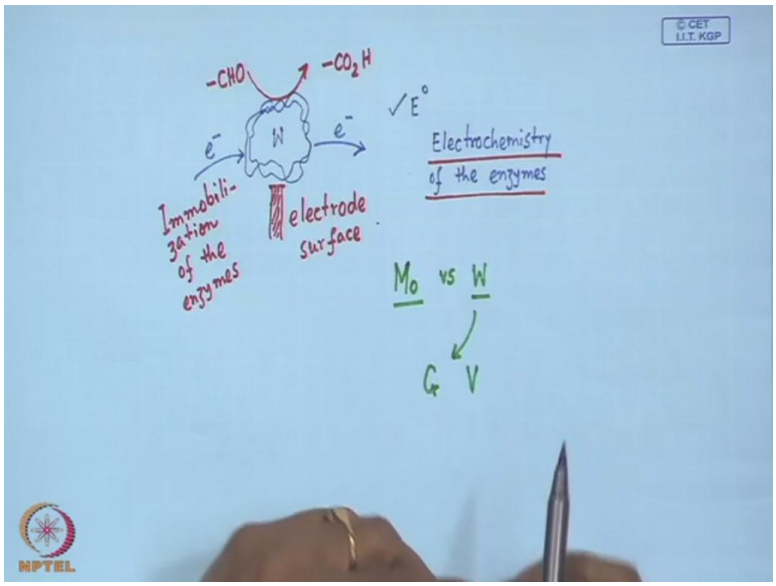

So, if we have the typical enzyme environment and if it is in the huge polypeptide chain and this polypeptide chain and within that polypeptide chain we have the tungsten unit present and we are thinking of some electron in and electron out business. So, electron is entering or electron is leaving from the system for that catalytic reaction and the E 0 value. We have determined for the particular transfer and the corresponding kinetic barrier. We should also determine that the rate of that electron transfer So, the rate of the electron transfer is also important.

So, if we are able to overcome the corresponding kinetic barrier utilizing this enzyme we also get a very fast reaction based on this particular tungsten center. So, this determination of E 0 values, because we can have some measurement some instruments are also available. So, those instruments like the cyclic voltammetery. So, cyclic voltammetery can immediately determine the corresponding E 0 values for this enzymatic. So, electro chemistry for these enzymes is very important So, our electro chemistry of the enzymes are very important.

And these electro chemistry on the electrode surface what we do and those electrode surface and which is sometime bound with the immobilized enzyme. So, this enzyme can be bound to the electrode surface So, we have this electrode surface, and on that electrode surface we basically go for the immobilization of the enzymes. And we have to

see the corresponding substrate what is getting oxidized that means you can have the corresponding substrate as the corresponding aldehyde or the acid.

So, all these things basically can comment on whether we have a very useful enzyme based on tungsten and whether we have a very useful enzyme which is based on the molybdenum. So, these particular things can basically utilize for the comparison of this molybdenum verses tungsten. So, next day will just see, whether this particular tungsten enzyme can also be useful some other system. And in our some our future classes will just slowly correlate that whether the chromium can have some biological role as well as the vanadium in this particular environment. Because we have seen that all these 4 metal ions can give rise to the corresponding oxo functions very easily.

Thank you.