Bioinorganic Chemistry
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Lecture - 34
Vanadium Enzymes - II

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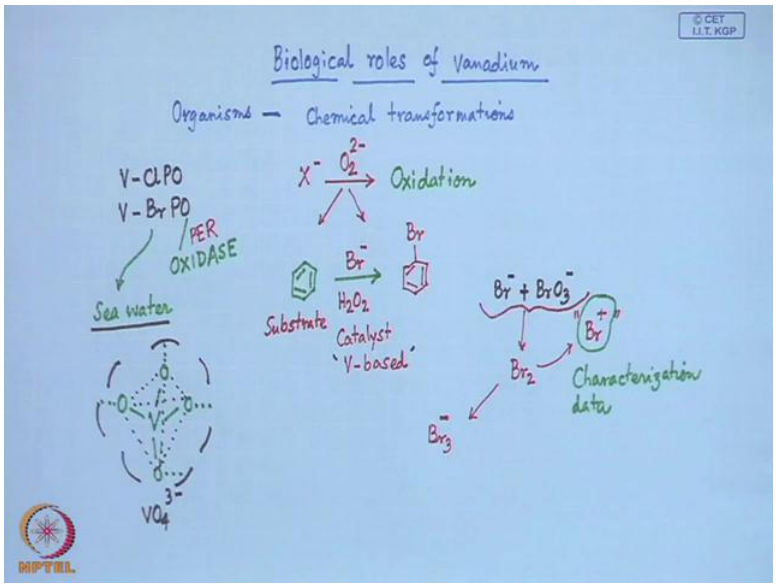

Hello, good morning everybody. So, today we will still continue the most significant role of vanadium in biology. So, we will try to find out some of the biological roles that vanadium can play, and different organisms, because it is not very much available in human system, but it certainly beneficial to some of these different organisms. And these organisms can see, whether it can be useful for some chemical transformations also; that is why today we will be just talking about this second part of this system, which is the Vanadium Enzymes.

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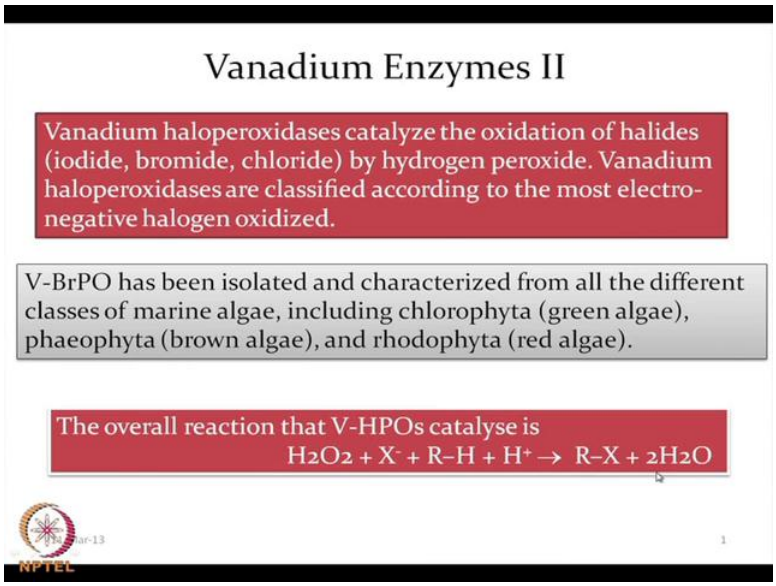

And this vanadium enzymes second part will deal with some of the things what we have already discussed, that the vanadium haloperoxidases which catalyze the oxidation of halides. So, if we have some vanadium bearing, chlorine peroxidase or vanadium bearing bromine peroxidase, so definitely they all will have the corresponding oxidase activity, which is very important. So, how nature is performing this dual role of activating some of the substrates, in presence of the halides, which are very much available in marine system or in sea water, so we all know that in sea water, large amount of (( )) halides, whether they are bromide, chloride or iodides are available.

So, if we have any such thing that means, whether you can have bromide or chloride or iodide, and how we can show some oxidase activity or peroxidase activity rather, so this is basically not the typical oxidase activity, these are the corresponding peroxidase activity. So, if the thing is reacting with O 2 2 minus, so what is happening, therefore we know that this peroxides can function in both ways, that means it has intermediate oxidation state compared to molecular oxygen, and the super oxides when you can have. So, this basically when can go for this disproportionate reaction, it can go for oxidation as well as reduction reaction.

But, since we are talking about the peroxidase activity, so we will be looking for the corresponding oxidation reaction on the halides; so if we are able to oxidize the chloride, the bromide or the iodide, we will be able to see some peroxidase activity, when

peroxidases is at present in the system. So, these basically use the hydrogen peroxide, and vanadium haloperoxidases are therefore, classified according to the most electro negative halogen oxidized, so if they are oxidizing chloride we call it as a chloro peroxidase, if it is oxidizing bromo it is bromo peroxidase like that.

So, depending upon the electro negative halogen that means, the halide anion, they have the different roles, and we all know that the redox potential for the corresponding oxidation of iodide, bromide, and chloride are different. So, we need different E 0 values, the redox potential values for the system, which is again getting activated only by the presence of peroxide anion. So, how these peroxide anions can be modulated for different types of oxidation reactions on chloride, on bromide, and iodide, that we will see which is interlinked by the presence of vanadium center.

So, one such example is that, our vanadium bromo peroxidase, which can be isolated from marine algae including chlorophyta, which we all know as the green algae and phaeophyta which is brown algae, and rhodophyta the green algaes. So, the origin is basically the algal origin, and this algae origin is therefore, useful if we want to see something that means, the reactivity related to the bromide anion. So, if certain reactivity we can talk in terms of the typical organic chemistry also, that whether we can go for some reaction, where we just simply see that means, we call in the model system that will see afterwards.

That if there is some model system where the system can show some bromoperoxidase activity that means we just simply supply or provide bromide anion, and the hydrogen peroxide, and some vanadium based catalyst. So, if that vanadium based catalyst can show some reaction on these, and we able to generate some bromonium ion, and that bromonium ion can attack the ring and can end up with some generation of some bromo benzene derivative. So, this is also helpful that whether, we should use some vanadium based catalyst for this sort of transformations.

Because, large number of reactions are already known in chemical literature for the organic transformation that means, the bromination of the substrate, any kind of bromination, chlorination, and iodination reaction. But when we find out something where the biological system is also helpful, for giving some of these derivatives, the brominated products, which we get from sea water, this brominated products how the

nature is doing for us all these algal bromoperoxidase or chloro peroxidases are doing that thing. So, that can be also utilized, if we can have a corresponding vanadium based catalyst in our hand, so all these brown algae, red algae, and green algae can give rise to some compounds.

Where we see that the overall reaction what we can have, for vanadium haloperoxidases, which basically catalyze the reaction of R H, transforming to R X that means, if we have benzene, the benzene will be converted to bromo benzene, with the reaction of bromine anion X minus will be there. That means, V R minus will be there, hydrogen peroxide will be there, and some protons source should also be there, so if it is present from the enzyme that means, some pocket is there, where the vanadium is nicely trapped inside.

And last time what we have seen in our last class that, these pockets basically are very much well fitting with the corresponding vanadate anion, so the vanadate anion and the corresponding surroundings can give rise to some of these; that means, the terminal ends of the enzymes of the protein chain can also provide some of these protons for this complete transformation. Because this X is incorporated nicely within the molecule R H, converting it to R X, and one molecule of hydrogen peroxide, with two other hydrogen atoms produced, two molecules of water.

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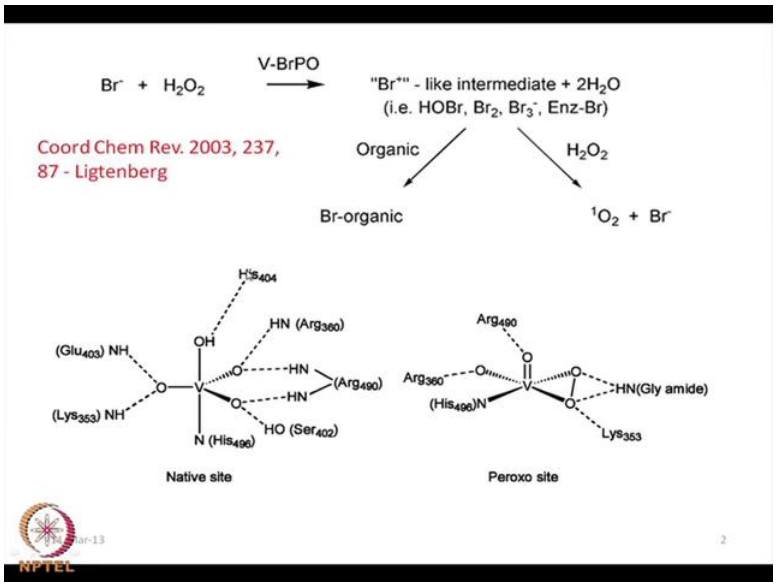

So, how we get that particular reaction, we will just see for a typical transformation, where we can have Br minus is there, which is reacting with H 2 O 2. And our catalyst is

vanadium bromo peroxidase, what is forming over there is Br plus, which is with within the inverted commas like that is like, Br plus like intermediate, because it is not that only Br plus is found. But some of other species like HOBr, where we can consider formally that HO is the minus part, the negative part, and Br is the positive part then Br plus; so these and other type of intermediates are forming along with generation of some amount of bromine.

Because, we all know that when bromide ion is getting oxidized we can produce in C2; that means, within the reaction medium, we can produce some of these free elemental bromine. Like that when we react Br minus with potassium bromate that also liberates bromine, and when this basically shows synthetically in the laboratory; when Br minus is reacting with some oxidizing agent like, bromide anion, say potassium bromide, so when it is reacting with potassium bromide, it basically liberates bromine. So, whether we can have the bromonium ion, what is written in the reaction or we have the free elemental bromine, which is utilized for the typical oxidation of the substrate, so you have the substrate.

And that substrate is getting oxidized and with the incorporation of bromine within the ring, so once this is a very straight cut and very simple reaction, we find in the laboratory when bromide is reacting with potassium bromine, bromine is getting eliminated. And if these two, when we have Br minus and Br plus, so that immediately gives us Br 3 minus also and some organic part which is also getting brominated. So, basically what we should focus our attention that whether, you can have these Br plus in the system, and how these can be identified.

So, any characterization pattern for that is we need some amount of characterization data, for the identification of Br plus, and this particular species is only responsible for the transformation or the reactivity or the catalytic reaction related to vanadium bromo peroxidase. So, when along with all these, like this Br plus as the intermediate or the other species is forming, we can have two different steps of reactions, when the organic substrate, this is the substrate like that of our simple benzene ring, when benzene ring is reacting whether, we will be getting some bromo benzene or not.

So, some suitable organic substrates like that, what we have seen in our previous class that all brominated marine chemicals, the marine natural products, we have they are

mostly and very useful, because they have the potential for use as from a good pharmaceutical chemical and good other molecule. So, which has the corresponding brominated organic compound, so if we have the substrate, so any useful substrate, any medicinally important organic substrate which can be more useful, if we can go for a typical bromination reaction.

So, this substrate is getting brominated, when the substrate is suitably available and it has certain sites where the bromination can take place; that means, it has some useful CH bonds available, where CH bond can be substituted by C Br bond. So, when substrate is present we get definitely, we will get the corresponding brominated derivative of the substrate, and in this particular case, when this substrate is not present and we have more amount of hydrogen peroxide as well as water, we can generate singlet oxygen from the reaction system.

So, basically this is also a conversion, if we want to produce some amount of singlet oxygen what we all know that the molecular oxygen in the ground state what is available in nature, in air they are all triplet oxygen. But for some transformations we want to convert the triplet oxygen to the singlet one, so this reaction also goes for the conversion of these triplet oxygen to singlet oxygen, and the Br is generated as Br minus again. So, this is the simple and straight cut reaction mechanism, for the conversion of this particular reaction, for the bromination, and we get this particular information from that coordination chemistry review reference of 2003.

So, now, will be interested to know that what type of environment, because last time what we have seen, that we just, so knew that we can have a typical pocket. And this pocket was there, and this pocket was surrounded by so many amino acids, so in that particular case what we have seen that, if we can have initially in the enzyme back bone, one pocket of vanadate anion can be formed, which nicely trap the tetrahedral vanadate anion inside. So, we can trap VO4 3 minus vanadium is present in plus 5 oxidation state, and these oxygens that means, the vanadium oxygen, so whether they will be further reacting with some proton or some other substrate that will be dependent on, how they are encapsulated by the different amino acid.

So, if we see that they are several amino acid residues, which can surround this particular pocket, so sometime we will find that 1, 2, 3, 4, 5, 6, 7, 7 amino acid residues with

terminal ends are sufficient to encapsulate the vanadate anion within. And this particular one definitely will show some interaction with the amino acid residues, so what are those interactions that will now see, that if we have these vanadium oxygen bond, and oxygen is rightly nucleophilic in character and it has some delta negative charges. So, it will start interacting with the amino acid residues such that, through oxygen it is basically getting stabilized within this particular pocket.

So, in the native state what will find that, you have this particular vanadate anion was there, and this vanadate anion was in tetrahedral geometry, and this tetrahedral geometry can go for some interesting conversions of structure. That means, it can immediately go for a trigonal bipyramidal geometry, this particular geometry around this vanadium is trigonal bipyramidal; so this is the trigonal pen, this O3, the three O O O atoms in this particular vessel plane is a (( )), and these are the two epical sides. When one epical side is occupied by the OH, and another one is occupied by the protein chain, through the nitrogen of histidine 496.

So, this basically the coordination of this particular nitrogen from the histidine side chain, which is very important and is well known and famous for that also that whenever we have a protein chain, and that protein chain can go for the corresponding coordination to the vanadium site. And this particular one, what we see also in case of hemoglobin molecule that in case of hemoglobin molecule, we have this particular porphyrins binding to the iron site, and the fifth coordination site was occupied by the nitrogen from the globins chain.

The same thing is happening that the histidine nitrogen, the imidazole nitrogen from the histidine residue of the protein chain is very much useful, for showing some strong interaction with this vanadium. So, this vanadium nitrogen bond is established, and that basically controls the reactivity pattern of the entire system. So, when it is forming an epical bond to the vanadium site, so this is the new bond which is forming apart from the four existing vanadium oxygen bonds. So, in the vanadate anion what we had, we had four vanadium oxygen bonds present there, and now the new bond is forming.

Since, the conversion is from a tetrahedral to trigonal bipyramidal, we do not have any other option that means; square pyramidal geometry is also not forming. And interestingly when we have this kind of arrangement, we expect that this vanadium

nitrogen bond can be little bit longer compared to the other vanadium oxygen bonds. So, this particular coordination is basically is very important, and is a vital coordination for converting the vanadate anion to a trigonal bipyramidal system or center based on vanadium, where it is establishing or showing a corresponding bond with that of the nitrogen from the histidine residue.

So, this was basically the other end of the epical bond which was our OH, and this has been converted through some proton delivery on the vanadium oxygen bond of the vanadate anion. So, it can come from the corresponding residues which is available from the protein chain, and then it can show since, OH is there, and since this histidine 404 which is quite far from histidine 496, that this histidine residue is not coming close in contact with this particular site such that, it can form some other type of bond with that of the nitrogen replacing, this OH as water molecule.

So, it can go for stabilization of this particular unit, so V OH part, this V OH part is getting stabilized through hydrogen bonding interactions with the histidine residues, so as we all know that histidine, in histidine we have that imidazole site chain.

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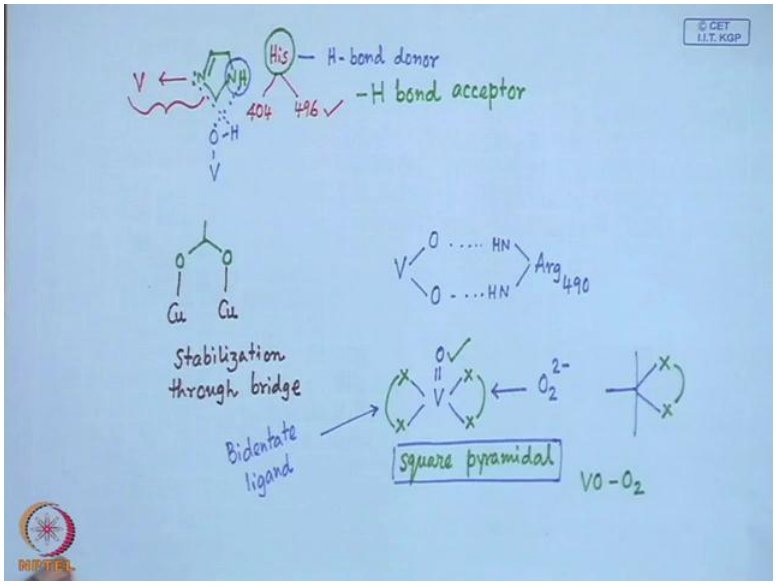

And this imidazole site chain, basically go for one as tertiary nitrogen, and one as NH, so this particular nitrogen with a loan pair of electron, most of the time it can bind our iron site, here also the way it is forming the bond, the vanadium nitrogen bond is forming by the coordinate bond coming from this tertiary nitrogen. So, this orientation of this

histidine residue, so is this histidine residue, whether it is 404 or 496, the number is 404 or 496 when it is 496, it is showing this particular bonding arrangement, when it is 404 it is useful for stabilizing the VO bond.

That means, if we have the V O H bond, if the available nitrogen is the same one, so it can show hydrogen bonding interaction with this hydrogen or this can use this entire NH function such that, it can go for some interactions involving this hydrogen. So, we can have two different options, whether this nitrogen is available which is not engaged in other kind of interactions such that, bonding with the vanadium, it can show hydrogen bonding interaction taking the hydrogen of the OH or this NH can show the same hydrogen bonding interaction with this oxygen.

So, this histidine can have dual roles to play, it can function as a hydrogen donor, hydrogen bonding donor, basically hydrogen bonding donor, it can function as hydrogen bond donor as well as it can function as hydrogen bond acceptor. So, whenever we have any histidine residues in the protein chain or the polypeptide chain, we know it is very useful to stabilize the vanadium center within the pocket. So, these two epical bonds are therefore, stabilized one through direct coordinate bond, and other through hydrogen bonding interactions.

And in this particular case these O O, so these two oxygen on the right hand side, would be stabilized by showing some bifurcated hydrogen bonding interactions the NHO and NHO. Bifurcated hydrogen bonding interactions we call, because simultaneously these oxygen is functioning to two hydrogen bond donors, which is coming from these arginase 360 and arginase 490, and this NH can show either interaction with this, and other end also can show interaction with these; so basically this is a rigid back bone on arginase unit like urea or biguanide.

This is the biguanide end, so this biguanide end basically functioning as a bridge like that of bridging two ends, we all know that some of the bridging groups are available all the time, like the simple copper acetate binding, when we have acetate groups CH 3, COO minus. So, this acetate groups can be a very useful bridging unit to binuclear system, if it is present in copper acetate, it can show the bridging interaction between these two copper centers. So, this particular entity is a very stable entity, so this particular motive is getting stabilized through bridge, so we can call it as stabilization through bridge.

So, this stabilization through bridging interaction is also seen, in case of this vanadium, so vanadium as these two oxygens, these two oxygens is already attached to vanadium. So, this dioxo vanadium unit in cis positions, how we can stabilize this, so this can stabilize with this NH unit, NH unit of arginase 490. So, if we a have a rigid backbone on arginase unit, which is very much similar to that of bridging unit of the acetate group, then this particular entity is getting stabilized through hydrogen bonding interactions with these NH functions.

So, this biguanide type of part on the arginase unit is very useful in stabilizing the adjacent oxo units on vanadium that means, the six dioxo unit is getting stabilized through hydrogen bonding interactions. So, this part is stabilized and it can show also for some more interactions with serine residue, serine 402, and the other oxygen is showing again bifurcated interactions with NH of lysine, and NH of glutaconic acid. So, all these hydrogen bonding interactions, so four of these oxygens are stabilized through hydrogen bonding interactions that means, they try to remain on the vanadium center.

Only this vanadium nitrogen interactions is unique one is a different one, which is showing a direct vanadium nitrogen coordinate bond, and this particular bond is therefore, the most stable entity which can hold these vanadium to the protein chain. So, during the reaction within the catalytic cycle, what we will see that during the reaction this, all these are vanadium oxygen interactions when we have the vanadate anion. We consider the bonding between vanadium and oxygen is less than vanadium oxygen double bond; it is more than vanadium oxygen single bond, the bond order, but it is less than vanadium oxygen double bond.

But, in this particular case when the catalytic cycle is on, the catalytic cycle is operating and it is mostly stabilized through this and still, you have this still histidine 492 nitrogen interaction with the vanadium. And we have arginase 360 this interaction, in arginase 360 is also interacting with this oxygen, but all other oxygens are not present, instead we have a peroxo linkage, so within the catalytic cycle we have the intermediate as the peroxo intermediate. So, vanadium is showing the peroxide intermediate through the reaction with hydrogen peroxide, and that vanadium peroxide unit rather than venodil peroxide unit can be very easily identified through NMR experiments also, vanadium NMR we can do.

And that vanadium NMR can establish that both the venodil oxygen is present, which is stabilized by hydrogen bonding interaction, as well as the peroxo unit which is also stabilized by the glycine amide backbone of NH, and the lysine 353. So, the conversion from the native state which is trigonal bipyramidal geometry to a square pyramidal geometry, this geometry is completely different, and is also well known and well established geometry for, so many vanadium sites where we have the venodil as the species. So, we can have in synthetic molecules also, when we go for studying the model compounds.

We will see that these two are the corresponding bonds, which are basically the cis bonds, and this is also another cis to cis bonds basically, giving a square pyramidal geometry. So, any bidentate ligand, like acetyl, acetone or any other, so this can be occupied bidentate ligand, and this can be by O 2 2 minus the peroxide ligand. So, we immediately get a corresponding square pyramidal geometry from the native geometry, which is trigonal bipyramidal in nature.

And because we can have this particular arrangement that means, this arrangement is already there when we have the trigonal bipyramidal geometry, because this is the trigonal bipyramidal geometry, it has also the two cis positions. So, if we can have these two cis positions, so we can justify that these two cis positions can be occupied by bidentate ligand or the peroxide linkage, only the rearrangements in all other sites are taking place, because this is the new connectivity the venodil ion. Such that, within this species we have this interaction, the venodil plus peroxide interactions which can be found out from NMR study also.

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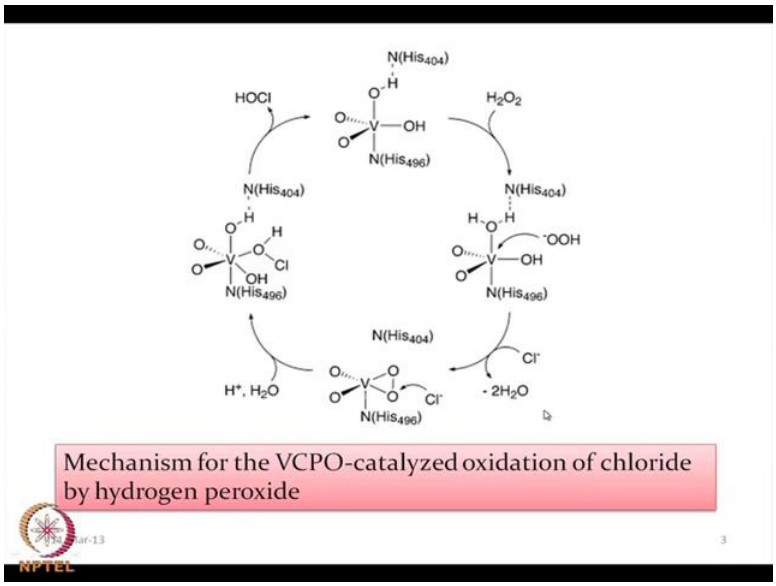

So, after identifying these two centers, one is the native center and another is the reactive peroxide. We can go for getting the entire catalytic cycle which is very important to know that when we have that means, this is the well known thing that means, what we have this is the thing that means, that trigonal bipyramidal geometry, this trigonal bipyramidal geometry we can start with; so we are not talking in terms of the corresponding hydrogen bonding interactions, through all these oxygen atoms. So, this is the corresponding trigonal bipyramidal geometry, and this particular geometry can show very useful interactions with that nitrogen already.

So, this particular bond basically controlling, the reaction pattern on the vanadium center, so we have this hydrogen bonding interactions with this OH also. So, when it starts reacting with the hydrogen peroxide, so showing the peroxidase activity, so this particular peroxide can interact, that means, this goes for reacting this vanadium site through this particular position. Because we can have more vacancy on this particular site, because this site is little bit crowed, so this site we can have and during this particular reaction with hydrogen peroxide.

The one proton from the hydrogen peroxide unit is coming, and that proton is going to attach this particular O H function on the vanadium making it; vanadium hydroxide bond to a vanadium aqua bond. So, this O H function attached to the vanadium is also basic function, so (( )) of this O H groups is such that, it can abstract one proton from

hydrogen peroxide to convert this vanadium hydroxide linkage to vanadium aqua linkage. So, this proton from hydrogen peroxide goes on this oxygen, and we are remaining with hydro peroxide anion, so this hydro peroxide anion then just go and attack this particular vanadium site.

So, this particular attack on the vanadium site is that is already protonated, so this water molecule will go immediately from there, and this OH function will also go through another step of protonation, this hydrogen will go and attack this particular OH and it can go for another water molecule, so removal of the V O H bond. That means, the vanadium oxide bond, vanadium oxide bond is very useful because the basicity of this OH functions attached to the vanadium is more, and this particular basicity can also transfer the second proton from the hydrogen peroxide to this OH group.

And it is converted to water molecule, as soon as it converted to water molecule it goes out from the vanadium coordination environment, because we have the competitive peroxide anion on our hand. And that peroxide anion can basically remove this particular bound water molecule, which is loosely bound to the vanadium center, and we expect what we see here also that, it can go for the corresponding ligand exchange reaction. So, the ligand exchange reaction for this OH by peroxide anion is taking place, and we are ending with the corresponding square pyramidal geometry, what we have just now we have discussed, that this is the vanadium square plane, where we have the already present O O bonds were there.

And two new bonds from the peroxide unit is forming, and the epical site is occupied still with that of the nitrogen of the protein chain of the enzyme, so this is the peroxide intermediates. So, for all this reactions the basic step for the formation of this is, that we should generate the corresponding peroxide intermediate to react it with the chloride anion. So, vanadium can show the mechanistically, the vanadium chloro peroxidase activity can be seen, when hydrogen peroxide is first reacting with the vanadium center.

And this reaction with vanadium center can give rise to very nicely, the corresponding vanadium peroxo compound, which is well known to the laboratory also in the analytical chemistry laboratory. We see that, vanadium peroxo compounds are very characteristic having, very characteristic color, it shows some beautiful coloration reaction with the peroxide anion; that means, the vanadium always has the tendency to form vanadium

peroxo bond, whether it is forming in the enzymes state or it is forming in the test tube in the laboratory.

So, in the next step, once the vanadium peroxo linkage is formed, the chloride anion from the water source, from the sea water also that large amount of sodium chloride, potassium chloride, magnesium chloride we can have. And all these chlorides as the corresponding chloride anion is, then reacting on this O O peroxide linkage on vanadium, and is basically at this particular point, because here we have the trigonal bipyramidal geometry. And that particular bipyramidal geometry has been squeezed basically, to a square pyramidal geometry, and for that purpose these two coordination positions has gone instead of that we have in plane coordination which is forming forcefully.

Because, the peroxide linkage has only this alternative, because it cannot have (( )) epical bonding only it can have both the vanadium oxygen bonds in the (( )) position, so this O O bond which is in the straight form, because of vanadium oxygen oxygen is a three member ring which is not very stable and not very strong. So, one C l minus can go and attack this particular peroxo linkage, the O O bond is getting cleaved, and once these O O bond is getting cleaved, what we find that this oxygen is again reward back to the original epical side, and this oxygen is taking the position of this trigonal phase.

So, this if this is the driving force, but is not happening altogether what is happening that this O O bond is getting cleaved and forming this vanadium OH bond, and vanadium H O C l bond, because this H is coming, and we can get the corresponding reaction with water molecule. So, this water molecule providing some extra proton because we need, so many protons as well as the hydroxide groups, so in a single step when vanadium peroxo compound is getting cleaved by the attack of the C l minus, in presence of proton as well as water molecule. We get this particular step or this particular intermediate followed by the peroxo intermediate, where hypo chloride; that means, in the form of hypo chlorus acid H O C l is the hypo chlorous acid.

So, hypochlorous acid is generated from the reaction of hydrogen peroxide and C l minus, and one such bond is converted to O H, and another one is coming from the water molecule as O H. Because what we have in the native state is the O H bond connected to vanadium in the epical side, so this particular part is getting regenerated, similarly these

two oxygens we are not touching, so they are the silent spectators in the catalytic cycle. So, we are not touching these two oxygen, so they remain as this, only the nitrogen is also controlling the entire reaction process, so you can have these three sides.

So, in the case of model compound development, the model studies will find that if we can have one particular ligand system, where all these three positions this facial positions this nitrogen oxygen oxygens, these are the facial positions. So, if we can occupy these three facial positions for these reactions, we can expect we will see how definitely for such reactions on vanadium model compounds.

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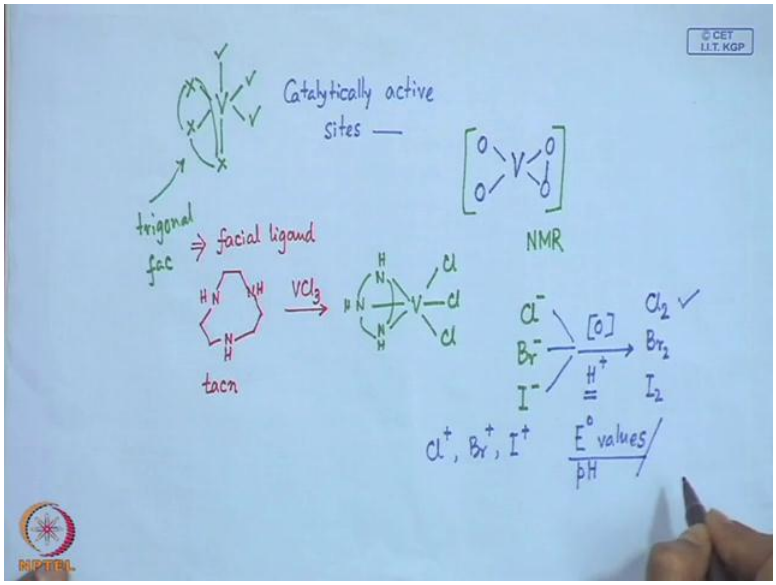

So, if we can have these, so if this is the positions, this is the position, and this is the position, so so one face this is the face, trigonal face which can be occupied; and once the trigonal face is occupied, we use one particular type of ligand, and we call it as a facial ligand. Like, it is very useful facial ligand is the triazacyclononane tacn, we call it as the tacn ligand, triazacyclononane ligand when it reacts or giving some vanadium compound, so definitely when it reacts with a simple vanadium tri-chloride what compound we do expect.

Because, we will seen our some next classes that, how these model compounds can be generated and can be a very good catalyst, so we have three nitrogen donors from here, rather NH donors these three are forming on a face three bonds on the vanadium. And we can have three chlorine bonds to the vanadium, giving rise to a beautiful vanadium

compound bound to tacn ligand and the three chlorine unit. So, this is the tacn part, so if this is the thing that means, these three positions we require, so these three are catalytically active sites.

So, they are because all these peroxides and all other groups are OH functions, they are mostly covering these three sites, so they are the catalytically active sites, and all the reactions are going from these three positions; that means, the other face of the octahedron. So, if we consider the basis forming from the octahedral geometry, so this basically going for this particular type of octahedral geometry; so other face, this is one face, and this is the other cis three face of the octahedron. So, other cis three face of the octahedron is basically leaving that particular type of reactivity.

So, in the final step therefore, for the catalysis, we just can go for the removal of H O C l, so H O C l is removing hypochlorous acid is removed, and the original catalyst in the native state is getting regenerated. So, if we see the entire catalytic cycle the mechanism for the vanadium (()) peroxidase activity, we see that when we do not give any other organic substrate or the very complicated organic molecule from the marine region, we do not get the corresponding chlorinated product of the organic compound.

So, if the organic substrate is lacking that means, the organic substrate is not there, what we see that the water molecule is getting attacked by this particular reactions of this thing; that means, when hydrogen peroxide was there the C l is there, so H O C l is forming over there. And this H O C l is not getting any organic substrate to react to get the corresponding chlorinated organic product, instead this hypo chloride as it is living from the medium as H O C l, from the reactions system and the native catalyst is getting generated.

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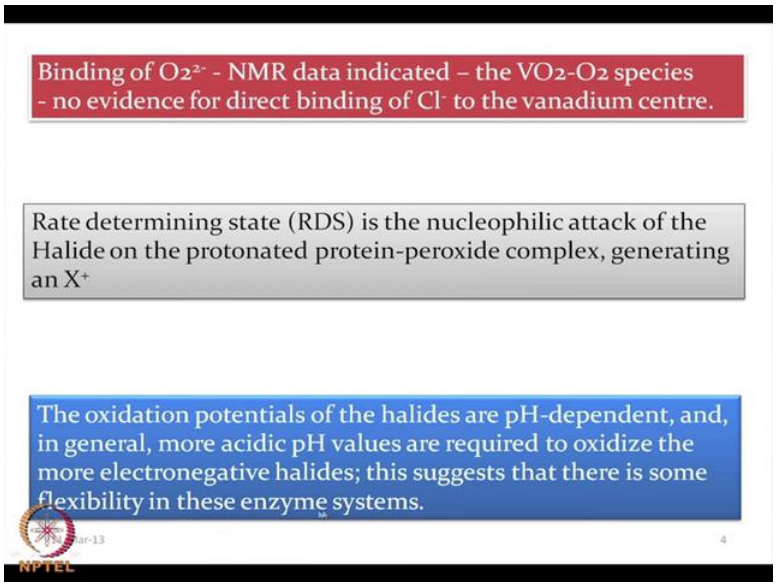

So, this particular one when we see that this cases that means, the vanadium site is there, so the important step for these reactions is that vanadium is forming the corresponding peroxo linkage, so which is very important to note. So, the formation of these vanadium peroxo linkage that means this one, as well as sometime this is forming the corresponding oxo form that means, whether this oxo form is there that means, this one is in the form of oxo.

So, V O 2 and the peroxo linkage, so the NMR data what we can have, because these two oxygens is what we have, these are not changed, so dioxo vanadium center, when attached to the peroxo species, so this particular entity the entire, this particular entity this particular entity can be analyzed through the vanadium 51 NMR. So, if we can study the corresponding vanadium 51 NMR, it can immediately indicate, that this vanadium center is bound to the peroxo linkage as well as two oxo as the vanadium dioxide.

So, any one data is definitely indicating us that we have the definite binding of the peroxide, and the vanadium dioxo unit is attached to the O 2 species. But how it is reacting with the C l minus, so how C l minus is reacting with the active vanadium center attached to the peroxide is not known from the NMR date. So, we do not get any direct evidence for binding of the C l minus to the vanadium center, because at this particular point the C l is not attacking to the vanadium center, and it is at the same time not giving the vanadium C l bond.

So, at the rate determining step or the rate limiting law what we can have is found where, the nucleophilic attack of the halide on that means, this C l minus is only functioning as a corresponding nucleophilic attack on the protonated protein-peroxide complex generating X plus. So, at this particular point only the step which is very much dependent on the formation of the vanadium peroxo species, as well as the attack of the halide anion; and we have the corresponding protein peroxide complex that means, the protein which has the vanadium center, and that vanadium center is responsible for the formation of the corresponding peroxide linkage.

And that is then reacting with C l minus and forming C l plus, this C l plus is basically reacting with the hydroxide anion forming the corresponding H O C l or the hypochlorous acid. So, during this oxidation process which can be also true, if our X is V r or X is I that means, for bromide oxidation or for iodide oxidation, the same peroxide we are utilizing. So, if we compare the corresponding redox potentials for the C l minus Br minus and I minus, we find that the corresponding oxidation potentials of the halides, are p H dependent.

And in the catalytic cycle for all these reactions also we see, that the p H dependency is also very much crucial, whether we are going for protonation of the vanadium O H bond or the corresponding thing what we are getting that the (( )) of the O H from the vanadium center to giving the vanadium oxo center. So, more acidic p H values are definitely required, when we try to oxidize the more electronegative halide. So, if we can have the species that means, C l minus Br minus and I minus which we all try to oxidize, so we basically try to oxidize all three in presence of the H plus.

So, initially we all know that, if some suitable oxidizing agent is available, we get chlorine, we get bromine, we get iodine, not only these, but also we want to have Cl as Cl plus, Br as Br plus, and I as I plus. So, these formation of these things, so they are very much dependent on the different E 0 values, and these E 0 values are also dependent on the condition that means, the H plus availability that means, the p H of the medium.

So, when we try to oxidize the Cl minus to get Cl 2 or Cl plus, we should have a corresponding very acidic p H value, for the more electro negative halide oxidation. So, the enzyme must have some suitable mechanism it has some flexibility, in these enzymes system that, they basically modulate the different E 0 values, because the reagent is fixed

we have the vanadium center we have the peroxides. But the enzymes should have some flexibility such that, depending upon the enzyme, it definitely modulate the different E 0 values, so for the different enzymes we have different E 0 values, for the oxidations of Cl 2 Br 2 or I 2.

Thank you.