NMR spectroscopy for Structural Biology
Prof. Ashutosh Kumar and Prof. Ramkrishna Hosur
Department of Chemistry
Indian Institute of Technology - Bombay

Lecture: 28
Application of NMR in the Area of Structural Biology: Structure of DNA and RNA 5

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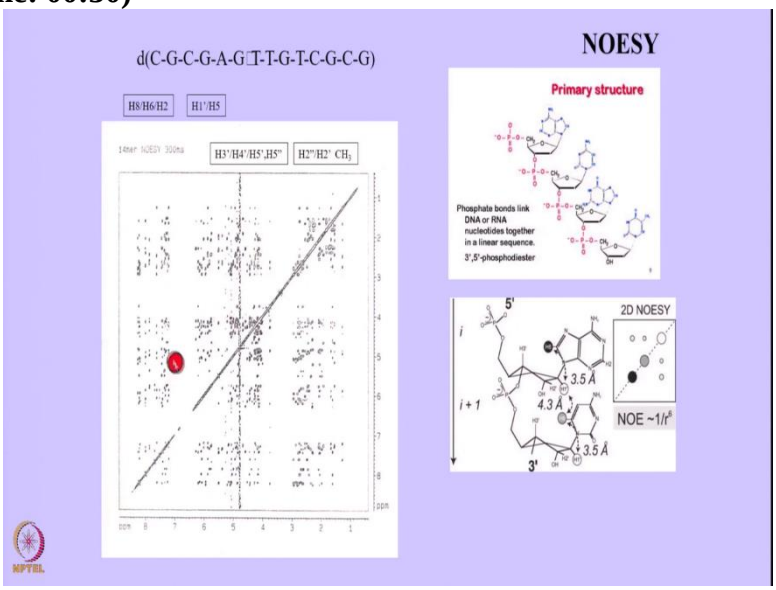

So, we will just do a recap of the assignment process in the case of nucleic acids and by and large we do this by the NOESY experiment which we discussed last time in greater detail and here is just to show that once more this is a 2 dimensional NOESY spectrum of a particular oligonucleotide whose sequence is given here on the top. So, this has; 3 thymines here and are very well characterized by the presence of the methyl Groups in the base.

So, here you have a methyl group in the in the thymine base and that is very distinctly seen at the top here. So, that actually fixes the positions of the thymine residues. So, and as you know this one is the area which corresponds to the base protons and the base protons the NOEs are there to the sugar rings these are the H1 ‘ protons of the sugar ring and then you have these are to the H3 ‘s and these are to the H4 ‘s 5 ‘s.

And then these are the 2 ‘ 2 double ‘ areas from the base protons H8 and H6 to the base protons that is indicated here H8 H6 H2 but the NOEs come from the H8 and H6 you not see. So, much from the H2 protons all of these are base protons. And then you have here very characteristically these are the thymines. So, therefore this fix these are the NOEs from the methyls of the thymines to their own H6 protons.

There are 3 of them here you can see them very strongly here these 3 and therefore this fixes the positions of the thymines here. So, everything what you do should be consistent with that that actually provides a very good starting point here because you see I will illustrate this to you in this particular sequence here we saw this in the last term but I will go over it again you start with the T at the 5 ‘ end then the T at the 3 ‘ end.

So, what we have seen earlier was that you will see from the base proton noise to the sugar protons of the same nucleotide and to the sugar protons of the previous nucleotide that is at the 5 ‘ end. So, therefore this T does not have any one on the top there.

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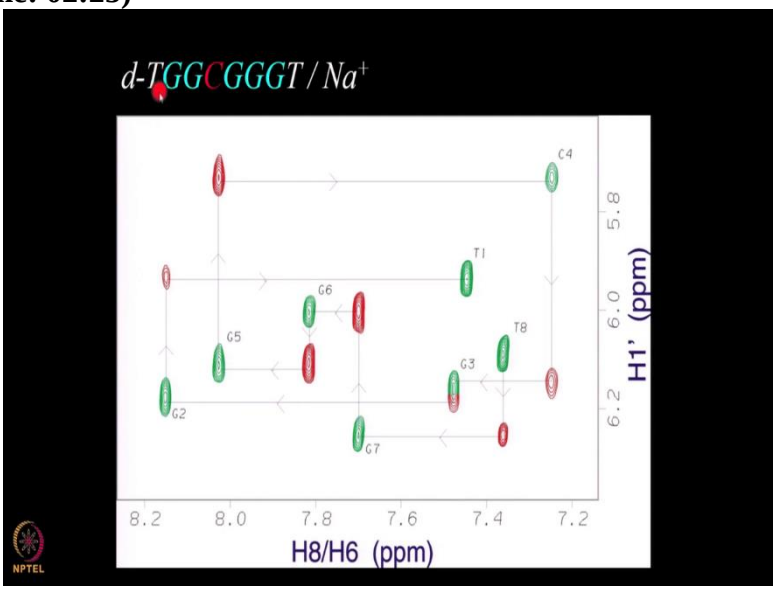

Therefore this thymine base will have only one connectivity from its base proton. Secondly we also know where are the thymines? The thymine positions are fixed here. In this particular sequence there are 2 thymines and the NOEs to from the methyls to the base proton fix the 2 thymines to be here. So, therefore one of the thymine is here other thymine is here. Now if you look at these 2 thymines one of them shows a sequential connectivity here to the 5 ‘ end.

Therefore obviously this must correspond to this whereas this one does not show any sequential peak therefore this must correspond to this. Now you can start from either end of these ones and you can walk and ultimately you have to reach this place. Therefore you can see from T8 self you see to the next one G7 from G7 it go you go to its own base from the G7 you go to the G6 this is the sequential peak then it will go to its own base.

Then it is this G6 here G6 you go to it is the sequential one you go to G5 then from G5 you go to the C4. Now that is here there is a sequential this is the self from C4 you go to the G3 and then that is the sequential connection here to this center here. Now you notice that this G consists of 2 peaks here. So, there is one sequential which is connecting the self peak is here and its sequential is also in this area.

And this connects to the G2 here from G2 you have you go to T1 and you reach here therefore you see the sequential walk is easily done in this entire sequence. Once you have this 1 ‘s identified you will also identify the 1’, 2’, 2” and the 2’, 3’ peaks from the NOEs which are occurring in this area. So, you have this one 1 ‘r identified here you see and we is to the 2’ in the 2” of the individual nucleotides.

And also to the 3’s here and then to the 4’, and the 5’s here therefore once the 1’ are identified you connect them to the one 2’ and 2” these are also seen in the COSY spectrum 1 ‘ 2 ‘ peaks will also be seen in the COESY spectrum therefore once you have the 1’, identified you will have the 2’, 2” identified there you will also see the 2’, 2” cross peaks here and this we are clearly distinctly seen at a different place.

And then you will have from the 1 ‘ you will have the 3 ‘ 4 ‘ peaks which are present here in this area. So, the whole spectrum therefore can be analyzed by once you get these 1 ‘ assignments here and you also have these base to base assignments here in this area.

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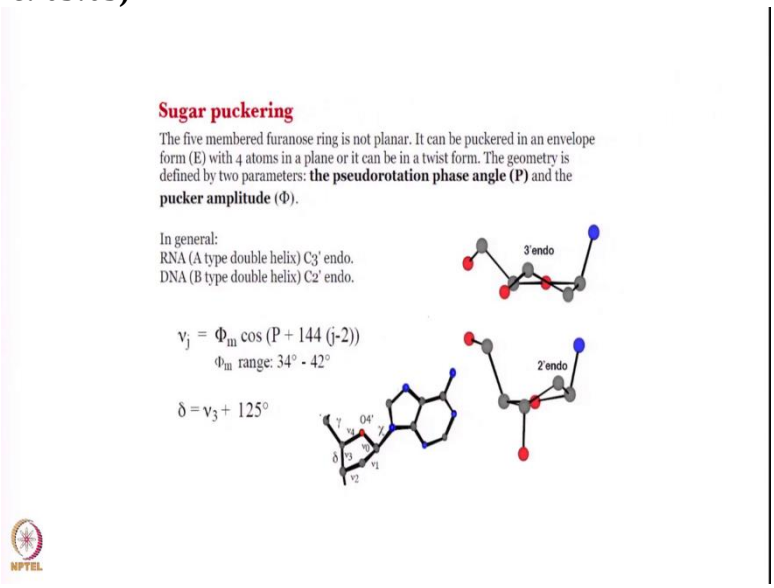

So, having done these assignments now you have the full assignments of the entire nucleotide. Now you have to go to the structure the structure as we said consists of the backbone structure and then the sugar ring structure. The sugar ring we have seen earlier is called the sugar puckering it is described by the pseudorotation angle P and we have all the sugar puckers are classified into 2 categories north and the south which we described earlier.

The north corresponds to the C3 ‘ endo geometry which is the center of the north region and this happens in the A type DNA helix or in the RNAs and in the B type double helix you have the C2 parameter geometry these are characteristically shown here what how do they look like the sugar pucker is in this in this way. And then you have the glycosidic torsion angle here which determines how the base is oriented with respect to the sugar ring there.

So, now we have to determine the sugar geometry but there is only one variable here that is pseudo rotation angle and how do we determine the sugar geometry what are observables from the NMR point of view. And that is the sugar ring as you know has many protons and all these protons are coupled J coupled. So, you have this 1 ‘ on every position you have the protons here 1 ‘ 2 ‘ 2 double ‘ then you have the 3 ‘ the 4 ‘ and the 5 ‘ it goes like that.

So, far the sugar ring is concerned we have these couplings which are present here one bond 3 bond couplings and those ones are indicated here.

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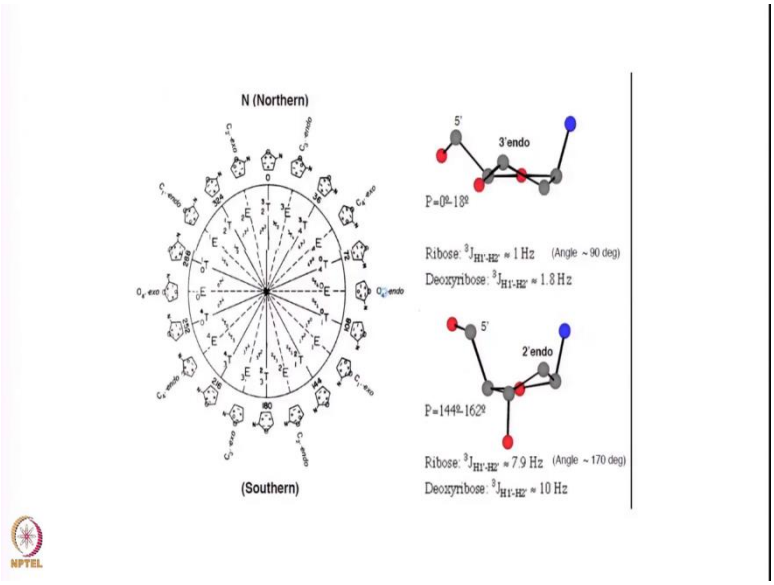

So, you will have the ribose ring you will have this coupling constants here I will show you the coupling constants a little bit more detail and this is once again the pseudo rotation circle. So, you have this in see the southern part this is the so called C2' endo region. And this is the northern part this is the C3' endo region all other intermediate puckers which occur in this area.

Now how do we characterize these sugar rings what are observables the observables are the coupling constants the coupling constants are indicated here.

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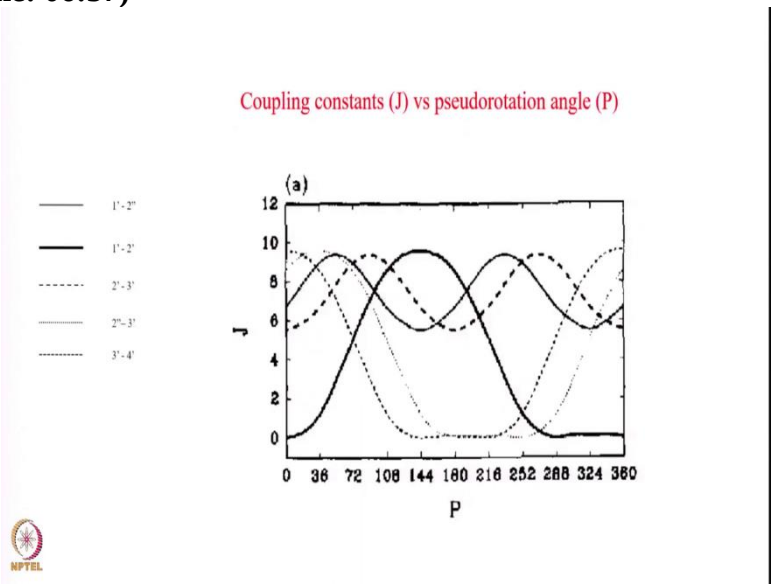

Now the coupling constants depend on the sugar geometry what is plotted. How many coupling constants are there we have 1 ' to 2 double ' then 1 ' to 2 ' 2 ' to 3 ' 2 double ' to 3 ' and 3 ' to 4 '. So, we have 5 different coupling constants which can be used to characterize the sugar ring and these ones depend on the confirmation of the sugar ring and that you can see from here.

These are plotted here the coupling constants vary with P in this manner this big one here this one this is the 1 ' 2 ' coupling this peaks here at this about 10 hertz is the coupling constant here for the C2 ' endo geometry. For the C2 parameter geometry this 1 ' 2 ' coupling is 10 hertz. So, and we can write here some of these things. So, for the C2 ' window for the C2 ' endo geometry you have the 1 ' 2 ' is approximately 10 hertz.

And you can look at the other ones the 1 ‘ 2 double ‘ 1 ‘ 2 double ‘ is approximately you can see which one is that that is the one which is below there. So, that is approximately 6 hertz that is here. So, in this area this is 10 hertz this is 6 hertz and this one is my 2 ‘ 3 ‘ what is 2 ‘ 2 ‘ 3 ‘ this is the third one and that is approximately 7 hertz . So, that is about 7 to 8 hertz 7 to 8 hertz.

And what about 2 double ‘ 3 ‘ 2 double ‘ 3 ‘ is this one we are talking here. So, this is about 2 hertz or less than 2 hertz 1 to 2 hertz and 3 ‘ 4 ‘ 0 and these are very characteristic coupling constants and they will show up in the cross peak fine structures in this we are going to discuss. Now what happens in the case of C3’ endo let me write here the C3’ endo geometry.

So, you can see what are the coupling constants C3’ endo means we are here we are here. So, here 1’, 2’ is 0 and all others are relatively larger all others are greater than others all others greater than 6 hertz. In this area you can see this one is very small this is the C3’ endo area the 6 hertz and this is about 7 hertz then you have ten hertz among these the strongest ones are the 3 ‘ 4 ‘ this is the 3’, 4’ this is the highest value for 10 hertz and then the 2”, 3’ is also around 8 to 9 hertz.

So, that is this one here. So, therefore these are very distinct coupling constants which will allow you to distinguish between the sugar geometries. Now how do they show up in the sugar geometry. So, we will have to calculate the sugar geometry fine structure for this. Therefore we will try and calculate that in the COSY we will have to use the COSY spectrum.

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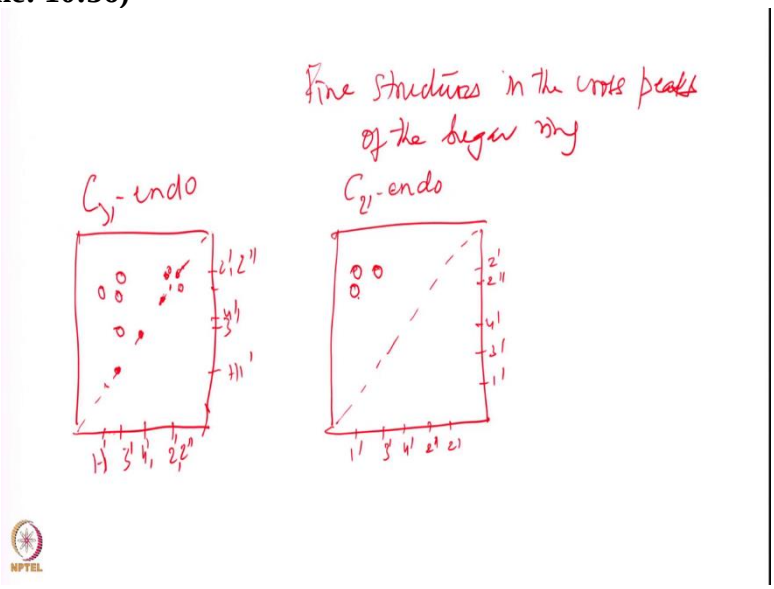

So, fine structure in the cross peaks of the sugar ring. First of all let me draw the schematic of the COSY spectrum what all you are going to get. So, let us say I have this is the C3’ endo let us say this is my where are the 1’ let us say this is the H1’ then I have 2’, 2” here then I have the 3’, here then these are the 4’, 5’ final problem 5’ we will not go.

So, up to the 4’ one can go. So, what do we expect here from the 1’ this is the diagonal. So, you have the 1’ to 2’, 2”. So, you will let us say this is my I will draw that here 2’, 2” there are two here then I will have the 3’ and this is the 4’ here and then another 1’ here. So, this is the characteristic. Now what cross peaks I will have of course the diagonal will be present here.

1', to 2', 2" because I will have I will have 2 peaks here schematically in the COSY spectrum. So, 2', 2" of course you will have the cross peak between these 2', 2" this will be present. Now 1', 2' peak will it be present in the C3'endo geometry for a general thing all these peaks will be there but will this 1' 2' will be present will it be present.

So, let us look at that. So, 1', 2' coupling is zero right. So, for the C3' endo geometry this peak will be absent 2'' will be there. Now all other peaks will be there. Now 2', 2' to 3' peak will be there though. So, 2' to 3' peak will be there and 3' to 4' that is here 3' to 4' will be there this is also very strong peak.

So, on the other side I am not writing this symmetrically they will also be present here. So, I am not writing on the other side. So, these peaks will be present you will have 1' to 2" and 2' to 3' this is 2' to the 2" to 3' will it be present? 2" to 3' will also be present and that will also be present here 2" to 3'.

And 3', 4' will it represent yes that will be present and that is this, this is 3' to 4' this is the diagonal of the 3' this is the diagonal of the 4' and these are the diagonals of the 2' and 2" 2 and 2's there. So, these mini peak these 4 cross peaks will be present in the case of a C3' in the geometry. Now let us do that for the 2 ' endo geometry.

So, right here C2 ' endo geometry. Now let me do the same thing here you have the 2', 2" then you have the 4' then 3' then the 1'. So, let us draw the same thing here 2', 2", 4', 3' and 1'. Now what peaks will be present here for the C2' endo geometry go back and look here we wrote those ones here what are the coupling constants 1', 2' coupling is very large.

Therefore I will see the 1' 2' cross peak what I will not see what I will not see is I am indicating below the 3', 4' cross peak will not be seen because that coupling constant is zero. See this coupling constant is zero 3', 4' and the 2", 3' is also very small 1 to 2 hertz therefore you may not see this one also. So, what you will see you will see these 3 couplings these 3 couplings only will be seen.

So, let us draw that here. So, therefore so, I will have 1', 2' coupling this peak will be present 1', 2" also will be present and what about 2', 3? 2' 3' will be present 2", 3' will not be present and 3 ' 4 ' also will not be present. So, therefore I will have only 3 peaks here on both sides of course correspondingly there will be symmetrically they will be present here as well.

I am not writing on this side. So, basically on one side these are the distinctively different kinds of patterns you will get in the 2 kinds of sugar geometry. By looking at this kind of a peaks itself you will be you will be able to figure out whether I have the 3' endo geometry or the C3' endo geometry which is pure in the case the pure geometries. But of course if there are equilibrium mixtures of the 2 then you may find the peaks present for the other ones also.

So, this we can figure out by actually measuring the coupling constants. Now how do we measure the coupling constants the fine structures contain this information all of these peak they have fine structures. And the fine structures will have the information about the coupling constants. So, how do you measure these coupling constants? In order to do that we must be able to understand what are the fine structures in this peaks.

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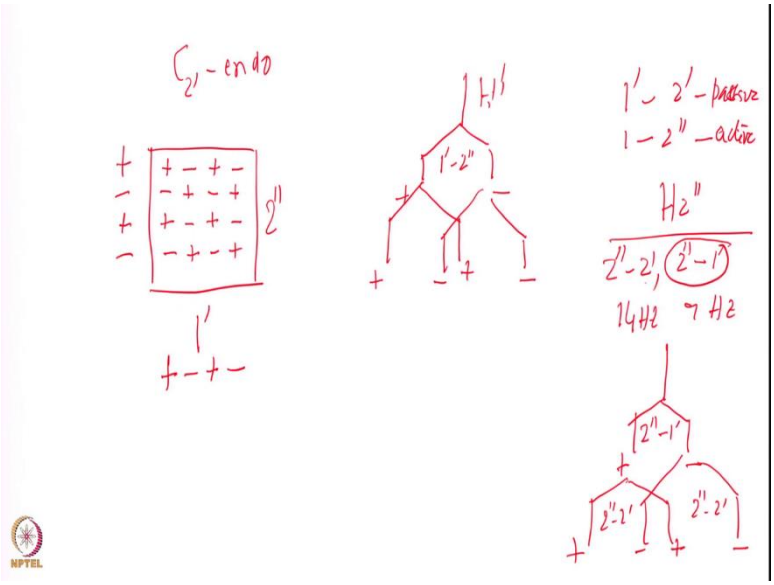

So, let us take a particular peak let us say cross peak 1' 2" let me consider one particular peak 1', 2" for a C2' endo geometry for C2' endo geometry. So, we have seen this how to calculate this fine structures but so, let us do that once more here. So, let us say you have the H1' chemical shift here this is the H1 ' chemical shift this is the H1 ' this is the single line this is the chemical shift here.

What are the coupling constant this will have this will have which one H1' has 1', 2' and 1', 2" these 2 couplings are there. Now if you are looking at the 1' 2" cross peak. Now I will have an active coupling and a passive coupling which is the active coupling here? Because I am looking at the 1' 2" cross peak therefore this is my active coupling.

And this is my passive coupling what is the consequence of this the actual coupling will lead to plus minus splitting. So, I will put this split this here 1 plus minus this is the 1', 2" coupling. So, now we notice here in this 1', 2" coupling is of the order of 6 to 7 hertz. Now 1', 2', coupling is much larger that is about 10 hertz. So, therefore this will further split into 2 and they will go like this.

But this since is the passive coupling I will have this plus and plus here and minus and minus there. So, this will produce me a structure plus minus plus minus. Let us look at the 2's the 2" peak. Now if I draw the 2" peak here H2" what are the coupling constants for this? It has a 2', 2" coupling 2', 1' coupling and 2", 3' coupling. Now this one has 3 couplings.

So, this one has 3 couplings it has 2", 2', 2", 1' and 2", 3' but 2", 3' is very very small. So, 2", 3' is almost close to 0. So, therefore we will not see that right. So, 2" has 2", 1'coupling 2", 3' coupling which is small for the C2' endo geometry and the 2', 2" this is a large one.

This is typically of the order of 14 hertz two double this is the geminal coupling and this is of the order of 7 hertz . Now therefore what is the kind of a splitting here? So, now let us draw this pattern here. So, what is the active coupling? The actual coupling is this one this is the active coupling right. So, therefore if I want to draw first the splitting due to the active coupling this will be plus minus and this will be of the order of 2.

This is 2 double ' 1 ' coupling and this will be further split into 2 because of the 2 ' 2 double ' coupling therefore this will be plus plus minus minus this is 2 double ' 2 ' coupling 2 double '

2 ‘ coupling. So, this also will be plus minus plus minus. So, what will be the fine structure. So, therefore I will have here plus minus plus minus and here also I will have plus minus plus minus.

So, if I multiply this how many components I will have I will have 16 components this peak will have 16 components. Therefore I want to write that here inside plus minus plus minus then I will have minus plus minus plus this will be plus minus plus minus minus plus minus plus. So, 16 components this is the kind of a structure that will be present in the 1’, 2” peak.

So, if you look at the 1’, 2’ peak of course then you will have to consider differently in the case of 1’, 2’ peak what are the do you have like 2’, 3’ coupling also in addition.

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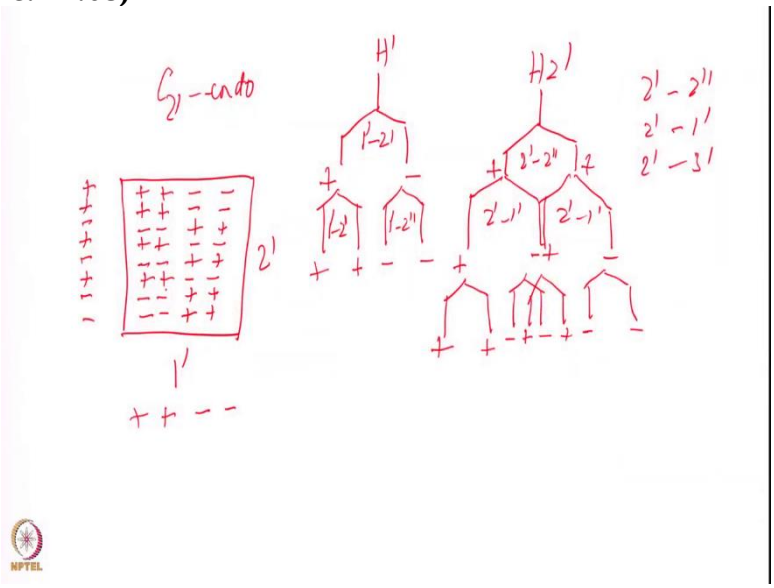

So, go to the 1’, 2’ coupling the same geometry C2’ endo. Now I want to see what will be for the 1’, 2’ peak 1’, 2’ see the 1’, 2’. Now what are the actual coupling here let us split that here 1’, 2’ coupling is very large 1’, 2’ coupling this is the active coupling. Now 1’, 2” coupling will become smaller that is the passive coupling.

So, this is this will be plus minus and 1’, 2” coupling which is smaller. So, therefore this will be like this therefore this will be plus plus minus minus. Now let us look at this is the one this is the H1’. Now let us look at the H2’. H2’ once again I will write 1’ 2’ is the active coupling this is what are the coupling constants here. So, in this case I will have 2’, 2”, 1’ and 2’ 3’ there are 3 couplings.

And the 2’, 3’ is not 0. So, therefore let us start with this big one 2’, 2” that is the big one there. Let us say this is my this is plus plus because this is the passive coupling. I am writing with the plus password coupling there and now what is this one. So, this one is my 2’ 2” and then I will have 2’ 1’. Now this is my active coupling therefore this will be plus minus plus minus this is the 2’, 1’, 2’, 1’ and 2’, 3’ is smaller than 2’, 1’.

So, therefore this will be again another passive coupling. So, now we see I will write here plus plus and this is minus minus this will be plus plus and this will be minus minus. So, what you got here, you got 8 of them plus plus, minus plus, minus plus, minus minus. So, 8 components therefore this will have how many total in the structure there will be 32 components 4 into 8.

So, here I will suppose I want to write here. So, this is plus plus, minus plus, minus plus, minus minus. So, and this side I have here plus plus, minus minus. So, we put that here. So, plus plus, minus minus, plus plus, minus minus, minus minus, plus plus, plus plus, minus minus, minus minus, plus plus, plus plus, minus minus, minus minus, plus plus, minus minus, plus plus. So, you will have 32 components and all of these coupling; this will of course will have the separation there as we indicated here.

So, which is the cup this is the coupling this is the 1', 2" , 1', 2". So, by looking at these plus minus separations you can figure out what are the coupling constants in each one of those. But often you may not be able to resolve the peaks in this manner. So, what will happen there will be cancellations in the middle all pluses minuses and things like that will cancel in the middle then you may not be able to find all the peaks.

So, in such a situation what do we do? When we do not have this of course you will have to calculate all of this. Similarly you can calculate the fine structures for the 2', 3' peaks and then the 3', 4's you can calculate the fine structures for the individual sugar geometries.

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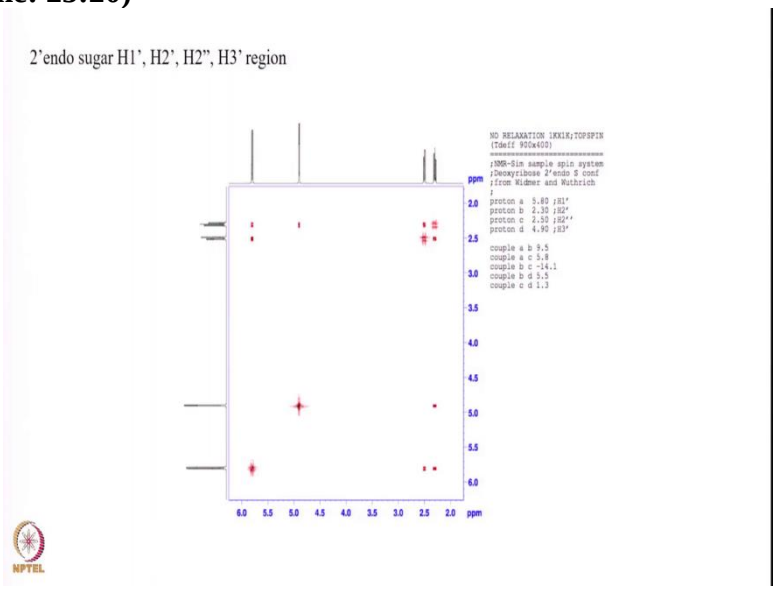

So, when we do that this is an experimental spectrum of the pseudo prime into sugar geometry which is shown here. Now what do you have here. So, you have the 1' which has already discussed with you the 1' peak is present here these are the diagonals here and then you have the 2', 2" cross peak present here . So, let me write here this is the 1' and this is the 3' this is the 2" and this is the 2'.

So, you also therefore you see 1' to 2" and 2' and then you see from this is the 3'. So, 3' to 2' notice 2", 3' peak is absent. This is what I showed you earlier 2" because that coupling constant is very very small. And then you of course you have the 2", 2'. Symmetrically you will also have these peaks here.

Now these ones as you can see have a fine structure these ones have a fine structure this is what I was discussing explain to you just now.

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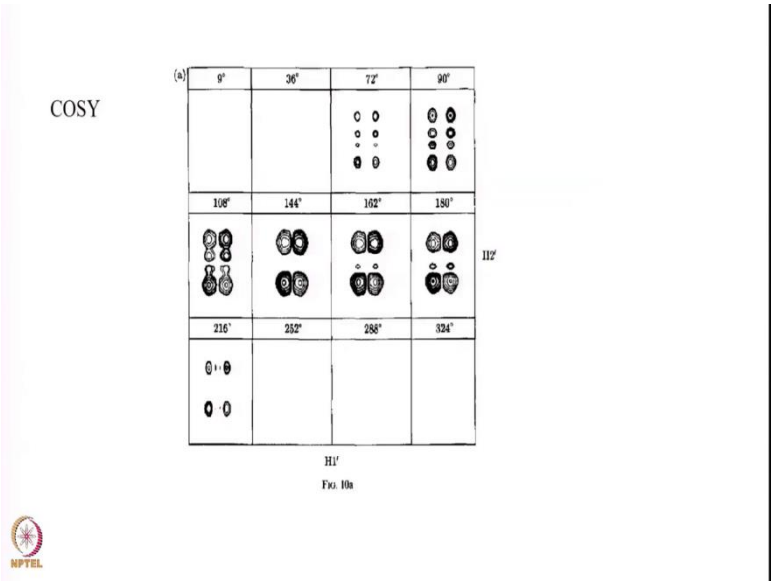

Fig. 10a

And we will see how one can calculate these. Now you see these are the simulations. So, it seems because since there will be cancellations in the peak intensities you cannot do it by simply all the all the components are not visible to you. You have an experimental spectrum you put in these numbers certain numbers for the particular sugar geometry here what is calculated is for all the sugar geometries starting from the pseudo rotation angle 9 degrees 36 degrees 72 degrees 90 degrees.

And this is my C2' endo geometry and we had instead of see how many cross peaks are seen here you only see 4 components all the other com there are lots of things which have merged they have cancelled or merged and things like that. So, in this particular case we see more components if you see here there is some cancellations here some residual things are present here.

So, when you come to this area then you don't see this cross peaks at all because this is the 1', 2' peak 1', 2' peak peaks around the coupling constant peaks around hundred and forty 4 there is 0 here and 0 here. And therefore you will not see the cross peaks for the pseudo parameter geometry for these values of the pseudo rotation angle. Now therefore these are the patterns which are calculated and simulated keeping in mind the resolution that are there and the line weights.

So, once you have those ones we get these patterns. So, experimentally what we have to do is you will have to simulate and compare with the experimental spectra to estimate the correct values of the coupling constants.

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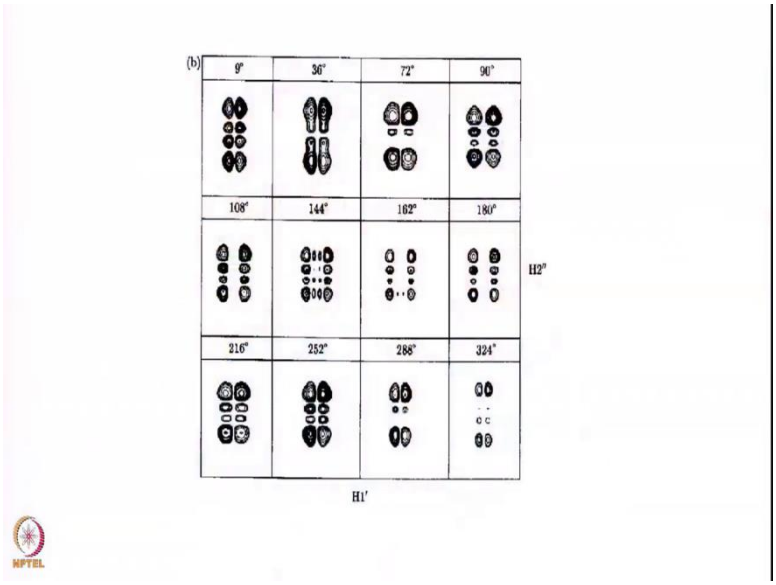

Now this is a similar calculation simulation for the 1', 2" peak. So, this is the 1', 2" peaks here. Now you see there are many more peaks which are visible here because this one has only 1', 2' coupling absent 1', 2' coupling is absent but all other couplings are there. See because of the 1',2' coupling is absent you see this peak appears narrow along this axis.

This for the various values because at this point at 9 degree the 1', 2' coupling is zero right for this and therefore at this value you 1', 2' coupling is zero therefore this side splitting is only two 1', 2", coupling therefore this is narrow. As we keep increasing you start getting contributions from the 1', 2' coupling also this actually becomes wider you see this width becomes more this becomes more further more.

And it has the largest see here is the largest width here because the 1', 2' coupling is large and 1', 2" coupling is also available here therefore this has a larger width along this axis. There are 4 components along this axis that you can see that here clearly in fact in this one you can also see all the 16 components so to say right centrally there are some cancellations at this point.

Because of the plus minus and you keep increasing the value of the P go over to C2' endo geometry and then you go over towards the end once again it becomes narrow here this is similar to this. This is again back into the north regime not the regime has a 1' 2' coupling is going to zero. So, therefore this is becoming a narrower one here. So, therefore from the looking at the patterns of the cross peaks in the in the COSY spectra you can actually calculate the; determine whether it is the geometry is in the north domain or the south domain. So, I think we can stop here.