Bioinorganic Chemistry
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Lecture - 33
Vanadium Enzymes - I

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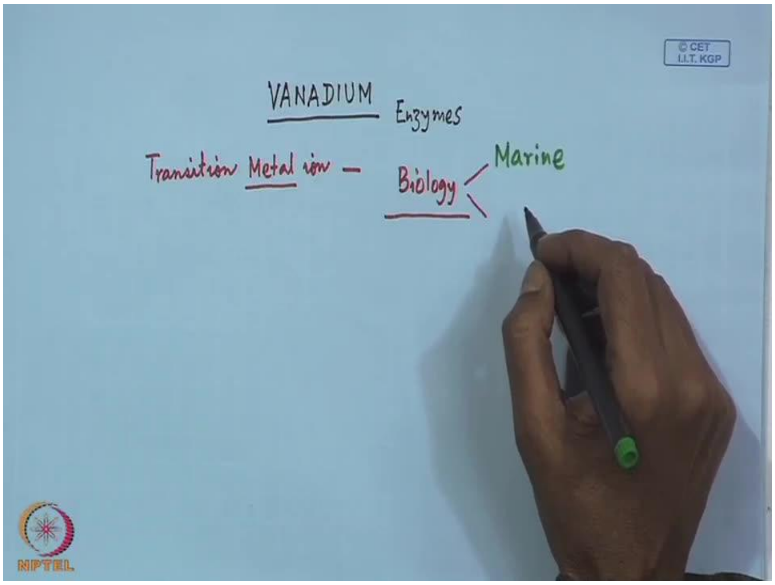

Hello, good morning everybody. So, today will just simply move from tungsten to another group of enzymes those are our Vanadium Enzymes. So, this Vanadium those not very much known about the biological role of this Vanadium. So, when we study this vanadium chemistry systematically. We should also little bit know about the corresponding it is property related to the Transition Metal.

So, it is the transition metal ion of the first transition series. Where this vanadium is coming after scandium and titanium, And this transition metal ion like other transition metal ion which can show some behavior in the biological system. So, How the biology can be benefited from the knowledge of the corresponding chemistry of vanadium particularly the coordination chemistry or the complex chemistry. Because the human being are not dependent on vanadium mass. But. some of this oceanic environment. That means, the Marine chemistry. The marine organisms are slowly depended on some these species where vanadium play some important role to survive.

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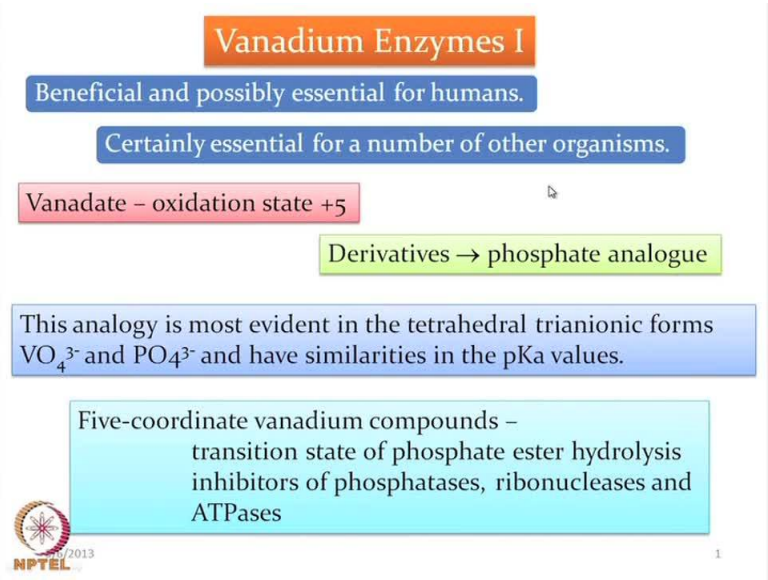

So, when we talk about these in this first class on Vanadium Enzymes, which we consider that this is beneficial and possibly essential for human, because we do not know anything related to the corresponding behavior in human system. But we expect that since it has some similarity with other anionic groups like phosphates it can have some role to play if not we can think of something where the vanadium when available to the human system in some point it can function like the corresponding phosphates. And this phosphates we all know are very much essential to some of these systems. Where we can have the corresponding behavior related to the phosphate transfer chemistry. And it is essential therefore in certain number of other organisms like these marine organisms.

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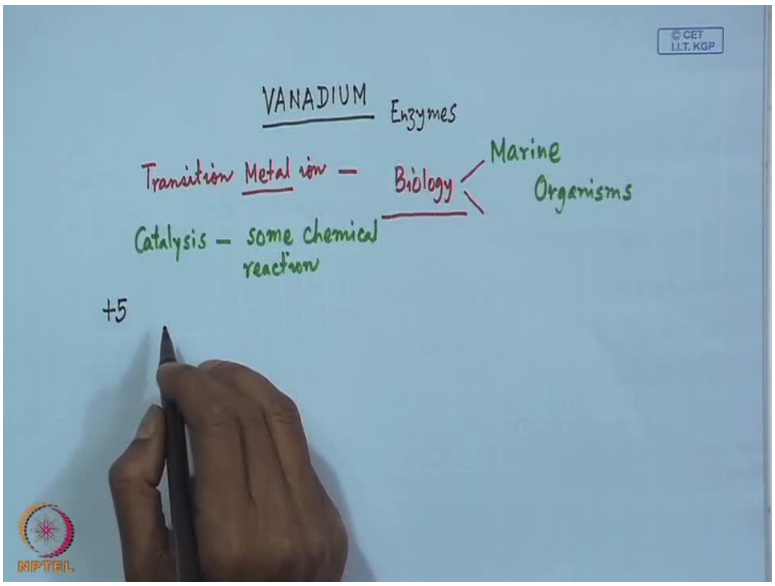

So, the most of the chemistry what will find related to the Vanadium Enzymes is due to the Marine Organisms. How the marine organisms is doing all this reaction and whether it can catalyze some important reaction. So, the Catalysis for some important chemical reaction if we can know for that so our knowledge about.

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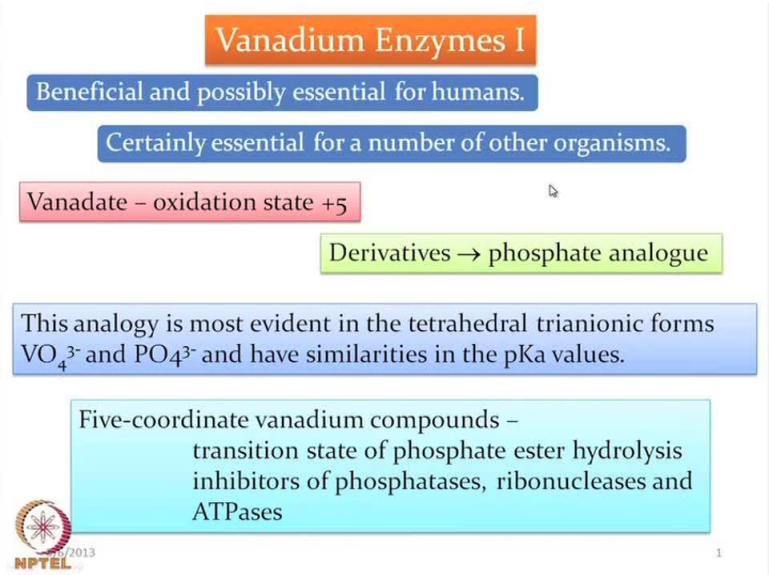

All these things particularly in biological system would also be benefited. So, the most common oxidation state what vanadium can have. So, vanadium depending upon the number the unpaired electrons present in it are elemental state or the metallic state. The

most common oxidation state of vanadium is plus 5. That means, including the 4s electron. It can have the corresponding 3d electron after scandium, titanium and vanadium. So, in a metallic state it has a corresponding electronic configuration of 4s to 3d 3. So when it forming the corresponding Vanadate in it is corresponding oxidation state. That means, it is mostly stabilized in plus 4 oxidation state. And we call that particular species as the corresponding Vanadate species.

The way we call sulphate, nitrate this ate nomenclature is related to the corresponding highest possible oxidation state of vanadium which we can have and if these and some of it is other derivates which can be the corresponding phosphate analogue species. That means, where the vanadates are there. You can consider that some of them related species are also the phosphates. And when this analogy we can extend due to the replacement of some of this phosphate ends by the vanadate ends, which is also similar to some of these reactions, where we know that the sulphates can also be attached to some of the phosphate ends in ATP and ADP molecules. But initially How we compare these two species? How we compare the vanadate in plus 5 oxidation state with that of our phosphate molecules is that both of them have a tetrahedral form.

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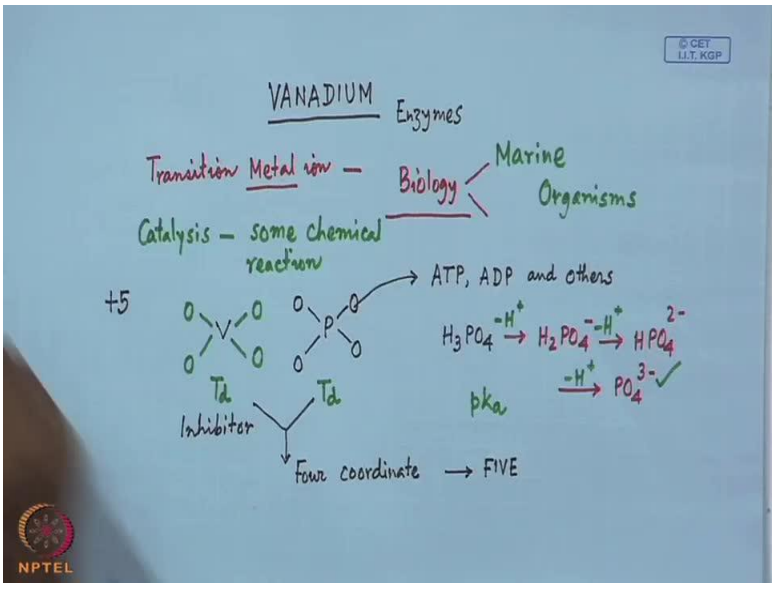

That means, when we consider that vanadium is present and we consider that phosphors is present and phosphors. We all know including the human body also that they are the most important fragment for all the bi-minerals species and some other species like ATP,

ADP and others. So, initially what we think of that when we talk in terms of the corresponding phosphate anion. So immediately, we can see that phosphate will have 4 phosphors oxygen bonds. And it can be derived from very simple one acid which is the corresponding phosphoric acid.

So if this phosphoric acid goes for deprotonation. It can go for H2 PO4 minus then HPO4 2 minus, and lastly PO4 3 minus. So, due to the 3 step deprotonation it is the corresponding tetrahedral species. So, this particular anionic tetrahedral and it has 3 negative charge. That means, it is a tri-anionic species because, the charge is also very important to play some role for all these biochemical reactions and biological transformations. Because, the very simple thing that when we do not want that much charge on the species. We can protonate that particular species to this or to this when you need only one negative charge it can be.

Doubly protonated, when you need two negative charges it can be singly protonated. And when you need the highest possible available charge on phosphate anionic go for complete deprotonation of the species for phosphate tri-anionic form. So similarly, the vanadate is also can have 4 vanadium oxygen groups.

And if we just compare the size of these two. They are also comparable to each other and the corresponding electrophonic equilibrium related to the corresponding proton transfer from one such species is also possible when, you go for the corresponding acidic form of the vanadate anion. So, when we consider the three steps of proton transfer this is minus H plus, this is the second step of proton transfer.

And this the third step of proton transfers. So, we can have 3pKa values for both the two species. That means, related to the vanadate ion and related to the phosphate ion. So if they have some close values of pKa then, we can also think of their similarities with respect to the phosphate anion. And we can substitute some of the sides where phosphates are playing some important role. Like that of the formation of ATP and ADP molecule can be substituted by vanadate group. But, interestingly the corresponding hydrolytic behavior related to the phosphors oxygen bond is completely different to that of our vanadium oxygen bond. But, when we substitute some pocket which is bearing that phosphate anion if it can be substituted by vanadate anion it cannot.

Reproduce the function for the phosphate anion can give to the system whether it can go for some type of inhibitory reaction and these vanadate ions can function as good inhibitors in that particular case. So, when it is functioning as an inhibitor. We should also know whether there is a similarity between the phosphate and the vanadate anion. So though the human being are not much effected due to some important biochemical reactions of this vanadium center but, if we inject somebody or if we go for the substitution of phosphate concentration by vanadate concentration. It can function as good inhibitor on some phosphate bearing important groups. So, when we have this tetrahedral species definitely they are both of them are.

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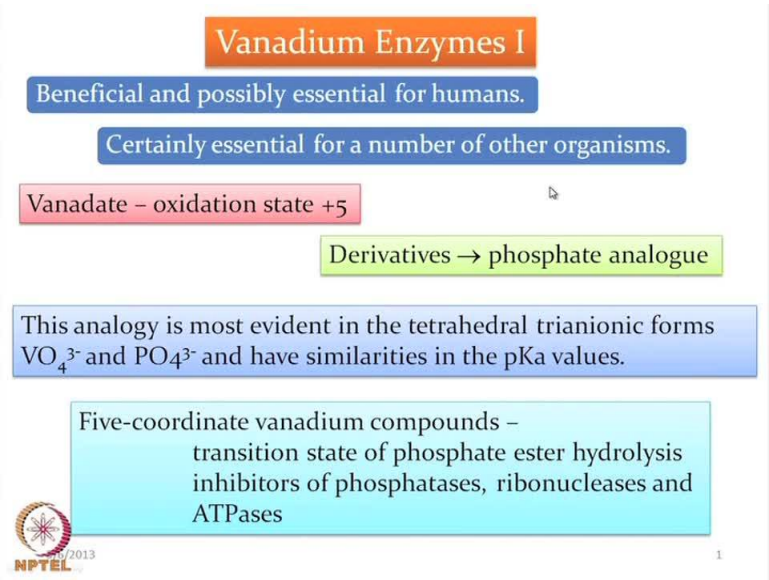

Four coordinate and this particular species can react with some other important donor atom. And it can go to some species which can be Five coordinated one. So, this change from a four coordinate species to a five coordinates species. And the corresponding geometrical chain also related to the coseniority of the vanadate with that of your phosphate. So, if we can have some five coordinate vanadium compounds synthetically. Also when will study the corresponding part as the corresponding module compounds. We will find that the five coordinate vanadium compounds are also very important and with the relation to the corresponding phosphate ester hydrolysis reaction.

If, we consider that this particular species that means, the phosphate groups are involved there for the corresponding phosphate ester hydrolysis reaction. But, in the transition

state when expansion in coordination number is taking place where will find that the four coordinate vanadate group can go for a five coordinate species. And it can go for inhibitors of phosphates. So, some zinc bearing or other metal ion bearing phosphates are there which are responsibly we all know that which are responsible for the hydrolysis of the phosphate esters. So, if vanadium compounds are given to that particular space and they are substituted the corresponding phosphates. Then, there will be definitely inhibition for phosphate ester hydrolysis.

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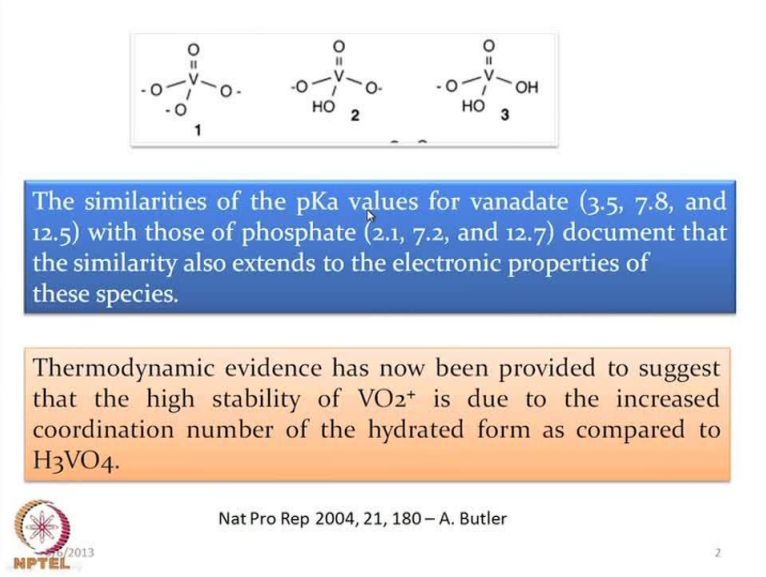

Similarly, the ribonucleases are there. They are responsible for the hydrolysis of the pop bond, the phosphate ester bond ATPs are also there. These ATP are responsible for the hydrolysis of ATP to ADP and inorganic phosphate. So, if vanadium is present and that vanadium is taking part in the inhibitory reaction of all these three groups of enzymes. Then, vanadate inhibition can also be studied very nicely in comparison to that of our phosphates, ribonucleases and AT Pases. So, what we see that when we have these tetrahedral vanadate groups and these groups already I wrote that this is the tri-anionic form, this is the di-anionic form and this is the mono-anionic form.

Depending on the level of protonation on the tetrahedral vanadium group. And this tetrahedral vanadium group is there important and will find that how this particular protonation is important to go for some of these reactions. Particularly if we consider that there is some electron transfer reactions because, these vanadium is in plus 5 oxidation

state is divide of any d electrons. So is a vacancy is there a3d 0 system but, if we want to reduce it to vanadium 4 or vanadium 3 or vanadium 2. We want to put electron to the vanadium d level. We sometime we find that which is easier or difficult when the protonation levels for those vanadium species are different.

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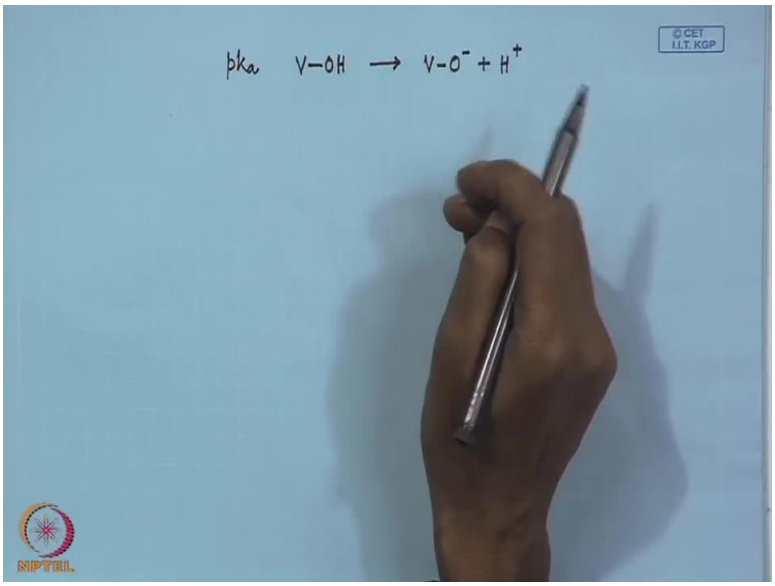

So, if we just compare not only in terms of the corresponding safes for the vanadate. And phosphate when we consider the corresponding pKa values. So these pKa values the dissociation constant values of negative logarithm in terms of the proton dissociation from some of these species where we can have the corresponding V OH bond which is going for the corresponding V O minus bond and H plus. We find the corresponding equilibrium constant value for that deprotonation state. So, we find that in case of vanadium and in case of phosphate group. These two values are pretty close in case of vanadate these values are 3.5, 7.8 and 12.5.

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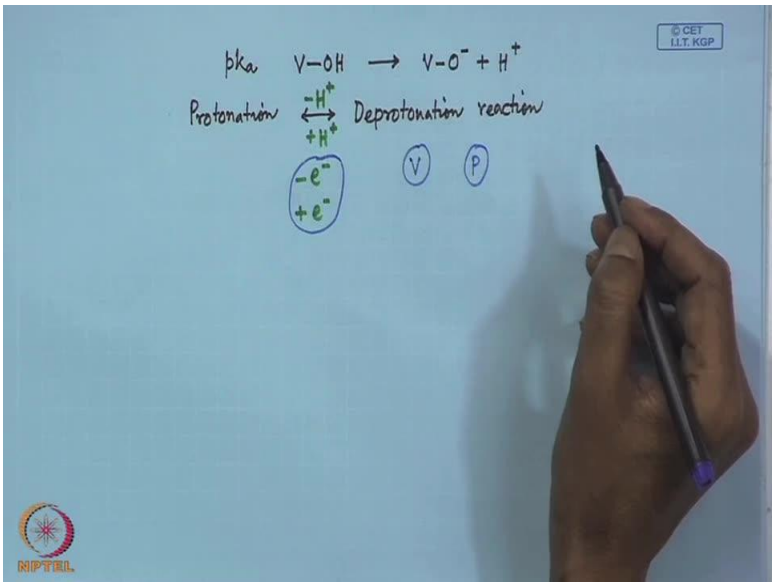

And in comparison to phosphate though first one is 2.1, second one is pretty close compare to 7.8, it is 7.3 and third one is also pretty close to 12.7 compared to for vanadate is 12.5. So, these particular values that means, the protonation deprotonation reactions. So, if we just consider the protonation and the corresponding deprotonation reaction in terms of something where we can consider that, not this is not the driving force for protonation and deprotonation reaction. We just consider that if we go for the deprotonation here this is the protonation level. So, we can have also the electron removal and electron addition to these steps.

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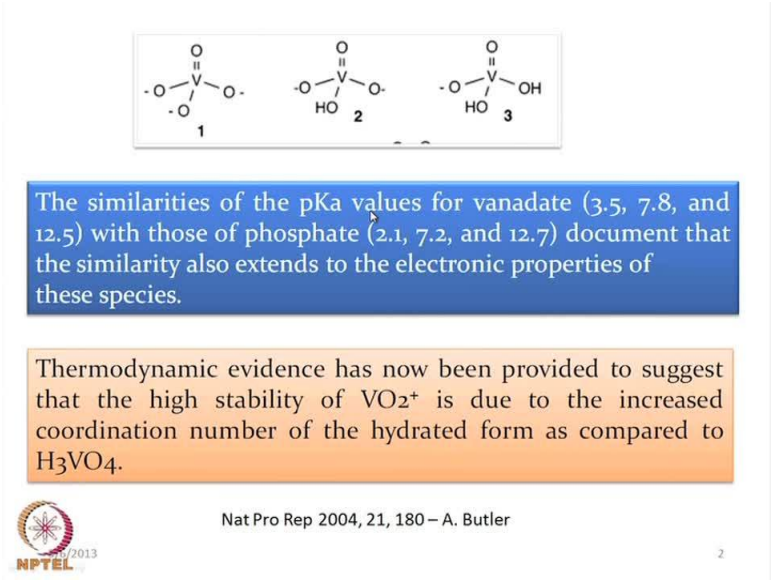

So, depending upon these that means, whether we can go for the corresponding electron transfer reactions we can have the required number of proton transfer can also take place from there. So if we just consider the corresponding electronic properties so vanadium as well as phosphors center that electronic properties related to the pKa values are also similar. That is why we are getting three closely related values for their pKa. So therefore, it can extend not only for the structure but, also from the electronic point of view the electronic structure point of view that these are very much similar. So, when we can have the corresponding electron transfer and the proton transfer behavior the thermodynamics also tells us that not.

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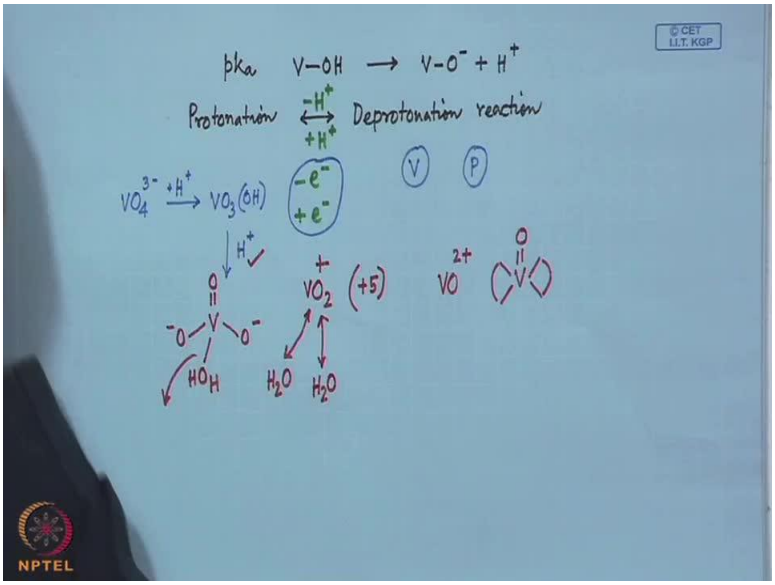

These species but, only the VO 2 plus species is there, where this V 2 VO 2 plus is forming there. Due to the increased coordination number of the hydrated form as compared to the corresponding protonated form the vanadate ion. So, when we have the VO4 3 minus and if, we can go for the corresponding protonation. That means, if we just go for protonation for that and we go for that VO 3 and OH is present over there and this basically continues because, this when this OH is forming and this particular species OH can go.

So when the second proton is coming into the system. What is happening there, that if we have this vanadium and this is the corresponding O minus, this is O minus and this is HO. And so this particular proton what is here it is come and attach either to this as the O

H 2 or to this OH. So ultimately, what is happing that in one particular case and when this is going for a corresponding water molecule. Then, we can have some species at one step which is the VO 2 plus which is also present vanadium.

In plus 5 oxidation step. So, when we have two of these that means, when we have these two as the corresponding VO form and two other are present as the water molecule. So, reversibly these VO 2 plus can take up water molecule and can go for the dissociation. So, when this is getting protonated then this is forming water and water molecule can go out similarly, this can be protonated in step wise fashion. First this proton is coming to this O minus, then, second one is coming for this and water is going away so will be remaining with VO 2 plus species. So, VO 2 plus when it is present like our VO species vanadial species. So, VO species which can be in the 4 plus this is 2 plus.

So, in the vanadial system that means, the most stable compounds in this particular form are also available. So, this is one from when only one oxygen is attached to the vanadium center and is known as the vanadial form. And we can have two ligands attached to the vanadium. So, it is the scropio metal environment around vanadium. When vanadium monooxo form is present and which is the dioxo form of the vanadium in the pentavalent state. So, it can go for several other water molecules also which have been dissociated from there during the transformation of this vanadate group to this particular species. So, this one when goes for interacting with some more water molecules.

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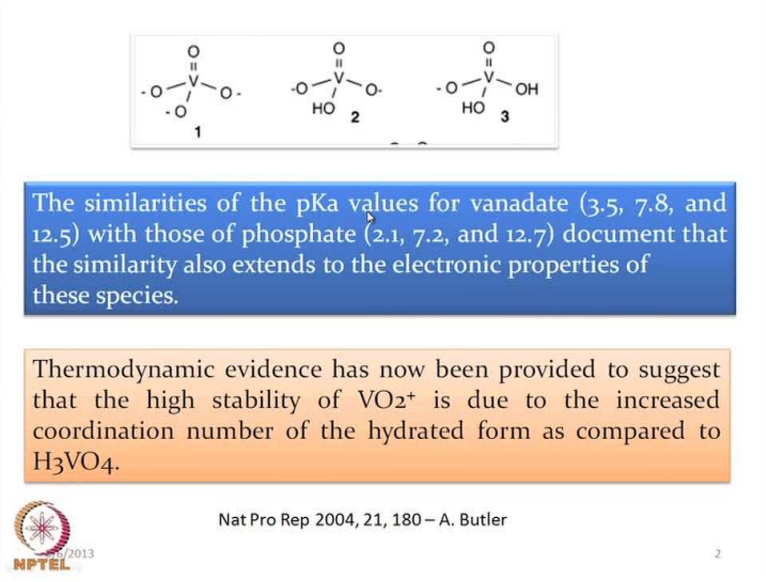

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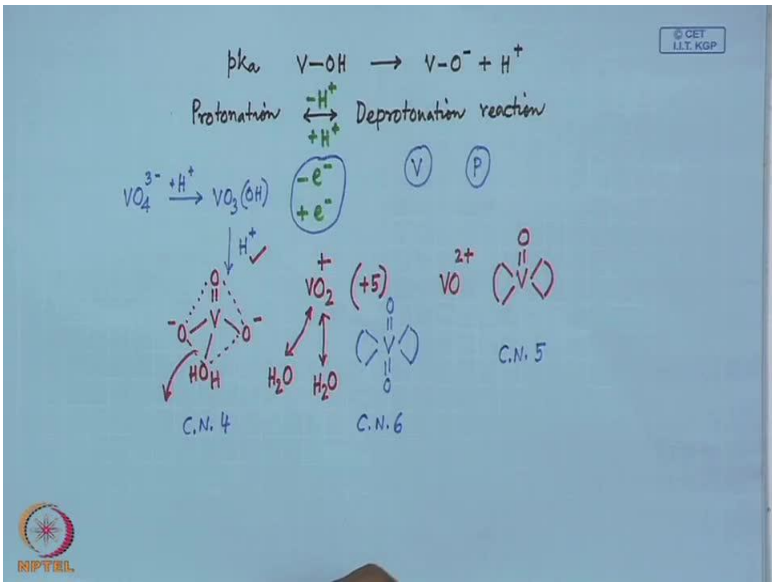

We can consider that is like this vanadial species or dioxo vanadial species. There we have the corresponding coordination number can be satisfied through the introduction of two or three water molecules surrounding this vanadium. So, How we consider that? We have some stability of VO 2 plus. So, VO 2 plus species if we can generate the VO 2 plus species can be hydrated. And it can have some higher number of coordination and it can be available if we just go for some higher coordination number compare to H2 VO 4. So, when we have H3 VO 4. That means, vanadium is present as the tetrahedral vanadium center.

So, when it is in tetrahedral vanadium center our coordination number is restricted to 4. And we have seen the example where we can move from that coordination number to coordination number 5 and in some cases we can go for the coordination number of 6. So, if we have the VO 2 plus species and if this particular one is like this it can be trans or it can be CIS also. But, in terms of the corresponding available donor points so if we can occupy these two by some bidentate ligand. We can go for the coordination number. So, the enhanced stability for more coordination number that moving from a coordination number of four to five to six is due to the changing the corresponding species.

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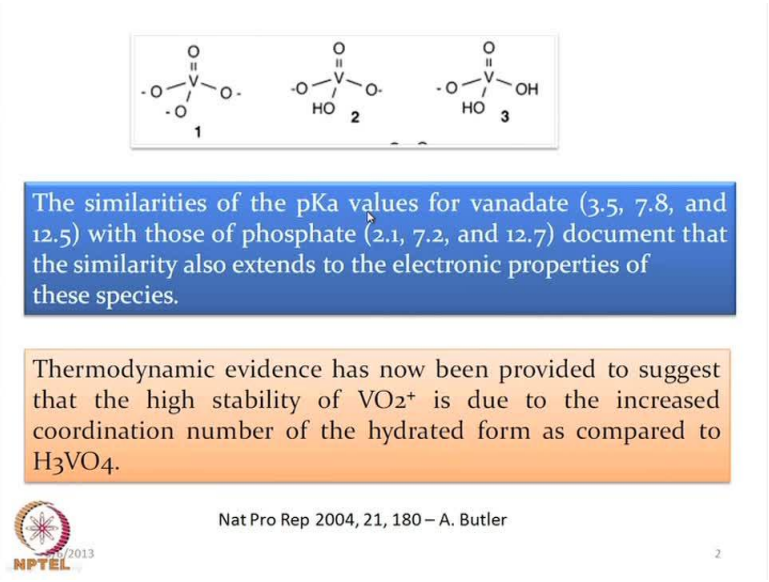

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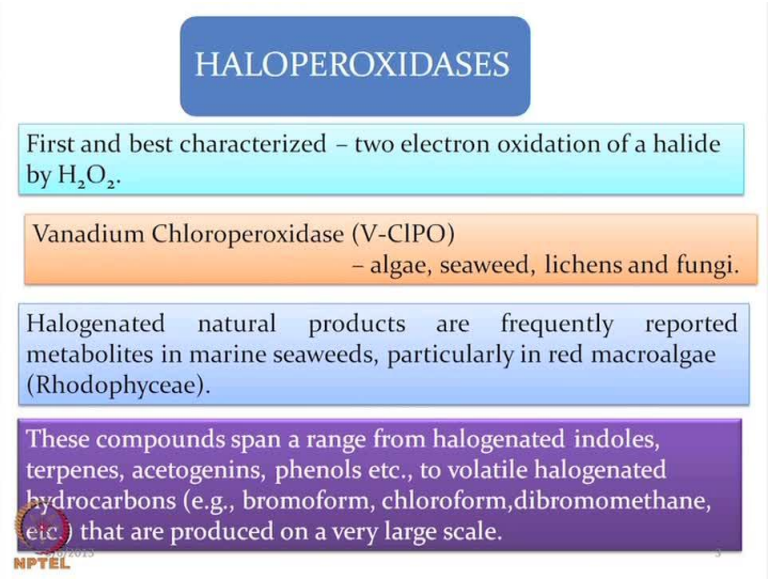

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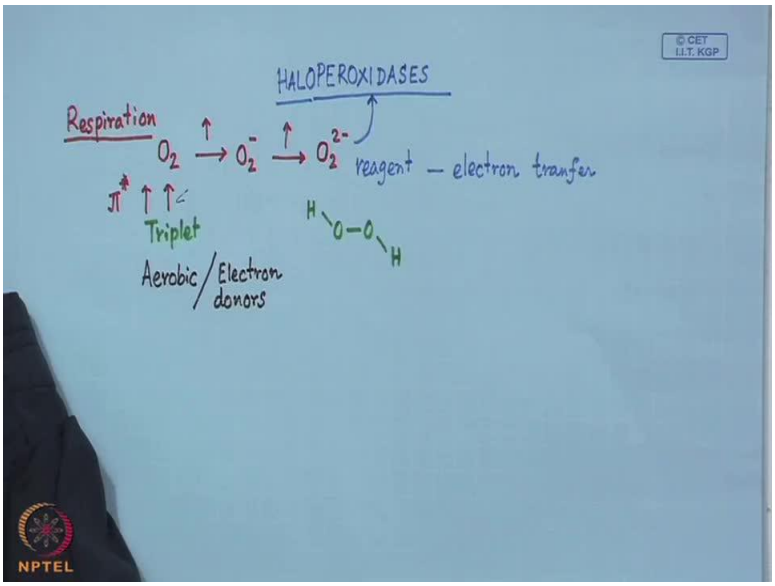

What we are getting which is not available from the typical vanadate form. So, vanadate form always try to abstract one water molecule or hydroxide group to go for it tetrahedral arrangement to a pentacoordinate species. And that pentacoordinated species can go for to another hexacoordinated form. So, the first and the best characterized form of vanadium which is present in biological system which is in the form of some peroxides. So, if we little bit if we recall what we know about this biological inorganic chemistry related to peroxides.

So, peroxides we all know very much simultaneously with that of the catalysis that when we basically the human system. The human body when takes off due to the respiration or anything we take this O2 molecule and this O2 molecule can take up one electron and it can go to O2 minus, which is a super oxide anion and that super oxide anion can take the second electron to give us the peroxide. So, during respiration so what the human system or any living system utilize dioxygen for respiration and at that time if the electrons are failed into the.

Antibonding molecular orbital the pi star level those are the corresponding pi star level for the O 2 molecule. So, one electron is fit at this point and another electron is fit on this electron to give you the corresponding peroxide species. But, this particular one though it has two unpaired electron and it is therefore, known as the corresponding triplet ground state of molecular oxygen. So, this is the triplet. So, the triplet form of molecular oxygen

when it accepts one electron it is bond order from two to basically reducing to 1.5. And when the second electron is feeding into the pi star level of this molecular orbital's of the molecular oxygen we get the peroxide.

When this oxygen bond order is again changing and we have one O. O bond and which is related to our well known species hydrogen peroxide. So, this super oxide as well as peroxide are always harmful to the biological system. So we want to have some mechanism for destroying them and in the human system all these can be taken care of by the different catalysis. For catalytic catalysis degradation path and some are iron bearing peroxides. Such that we can destroy the accumulation of hydrogen peroxide or the peroxides any alkyl peroxides can also form into the system by suitable catalytic path where the peroxides are degraded this is not only the one thing.

But, also we can study this out of reactions whether in all chemical reactions because most of the chemical reactions particularly in oxidizing environment the peroxides are very good reagents also. So, when they function as a reagent what we can study based on their electron transfer property or electron transfer behavior. So this electron transfer as well as electron settling behavior from one point to the other can be known. If we are able to know the handling of this peroxide anions by peroxides and in those cases we know we can take the help iron. So, iron bearing catalysis and iron bearing peroxides in human systems are well known.

So, in this particular case what we see that now vanadium is playing the important role for there peroixdase behavior. And if that peroxidase behavior on some halides we call them as HALOPEROXIDASES. So, the system will have a different nomenclature for that and we get these as halo peroxidases. So, these are attacking on so this hydrogen peroxide whatever we can have which can be very easily derived from the molecular oxygen in aerobic environment. So, if we have something any biological system which is present in aerobic environment molecular oxygen is plenty there, molecular oxygen concentration is very high.

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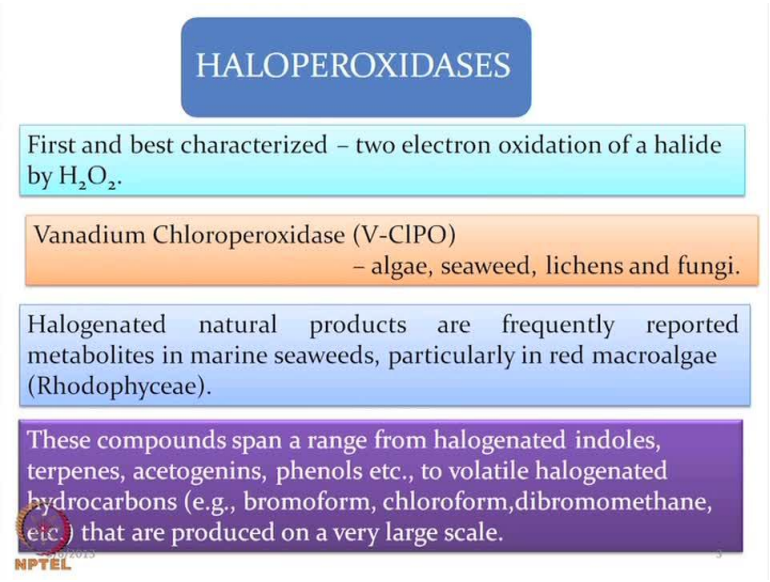

And if we can go for some electron transfer to that di oxygen molecule which can also be mediated by some other electron donor system? So, we all know that very good biological electron transfer donors are also available. So biological electron donors should be there and those electron donors can put the electron to the system, where it can be converted to O2 minus and then O2 2 minus. So for these vanadium compounds the most well known. Now, is the well characterized halo peroxidases which can be useful for two electron oxidation of any halide. It can be chloride, it can be bromide, it can iodide for using this hydrogen peroxide. So, it showing the corresponding peroxides type of behavior.

That means, if there is some accumulation of this particular hydrogen peroxide in to the system. We get that particular hydrogen peroxide and we can oxidase this particular halide present over there in the different marine organisms. So, vanadium chloroperoxidase which is also abbreviated as V-CIPO. So, V-CIPO which is available from the different algae, seaweed, lichens and fungi. So, they are basically the different microorganisms which are available in the marine environment. But, what we want to study from there? That how vanadium is doing some important reactions by doing some chemical transformation on the chloride ion which is available.

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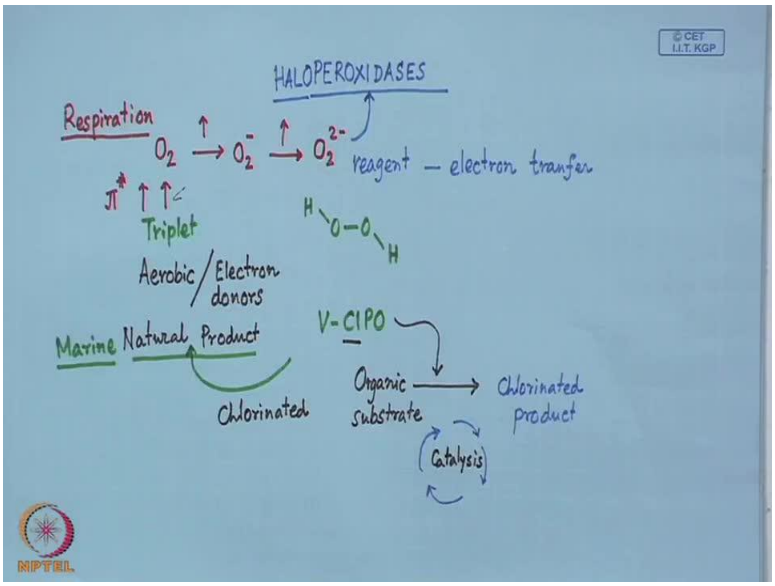

Or the bromide ion because, large amount of these halides are always present in marine environment because we all know that the sodium chloride, sodium bromide and sodium iodide can be obtained from the sea water very much. So, if the concentration of these halides are pretty much there and if the vanadium is available. And you could have some availability of the corresponding hydrogen peroxides there or the peroxide species. What type of transformations we can have such that due to that transformations we get some of these as the halogenated natural products. So, these is the corresponding discovery particularly the organic chemistry what we all do is that study of the different natural products.

So, the natural product chemistry is well known and well established now and when we have the product which is coming from the marine environment. So, the marine natural products how we can get and when we talk in terms of the corresponding dealing with the vanadium say chloroperoxidase. So most of these natural products are very useful to know them as that corresponding macroloids which are available and the corresponding pharmaceutical importance also. So, these natural products if we can utilize this and if when we are handling some chloroperoxidase that means, we get some chlorinated.

Chlorinated natural products. So, this chlorination reaction is very important and that also lead us to some important understanding. If, we can study the natural reaction the how nature is doing that simply chemical reaction under the sea, that how this particular

type of chlorination reaction can take place on some beautiful or interesting organic substrate. If, we have the organic substrate in our hand and we just put the corresponding environment; that means, percent naturally occurring vanadium chloroperoxidase and we get the chlorinated product.

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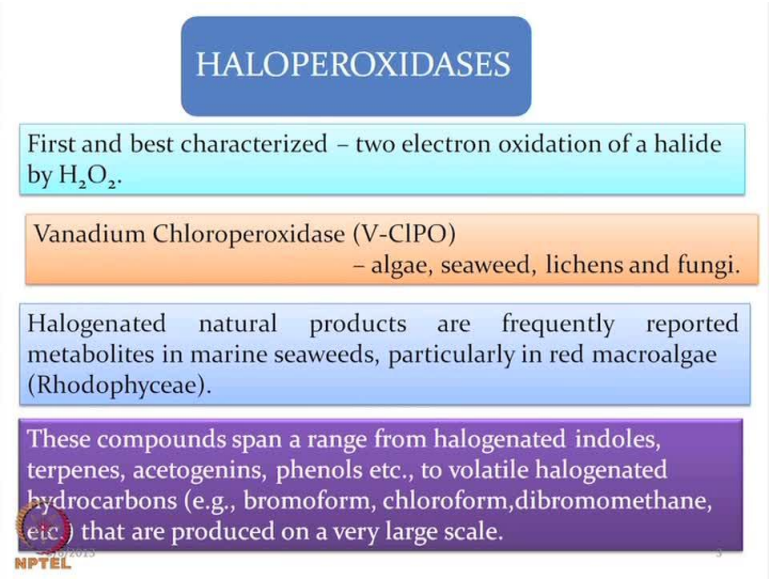

So, we get in the chlorinated product, so is very simple and straight cut reaction. So, we also know we should be able to know also, that how these reactions is going on. And if we are able to study the corresponding intermediates what is happening over there due to that transformation. So, if we should we can able to track all these so we should know little bit about the corresponding catalysis also. So, the catalytic cycle can also be well known for us for this very simple chlorinated product reaction.

So, when we go for these halogenated natural products in the different metabolites. So, in marine seaweeds particularly in red macro algae. So in red macro algae which is the rhodophyceae and this red macro algae what we get that some of these metabolite what is coming out, they can have some medicinal importance. They can be useful for medicine purpose and they are basically some important molecules where this species has been chlorinated. So, if have some in dole type of molecule, then terpene type of molecule, then acetogenins phenols so on. So, getting these products basically from the marine environments so this is again another naturals force of getting compounds. So, if we have phenol so the phenol what we are getting from the marines source that means, from the

sea water. So, if that can be halogenated we get the corresponding chloro phenol or the bromo phenol from the medium due to the presence of corresponding enzyme.

Vanadium dependent enzyme that means, the corresponding presence of chloroperoxidase or bromoperoxidase can give rise to the generation of halogenated phenol. Similarly, acetogenins can be terpene can be or in dole can also be halogenated and some other volatile halogenated. Hydrocarbons though it is sometime difficult to trap or difficult to get these species like bromo form, chloroform, dibromo methane but, these are the very simple reaction. Basically, what we get basically when one single carbon species can be brominated for these and they chloroform is also ch cl 3's, cb rc l 3 that whether we can get the all these things like that corresponding species as the bromo form and the chromo form chloroform these halogenated.

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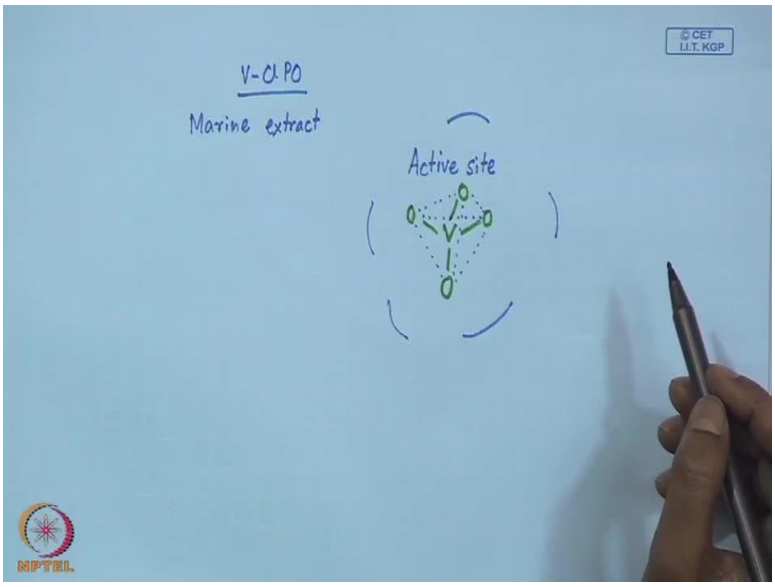

Hydrocarbon can be produce on a very large scale. So, whether this sea water can be useful source of all these important molecules that can be known, if we want to study these vanadium chloroperoxidase or vanadium bromoperoxidase nicely. So, what we have in the marine extract? So, if we have in the marine extract that chloroperoxidase. So vanadium chloroperoxidase if it is available there in different marine extract.

So, first thing what we can be able to know is the identification of the Active site that means, the vanadium active site. So, as we have seen that this is basically a simple tetrahedral vanadium site which would be responsible for giving rise to this reaction. So

active site should definitely be tetrahedral one, which can be converted to a pent coordinated form also like that of our trigonal bipyramid. The most common one compared to square pyramid geometry.

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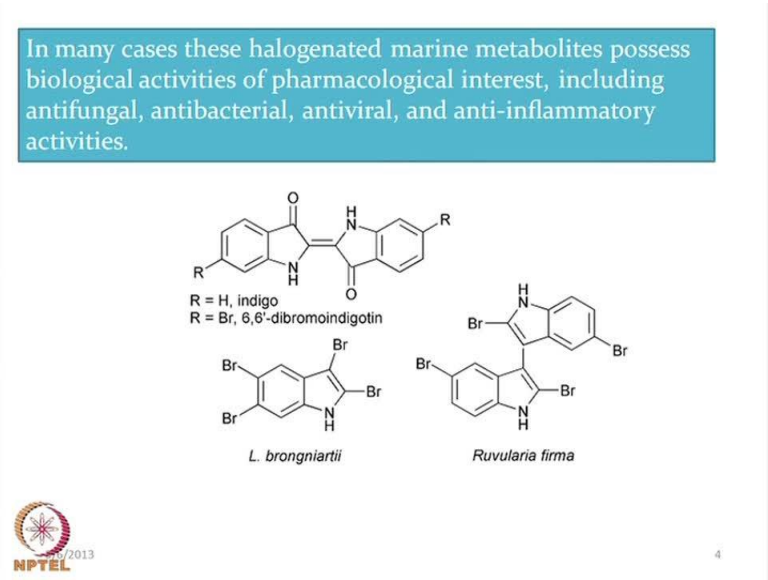

So, this is the tetrahedral one and we can have all other amino acid side chains or amino acid residues surrounding this particular active site to the active for the generation of many such halogenated marine metabolites. And they have beautiful interest for different pharmacological activity. So, some of these molecules have been identified from there and which have been tested for their pharmacological interest including their different behavior for their activity as antifungal activity, antibacterial activity, antiviral and anti-inflammatory activities. So, the marine metabolites are therefore a very good source for all these cases. Where we can find that if we can isolate or identify and then, characterize these particular molecules will find that some of these molecules would be definitely useful for their pharmacological values.

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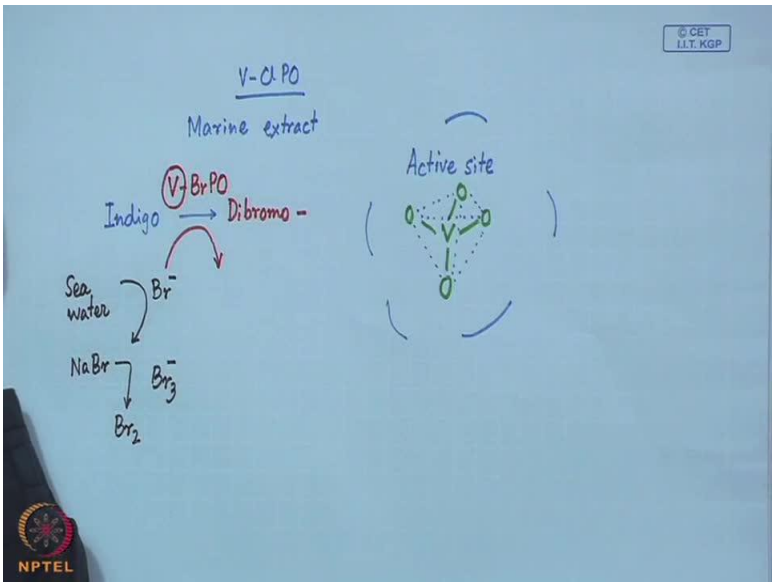

So, one such molecule have been identified as the Indigo. So, this is the corresponding indigo molecule and this indigo molecule what we see that you have these corresponding particular units, which is sometimes very much colored also. And these positions these two positions so when we get the indigo. Indigo in the laboratory we can make is a very useful compound and when we get indigo from the marine source not only the indigo molecule what we are getting from there is very important and very complex molecule also but, at the same time two specific positions the six and six prime positions of the indigo can be brominated with the help of the vanadium bromo peroxides.

So, if we can have so dibromo indigo, so we have indigo in one hand and that indigo can be brominated for its dibromo derivative. And now we are talking what in terms of the corresponding oxidation of the bromide anion B r minus. So, we are taking the help of vanadium bromo peroxidase to get the dibromo derivative and what we expect from these peroxidase activities that is basically working of functioning on Br minus. So, large amount of bromide ions are there in the sea water so it is available from sea water. In sea water it is mostly available as sodium bromide.

So, there is no need to supply these particular species and sometimes these can utilized for the corresponding oxidation to release the Br 2 also. Because, the bromine can also be extracted from sea water and when these two are combined we can have some species like Br 3 minus also.

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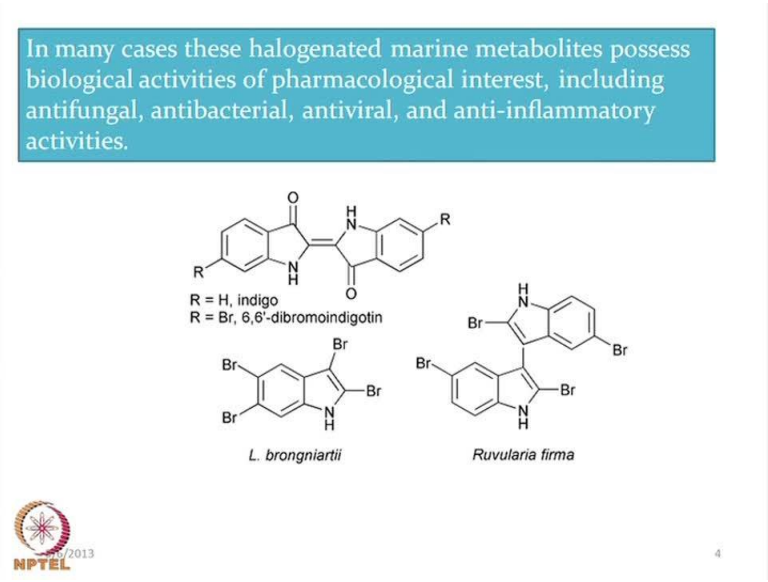

So, this Br minus Br 2 and Br 3 all in the environment of bromine and in that particular environment we need the help of the catalyst. And the catalyst is vanadium catalyst and that vanadium catalyst is responsible for the bromination reaction on the indigo molecule at some specific positions. So, we get dibromo indigotin from indigo molecule.

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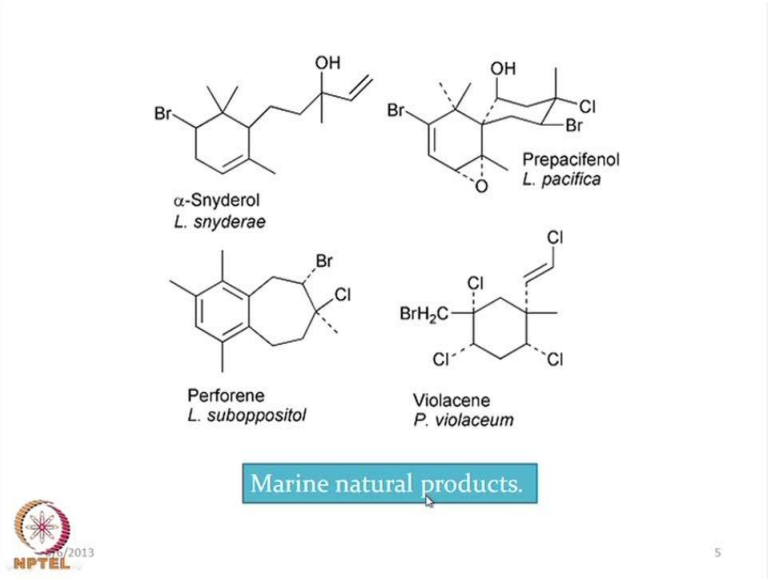

Similarly, this particular molecule which is some similarity with that of our indigo back bond and this can also be brominated at four different positions. So, bromination is an important step in terms of the corresponding availability of the bromide ion and the

peroxide. Another molecule based on the 1 by 2 of the fragment of the indigo is this particular one and these are the corresponding biological species. Where from we are getting these molecules and here the single molecule is brominated at four different positions.

So, these four different positions are brominated and due to that we are basically adding something that means, we call it as a value added product. That means, the brominated derivative sometimes is very difficult to make and they are also very much costly in the market if you want to buy. But, very simple and natural reactions related to some the natural product formation. We get the corresponding brominated and highly brominated species very easily form the marine source. Some more examples are there which is there that means, this particular can be terpeniod molecule, then we the alkaloid molecule.

So, these are all very important biological molecules available from the marine source. So, snyderol, so alpha snyderol, so is a snyderol molecule which is available there. And then perforene and the violacene and prepacifenol. So, these phenol molecule of this type there is all is there for this apoxide formation from there but, one important thing is that not only the parent molecule what we are getting from there. But, it is the corresponding system where some specific positions are halogenated.

So, not only isolating these things because, the solubility wise are some others environmental change. We get that the parent compound is very difficult to isolate from the medium, but we can isolate the corresponding dominated form.

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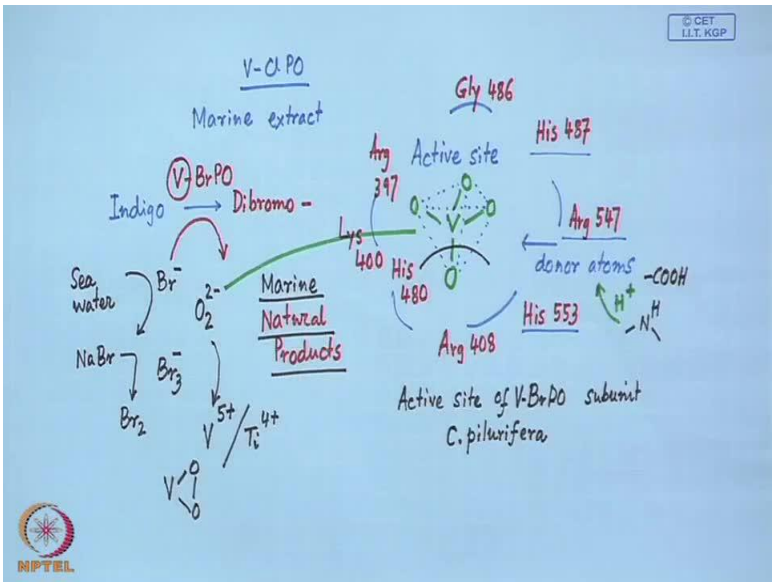

So, the brominated derivatives the brominted form of all these natural products from the marine source can be isolated very easily compare to the non-brominated form. So, it has advantages for isolating these molecules and its corresponding brominated derivative. And at the same time some big un complex terpiniodes or any other type of molecules alkaloids sometimes can be identified as the corresponding marine natural product.

So, we know all that in organic chemistry is a very good area is the corresponding knowledge on the natural products. So, studies on natural products are there and with the help of vanadium as vanadium enzymes related to those molecules. We now have something which are obtainable from marine source. So, we get marine natural products which can be very useful for the transformation of simple or complex biological molecule to its corresponding bromianted or chlorinated form.

So, when we have these active site that means, the vanadium site should be their which can activate not only the Br minus available there. But, also you can activate the O2 molecule as its peroxide form that means, O2 2 minus should also be available.

So, the uniqueness of the site the uniqueness of the vanadium site is two four. First it can go and simply react with the peroxides and from our knowledge of the common analytical chemistry lab is that the peroxide is also a very good reagent. So, is the identifying reagent to identify vanadium in the system. So, it can be vanadium 5 plus, it can be vanadium 4 plus or 3 plus. So, any unknown sample containing vanadium 5 plus

can be identified by testing it will peroxide, the way we also test titanium, both these two the titanium and vanadium form beautiful and colored peroxo species.

That means, it is well known to us that both titanium and vanadium can react nicely with peroxides species or peroxide anion to give us some beautiful colored. Or, the color reaction is such that we can identify in the semi micro level also in some plates that the color reaction is taking place which is identifying the formation of vanadium peroxo compound or the titanium peroxo compound. So, these active site should be designed in such a way that we can have the corresponding aims basically and the amino acid aims which are facing towards these vanadium oxygen bonds are here it is the glysine 486.

So, the glysine residue is coming and which is interacting with these particular sites and sometimes it is not forming any bond with the vanadate ion. But, the vanadium anion is basically trapping is trapped within the environment created by so many amino acid residues from the protein environment. So, is a histidine 487, then argenase 547, then, one more histidine 553, then, the other side of this oxygen we have argenise 408, then histidine 480, then lysine 400, then numbering on the polypeptide chain and lastly this side argenise 397.

So, all these basically play some important role to accommodate this vanadate try negative anion within this particular pocket and they basically afterwards catalyze the reaction. That means, the interacting with the peroxide anion, then interacting with the Rr minus. And finally, with the substrate for the catalytic transformation for the reaction were we get the corresponding brominated products. So, this is basically the corresponding active site what we write here also is the active site of now, it is bromo peroxidase Br P O. This is vanadium bromo peroxidase sub unit. From the biological origin is pilurifera c clustypilurifera.

So, this particular unit when we get that these has some pocket forming affinity for that that one particular pocket is forming and this vanadium when reacting with all these species can cycle from once state to another, and these particular amino acid residues. So, all these amino acid residues whatever we can have these amino acid residues can supply good donor atoms to coordinate to the vanadium site. But, what you have seen earlier those in terms of the corresponding protonation. That means, these particular residues are all corresponding amino acid residues that means, we all will have some

pendent groups available, where we can supply from where we basically give or donate through the system some protons.

So, depending upon the different matching for the pKa values, these protons if they are supplied by the surrounded polypeptide environment or the protein environment they can basically go inside. And tunnel inside to protominate these vanadium oxygen bonds and these vanadium oxygen bonds are basically go for protonation. And for the second level of protonation it can go out and vanadium can accommodate further species that the further new donor atoms like it can interact with these particular peroxide these O2 2 minus. It can react with this O2 2 minus.

So, what we should have there that we can generate some vacancy from there that means, if we can take out these particular oxygen vanadium oxygen bond if we are able to break. That means, in the simple way to do that is that if go for the protonation of this, this oxygen as the vanadium oxygen bond as the anion it initially protonated as hydroxide anion. Then, as water and it goes out and this hydrogen peroxide can enter to this situation and if it can form the direct bond to the vanadium site. It can give a corresponding species related to that of our testing like vanadium peroxo bond can be formed over there or sometimes due to this deprotonation.

If, these protons are available on some nitrogen sub unit NH sub unit and this is delivering to the system as H plus. So, nitrogen will remain there on the amino acid back bond like histidine back bond where, we have the imitactual unit. And that imitactual unit contains the NH function and that imitactual unit when gives goes for the deprotonation. It basically going for a corresponding charge and that charge is basically now well suited to attack to the vanadium site.

So, it can bind to the peroxide unit through the creation of the vacancy or expansion of the coordination number. Or it can go for the coordination from the deprotonated amino acid site chain. It can also go from other carboxy side chain also, so the next day what we will see that how the initial entrapment of the vanadate anion can be modified through the change in the coordination points. And from that how it can bind this peroxide unit or it can go for the activation of the Br minus and ultimately, that is transforming to the bromonium ion. And that bromonium ion can use for the bromination of the indigo type of molecule for useful bromination reaction.