**Metals in Biology**
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**Lecture – 32**
**Photosynthesis Part I**

Hello. Welcome to Metals in Biology. Today's topic is Photosynthesis. You will be amazed in knowing how great this reaction could be where we will be converting water into oxygen. Now, overall if you see the all the transformation, perhaps two reaction stands out. Of course, there are many reaction which stands out including the methane to methanol.

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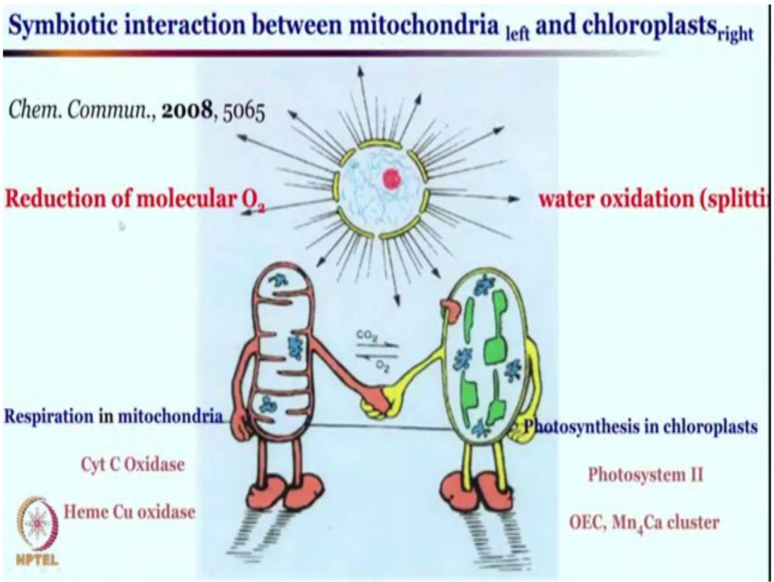


Perhaps two reactions which are complementary with respect to each other and they are very much fascinating and that is oxygen to water formation as well as water to oxygen formation, life comes to a circle I would say. So, reduction of molecular oxygen to give the water and water oxidation to give you the oxygen I think are the two most important processes that is keeping us alive.

So, it is happening this oxygen to water is happening at mitochondria, but where you have seen the cytochrome C oxidase, the heme copper oxidase and the intricate details of it that you have seen how oxygen is converted to water by the heme copper oxidase which is quite fascinating. This is the mitochondria where things are happening the cytochrome C oxidase heme copper oxidase is getting involved in there to convert oxygen into water.

On the other hand, in chloroplast in plants we have this photosynthesis where you have photosystem II the oxygen evolving complex we will see in a moment manganese IV calcium compound or the structure cluster will be involved in the water oxidation. So, water will be converted to oxygen at chloroplast over there. These are complementary reactions and these are right diagonally opposite reaction. These of course, required 4 proton and 4 electron for converting oxygen to water and the reverse will be true for the water oxidation to make the oxygen molecule.

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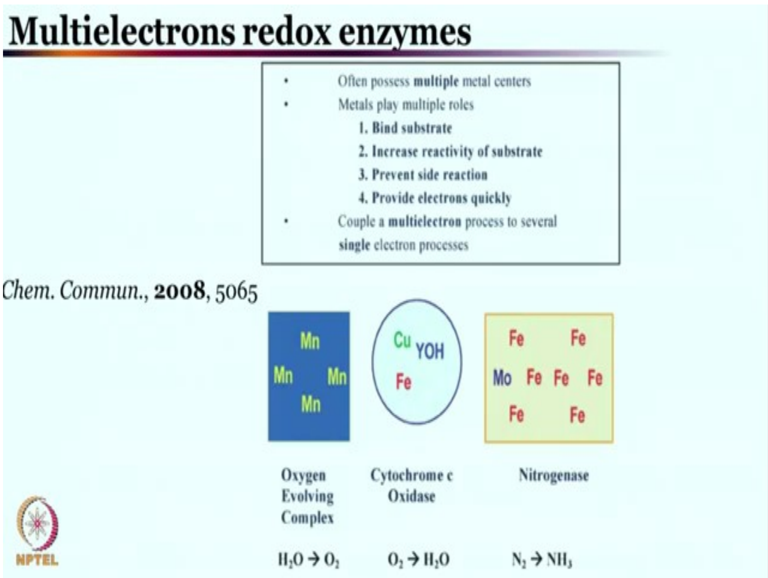


This transformation required both this transformation require multiple metal center. So, that is quite fascinating. You see the metal is everywhere; of course, more than 50 percent of our body weight is metal, but more importantly wherever there is a difficult reaction or difficulty arising nature has always trusted on metal. Metal is the place to go when you have seemingly nowhere to go. Metal makes the reaction feasible. Metal play multiple roles in these enzymes both I mean in these today's case let us say heme copper oxidase and the oxygen evolving complex chloroplast.

Metal plays multiple roles such as it binds with substrate; substrate meaning in these cases let us say oxygen or water. Increase reactivity of substrate of course, increase the reactivity of oxygen or water in this case it would be water activity, water binding and water activity will be improved. Prevent side reaction which is important and essential because these biological processes are completely bulletproof right. These are the reaction which happens no matter what this is one-dimensional, single way, one way, one product formation or the desired product formation only. There is no detour almost no detour nowhere and then almost no other side product formation happening.

Of course, depending on the concentration of the materials things can vary. In few cases that we have seen for example, peroxidase catalyzed cases we have seen, but otherwise it always prevent side reactions. Provides electron quickly that is one of the great thing about the metal center. Metal centers are used very cautiously and very carefully particularly wherever required to provide the electron. It can couple a multi electron process that is also important of course, at a time only one electron transfer occur, but it can provide multi electron process to several single electrons poses. It can have multi electron transfer at a time or several one electron transfer at a time or at times.

So, you will see in a moment that this OEC oxygen evolving complex will have four manganese center. You have already seen in the previous classes where cytochrome C oxidase you have iron copper site along with a phenol or tyrosine cross linking over there. So, this manganese four manganese calcium one calcium structure we will see today for the oxygen evolving complex or for the photosynthesis where water is converted to oxygen. The diagonally opposite exactly opposite cytochrome C oxidase confirms transformation of oxygen to water.

Of course, another very important enzyme is this nitrogenase which can convert nitrogen to ammonia. These are fascinating enzyme. These are fascinating time, fascinating transformations. We will come back to that a moment. Let us look at the crystal structure of the photosynthetic oxygen evolving center or oxygen evolving complex.

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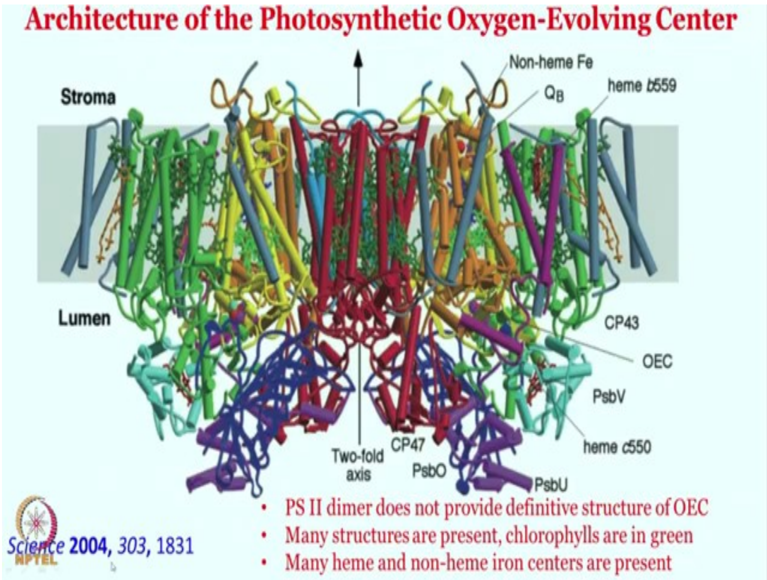


This is back in 2004; this is the crystal structure that you see is reported. Many structures are present as you can see non heme iron center, heme center and cytochrome center many different centers are present many heme and many non heme centers are present.

Many structures are present like this sort of different structure is as you see the one in the green here is the one which is the photosystem II and this is a great structure for the photosystem II. As you see this is the oxygen evolving complex or oxygen evolving center this is the chloroplast structure in green over there. Of course, this photosystem II dimer structure is existing, but it does not still provide the definitive structure of OAC.

So, what happens here is this crystal structure which is phenomenal and highly informative, but still it cannot give you the resolution at a level what a inorganic chemist would like to have perhaps right. This crystal structure are fascinating many different iron center heme iron, non-heme iron center, many different metal centers are present despite the presence of these many different centers we see that still it cannot gives the give the clear idea about what is happening at these metal centers and what is the metal what are the exact association of these metal centers ok.

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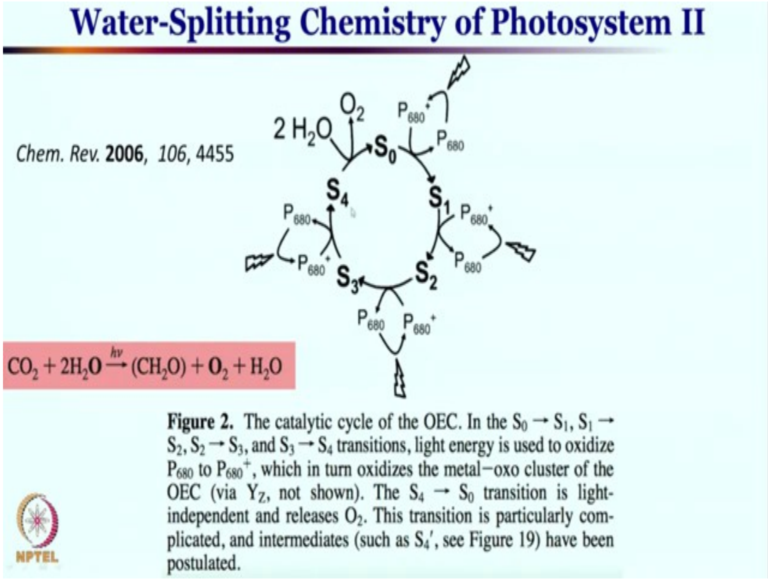


So, we will see this OEC oxygen evolving complex over here oh in a moment we will be coming back, but what has been known or what has been understood so far is these four manganese one calcium structure which will start at S0. We will come back to that in moment also. It is photosensitized to give you S1, then subsequently S1 can form S2 um. Once again it these are the subsequent redox reaction each of the step involve one electron processes the catalytic cycle of OAC is right over here. It is going through S0 to S1, S1 to S2 and S2 to S3 and S3 to S4. So, all these steps are dependent on light and it is it is in turn oxidizing metal oxo clustered. In each step metal is getting oxidized further ok.

Finally at S3, S4 the catalyst or four manganese center one calcium center is getting ready to react with water to give you the oxygen molecule the S4 to S0; that means, the final step where water is converted to oxygen is light independent. This step is light independent and religious oxygen. This overall transformation is very complicated and intermediates all have been postulated not direct evidence I mean some evidences are there that we will see in a moment, but these are not one hundred percent clean and clear informations.

But, most importantly as you know by doing or by converting water into oxygen this whole process is also coupled with the carbon dioxide to form the glucose which is the pretty important molecule for our for our existence right. Water to o oxygen conversion is also coupled with carbon dioxide that is I think you are perhaps more familiar with carbon dioxide to the glucose formation. These whole process are coupled and we will see stepwise what perhaps is happening over there.

I think one thing for sure unlike many other enzyme that we have seen in this class, this enzyme is perhaps the most complex and very little understood. It is not at least I mean in some it is understood well, but it is not up to the mark what we would like to have because the thing is these are very sensitive metal cluster that is involved that we will see in a moment. There are many proposition that what would be the relative disposition of this different manganese center, but no direct or no clear indication exist till that ok.

There are proposals their argument in favor or disfavor in one of the structure, but, but nailing down one structure had still been inconclusive I would say still lot of studies are going on these are fascinating structure both in terms of the enzyme study as well as the as well as the synthetic studies has been done quite a lot.

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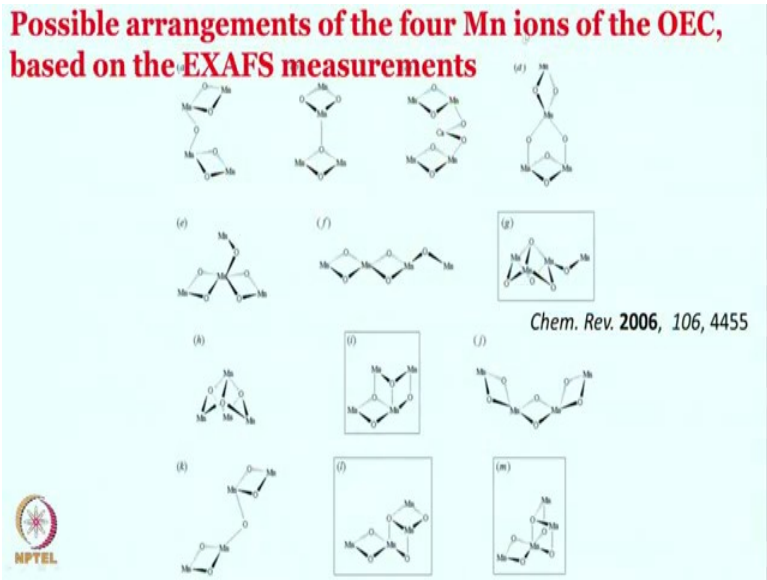


So, the possible arrangement of the four manganese and that we are talking are many and some of them are soul over here as you can see many of them are having the butterfly like structure and these are the structure in the box which are getting support from the EXAFS measurement. So, still from the EXAFS also many structure are proposed it is not really one definitive structure or one clear structure that can be proposed.

But, for you know the course purpose or keeping it simple we will slowly evolve into one structure and try to say that let us try to follow that rather than trying to follow many structure. Let us keep one in mind knowing very well that that structure may or may not be the correct one because there are many structure as you see the different permutation and combination is possible between the four manganese and one calcium orientation and at least if you are just focusing between four manganese 1 2 3 4 manganese, you can have all these different structure orientation bridged between by the oxide right.

So, manganese and calcium also comes into the picture how they are bridged, how they are helping that can be debated. It is still debated no clear solution a once again exist for this beautiful enzyme and most effective one of the most effective enzyme that is converting your water into oxygen right. That is fantastic. We will try to see what is understood, what is evolving and how we perhaps will be able to understand it better in future.

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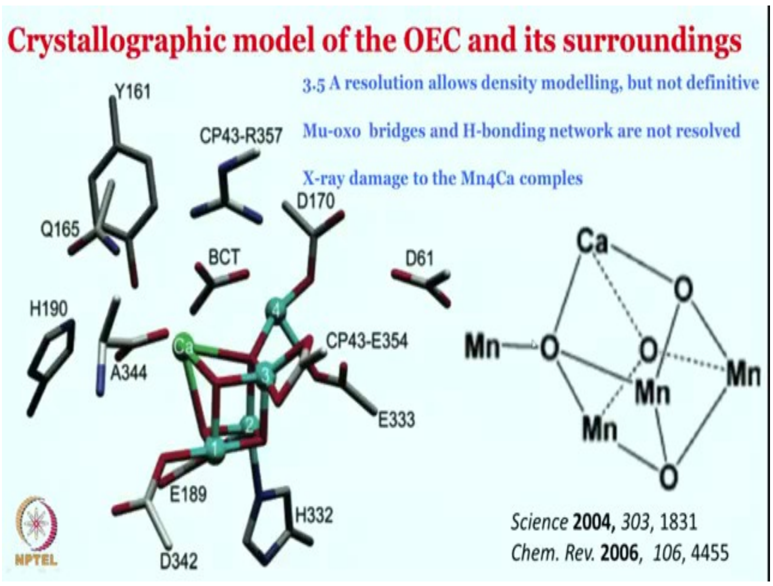


So, let us get back to that crystal structure that OEC crystal structure, three slides back we have seen this is the crystal structure. If you zoom down over here further if you zoom down in this region you see that these are 1 2 3 4 these are the four manganese clusters that we were talking about this is the calcium over there. So, what is the debate about?

Well, although this is looking like a definitive structure people do not believe that that is the original structure. What has happened during the crystallographic characterization or crystal data collection people believe that this cluster is broken and rearranged so or I mean you know this is not actually is the real structure. So, X-ray damage to the manganese four calciums complexes has happened already. Although this is a decent 3.5 resolution structure which allows the density modeling, but it is still not definitive ok.

The muoxo bridges that is present over there and the hydrogen bonding network are not really well resolved and this remains one of the most debated structure most debated most debated active site so far. So, the structure perhaps that we can move along with is this one 1 2 3 4, these are the four manganese bridged by these four oxo and also it is shared by the calcium. These are the two references you can study which are which are quite informative.

In any case, but still the debate remain what would be these four manganese doing and of course, their role is little understand. But, how they are relatively position you see these as if like these our di-manganese, dioxo structure they there are different way you can look at this structure this is perhaps the one if you want to remember something perhaps this is the one from the crystallographic characterization.

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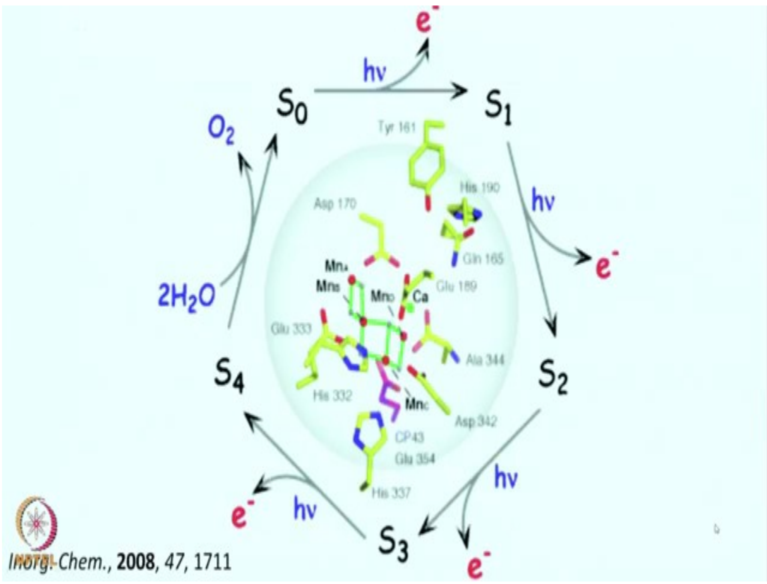


Let us look at a little bit more into that electronic properties of these intermediate. So, you start with S0. Of course, all these intermediates are having four manganese one calcium structure. So, from the S0 which is the fully reduced form it is getting oxidized at each step each of these four steps are dependent on light, but the final step is not dependent on the light that is converting S4 into S0. This light dependent step oxidize the manganese core to give you a S1 intermediate which is oxidized by one electron at every step one electron oxidation is happening.

We just do not know or for sure what was the manganese four different manganese oxidation states are, but none the way nonetheless these will be oxidized by one electron. These will be also oxidized by one electron S1 to S2, S2 to S3 another electron goes out in presence of light S3 to S4, another electron goes out. This is the fully oxidized form; this is the fully reduced form. The fully oxidized from now will react with water to give you oxygen and reforming the fully reduced from S0 and that is how the catalytic cycle would go like right.

You have seen the cluster what perhaps it could be talk over here although knowing very well that is not the right representation of the structure that one would like to have.

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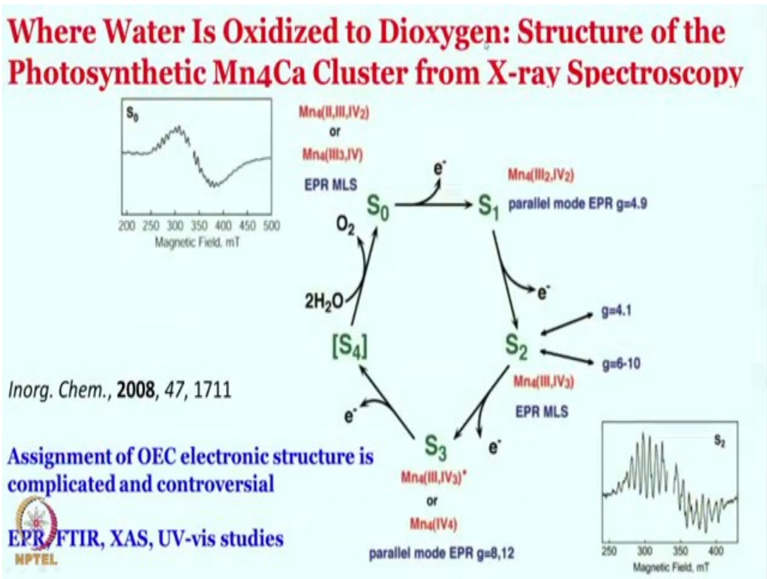


So, let us look at the same thing once again. We are trying to understand where water is oxidized to oxygen and the structure of the photosynthetic four manganese one calcium cluster from X-ray spectroscopy that has been done and then what are the caveats remain. This is a fantastic paper to read if you are trying to understand, but let me tell you that many things remained unclear. These are some of the proposition, still many evidences are required to nail down it further.

So, the S0 state as you can see over here these are the proposed structure or proposed oxidation state assignment by the EPR multiline spectrum, but. It fits with both one manganese being II; one is III and another being plus IV or another two being plus IV. II plus II plus III and plus IV, two of them is proposed or alternatively it could be three of them is in plus III oxidation state and one of them in plus IV oxidation state this is the fully reduced form.

From there on one electron oxidation occurs and this is quite definitive and that is that the upon oxidation of one of these two intermediate perhaps one of them these two are correct which have spectroscopic evidence. We will see that it would be manganese III, two of the manganese will be in plus III oxidation state and two of the manganese will be in plus IV oxidation state ok. So, this gives the EPR and EPR very characteristic for these species.

Further oxidation of this intermediate gives rise to a situation where once again one of the manganese remain in plus III oxidation state another manganese which was in plus III oxidation state now oxidized to IV plus IV oxidation state. So, three of them three of the manganese is in plus IV oxidation state, one of the manganese is in plus oxidation state. Once again this will give rise to the multiple EPR, multiple line spectrum with two different g values, one for the manganese III plus and another for manganese IV plus.

These are these are quite significant understanding what we have from S2 to S3 then it is further oxidation; that means, you know we have a all manganese IV plus oxidation state or a radical intermediate where manganese III and manganese one manganese III plus and three of the manganese in plus IV state as well as a radical formation ok. So, these are the perspect EPR spectra study.

Now, now the assignment of oxygen evolving complex or center electronic structure is complicated and controversial ok. Let me get to that once again that these are putative assignment perhaps some of them are correct, but definitely not all of them are correct. And, there are open discussions on this there are open debate on this still ongoing in the literature. These different structure or the different oxidation state assignment was possible by diff various EPR studies. These are complex EPR studies, one has to be really expert in EPR to study and conclude or try to propose some of the valid oxidation state from this EPR studies. FTIR studies, XAS – XAS studies and UV-visible studies were conducted for these to gives rise to the original proposal that we have seen over here.

So, finally, once you have this S3 state one more electron transfer one more electron oxidation and subsequent water to oxygen formation is going on. We will we will see these in a chem draw format real sure ok. So, what we have seen so far then that is saying that perhaps there is a manganese V-oxo formation as you see if you just for simplicity pick up only one of them let us say you pick up this one; so, 4 manganese IV plus all of them are IV plus. So, further one electron oxidation has to have at least one electron oxidized to manganese V plus. So, these perhaps will have one manganese V plus other three manganese could be in manganese IV plus.

So, you can you can remember one of these oxidation state in each of them. So, although these two are possibilities these two are possibilities, but S4 will definitely have a manganese V plus.

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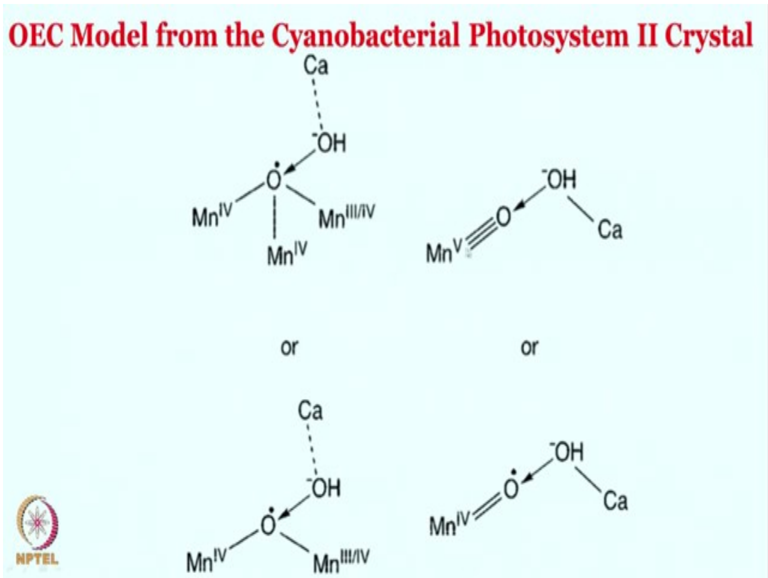


So, that manganese V plus oxo would be would be quite electrophilic in nature and quite naturally calcium hydroxide which is generated from aqua or water molecule can attack on this as a nucleophile is attacking on the electrophile to form to form the oxygen-oxygen bond. So, this is the most critical bond we are looking for because water to oxygen formation would require not the breaking of the bond, but the formation of the bond and this is where perhaps that oxygen-oxygen bond formation is happening.

This is the terminal manganese V oxo species we are talking about. We will come back that to in a moment, but it could be also the bridging oxo the bridge between the three manganese center can also be possibly forming the oxygen-oxygen bond. This are the structure or of course, different orientation would be possible instead of manganese V oxo in this format one can have manganese IV O dot radical where OH will be attacking the oxygen calcium hydroxide is also will be attacking the oxygen in these cases ok.

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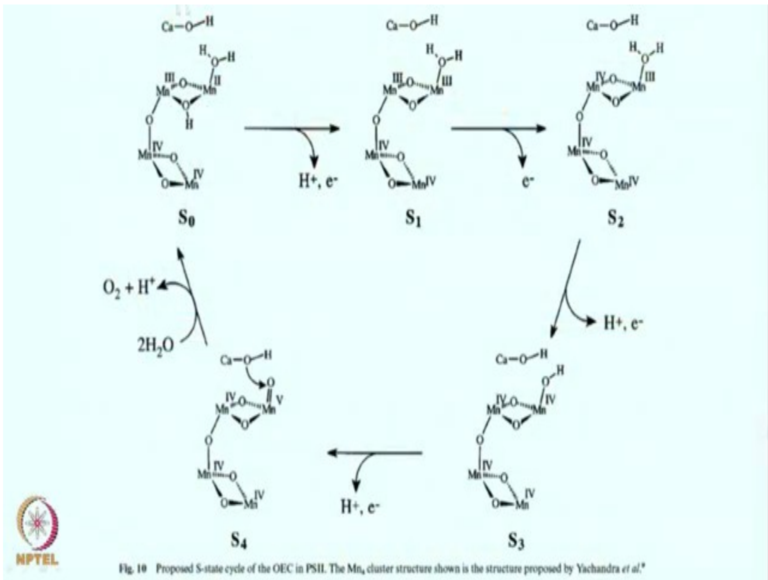


So, this is what we have come to. This is again a simplified one of the cluster we are picking up not every cluster we are talking about. One of the cluster which is proposed one, but it could be very well be wrong, but for simplicity let us try to remember this where we are saying that two of the manganese center are always is in plus IV oxidation state that although we did not say like that over here. Let us say we are ruling out this as a possibility. We are just for you know these are still argumentative we are just for clarity just taking this one 2 manganese is in plus IV oxidation state and another two is in manganese II and manganese III oxidation state.

What we see over here, this center will remain constant, nothing will happen to this structure throughout the catalytic cycle. All this is again a presumption not direct evidence are existing, but it is becoming simpler if we are trying to think like that. Two manganese center is in plus 4 oxidation state bridged by oxo just like bismuoxo intermediate. What you have seen in the last class in case of let us say intermediate Q in the in a in that your methane monooxygenase cases right this was iron IV-iron IV oxo-oxo.

This is now a constant site or the site which is not involved into the oxygen-oxygen bond formation. It is linked with another di-manganese center which is of course, it will be setting the redox potential really correctly. In any case this is bridged by another oxo molecule and from there you have a one manganese II and one manganese III center. It is proposed that in this case you will see one of the water molecule will be bound with the manganese and therefore, this calcium hydroxide which is right over there will be then subsequently interacting in the future cases.

So, in the next class will be discussing the details of these steps, how the step wise mechanism is giving you water to oxidant oxygen. But, it is important to understand that these once again the mechanism are not mechanisms are not really crystal clear. We really need to do much more studies to better understand this OEC or the photosystem II. One thing is for sure these are four-manganese one-calcium cluster and these are going to be the most fascinating and perhaps the longest unsolved enzyme so far where we despite having the crystal structure still we are not sure what is going on.

We will see how the oxygen-oxygen bond formation is happening in the next class. Keep studying photosystem will be back soon.