Metals in Biology
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Lecture – 33
Photosynthesis Part II

Hi, are you ready for today's class on Photosystem II? I hope so.

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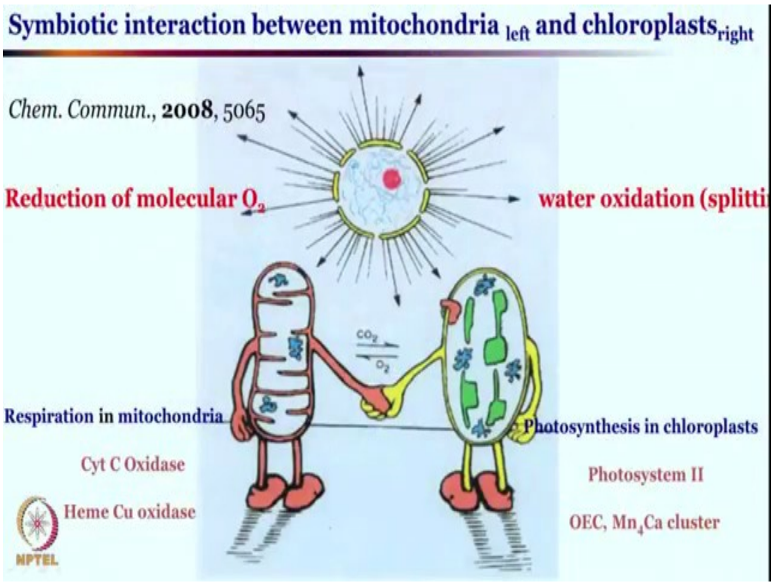

So, let us look at the photosystem II. In the last class we have discussed that reduction of molecular oxygen takes place at heme copper oxidase and that is the cytochrome C oxidase. And, also we were mainly discussing the water oxidation by which 2 molecule of water will come close together, and form the oxygen molecule that is in photosystem 2; oxygen evolving center is involved, and 4 manganese 1 cluster is involved right.

These two reactions are almost microscopic reverse of each other right, oxygen going to water and water coming to oxygen. This is what perhaps one would dream of in their wildest life right.

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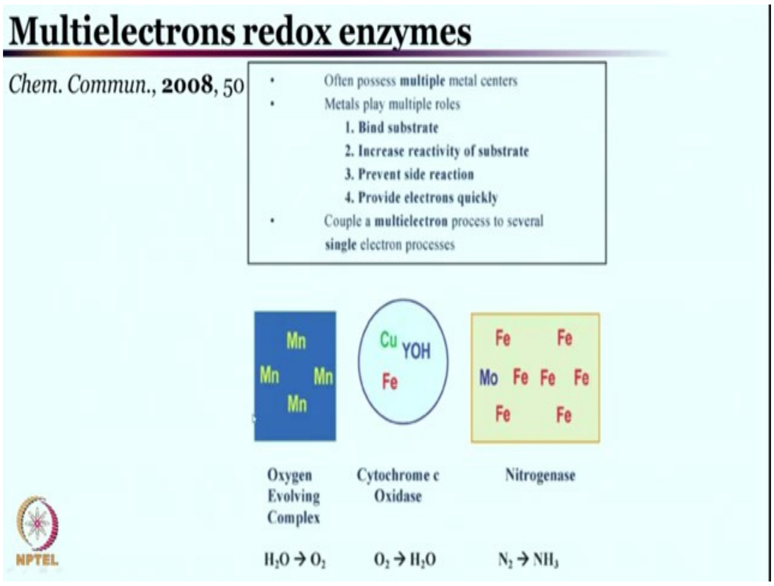

So, this enzyme, which is involved in to water to oxygen is called the Oxygen Evolving Complex: OEC or oh OEC for the center right; 4 manganese center 1 calcium is involved for such transformation. And, we have seen earlier that cytochrome c oxidase can be converted to; converted to water for from oxygen right. So, overall these two are very important enzyme, and they are involved in multi electron processes.

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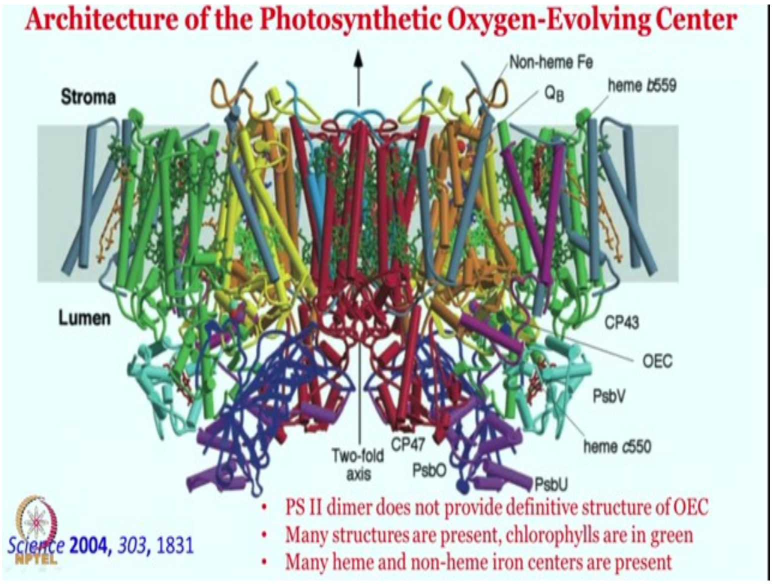

Now, the crystal structure of OEC is very complex as we have discussed in the last class, but major interesting point for us is this OEC oxygen evolving complex or oxygen evolving center, this structure has many problems.

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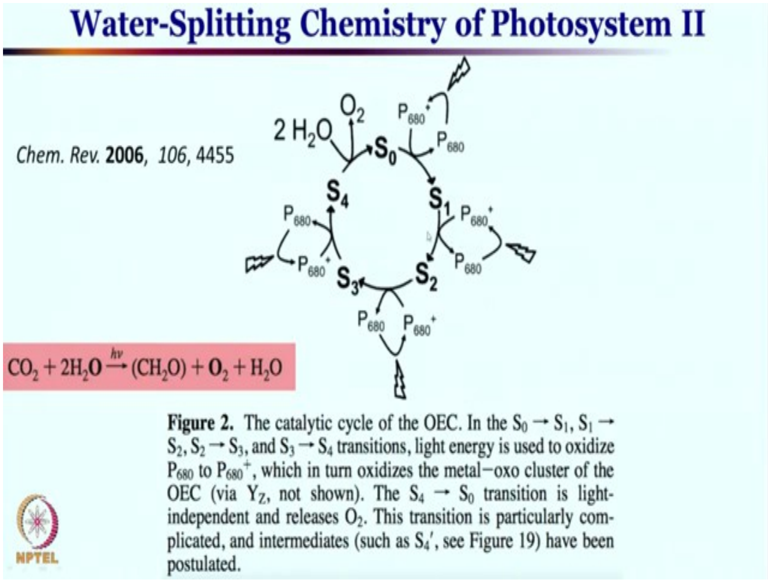

But it can all give rise to a situation, where all these steps are happening very happily perhaps.

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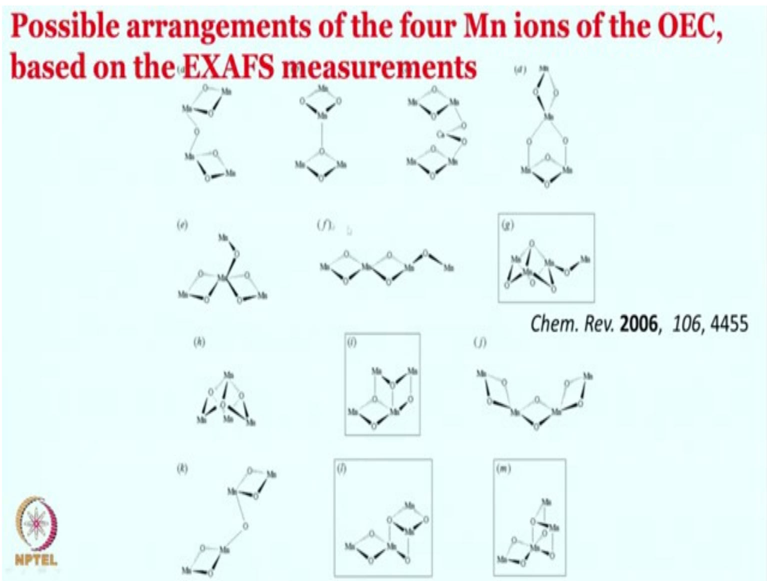

And, the crystal structure does not give the clear evidence or clear indication, what exactly the manganese structure is, but these are many different proposition that exist in the literature. All of them are valid, but it is going to be only 1 center that is involved what that is I think nobody knows sure 100 percent. Now, these are the one or these are the structure which got support from the EXAFS study.

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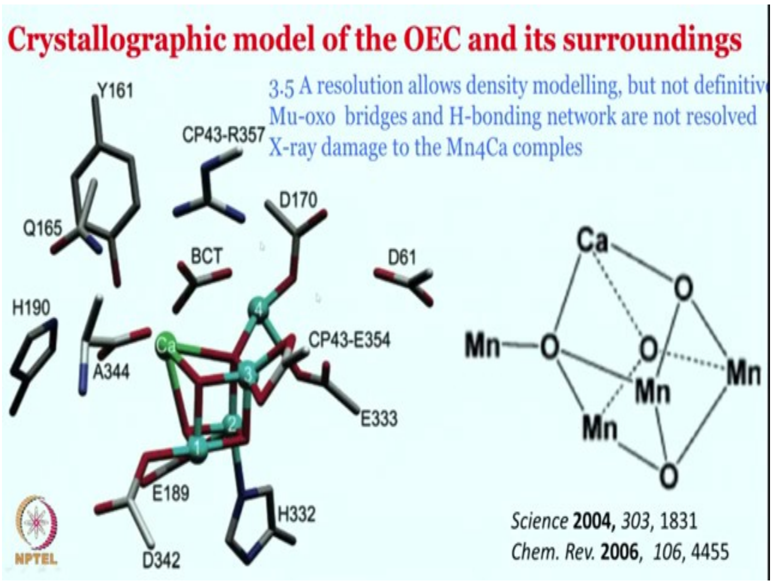

If you look at the crystal structure in that science paper, this is the center where you see the 4 manganese center 1, 2, 3, 4 are situated as is here. Calcium is bound in between, but more importantly these structure people believes that is not the right structure. Because X-ray is damaged; damaging this manganese calcium and manganese oxygen bonds right.

So, nonetheless although, this is not reliable data or reliable structure for 4 manganese 1 calcium, but still people believe that, it would be some combination of these species, that is involved into the oxygen evolving center. Although this structure is quite quite interesting, but we have to keep this in suspense right now.

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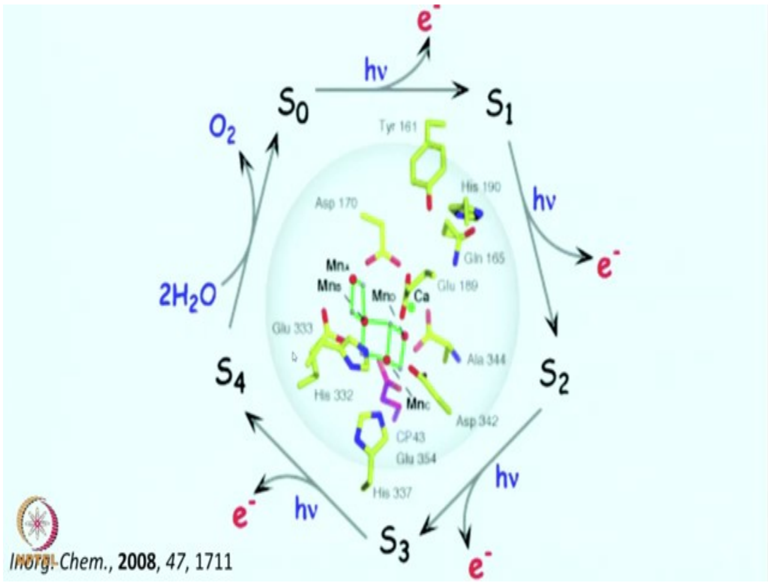

But one thing for sure throughout the literature, what we learn is S0 the fully reduced form will be oxidized to S1, and then to S2, then to 3, and S4 in each step 1 electron 1 proton is involved. And, overall the fully oxidized S 4 oxidation state will be converting water; 2 molecule of water into oxygen. And this is where mainly; we were interested in learning what happens at this S4 so, that water can be converted to oxygen right.

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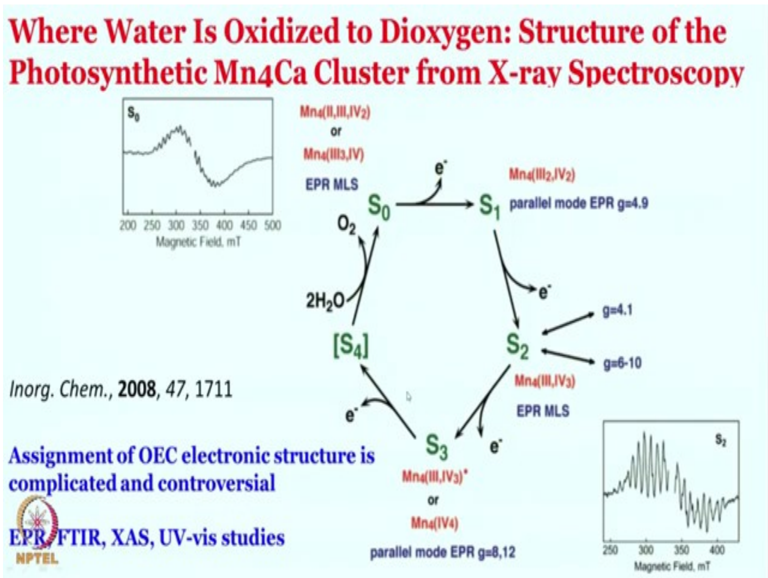

If you look at this S 0 to S 4 overall catalytic cycle, you will see that manganese oxidation states are varying, and these are the two possible assignment for S 0. This is the single possible assignment for S1; this is also the single possible assignment for S2, and then these are the two possible assignment for S3 and subsequently S4 which is the fully oxidized form.

But more importantly 1, 2, 3, 4 these 4 steps are light dependent or photo dependent, the final step is photo independent. Many different studies or many different spectroscopic techniques, such as EPR, FTIR, XAS, UV visible studies are employed for characterizing these intermediate ok. So, today let us try to see, what happens to these intermediate. What are the oxidation state, what is the proposed intermediate for these; for this water to oxygen formation.

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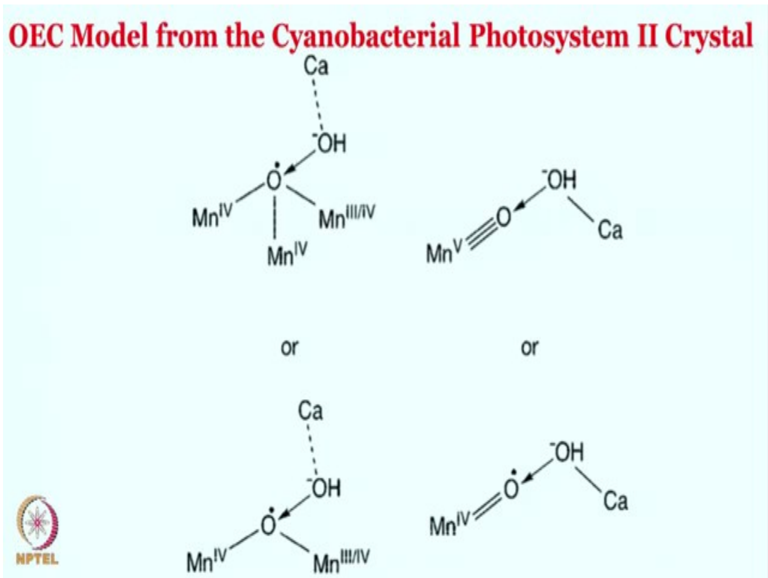

Well, I think one of the key step that we are interested in is the oxygen-oxygen bond formation because, if water has to give oxygen, there would be a step where O-O bond formation take place. Oxygen-oxygen bond formation takes place. And it is believed and with some evidence that this is the calcium hydroxide, which is the nucleophile here and the manganese oxo centers can be acting as the electrophile.

For instance, as you see in the right. So, the calcium hydroxide which is up arising from water molecule and this oxo which is also arising from water molecule. We will see the mechanism of how water is giving rise to oxo in the next slide. So, this calcium hydroxyl can attack on the manganese V oxo bond to form the oxygen-oxygen center or oxygen-

oxygen bond of the dioxygen molecule ok. It could be in this form or it is a radical form as is shown over there manganese IV O dot.

Alternatively, calcium hydroxide can be attacking a bridged oxo species as is shown over here and then, form the oxygen-oxygen bond. This rearrangement can be between the three manganese one oxo or two manganese one oxo. See, the clarity at 100 percent level or for a synthetic chemist level, for a bioinorganic chemistry level are not really there. So, therefore we will discuss the possibilities and we will assume some of the possibilities to be actually happening and then, move on from there. This is the model from the cyanobacterial photosystem II crystal structure; the crystal structure we have seen in the science paper.

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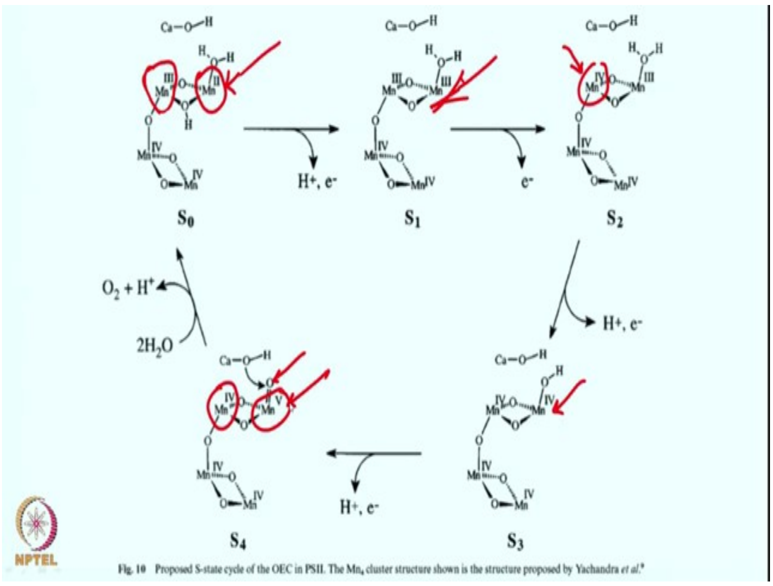

One many different studies are known right; this is one by Yachandra et al. The proposal simply is that part of the four manganese calcium structure remained intact throughout the catalytic cycle. Only two manganese center is varying the oxidation state. And therefore, they are involved into the oxygen-oxygen bond formation process. The part which remained completely constant is shown in the bottom in here, if you look at S0 to S1 to S2, S3 and S4 this part remained constant. So, in all the cases, it is a di manganese IV bis mu oxo intermediate; di manganeseIV bismuoxo intermediate. See, di manganese IV bismuoxo intermediate.

The part which is varying is this di-manganese with a lower oxidation state. To start with it is proposed by a Yachandra et al along with the experimental evidences that it is S0 where, we have a manganese II and manganese III center. And this is the aqua molecule that we are talking about and this is another water molecule aqua molecule which is with calcium. So, the oxygen-oxygen bond will form between these two oxygen center.

This manganese II plus will be oxidized by photon to give you the oxidized manganese III plus a proton and electron release from the center will give rise to the manganese III aqua complex this OH bridging hydroxyl can give rise to the H dot. As is in here overall H plus an electron and then this electron that is getting generated, O dot is getting generated that can be reduced further, by the manganese center to form O2 minus. So, that is going to be the oxo.

So, manganese III, manganese II, oxo hydroxyl species becomes manganese III, manganese III, dioxide intermediate. So, these are also di oxo; the only difference is these are in manganese IV plus oxidation state over here. This is a di-manganese bismuoxo species. So, oxidation state is only varying, but remaining things are the same. At this position or at this point a yet another electron oxidation of this score will happen or will take place where this manganese III will now be oxidized to manganese IV plus. Now, this is a mixed valent center manganese III, manganese IV, bismi oxo intermediate, water remains as it is, calcium hydroxide remains as it is.

Subsequently, from this S2 state as you see S0, S1 and S2 all of them are spectroscopically characterized specifically; if your multi line spectrum will be informative although very complicated. Now, this manganese IV, manganese III center as you can see over here can undergo further, one electron oxidation reaction in presence of light to give the manganese IV hydroxyl center. So, this H dot from this aqua molecule can come out; H+ and electron in the form of H dot or overall can come out to give rise to the HO dot, which can be converted to HO minus by transferring one electron from the manganese site to give manganese IV HO minus. So, this is where, manganese IV HO minus is there.

And therefore, overall you see we started with manganese III, manganese II; then, III III; then, III IV; then, IV IV overall manganese di or bismuoxo species with di manganese center is forming along with that there is a hydroxyl, which is essentially is ready now to form perhaps the oxygen-oxygen bond. Not yet right now it has to be oxidized further. So, it can give rise to again an H dot. H dot will leave Mn IV O dot, which is nothing but oxidized form would be manganese IV is giving one electron to O dot to make it O2 minus; so, oxo 2- and manganese V oxo that is forming. So, this manganese oxygen S double bond can be formed right over here; right.

So, at this stage it is believed that this hydroxyl, which is sitting very close to this manganese oxo now can attack this nucleophile can attack on this nucleo electrophile to form the oxygen-oxygen bond and therefore, overall two water molecule is giving rise to the oxygen and proton and this overall process will convert S4 to S1. So, what you have seen right now is quite fascinating reaction where, these manganese centers are involved into the oxygen-oxygen bond formation reaction.

Not all of the centers apparently are involved in this process; it is the terminal manganese oxo bond formation which is the key and subsequently calcium hydroxide or calcium aqua complex will be giving rise to the desired oxygen-oxygen bond formation to give you the molecular oxygen from water. What a effective reaction it is, you see it is fascinating as I said this core remained completely constant throughout the catalytic cycle. Only this is the core or only this is the center, which is getting getting oxidized manganese II manganese III we have started with in the final oxidized trump; it is becoming manganese IV, manganese V oxo right. So, this is the change, only change that you see is this manganese IV, manganese V oxo and that is happening quite easily right.

So, what you have seen over here is manganese III and manganese II over here. You end up having manganese IV and manganese V center along with the oxo and these oxo is the one, which is responsible for formation of the oxygen-oxygen bond. As you have noticed this manganese II first goes to manganese III. This manganese III remain constant and then, this manganese IV center is happening. So, and finally, and then, another water H atom transfer it gives rise to the change in this manganese center. Finally, that is the again the manganese center which is undergoing.

So, overall in this step if you see, this is the center terminal center, which is responsible for the oxidation almost on at all the step except in one step the second manganese is involved. So, if you are looking at the overall crystals or overall structure or overall chem draw of this proposed mechanism. Then, you will immediately realize that it is this center, which remained completely constant or almost constant except one time oxidation from manganese III to manganese IV, but otherwise, this is the center which is the one responsible for chemistry. So, manganese aqua complex is converted to manganese oxo complex.

Now, I would say that same really fascinating example, what we are overall saying then, is well it is transformation into manganese oxo.

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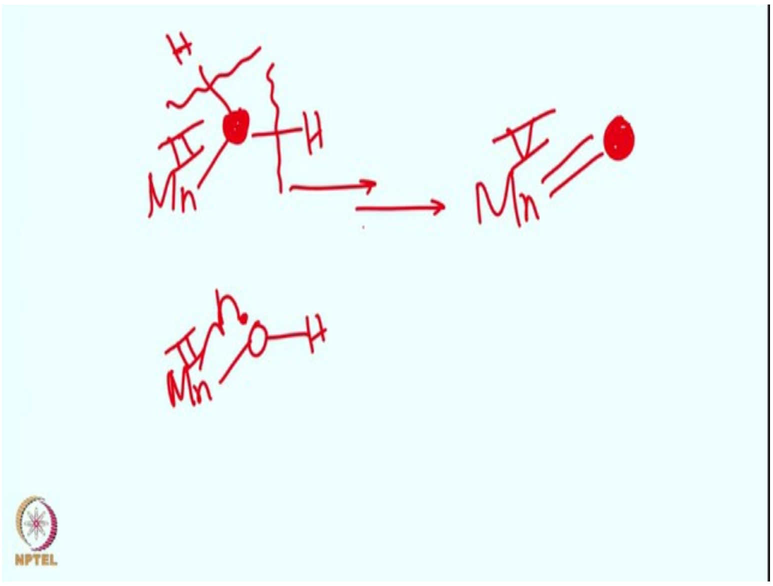

So, we are starting from manganese aqua complex; overall with steps manganese II aqua complex. This is now forming manganese II oxo; well. This is quite a phenomenon right. So, you can break this a OH bond, you can break this OH bond overall you can make it. Let us say manganese oh minus or dot. So, its each step these manganese II let us say was there and then, it can keep on ox getting giving one electron to form a bond or one electron to make it hydroxide overall it will reach to manganese V oxo, when, we are trying to trying to form these manganese II to manganese or V oxo bond formation this proton and electron transfer will be the key transformation in these cases, right.

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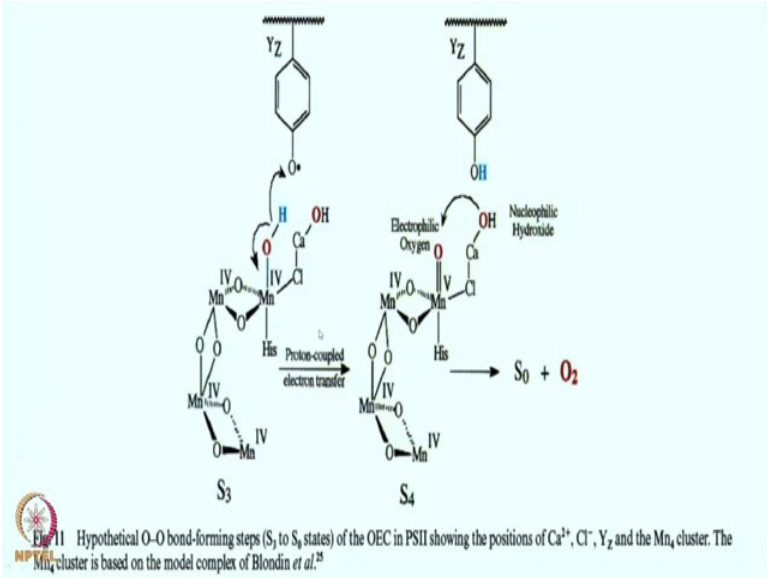

So, let us look at some other proposal that is existing over here. Let us look at in this you know quite interesting another; yet another proposal where, it has been discussed by Blondin et al that this is actually the tyrosine radical, which is from tyrosine phenol unit is involved into the overall manganese oxo species formation; for instance, this manganese IV hydroxyl that is generated into the process.

So, we are looking at manganese manganese IV IV oxidation state with oxo bridge these as I said this remain constant. And the terminal case is over here, this is where manganese hydroxyl is forming and then of course, there are other ligand involved histidine is also involved. This manganese hydroxyl is the focus; this manganese hydroxyl that H dot formation is facilitated by or O dot formation is also is facilitated by these tyrosine radical.

This proposal is I think quite interesting in a sense that this gives rise to a direction from to where, the h dot will be transferred. This phenoxy radical formation is quite similarly quite exciting for the generation of manganese oxo radical. Overall, this process helps you in forming the manganese V oxo intermediate from where, calcium hydroxyl will be able to attack electrophilically on this oxygen atom ok.

And therefore, we will see that the oxygen-oxygen bond forming process will take place to give rise to the dioxygen molecule right. So, that is quite fascinating and we will be keep seeing these mechanistic details or change of the proposal from different groups and all these studies are mainly based on the enzyme. The synthetic studies are also done quite a few, quite a lot, but the problem is without knowing the enzyme structure properly or without having a clear cut manganese IV for calcium structure, I think it is it is getting very difficult to assign what type of chemistry is happening in the enzyme. And therefore, trying to mimic this enzyme is always much more challenging, but once again these has to be understood at a molecular level if the mankind has to understood the biological processes and our very existence; I think these processes at a molecular level should be and must be addressed.

Let us look at some more proposal. So, once again these are many different proposals exist in the literature for which we do not have a crystal clear idea although the crystal structure exists, but the reliability of those crystal data can be questioned, because the X-ray damage of those crystal structure. Now, more importantly these studies are mechanistic proposal are based on some of the experimental of the reason in terms of excerpts, in terms of UV visible, EPR and FTIR studies.

And therefore, one can think perhaps they all are; you know all of these mechanism might have relevance in the case of the ever important the oxygen-oxygen bond formation reactions ok. The fact that even the tyrosine radical is involved or believed to be involved in such process makes it even more exciting ok.

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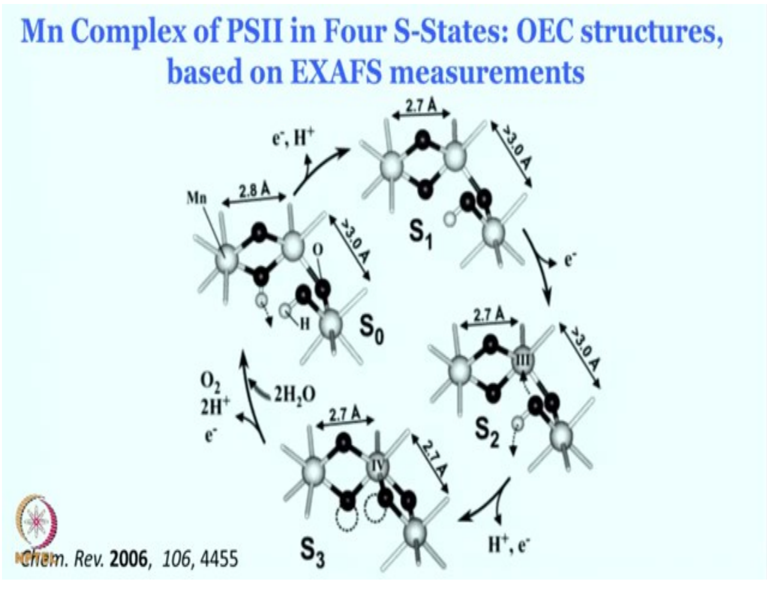

Let us look at a proposal which is based on that EXAFS study, here again we are going to look at S0, S1, S2, S3 and S4 without a detailed characterization. These data are based on or these sort of proposal are based on the EXAFS measurement on manganese complex of photosystem II in 4 S states ok. So, these are the 4 S states that is referred over here.

Now, if you look at the manganese distance between these sites are 2.8 angstrom right. So and the second manganese to third manganese distance is 3.0 angstrom ok. These are part of the cluster we are looking at, part of the four manganese one calcium cluster we are looking at. And that is the site where, water binding, water activation and the oxygen-oxygen bond formation will take place, as we have previously said these score remained somewhat constant throughout this process.

Now, this overall S0 state can then be oxidized or hydrogen atom leaves this center overall to make it a S1 center where, once again 2.8 angstrom distances only changing up to 2.7 angstrom. These accepts measurements are can be quite accurate; the distance between these two manganese center can be very critical and as you see only very slight change in the manganese distance take place.

Subsequently, these second and third manganese distance is turning out to be 3 angstrom, which is remaining constant even after this electron transfer and proton transfer processes. This is quite phenomenal because one should understand that this shot of electron and proton transfer does not make much of the change in the code structure and that is what is observed by the EXAFS study. We can further oxidize this center by one more electron wherein, you see that di magnet center is isolated by 2.7 angstrom and the third center is it separated only by 3 angstrom as you as you see in all other cases.

From this S2 state it is also possible to remove yet another electron as you have seen earlier, but in this case you will see the manganese this center and then, second and the manganese third center distance between them decreased significantly from 3 angstrom to 2.7 angstrom. These are little greater than 3 angstrom or greater than 3 angstrom and then, these are going to be 2.7 angstrom.

As you see now these manganese distance and these manganese distance become equal. So, this is the S3 species from which, the fully oxidized S4 oxidation state will be created and then, water will be converted to the oxygen molecule ok.

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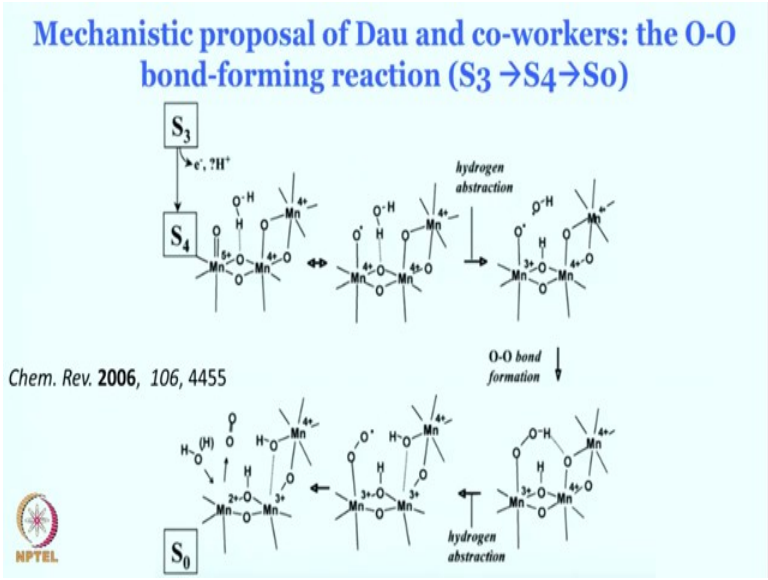

So, in the next slide as you will see that there is other proposal by which people believe that this oxygen oxygen bond formation is taking place. So, the mechanistic proposal by Dau and co workers show shows that a complete or little bit different structure as you can see these we are looking at here. The manganese S4 oxidation state or S4 state, where manganese IV and one calcium structure is there.

Now, they are according to this proposal, this is going to be the reaction between these manganese 5 oxo that the one we were discussing to slide back; manganese V oxo and bridged by this di manganese di oxo intermediate.

This manganese di oxo intermediate actually is participating in activating the OH bond unlike, what we were saying that this is the calcium structure that is involved, but this is the aqua molecule which is getting involved not the calcium hydroxyl. But the aqua molecule is getting involved it in this stage it obstruct hydrogen atom from this water molecule slowly, but steadily deprotonation and electron transfer overall occurs to gives rise to the manganese this di manganese center with an HO dot radical right.

So, this manganese center will first then create a manganese IV and O dot which is nothing, but manganese V oxo no problem in there, but most importantly this aqua molecule is interacting with this bismuoxo species. Now, this bismuoxo species will subsequently give the hydroxyl radical. This hydroxyl radical upon removing one H dot. So, this hydroxyl radical and manganese oxo radical will then combine to each other to form the oxygen-oxygen bond. As you can see, this is a manganese V oxo which is nothing, but manganese IV O dot that is fine, but these H dot transfer will reduce the manganese IV center to manganese III by and then, hydroxy or intermediate.

These electron transfer from H dot from this oh bond to manganese center will make it manganese III plus O dot and this or dot and this O dot and this OH can then combine to give the OOH ok. Which is then can undergo an OH bond cleavage or activation to give the O-O dot; this O-O dot, then can form the pi bond as is shown over here and then, gives rise to the S0 state.

So, overall in this slide, we were looking at the details of the oxygen-oxygen bond formation step. Here, how S4 is forming S0 is considered and it is quite amazing that that step wise mechanism can be suggested so nicely, but without involving the calcium intermediate. Once again these proposals although remain quite exciting all these proposal, but there is a missing consensus in the community that, which is the real you know mechanistic understanding for the oxygen-oxygen bond formation process that once really still need to discover and convey to the scientific community.

So, overall what we have seen so far are little variation of the reaction mechanism, but nonetheless it is the manganese terminal oxo center, which is involved in to the oxygen oxygen bond formation or the bridge manganese oxo species involved in the oxygen bond formation, which is the critical step in the formation of the oxygen molecule from the water molecule right. So, water is forming the oxygen dioxygen molecule with the help of this manganese oxo.

The details of this procedure still remain unclear. These sort of studies or the studies both in the enzyme as well as the deputy studies and in conjunction with the mechanistic studies as well as modeling studies from bioinorganic chemist are quite crucial. In setting light into this highly complex intermediate and I think the study has must be go must be going on to better understanding procedure.

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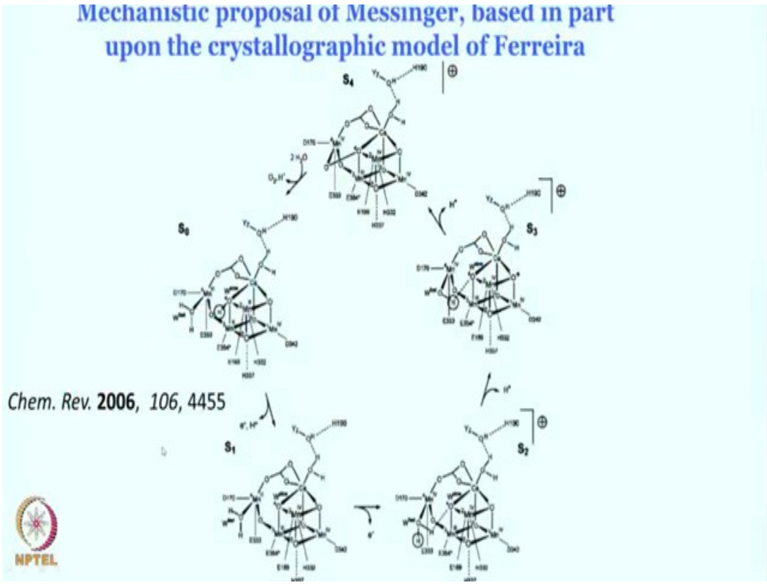

I will not discuss this one; this will be there for you to further divulge into it. So, this is another study based on that crystallographically model of Ferreira that science paper, it is little more complicated let us not get into that. So, let me then, once again summarize by saying that there are many different cluster that is proposed. Still some of them perhaps can be ruled out, but many of them cannot be ruled out and.

So, are the many proposals that are existing in the literature still cannot be ruled out, there exist little bit various and among these proposal, but nonetheless it helps us understand, how perhaps the oxygen oxygen bond is forming in the experimental studies or in research you do not have to understand everything right on day one. Sometimes it takes decades, sometimes centuries to completely understand a mechanistic proposal, some time perhaps it would never be understood because these reactions are so fast understanding each and every intermediate becomes next to impossible.

But efforts are all many synthetic studies, many experimental studies both in enzyme as well and synthetic setup are on the computational studies are the amount effect having are having great contribution in this field to better understand these oxygen oxygen complexes or oxygen evolving complexes and the oxygen oxygen bond formation reaction. So, we will come back with more discussion on these and mainly, we will be next summarizing what we have learned so far in this topic. Thank you very much; see you soon.