Circular Dichroism and Mossbauer Spectroscopy for Chemists

Prof. Arnab Dutta
Department of Chemistry
Indian Institute of Technology – Bombay

Lecture – 29
Applications of CD Spectroscopy

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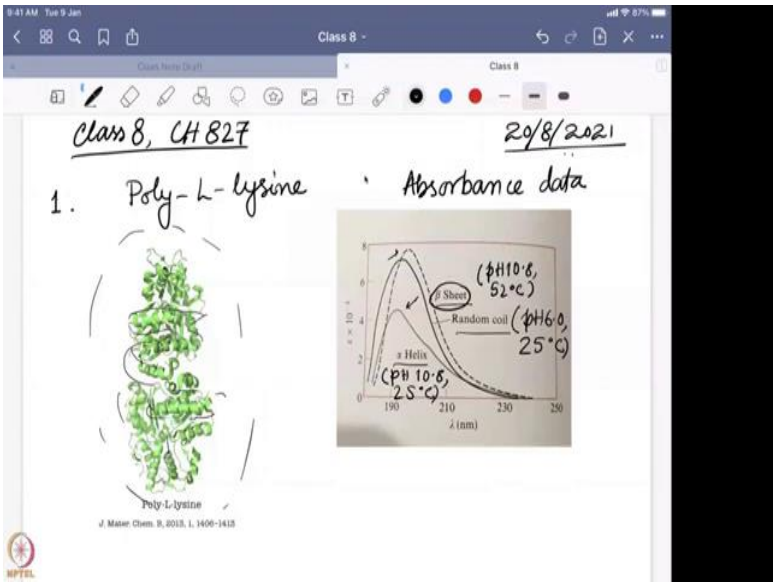

So today, we are going to talk about the some of the examples of CD spectroscopy, as it can help us. So, the first example we took that of poly-L-lysine. So, over there let me first find out what is the structure of this poly-L-lysine? okay So, this is the structure of poly-L-lysine which is actually, nothing but a continuous sequence of lysine all together. So, like hundreds of lysine next to each other.

So, if you try to draw the primary sequence of it, so, you will see it is lysine, lysine, lysine, lysine all these things. And then people try to find out what is the effect of this lysine on it is different conditions of pH and temperature and for that what they did they tried to figure it out how it looks like in the different temperatures and that is the data they got. So, you can see so, there are some difference, so, this is just a simple absorbance data.

This is the absorbance data for this poly-L-lysine drawn over here and what you can see that the bands, although changed a bit but it is not showing a huge difference over there and that is a little bit uh tricky to understand what is actually happening just by absorbance data. And let me tell you what are the difference… (02:12). So, over there as it is shown that we already written it is alpha helix beta sheet and random coil.

That is the data we got from other experiments, not from the direct optical spectroscopy and what are the conditions they have actually, have used. So, for this data which is shown random, coil, the condition was pH 6 & temperature 25°C. And then they changed the pH to 10.8 and this find that it is actually, forming an alpha helical formation. So, you can already see a lot of alpha helix present in this structure a huge number of alphabets.

So, at 25°C at a very high pH of close to 10.8, all the random coil automatically forming alpha helices and then when they keep the pH same but change the temperature to 52°C most of the alpha helices turn to beta sheet. So that is the change they have got. So, in the studying of the structure, it is found that it is not a simple absorption spectroscopic data cannot give you all the information that is required.

So, you need to have some other contemporary data to support what is actually, happening. Simple absorbance data, although they are showing some changes, some shift in the bands and all those things but they are not any characteristic change. right You can follow it that whether it is alpha helix or beta sheet or anything else. So, in that case it is found that the CD spectroscopic data is actually, the most helpful one.

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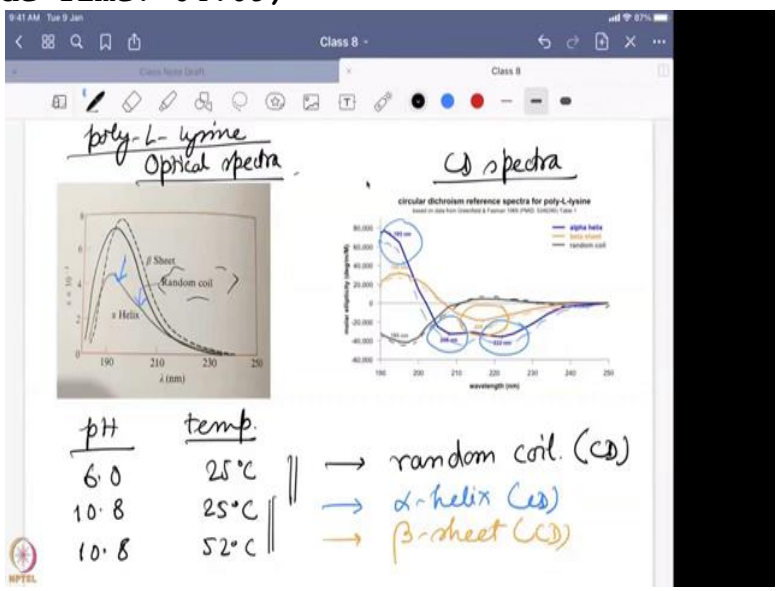

So, let me draw the structure draw this data one more time the optical spectral data and let me show you also how the data looks for the system. So, this data that I have shown you earlier that is from the same poly-L-lysine at this different conditions. So, what it is found that if you have a poly-L-lysine and you put that in different temperatures, as you value the pH and the temperature. What is the thing you are going to get at pH 6 at 25°C?

You find that this is the optical spectra of it and this is the CD spectra when you run that at pH 6 at 25°C. you get this black line over here. So, it clearly suggests that it is actually, nothing but a random coil and it is full heartedly support from the CD spectra. No other things are presents that is the inference you cannot draw just from the optical spectrum on the left hand side.

Then they change the pH to 10.8 keeping the temperature same and what they found that the data is this blue data shown over here with the characteristic positive peak at 193 and two humps in the negative region 208 and 222 nanometre. So that clearly suggests that now, you have an alpha helix furnished and that is also supported by the CD spectra and over here you can see the random coil to beta sheet.

The only change you are seeing some drop in the absorbance with very minimal shift in the actual fix. So that is not a good, conclusive data to support that you have such a change from a random coil to alpha helix. And then, when you go to a higher temperature 52 degree centigrade over there what happens? That you find this is the new band coming up which is shown by this brown colour data.

Now, instead of two humps at 208 and 222, now, you have only one hump at 218, so that suggests that now, you have a beta sheet character. So, all this data is supported by the CD spectra, so, CD spectra is much straightforward data that you can use to find out exactly what is happening? And you can see there is a huge change happening in the solution the structure of the system it is going from alpha helix to beta sheet and random coil.

All these huge changes are happening with respect to pH and temperature and CD spectra can undoubtedly give you the idea like what is actually, happening over there. So, in this case of poly-L-lysine, we are taking this example because it is a clean and clear-cut change. It is going from 100 alpha helix to 100% beta sheet in this region, 100% random quality to 100 percent alpha helix in this particular region.

So, it is not a mixture of anything, so, it is a clear cut change and that is why it is one of the data is always used for an example which actually, shows a clear-cut change in the overall structure. So, this is one of the example we like to follow and it also emphasize how important CD spectroscopy can be to understand what is the overall structure of your protein structure.

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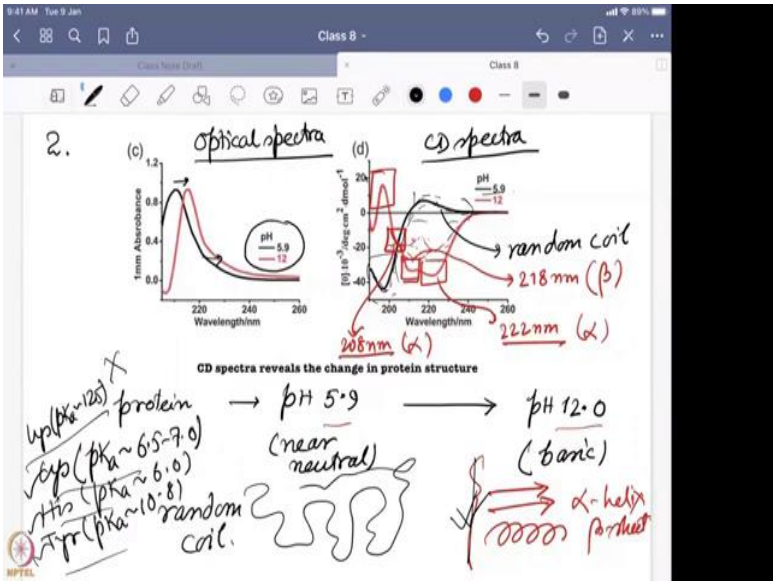

After that we take an another example over here I am going to again through a another spectral and CD spectral data, optical spectrum cd spectral data and show how the changes are happening over here. So, it is actually, also for two different uh one particular different protein absorbed uh in two different phs. So, there the pH is given over there pH 5.9 and pH 12. So, you can see whatever the protein is the protein structure we have.

We take that at pH 5.9, you can say it is close to near neutral condition that is close to pH 7 and then we actually, make the solution alkaline and we are going to basic condition. And now, we go to pH 12, pH 12 is quite a basic condition. So, you can say it is basic condition. Now, the question is if I change this ph, what is the effect is happening on the protein structure? And over there you can see a simple optical spectrum comparison.

It is showing that yes, there is some changes you can see some shift over here some change over here but it is not a clear cut change. It is not conclusively saying that this is the change what you are seeing. On the other hand, if you look into the CD spectrum. What the change you are seeing? So now, take a look at pH 5.9 data pH 5.9 data you can see a negative band over here and a small positive hump around 210.

So, what that data suggests that this black coloured line the black trace over here it is suggesting that at pH 5.9, it is actually remaining as a random coil. So, you can see there is no particular bonding in this particular protein at pH 5.9 so, it is actually a random coil. And not only that it is also showing how much of it is actually, random coil? You can see it is 100%. There is no hump on the positive side, no harm in the negative side.

Over here no positive hump on this side. So, it can clearly says that you are seeing 100% random coil. So, in the near neutral condition. This protein is such that it is assuming no particular structure it is randomly oriented it is a linear random coil. However, as you go to basic condition, you get this particular red colour spectrum and whatever you are seeing over here, a positive band over here.

You can see a hump over here, a hump over here and another hump over here and if you combine all those things what you can say. So, this hump over here, it is coming at 208 nanometre. On the other hand, this one is at 222 nanometre. And this one in the middle it is at 218 nanometre. So, all together what we can say. So, it is not a simple alpha helix of beta sheet but a mixture of it.

Because we have both the humps for alpha helix is here and also the hump for the beta sheet. So, you can say that over here, when we actually, put this at pH 12 condition, it is actually showing me a mixture of alpha helix and beta sheet. And then, as we are discussing last time that we can actually convolute that that how much of it is actually alpha helix and how much of it is actually beta sheet?

We can actually, do a calculation and fit that data to find out how much it is alpha helix? How much it beta sheet? So, over here you can see the alpha helix is a little bit higher in concentration because that is creating the background of it. So, probably 60%, 70% is alpha helix and 30% is beta sheet. And in the calculation, you can also include some of the random coils to see if it is uh participating in the overall structure, you might find a little bit of it.

But overall, the CD spectra is clearly saying what is the start change it is happening over here? You are going from a directly a random coil structure to a very much well structured, alpha helices and beta sheet, as you are going to a basic condition. Now, the question might come that why we are seeing such alpha helix and beta sheet in the pH 12 which is not happening in neutral condition?

So, you can imagine that what happens? What are the changes are going to happen if I go to a basic condition, so, there are two important parameters one is the amide backbone. So, there is no particular change is going to happen in the amide backbone with respect to the pH in this particular region 6 to 12. However, the side chains, the side chain residues, might have a huge role to play and over there which are the group which actually, can be affected in this region, pH 6 to pH 12.

So, over there those groups will be affected which has a protein group or a proton exchanging group that has a pka in that region. So, what are those groups you can have cysteine over there which has a pka around 6.5 to 7? This is the range because it also depends on the overall environment if it is more hydrophilic or hydrophobic, depending on that and the cysteine has a thiol chain which actually, can have this particular pka.

You can also have the histidine group which can have a pka around 6. The metazone group you can have the tyrosine group which has a phenolic OH group which can have a pH around 10.8. And you can also have lysine's which has a decay around 12.5. So, if you go too close to 12 that means you have started affecting it. So, all these things can come together and play a huge role over there.

So now, you just take a look into the primary sequence of the protein and then you can actually predict what are the changes might be happening which is triggering such a change from a totally random coil to alpha helix? Now, imagine a random coil means there is no particular structure and then, in the basic condition it is getting structure. So, it is creating some more interaction.

So, you can imagine that a tyrosine group which is actually, protonated in the neutral condition and it will be deprotonated into basic condition that might play a role that once it is deprotonated, it is probably participating in certain kinds of salt breaches like a ionic bond with an another cation and that is probably helpful. The lysine should be positively charged in the neutral condition close to 12 it should be neutral.

So, it is probably breaking down some of the bonding. So that might not be the case because in the well-structured system you need more well-structured bond and a positively charged lysine effects better than the neutrons. So, probably lysine is probably not the cysteine which is affecting this chain. Cysteine can, histidine can, tyrosine can so, by that slowly looking into one and each of the possibilities you can even sort it out which are the amino acids are probably playing a huge role into this particular change.

So that is how you can use CD spectroscopy to find out what are the changes are happening into the overall structure of the protein? And how it is connected to the change in the structure? With respect to the different parameters pH the temperature, all those things can come into play over here, so that is one of the examples I would like to show.

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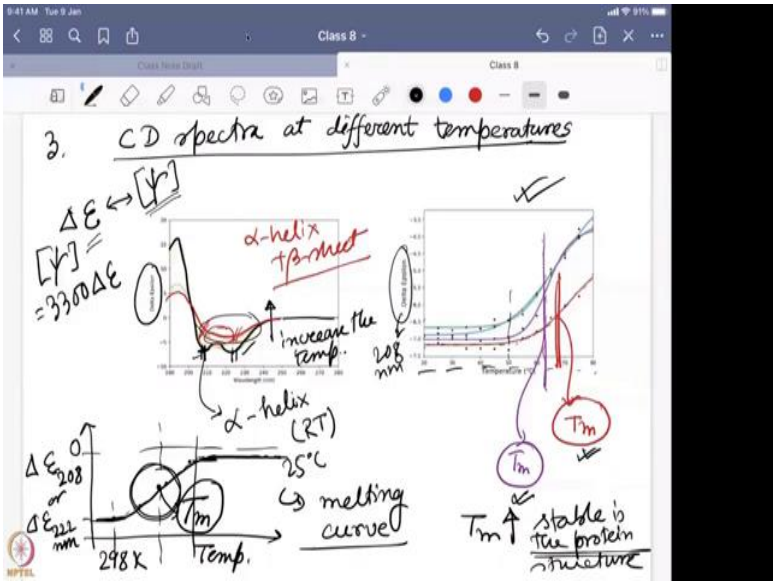

Then comes the other example. So, let me find out the figure first. So, we have discussed that we can actually, look into the stability of a protein at different temperatures and if the protein is destabilizing, we can clearly see that with respect to the effect of our with respect to the CD spectroscopy. So that we would like to find it out. So, over here, what you are actually, trying to look, we are looking in, so, what we are actually, doing.

We are taking a protein structure which is starting from this green line over here which is shown over here this green line. It is actually, the first spectra we recorded at room temperature and over here you can see it is very nicely showing that it has a very clear cut alpha helical structure at room temperature,25°C. And then slowly you started increase the temperature. So that we are actually, doing it over here.

So, we started increase the temperature and what we are actually, seeing over here. You can see the data is slowly losing it is alpha helical structure and going mostly to a mixture of alpha helix and beta sheet over here. So, it is not clear cut alpha helical humps you can see anymore. What you are actually, seeing at the end? Let me go in the red line. What you are seeing over here? It is kind of a mixture of beta sheet and alcoholics.

You still have some of the signatures over here and here it shows that it is not 100% leadership but beta sheet is right now actually, uh the major component of the overall protein structure. So, as we increase the temperature, it is going to a mixture of an alpha helix and beta sheet and again you can do the calculation with respect to the computational calculations. And you can find out how much is beta sheet? How much is alpha helix?

And you can see, majority of them is beta sheet now but the important part what it is saying that it is losing it is shape. It is going from alpha helix to beta shear. Now, what I want to have like what is the effect of it? What is the changes are happening over here? So, how I can do that so, this alpha helical positions, this 208 or 222 nanometre. I can plot what is the actual value? So, over there take a look into the unit it is given as delta epsilon which is nothing but delta epsilon.

And we have discussed over there that this delta epsilon is connected to the phi value. So, phi value means molar phi value. To be honest, it is connected to the delta epsilon value like this. And delta epsilon means difference in the molar extension coefficient for the right hand and left hand circularly polarized. So, we put that in delta epsilon because with that if I put that in delta epsilon, I am taking care of the concentration.

Because if I do this experiment in different places with respect to different concentration, the actual chi values will be different. Actually, electricity values will be different but if I normalize that with respect to concentration that means molar electricity or better to go with the delta epsilon then it is becoming a parameter which is concentration independent. No matter what the concentration you use at any place of the world or galaxy.

Say someone is using that in US at 1 millimolar, say someone is using in India at 10 millimolar. You should see the similar amount of change because delta epsilon takes care of the concentration. So, if I try to find out what is the change of delta epsilon value at 208, nanometre or delta epsilon value at 222 nanometre? But I am going to get so, you can see it start from a very negative value because it is 100% alpha helix.

So, you start from somewhere over here and then I am going to measure at different temperatures. So, it start from very say this is the room temperature 298 kelvin and I got a very negative value. So, say here is the 0 value. And now, as I increase the temperature, the value slowly goes up and then it normalizes because after a while there is no such change. So, whatever the change should happen in the structure of the protein, it is already done.

So, over there I am going to get this kind of data with respect to temperature and this data is showing that at what particular point of time, the molecule or the protein is breaking down after that no such change. That means whatever the change supposed to happen it is already gone. So, over

there, this is the change. What in measure and this is known as the CD melting curve.

Because we assume the protein is a globular structure which actually, melts or loses it is 3-dimensional structure as we increase the temperature very similar to melting of an eye. So that is why this term melting comes over here. So, it is the melting of the solid arranged structure of the protein to a unstructured condition or a different structure of the condition and over here we take when it started and when it finished at the middle point.

This middle point will say that is the melting temperature of Tm and that will define how stable a protein structure is and over here in this particular data. That is the real life data that we are seeing so, over there you can see these black dots are the lines that the data are collected and the different traces are for different proteins and we try to find out as you are changing the temperature.

You can see what is the change happening in the delta epsilon? They did not mention it but assume it is measured at 280 nanometres. So, you are looking at a change of alpha helical signature. And you can see it is staying as it is up till this point up to 50°C. It is fine and then it started showing a change. For example, this red line you can see the maximum half maxima is around here, so, this will be the T melting for this red colour protein.

Whereas if I go to this purple colour one, this is actually, happening a little bit earlier. So, now, by looking into this data, what we can say that this red colour protein is actually, more stable compared to this purple colour one. So, higher the melting temperature stable is the protein structure and that we can do it very easily by using a CD melting curve. What you have to do?

First record the CD find out some signature that you want to follow at that particular point you are going to measure what is the absolute value of the delta epsilon or the molar ellipticity. And then measure at the different temperature plot that value with temperature versus Δ□□or molar epsilon molar electricity and find out what is the trend. And from there see where the changes are happening find out the 50% or the half maximum that will be your melting temperature.

And then, if you are comparing different proteins, you just compare the different proteins and find out who has the higher temperature of melting that will be the most stable. Whoever has the lowest that is the least stable and why it is important because different protein structure actually, plays

different role in biology and their structure controls the reactivity? If a protein loses it is structure, it might loses it is activity.

So now, coming back to this overall structure so that is how a change in the protein structure is happening and it is very much important. Because as I was saying that some of the protein, for example, we as human being, we are actually running at a temperature within the range of 25 to 40°C as if we want to measure the body temperature, it comes out around 37-38°C.

That means our blood is actually, probably at the same temperature. That means all the protein which is running through the blood lying on the tissues is running very well. At 37-38°C. Now, if I put someone in a very high temperature what we have at what point of time we start degrading our protein at what point of time will probably die even. So, for example, if someone is getting a heat stroke, what happens actually, at that point?

So, for that if you want to know actually which protein is probably getting affected, much to find out a solution for heat stroke or find out the condition where human can probably still survive. So, you want to know actually which particular temperature the proteins are getting degraded. So, I want to know the melting temperature of the most important food is one example for us, probably haemoglobin, myoglobin, all the metallo-enzymes, all the tissue proteins.

All those things we want to know what is the melting temperature? And from there we can figure it out which of the proteins are actually, falling out with respect to the increase in temperature. And that would give us an idea which are the things we need to fix. If I want to go beyond whatever the evolution has bring us to. So, to give you an interesting example for an example, we generally do not survive more than 50 to 60 degrees into it.

So, for example, if you put your hand in a boiling water, you will like to get a heat shock and you are going to remove your hand as soon as possible. What is actually, happening over there? Obviously, heat signals are going through your neurons it is showing a please remove it. If you do not remove it, you are actually, going to be a boiled egg at the end. And over there your protein structure will change. Just take a look into an egg.

So, if you take an egg before boiling after boiling, you can see a liquid structure then becomes solid. What is happening there? It is a protein denaturation. It is a melting

temperature we are just crossing for the albumin which is present in the white liquidy but it is actually, getting solidified. It is changing it is structure, it is melting, it is losing the overall structure. Now that is going to happen to us also, if we actually, put ourselves in boiling water.

However, it is believed that the first point of the life during the evolution is actually, formed very close to boiling water, where, where the it is, the water is getting connected very closely to a lava holes. So, in the beginning of the world, our world is much more inhabitable condition that what we are seeing right now at that time world was very young and we have at different positions in the world.

The lavas are coming out and getting connected to the atmosphere, the atmosphere I mean the aqueous atmosphere and also with the aerial atmosphere. We still see some of these volcanoes where it is actually, emitting the lavas and you can see what are the changes are happening. And those extreme conditions are actually, believed to one of the parts where the first hints of the biomolecules are probably formed.

One of the first nucleic acid or the first amino acids, probably formed over there because that is actually, enough energy to form carbon-nitrogen, carbon-carbon bonds coming out of carbon dioxide, nitrogen and all those things. And one of those places which is believed to type the la to or support the life is actually, volcanoes which is sitting under the sea. So, there are also volcanoes under the sea we do not see it from outside.

But it is also leaving lava all the time. And over here there we have this hypothesis that that can be a hot bed for life because we still find different kind of life forms that is surviving over there very near to that where the lava is meeting that sea water. The water is close to 100 degrees centigrade is boiling. Because it is almost 500 degrees centigrade or even thousand degree centigrade lava is getting connected to the water.

The water is boiling over there and still we found that there are some life forces actually surviving there. Now, the question is if there is a life form, they should have protein structure and how their food is such as surviving. And then people look into their protein structure and they found very interestingly they have a very suttle change in their protein structure and by those suttle changes they actually, stabilize their overall protein structure, even at high temperature.

And over there they found their metal enzymes are worthy even at 100 degree centigrade because the melting temperature of

their protein is beyond 100 degrees. Whereas most of our proteins in the other parts of the world is melting within 70°C. So, CD spectroscopy was a huge player in that particular way to find out is it really the protein structure is playing a role.

And when they did the CD spectroscopy at this temperature they found? Yes, the melting temperature, measured from the CD spectroscopy, is showing a huge difference when it goes to this particularly different biological system surviving underwater volcanoes and the other part of the light. So, you can take a look into all these different life forms and find out what are the differences they have in their protein structure.

That actually, allows them or I should say, allowed them to survive all these very uh hazardous and extreme conditions. So, take a look into it, so that is one of the examples I wanted to give it to you.