Circular Dichroism and Mossbauer Spectroscopy for Chemists

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Lecture – 60
Mossbauer Spectroscopy: Mixed Valent Complexes III

Hello and welcome to the final segment of CD and Mossbauer Spectroscopy of Chemist. And over here we are going to discuss today the Applications of Mossbauer Spectroscopy, where we are trying to find out a Mixed Valent state in a Biological Sample. So, let us start that.

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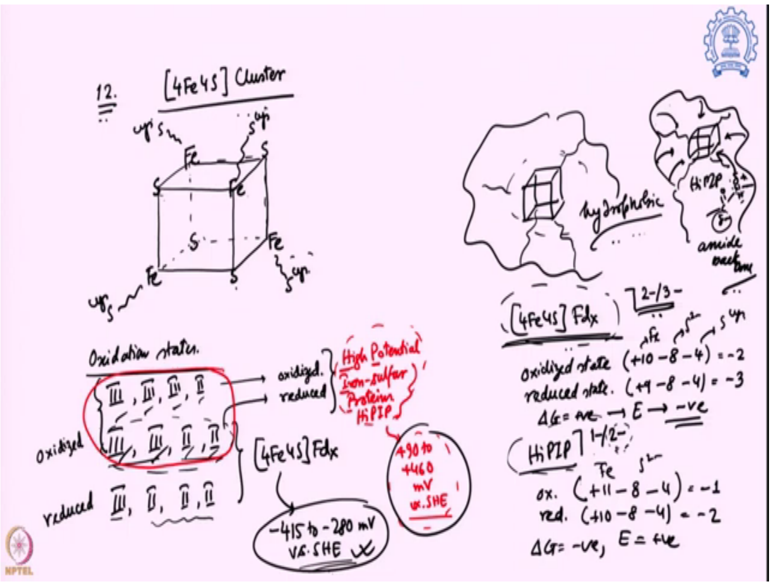

So, this is example number twelve that we are covering and today we are going to talk about a 4 iron 4 sulphur cluster. So, you can start from the beginning one more time. So, we are going to talk about this [4 Fe 4S] cluster today which is having a structure of a cubane and the vertices we are having a Fe and S on the alternate spaces. So that is how the structure looks like a [4 Fe 4S] cluster that is the core of it.

So, four S^{2-} and 4Fe centres and now, iron you can see it is coordinated with three S each of them but Fe need to have four coordination, so that last one is actually coordinated with a cysteine. So that is, what is the overall structure of [4 Fe 4S] cluster. Now, once we have it the question is what is the oxygen state? It actually varies and we found that there are actually different situation possible, two different situation.

So, there are 4 irons so, I am just writing the oxidation state So, one of the possibility is the most common one is two of them in +III, two of them in +II state that is the oxidized form. And the reduced form is one III and three of them in +II state that is the reduced state that is actually happening over here. And this one is known as [4 Fe 4S] Fdx, where we are seeing two of them in +III, two of them in +II the oxidized state.

And one +III and three of them +II is the reduced state. And then there is another possibility that some of the [4 Fe 4S] cluster remain in three of them +III state and one is +II that is the oxidized state. And its corresponding reduced state is two +III and two +II. And the protein which actually follows this particular set of redox system. So, these two it is known as high potential iron sulphur proteins and if I take this letters it is short from it is HiPIP.

So, this HiPIP proteins also use the same template of [4 Fe 4S] cluster but it is using different set of oxidation states. Whereas, the common [4 Fe 4S] cluster they use the different set of oxidation state, this is the common one. So, it is oxidized state for [4 Fe 4S] cluster it is the reduced rate for the HiPIP. So, these are the different oxidized state possible, what is the difference between them?

Difference between them is the oxidation potential, [4 Fe 4S] cluster varies it is potential from –415 to –280 mV vs. SHE that is the potential it looks for. Whereas the [4 Fe 4S] cluster which is forming this high potential ion sulphur proteins (HiPIP) that actually varies from +90 to +460 mV vs. SHE. So, you can see the difference it is more on the positive potential.

So that is why it is known as high potential iron sulphur proteins and this is commonly figuring out a position on the negative side for the typical [4 Fe 4S] clusters, so that is the big difference. Now, question is why there are such difference in the potential? So, [4 Fe 4S] cluster are majorly found in a hydrophobic media. So, this cluster we are saying it is coordinated to a hydrophobic motif.

If it is in a hydrophobic motif, what is the overall change in the charge for this particular molecule? So, if it is a [4 Fe 4S]Fdx protein, let us calculate the overall charge. So, in the oxidized state, you can say it is two III+ and two II+ so, total +8 charge from there. And there are 4 S^{2-} so that will be –8 charge over here and along with that we also have the charge from the cysteines.

So, all of them if we combine together, what is the overall charge we will see in the oxidized state? And what is the charge we are going to see in the reduced state? So that we are going to consider while we are calculating the overall charge of the [4 Fe 4S] cluster. And if we did that for this particular system, we see that reduced rate and oxidized state, we write typically write oxidized state versus reduced it varies from [$[4\,Fe\ 4S]\ Fdx]^{2-/3-}$ charge.

That is how it is actually varying over here and the same thing because over here it is actually two Fe^{III+} and two Fe^{II+} so, total +10 charge and –8 charge for this for S^{2-} and –4 charge for the cysteines this is for the Fe, this for the S^{2-} and this is for the cysteine. So, you can see the overall charge is –2 so, the reduced state obviously is going to be get one further negative so, –3 so,
–2 to –3 is the charge.

So, over here it is going to be more negatively charged as it is getting reduced. And if it is in hydrophobic media, hydrophobic media does not really favour the stability of a negatively recharge system. And if you are going more negative the ΔG is going to be positive because it is unstable and that is going to show in the E value it will be more negative and that is what is shown over here.

Now, what happens for this HiPIP was? In HiPIP what is the oxidized and reduced state? In oxidized state you can say is +3 Fe, three of them and one + 2 Fe so, it is +11, for Fe, –8 charge for the S^{2-} and –4 charge for the four cysteines coordinating the iron centres from the protein. And all those things together you can see what will be the overall charge of this system? And you can see it is a charge of –1 and the reduced it is the (+10, –8, –4) which is –2 charge.

So, it is going from 1– to 2– charge and this HiPIP is also found in a hydrophobic media but over here one important thing is that this background of this protein they have amide bond. And this amide bond has a dipole, $\delta +$ on the NH and $\delta -$ on the carbonyl. And the $\delta +$ is actually situated directly towards the [4 Fe 4S] cluster and a multiple of them.

And those are actually positive charge and stabilizing this negatively charged system –1 versus –2. And over here –2 will be more stabilized because more negative charge and more ionic interaction dipolar and interaction coming from the amide backbone. And that actually brings the stability towards the more reduced state and that is why the ΔG for this one will be negative, the E value to the positive direction and that is what we found over here.

And that is what is happening in the HiPIP and the [4 Fe 4S] cluster. Now, all these things now understood and we kind of know the overall oxidized state now, take a look into the Mossbauer spectra of this particular system.

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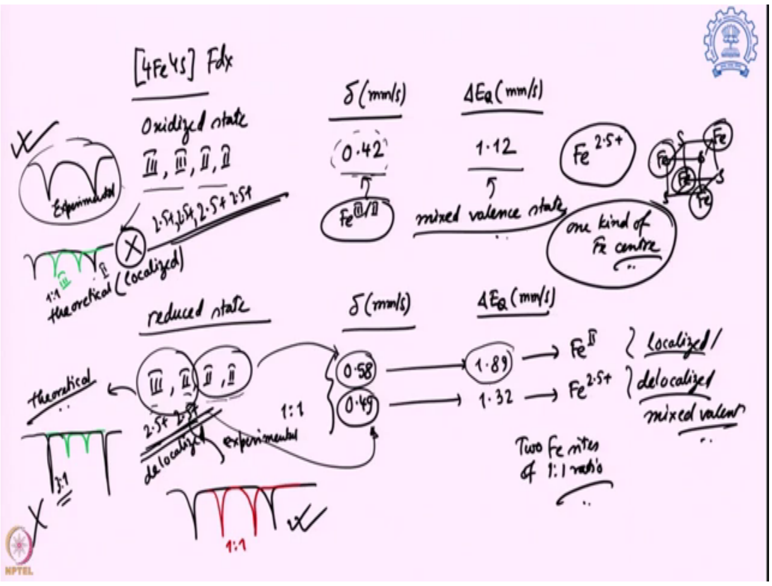

So, first we will try with [4 Fe 4S] cluster, what is actually happening in it is oxidized state? And as we said it is a charge of +III, +III, +II, +II. So now, let’s say what is actually happening in the Mossbauer spectroscopy? What is the isometric shift? What is the quadrupolar splitting? that we are observing over here. So, we found only one set of data over here which is actually one set of data like this.

And over here this is the value ($\delta = 0.42\, mm/s$) of isomer shift and the quadrupolar splitting ΔE_Q is 1.12 mm/s. Now, previously used remembered if it is a +III state for the iron sulphur cluster, the value of isomer shift is around 0.3 and if it is a +II study around 0.6 so, where right now, we are in middle of somewhere. Because what we expect this is the experimental? And what is theoretical if it is remaining +III and +II?

We expect two sets of data one for the +II and one for the +III. So, +III and +II so, two sets of data we expect at the ratio of 1 : 1 but what we are getting only one set of data. And this is the value we are getting (0.42) which is in between iron III and iron II. So, this data suggests that iron actually seeing a state of Fe $^{2.5+}$ for all the iron. So, in the answer for cluster in a state over here in this particular geometry where they are in a cubical form.

The iron centres are actually exchanging electrons through these sulphurs and gaining a mixed valence state. And that is what we are getting from this particular Mossbauer spectra is clearly speculating that. And this is also supported by the EPR study and magnetic study but Mossbauer spectroscopy give us the most cleared data. That yes, it is in a mixed valence state and only one kind of Fe is present over here.

Not two kind of Fe centre that we would get if it is delocalized sorry localized. So, if it is delocalized, we are getting to see only one kind of Fe centre. Now, what happens if we go to the reduced state? So, over there we are going to see +III, +II, +II, +II what is the theoretical thing we are expecting if it is localized? So, it is also the theoretical one previously if it is a localized sample if we would be getting and that is not we got we got this one.

So, over here let us say theoretically what we say? We should see one III+ and three II+ so, III+ we know it is going to show very narrow down signal. And II+ we know a large signal like this and the ratio should be 3:1 that we expect. Now, let us see what are the values we get? And what was their ratio between the different signature? So, we got two peaks one at 0.58 and one at 0.49.

And the 0.58 signal has a quadrupolar splitting of 1.89 and this one (0.49) has a quadrupolar splitting of 1.32 and very interesting the ratio was 1 : 1, it is not 3 is to 1 that we are expecting. So, we get two signals actual experimentally, one is splitted and the other one somewhere in between but it is also similar ratio, so 1 : 1 ratio. Now, the question is why it is so? So now, again we look back to the values.

So, 0.58 and this splitting up to 2mm/s, so that is kind of saying it is a Fe (II) centre. But again 0.49 it is somewhere in between 0.3 and 0.6 that we expect for Fe(III) and Fe(II) respectively. So, this is actually showing a Fe$^{2.5+}$ charge system. So, again over here what happens between these III+ and II+? They become mixed balance and delocalized and form this 2.5+ and 2.5+.

So, at the other Fe (II) remains Fe(II) which is showcase over here, the delocalized one is shown over here. So, we have a mixture of delocalized and localized system over here. But in reality we can say we are having a mixed valence system which is quite obvious from the Mossbauer spectra of the reduced state of the [4Fe 4S]Fdx. So, in the ferredoxin if we look into the experimental what we are seeing?

Is not different sets of signatures with different ratios in the reduced and similar ratio in the oxidized form, what you are actually looking? Only one set of signals in the oxidized state and two set of signals in the reduced state but the ratio is 1 : 1. So, it says only one kind of iron in the oxidized form and two Fe sites of 1 : 1 ratio in the reduced state. And by that we now, know the over oxidized state that is really present on the state of ferredoxin is all $Fe^{2.5+}$, $Fe^{2.5+}$, $Fe^{2.5+}$, for the oxidized state.

And the reduced state is $Fe^{2.5+}$, $Fe^{2.5+}$, and the rest of them is Fe^{2+}, Fe^{2+} that is how it is behaving for ferredoxin.

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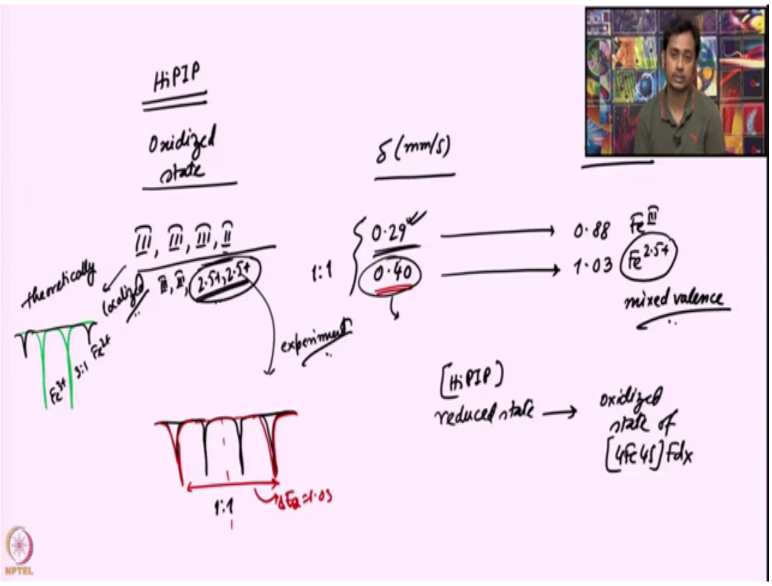

Now, we look the HiPIP which is in its oxidized state. Here three of them are Fe^{3+} and one of them is Fe^{2+} so, what do we expect over here, theoretically? Theoretically you expect that there will be three of them III+ so as know III+ is actually much more narrower splitted so that will be there. And along with that we are going to see one II+ so, it will be largely splitted and the ratio would be 3 : 1.

This is the iron Fe^{3+} and this is the iron Fe^{2+} that we expect. Now, let us see what we actually observed in Mossbauer spectroscopy of this HiPIP oxidized sample. So, over here we again got two signature one at 0.29, one at 0.40, 0.29 was splitted 0.88 mm/s and the 0.41 is a little bit largely splitted. But very interestingly the ratio of the pixel 1:1 so, what we are actually seeing in experiment it is?

Again, not exactly what we are thinking theoretically if it is a localized one. So now, what we are seeing? Is actually closely splitted thing and along with that is the largely splitted thing and the ratio is 1 : 1. And over there we are seeing this largely splitted thing this is the largest splitted thing the ΔE_Q of 1.03 and there middle point the average value of the δ is 0.4.

So, what we are seeing over here? That is you can see again it is failing in between a typical Fe^{3+} why do we see 0.3 which is actually shown over here. So, this is obviously Fe^{3+} system. But this one we are seeing it is neither Fe^{3+} because it is not 0.3, neither and Fe^{2+} which some where it comes around 0.6 it is on mid value in between it is coming because it is again a mixed valence $Fe^{2.5+}$ system.

So, what we are actually seeing over here? We are seeing a ratio of 1 : 1. Because now, over here it is a delocalization happens. So, two of them remain Fe^{3+} , Fe^{3+} and the rest two of them do the quadrupolar splitting such a way and isometric shift such a way that it is actually doing a mixed valence and it is coming $Fe^{2.5+}$ and $Fe^{2.5+}$ and that is what we are seeing over here and it is a mixed valence system over here.

So that means in HiPIP oxidized state we are seeing mixed valence and we are seeing a set of two Fe^{3+} and a set of two $Fe^{2.5+}$ systems 1: 1 ratio all of them. And the reduced state it is going to be exactly the same of the oxidized state of ferredoxin. So, it is reduced state is exactly the same for oxidized state of [4Fe 4S]Fdx. So, we have just discussed that few minutes back.

So, we are not looking back into that this is what is actually going to happen. So, by that you can see Mossbauer spectroscopy can give us very detailed analysis, how it is actually interacting between the different oxidation states of iron in this answer for clusters which are very crucial biological entity for electron transfer? And we know exactly where the electrons are transferring and what is their particular oxidation state?

So, if that would like to conclude over here, the applications of Mossbauer spectroscopy. Thank you, very much.