Bioinorganic Chemistry
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Lecture - 26
Molybdenum Enzymes-V

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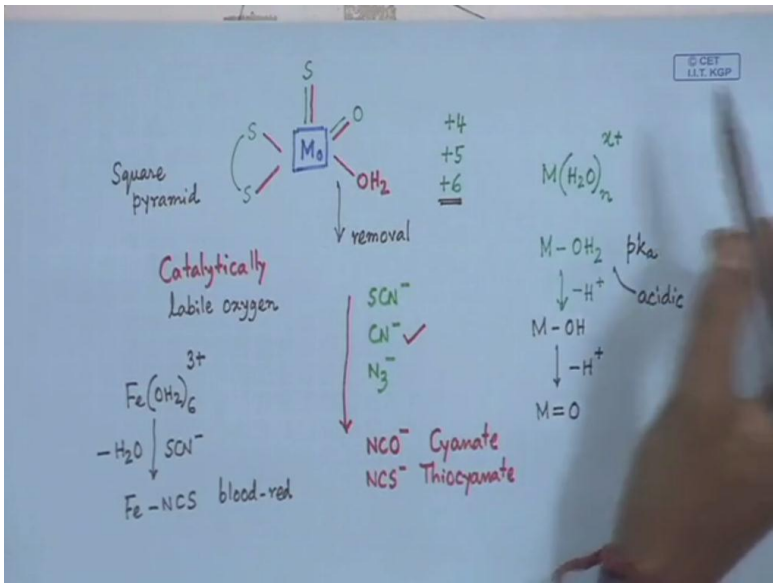

Good morning everybody. So, we are almost reaching towards the end of the enzymes bearing this particular metal center which is very interesting starting from their corrosion chemistry and all other aspects. So, this molybdenum center when we can have certain coordination positions which are occupied and we find that in some of these cases you have loosely bound water molecule bound to it. And this water molecule together with some other strongly bound groups like oxo or sulfido in its plays some important and interesting role towards there catalytic activity.

So, when we talk in terms of their corrosion chemistry of molybdenum in the 3 possible oxidation states from plus 4 plus 5 and plus 6. And the relative binding properties of all these groups, here we all know now that these two are the sulfur bearing bidented lignite of molybdenum class. So, if we consider these 3 groups and the relative binding properties towards the metal filter either in the lower oxidation state like plus 4 or in the higher oxidation state like plus 6. We just simply consider that in some cases the binding is strong, and in some other cases the binding is weak. And if we just simply consider that binding of any metal ion; it can be iron, it can me nickel, it can be copper or simply

the molybdenum of center when any of these salts which dissolve it in water we generate these water bound or echo spaces in solutions.

And in all these cases depending upon coordination number n and the oxidation states x we see that in all these cases at least one of these bound is M OH 2 bound. And how strong this M OH 2 bound will clearly dictate us how quickly we can remove this thing by some other group or how quickly we can go for deprotonation. These are very important states for all these molybdenum enzymes, because if we just simply go for deprotonation from here will be reaching somewhere where we can have M OH. And after another step of deprotonation, we get that oxospecies if the center n is the molybdenum we get molybdenum oxospecies.

So, most possibly in all these cases if the metal center same the molybdenum center is present in highest possible oxidation state that means the plus 6 oxidation state it is little bit strongly bound to that OH 2 and it can change its corresponding p k a halloo of the water molecule. And we all know we have a standard p k a halloo for the isolated water molecule which is not bound to any metal center, but these water bound molecule can be acidic when they are bound to the metal center and which is in the little bit higher oxidation state.

So, if the p k a is less these bound water molecule will be acidic in nature and since they are acidic in nature it undergo deprotonation and we get molybdenum hydroxide for complex if the metal center is molybdenum. And again we just can go for the deprotonation we get the molybdenum oxospecies. So, slowly moving from these two steps we can have these particular binding point it can have the molybdenum OH 2 bound that means the molybdenum aqua bound or molybdenum water bound it can simply go to for the corresponding molybdenum oxospecies. So, at the same time if you want to if you want to remove this water molecule.

So, the removal of this water molecule is important, because we can have the sub state molecules and those sub state molecules are coming into the picture in the close disunity of the molybdenum center. And those sub state molecules will try to remove this water molecule and will go for the corresponding coordinate bound to the molybdenum center. So, if you want to have the removal of these water molecule we should have a correspondingly with water bound to the metal center. So, if we have a catalytically

labile oxygen. So, you call this as catalytically why it is catalytically? Because it will participate in the corresponding catalyses so catalytically labile oxygen we can have. So, we can have a corresponding labile center attached to the molybdenum so if this particular coordination environment is square pyramid in nature.

So, the geometry will immediately tells us that you can remove this loosely bound water molecule and this particular site can be useful for binding some other group that means the sub state if when we are talking about the corresponding oxidation reduction of the aldehyde or alcohol or any other sub state. So, this particular sub state will come and bound to this particular site to be view the corresponding catalytical activity. And another case is also is important to know here that in all this cases that means if we have a metal center like say iron when we dissolve it in water say ferric chloride. If we have ferric chloride and the ferric chloride is dissolving in water we get the corresponding hexa aqua complex of the ferric ion and these hexa aqua complex of ferric ion, can we all know that the corresponding determination of concentration of iron can very easily be done by using thiocyanate ion, because this particular bound which is forming there that means the thiocyanate bound by removal of one of the water molecule.

So, you remove one of the water molecule and thiocyanate is going there and attaching to the iron site. So, this particular thing is highly colored so it is blood red in colored and we can determine the corresponding concentration of the unknown iron present in the system or unknown concentration present in the system. So, the formation of this particular type of bound which is nothing but the removal of the water molecule and introduction of some other group to that position has there the corresponding iron nitrogen bound is stronger compared to our iron oxygen bound such also happening in case of this molybdenum base complex or molybdenum base enzymes. So, if we just simply react with these and if this catalytically labile site is reacting with if any of these anions inorganic anions say thiocyanate anion or cyanate anion or nitride ion.

All these things are very useful in studying all the different types of metal enzymes, because if we have a loosely bound site or a water bound site it immediately go and replace those water molecules. And occupies those positions, because the molybdenum oxygen bound is little bit weaker that is why we are talking in terms of the corresponding not in terms of corresponding weakness or strength. But also in terms of corresponding activity in the kinetic term that means which is not in odd but it is labile. So, the liability

of the complex corresponding complex is important, and we get the corresponding binding of the molybdenum site by the nitrogen of thiocyanate or nitrogen of cyanate, nitrogen of azide. So, due to that substitution, so these can go and bind to the molybdenum center.

So, due to that substitution what we find that this catalytically while oxygen is removed that means the water molecule is removed and this position is occupied by some small inorganic anion. So, these small inorganic anions if after binding to the molybdenum center if we see that the same molybdenum enzyme is not catalytically active for the reaction we can say that these are strongly bound to the molybdenum site. And the substitute is not able to reach to the molybdenum site and is therefore, getting inactivated. So, we can say also that innovation of corresponding enzymatic activity is done. So, this is one way of replacing water molecule and that can also establish that how important is these catalytically labile site that means why there should have a bound water molecule to the molybdenum site catalytic activity or not. And the second case that means how these two positions, because these are the 3 positions of deviate that whether the oxosite or the thiocyte is are equally important to go for corresponding catalytic activity.

So, if we cannot see the removal of the these water molecule, but we can go for some of these that means these groups particularly these CN minus this can also very well attack on the corresponding oxygen or corresponding sulfur on the molybdenum site what happens there then they are producing CNO if CN is attacking on this oxo bound to the molybdenum center. So, it is forming cyanate anion or this CN minus is attacking to the sulfur it is forming NCS which is thiocyanate. So, these two informantions that means whether you can have a simply a cyanate bound molybdenum center or thiocyanate bound molybdenum center from the introduction of CN minus to the reaction site.

We can immediately say that this oxygen or this sulfide has been comforted to the cyanate and the thiocyanate. And during that sort of coordination the molybdenum site reactivity can also be lost and its corresponding enzymatic activity can also be lost. So, we can have two such innovations; one is the direct innovation by removal of the catalytic water molecule or the kinetic site is not there in some other position are occupied by some other groups or the conversion of the cyanate group to cyanate and

thiocyanate. So, that also tells us that how these two sites are also important for the catalytic activity. So, the inorganic anion like the cyanate azide or thiocyanate.

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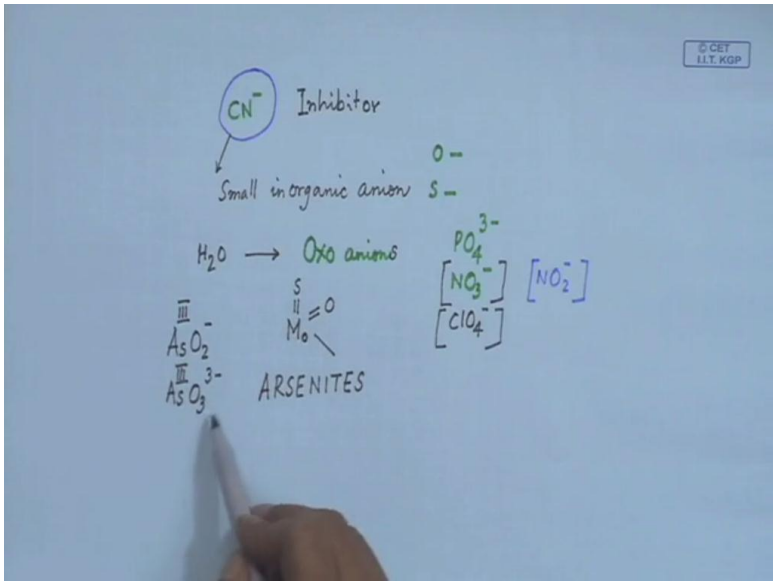

So, this CN minus can be a very good inhibitor for this molybdenum site so is inhibitor so this inhibition we are getting from small inorganic anion, inorganic anion. But also we see that since the site is we are talking about, and we are talking about some removal of water molecule by some group or the conversion of some of these group to useful to oxygen or sulfur deride inorganic anion, because we can have oxygen deride and sulfur deride cyanate and thiocyanate anion. And these two oxygen and sulfur source are found from the molybdenum site. So, you can also use here the corresponding oxo anion and this particular anion in any oxo anion so can be avoid phosphate then or cyanate it can be nitrate it can be chloride. So, these are the different oxo anions. So, these oxo anions can also go to the metal center and bind there.

So, if we have the molybdenum site and already we have seen that water is bound to this site, but will find that compared to water some of these oxo anions are weakly coordinating. And these weakly coordinating groups will not replace the water molecules, because they will also remain as the corresponding typical anion outside the COD sing spear. And we get the corresponding connectivity halloo related to the non coordination of the nitrate anion and non coordination of the part fluid anion to the molybdenum center. So, we are having this thing that means we have this corresponding

sulfur and the corresponding oxygen groups. And this particular one if we just think of all the corresponding arsenite based anion which is arsenite plus 3 so which is a cyanate. So, these arsenite again another form is A s O 3 C minus.

So, these are arsenites which are different from corresponding oxidation state of plus 5 which are arsenites. So, between these two spieces we see that the corresponding variation in the, because arsenite oxidation state in both these two cases are plus 3. It is only the change in the oxygen content of the corresponding oxo anions like that of is nitrate and another well known species we know that is the nitrite.

So, the nitrate and nitrite both are oxo anions, but having different nature of the nitrogen atom as the center of that oxo anions. So, these things if we just now extend our idea what we are discussing for last 7 minutes that when we can have this binding for this CN minus which in inverted it can go and directly bind to the molybdenum center or it can interact with the oxo anion or thio anion bound to the molybdenum site. So, the binding form of the corresponding oxo anion to the molybdenum site is important. And if we find that these oxygen is involved to react with A s O 2 minus and converting the entire species as A s O 3 minus, because these oxygen can now have the competition of sharing between the molybdenum site and the arsenite site.

So, what we are doing? We are doing something that we have the labile position here we have oxo site and we have the thio site. And these groups particularly these oxo anions of arsenic or the molybdenum, because the molybdenum has equal affinity for binding to sulfur as well as oxygen, because molybdenum we all know that molybdenum can form tetra oxo species or molybdenum can form tetra thio species. So, these two immediately tell us that whether this particular anion or this anion see this one having less amount of oxygen, because we are providing we think that we are providing either oxygen or sulfur from the molybdenum site and it is not going to bind alone from this labile site.

So, the change in this thing that means the change in nature of corresponding group that means wherever we are using A s O 2 minus and converting back to A s O 3 3 minus and with the donation of these oxygen this oxo anion from the molybdenum site, what we are doing? We are engaging this particular site as well as this labile site, because the water will be removed ultimately and the entire arsenic site the arsenite anion, arsenites anion will bind to that molybdenum site. So that soft’s the path pass of the placing this

particular catalytically labile site as well as engaging the oxygen for the corresponding attachment to the arsenite site.

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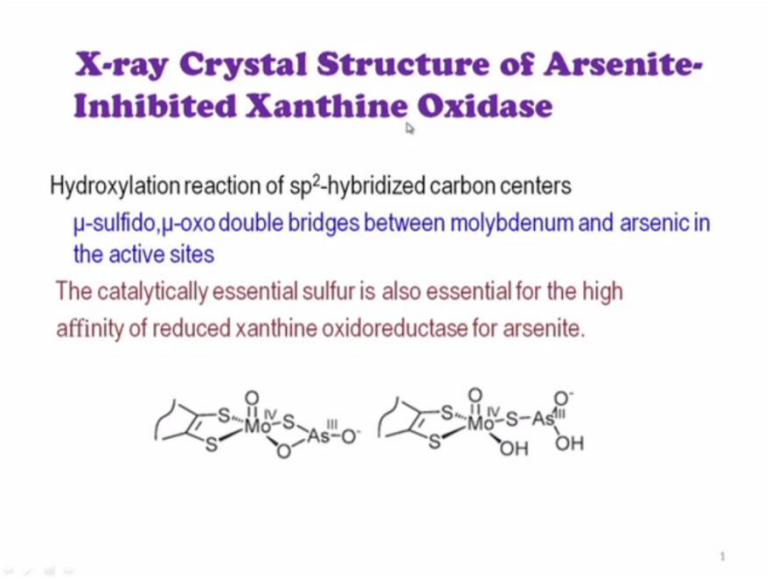

So, that we have seen from our last days thing that what we started our discussion there that arsenite is also available there and this arsenite anion can in aviate xanthine oxidase. So, we have these arsenite in the plus 3 oxidation state and when sulfur is involved so between these two that mean molybdenum and arsenite we have the corresponding competition for the sulfur binding, because this entire group what is there that means arsenic one sulfur and two oxygen can be a very useful mixed oxo thio anion of arsenite which can bind in these xanthine and can remove from the system. Also the same arsenic that means A s O 2 minus and directly bound to the corresponding sulfur site and providing these particular site and the porhonated site. So, whether the essential site is the oxo site or sulfur site so the catalytically essential sulfur is also essential for the high affinity of reduced xanthine oxidoreductase for arsenite. So, this sulfur is essential for our catalytic activity and this sulfur is also essential for arsenite that means this sulfur is helping these binding of this arsenite group to the molybdenum site.

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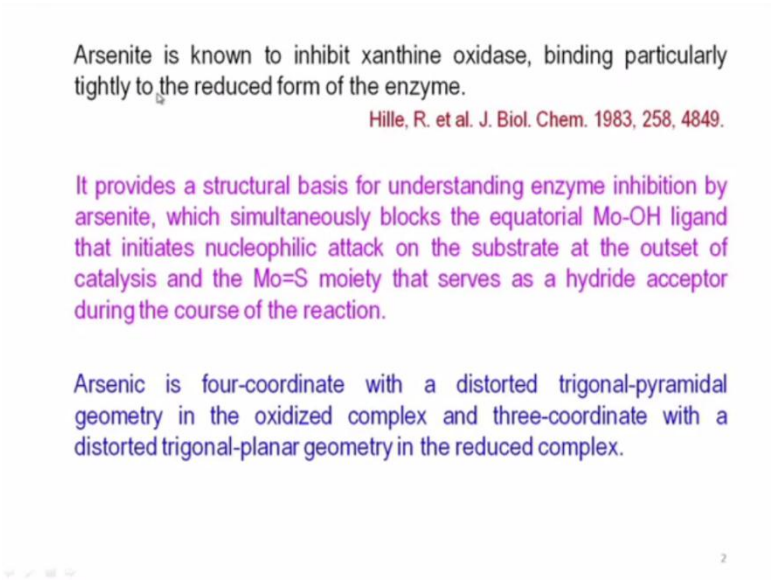

So, this is well known that means the arsenite is known for long time that it can come into the picture and just go for the corresponding xanthine oxidoreductase activity reduction. And since, it is tightly bound compared to the loosely bound to water molecule or loosely bound any other sub state. This particularly tightly bound to the reduced form of the enzyme which is very important the reduced form.

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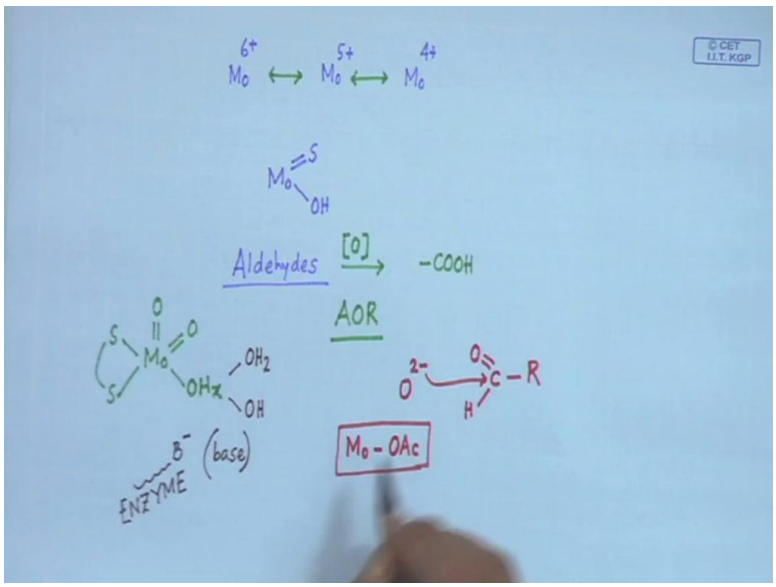

So, the enzyme what we are talking about in all these cases the molybdenum in 6 plus molybdenum in 5 plus and molybdenum in the oxidation state of 4 plus. So, all these

cases as I told previously that this particular system that means whether the binding of any other group whether it is forming the oxo anion whether it is forming the thio anion or the binding of the sub state molecule or binding of the water molecule to the system is depends on the corresponding oxidation state. So, this arsenite basically when you it goes to the particularly binding tightly to the reduced form of the enzyme where the molybdenum site has been reduced to plus 4. And at the same time it provides the corresponding structural basis for understanding the inhibition of the enzyme where able to inhibit the corresponding activity of the molybdenum enzyme for this by attaching arsenite to this. And it simultaneously blocks the equatorial Mo OH ligand that initiates the nucleophilic attack on the substrate at the outset of catalysis.

So, when we start the catalysis based on molybdenum we can have the molybdenum OH bond also and we can have the molybdenum sulfur double bond. So, the molybdenum sulfur moiety serves as a hydride acceptor during the course of the reaction. So, if this particular Mo double bond if it is getting comparted to Mo single bond S H group that means this sulfur functioning as a very good hydride acceptor for these particular reaction, because the initial activation of any substrate species or any other species enrolls the attachment of this hydrogen atom in the form of a proton or hydride to any of these groups. But the molybdenum site does not have the corresponding affinity for binding proton or binding hydride iron. It is the group which is already attached to the molybdenum site that means the sulfur or the oxygen site which can take care of the hydrogen atom or ion in the catanic form like the proton or in the hydride form as anion.

So, ultimately what we get? We get this particular arsenic site. So, arsenic is tri coordinated so arsenic site is little bit this planar arrangement. So, this plane this arsenic plane is coming close to the another plane where the molybdenum site is there which is in a square base 3 sulfur and one oxygen little bit above the square base. So, these two planes basically this plane S 3 O plane around molybdenum and this O 2 plane around arsenic they are almost co planar. So, they have this thing that means in case of arsenic when it can expand for a four coordinated geometry by attaching some other group in this particular form can be trigonal by pyramidal geometry. And in the 3 coordinate form the trigonal planar geometry in the reduced complex.

So, the reduced complex means the molybdenum site we are not talking in the terms of the corresponding inivitor which is coming as molybdenum on the surface of the

molybdenum as the arsenic. So, the arsenic is already in the reduced form that is why we are talking in terms of arsenite in a vision not the arsenite in the vision. But the enzyme is also in the reduced form that means this enzyme can be in the plus 4 and for settling it can be enzymatic activity it can go up to plus 5 oxidation state from the plus 4 oxidation state.

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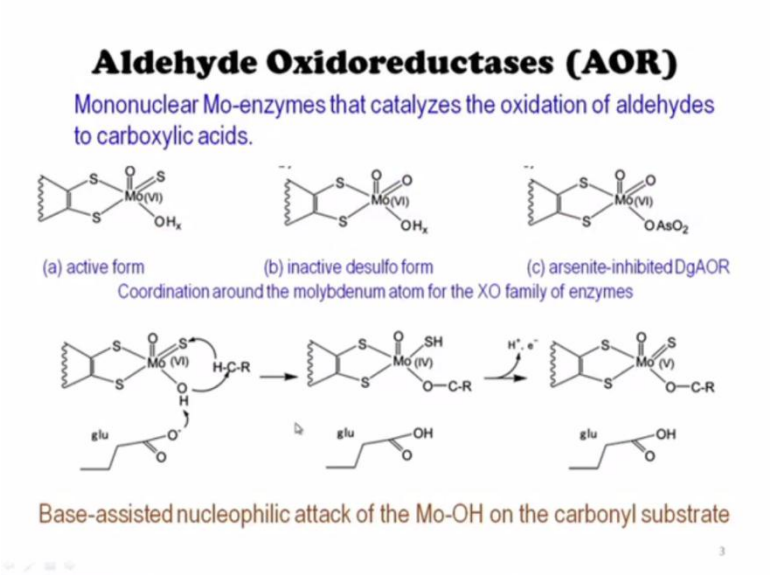

Now, we just see the corresponding handling of these enzymes by aldehydes. So, how aldehydes can be there and how aldehydes can be useful for the corresponding oxidation to the center? So, aldehydes can be every easily take up one of the oxygen at once what we are talking about the corresponding hydroxylation in case of xanthines oxidizing. So, aldehydes can very easily be oxidized to form the corresponding carboxylic acid group. And now this oxygen based on the molybdenum site can very easily transfer that oxygen to the aldehyde site, so one group of these molecules which are known as AOR. So, aldehyde oxidoreductases, so this aldehyde oxidoreductases wearing same molybdenum site can go for the reduction and those reductions still involving these 3 oxidations from molybdenum which are very easily accessible to us. So, these AOR families like xanthine oxidation family which are XO familiy.

So, these are another group of molecules which are aldehyde oxidoreductases, we call it as AOR. And when we consider this depending upon their different source that means the biological source what can we have suppose we can have the desulfovibrio gigas. So,

the desulfo function based on the molybdenum is available. So, we just level it so which is DgAOR. So, desulfo hydro gigas would be DgAOR that means it is still aldehyde oxidoreductase responsible for the oxidation of the aldehyde function. So, immediately we identify that this also contains a molybdenum site. So, identification of this site as molybdenum is important which catalyzed the oxidation of the aldehyde, just now what we have seen that it can very easily be converted the corresponding carboxylic acid.

So, some of these groups basically, if we see that what we are just talking right now that you can have this aldehyde oxidoreductase family, we are not talking not in terms of 1 sulfur, 1 oxygen what is the molybdenum dioxide which is well known to us also from the days of corresponding complexes the coordination complexes of molybdenum what we have seen that the molybdenum acetyl acetone complex. So, these are also well known compounds in the 6 varieties that means these two molybdenum oxygen groups are in 6 positions. So, they are roughly 90 degree a part so one more site is available for this and two other forms the terrine part that means the sulfur bearing molybdenum terrine part is coming to the picture. So, this is has I told you just now that this desulfovibrio gigas which is the biological for naming of the origin of these molybdenum enzyme. And the family belongs to AOR that means aldehyde oxidoreductase.

So, just now we have seen also that arsenite innovation how it does show in the plus 6 oxidation state though it is very easily binding to the molybdenum in plus 4 oxidation state. But it is still bound to the molybdenum site and the plus 6 oxidation state. So, this two oxo groups available and the third one is the corresponding arsenite group attach to the molybdenum site. We can have also this is typical innovation with respect to the arsenite. Then we have the desulfo form that is no sulfur group is attached there. So, it is known as inactive desulfo form and the active from also. So, to begin with the catalytic cycle it starts simply with 1 oxo 1 sulfur form. So, this 1 oxo 1 sulfur form when it goes to a dioxide form, we consider as a inactive disulfo form that means this has lost the corresponding activity towards aldehyde oxidoation.

So, which means immediately to us that the presence of this sulfur is therefore, very important, without this sulfur we cannot see the corresponding catalytic activity. So, when we remove this sulfur your activity is lost. So, the relation to the corresponding molybdenum sulfur double bound or molybdenum thio form. So, this molybdenum thio form should be there in the active form, but if we can convert into a desulfo form it

would be inactive. So, the presence of this sulfur group is important for the catalytic activity. As well as the presence of this labile site that means OH x binding of OH x, so here what we are seeing that we are binding something which we are labeling as OH x, why OH x? OH x means during the catalytic cyclic it can be OH 2 that means the water molecule attach to the site or it can be OH that means during this particular point.

That means depending upon molybdenum oxidation state whether it is in the plus 4 oxidation state or some other oxidation state the bound water molecule can immediately can go for the depotonation and you can have a hydroxide function attached terminally to the molybdenum site. But the catalytic activity for these two groups that means the catalytic activity for water bound molybdenum site and hydroxide bound molybdenum site are rectified. So, initially identifying that immediate position that means we can have for a active site a bidented sulfrutuation (( )) from the bidented group, oxo function xanthine function and the OH x function. So, we can immediately write the coordination environment as a typical coordination chemistry always interest to know what are the immediate groups which are bound to the metal site which are bound to the molybdenum site.

So, the coordination around the molybdenum atom for the xanthines oxides family of enzymes is therefore, we have this OH x is considered has OH after deprotonation from here. And this is something where the enzyme is providing a typical base. So, nearby this sometime we label it as B minus or B H plus so B minus which is the base originating from the enzyme environment that is very important, because this particular base like the way we do the corresponding chemistry in the laboratory we externally add some base for deprotonation of the ligand or deprotonation of the water molecule which is attached to the metal, site but when we are talking in terms of enzymes. So, this particular B minus is of enzyme origin so it is coming from enzyme so this particular one is therefore important, because it can take up these proton form water molecule or another proton form the hydroxide anion to control the activity.

So, what is happening there that we are talking in terms of the corresponding oxidation of the aldheyde function. So, aldehyde will be there so that aldehyde is written in terms of RCH without showing the oxygen already present in the aldehyde function, because if we consiter that RCH has the aldehyde it would be very similar to that of our sub state what we are earlier seen in case of xanthine family that means what we are doing there?

We are doing something which can again be consider as a hydroxylation reaction that means already we are having 1 carbon oxygen double bound. But still we are able to go for another hydroxylation level that means another oxygen can be attached to this carbon to make this carbon as the carboxilate group. So, when this OH is attached to this molybdenum site, this glutamate anion which is the basic site provided by the enzyme? So, the glutamate anion that means the residue is based on the glutamic acid. And the glutamic acid in the depotonated form that means O is present as minus O minus.

So, glutamite anion is there and that glutamite anion will have some affinity for the proton which is near by attaching to the molybdenum site. So, reversibly this can takeoff these hydrogen ion as proton to the oxygen site as O minus that means the basic site of the glutamate anion coming from the protein in chain. And when it is coming to this oxygen this O is getting some accumulation of negative charge that means it is become O minus. But at the same time it is not forming the corresponding double bound to the molybdenum center. But this corresponding basicity or the nucleophilisity will allow this oxygen to attack the carbon of the carbonic function of the aldehyde.

So, this is facilitated by the base that is why we consider these as which is very important statement like organic statement that base assisted nucleophilic attack. So, base is there which is assisting the liberation of proton from the OH function. So, OH having lone pair of electrons in fact, it is there also in water molecule also water molecule also bear some amount of lone pair that means two lone pair electrons are there on the water molecule. So, the availability of those two lone pairs or any kind of nucleophilic attack is there, but which can be assisted that means which can be enhanced the nucleophilic corrector can be increased. If we just go for the corresponding deprotonation of one proton from water molecule to hydroxide anion or the second proton to go for its corresponding O 2 minus. So, this O 2 minus basically will have higher nucleophilic corrector to attack the carbon center on the aldehyde function.

So, you have C OH and R group of the aldehyde group. So, this will basically go for the nucleophilic attack on the carbon center. So, that attachment of this proton to the glutamateisit will assist the nucleophilicity or increase the nucleophilicity of this oxygen to be attack on the carbon. Since it is a nucleophilitic attack for that so electrofillicity of this particular carbon that means the carbonyl carbon should have to be increased. If we can take out this particular hydrogen attached to this particular carbon center. So, if this

we can take out as the hydride anion. So, this can go to the sulfur nearby already attached to the molybdenum. So, this sulfur can take up the hydride anion so it is basically loosing this corresponding hydride to the sulfurs this hydrogen is moved from this carbon to sulfur making this sulfur has SH group.

So, when it is moving the SH group function this oxygen already attached to the molybdenum site. But can no noisily attack this particular carbon of the carbonyic function of the aldehyde group. So, we can have this particular oxygen in between molybdenum and the carbon site. And in that way we have transferred this oxygen from the molybdenum site to the carbon site that means we have formed a new carbon oxygen bound mediated by molybdenum oxygen bound.

So, we can have the bridge between molybdenum and carbon site by oxygen which was already present as OH function attached to the molybdenum. So, we have these that means RCO. So, basically RCO HRC was the aldehyde. So, this has been converted to a carboxylate function. So, this carboxylic group and the attachment of the SH we need the transfer to 2 electrons also and those 2 electrons are basically donated to the molybdenum site and molybdenum site is reduced from plus 6 to plus 4.

So, this particular site is now very important that molybdenum 4 is attached to the carboxilate function. And this particular one is a catalytically transient state that molybdenum 4 is there an SH function is attached to molybdenum 4. And this molybdenum 4 will have some immediate tendency to the oxidize to molybdenum 5, because the corresponding (( )) is less for the corresponding electron transfer couple a half way potential between this molybdenum 4 and molybdenum 5 is less. So, it can go for first electron transfer followed by a proton transfer from this sulfur. So, it has been accepted this hydrogen has hydride anion, but now it is losing this as a proton H plus such that you can have S as SH minus and it will revert back to original sulfur double bound molybdenum. And your molybdenum site is being stabilized here in plus 5 oxidize state.

So, if the carboxine so carboxiline bound molybdenum 5 is the typical the mediate form where we have converted the aldehyde function to the carboxilate function. And after that conversion it is still attached to the molybdenum site and which is very much similar to the binding of several other species where we know that the metals solves we can have

the corresponding carboxilates. The typical examples are has the metal acetates like corresponding copper acetates, corresponding chromium acetates and all this. So, if we can have the corresponding molybdenum is there. And we consider it has the corresponding OA c type of thing. So, this is a useful arrangement for binding for acetates or any other carboxilate function.

So, carboxic groups can go and bind to the molybdenum site. So, this can function has a weakly bound ligand center to the molybdenum site. So, when it is attaching to this particular site our corresponding enzyme function based on the corresponding glutamine base is still they are in the acid form. So, is in the glutamic acid from so Mo OH function on the carbonyl sub state attack that means Mo OH function in the carbonic sub state ultimately producing that caoboxilate group attaching to the molybdenum site. And we have the glutamic function presiding there close by at the corresponding, corresponding acidic form. And this acidic form glutamic acid which is not a part of the molybdenum site in the catalytic site, but this is nearby. Now, it is acidic in nature when it is converted to the corresponding carboxilate function it can immediately supply this particular proton to this bound carboxilate anion.

So, like acetate anion which can be bound to any metal center these carboxilte site cannot be stabilized as a bound site to the molybdenum when we can have a nearby 3 acidic site. So, the 3 glutamic acidic site from the protean site chain is more acetic and can supply this proton to this carboxilate function to make it typical acid. And once it is protonate it will not be attach to the molybdenum site and it will simply remove from the molybdenum site. And one vacancy will be generated over here and that generation of that particular vacancy will next be occupied by simply water molecule to that particular site. So, our catalytic site can be regenerated by transferring this molybdenum site to back to molybdenum 6 through this removal of this proton form here as OH.

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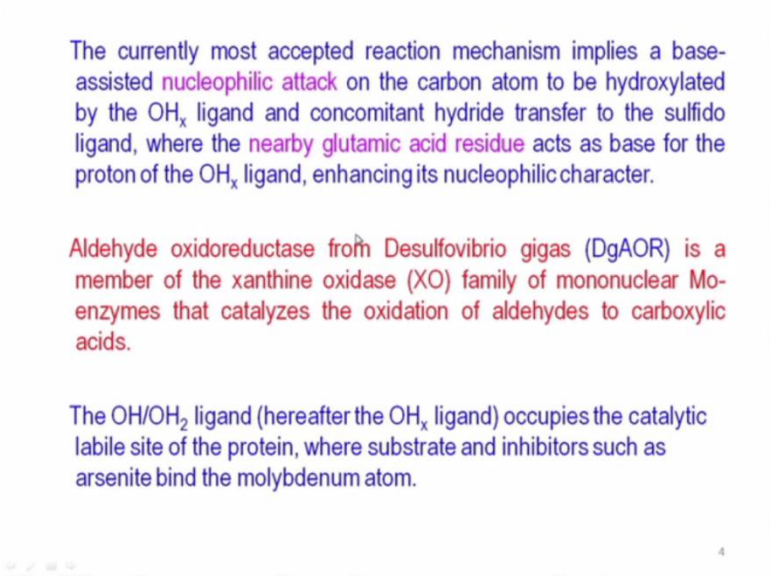

So, what we see here that the most accepted reaction mechanism what we have seen just now that the corresponding reaction mechanism implies that we should have the base assisted nucleophilic attack. So, definitely we should have a attack which is necleophilic in nature, but which should be assisted by the corresponding glutamic base nearby. And the carbon atom of the aldehyde group is hydroxilated by the OH x ligand and concomitant hydride transfer to the sulfiodo ligand these all I just now I told you where the nearby glutamic acid residue acts as base for the proton of the OH x ligand enhancing it is nucleopilic character.

So, how nearby glutmic acid can enhance the corresponding nucleophilic character of the bound ligand to the molybdenum site. So, what we see which is very interested to note here that this OH x ligand; this OH x ligand is nothing but our reagent for the corresponding reaction that means the oxidation of the carboxy function of the aldehyde to the carboxyl function of the acid. So, if we have the origin is desulfovibrio gigas and AOR is coming out from that. So, it also belongs to the bigger family of xanthine oxidase wearing the mononuclear molybdenum center.

And now the catalyzes is different it is involved in the oxidation of the aldehyde to carboxylic acid. So, together OH and OH 2 ligand we can call it as wherever we find these on the molybdenum. We can consider it is OH x ligand; OH x ligand means that it can be OH or it can be OH 2, because some time it is very difficult to identify whether

the center is OH or OH 2, because if we can go for the corresponding protein (( )) also. And through x a diffraction it is very difficult to identify the number of hydrogen atoms attach to the oxygen site so is very difficult to identify whether the group is OH or OH 2.

So, some other in direct evidences we must sort for to clearly identify the nature of these groups. And already we have discuss we have establish that the binding of the OH and OH 2 ligand to the molybdenum site is definitely the corresponding labile site of the protein. And when substrate on inhibitors it can come, it can bind that particular site only to the molybdenum. So you can ask the question that which particular site is there for responsible for binding of inhibitor for binding of the substrate and what are the groups invalid for the corresponding catalytic activity, because is very much complicated one for the proton abstraction as well as the hydride transfer for the molybdenum the corresponding subtraction molecules.

So, all these things together you can give us some very good idea about what is happening they are for the corresponding simple case that means the corresponding simple oxidation of aldehyde, because aldehyde oxidation is a not a very difficult task in air also synthetically prepared or laboratory available aldehyde molecules can go for immediate oxidation through the air. But when we are talking in terms of the corresponding concentration in the biological system in the living organism the corresponding reaction is little bit complicated where we take the help of molybdenum.

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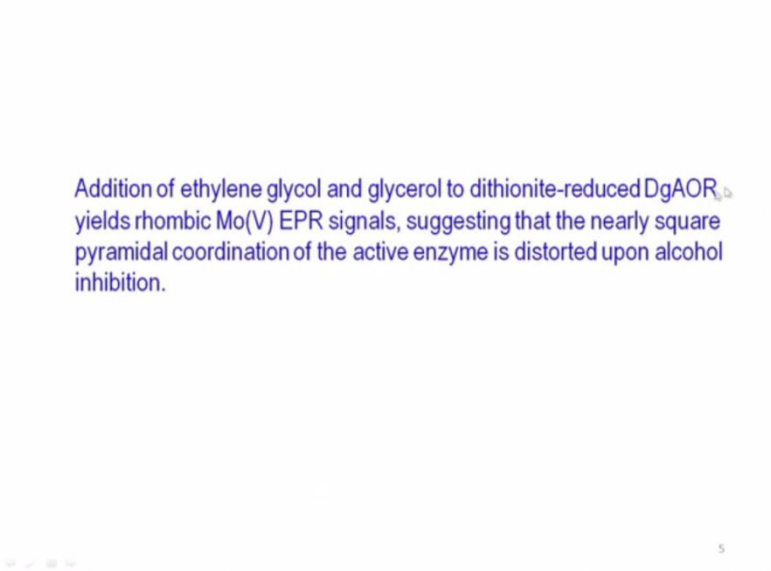

So, in all these cases, all if we take that particularly desulfovibrio gigas AOR and if we want to have some informations whether your molybdenum site is stabilized or your molybdenum site is there in the catalytically end point whether that is a molybdenum 4 or a molybdenum 6 or really a molybdenum 5.

So, you can use something that means the addition of ethylene glycol and glycerol to the system where we can have dithionite group. So, the dithionite is a very useful reagent for reducing this particular site that means the molybdenum site, because not all chemically available or bio chemically stable reducing agents can reduce the corresponding molybdenum site from molybdenum plus 6 to molybdenum 5, because in all these cases particularly in the biologically environment we can have the corresponding E 0 values for the reduction of molybdenum plus 6 to molybdenum plus 5.

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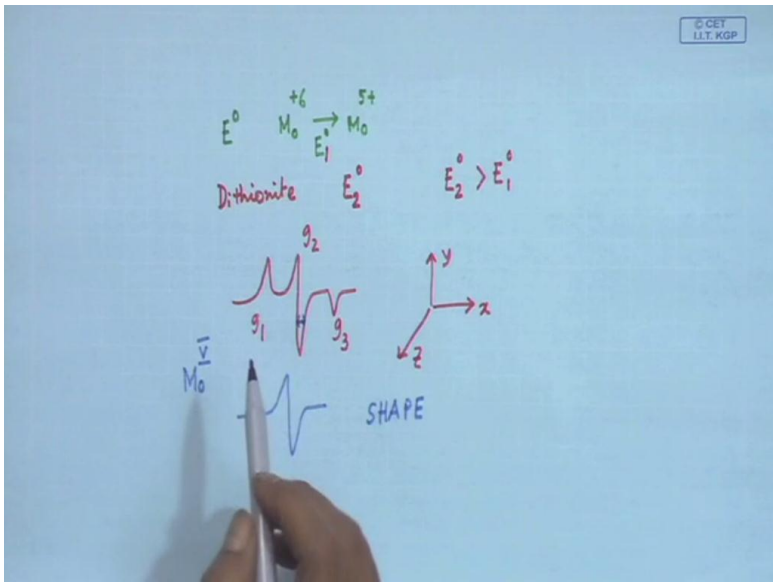

So, we have the corresponding E 0 value for the reduction so it can be some Millie volt only. So, depending upon the corresponding available potential for molybdenum we can have the dithionite anion so dithionite which is little bit a strong oxidizing reducing agent compare to other reducing agent. So, this dithionite also can have the corresponding reduction potential E 0 value. So, if these two potentials the potentials for molybdenum center reduction and potential for dithionite based reduction is matching. And if this is higher if this is E 1 and this is E 2, so if E 2 0 is higher than E 1 0 on the redaction site is are able to reduce the molybdenum from plus 6 to plus 5.

So, the molybdenum center what we are getting? We are just simply trying to establish their site has available oxidation state or molybdenum during the catalytic turn over whether the particular molybdenum site we are getting is EPR active. And between these 3 oxidation states of molybdenum 5 having one particular unpaired electron which is EPR active and it gives a corresponding rhombic nature of the signal. Rhombic nature is nothing but we can have 3 different g values basically one is known as g 1, another is known as g 2 and third is known as g 3.

So, basically your all these 3 different sites that means x y and z are different around molybdenum So, we get the corresponding z tenser in the EPR spectrum different. So, instead of having one particular g value which we call it has the corresponding isotropic signal we get a speeding of that particular isotropic signal into 3 and the nature of this signal can be known by knowing the corresponding values of g that means what is the value of g 1 g 2 and g 3, and their corresponding line weaved also their typical positions and the set which is very important?

So, for this molybdenum 5 site, which has a very good data base for knowing the corresponding shape of the typical molybdenum 5 sites, so the biological sites if we get the similar type of spectral signature we can immediately say that you see that molybdenum 5 is involved in the catalytic cycle, because it has a similar EPR signal what we know from other synthetically prepared molybdenum site or other biologically known molybdenum site like that of our xanthine oxidase. We can say that this signal having some values of g 1 g 2 and g 3 and the corresponding shape which we are talking in the terms of rhombic which is not isotropic; which is not axial, but it is rhombic that immediately confirms something that it has a nearly a square pyramidal coordination geometry of the active site and it is distorted upon alcohol inhibition. That means when we use glycol or glycerol we just looking for the binding of the molybdenum site by replacing the weakly bound water molecules by the OH function of the glycerol or the OH function of the glycol so that alcohol inhibition is still there.

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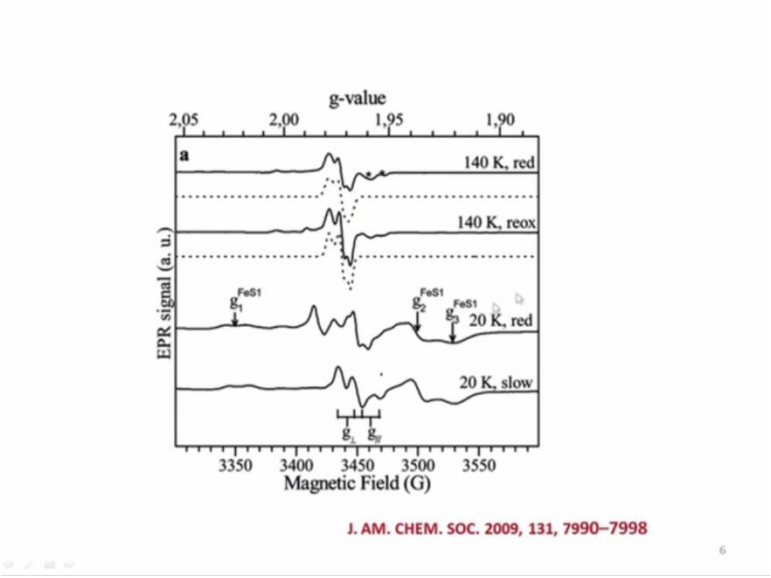

And that alcohol inhibition is responsible for the signature what we are getting. So, the EPR spectral studies is little bit complicated it has taken from jabalan American chemical society of issue of 2009. So, which gives us some example that what are the typical values in this cell of 1.90 to 2.05? And this is the first signal; this is the second signal and this is the third signal. And how they are changing and the dotted line are also we can go for the simulated one that means theoretically we can predict these values for changing the different parameter.

And sometime low temperature is also required like liquid nitrogen temperature or liquid helium temperature. And this is the corresponding signal for the reduced form and this is the deoxidized form. So, these two things are basically sometimes changing and apart from the positions for molybdenum. So, this is g 1; this is g 2, and the third one is g 3 and not like these, what just now we have seen they are little overlap in nature that means the values the difference in g 1 g 2 g 3 are less. And there are several other signatures also available if we can have other paramagnetic sites. So, in all these cases some iron sulfur bond systems are there in which are responsible for giving electron transfer to the molybdenum site.

So, at some other lower temperature where we can go for 4 Kelvin in liquid helium temperature, so this particular one can also show some other values and those particular positions can be identified nicely as the corresponding signature for the molybdenum

sulfur clusters which are parametric in nature. So, the entire EPR signal which is getting complicated and which is also getting disturbed little bit if we have some other nearby paramagnetic site like that of iron sulfur site. So, simulation of this halloo basically that means involving iron site or involving the molybdenum sulfur site can tell us some idea about the corresponding g halloo that the, what are the corresponding g halloo which can immediately tell us that whether we can have 3 signals or 2 signals. So, next day we will just continue from here how this EPS signals apart from this structural identifications are useful for our identification.

Thank you.