Chemical and Biological Thermodynamics: Principles to Applications

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Lecture - 50
Calorimetry in identifying partially folded states of proteins (Molten Globule State)

Today we will discuss calorimetry in identifying partially folded states of proteins. We have earlier discussed the significance of understanding the protein folding problem. Since we do not know the protein folding code therefore, it becomes very important to understand about the intermediates which occur during the protein folding. And as we discussed earlier lot of efforts are being directed towards that, and slowly and slowly we are understanding more and more about these protein folding problem and if we can understand the intermediates which occur during the protein folding pathway in a qualitative and quantitative manner. That is a contribution towards understanding protein folding problem.

The protein folding is quite fast process. And therefore, studying the kinetic intermediates which occur during protein folding becomes relatively difficult. It will be very important if we can isolate such intermediate states under equilibrium conditions. And then study these intermediates both qualitatively and quantitatively we can get lot more information about these intermediate states. One such intermediate state which became very popular in late 80s and early nineties was molten global state. And today we will specifically focus on the molten global state or partially folded states what are these states, and how we can use the concepts of thermodynamics in identifying these states and getting qualitative and quantitative information about these states. Let us discuss this in details.

Let us take a look at this slide.

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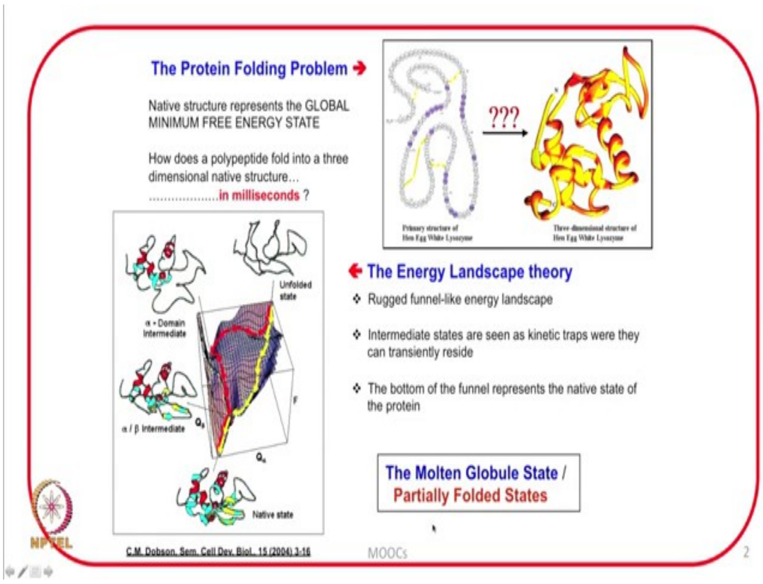

We are revisiting the protein folding problem. The protein folding problem is how this primary sequence folds into a unique 3 dimensional confirmation this question mark is the protein folding problem. Why it folds into a unique 3 dimensional confirmation? And then we discussed that one of the most popular model which has been used in explaining the protein folding is energy landscape theory. And there I highlighted the molten global state slash partially folded states. This becomes more clearer in the next slide that after the protein is synthesized from ribosome's.

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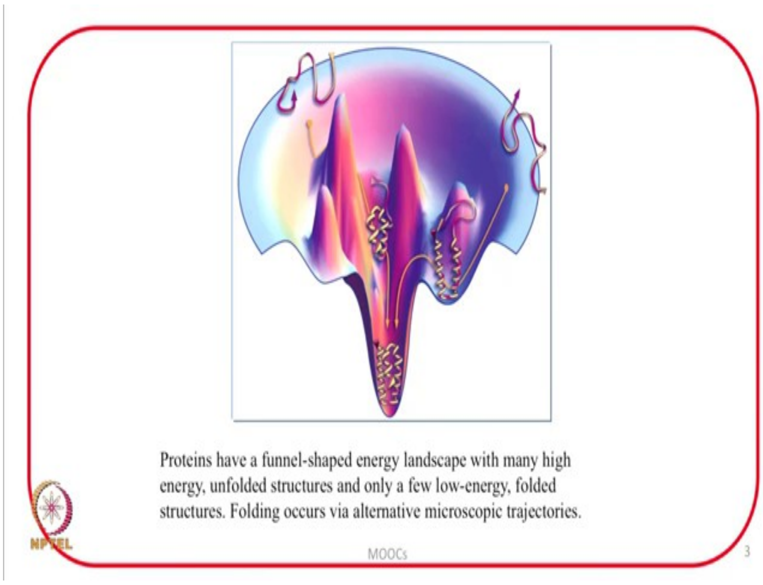

And this primary sequence of amino acids it has to adopt secondary structure and finally, get the minimum energy 3 dimensional confirmation which is the native state of the protein and it goes through several intermediate states.

Today we are going to discuss that if we can get qualitative and quantitative information about such intermediate states, then it is a contribution towards the protein folding problem.

Now, as I mentioned in late 1980s and early 1990s a term molten globule state became very popular.

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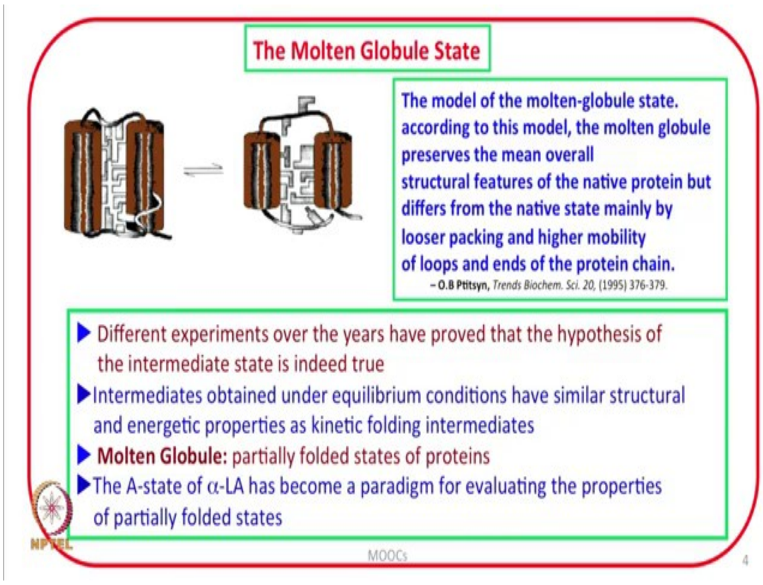

As the name itself suggests molten globule state it is the molten form of the globular protein. Molten in the sense that it is not a rigid 3 dimensional structure, but it can be in between the native and denatured state of a protein. Let us take a look at this figure. This figure represents a cartoon picture of the formation of molten globule state, and this was proposed by Ptitsyn in 1995 were this part of the figure represents the native state and this part of the figure represents the molten globule state.

And the difference between the 2 is some of the structural features of the native state are retained, but some of the structural features of the native state are lost. So, this is a relatively molten form of the native state. And that is what is commented over here, the model of the molten globule state according to this model the molten globule preserves the mean overall structural features of the native protein, but differs from the native state mainly by looser packing and higher mobility of loops and ends of the protein change.

Before we actually start discussing the thermodynamics of the molten globule state it will be very important to first understand; what is molten globule state. Let us discuss a little bit more about this and also what is the relevance of the molten globule state with the actual intermediate states which are observed in the protein folding pathways. Let us take a look at the comments. Different experiments over the years have proved that the hypothesis of intermediate state is indeed true. Several groups have been working. From different angles in understanding the protein folding pathways, and hence the protein folding intermediates. Lot of spectroscopic techniques have been applied to get qualitative information on the protein folding intermediates; however, along with the qualitative information getting quantitative information becomes very, very important. Direct application of calorimetry can give quantitative information. We will discuss how that information can be achieved in today's lecture.

Let us take a look at the next comment; intermediates obtained under equilibrium conditions have similar structural and energetic properties as kinetic folding intermediates. This comment is based upon the literature information. Since the protein folding is a very fast process, if we can isolate the intermediate structures intermediate states under equilibrium conditions, it becomes easy to study those literature suggests that it is possible to obtain intermediate structures under equilibrium conditions. And we will discuss a little bit more about that a bit later.

Now, let us take a look at the next comment. Molten globule partially folded states of proteins. Let us discuss; what is a traditionally accepted definition of molten globule state. Several researches defined molten globule state of a protein as a state in which the secondary structure is similar to that of the native protein or even strengthened, but the tertiary structure is poorly defined or tertiary structure is lost. So therefore, the molten globule state a sort of became a specific title for a state in which the secondary structure is retained, but the tertiary structure is lost let me also make it very clear over here.

Since we are discussing about some specific research area today it is not always true that a given terminology is accepted by different authors or different researches. Molten globule state anyway is a state which is in between the native state and the denature state.

And as I said that several researches define the molten globule state, as a state which specifically has no tertiary structure or poorly defines tertiary structure, but the secondary structure almost same as the native protein. Whereas, some sort of researches believed that any intermediate state which is in between the native state and the fully denatured state is an intermediate state, and getting information about those intermediate states is anyway important, but they did not believe the concept of the molten globule state.

So, this controversy one may find in literature, but never the less there are several reports which have demonstrated that such intermediate states do exit is in which the tertiary structure is lost or poorly defined, but secondary structure is intact.

Now, let us take a look at the next comment the a state of alpha lect albumin la I am using abbreviation of alpha lect albumin has become a pyridine for evaluating the properties of partially folded states. I will take several examples by using the protein alpha lect albumin. Alpha lect albumin is a milk protein. It has calcium in it. What happens is that if you remove calcium from alpha lect albumin it goes to an a state it is called an a state which is the molten global state; that means, if you remove calcium from alpha lect albumin the tertiary structure becomes poorly defined, but the secondary structure remains intact. We will discuss more about this.

Now the question is that if we want to generate these partially folded states or so called molten globule state elaborately, what should be the experimental conditions what kind of additives we can use to generate these partially folded states? We know that urea can denature the protein at a certain concentration or above a certain concentration. Usually, 6 molar or 8 molar urea is sufficient to denature a protein. Similarly guanidinium hydrocolride is a protein denaturant. So therefore, if we use an additive or a co solvent which can partially destabilize the protein partially denature the protein should assist in pushing the native state to an intermediate state. And let us take ax let us take a look at slide see what kind of additives can be used to generate partially folded state.

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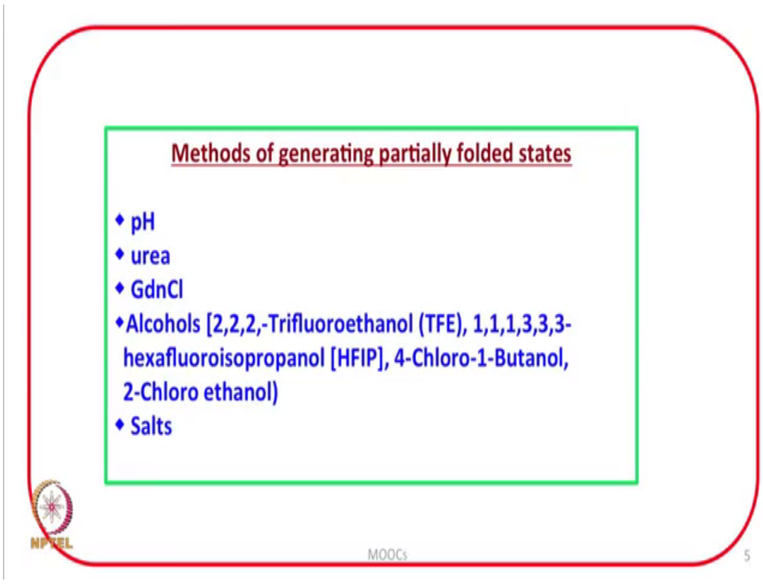

One is pH, we have discussed pH at length, and how pH can affect the conformational stability of a protein. Suppose if we keep on increasing the pH or decreasing the pH, both extremes of the pH can lead to denaturation of a protein. For example, if we keep on decreasing the pH, at very low pH let us say at pH 1.5 or one the proteins becomes very, very strongly protonated and there is a lot of positive charge. And due to the positive charge repulsion the protein may unfold it may undergo denaturation. And the same thing can be expected when the pH is very, very high. So therefore, by altering the pH some proteins may show a confirmation which resembles a molten globule like confirmation.

Now, let us say take a look at the next possibility urea. Urea is another molecule which affects the protein confirmation. It is believed that urea can affect the protein confirmation by altering the structure of water, because urea can form hydrogen bonds with water it will alter the structure of water to large extent, an alteration in structure of water can ultimately affect the protein confirmation. Urea can also disrupt the protein structure by directly interfering in the intra molecular hydrogen bonding in protein. So therefor urea is another molecule which can push the native state of a protein to an intermediate structure.

Now, let us take a look at the next one, next is guanidinium hydrochloride or guanidinium chloride. Guanidinium chloride is another molecule which is known as a protein denaturant. Literature search suggests that there are several alcohols for example, TFE 2 2 2 trifluoroethanol or 1 1 1 3 3 3 hexafluoroisopropanol which is abbreviated as HFIP. These 2 proteins have been extensively used in literature to generate the molten globule like structure. This trifluoro group or hexafluoro group actually acts as a hydrophobic group, and what has been observed in literature that at certain concentration of TFE and HFIP the tertiary structure of a protein is lost and the secondary structure of the selected proteins remain intact.

And this is the confirmation in which the tertiary structure is poorly defined or tertiary structure is lost, but secondary structure is intact is termed as molten globule state. And let us again go back to the slide. The other alcohols which have been used to generate the molten globule like structure is 4 chloro butanol and 2 chloro ethanol.

The next category can be salts. Let me discuss how salts can be used to generate the partially folded state or molten globule like state in a protein. I just discussed that if we keep on lowering the pH, at very low pH extensively positively positive charge will be there on the protein because there prots because of strong protonation. And that excessive positive charge into positive charge repulsion the protein may denature.

Now at very low pH if we introduce large anions, these anions can neutralize the positive charge to certain extent and will induce refolding in the protein. And this refolding can induce a molten globule like confirmation. And in other words the refolding will lead to formation of certain intermediate confirmations that is how salt becomes important. And similarly there can be other variety of experimental conditions which can help in generating the partially folded states of the protein.

Now, the next question is before we actually address the thermodynamic aspects. First we need to have characterization of these partially folded states. What kind of methods can be used for a qualitative characterization of these partially folded states? And if we are interested specifically in addressing the molten globule like confirmation in which the tertiary structure is poorly defined and secondary structure is intact, we should use a method which can directly give us information about the secondary structure and tertiary structure of a protein. And there are spectroscopic methods available. One such spectroscopy which directly gives information about the secondary structure and tertiary structure of a protein is circular dichroism spectroscopy. The principal of circular dichroism spectroscopy you will learn somewhere else, but let me very quickly tell you what the circular dichroism spectroscopy measures and how we can use it like a finger printing technique to get information about the secondary structure and tertiary structure of a protein, it not only gives information about the proteins it can also be extended to other biological systems including DNA.

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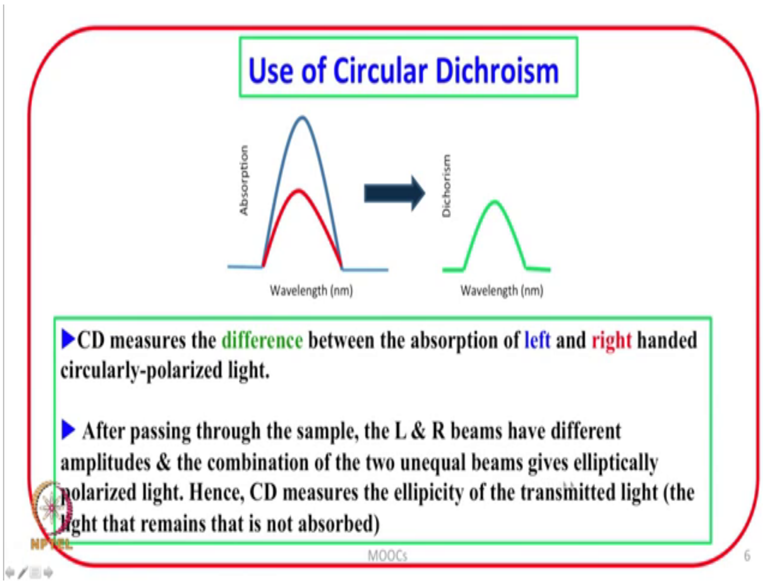

In circular dichroism spectroscopy, the sample the chiral sample the sample which have molecules have having chirality, will absorb left circularly polarized light and right circularly polarized light to different extents. And it is the difference in the absorption which is measured in the circular dichroism spectroscopy. Else we can see in this figure. Let us say one of these absorption we take as left circularly polarized light and the other is right circularly polarized light and the difference is measured in circular dichroism spectroscopy.

The main features that we need to know, are that CD measures the differences between absorption of left and right handed circularly polarized light. And let us keep in mind the next comment also, that after passing through the sample left and right circularly polarized lights have different amplitudes and the combination of the 2 unequal beam gives elliptically polarized light. I suggest you to go through the principle of circular dichroism spectroscopy if you would like to learn more in details how the elliptically polarized light is obtained. And hence the CD measure the ellipticity of the transmitted light the light that remains that is not absorbed and pictorially it is represented in this figure.

Now, this ellipticity as a function of wavelength can be correlated to the secondary structural and tertiary structural content of a protein.

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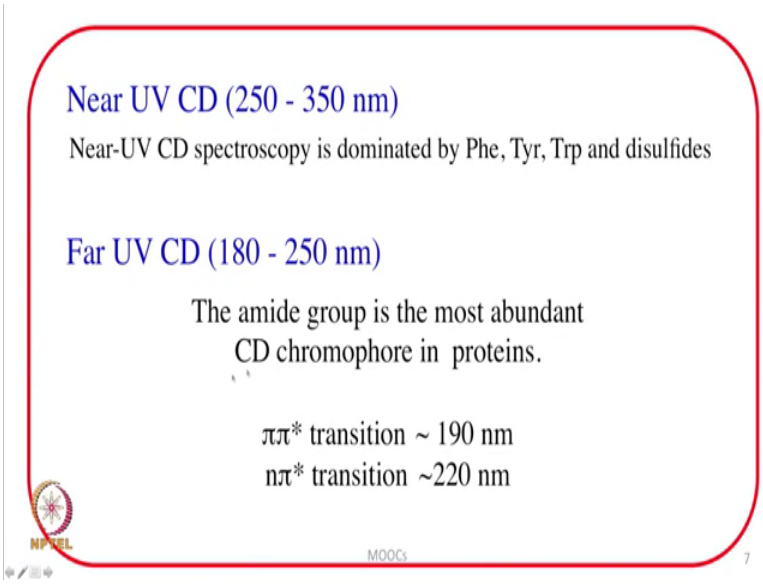

Let us quickly take a look at near UV CD and far UV CD features. Near UV CD which is in the range of 250 to 350 nano meter is dominated by phenylalanine tyrosine tryptophan and disulfides. And the phenylalanine tyrosine tryptophan disulfide basically define the tertiary structure of a protein; that means, near UV CD signals will carry information about the tertiary structure of a protein. And the far UV CD which is in the range 180 to 250 nano meter.

In which amide group is most abundant chromophore CD chromophore in proteins give rise to bands. For example, pi pi star transition will be observed at one ninety nano meter and n pi star transition observed at 220 nano meter, but what is more important at this point is for understanding qualitatively the secondary and tertiary structure of a protein the numbers presented in this table are important.

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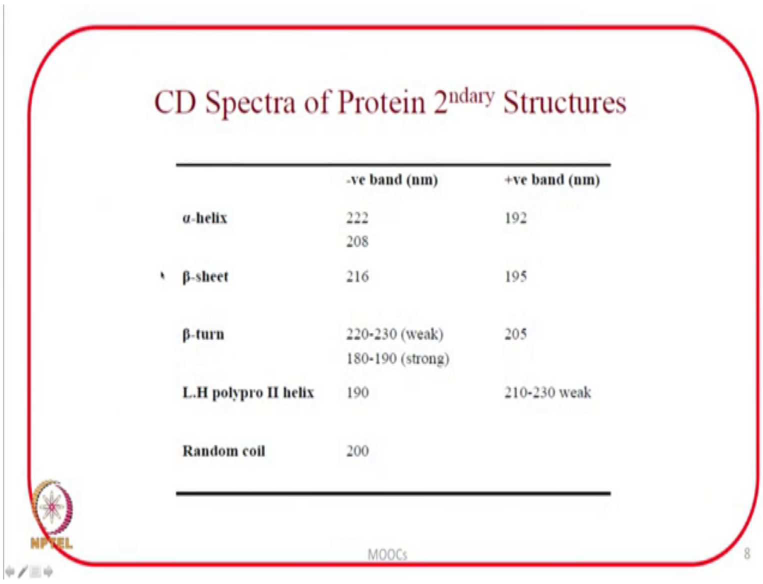

For example, the different secondary structural elements for example, alpha helix will show a negative band at 220 nano meter and a positive band at 192 nano meter. And alpha helix is also accompanied by a negative band at 208 nano meter. So, the bands at 208 222 negative bands and a positive band at 192 nano meter will be characteristic of alpha helix. A negative band at 216 nano meter and a positive band at 192 nano meter are characteristic of beta sheet. Beta turn is accompanied by weak negative band in this range 220 to 230 nano meter and a strong negative band 180 to 190 nano meter and a positive band at 205 nano meter. Similarly a random coil will show up a negative band at 200 nano meter.

So, these numbers can help us in qualitatively establishing the secondary structure and tertiary structure of a protein. And the changes in ellipticity as a function of temperature or as a function of the concentration of additive can tell us that how relatively the extent of secondary structure or tertiary structure changes when the environment is changed.

Let us take a look at the next slide.

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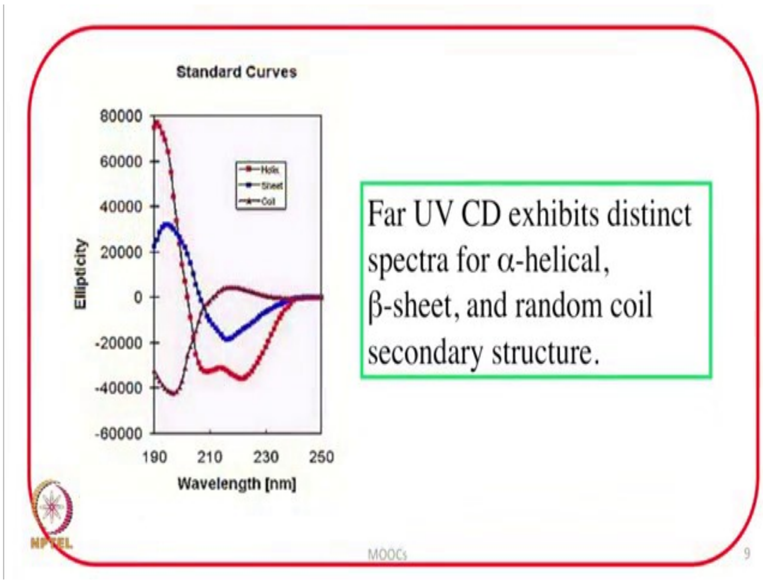

Now these are the far UV CD characteristics for alpha helix the red one, you see 2 bands 2 negative bands these characteristic this kind of spectra is characteristic of alpha helix. Beta sheet is showing one negative band one positive band here. And the random coil is showing a negative band the numbers where given in the table that I showed in the previous slide.

Now, let us take a look at the next slide.

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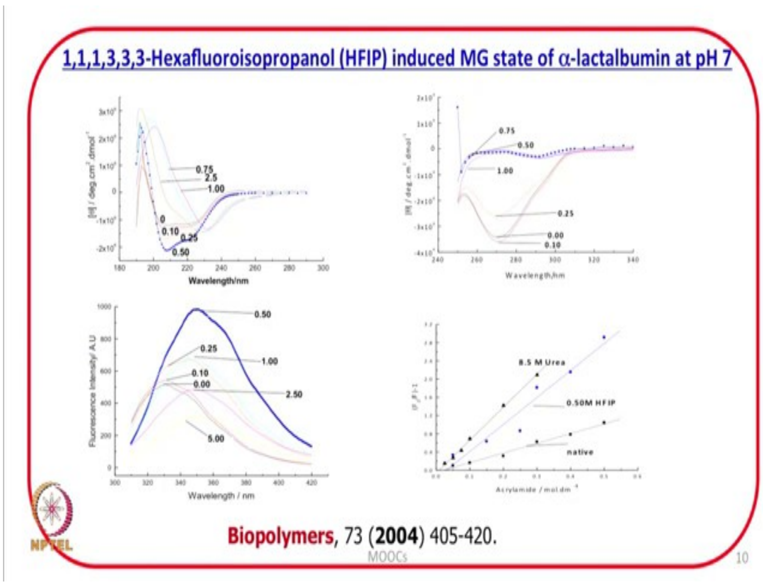

We will discuss about the molten globule state formation and it is thermodynamic characterization in the next lecture, but alpha lect albumin at pH 7 without the removal of calcium ion shows a molten globule like state in presence of 1 1 1 3 3 3 hexofluoroisopropanol. And we have just discussed that how the circular dichroism spectroscopy can be qualitatively used in establishing the molten globule like state if we look at the upper panels these are the far UV CD and near UV CD spectra of alpha lectalbumin in presence of different concentrations of hexafluoroisopropanol. For the time being if we just concentrate on the blue line, it shows 2 negative bands at about 205 and 222 nano meter characteristic of alpha helix.

Very strong secondary structure just similar to that of the native one native one is shown over here 0 mil 0 molar; that means, the native the secondary structure is relatively stronger than that in the native structure, but look at the blue line here the ellipticity is almost 0 in presence of 0.5 molar hexofluoroisopropanol tertiary structure is lot. In presence of 0.5 molar hexofluoroisopropanol secondary structure is strengthened tertiary structure is lost and therefore, this concentration induces molten globule structure molten globule confirmation in the protein. The lower panel is fluorescence characterization and that we will discuss a little later, but the circular dichroism spectroscopy is directly telling us that 0.5 molar hexafluroisopropanol induces a molten globule state in the protein.

So, what we have discussed in this lecture is that; what is the significance of studying the partially folded states of a protein, what is a molten globule state, how to experimentally obtain a molten globule state under equilibrium condition, and how to characterize a molten globule state in a qualitative manner? That characterization we have discussed only by using one technique as so far. Now that is the circular dichroism spectroscope.

We will try to understand more about quantitative aspects and associate the thermodynamic parameters along with it, and discuss how to obtain those thermodynamic parameters how to get quantitative information about the molten globule state and such partially folded states in the next lecture.

Thank you very much.