# Bioinorganic Chemistry

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## Lecture - 23
## Molybdenum Enzymes – II

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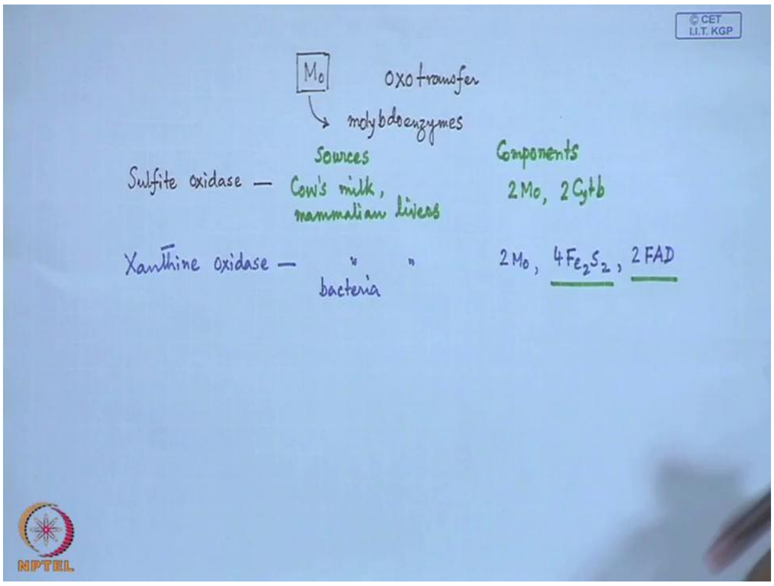

$\boxed{Mo}$ → molybdoenzymes

Oxo transfer

| | Sources | Components |
|---|---|---|
| Sulfite oxidase – | Cow's milk, mammalian livers | 2 Mo, 2 Cyt b |
| Xanthine oxidase – | " " bacteria | 2 Mo, $\underline{4 Fe_2S_2}$, $\underline{2 FAD}$ |

Welcome. So, today we will still continue with that enzyme which is taking the help of molybdenum, and we are talking about some reaction which is a most fundamental reaction that is the Oxo transfer reaction, and since molybdenum bearing enzymes are involved. So, we call them as Oxo transfer molybdo enzymes. So, last time we have seen about that DMSO reductase. Today we will first talk about the sulfite oxidase and these are very important group of enzymes, and they have several sources where from we get these sulfite oxidases, and the basic components; because while talking about this molybdenum center you should not miss out what are the other components also present along with this particular center.

So, the most important source is the cow's milk, mammalian livers and in case of this sulfite oxidase two molybdenum centers are present along with cytochrome B. So, other electron transfer agents which is cytochrome b. So, these cytochrome B centers basically they all we know as that their iron centers and those iron centers will be responsible for transferring electron to the molybdenum center, and this molybdenum center ultimately taking part in the Oxo transfer to the sulfite group. And in the next one which we will talk about is xanthine oxidase having similar sources as well as we can get it from some bacteria’s also and here also two molybdenum centers are present, and four iron sulfur clusters histidine of cytochrome b; we have iron sulfur clusters, and flavin adenine dinucleotide. So, these are nothing but they provide the right number of electrons at right potential to the molybdenum centers. So, these are basically the centers which will be responsible for transferring electron to the molybdenum centers.

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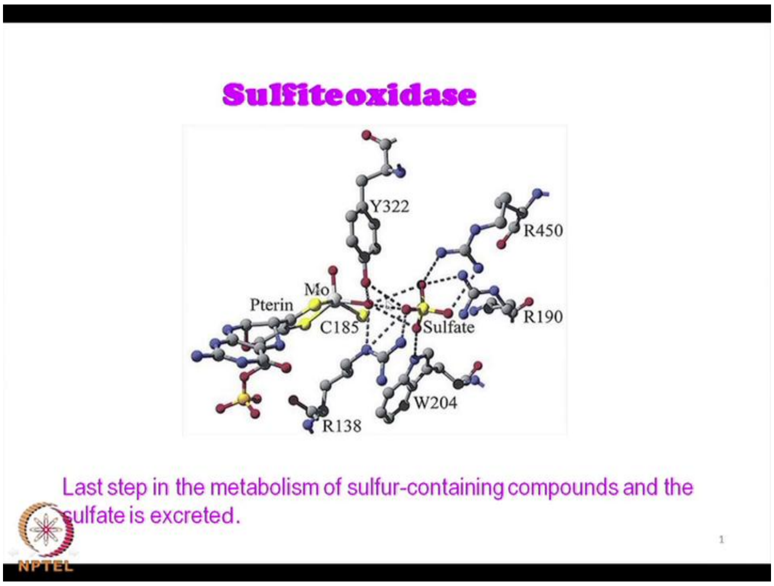

## Sulfiteoxidase

Last step in the metabolism of sulfur-containing compounds and the sulfate is excreted.

So, when we talk about this sulfite oxidase, we will see that the individual centers what is present this I also showed you last time that after this conversion; that means, the molybdenum is there and some groups basically these red bolls all your Oxo groups. So, you have this thiocholine type of di-sulfur coordination then cysteine sulfur.

So, this is also cysteine sulfur and this particular Oxo function. So, this Oxo function and its transfer, it is all present in DMSO reductase, nitrate oxidase, aldehyde oxidase and everywhere this molybdenum centers whether we can go for a dioxo system to a mono Oxo system, and a non-Oxo system. So, if we go for a dioxosystem to a mono Oxo system that particular oxygen can be transferred very nicely to that sulfur center bearing that sulfite anion not the DM center or the dimethyl sulfate center. So, this is the last step in the metabolism of any sulfur compound which can be your drug molecule which can be any other sulfur bearing molecule where you want to oxidize the sulfur center, because sometimes we want to make in the synthetic chemistry also in the chemical syntheses what we find that if you have a sulfur bearing center, we want to make it in the sulfond or sulfoxide groups and ultimately if we can break something; that means, the carbon sulfur bond if we want to cleave, if we can go for the carbon sulfur cleavage where you the sulfur is attached to the organic part. So, that is removing as the corresponding sulfur bearing anions.

So, it can be sulfide, it can be your sulfate and interestingly this substrate hold; that means, where you bind this sulfate group close to the molybdenum center, because you cannot have a very long range electron transfer; because all the iron bearing cytochrome b or pyridoxal molecules or the F a d types of molecules which are involved there. So, these two centers are close to each other and several other residues are also involved for interacting with the substrate. So, this is the substrate binding pocket and this is your molybdenum site.

So, flow is showing the corresponding close proximity between these two centers and some time we will find that this molybdenum Oxo centers the suitable positioning of this particular Oxo compare to the other one, because if you in the most cases these are not in trans orientation, but when a cisdioxo molybdenum center even in synthetic compound or model compounds we will find they are oriented in the cis positions. So, when they are holding in a cis position and if the molybdenum center is penta coordinated, it is closed to a distorted square pyramidal geometry.

So, you have a square base which is also a molybdenum is sitting above this square base and one Oxo is the epical site, but this particular one is the bassel site and this Oxo is close to your sulfite site, and these Oxo center is responsible for transfer this oxygen to your original sulfite group. So, when it is oxidized and formed a sulfate group also. So, this particular one when it is oxidized compounds can be excreted from the system. So, sulfite initially after the sulfate formation, it also binds within the pocket of that particular oxidase enzyme. So, later on it is then removed from the system.

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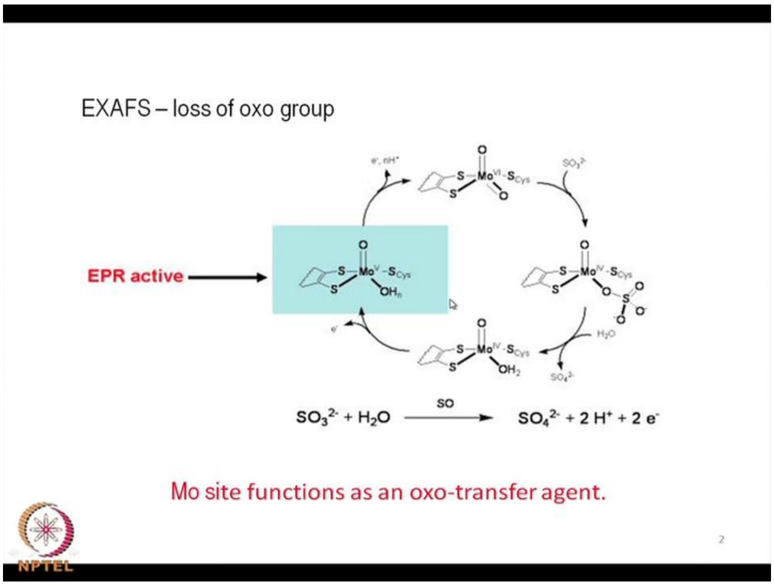

EXAFS – loss of oxo group

EPR active

$SO_3^{2-} + H_2O \xrightarrow{SO} SO_4^{2-} + 2\,H^+ + 2\,e^-$

Mo site functions as an oxo-transfer agent.

So, these are the corresponding sides we have already seen that how the different sites are involved. So, when we have this dioxo site, you have this dythaline part. So, it is in the indythole. So, you have in function one carbon double bond which is giving you the sufficient rigidity to the dythole coordination and the molybdenum one side you have the cysteine sulfur, and the two oxygen. And this particular one though they are in sys orientation, but this angle is greater than ninety degree; this is above hundred degree sometime. So, when it is going there. So, when this formation of this dioxo species in class six oxidation state is important like that of our molybdenum di Oxo acetyl acetonate compound.

So, if you go two of these two sides are coordinated and other two sides are also coordinated. So, that will be hexa coordinated for the synthetic molecule, but here it is 5 coordinated only and you see this not this oxygen, but these oxygen is interacting with your sulfite anion. So, that is why these two centers are sitting within the enzyme pocket nearby. So, what is basically happening there in this particular center? So, cysteine sulfur is still attaching there. So, it is basically going from molybdenum plus 6 to molybdenum plus 5. So, this molybdenum all the time even in the synthetic compounds will find that whenever you are handing something; that means, the molybdenum mononuclear system. So, if you have a mononuclear molybdenum system then when it is attaching through this sulfite groups; it is basically forming though this part is basically a an ionic part, but you have something; that means, you have this bridge between this molybdenum and sulfur through one of the oxygen which is important. So, if you can go for some bridging unit. So, in the intermediate state this molybdenum can interact with some other species and it can go for some interaction where you can have a molybdenum sulfur dinuclear entity through bridging oxido group. So, when it is bridged then these oxygen will be there will be competition between this molybdenum center and sulfur center for this oxygen, and this molybdenum is in the reduced form. So, when this molybdenum is in the reduced form, it will have less affinity for Oxo function.

We all know that the oxido group or the oxido function is very useful for stabilizing the molybdenum center or any other metal center in the highest possible oxidation state. So, if you are able to reduce, its affinity for Oxo would also be less. At that particular point only this molybdenum is providing this oxygen to the other anionic species. So, this is also very interesting to know that some other compound or some other molecules you can consider it also that if you have the metal center you reduce it and it will just basically donate that oxygen to other substrate or the parent molecule. So, this Oxo transfer reaction is very fissile in that sense that if you have just reduce it and that oxygen can be transferred to the sulfite or any other substrate.

So, this is the entity where the sulfate group is oxidized form and it is still bound to the enzyme function. So, this is this thing what we have seen in this particular one; molybdenum is there and you have the sulfate, and already you have the sulfate then you have the transfer then Oxo form is again formed over there, but still it is attaching to the system. So, either it will be attaching to the enzyme center or it can leave from the system, because we are not showing the protein envelope. What we are showing? We are concentrating our attention only on the molybdenum center or the sulfur center, but it is not like that you have the huge envelope surrounding the molybdenum side as well as your sulfate side. So, when we are focus or attention on the metal center for the catalytic point of view, we only focus our attention on the metal center molybdenum and the sulfur center, but we are not focusing our attention on the entire protein envelope. So, protein envelope will be there. So, that will be basically stabilize this formed anion. What is formed anion? Formed anion is your sulfate what is formed from the sulfide.

So, there are some others positions also for the protein chain or the protein cluster that this sulfate group then ultimately will leave the corresponding enzyme function and you will be leaving behind with that again a penta coordinated molybdenum center, but your

Oxo site will now be occupied by O H 2; that means, water. So, initially it is forming as a vacant site, but molybdenum will try to pay for or try to go for a penta coordinated species in plus four oxidation state. So, it will attract water from the enzymatic system. So, then at this particular point again it will go for some amount of this particular oxidation. So, this molybdenum center when it is attaching to Oxo as well as water center, it can go for oxidation. So, one electron removal step can take place and you can generate something where you have this instead of water it can bound to water as well as hydroxide group, because this O H N that is why it is showing as O H N.

So, N can be your one or N can be your two. So, it can be water, it is very difficult to identify whether your water is bound to molybdenum center or hydroxide function is bound to the center. So, what we are talking about for this oxidation reaction is we are settling between two oxidation state which is plus 6 and plus 4, but the regeneration of the active state or the regeneration of plus 6 state is going through some intermediate where molybdenum center is present in plus 5 oxidation state. So, how we can prove this particular form; that means, here you have only a mono Oxo species. So, this molybdenum center is basically a monooxo center and one water molecule is attaché to that molybdenum center, and it is in the molybdenum 5 oxidation state which is a four d one system. So, you have one unpaired electron and that unpaired electron can be very nicely detected by EPR spectra. So, EPR spectrum will tell you that whether you are going from molybdenum four to molybdenum six; why a transient or very weak life time for the corresponding molybdenum 5 center.

So, that can be very easily detected when you pass from here to the other side. So, this spectroscopic technique for this particular one; that means, it should be EPR active. So, in synthetic molecules also whenever we make some molybdenum 5 compound and that can be immediately characterized; forget about its corresponding donor atoms or the ligand centers; you can consider it as that it is EPR active; that means, it is in the molybdenum 5 oxidation state. And this particular loss in corresponding Oxo group; that means, the molybdenum di Oxo species to molybdenum mono Oxo species can also be examine by EXAFS; Extended x-ray absorption fine structure.

So, immediately that is changing the corresponding oxidation state we know that EXAFS technique is very useful to know the corresponding oxidation sate as well as its immediate environment. So, when your immediate environment is changing for a di Oxo form to a mono Oxo form your corresponding feature for x-ray absorption fine structure is also be different for molybdenum six and molybdenum four species. So, EXAFS technique, this specifying characterization technique using EXAFS as well as EPR would be useful to identify any molybdenum site, because in most of the cases they will be settling between these two oxidation states; one is plus six and another is plus four and at which particular form basically; that means, it is transferring this oxygen to the sulfide group and that particular species is responsible to know as this corresponding Oxo transfer agent.

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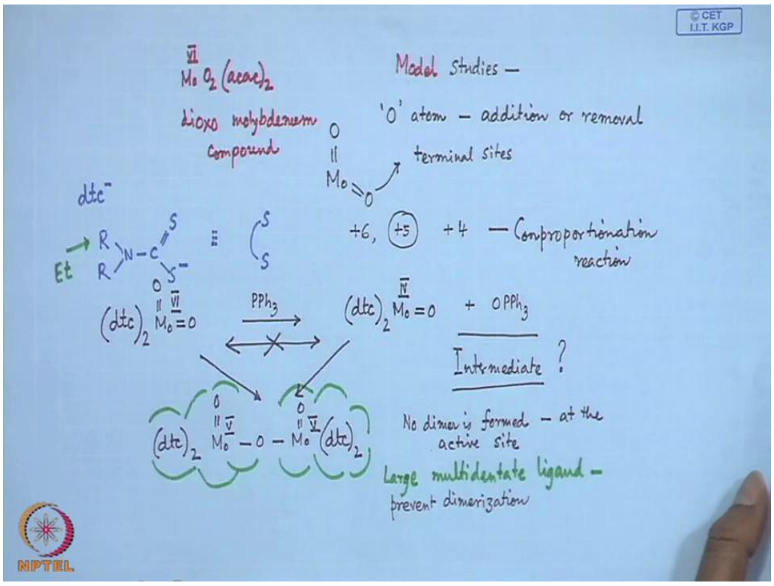

So, what we will see that in some synthetic molecules the most important example is M O O 2 acetyl acetone. So, M O O 2 acetyl acetone is a typical di Oxo compound. So, it is a di Oxo molybdenum compound. So, you can think of something that in all these model studies, because the model studies will be useful to know how good all these synthetic molecules also are useful to transfer this Oxo group. So, model studies will tell us for the fate of this oxygen atom whether it is going for addition to the molybdenum center or removal and since we are talking something where you have this molybdenum and cis dioxo from, and basically we are talking about something; that means, Oxo removal or Oxo addition from the terminal sites it is not where anything from the briding site; that means, that the intermediate what we are looking for the corresponding characterization of molybdenum site in the plus 5.

Oxidation state that is not giving you anything where you have Oxo molybdenum thing is forming; that means, the breezing oxygen is not involved there; the terminal sites are involved for transferring oxygen atom to any other substrate like your sulfite. So, like this acetyl acetone you can take some good example for another ligand is dithio carbamate; dithio carbamate we can make it from diethyl ammine. So, what we are looking for is that instead of not getting anything related to simple that dithiolen molecule, but a sulfur sulfur bidanted ligand is useful. So, at terminal sites when they are forming; that means, we are looking for some oxidation state of plus six and plus four, and at intermediate we are getting plus 5 oxidation state.

So, if we get that compound the DTC, the dithio carbamate through these two sulfur groups can attached to your molybdenum; it is a dioxo molybdenum in plus six oxidation state and what we are looking for? We are looking for something where you can get a corresponding Oxo transfer to any substrate. So, one well known Oxo transfer species is triphenylphosphine and most of the cases we take the corresponding, we can abstract this oxygen from the molybdenum center. So, this immediately can give rise to a compound where you can make the corresponding molybdenum four compound which is mono Oxo molybdenum N O p p H 3.

So, what you want to know that you can have this sufficient stability for the molybdenum in the plus six oxidation state as the molybdenum in the plus four oxidation state so but we all know that in case of say copper two and copper zero and all these. So, if you have these oxidation state one is plus six another is plus four and interestingly from the terminal site, we can expect something which we can call it as a comproportionation. So, whether we can get comproportionation reaction over here or not. So, conproportionation reaction that means if you have these two, when they interact to each other you expect something which is molybdenum a dimeric form. So, molybdenum 5 dimer. So, how these two can interact is molybdenum Oxo molybdenum dimer; that means, we are looking for in the enzymatic cycle; the intermediate. What is that intermediate?

So, if we get that this is the corresponding Oxo form and two DTC ligands are still attached to it; this is 5 and this is 5. So, from the two techniques what I just told you that one is the UDPR technique and another is the EXAFS technique, and these two techniques are sufficient to tell you whether a monomeric molybdenum 5 is in the active site or as the intermediate or a dimeric molybdenum Oxo bridge molybdenum 5 compound is the corresponding species which is there in the active site, but all these spectroscopic measurements will tell us that no dimer is formed at the active site. So, that particular dimer formation is prevented and in this particular case even if we just talk about the simple diethylcarbomate. So, if R is your ethyl, we get diethyl diethiocarbomate. So, at the terminal point; that means, this particular part; that means, if you give for this any of this Oxo center is responsible for the formation of this dimeric one, but in the enzymatics reaction you have the typical protein envelope is there and another protein envelope is surrounding this molybdenum site.

So, the steric bulk of this groups which are responsible to bound to molybdenum center will prevent the corresponding dimerization reaction. So, any synthetic molecule which bears a bidentate ligand of large size. So, large multidentate ligand some sufficient amount of steric bulk such that your one of the species which is forming over there and after reduction giving you the molybdenum four; these two cannot interact; this interaction between these two form can be prevented. So, the large multidentate ligand of steric bulk will prevent dimerization. So, that way we can prove that only the mononuclear system is present and mononuclear species is responsible for the corresponding Oxo transfer to the sulfite group. So, once this is formed; that means, the sulfide is therefore, attaching the sulfite oxidase and your sulfate is formed, and you are transferring nicely this Oxo group to the inorganic and ionic from; that means, the sulfite group.

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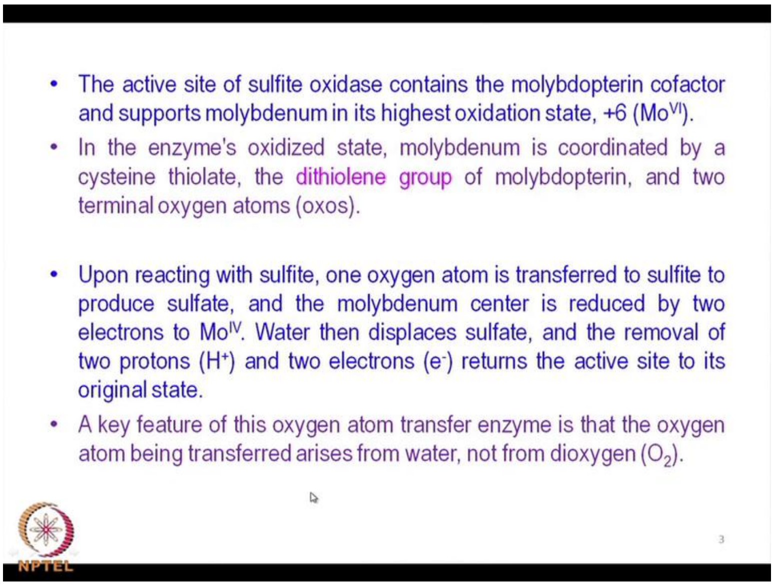

- The active site of sulfite oxidase contains the molybdopterin cofactor and supports molybdenum in its highest oxidation state, +6 ($Mo^{VI}$).
- In the enzyme's oxidized state, molybdenum is coordinated by a cysteine thiolate, the dithiolene group of molybdopterin, and two terminal oxygen atoms (oxos).
- Upon reacting with sulfite, one oxygen atom is transferred to sulfite to produce sulfate, and the molybdenum center is reduced by two electrons to $Mo^{IV}$. Water then displaces sulfate, and the removal of two protons ($H^+$) and two electrons ($e^-$) returns the active site to its original state.
- A key feature of this oxygen atom transfer enzyme is that the oxygen atom being transferred arises from water, not from dioxygen ($O_2$).

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And this we all summarize here there is active site of sulfate contains the molybdopterin cofactor in last class we have discuss that what is that your molybdopterin cofactor. And this particular sulfur coordination in around a double bonded in backbone is basically stabilizing the molybdenum in its highest possible oxidation state; that means, the plus six and the enzyme in the oxidized form is coordinated to the cysteine thiolate, and another interesting group we call it from the molybdopterin unit is your dithiolene group. So, what is that dithoilene group is very important to know is a huge group of molecules and people have studied started knowing all these things when this particular molecule is known let what these dithiolene group, because it has other immediate application. So, dietholene ligand is a separate class of molecules which can bound to molybdenum; not only molybdenum to nickel to any other groups, and they give rise to very useful materials. So, like that of your acetyl acetone or any other class of ligands system, dithiolene also form a distinct class of ligand system.

So, this dithiolene group of ligands are useful for the coordination of molybdenum and you already know that you have the two terminal oxygen atoms, and the manipulation of those two Oxo groups are basically giving rise to corresponding oxygen transfer to the sulfite function. So, is transferred to sulfite and sulfite is formed, and water is then displaces the sulfate, and the removal of two protons, and two electrons returns the active site to its original state.

So, how we get back the cycling and every time I am talking all about this that in all these cases your dioxygen is not taking part for this Oxo transfer reaction, you have to use of the corresponding water molecule. So, it is water mediated reaction that is why this reaction is also very interesting that this particular one is very useful and we are able to go for some oxygen atom transfer, and that oxygen is originated from typical water molecule. So, that is why it is very interesting to know that any coordination chemistry related to the metal center which is initially coordinated to the water molecule then it is coordinated to the hydroxide function and ultimately coordinated to oxido function.

So, how easily we can generate this oxido function from the water molecule that this catalytic cycle for this class of enzymes also show us that you generate the Oxo function; obviously, by taking the help of molybdenum center and that Oxo is very much useful to transfer to any other organic substrate. We do not require any such other reagents like this hydrogen peroxide or M c p b a and all these things to go for this corresponding Oxo transfer, but your source is simple water molecule. So, these basically what we have seen for these the different steps for the catalytic cycle’s and ultimately it is the water molecule which is taking part for Oxo transfer reaction. So, these group of molecules when coming to us will see that whether this particular Oxo group like that of your dimethyl sulfoxide deductase or sulfur dedactase.

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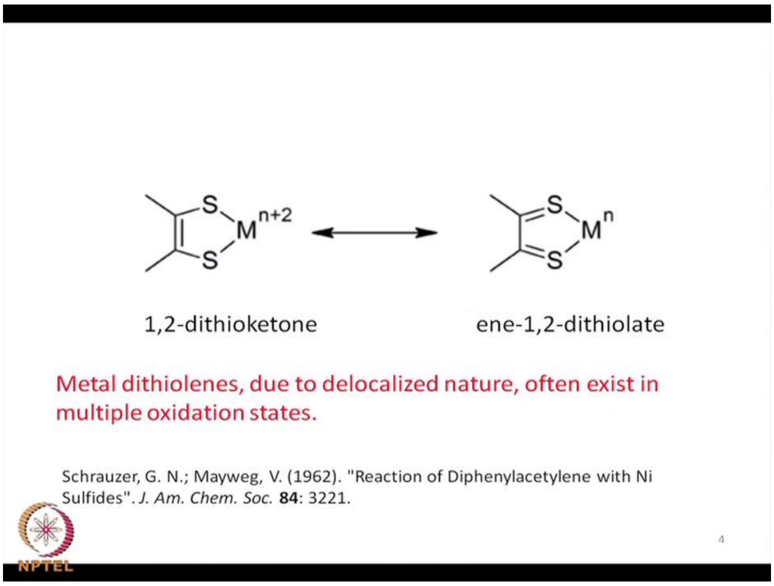

1,2-dithioketone

ene-1,2-dithiolate

Metal dithiolenes, due to delocalized nature, often exist in multiple oxidation states.

Schrauzer, G. N.; Mayweg, V. (1962). "Reaction of Diphenylacetylene with Ni Sulfides". *J. Am. Chem. Soc.* **84**: 3221.

So, these are some kind of organic solvent type of molecular typical anions of sulfite based type molecule, these particular group which is already we know that this dithiolene part. So, this is diethoketone; this is changed; 1, 2- dithioketone is this one; and in one two dithioketone, it is the left one. So, whenever we bind this particular one and molybdenum is in the particular form, and your sulfur is negative, and this sulfur is also negative, and you have molybdenum in the plus six oxidation state. So, why we are talking these are very interesting molecules? So, they give rise to some metal dithiolenes. So, these metal dithiolenes has tremendous applications also in some other areas and due to these delocalized nature exist in multiple oxidation state, because in sometime we talk about like some other ligand system whether your ligand is in the oxidized form or the metal center is in the oxidized form; like in the case of copper we have seen in the case of galactose oxidases the tyrosine radical formation. So, any organic part. So, this dithiolene part also we should be very much careful whether the metal center is bound in dithioketone form; this is dithioketone form.

So, dithioketone form or ene-1, 2- dithiolate form. So, this knowledge basically the identification of molybdenum in all these Oxo transfer molybdenum enzymes has generated tremendous interest to the scientist or some synthetic chemist. So, that is why it is a very old papers in a 1962 in Ajax paper; the reaction you can see this paper is a very interesting one is diphenyl acetylene with nickel sulfides. So, diphenyl acetylene is there and we reacting with nickel sulfides. So, these diphenyl acetylene these will be P H P H. So, you have the direct sulfur sulfur attachment to the triple bond or diphenyl acetylene that give rise to the generation of this sort of ligand system and which is already.

So, you have a modified ligand system based on nickel sulfides and you have the ene-1, 2-dithiolate group which attach to the nickel center. So, these dithiolene molecules are very interesting and they give rise to different interesting molecules to vanadium, to molybdenum, to copper and all this. So, depending upon the presence of this metal center and the corresponding delocalization, because these are very small molecule species. You can bound this at high temperature in the oven and you can get a different type of corresponding metal sulfites. What you are reacting is a one type of nickel sulfide then if you can burn it, you get a different type of metal sulfide which have some tremendous material’s application.

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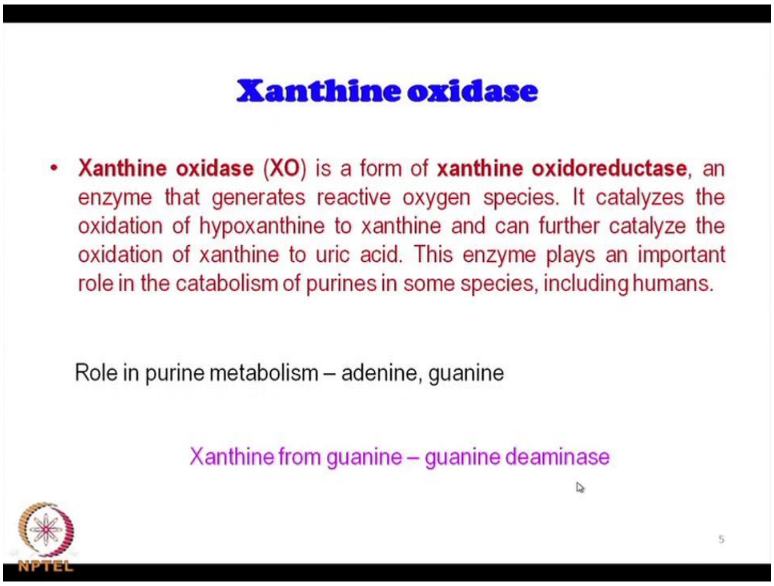

## Xanthine oxidase

- **Xanthine oxidase** (**XO**) is a form of **xanthine oxidoreductase**, an enzyme that generates reactive oxygen species. It catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. This enzyme plays an important role in the catabolism of purines in some species, including humans.

Role in purine metabolism – adenine, guanine

Xanthine from guanine – guanine deaminase

Then next we will just go for the corresponding system which is our xanthine. So, xanthine molecules what we get is the corresponding form what we get from guanine. So, xanthine we can make from guanine through the use of guanine deaminase.

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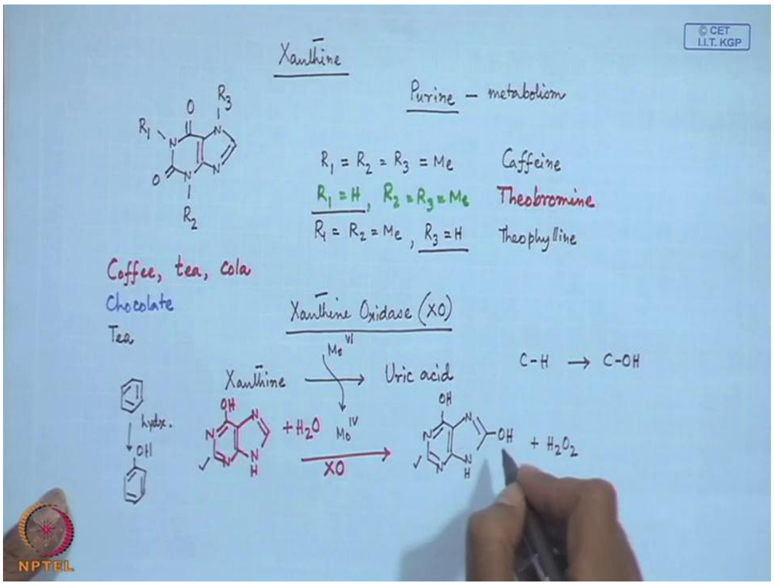

So, not only xanthine, there are several other groups of molecules which are also interesting and you can have three substitutions R 1, R 2 and R 3; these can be your hydrogen also. So, xanthine molecule will come there. So, related to xanthine molecule some other type of molecule are these. So, they are involved in related to our purine metabolism purine and pyramedine. So, purine and pyramedine are very important molecule.

So, these molecules when we go for the corresponding metabolism in our body also. So, purine metabolism and there are several other sources also present that is involved for the corresponding assimilation of these from natural sources. So, one such we can just simply know that when R 1 is equal to R 2 is equal to R 3; that means, when all the substitutions are methyl we get caffeine. So, your source definitely would be coffee, tea, cola etcetera. So, we get all these form coffee, tea, cola and other things. So, simple manipulation of these substitutions R 1, R 2, R 3, we just basic skeleton remains same like that of your guanine like that of your xanthine all are same.

So, they are all pyrimidine base. So, now, if we just simply make R 1 as H and other two R 2 and R 3 are methyl; that means, one of them we will make as the corresponding protonated form which is also has some additional value which is Theobromine; which is available from chocolate. So, and then the other one; that means, you change R 1 and R 2 what we had as methyl, but you make R 3 as H. So, one case R 1 is H, in another case R 3 is h which is theophylline; which has medicinal value also; which is also present in tea. So, if we want to metabolize these; that means, how we are metabolizing your sulfide group to sulfate? So, basically your any other sulfate sulfur bearing molecule can be oxidazed to sulfite and then to sulfate. So, we need some oxidation reaction. So, xanthine oxidase is that molecule these xanthine’s oxidase molecule is responsible for converting. So, if you have another related molecule which will be getting from the purine basis.

So, xanthine in that particular case will be converted to uric acid; that is why we always talk about uric acid and the block contained uric acid is also very important to know and this particular one is catalyzing by molybdenum. So, you have molybdenum six and molybdenum four. So, therefore, your xanthine oxidase as molybdenum center and which will be responsible for another type of Oxo transfer reaction. So, this can also be considered again as Oxo transfer reagent. So, this very good Oxo transfer reagent can be responsible for oxidation of xanthine to uric acid. So, this is a big group of molecule. So, xanthine oxidase is a form of xanthine oxido reductase.

So, we are talking about the molybdenum oxido reductase that means the molybdenum bearing oxido reductase, but this is a very specific one; we all know now that the corresponding coordination environment and what are the groups which are directly attached to your xanthine oxidase center, so depending upon that you get a corresponding oxido reductive which is known as xanthine oxido reductase. So, this particular one can be responsible for generate the reactive oxygen species ROS, it can generate and it can catalyze the oxidation of hypoxanthine to xanthine, and can further catalyze the oxidation of xanthine to uric acid. Not only the simplest possible transformation of xanthine to uric acid it is catalyzing, but also it can catalyze the corresponding formation of xanthine also from the hypoxanthine molecules. So, what we get? In our body also in some other all living systems we all have the purine basis related to your nucleoside and nucleotides.

So, all these purine basis we can have and these purine basis if they are getting oxidized particularly these two - the adenine and guanine. So, adenine and guanine can be giving rise to some xanthine molecules and those xanthine molecules can ultimately could be converted to uric acid. So, how we get? That is also another biosynthetic pathway or biosynthetic process where you get xanthine from guanine. So, your source is the guanine molecule and that guanine molecule is giving rise to xanthine molecule. So, structural coordination between all these groups all these purine basis, we should know little bit that what type of transformation is taking place when you move from guanine to xanthine. So, if it is simply going for some removal of N H 2 function, we call it that is the guanine deaminase.

So, if we just simply look at the corresponding reaction that one extra N H 2 group is going away and that particular group either it is in the deamine form or it can be attached to something where you have the Oxo center there. So, molybdenum we all know now that it is a very good reagent, very good species like that of your sulfite to convert to a sulfate species it can go for corresponding hydroxylation on the carbon center; that means, the carbon base hydroxylation is also possible over there and that carbon based hydroxylation reaction is also giving rise to corresponding uric acid. So, in this particular case how you get xanthine to guanine is the guanine deaminase reaction and in this particular case we are not talking about guanine deamenase, but we will be just concentrating our attention on how your xanthine molecule is getting converted to your uric acid molecule.

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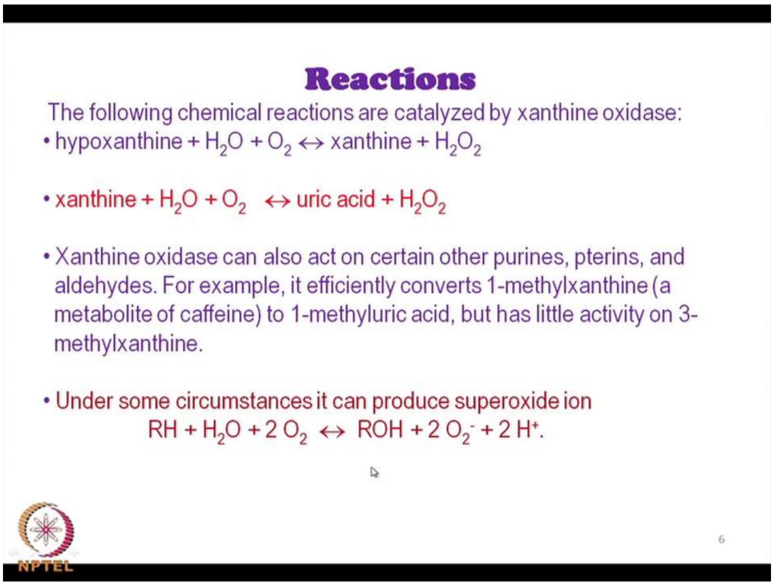

## Reactions

The following chemical reactions are catalyzed by xanthine oxidase:

- hypoxanthine + $H_2O$ + $O_2$ ↔ xanthine + $H_2O_2$
- xanthine + $H_2O$ + $O_2$ ↔ uric acid + $H_2O_2$
- Xanthine oxidase can also act on certain other purines, pterins, and aldehydes. For example, it efficiently converts 1-methylxanthine (a metabolite of caffeine) to 1-methyluric acid, but has little activity on 3-methylxanthine.
- Under some circumstances it can produce superoxide ion

$$RH + H_2O + 2\,O_2 \leftrightarrow ROH + 2\,O_2^- + 2\,H^+.$$

So, the basic reactions these are the several reactions what we can have; that means, you have the xanthine and the xanthine you have certain number of oxygen groups attached to this particular center, and which is converted to your uric acid. So, basically we are going for some oxygen atom transfer to the substrate system. So, what is our xanthine? So, xanthine molecule is nothing but related to this particular caffeine theobromine or theophylline type of molecule. So, is again something derived from your purine base, it is the aromatic. So, you have this is hydrogen, R 3 is hydrogen and this is O H. So, you are using xanthine oxidase and that xanthine oxidase in all cases we are discussing so far is the oxygen atom what is coming over there is derived from your water molecule. So, you are reacting it with water.

So, this O H in one form you can write in double bounded O and N H function. So, these basically going for its corresponding uric acid; this uric acid form plus iodine peroxide. So, in some cases also you have another site, this particular site and this two particular site; those can also be hydroxylated. So, basically what we are looking for? We are looking for something where we have all know that aromatic hydroxylation reaction. So, benzene to phenol or phenol to catcall type of conversion hydroxylation reaction, two hydroxylation reaction such that you get A C H function to A C OH function.

So, once this uric acid is formed and this uric acid formation to us to any one’s bodies forming a corresponding state which is related to your route formation, because the uric acid crystals are forming there and that is giving us to severe pain within the bone joints. So, this particular one; that means, typically now in this particular case of molecules we can transfer this oxygen, we can go for the typical hydroxylation reactions what we see in case of copper mediated reactions or any other cases. So, you have xanthine. So, therefore, you straight away we write it xanthine plus water plus oxygen giving you uric acid and hydrogen peroxide, and the formation of xanthine is also in the similar fashion; it is catalyzing by water and oxygen and forming hydrogen peroxide over there. So, xanthine oxidase can also act on certain other purines. So, this is interesting.

So, any kind of purine like substrate if we can have that gives us some important clue that what type of molecule you are taking over there and that is responsible for the corresponding hydroxylation reaction or the pterins or the aldehydes. So, xanthine oxidase is effective for some kind of other purines, pterines and aldehyde molecules. So, if the substrate molecules like xanthine’s are available, it will immediately convert those xanthene’s to uric acid, but if your substrate concentration is not that much; it will just act on the other molecules available if the aldehyde function is available, it can be oxidized that aldehyde to acids and other oxidized form. So, some selectivity also there, it efficiently converts one methyl xanthine.

So, one methyl xanthine is already we have seen the caffeine is there; all the three positions are the methyl function. So, one of them is the one methyl xanthine is the metabolite of caffeine molecule. So, metabolite of caffeine molecule is there and that can immediately converted it to one methyl uric acid. So, you see that if you have caffeine in your body source what you are increasing by all these type of molecules not only that you have the purine base from ATP and all other things. So, that particular one’s; that means, if you get these through some other sources particularly the food sources that will also be converted to some other molecule which is related to your corresponding increase in concentration of uric acid; it is only a substituted version of uric acid. So, you will be able to form methyl uric acid, but it is not active on three methyl xanthine. So, it is only active on one methyl xanthine.

So, there is some selectivity definitely that one particular substrate in the biological system the selectivity is much more which we cannot get in the laboratory and in some cases we find that when you have the corresponding reaction on some alkane type of thing that methyl methane hydroxylation to methanol that it can produce in some cases, it can also produce some super oxide anions also. So, basically in these particular cases if you have the dioxygen available and the dioxygen is taking of that electron and you are getting some super oxide anion, and that super oxide is involved for further transformation.

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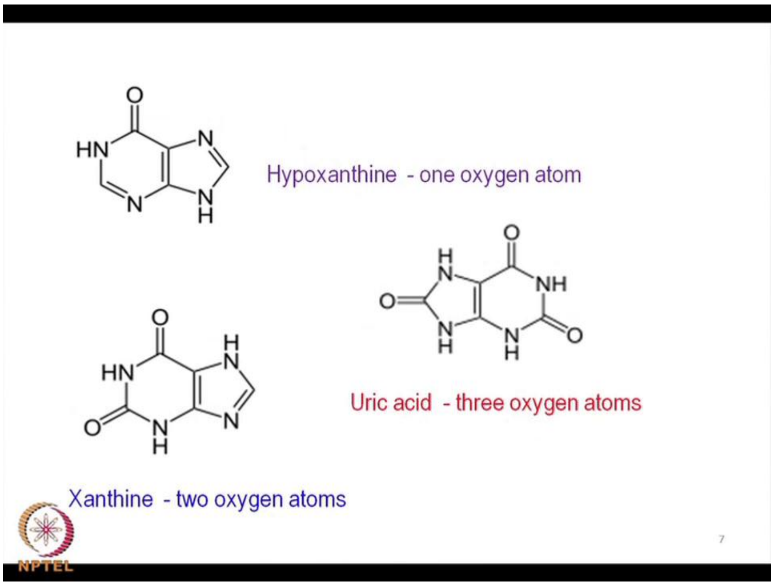

Hypoxanthine - one oxygen atom

Uric acid - three oxygen atoms

Xanthine - two oxygen atoms

So, now we just simply compare, because we know now what the caffeine theobromine theophylline is. So, what is that your hypoxanthine? So, now you can corresponding relationship with the guanine molecule, you should know that a typical guanine structure. So if you have this N H 2 function and one more N H 2 function. So, you just simply remove the N H 2 functions. So, N H 2 functions are removed, it is not remaining as it corresponding C H or C H 2 function, but some oxidation reaction is also taking from there. So, these three molecules basically we can correlate that in this particular case that the hypoxanthine has one oxygen atom; that means, you have transferred one oxygen atom to the parent molecule related to your purine base; that means, the guanine where you have extra N H 2 functions also.

If you can go for two such points; that means, this carbon is going for the hydroxylation reaction; if you go for the second carbon you get a molecule which bears two oxygen atoms which is your xanthine and if it goes for the third one you get the uric acid. So, one particular substrate, the enzymatic reactions are important that one after another you can insert one oxygen atom or you can insert two oxygen atoms and you can insert three oxygen atoms.

Ultimately, the corresponding one; that means, the crystallization of uric acid because their solubility and the corresponding anionic form dependence on P H, all these things which depend on how much uric acid is there in your body or in your blood and that will also be related to your gause formation or diseased condition depending upon your uric acid formation. So, molybdenum will definitely be important their and the molybdenum is responsible for giving rise to this particular reaction where you get the corresponding hydroxylation reaction.

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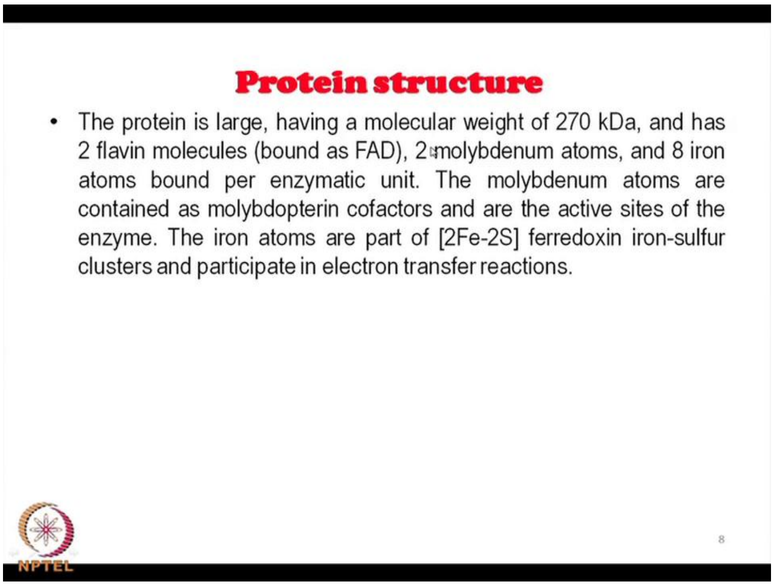

**Protein structure**

- The protein is large, having a molecular weight of 270 kDa, and has 2 flavin molecules (bound as FAD), 2 molybdenum atoms, and 8 iron atoms bound per enzymatic unit. The molybdenum atoms are contained as molybdopterin cofactors and are the active sites of the enzyme. The iron atoms are part of [2Fe-2S] ferredoxin iron-sulfur clusters and participate in electron transfer reactions.

So, how we get; that means, there are something; that means, you have the entire protein structure and we are writing something that you have iron also, and you have the FAD molecule also. So, the entire protein structure which is definitely a complicated one, because it has a very huge molecular weight of 270 kilo Dalton. So, it has two FAD molecule, two molybdenum center and eight iron atoms per enzymatic unit. So, we are talking about one molybdenum center and this molybdenum centers which as your molybdopterin cofactors factors an active sited enzymes, and it is accepting the electron from your iron centers. And this iron centers will provide you the right number of electrons at right potential; your E 0 values which are important at what E 0 value you will be able to reduce the molybdenum center. So, that is why we are talking about the molybdenum center with different environment.

So, if you move from one environment to the other particularly that typical one which we are seeing the sulfur environment; the diethiolene environment. So, is this diethyl environment is corresponding diethiolene environment depending upon its electronic structure. This dyethiolene environment is completely different compare to your cysteine environment; that means, it cannot be equivalent to two cysteine residues related to your molybdenum center.

So, if you are able to make synthetic molecules also bearing those sulfur groups from this cysteine sulfur or any other sulfur from that corresponding thyphenol and all this we will find that that corresponding double bond the ene diethyl unit. So, that electronic structure or electronic environment related to that ene unit of the diethyl is different that is why if you measure the corresponding E 0 value; the diethilene ligands give you some other E 0 values.

So, it is giving most of the time, it is a very low potential for the corresponding center oxidation; that means, its corresponding charge density or electron density on dithylene is much more compared to its other corresponding diethyl ligands. So, iron sulfur clusters are responsible; so peridoxin type iron sulfur clusters are responsible for electron transfer. So, obviously, this particular iron center at particular E 0 value is used for transferring the electron to the molybdenum center and molybdenum center will also be known at what potential it can be reduced depending upon its corresponding environment whether you have a dythylene environment or any other environment.

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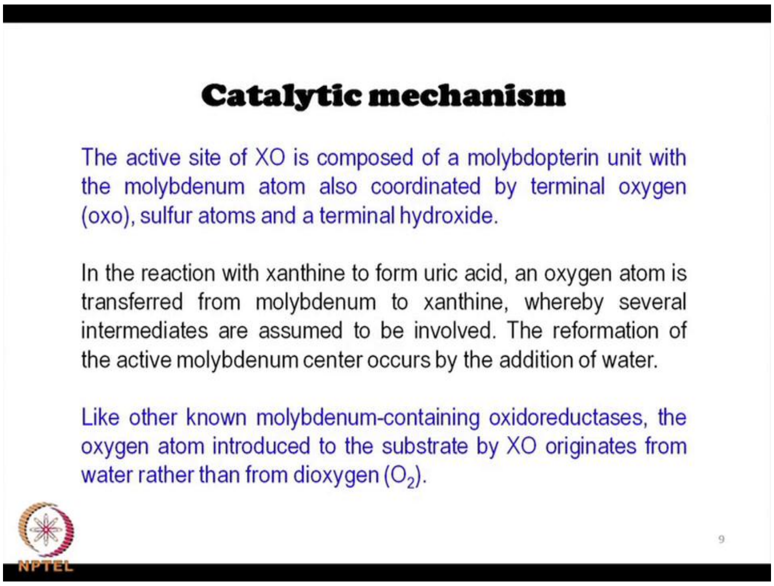

## Catalytic mechanism

The active site of XO is composed of a molybdopterin unit with the molybdenum atom also coordinated by terminal oxygen (oxo), sulfur atoms and a terminal hydroxide.

In the reaction with xanthine to form uric acid, an oxygen atom is transferred from molybdenum to xanthine, whereby several intermediates are assumed to be involved. The reformation of the active molybdenum center occurs by the addition of water.

Like other known molybdenum-containing oxidoreductases, the oxygen atom introduced to the substrate by XO originates from water rather than from dioxygen ($O_2$).

So, the active site of the xanthine oxidase is again similar to that of your sulfide oxidase and you transfer this particular one from molybdenum to xanthine; again is a typical that Oxo transfer reaction that of your sulfite and you just regenerate back the corresponding active molybdenum center which is generated from there with the addition of water molecule. And other known molybdenum containing oxidoreductases, as we are talking all the time the same thing; that means, it is the water molecule which is providing that corresponding oxygen to the molybdenum Oxo center.

So, any molybdenum Oxo compound if we can consider whether it is a mono Oxo molybdenum compound or di Oxo molybdenum compound, if we are able to reduce that center which is important that whether you are able to reduce that particular; that means, use of that thing in presence of some reducing agent. So, we will be using some reducing agent and we reduce the molybdenum center to either to molybdenum 5 or molybdenum four, then you can take up that oxygen from the molybdenum center for some suitable conversion whether it is a conversion from the sulfite or it is a conversion on the xanthine molecule to your uric acid. So, this molecule have some relationship with that of your structure of the xanthine molecule. So, if we get the corresponding structure of that xanthine molecule what we have seen that corresponding xanthine molecule; that means the purine base.

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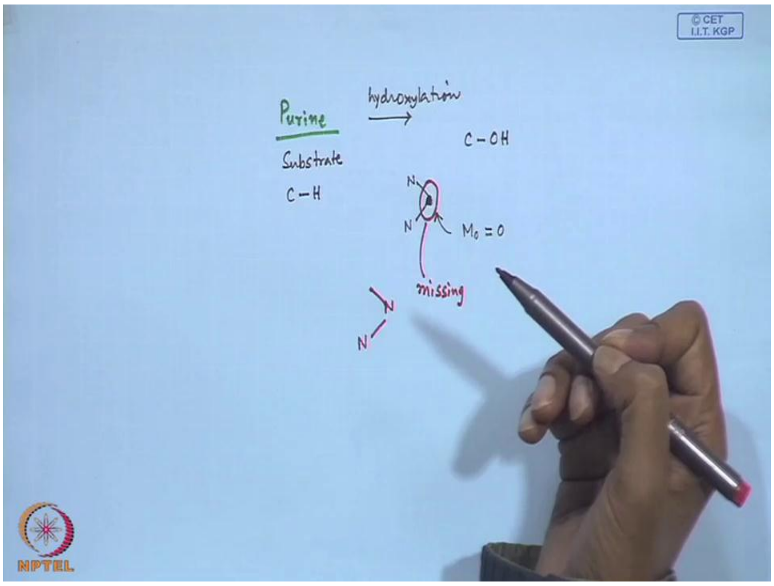

So, that purine base we can have and that purine base basically is our substrate molecule and we are going for the corresponding hydroxylation reaction. This purine molecule will have C H bond and this will be converted to C OH, and the corresponding specificity of the enzymatic reaction will immediately tell us that one particular position this nitrogen nitrogen position.

So, this carbon is your center where you attack by this corresponding molybdenum Oxo center. So, this oxygen can be transfer to this carbon center which will be utilized for this corresponding hydroxylation reaction. So, uric acid has that particular structure also; only thing that you have the corresponding hydroxyl function on this carbon. So, that corresponding remedy for this corresponding accumulation of uric acid from purine metabolism. How you stop the accumulation of uric acid? So, you have to find out something where you can have low concentration of uric acid; the generation of uric acid should be less there.

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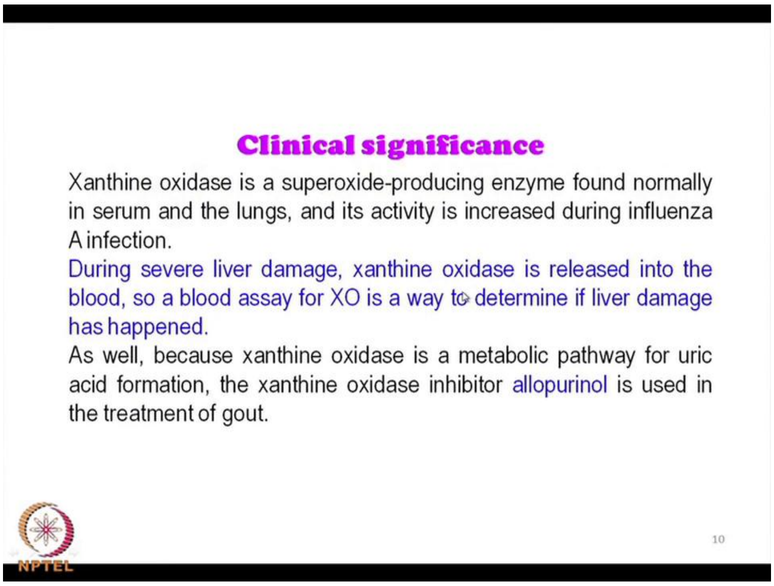

## Clinical significance

Xanthine oxidase is a superoxide-producing enzyme found normally in serum and the lungs, and its activity is increased during influenza A infection.

During severe liver damage, xanthine oxidase is released into the blood, so a blood assay for XO is a way to determine if liver damage has happened.

As well, because xanthine oxidase is a metabolic pathway for uric acid formation, the xanthine oxidase inhibitor allopurinol is used in the treatment of gout.

So, if you have something; that means, xanthine oxidase also can produce superoxide that we have seen when it is attacking to your dioxygen molecule. So, we all know that the superoxide is also a very deadly system or deadly molecule and which can damage your serum and lungs, and activity is increase when we have the influenza infection. So, the corresponding reactivity pattern for xanthine oxidase where the first thing what you should know that the amount of xanthine oxidase in our system.

Any living system, how much xanthine oxidase you can have which can oxidize your that purine molecules to your corresponding oxidized form and at the same time, because it is producing hydrogen peroxide as well as it is activating your dioxygen molecule to superoxide; because it is also related to some other diseases; that means, that is corresponding marker for the concentration of xanthine oxidase can also tell us that how much activities for generating your superoxide molecules.

So, during this particular cases; that means, this is the diseased condition your xanthine oxidase can be released into the blood. So, blood assay for xanthine oxidase. So, there are two typical thing that we will discuss in our next class that if you can have the blood assay for xanthine oxidase; that means, what we are measuring your level of xanthine oxidase in your body. So, depending upon the concentration of the available xanthine oxidase in your body and depending upon the purine concentration; that means, the guanine concentration, you can tell that how much uric acid will be produced by the concentration of the available xanthine oxidase.

But in some cases, if we want to stop or if you want to inhibit the corresponding reactivity of this corresponding reaction of this xanthine oxidase for the uric acid pathway. So, this we call is as some inhibitor design. So, all these medicinal chemistry basically related to some designing of inhibitors. And inhibitor designing is very simple that we will see in the next class that one such molecule is allopurinol which can be used for the treatment of gout which is nothing but this particular structure will have very much similarity to that of your xanthine molecule; only thing that it cannot be hydroxilated.

So, you have the substrate where this function; that means, this C H 2 function is this particular backbone is missing. If you do not have this particular carbon; that means, if you just bring this nitrogen over here; that means, you carry this carbon over here and make these two nitrogen’s. So, you see the selectivity such is an interesting one; that means, once you make this carbon as nitrogen, it cannot react with the molybdenum Oxo form to get it hydroxylated by N hydroxylation reaction. So, N hydroxylation reaction is not facile there and it will not form there to give you some N hydroxilated product, we will just looking for uric acid formation the C hydroxylation reaction. So, C hydroxylation reaction is important. So, anything any molecule related to your xanthine which can stop or which can inhibit the reaction of xanthine oxidase will solve your purpose and give as an inhibitor, and your corresponding medicine for the treatment of gout.

Thank you.