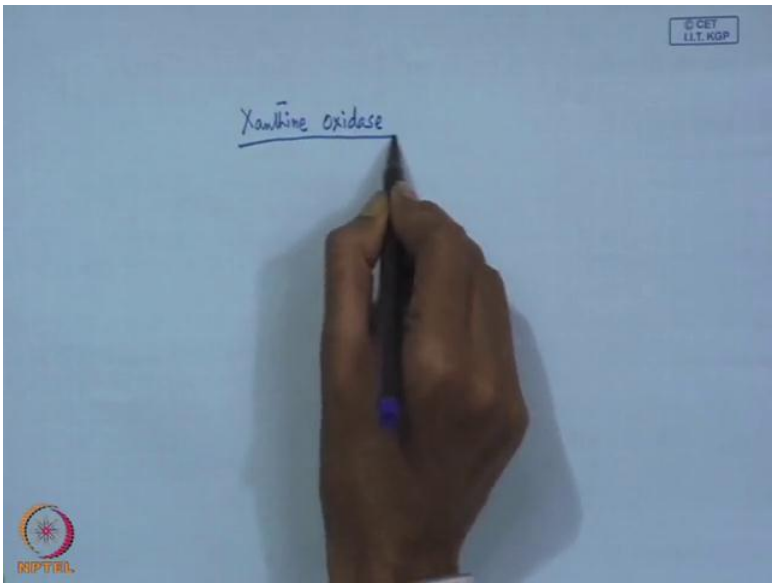
Bioinorganic Chemistry
Prof. Debashis Ray
Department of Chemistry
Indian Institute of Technology, Kharagpur

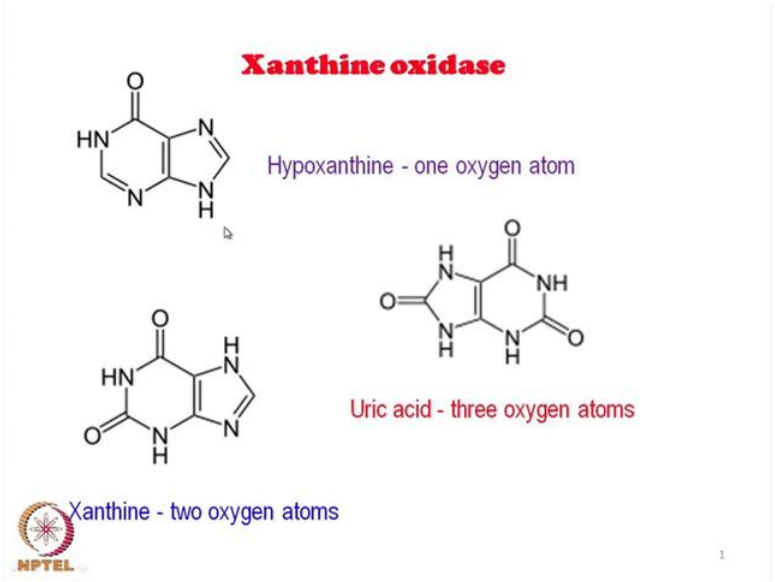
Lecture - 25
Molybdenum Enzymes-IV

(Refer Slide Time: 00:31)



Good morning, so today will still continue with some of the aspects of ours Xanthine oxidase and other related oxidoreductases.

(Refer Slide Time: 00:49)



So, as we all just looking at xanthine oxidase which is operating on hypoxanthine xanthine and ultimately producing uric acid. So, if we considered as already we discussed earlier that all this three molecules are different from the basic point of view. This skeletons are all same only thing that is hypoxanthine has one oxygen atom attach to this particular carbon. On this six members heterocyclic, two nitrogen bearing ring were has xanthine has two oxygen atoms and uric acid has three oxygen atoms attach to this carbon. The second one and ultimately between these two nitrogen this carbon, so if we considered that the transformation mediated by this molybdenum bearing enzyme.

Xanthine oxidize is taking place from the conversion of hypoxanthine to xanthine and then xanthine to uric acid which is deadly for the living system and also for the human being that accumulation of uric acid in the blood as well as in the body. It has some save here consequences after word because this particular species compare to hypoxanthine and xanthine is much soluble in water. It can crystallize from aqueous medium depending upon the ph of that particular medium.

So, crystals of uric acids are also forming in some of this cases, so basically we will just focus our attention on this particular conversion because the conversion of this xanthine two uric acid is really very important to study. This hydroxylation reaction and that oxygen atom transfer reaction is taking place on this particular carbon. So, the positioning of this carbon when we go from hypoxanthine to xanthine that means the insertion of the oxygen atom on this carbon is little bit different.

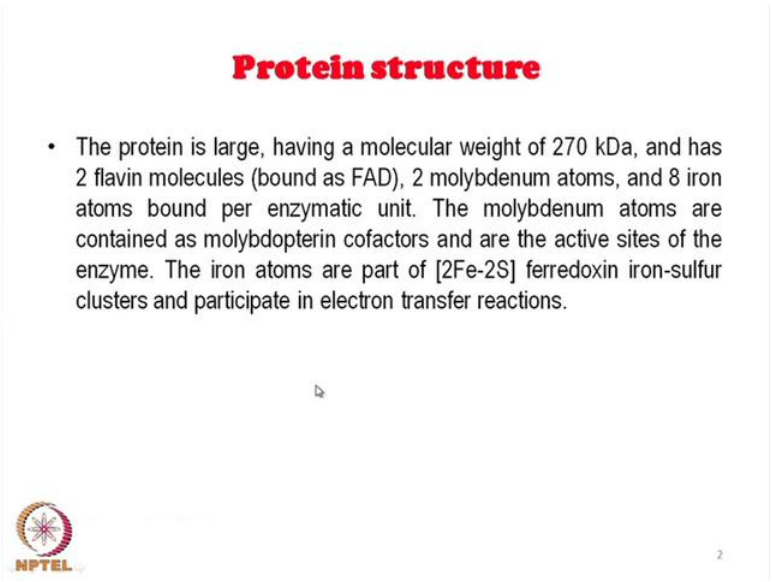
Getting xanthine from hypoxanthine compare to that oxygen insertion for the last tape were we get the uric acid bearing three oxygen atoms. That means, particular carbon center and all this cases the basic aspect what we can see is that since we are talking about a molybdenum bearing enzyme. So, molybdenum oxo species if in the intermediated is forming and which we have seen earlier that it can be transferred from a non oxo molybdenum centers species to a oxo molybdenum.

Then, ultimately to a dioxide molybdenum species that this oxygen atom is getting activated due to the coordination to the molybdenum center and ultimately that oxygen is transfers to hypoxanthine to from xanthine and ultimately to hypoxanthine to uric acid. So, it is the activated molybdenum enzyme here we have the molybdenum oxo form is present that is utilized for the hydroxylation reaction on this particular carbon of xanthine. This particular

hydroxylation reaction is little bit different from the conventional other type of hydroxylation reaction based on other metal aliens like iron copper etcetera are little be different.

In this particular case, xanthine is a also a very good heterocyclic and if we considered that is particular molecule I mean four nitrogen bearing centers, only this is the nitrogen which do not have any hydrogen attach to it. So, it is coordinating ability is much more, so this particular nitrogen can go and bind to the molybdenum center very easily. So, the substrate if we considered that this xanthine is the substrate, so substrate can go very nicely on the molybdenum center. That molybdenum center bearing the oxygen atoms means the molybdenum oxo site, that oxo group if it possible to transfer on this carbon were this carbon is in close facility of the molybdenum side, we get that difficult hydroxylation reaction.

(Refer Slide Time: 05:28)

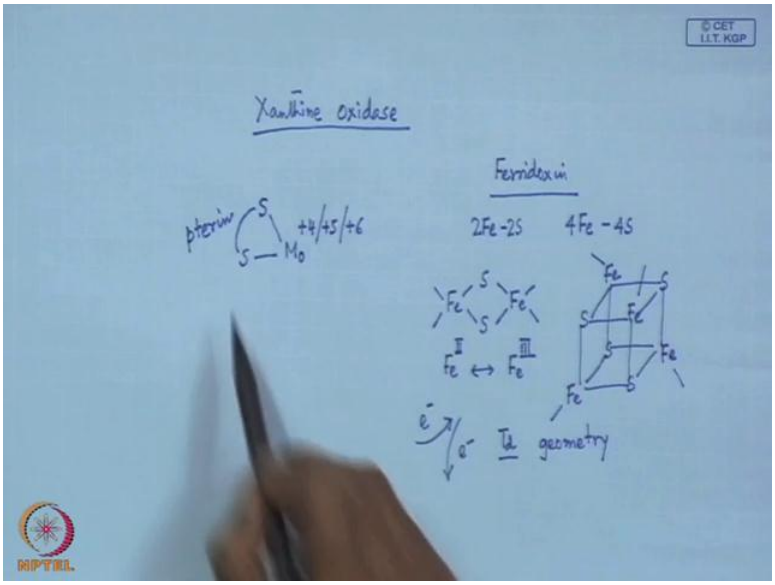


So, in a big protein structure where we have several other parts also, little bit we should also know what the protein structure which is a large one is and have a several molecule weights of a 270 kilo Delton and apart from molybdenum atoms. So, analytical part that means the how many molybdenum centers what other per which is taking part in oxidation and deduction reaction we should also know. So, it has 2 flavin molecules, also those flavin molecules bound as FAD fadalin adenine dynucliotite, then 2 molybdenum atoms that we are seeing, the molybdenum centers which is responsible for the oxygen atoms transfer and 8 iron atoms per enzymatic unit.

So, for the molybdenum center to work and so the enzymatic action on the xanthine molecule what we see that the iron bearing centers whenever we have some iron bearing centers like that of our ferredoxin or other related iron sulfur proteins. We will see that these particular iron centre when they settle between the two oxidation states commonly available for iron that means the ferra state and the ferric state can give the electron to the molybdenum side at right potential because we want to transfer.

We want to move this molybdenum center from plus 4 to plus 5 and then to plus 6 because the catalytic reaction. This molybdenum centers are sate ling between plus 4 and plus 6 oxidation states, so how we bind this molybdenum sides when the within the protein structure they are contained in moybdopterin cofactor and are active sides of the enzyme.

(Refer Slide Time: 07:33)



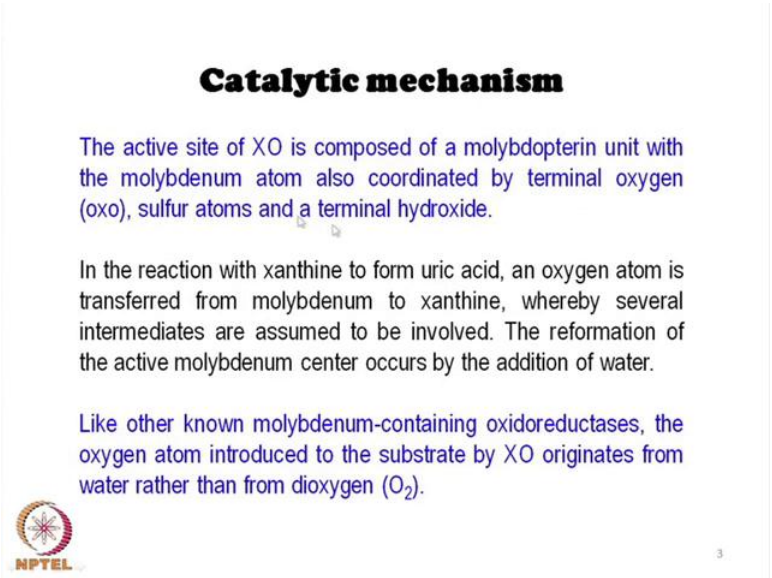
So, that we have an earlier seen that if we have two sulfur parts which are coming from moybdopterin part. So, basically these two sulfur groups are being utilize to buying this molybdenum site, so this particular molybdopterin unit. So, when this terrain unit is bound to the molybdenum site to a, we consider it has a molybdopterin cofactor and there for that molybdopterin cofactor is the active site of the enzyme and what is the role of this iron atoms. So, iron atoms as part of two iron two sulfur ferridoxin type of thing and there for this particular two iron ferridoxin or four iron ferridoxin. So, when we have start in ferridoxin molecules, so it can be two iron ferridoxin or four iron ferridoxin.

So, when we have two iron, two sulfur ferridoxin or four iron, four sulfur ferridoxin, we all know that this two iron sides are in tetrahedral geometry. You have this two sulfur and this two that means in all this cases is particular center is sate ling between Fe 2 and Fe 3. So, you can take out electron and you can put the electron to the system, but this one is giving much mod electron where you have a cube type of arrangement. So, we call is a cube like four iron ferridoxin molecule, so this particular one, so alternate corners are occupied by iron atoms and other four positions belongs to sulfur, so this iron again we have all iron in tetrahedral geometry.

So, this particular geometry is important and from that geometry it can give lies to this two oxidation stress iron to analytics, so basically it can give up electron to the system were you have this molybdenum. So, this molybdenum you can have plus 4 plus 5 and plus 6, so these are the electron pull basically, so we are having some electron pull based on two iron, two sulfur system or four iron, four sulfur system which is being donated to the molybdenum site. This can ultimately go for the corresponding oxygen transfer reaction, so this is therefore, a part of the iron sulfur clusters and participate in electron transfer reactions.

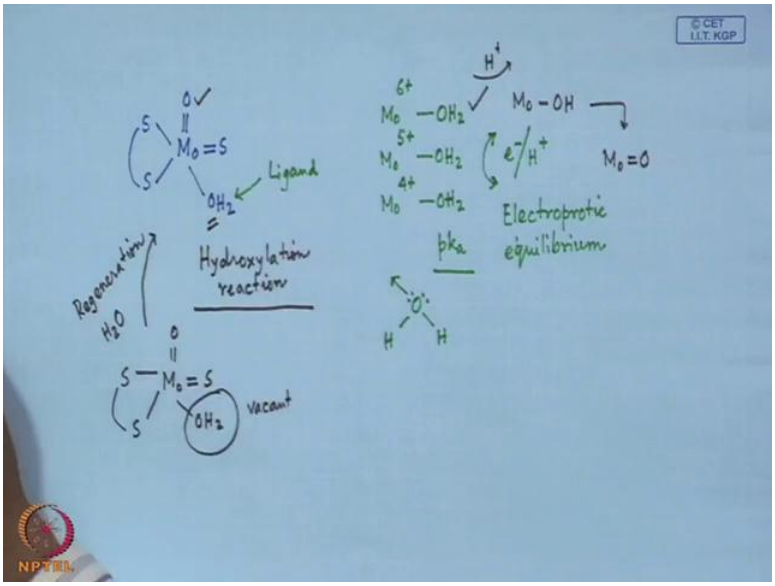
So, basically when we are talking about some reaction were electron transfer reactions are taking place, but some of these redubs reaction are taking place based on FAD like NAD also. Based on ferridoxin, this can be a good Supplier of a electron or which can take up electron from the system as an when required.

(Refer Slide Time: 11:24)



So, if we have see that the what is happening there in the catalectic mechanism the active site of xanthine oxidize is already we have seen is composed of the molybdopterin unit with the molybdenum atom also coordinated to terminal oxygen. That means the oxo sulfur atom and a terminal hydroxide group and interactively at this particular point is important to know that if we have that molybdenum site which is bound to the oxo which is bound to the double bound sulfur also suloxide also. Then, terminal hydroxide because this particular terminal hydroxide function can generate very easily on the molybdenum center.

(Refer Slide Time: 12:10)



If we considered that molybdenum is there which is bound to our oxygen as well as sulfur and two sulfur from the terin unit and bound two water molecule. So, in this panda coordinate form, initially we have this water as the very use full ligian or very good ligian to bound to molybdenum site and this molybdenum site can have plus 4, plus 5 or plus 6 oxidation state, so this particular one. So, if it goes or if it bounds to the molybdenum in the highest possible oxidation state in this form that means in the plus 6.

So, you can think of that molybdenum 6 plus you can have molybdenum 5 plus you can have and molybdenum 4 plus you can have and we are talking about something were you have all these centers bound to water molecules. This is true for all other metalize, also when we dissolve any typical metals all in water we always get a corresponding compress species like that of in that. When we dissolve predict chloride in water, we get the corresponding hexa

aqua ferric iron in plus three oxygen state. So, initially when nothing is available, so we have the loosely bound water molecule it forms the coordinate bound to the molybdenum center.

So, depending upon is transfer that means how its sate ling between this electron change it can also considered the corresponding equally be on related to proton transfer and these to transfers are simultaneously related to each other. They are engaged in sum equilibrium which we considered as electroprotic, so this equilibrium will dictate that when a metal center in different oxidation states are bound to these water molecules. We can considered the corresponding pka values of the water that means the free water which is not bound to anything.

So, we all know the corresponding p k a value of these water molecule and when any one of these loan pair of electrons are both of them are utilized for the co ordination to the metal center. We get something and due to this donation to the metal center these p k value is changes and we are getting more and more acidic proton. So, p k a value is trooping down as we move from a oxidation state of plus 4 to plus to plus 6. So, this water molecule means hydrogen iron that attach to water molecule are more and more acetic as we go from a lower oxidation states to higher oxidation states.

So, for that reason, we get the corresponding information that these particular one when p k is lowering, so this is the thing that means the molybdenum in plus 6 in oxidation state. It can immediately go for a proton loss such that you can have a corresponding molybdenum binding for OH and will the loss of one proton. So, instead of water bound to the molybdenum center, we have the hydroxide group bound to the molybdenum center. If there is a reduction in oxidation states that means during this process, if it going down to plus 5 or plus 4 oxidation state, the p k value of this OH group attach to the molybdenum center is for that change.

Otherwise, it can go from here to a molybdenum oxo spaces, so these particular wants to attachment of this water molecule to the molybdenum center is a very important attachment. This ligand after losing one proton it can go to hydroxide functions or it can go for to molybdenum dioxo spices. So, that particular formation, so very easily in water medium when plenty of water is there surrounding the enzyme surrounding the protein structure. It can go and attach to the molybdenum center and depending upon its reactions of all these it can move to molybdenum oxo form because whatever we are looking from here is that.

Ultimately, we can transfer these oxygen to the xanthine molecule for its corresponding hydroxylation reaction.

So, either these oxygen or these oxygen from the water molecule will be utilize for hydroxylation reaction on the substrate molecule hydroxylation reaction on the substrate molecule. So, when our substrate is xanthine which is be utilize for the formation of uric acid, so what we get we did have to transfer one oxygen atom from molybdenum to xanthine. In that process, several intermediates are assumed or several intermediates some formed, so involved from the intermediates are very important to know to know the correspondent catalytic cycle.

If we are able to know what the corresponding nature of these intermediates are, then only we can have some idea that what type of transfer is taking place from molybdenum to xanthine molecule. Weather it is from the water molecule attach to the molybdenum center or already the dioxo function what is present on the molybdenum that is getting transfer to the xanthine molecule. In the intermediates form which is very important for all the metalize enzyme that whatever intermediates enzyme sub form. If we can identify the nature of molybdenum at the starting point that means that catalyst at the resting states if we know that.

Then only, we can start thinking or start forming that entire catalyst cycle depending on the oxidation states of that particular transition metalize. So, when we have the molybdenum, immediately the crystal structure for the corresponding metal enzymes available. We can know the corresponding environment that means what are the donor atoms attach to molybdenum and the corresponding oxidation state of the molybdenum. During the transformation, how the molybdenum oxygen states is changing and it is not always possible to identity the corresponding crystal structure.

For all the molybdenum center that means the molybdenum in plus 6 plus 5 or plus 4 oxidation states which can be crystallized and we can determine the corresponding x structure based on hole sides. So, we can take the help off the spectra off techniques, so among the three oxidation states involved plus 6 plus 5 and plus 4, some of them particular the molybdenum 5 is well established as it is corresponding epr signals. So, EPR response if we can monitor in the solution state the corresponding EPR response and if we are able to dictate the corresponding EPR pattern for the plus 5 oxidation state of molybdenum.

We can think of that during this catalytic cycle molybdenum is getting reduced from plus 6 to plus 5 oxidation state and in that particular process the molybdenum is also activating the oxygen atom for transfer to the xanthine molecule. So, during this transformation whatever molybdenum centers is active in the resting state after the transfer that oxygen atoms which is already attach to molybdenum side. We are giving the particular oxygen to xanthine to produce the uric acid, so you have the molybdenum center already there so that particular side will be vacant.

So, if this water molecule is getting activated for hydroxylation reaction and at the end were you have they transfer off these oxygen safe from this water molecule is taking place to xanthine molecule we generate some vacancy over here so these will be vacant. So, to get back the original catalyst in that active side so that is means regeneration, so regeneration of that active site is possible through water co ordination which is available in the system and those water molecule. So, vacancy is created because this is transferred to the xanthine and large number of water molecule are available in the enzyme in surrounding and that is water immediately go and mind to the molybdenum side.

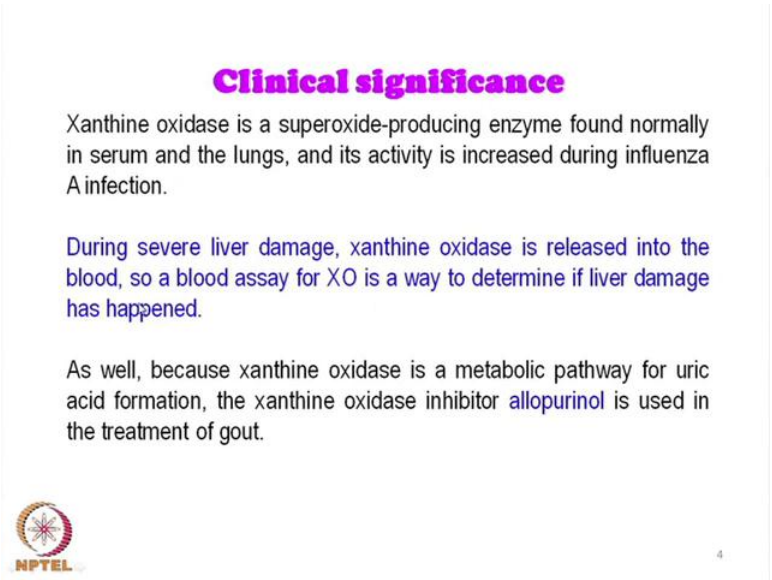
So, this basically tells us that reformation of that active molybdenum centers which is catalytic active there for occurs through the addition of water molecules which are available surrounding the enzyme system. That means large number large numbers of water is there and from that water molecule in dually can go. One vacancy is created the molybdenum side because the molybdenum, if it is stabilized in the panda coordinated form. So, it cannot be in a coordinated form, immediately the water will go and bin that particular vacant side and make that molybdenum center site again pent coordinated.

So, in all these oxidoreductases when we have some oxygen introduce to the molybdenum site and molybdenum dioxo form is forming and xanthine oxidase is generating. So, during the formation and xanthine oxidase we find that the molybdenum is getting activated through water coordination as molybdenum oxo compound. That molybdenum oxo compound is transferring this oxygen to the xanthine molecule giving xanthine oxidase but, in some cases with know that the this particular dioxygen is not in there.

Like that particular case, our iron bearing and copper bearing system where iron is also bound to the dioxygen molecule and copper is also bound to dioxygen molecule for the hydroxylation reaction. This particular dioxide hydroxylation reaction is pretty interesting

because it is only that lasing through water molecule. So, water molecule is informed they are and oxygen of the water molecule are corporate on the molybdenum sits and that is ultimately transfer to the xanthine molecule. So, it is not that the dioxygen molecule is form there are for this particular hydroxide, so it is water mediated hydroxylation reaction based on molybdenum enzyme on xanthine molecule.

(Refer Slide Time: 24:50)



So, why we want to know this that why this are so important so if we considered the corresponding the clinical bio chemistry what we know from the understanding that how much uric acid is forming in one person's body compare to the other. When it increases, a particular level we have several other thing happening on this particular patient, so clinical significance is there for important. In some cases, it is hydroxyl ting the substrate like xanthine but, in some can other cases it can also produce super oxide also from dioxygen molecule.

So, if the electron is transferred to dioxygen molecule itself, it is producing a super dioxide. So, we can considered these as well an superoxide producing enzyme, so they are found in a serum and the lungs and its activity is increased during influenza a infection so xanthine oxidase so during some ab normal condition influenza flu. A person suffering from influenza an infection the xanthine oxidase present in his or her body is little bit active. This activity means if this is active it can work well on the xanthine molecule and xanthine oxidase is active and it is producing more and more uric acid.

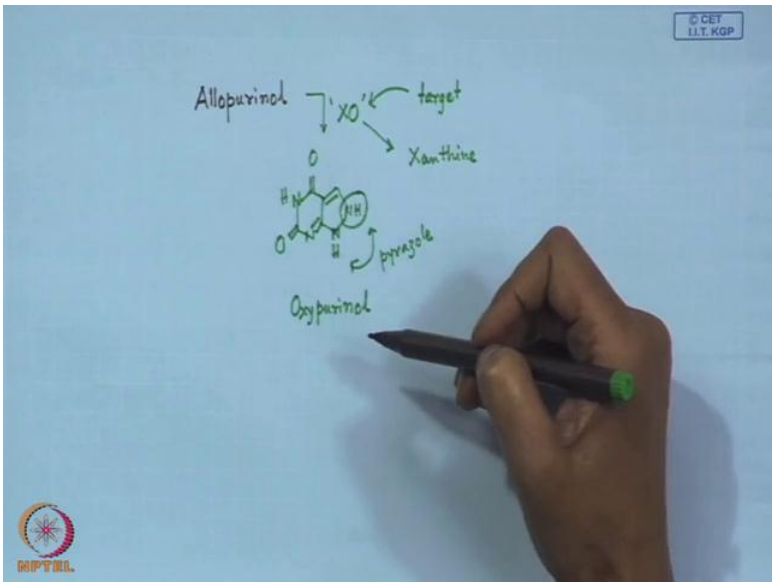
So, accumulation of the uric acid in the body would be more and more some disease condition also in some savior liver damage some cases when people suffering from a damage in the liver xanthine oxidase is also resealed in the blood. So, a blood assay xanthine oxidase can be utilized for the determination of the condition of the liver damage or some disease related to liver. We come to know well known is this pharoses one and the condition of the liver and how much damage is taken place on the liver related to the concentration of xanthine oxidase available in the blood we can considered some information.

So, in all these cases weather the xanthine oxidase is little bit active more or in some other cases were the liver is also getting influence or its getting damage due to the presence of this oxidase. We want to do something to reduce the corresponding activity of these so this what is we are telling here is that activity is increased. So, in some condition when the activity is increase we have to slow down it is activity fast, we do not have at the correspondent increase income concentration of the uric acid is in the blood. So, in blood some inhibitor which can go and buy into xanthine oxidation system that means go and buy into the molybdenum center function has a xanthine oxidase inhibitor.

So, this inhibitor are nothing but which can inhibitor corresponding activity of any enzyme which is a common terminology for all this inhibitor design people can design as this in inhibitor design. So, they are very good medicines also for reduce the activity xanthine oxidase in our body, so in a metabolic pathway when we want to inhibitor corresponding action of xanthine oxidase on xanthine to reduce the formation of uric acid in our body. We use in one such inhibitor which is known as allopurinol, so which is utilize for the treatment of gout because the gout is nothing but it is the correspondent manifestation of the large amount of accumulation of the corresponding uric acid.

It is getting crystallized in the bone joint which can give several pain to the patient, so if we want to reduce the correspondent crystallization of uric acid. We want to reduce the correspondent concentration of uric acid that means we should have such in condition where the activity on xanthine oxidase can be reduce, so these reduction can be taken place by a suitable thing which is our allopurinol.

(Refer Slide Time: 29:32)



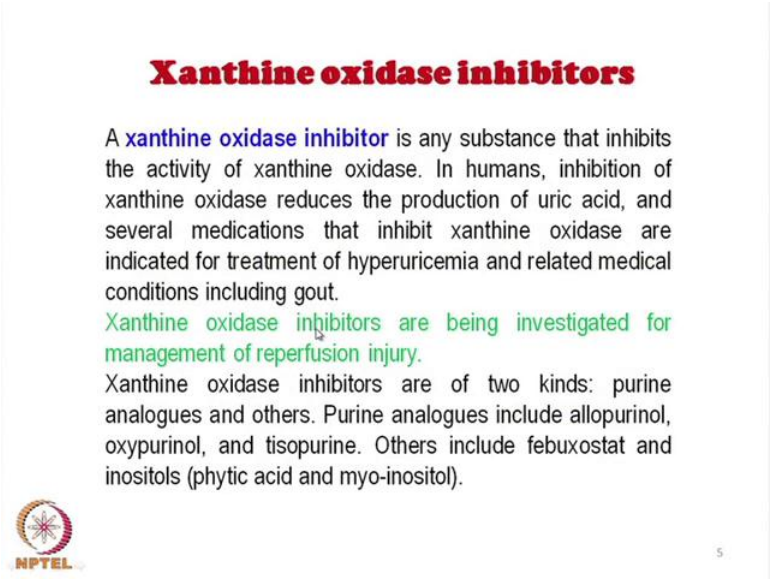
So, this allopurinol will see what that particular molecule is, so this allopurinol will be some molecule which will be related to xanthine itself, so this particular allopurinol will be acting on xanthine oxidase. So, because this xanthine oxidase is the target because for the different targets so the allopurinol will come and bind to this particular system. The xanthine oxidase instead of working on xanthine because this xanthine oxidose can work on xanthine itself to get is oxidised uric acid. So, it cannot work on xanthine because instead of xanthine, it can bind to allopurinol and oxidation of xanthine to uric. The allopurinol will be oxidised to something else related to the same type of reaction.

So, if we know the entire structure of these that means this is our allopurinol and these particular differences these particular one for that means in xanthine. We have nitrogen over there and this particular center was our carbon center, but in allopurinol this two nitrogen are adjusted in nitrogen. So, it is again a design type of molecule on the five member ring, but this particular part is basically related to when we have two adjust in nitrogen in any pentagonal or cyclic system. We know that this is our correspondent phrasal part so it is very easy to synthetize the synthetic chemistry no how to synthetize this particular parts this is basically phrasal part.

In case of xanthine, when these nitrogen these two nitrogen are not adjusted in between we can have a carbon that is known as immeduial part. So, if this particular ring immeduial parts is substituted by perosol part, this can function as a drag instead of functioning as a substrate

xanthine oxidase. This xanthine oxidase can work on this particular drag on allopurinol and this allopurinol is getting oxidized on this carbon. Now, giving us the oxiperinol, so the product of thing is the oxiperinol, so the different course of reaction on substrate molecule this allopurinol is there for functioning a correspondent telemeter of xanthine oxidase.

(Refer Slide Time: 33:06)



So, when we use this allopurinol has the corresponding drag for treatment of gout we get a general class of different xanthine oxidase inhibitors. So, in general form which are the molecules which can go and bin to molybdenum sites bin xanthine oxidase. We see that xanthine oxidase inhibitor is there which will definitely go and bin to the molybdenum center and reduce the activity of xanthine oxidase in our body when introduces the corresponding production of uric acid and other medications. In it also, xanthine oxidase are indicated for treatment of hyperuuricemia and related medical conditions gout. So, gout as well as hyperuricmia the concentration of uric acid is in much more compare to the standard level.

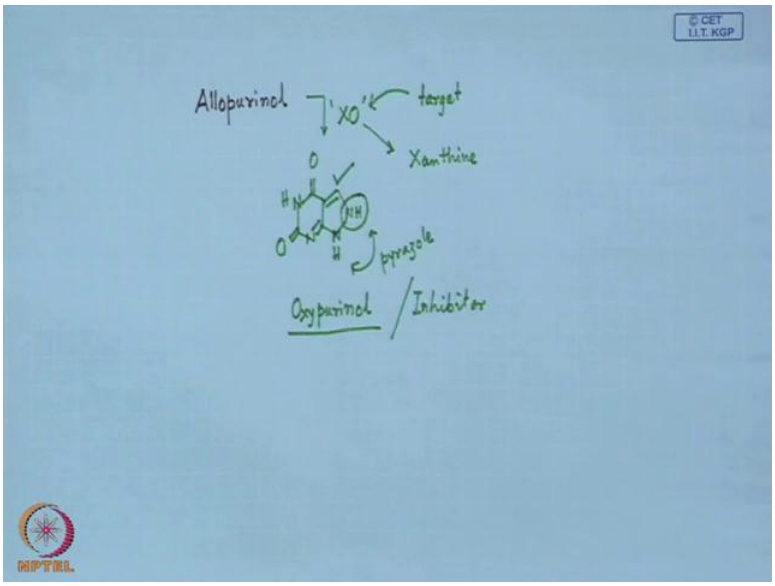
We know that xanthine oxidase is much more active and which is acting on the xanthine molecule producing large amount of uric acid into the blood. Ultimately, the urine, so we design large number of inhibitors and we can use these as they are potential as a inhibitor and which can also, which can study or which can investigated for management of reperfusion injury.

Also, we can have the mode active xanthine oxidase diseases, so we can have two different kinds of this purine analogues and the other. So, since we are talking about this performing

oxidase activity on purine type of molecule some purine related molecule because we do not want to change the entire skeleton which is our soft strait much more. Otherwise, if we can have a different skeleton compare to our purine molecule, we find that particular thing cannot function as a very good or a useful in a inhibitor because it can go and bin to the molybdenum sits easily.

Little bit if we can modify like that the formation of the alloupurinol compare to our xanthine molecule, we see that the simple change that mean the carbon is changing to nitrogen and another carbon is that is nitrogen is getting change to carbon. So, little bit modification of the parine, soft strate molecule can give a rise to the design of the inhibitor of molecules. So, one such analog of purine which is our substrate is allopurinol then what is forming from allopurinol, just we what we have seen that is our Oxy purinol.

(Refer Slide Time: 36:03)

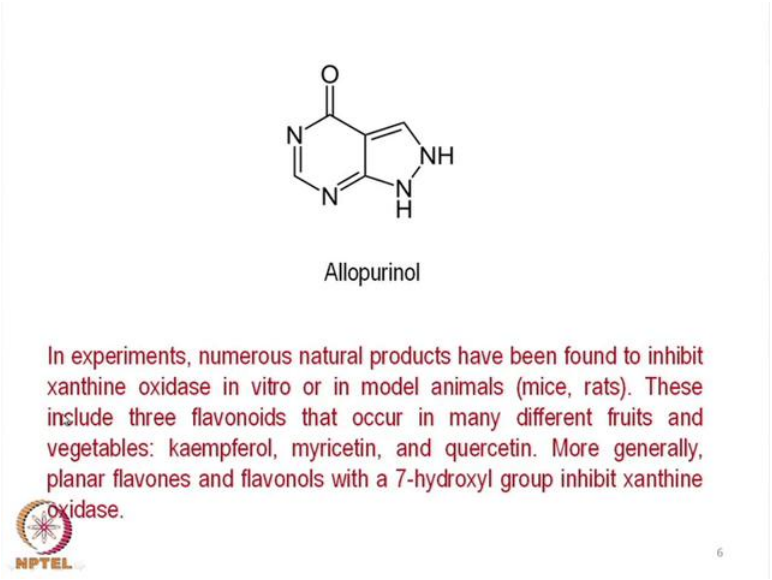


So, this particular oxy purinol can also function as inhibitor again because it has also several nitrogen donor points. So, donor groups, so this nitrogen donor groups are go and been to the molybdenum sits and it has still some position available which can go for hydroxylation oscillation. So, if the carbon atom is still available for hydroxylation reaction we can go for another oxygen oxidation reaction. Already, we have seen that they production of uric acid, we can have three oxygen atom the entire molecule.

Here also, you can have the possibility of inserting the third oxygen atom on the oxyperinol molecule. So, the second category oxyperinol, then tisopurine is also another purine based

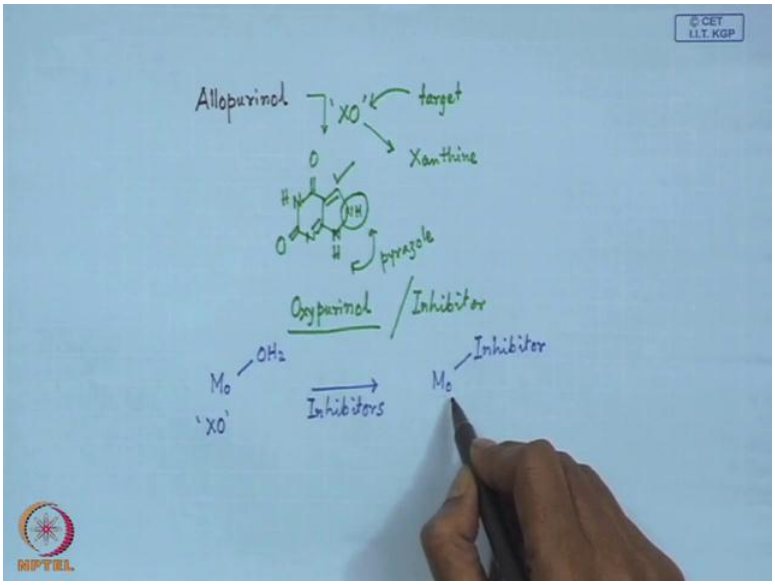
molecule which can function as a very good in embitters. So, these are the inhibitors which can work on this xanthine oxidase and function as very good oxidase inhibitors. Then, in the dark marketing very huge and other molecules are also people are tasting in potential also utilized they can be non perial type of molecule. So, that include febuxostat so that inositols, so they are corresponding other diver tic inostols can also very useful to beyond to the molybdenum sits it can protect that molybdenum sits from binding to the xanthine molecule.

(Refer Slide Time: 37:43)



So, these already we have seen this allopurinol so they are several other group of molecules.

(Refer Slide Time: 37:57)



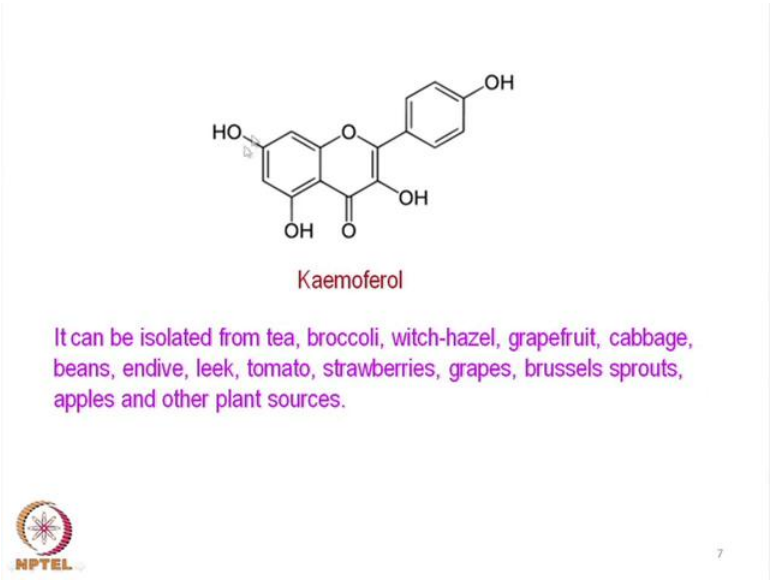
So, when we have the xanthine oxidase we can react basically is very simple reaction what we can study because what we are studding here is that particular molybdenum sits which is there as the xanthine oxidase. So, molybdenum sits is available and that molybdenum sits which is loosely bound to the water molecule so that particular sit available so due to this displacement of this water molecule. We can put some different type of inhibitors such that the same molybdenum sits is bound to the inhibitors.

So, the binding position, so this nitrogen is available on this molecules like allopurinol is go and bind to the molybdenum sits. So, different other groups, so inhibitors study or in model animals like mice and rats several flavonoids which are coming from different fruits and vegetables. So, kaempferol and myricetin and quercetin, so these are from the biological origin like that of our fruits and vegetables, so fruits and vegetables origin is also useful to know because some time.

We are consuming because allopuriniol is synthetic molecule, if it is not available naturally you can have to consuming at a drag, but this particular things if we can study this that is simple molecules from fruit origin or vegetable origin can go. Also, coordinate molybdenum sits and once they are coordinating, if they are binding constant is pretty high compare to the binding of the corresponding xanthine molecule. We can find that is particular molecules can inhibitors the corresponding activity of the xanthine oxidase.

So, they can function very as some good and useful enzyme inhibitors, so this and some other planar flavones and flavones, so flavones and flavones with a seven hydroxyl group inhibit the corresponding xanthine oxidase disease. So, there are large numbers of molecule apart from the allopurinol is also available to function as some kind of corresponding inhibitors for xanthine oxidase.

(Refer Slide Time: 40:31)



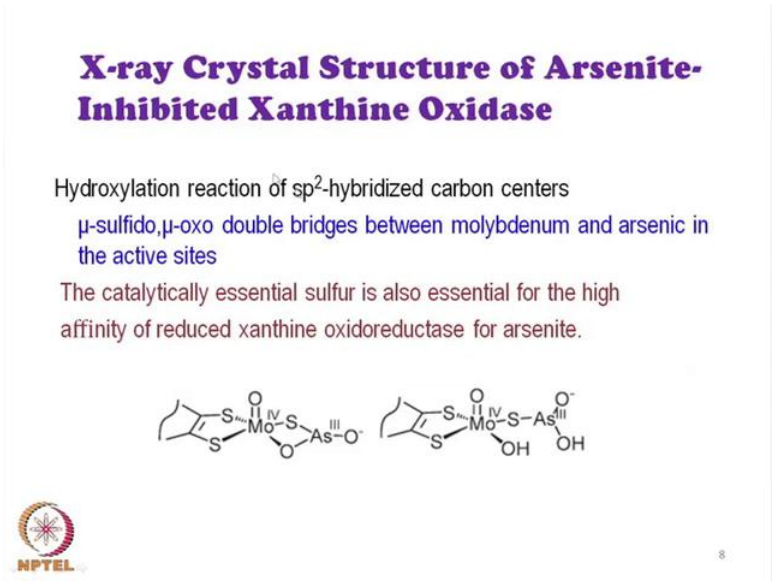
This is the corresponding structure, so basic structure and basic skeleton for this molecules is that is basically kind of molecule and we have several OH each function this is not like allopurinol. So, kaemoferol is such a molecule that we have several oxygen and OH groups, so these are basically same in equal type of fragment when we have two adjust in oxygen atom and in a form. Here, this particular function can behave as a such a lesson to the molybdenum sits, so its binding constant should be more and this particular warm when it enzyme to the molybdenum sit it very easily, go and sit on the molybdenum sits.

This particular molybdenum sits on the xanthine oxidase is not be available to enzyme xanthine molecule there for this kaemoferol. We can use full as a very good xanthine oxidase inhibitors and this particular molecule which is of natural origin also large numbers of food materials. That means the several plant sources are available such has it can be available from tea is available from broccoli, witch hazel, grapefruit, cabbage, beans, endive, leek, tomoto, strawberries, grapes, Brussels, sprouts, apples and other plant sources.

So, this is a very useful xanthine oxidase inhibitors when we get all this from tomato and all these fruits which we take day today, but it depends on the concentration because since we are not consuming in the pure form because as a drag is concentration. That means it is bound in different form in this food materials, so this should be available to go and enzyme xanthine oxidase. Then only, it can inhibit the corresponding activity of xanthine oxidase, those they are very much available from the food material. So, the flavones and flavones type of

molecules which we know is plenty in all the food materials is a very good information, this can also function as a xanthine oxidase inhibitors.

(Refer Slide Time: 42:58)

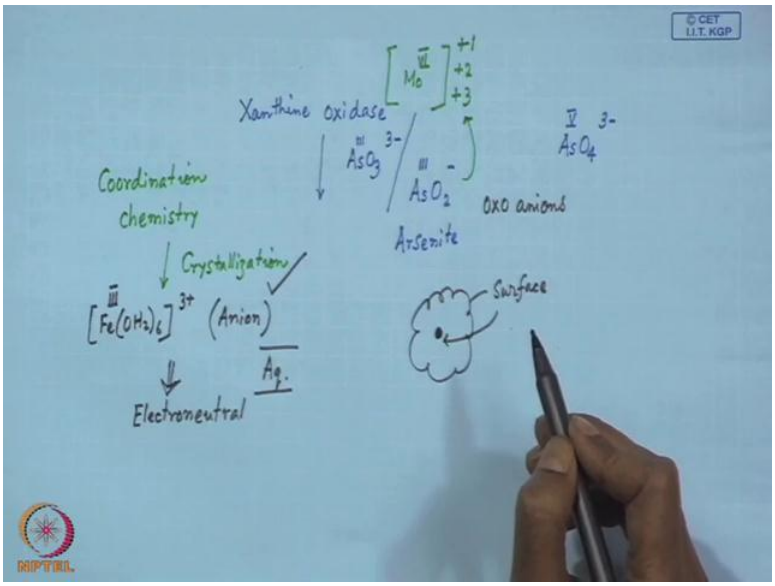


So, one such examples of this inhibited design tells us that that if we just go for a corresponding xanthine oxidase which is a very recent of starvation people can go for. This particular metalize enzyme sits which is molybdenum based enzyme sits and sometimes it is very difficult to be crystallized that means if we want to determine to corresponding crystal structure of this molybdenum sits. So, it is difficult some time to crystallized in a good crystal because it diffracts the corresponding diffraction, but another way of learning particular learning form that.

If we use some iron, other groups because inhibitors whatever we have discuss, now that the corresponding allopurinol and this corresponding this different flavones and flavones. So, those in the bound form to the molybdenum sits is also difficult to crystallized, but if we can go for some in organic oxo an iron, so in organic oxo iron like that is of our arsenite.

So, if we go for crystallization of this particular spaces were a arsenite and iron has been taken and this arsenite were arsenic is present in the low oxidation states not in five oxidation states. That means we have two combination of this arsenic oxo an iron one is arsenite another is arsenate, so this particular arsinite, so simply reacting this corresponding oxidase states.

(Refer Slide Time: 44:55)



So, if we have xanthine oxidase and if we are trying something were we have AsO3 which is three that means three minus and we are not using and spices which is related to arsenate. So, arsinite will be there, so between this arsenate and arsenate, we have a separation form to this particular oxygen atom this can also produce some other spices which also arsinite base which is AsO2. So, in solution any of this two spaces which is based on arsenic three oxidations states compare to oxidations five. So, they are different arsinite spices and basically what we are doing we are doing a very simple in a organic chemistry or coordination chemistry this particular point.

So, that there are taking the help of typical coordination chemistry, so what we can do is basically the crystallization since we are having with xanthine oxidase which if say is considered is a molybdenum six diseases. We have the legend from the there in part we have the oxo form and all other thing still we can have from this coordinate's we can have some charge.

So, if we have a cationic charge on it plus 1 or plus 2 or plus 3, so you can have different cationic chargers on the protein in well and enzyme well. So, definitely during crystallization it will definitely go for attracting the corresponding an irons, so if arsinite is utilized it can go for corresponding crystallization because you can have the charge utilization the way we go for the typical crystallization. That means if we have simple Fe axo iron three in cationic form.

So, we can supply sum an iron such that we get some molecule which is electro neutral, so the electro neutral definitely tell us something that when we go for electro neutrality because you have the medium as the aqua's medium or water based medium. So, if we are able to neutralized the positive charge accumulation on the protein surface or the enzyme surface by some anions providing suitable amount of on which we are reducing corresponding cationic charge accumulation on the protein surface. So, the corresponding tendency for crystallization would be much more and at the same time this oxo anions so this are typically different oxo anions which are oxides base.

So, this are oxo anions base oxo anions hence can have another tendency not only bin to the corresponding protein or the enzyme surface because on the surface. We have some cationic spaces like the nitrogen bases better iron like all this, but it striate can go inside and can approve to the center were you have the molybdenum. So, what we can think of that this particular thing well known that it is responsible for the hydroxylation reaction of sp 2 hybridized carbon center not sp 3 hybridized. So, it can go for the corresponding hydroxylation on the sp two hybridized carbon center on xanthine.

It can go for the corresponding phenol type of thing but, it is not going for the methanol which is sp 3 hybridized, so what we get during the reaction of this arsinite thing we get a mu sulfide mu oxo double bridge. So, already we can have this terminal groups already we know that molybdenum oxo and molybdenum sulfide groups are present. This was basically water molecule if we recall the corresponding the active sits of the enzyme. This was our water molecule this is the corresponding the oxo and this is the corresponding sulfur system. So, if we just simply attach with that of your arsenate based on AsO2 which is coming from AsO3 spaces.

So, we get something because this arsenate already because oxygen is present this particular sits us oxygen is present with this arsenic. It will immediately remove the water loosely bound to the molybdenum sits and it established new bond which is from this oxygen of arsenate to the molybdenum sits and this arsenic also have some reminders tendency to bin to the sulfur. Also, at the same time it can also bin to sulfur because arsenic has a affinity binding or coordinating to both oxygen and sulfur.

So, basically it can established a double bridges arrangement it is basically a system where we have best on that Melton enzyme, but is a molybdenum arsenic dynamic form. So, that

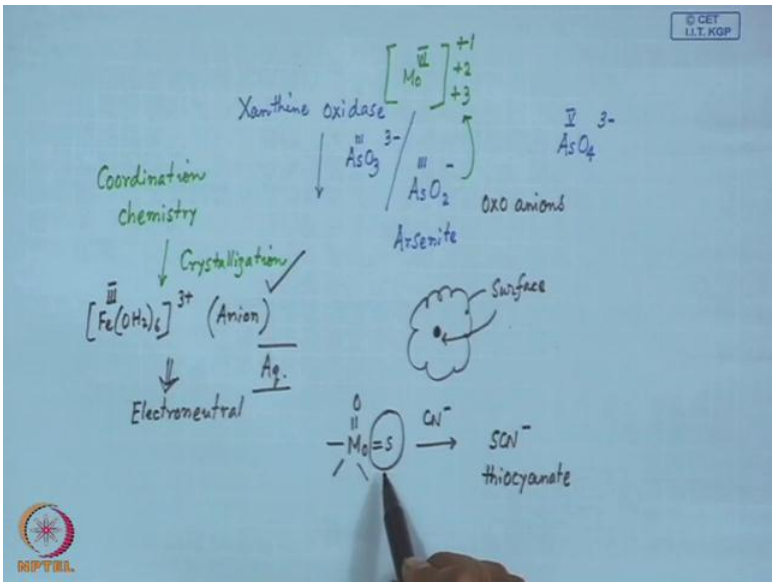
dynamic form what we are getting we are getting that due to the establishment of molybdenum sits molybdenum and arsenic between this two. This is one particular form and this is what we have seen in the other form means when we have this water also here this water can go for depot nation and arsenic when approaching the molybdenum sits.

It can either go through the sulfur for or through this oxygen so if we establish the first thing that means if we can go to sulfur. Next step, it can bin through this oxygen through the removal or one water molecule, so this catalytically essential sulfur is the high affinity of reduced xanthine oxidase dedated for arsenite. So, what we see that due for this particular reaction the molybdenum center what is present this particular molybdenum center is useful required, but not that molybdenum but also the sulfur center.

Also, is useful because the quadratic function is dependent on presence of sulfur, but when we are trying to block this particular reactivity of molybdenum as well as the molybdenum sulfur assembly. Through the attachment of the arsenate group attach to that, we see that basically block the particular site in this form that means this oxygen is also not available for binding to the xanthine molecule. We are blocking this particular assembly, so arsenate is definitely there for a very good inhibitors.

If we can consider that this oxygen is available and this oxygen is responsible for transferring the xanthine molecule, so what is the role of this sulfur that people have. Earlier, established that when the presence of first this oxo group and the sulfido group on the molybdenum sits was established. This has been established through the removal of this sulfur group from the molybdenum sits by doing is very simple reaction.

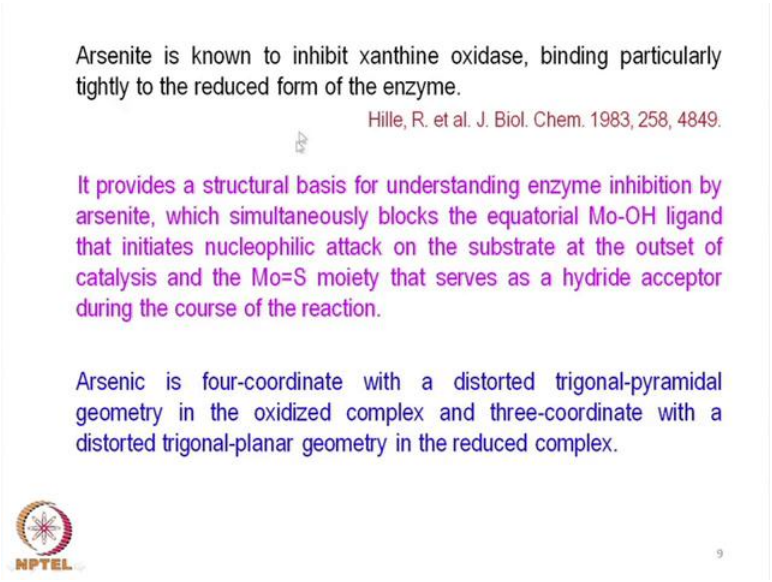
(Refer Slide Time: 53:51)



That means, if we have a MOS, how we can established this a m o s functions from this particular side we just simply reactive this particular signed iron. So, any form of this iron inform of this sodium iron potential sodium iron solution, it can very easily removed thiocynate an iron from the system. So, thiocynate group are removed from the system which is basically removing this particular sulfur function from the molybdenum.

This is getting deactivated through the removal of thiocynate molybdenum sit we see that the corresponding cathartic activities also changing and that catalytic activity reduction will also tell us that the presence of this sulfur. This was important for the corresponding action of that xanthine oxidase, so this particular sulfur is there for important to be a part of these activity related to molybdenum sit.

(Refer Slide Time: 55:06)



This, we see that this can inhibit Xanthine oxidase particularly it is binding in the reduced from the enzyme and this very good account you can find from a geranial page or the geranial biology chemistry. So, this basically tells us that the structure bases of enzyme that means so that MOOH ligand that the initiates nucleophilic attach on the substrate molecule or MOS moiety that serves as a hydride acceptor during the course of the reaction.

That will see that the between this two things when we have the molybdenum function attach to the water molecule and molybdenum function attach to the sulfur unit what type of nucleophilic attack. We can think of on the substrate molecule so the positioning of this two groups that means weather the particular MO OH bond is present is in the equatorial site. The excel site is important that means the availability of the mo oh ligand from the equatorial site which is important. That means it is not operating from the excel site for the nucleophilic attack that means MO OH which is attacking in the xanthine molecule for the hydroxylation reaction.

The MOS moiety that is basically serving as a hydride acceptor during the course of the reaction so this particular MOS group which is when it is taking of the hydride and it is going from the MO double bond S 2 MO OH. So, during that part of also when it is functioning hydride acceptor, so this cannot function as a nuclear for to attack on the substrate molecule, but this particular MO OH function can a very good nuclear file. This MO OH is can a which is originating from the water molecule and that ultimately delivering this particular oxygen

atom to the substrate xanthine molecule is important to know the corresponding path way for the catalysis reaction.

So, this continue this particular part also because the arsenic inhibitor form which is four coordinate one and in a typical geometry in the oxidized form and the three coordinate is also available distorted tri gonal planar geometry in the reduced complex. So, what there are basically in the form that means this is the tri gonal warn and this is the type of binding. So, what that means this two forms is four coordinate between tri gonal pyramidal geometry in the reduced complex because these two type of geometry around arsenic. Also, important because we all know that the geometry is important for stabilizing a particular oxidation states.

Thank you.