Circular Dichroism and Mossbauer Spectroscopy for Chemists
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Lecture – 33
Applications of CD Spectroscopy - V

Hello and welcome to this new segment of CD Spectroscopy and Mossbauer Spectroscopy for Chemist, my name is Arnab Dutta and I am an associate professor in the department of chemistry IIT, Bombay.

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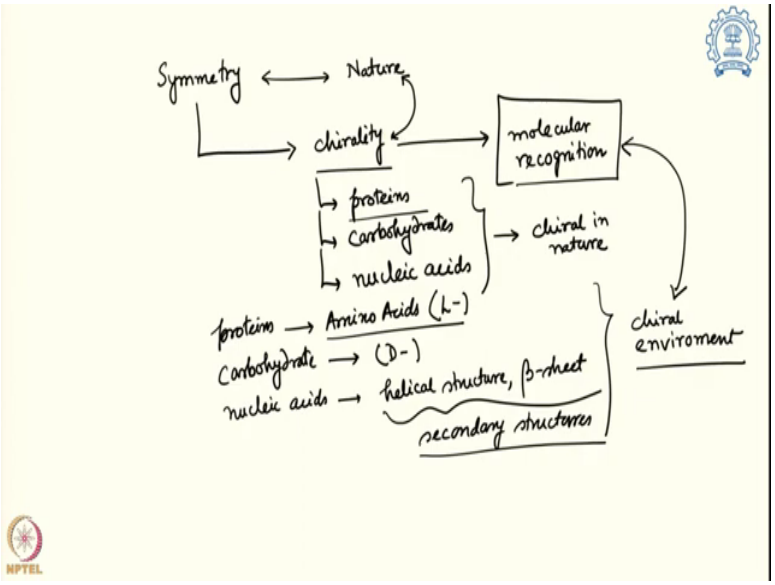

So, over the last few classes we are discussing about symmetry and how it is an integral part of the nature. Because one particular portion of the symmetry is chirality and nature uses chirality as one of the tools for molecular recognition. And over here when you are talking about molecular recognition this chiral molecules in the forms of proteins in the forms of carbohydrates and nucleic acids they all became important.

Because all these molecules which are actually an integral part of the biological segment is chiral in nature. For an example, proteins when you talk about proteins are made out of amino acids which are the building blocks for it and these are mostly L-amino acids in nature. Whereas other hand when you talk about carbohydrate they are D carbohydrate in nature. So, this D and L are talking about their absolute configuration in the three dimensional space.

On the other hand nucleic acids itself are not chiral but when they form this helical structure or the other secondary structure like beta sheet and all the systems the secondary structures are all chiral in nature. And when biological system are talking among each other or actually interacting with other molecules this chiral environment becomes the key. And that actually signifies which particular amino acids is going to interact?

Which particular carbohydrate is going to interact? And how it recognize them? So that particular system the molecular recognition and one of the important factor of that is the chiral environment. And that is why we are interested to know about the symmetry and chirality.

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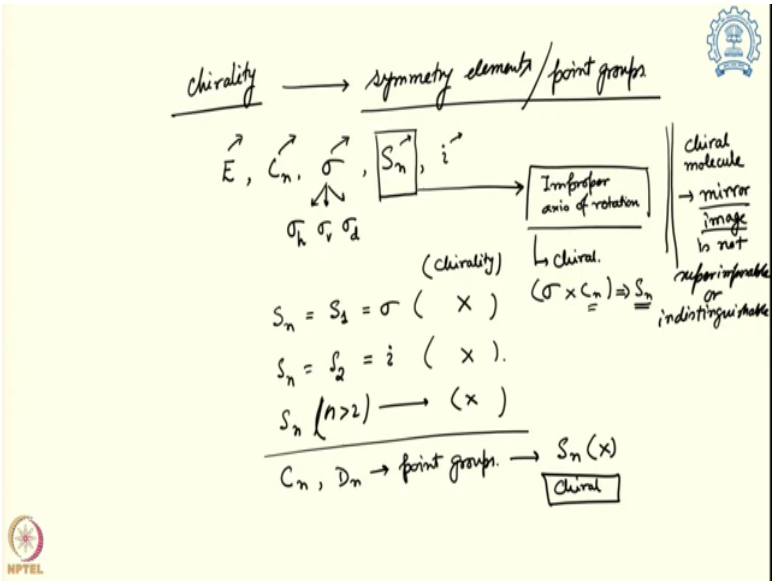

And then when you are talking about chirality, we found we can define it in the form of symmetry elements and also point groups which we have derived from mathematics. And over there we found there are five symmetry elements can be found which can be E or identity operator C_n, rotational axis σ, reflection plane which can be σ_h, σ_v or σ_d.

Depending on it is interaction it is positioning with respect to the principal axis C_n and improper acceleration S_n and centre of inversion i. And among them all those things can be present in a molecule this particular symmetry element S_n or improper axis of rotation is the key to find out whether my molecule will be chiral or not. And over there we found that when you are talking about improper access rotation why it is important?

Because by the definition of chirality or a chiral molecule or chiral object we say the mirror image is not super imposable or indistinguishable. So over there what does it mean? That I am doing a mirrored image that means I am doing a σ operation. And then I am doing some rotation to find it out whether it is matching or not? So, I am doing σ and C_n together which is nothing but give me S_n axis of system.

And that is why we make it much more simpler when $S_n = S_1$ that means say n = 1 that is nothing but a σ. Because I am doing a C_1 rotation which is nothing but an identity operator or leaving the object as it is so, in S_1 is nothing but σ so, if you have a σ you cannot have chirality. If you are S_2 when you are talking about $S_n = S_2$ it can be written as i, centre of inversion.

So, if you have a centre of inversion or S_2 it cannot be chiral. If you have any other S_n where $n > 2$ even then your molecule cannot be chiral. So that is the mathematical interpretation of chirality, when a molecule can be chiral. And if I try to bring that in the point group we have the C_n and D_n point groups where we found this particular point group does not contain any S_n axis so, they can be chiral in nature.

So, in simplistic form to find out a molecule or an object whether it is chiral or not? We have to simply just find out what is the point group of it? If it belongs to C_n or D_n it will be chiral in nature.

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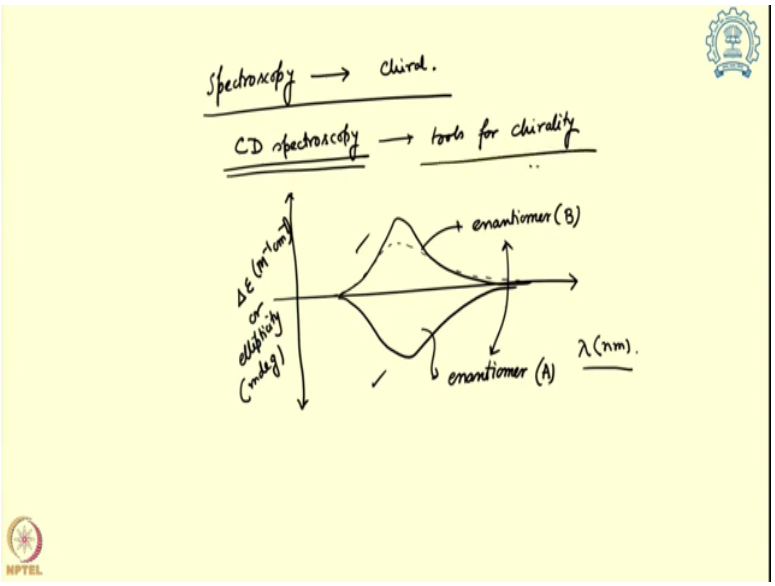

And then we try to find out which particular spectroscopic technique we can perform to find out whether the molecule is chiral or not? And we find out there are multiple options and among them CD spectroscopy is one of the leading one. We also have optical rotation along with it but series spectroscopy is a crucial one. Where we found that we can observe it is optical absorbance and if you have the optical absorbance band like this and if it the molecule is chiral.

And this particular band is chiral you are going to see some portion of it through chiral or it can be the opposite direction to which will define two different enantiomers. So, if say enantiomer A and enantiomer B are actually belongs to the same molecule that means they are enantiomer of each other. These two will show a CD spectroscopic curve where the x axis is the wavelength and y axis is the $\Delta\varepsilon$ $M^{-1}cm^{-1}$ or we can also put that with ellipticity which is given in mdeg unit.

And we can find yes, a molecule is chiral or not? By CD spectroscopy if you get a signature in the CD spectroscopy then you can say yes, my molecule is optically active. And very interestingly this chiral molecule if you are seeing in a CD spectra that CD spectra should always have a precursor optical spectra, unless you have optical active band you cannot have a CD active band.

So that is why first we perform an optical spectroscopy experiment figure it out which are the bands we are looking into? And then if your molecule is chiral then you will find the CD active band among all this available optical active bands. So that it is thing we found and that is why CD spectroscopy becomes one of the important tools for finding out whether my molecule is chirality or not?

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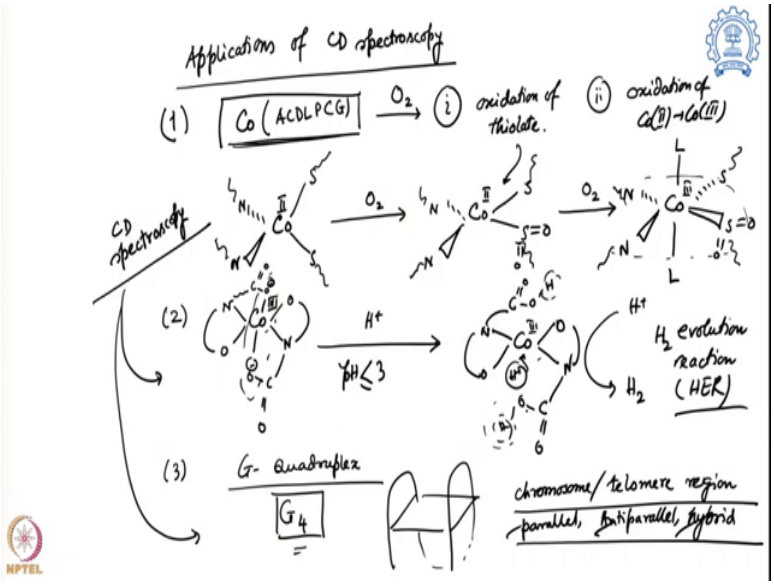

Next, we look into another system which is nothing but applications of CD spectroscopy. Next, we look into applications of CD spectroscopy. And over there so far we have covered three different examples the first one we covered it is actually a cobalt based molecule which is bind with a peptide A C D L P C G. And on the last class we have covered that or we found this molecule in terms of oxygen it goes sequential oxidation, first there is an oxidation of the thiolate ligand.

Because over here when you talk about this system it is found that it is being bound with 2 nitrogen and 2 sulphurs. And then the first molecule of oxygen reacts with it and in the Co^{II} state, the cobalt does not change it is oxidation state but the sulphur, one of the sulphur becomes sulphinate or SO_2. And then further reaction with oxygen then the cobalt becomes Co^{III} and along with it this sulphonate group comes into the plane of the 2 nitrogens.

And it goes to a ligand or solvent the axial ligation and it goes to a octahedral geometry. So, over there the first is oxygen of the thiolate and the second one is oxidation of Co^{II} to Co^{III} and that we have found over here. And this particular sequential oxidation we found with respect to CD spectroscopy. Because CD spectra give us the first hint that there is a change in the molecular primary structure.

Because all those things are actually part of this peptide which is chiral in nature. And as I am going through sulphur to sulphinate. Sulphur to sulfinate this actually triggers a change CD spectroscopy can monitor that. And then over here the Co^{II} becomes Co^{III} and it goes to an

octahedral geometry and again CD spectroscopy can configure that. Because the presence of this chiral ligand scaffold and it is interaction with the central metal atom.

Over here I want to mention one more time the metal itself is not chiral in nature, the metal active site. However, the presence of chiral ligand around it induces optical activity to this metal centre, specially metal centred optical absorbance band like d-transition band or LMCT band so, those becomes chiraly active. The second example we figured out in another sample with a cobalt salen ligand where this is actually bind with a nitrogen and oxygen molecule.

And over there we found this molecule is actually bound with the carboxylate ion and this is actually coordinating the cobalt especially in the Co^{III} oxidation state. And stop it from binding to a another protons to produce hydrogen and this can only occur if I acidified it enough and the pH goes to below 3. Only then this molecule actually opens up because nitrogen and oxygen remains as it is but this carboxylic acid group becomes COOH.

So, it does not bind and only at the same time this protons are actually also acts as a proton delay. So, proton can directly go and bind with the cobalt and it can trigger the proton to hydrogen transformation or hydrogen evolution reaction, in short from we call them HER. So that is possible to happen and we found it is happening over there because this is getting protonated and ligate off from this cobalt.

And again, CD spectroscopy is actually allowed us to follow that when we record the CD spectroscope at different pH's we found when I am crossing this barrier of pH 3 to lower than that. We get a significant and visible change in the CD spectra which signifies there is a change in the primary coordination sphere of the metal. Because this particular band is an optically active band in the visible region, not coming from the ligand itself but belongs to the metal centre.

And that becomes CD active not only that it shows as a change this alteration allows us to follow what is happening in the system. And the last one we followed with G-quadruplex which actually shows that the 4 guanidine can form a nice planar structure which typically remain in the bottom of the chromosome in the telomere region. And this particular G-quadruplex can form in different forms parallel, antiparallel and hybrid a mixture of these two.

And again, CD spectroscopy can tell us how this molecule is going to all these different kinds of possible orientation? And this has particular signature and we can easily follow that to find out whether the molecule the G-quadruplex is forming in the parallel, antiparallel, hybrid mode. And also, it will also allow us to find out whether I have a G-quadruplex or not?

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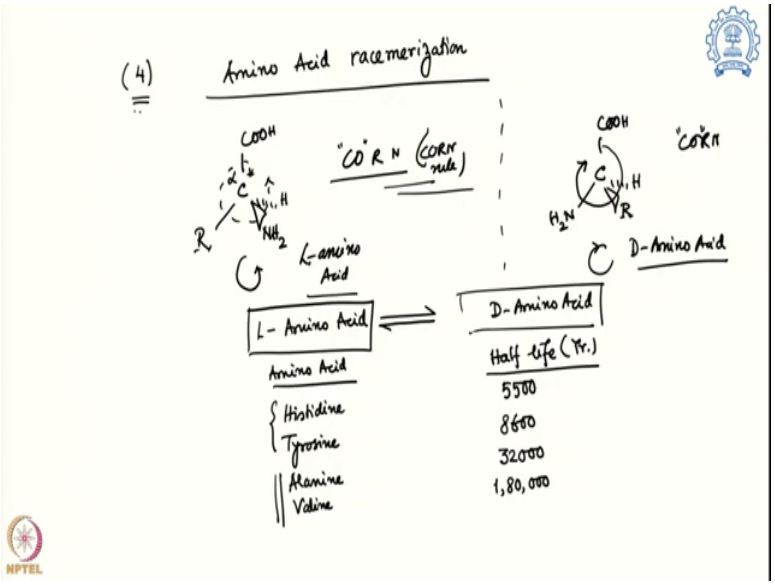

Then today we are going to follow an another segment about amino acid racemerization. So, when you talk about an amino acid, we already discussed that this this molecule is tetrahedral in nature around the α carbon or the chiral carbon. Where if you put the hydrogen at the back carboxylic acid over here the R group over here R is the changeable group of an amino acid and this is the amine group over here.

We find out how it is oriented the carboxylate group R group and N group which is known as the corn rule? And you can see it is oriented left and right so, this is the L-amino acid. And there is also possibility of presence of another molecule, where now I am putting R over here N in over here. Now, you can see the corn rule still applies here but now I am moving in the opposite direction right hand side direction.

So, this is the D-amino acid. So, we have discussed earlier L-amino acid is the most dominant form found in the nature when nature produces amino acid it is L-amino acid in nature. And this particular orientation why it is preferred? There are multiple versions of that why it is happening? And we have discussed even the possibility of an extraterrestrial material is

actually influencing that which actually triggers the formation of amino acid of a particular geometry or chirality is the L-amino acid.

Now, the question is what happens if I leave the L-amino acid for a long time in presence of water? It slowly converts to D-amino acid. So, I should put it this way so, it is reversible but it is slowly convert to D-amino acid. So, once but it is L-amino acid it can be D-amino acid in a while. Now, I am going to give you several example of amino acid and will give you a time scale in the terms of half-life which gives us an idea.

How much time it requires to produce an L-amino acid to D-amino acid? So, if you start with 1g of L-amino acid it will slowly start producing D-amino acid. And at one point of time you will find only 0.5g of L-amino acid is actually present the rest of them become D-amino acid. So, if you follow this reaction it will be a faster reaction in nature and from the faster reaction you can find out the half-life.

How much time it requires to get half of the amino acid to resemerize? And if we look into different amino acid, we will take histidine as an example and the half-life for L histidine to convert converted to D histidine how much time it takes it takes 5,500 years this is a huge long time. Then if I take tyrosine the time scale is almost 8,600 years it is longer. If I take alanine there is a polar amino acid it has a half-life period of 32,000 years say very long time.

And then valine and amino acid with particular hydrocarbon chain and it is one of the slowest to convert to D-amino acid at 1,80,000 years. So, you can see it is actually quite slow in nature now the first question is why there is a difference over here between these two versus this hydrocarbon one as side chain? And secondly how I can follow it up? How I can follow this racemerization is happening? First we try to understand why the racemisation happens? And how it is happening?

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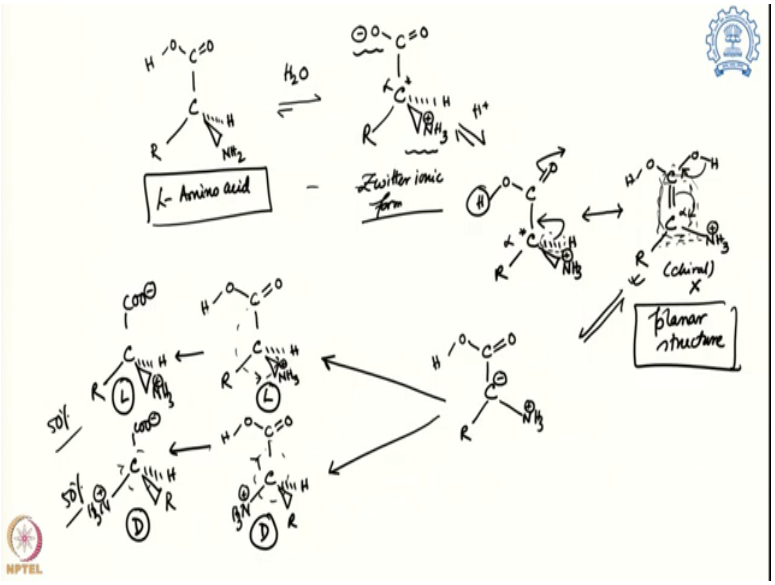

So, for that we will start with the amino acid structure so, again I am drawing L-amino acid this is the structure we draw. But in reality in presence of water what happens? They actually stay in their most stable form. The carboxylic acid group becomes carboxylate because in neutral water around pH 7, the carboxylate is the most stable form R group remains as it is the nitrogen the amine groups becoming NH_3^+.

Because that is the most stable form of any primary amine group in water at pH 7. So, you can see that one side of the system is a positive ammonium group one side is negative carboxylate group and this particular form is known as zwitter ionic form. This zwitter form is the most prevailing form for any amino acid present in nature and neutral condition and this is where all the reaction began when it start racemizing.

So, the next thing happens this particular molecule react with some acid and this actually form the protonation form. So, this particular group gets protonated carboxylate the rest of it remain as it is. So, only the protonation of the most charge group over there it is a carboxylate it becomes carboxylic acid. Then comes the next step this proton over here C-H bond folds out and create a C=C and if you want to create a C=C over here.

This particular group has to move and becomes O^- which actually takes this particular proton. And if this is actually happening then what we are going to see is the following it is more of an tautomeric form. Now, this any NH_3^+ group come into the plane of the molecule so that is what is actually happening when this tautomerism happens. And over here you can see now my α carbon was there previously it was terrible in nature.

So, it is holding it is chirality but now what happens? It becomes a part of a C=C. Once it form the part of a C=C it becomes planar in nature and once it form a planar structure then it loses it is chirality so, it is not chiral because this actually contain a plane of symmetry. So, now what happens? This becomes achiral and from here the molecule wants to turn back because this is not a very stable form.

However, there is a possible structure it can go through as a tautomeric structure. And how it is going to turn back? It is going to return back the electrons over there this will come over here and this becomes carbanion and this carbanion is going to form over here. And this carbanion is going to abstract the proton and form the amino acid back and because it is a carbanion derived from this C=C planar structure it can go either direction.

And there it forms either of the amino acid so, this is one side and there is also possibility of the other side. So, this becomes NH_3^+ this becomes R so, you can see over here carboxylic R amine. So, this is the L-amino acid and over here carboxylic R amine so, this is D-amino acid. And later on, you can lose this extra proton over here and from carboxylate R group NH_3^+ and H are carboxylate NH_2 sorry let us put R over here NH_3^+ and H and this becomes the D-amino acid over here.

So that is how starting material and an L-amino acid has to go through a planar structure where it can lose it is chirality. And from this planet structure it can again regain the chirality but over here if it is happening without any chiral environment in nature itself. But without any chiral environment it would produce both of them 50:50 and at one point time they will racemize. So that means I will see both the amino acid D and L present in the similar structure.

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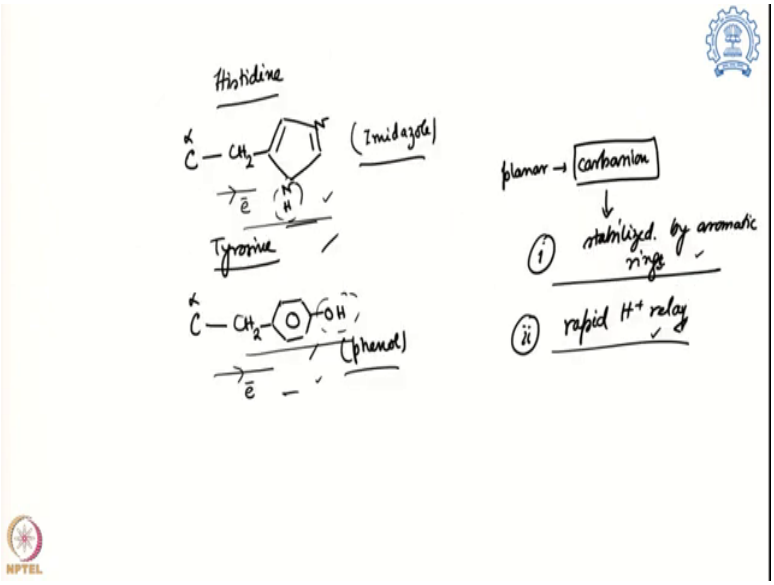

So, now the question is why histidine and tyrosine are better? So, for that I am going to draw the side chains of them. So, side chains of histidine is CH_2 five membered ring so that is how the structure look like for histidine. For tyrosine there is a structure. So, now over here you can see histidine and tyrosine this is actually imidazole ring this is actually phenol ring so, both of them are aromatic in nature.

So, they actually have a natural drift of electrons towards them. So, through this electronic movement that the electron is going to come towards this electron dense aromatic rings so, they can stabilize the extra electron. And this carbon if you remember it is bind with the α carbon which is actually forming the carbanion. So, this planar structure then it goes to this carbanion intermediate this carbanion intermediate is actually getting stabilized by this aromatic ring.

And that is actually one of the factor which stabilizes the carbanion intermediate with this particular group. And if it is stabilized more that means as we saw in the previous structure the possibility of coming from this part to this particular carbanion intermediate is high. So, you will end up at a higher rate in the racemisation region and that is why it is happening for histidine and tyrosine.

And one more factor also come into the picture that is due to the rapid proton relay. As you can see during this particular carbanion formation from the original structure this exchange of protons between the systems with the broad peak which is actually present in the vicinity.

And over here this particular tyrosine, phenol or imidazole they actually have some groups which can exchange proton very fast.

So, this actually create a rapid proton relay so, when this carbanion is forming a lot of proton has to be exchanged from this place to that place. And this particular groups provided the source of the proton which is very near to it. So that is why that is also an another addition to this whole process. So, it is stabilized by aromatic ring through -I effect and rapid proton relay, both of them affect the first formation of the carbanion intermediate.

Which is reflected into the first racemization compared to the other groups which do not have them. And that is why we find that the histidine and tyrosine is moving very fast whereas alanine and valine is pretty slow. Because they do not have a group which can stabilize the carbanion and not they have protons to exchanges first.

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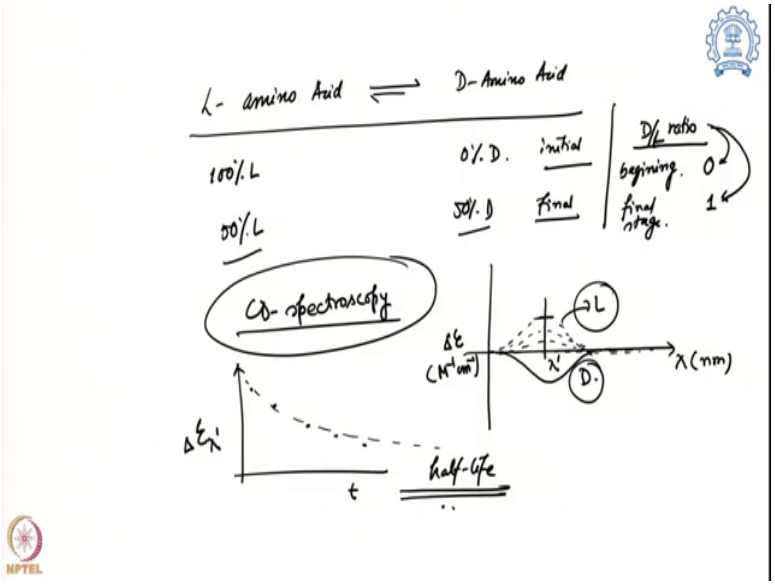

Now, the question is how do I find it? How do I monitor that my L-amino acid is converting to D-amino acid. So, first of all we have to find out that when we start this reaction, we have 100% L and 0% D. And at the end of the reaction when the reaction will be end, will be ending of 50% L, 50% D so that is we are going to finally get if we allow this reaction to go for thousands of years.

So, what we actually do we follow this reaction with respect to D and L ratio. In the beginning of the reaction there is actually no D-amino acid at all so, it should be 0 and at the final stage because there is 50% L, 50% D, D/L ratio would be 1. So that is what we are

actually going from D/L ratio 0 to D/L ratio 1. So, how do you monitor that? We can use again CD spectroscopy.

Where we can follow CD spectroscopy of the amino acids where we start say this is the L-amino acid and this is the D-amino acid. So, when we start we will see only L-amino acid and slowly with respect to time I start seeing this is actually going down. And at one point time it will be 0, there will be no signature. Because D and L will remain in the similar concentration so that is I am going to follow.

So, what I can follow is that particular wavelength over here. What was the Δε? And how it is changing with respect to time at this particular wavelength and with respect to time? So, this should slowly come down and that we can measure and extrapolate it to figure it out what will be the half-life of this reaction? And that is how it is being measured of thousand years.

And more than thousand years nobody is doing the experiment for thousand years running so it is an extrapolation of the result. And what is the advantage? So, as we know this an L-amino acid is slowly going to racemize to a D-amino acid so that means if I am doing this reaction even for a fossil an old stage sample. I can look into what is the D and L comparison? What is the absolute Δε value? I know the 100% L, 100% D value.

So, from there I can back calculate how long the system was present in a biological form where L-amino acid will be preserved 100%? Once it is tied it is out of this biological chiral environment, only then it starts to racemize and go to 50% L, 50% D. So, from there we can actually extrapolate that. So that is why CD spectroscopy can also be used in studies of old samples palaeontology and all those things, to figure it out what is the age of different fossils.

So that we would like to conclude our segment over here. Where we found CD spectroscopy can be a useful tool to find out the racemization rate of L and D-amino acid in natural phenomena. And by that we can follow the age and the reaction time of different old fossil fuel samples. Thank you. Thank you very much.