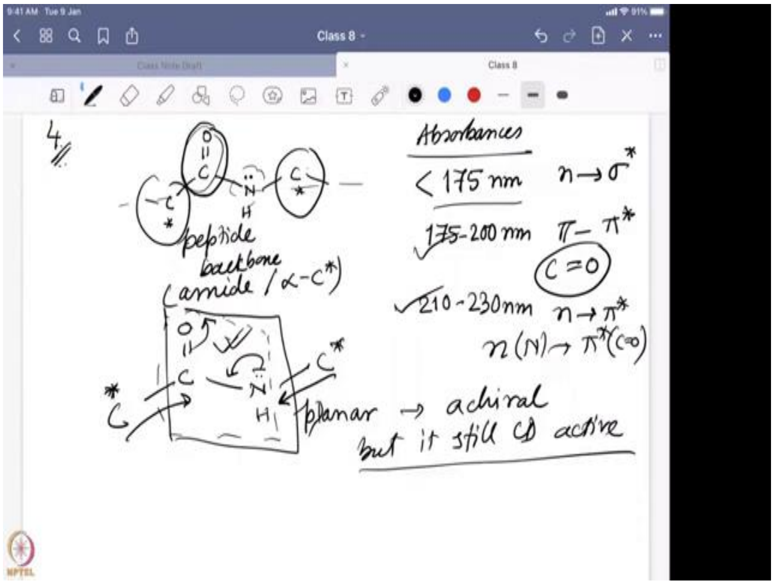
Circular Dichroism and Mossbauer and Spectroscopy for Chemists

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

Lecture – 30
Applications of CD Spectroscopy - II

(Refer Slide Time: 00:15)



How these things are happening? And now example number four is more not more of an example but a kind of answer to that quiz question that we have uh in the last class. So, the question was. We have an amide bond and these are the alpha carbons which are actually, the chiral. And if I try to find out what are the absorbances coming from a peptide backbone? So, this is we can say a peptide backbone.

And the peptide backbone is mostly amide and this alpha carbon. And the absorbances we can see we have absorbances below 175 nanometre which is coming from n to σ* interaction. What is the n? n is the lone pair coming from the nitrogen, mostly which is going to this n-C n to σ* bond which is coming below 175 nanometre very strong UV region. We can have some bands around 175 to 200 nanometre region.

Mostly around 190 nanometre which is coming from π to π^* transition, where this π to π^* transition coming this π to π^* transition is coming from this carbonyl stretching frequency. So, it is a carbonyl π to π^* transition and then we have this 210 to 230 nanometre region where the n to π^* transition happens. And that is mostly happening to this nitrogen lone pair to the π^* orbital of this C=O.

So, it is a lone pair of the nitrogen to the □□ of the C=O. So, this is what is actually happening over there? Now, the question is that okay that is only very fine but when we look into the structure very closely, we found this amide bond and as you have discussed earlier. Due to this lone pair interaction over there, it is actually, creates a planarity over there. And as we know, a planarity means that is not chiral.

So, how still a chiral band found over here? It is actually the CD active brands because when you look into the CD bands for example, look over here the CD bands over here. We are mostly looking at very strong CD bands in the region of 200 to 230 nanometre even 190 nanometre which are basically the regions of □ to □□ and n to □□ absorbance. Which forms this planar amide region which should be a chiral in nature but it is still CD active.

That means somehow chirality is induced over here and that is the question why it is happening? So, most of you try to write it in a way that it is somehow losing it is planarity. So that is not entirely correct because it actually holds quite a good planarity because of the strong amide bond. And especially when it is forming some of you write that when it is forming the alpha helix the beta sheet that is probably creating some strain on this amide region.

And that probably going out of the planarity okay it does put some strain over there but it never loses the planarity on it is own. So, one of you also discuss about the Ramachandran plot so, if you look into the Ramachandran plot you can find that there are two different dihedral planes we can talk about. One is coming from this amide nitrogen energy amide, one from the carbonyl energy amide and we found that two different planes.

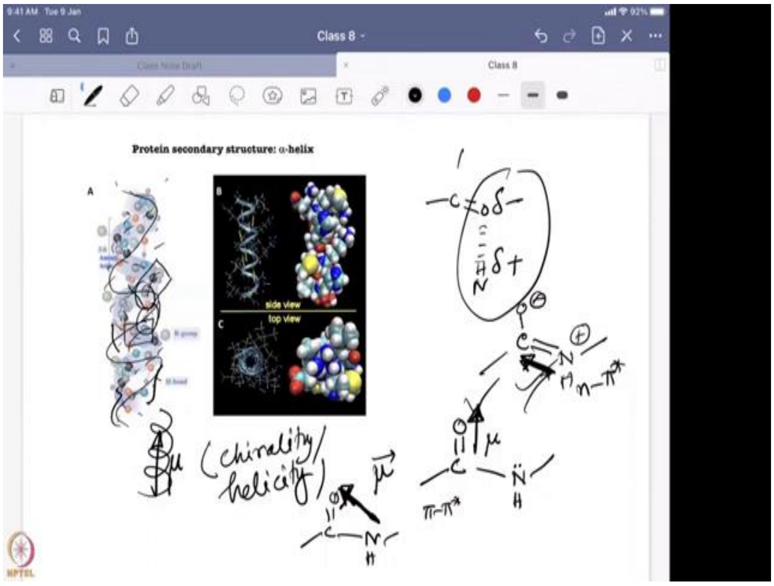
However, if I try to draw a plane considering these four groups that also forms a very good planar region. So, Ramachandran plot is a little bit different because we are talking about two different things not this amide bond all together. So, take a look into it later on. So, all together what I can say the alpha helix or beta sheet obviously it puts strains on that. How it is going to behave? How it is going to orient?

But it is not really stripping it out of the planarity. So, whoever wrote those particular answers, they got 8 out of 10. Now, some of you try to write that okay they are somehow the chirality is induced over there and the chirality is getting induced probably from this alpha carbon present over there.

The alpha carbon present over here it is somehow inducing some chirality over there. That is partially true.

That is somehow one of the induction of the chirality happens but exactly how? So, some of you, who wrote up to this point that chirality is getting induced they got 9 out of 10. And then a few of you wrote it very nicely that what is actually happen?

(Refer Slide Time: 05:48)



So, for example, let me draw the structure of the alpha helix that would be easier for me to explain. So, for example, this alpha helical structure so, over here what happens you can see? This amide bond is formed between this carbonyl and this amine but that does not mean that this amine and this next carbonyl is getting out of the plane or this nitrogen and this carbonyl next to it, it is getting out of the plane they are remaining in the plane on their own.

So, this is actually planar, this actually planar. However, it is still becoming carbonyl y that is coming because of the overall secondary structure. You can see the overall secondary structure it is formed over here it is creating a dipole. These are all dipole interactions right between the carbonyl and the amide group. So, this is there is a carbonyl group, there is a amine group, so, it is forming the hydrogen bond that means it is creating a dipole.

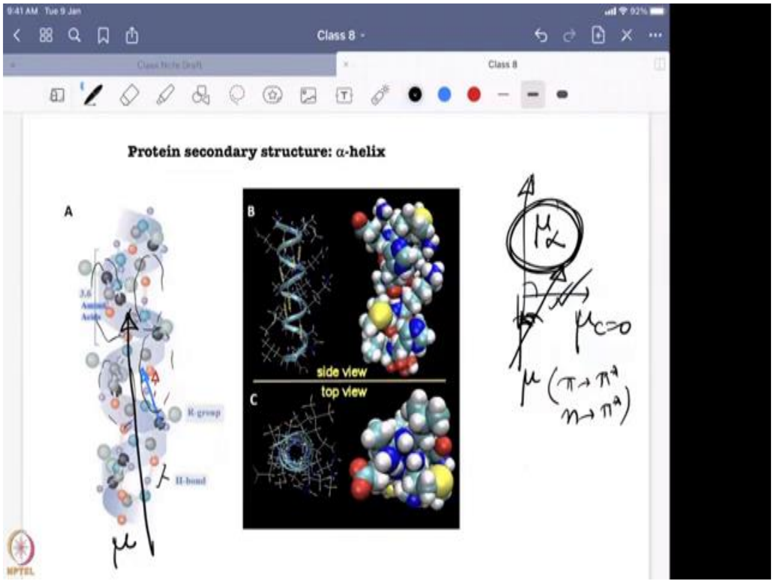
And this dipole has a direction and all this dipole if you consider together, it is going to create a helical motion of the dipole moment because that is how it is oriented. That means it is creating a dipole moment which is actually helical in nature. And as you know, once you have a helical in nature you are creating a chirality. So that is coming from the helicity motion the dipole moment is creating over there.

But how it is affecting that amide bond? That is affecting in the following way. So, as we know the nitrogen it is creating a amine bond over here. So, let me just move it over here a little bit. So, over here what happens? This carbonyl which is doing going for the □ to □□ star interaction will be going to be surrounded like this. This should be the direction of the dipole moment for the □ to □□ and n to □□, n to □□ will happen in the following way.

If I draw it again, it will be C double bond NCO – NH^+ . So now, the dipole moment for the n to □□ will be in this particular region, in this particular line and □ to □□ star in this line. However, those things because of their planarity and their conjugation, they are going to come together. So, you are going to have this particular direction and this particular direction all together. What we are going to see is the following?

We are going to see a conjugate emotion like this. This will be the dipole moment which will be affected by the n to □□ and □ to □□ it is one of these two are actually vectorially connecting together. So that should be the direction of the dipole moment change and why document change we are talking about? Because that is the operator for the transition. Now, imagine look into all these C=N's.

(Refer Slide Time: 09:19)



Protein secondary structure: α-helix

Let me draw it in a fresh one. So, if I now draw the alpha helix a little bit bigger now. Now, you can see the alpha helical motion it is actually, going through this. This is the dipole moment created by the alpha helical position for this hydrogen bonding network. And the C double bond n as I just said otherwise, it will be like this if it is □ to □□ star which will be out of the plane.

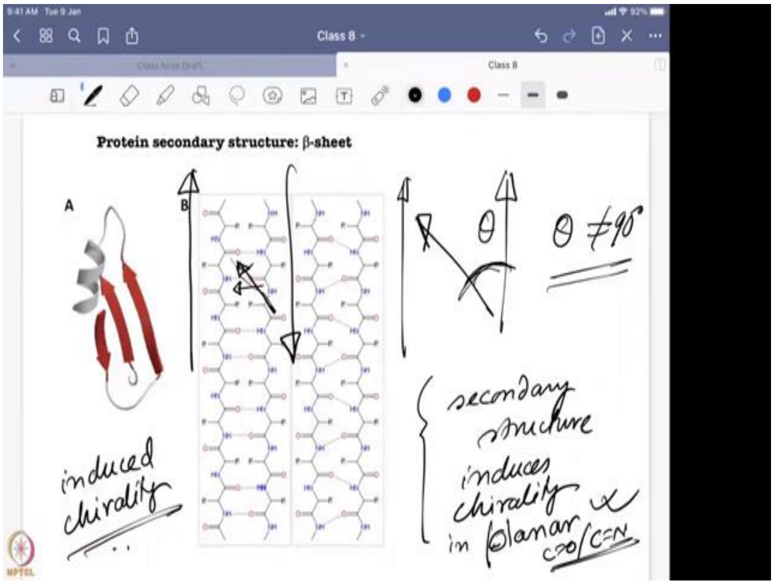
Let me draw that with the red line which will be perpendicular to this alpha helical motion we have created. But due to this carbon nitrogen interaction and n to □□ interaction that is going to be somewhere in between. So, instead of having say this is the alpha helical one and this is the C double bond of □ to □□□□ And what we are having something like that all together?

This is the real □ to □□ and n to □□ star interaction when both of them are interacting. And now, you can see they are not 90°to each other but some other angles. And if there is some other angle that means they can have a vectorial contribution together and that is why this alpha helical motion which is created by the secondary structure that is going to affect my alpha helical structure like this.

And over here, you can see or you can imagine depending on which particular direction it is coming you can have two different orientation and that is what it is believed? That why we have two different humps at 218, 208 and 222? Because of the two ends of the dipole a little bit differently oriented with respect to the alpha helix because the alpha helical position, this 1 to 4 is actually repetitive but they have two different orientations over there.

And that is probably the reason why we have two different humps over there. So, it is because of the secondary interaction and the normal magnetic moment is actually coming together and playing a role over here.

(Refer Slide Time: 11:45)



Protein secondary structure: β-sheet

$\theta \neq 90^\circ$

induced chirality

secondary structure induces chirality α in planar C=O/C=N

Now, what happens to the beta beta sheet? So now, if you look at the beta sheet. Now, you can be very sure what is actually happening? Now, take a look in the beta sheet when you form, where is the C=O on this side, C=N will actually going to bring that over here. And with that respect you can see the beta sheet this is parallel, one is antiparallel. So, this is going like this and this.

Now, they are not perpendicular to each other or in this case parallel and your C=N will be like this. So, they are actually, making an angle which is not 90° and that is why it is going to be non-orthogonal and will be going to interact with that? However, in the beta sheet you can see the interaction of this is mostly one particular angle. No other orientations are possible in this particular orientation.

And that is why it is believed there is only one broad hump the 218 nanometre for the beta sheet. So that is how the overall secondary structure induces chirality in the planar C=O or C=N and that is very much important for the CD spectra because right now we are measuring the CD spectra in the region of 190 to 230 nanometre region. Otherwise, if it is not possible.

We have to go to the region where the carbon bonds are actually absorbing below 175 and that is very tricky to do. Because over there all the gases started absorbing nitrogen, carbon so, it will be huge background spectra and it will be very tricky to measure your original signal from there. So that is why because of this phenomenon of induced chirality the systems that you are measuring especially for the protein structure is actually possible that we can actually, measure it.

So, this is known as the induced chirality and there will be a charity of the alpha helical structure of a polymer that you are taking and the polymer because of the alpha helical structure. It is showing some chirality or a system with a alpha carbon with four different groups it is showing some chiral signals that is known as intrinsic chirality which is automatically coming on it is own, not induced by other chiral.