Circular Dichroism and Mossbauer and Spectroscopy for Chemists
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Lecture – 28
Examples of Circular Dichroism - IV

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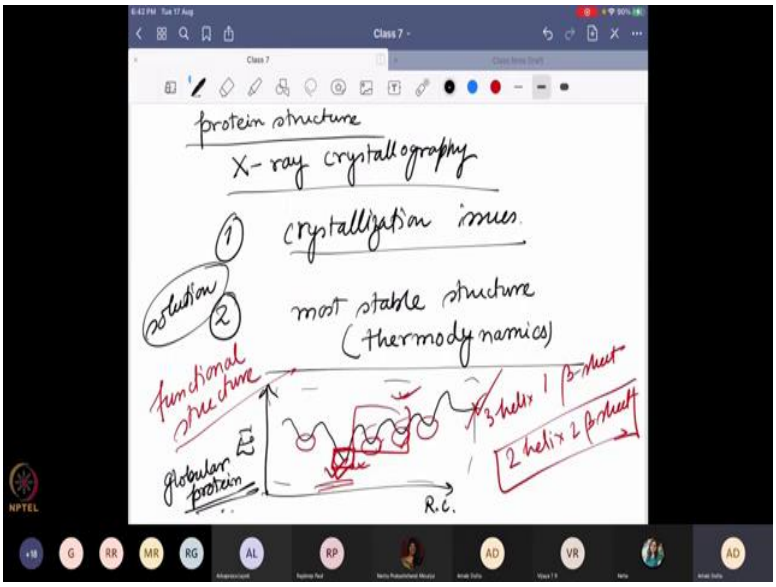

So that is why we need a system which can give me an idea, how the structure behaves in it is solution structure?

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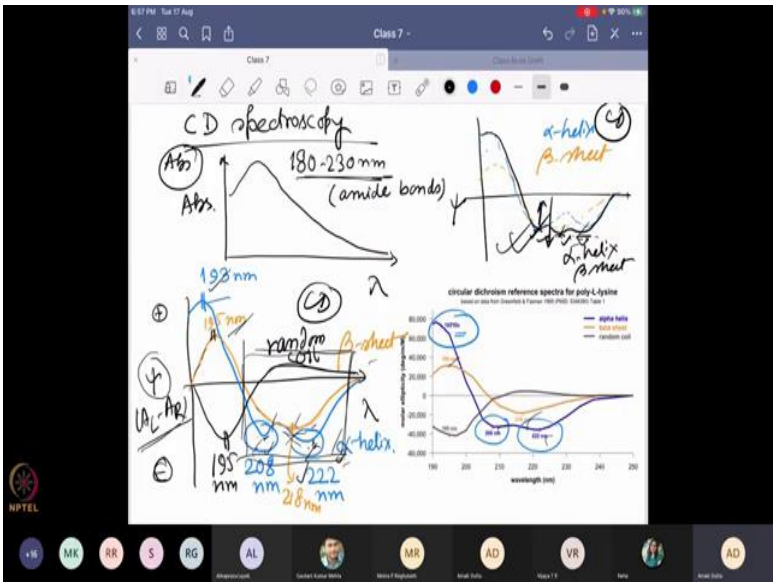

And for that we have CD spectroscopy which can give me a unique idea, how many alpha helix I have? How many beta sheets I have from a solution? How to do that? So, as we just said before, I do a CD I need to do a absorbance spectroscopy first, absorbance versus lambda. And over there say I have a

peak like this and this peak generally looks into 180 to 230 nanometre region.

Every protein have a band over there every protein because this band is coming from the amide bonds. And as you know amide bonds are actually, presented every protein this band will be always there and with respect to that if I now take a look into a CD spectra. How the CD spectra looks like? And here comes, the data how it actually looks like? That is how it does look like? So, let me go through one at a time.

So, first I look into the ellipticity positive, negative do not look into the actual value now look into the trend with respect to the lambda. First, if you have a alpha helix if you have a alpha helix, you are going to have a signal like this. A very characteristic signal same as the blue line over here. So, you have a positive band around 193 nanometre then a negative band it crosses and comes to negative.

And in the negative region you have two maximas one at 208 nanometre, one at 222 nanometre. If you have these three signatures 193 positive, 208 and 222 double negative. You can say I have an alpha helix. For beta sheet how it is coming? So, for beta sheet, you have a positive band around 195 nanometre. Then it goes to negative. However, instead of double negative, you have a very broad peak and the maxima comes around 218 nanometre.

And if you have that you can say I have a beta sheet, one positive 195 and one broad negative at 218 nanometre. And then if you have a random coil that means not really oriented in any particular way. It start with a negative value around 100 lower value and then it crosses and then it remains positive in the region between 210 to 230 previously which was in the negative region for both alpha helix and beta sheet now it becomes positive.

And there is no particular such peak it is mostly slowly going down. The peak only found over here, 195 nanometre but it is now in the negative region. If you have that you can say I have a random coil that means if you take a sample of only alpha helix, you should see a band 193 positive, 208, 222 negative. You say I have alpha helix, beta sheet, characteristic peaks 195 positive, 218 negative random coil like this.

And it is going to give you the data in the solution. Now, you can imagine that in a protein there is a mixture of alpha helix, beta sheet and random coil. right. So, what you are going to get it a mix signal? You are going to get a mix signal in CD. This is absorbance spectra, this is CD spectra I

am also looking to CD spectra now. And what you are actually going to get is probably something like this which shows that you have a mixture of alpha helix and beta sheet.

And you have the particular values and from there, what do you need to do? You just need to do some deconvolution, where you actually try to get the original data and try to match up with some expected value say I have 70% of my alpha helix and say 30% of that is beta sheet. If that matches then I can get this black colour original data. So, from there I can find out how much is my beta sheet? And how much is my alpha helix? You can find out.

And also, you can find out, how much is your random coil? And all those things you can imagine now you can be done computationally. You have to just record the original data and feed the data into a computer and ask them to run a logarithm to find out. How much is alpha helix? How much is beta sheet? And by that you can figure it out, what is the overall structure of a protein in a solution?

And not only that you can also figure it out, what is the change happening in that particular molecule? As you change the pH of the solution, as you change the temperature of the solution and that will be directly affect the alpha helix, beta sheet their orientation and the CD spectra will change accordingly.

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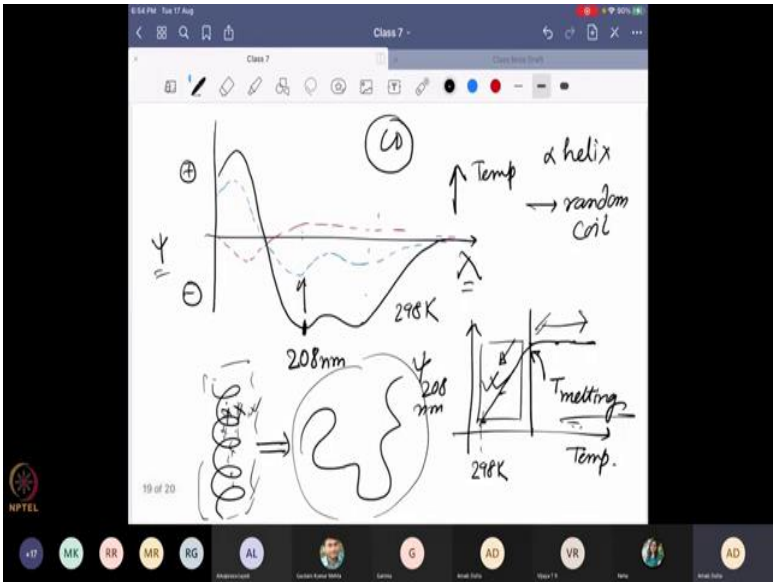

So, for an example say I have a protein structure with only alpha helix and I have a data like this. So, in anywhere in your life, you are drawing a graph make sure that you always draw the axis and write the proper axis like, what are those axis actually mean? So, in that I see this particular data at say 298K then say I change the temperature I am heating it and

slowly the same solution data looks like this then at one point of time it becomes like this.

That means what is actually, happening? As I increase the temperature my alpha helix structure is going to a random coil. So, what does it says? It says my protein structure is actually degrading. And if I can measure this particular data at different temperature and say I am monitoring what is the change happening at 208 nanometre? And plot that with respect to temperature, how my data will look like?

At 218 nanometre I have a very negative value at 298K then I go further temperature high and slowly and slowly it is going up. And at one point time it will remain same because at that point of time you actually already degraded the protein. So, from there this point I will say here the protein is breaking down. And this is known as the melting temperature of a protein, melting temperature means where a particularly nice helical structure with respect to temperature just defaulted.

And protein folding has a huge role to play with this function. So, with respect to CD data, we can very easily find out exactly where does it happen? And if I want to do an experiment at which particular condition I should do where the protein structure is still stable. And after what I can actually, break it down. So, what is the temperature? I should keep my protein. So, all this for example, right now we are looking into different vaccines and some of the vaccines are protein based vaccines.

And over there you can imagine that they always have to keep it at a particular lower temperature. Why? Because if you go to higher temperature, you can break down your protein structure and the vaccine will go bad. So, how to look into that? How to find it out which temperature would be the safest for it? So, you just do a simple CD spectrum find out where you see this melting point.

And you can specifically point out this is the temperature you have to keep to make sure that protein is stabilized. Now, the last part I am going to talk about. "Professor - student Conversion Starts" Hello sir, yes, sir you showed in graph that alpha helix is changed to that upper one graph in a random coil but what about beta sheet? Yeah,it I am just giving an example, it can go to a beta sheet, it can all the possibilities can be there.

Because I am just giving an example that one alpha helix is going to random coil. You can think about it can also happen sometime higher temperature can actually bring you to totally new structure. It can go to alpha helix to a random coil to a

beta sheet. And from the CD spectra you can follow it up like exactly, what is actually happening? It means after hitting beta sheet can be converted into random coil. Yes, it is possible, all possible.

So, random coil is generally the structure at higher temperature because it does not have any hydrogen bonding or anything over there. Because hydrogen bonding is actually stabilizing it right when you hit it you are, giving it enough energy so that you can go and break those bonds. Because entropically over here you are losing some energy but enthalpically you are getting some energy.

So that is why the ΔG is negative to form such structured structure in alpha helix or beta sheet. But in random coil you have huge entropy. So that is the favourable thing but there is no hydrogen bond or anything to enthalpically help it. ΔH is pretty actually non-existence over here. However, at high temperature you are giving enough energy from the outside. So only entropy can take care of the ΔG.

And you have to have enough delta uh enough energy coming from outside so that you can break these bonds and over there ΔH. So, from there you can even find out, what is the hydrogen bonding energy present in the alpha helix or beta sheet? And in some proteins it might happen you have five alpha helix or two beta sheet when you hit it only two of them breaks down not all of them.

And from the CD spectra you can find it out exactly which of them are actually breaking down. okay So, they are all different kinds of possibilities out there the main point is that from the CD spectra you can follow it like, what is the change happening by following the alpha helix beta sheet and random coil signatures? okay Because this particular data I just showed over there their signature no matter, what where you measure?

Alpha helix always will show this particular peak, beta sheet will always show this particular peak. Okay Any more questions? Sir, I did not get about the peak uh that is near 125 positive peak. These are 125 sorry 195 ah 195. So, what do you mean uh? So, what is the question 195? So that is actually positive signal. So that means over here, this is A_L minus A_R right a function of that that means over here A_L minus A_R is a positive number.

So that means your absorbance of the left hand circular polarized light is more than the right hand circular polarized light. And then you, when come to 208, 222 around this region A_L is actually absorbed less compared to the right hand

circular polarized light so that is becomes negative. So that is what we mean by positive and negative that is the direction of the value of the psi(ψ). Thank you, sir. Okay "Professor - student Conversion Ends".

"Professor - student Conversion Starts" Any more question anyone? Yes sir, sir from that value, how can we decide? That that is due that is for the alpha helix or beta sheet means. How we confirm that? uh Means that value but I want to know that how is the characteristics is decided from that value? Which value? That means that uh for the alpha helix it is decided that 193 or we will get that but I will confirm that that is due to the alpha helix only or in the first time we will get that the alpha helix. Yeah,

Mainly people actually look into this negative region to be honest because this 208, 222 there are very unique two humps beta sheet have a very broad peak and random coil is in the positive side. So, people mostly look into this region 200 to 230 region. And over there they you have a result like this and then you try to deconvolute it put different values for alpha helix and beta sheet and see which data by calculation is matching the original data.

And from there you can calculate like, what is the probable? Alpha helix or beta sheet Uh distribution for that particular protein? Does it answer a question? Yes Sir. for example For example, if you take 70% alpha helix and 30% beta sheet versus 50% alpha helix, 50% beta sheet the overall data will not look same Because these three different humps because there are three different humps when both alpha and beta helix are present 208, 218, 222.

And their contributions will vary with respect to the overall presence of the alpha helix, beta sheet. And that is what we actually follow that what is the distribution of these three different humps at 208, 220 218 and 222? And try to match it, how it matches with the actual experiment? And from there we find out how it actually happens? "Professor - student Conversion Ends".