Chemical and Biological Thermodynamics: Principles to Applications

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Lecture - 42
Explaining Differential Scanning Calorimetric Profiles (DSC Profiles)

We have been discussing the details of differential scanning calorimetry. And to begin with we have been discussing the examples of biologically important systems, specifically proteins. And since the biological systems mostly are active in aqueous media, therefore, in the previous lecture we also discussed the structure of water. Because any changes in the conformation of a biological micro molecule is going to affect the structure of water. Therefore, it is very important to understand how the structure of water is modified when the conformational transitions in the biologically important systems take place, not only that how the structure of the solvent is modified when even the host guests recognition in biologically important systems take place.

So, let us continue with our discussion on how the structure of water is affected, when different type of solutes are added to it to begin with let me recall back the structure of water that we discussed in the previous lecture, we discussed that liquid water is a mixture of unassociated water molecules.

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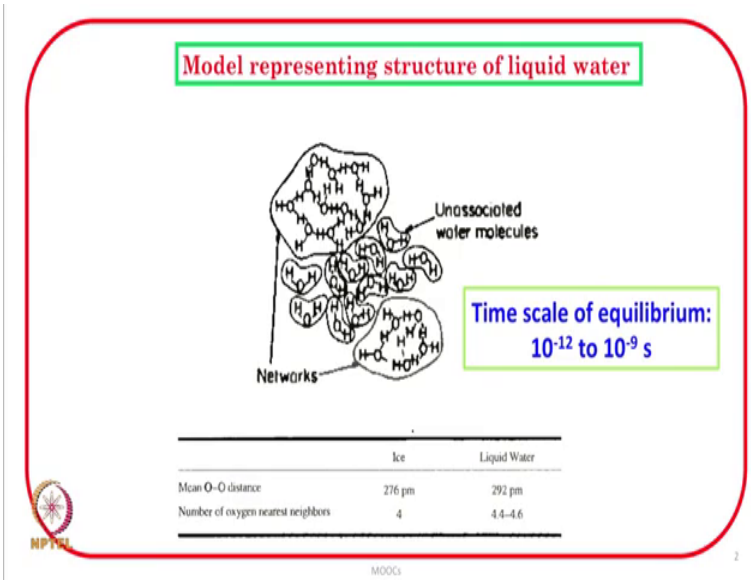

And a hydrogen bonded tetrahedral network in this tetrahedral regular network of hydrogen bonded water molecule there is extensive amount of hydrogen bonding. And we discussed about the time scale of equilibrium and other parameters, which describe the properties of water in the liquid form.

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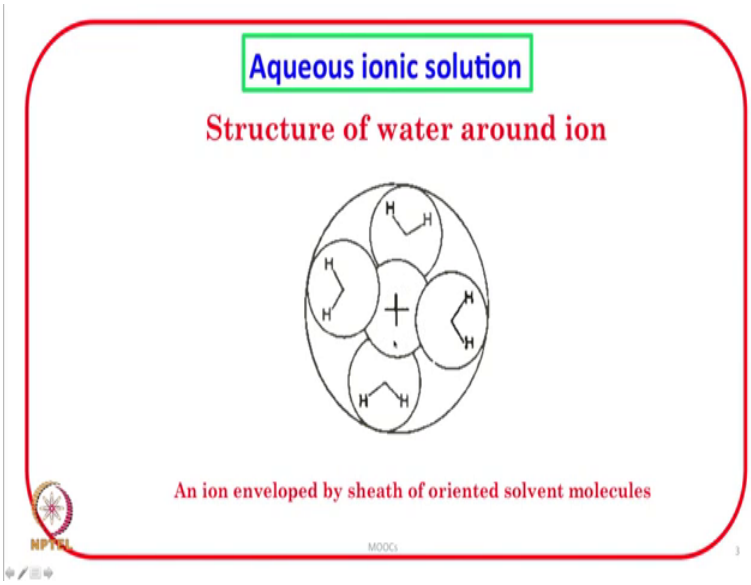

Now, let us first talk about how the structure of water is modified when an electrolyte is added to it. When we talk about the protein molecules, protein is made up of amino acids and amino acids are bonded to each other through peptide bonds. The protein may also have charged to amino acids or protein may also face an environment in which there are lot of ions. And therefore, it becomes important to understand how the structure of water is modified when an ion is added to it or when a hydrophobic solute is added to it.

Let us first talk about what is the structure of water around an ion. How do we generate ions in an aqueous solution. We can take an example of sodium chloride. When you take solid sodium chloride and add the crystal of sodium chloride to water sodium chloride ionizes. It ionizes in the form of sodium ions and chloride ions. Now let us to begin with start with only on cation, let us first consider only one ion in the form of cation and discuss that how that affects the structure of water. Let us take a look at the slide. Now let us consider all of a sudden this central ion appears. This is positively charged and; obviously, it will attract till negatively charged oxygen of water. So, under the influence

of it is electrostatic field the central ion will attract water molecules and through electrostatic interaction, it will be surrounded by a certain numbers of water molecules.

Now, let us remember that these water molecules, which are surrounding the central ions were earlier part of the tetrahedral network. And therefore, when these water molecules are surrounding the central ion. These are no more associated with the rest of the water molecules. In other words what I am trying to say is that these water molecules loose there kinetic freedoms to the central ion. Wherever the central ion moves in solution these many water molecules will move along with the ion. Please carefully listen to the word that I said that these many water molecules will move along with the central ion. When I say these many water molecules I am not saying that these only water molecules, what I am trying to say is it is possible that there may be 4 water molecules directly associated with the central ion I seen in the slide this number can variate.

The number at a given temperature may remain constant, but the residence may not be same because when this central ion is moving somewhere these water molecules may become a part of the tetrahedral network and replaced be replaced by another water molecule. So, these water molecules are not imprisoned with the central ion for the rest of their live, but these can be exchanged with other water molecules; however, the number of water molecules which are there in this primary hydration shell I am calling this as primary hydration shell in which the water molecule lose their kinetic freedom to the central ion. So, that is why this is an ion envelope by a sheath of oriented solvent molecules. These water molecules these number which lose their kinetic freedom to the central ion is called the primary hydration number.

And obviously, this primary hydration of the central ion has affected the rest of the structure of water and before we start connecting this disturbance is in the structure of water with the thermodynamic parameters. Let us discuss what happens beyond the primary hydration shell.

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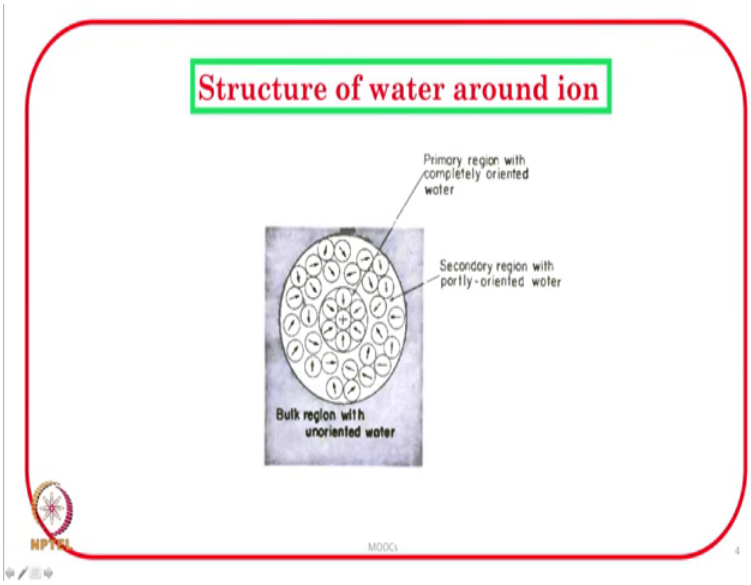

let us take a look at what happened beyond the primary hydration shell. I just talk to you about the central region, that is you have a positively charged ion and it is surrounded by the negative end of water molecules. And a certain numbers forms the primary hydration shell and primary hydration shell you say is clearly demarked from the rest of the water molecules by a solid boundary.

Beyond that the water molecules which are there will be under the electrostatic field of the central ion; however, the rest of the water molecules would also like that these water molecules should engage in hydrogen bonding. So, therefore, beyond the primary hydration shell these water molecules may compromise between the electrostatic fields of the ion and the hydrogen bonding ability of the rest of the water molecules. And therefore, you see their orientation is not exactly oxygen pointing out towards the central ion; however, they are in intermediate orientation and this is called the secondary region with partly oriented water.

The primary region is with completely oriented water secondary region is with partly oriented water and rest of the water is a bulk water, that is in this shaded region you have a tetrahedral network of water which is in equilibrium with the monomeric water; however, this one ion has altered the structure of water. And if you try to rationalize overall because when you add sodium chloride, you are not just adding one ion if you calculate how many ions of sodium chlorides are there in aqueous solution when you

prepare even 0.01 milli molar solution you can do a calculation and find out you will see the number of ions in solution are anormalsand we can appreciate that how those many ions will affect the structure of water.

And if we go back to the slide, suppose if lot of water molecules are oriented around the ion or lot of water molecules come under the electrostatic potential under the influence of the electrostatic field of the ion then it will lead to collapse a partial collapse of the structure of water. Let us keep this things in mind.

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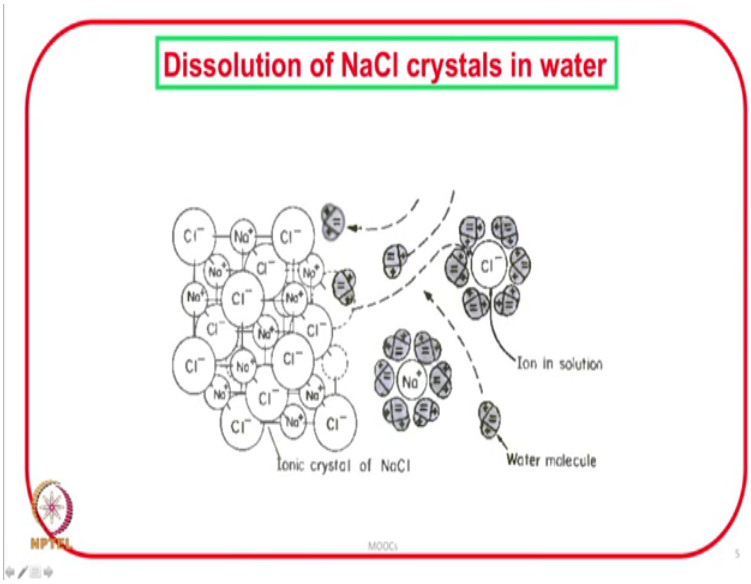

So, what happens when you dissolve sodium chloride crystals in water? Sodium chloride from the crystal is ionized and you see sodium ion is surrounded by water molecules where the positive charge interacts with negative end of water. Similarly chloride ion is surrounded by water molecules where this ion is interacting with the positive ends of the water molecule. So, this is the scenario of the solvent structure around the central ion, but when it comes to biological macro molecules, when we talk about the proteins or when we talk about the DNA or we pick up any other biologically important systems.

Let us first talk about the proteins what do the proteins have the proteins will have peptide bonds the proteins will have hydrophobic amino acid residues. And we know that when the protein unfold the hydrophobic content of the protein or hydrophobic groups of the proteins are exposed to the solvent. And the solvent in this case is water. And along with the hydrophobic groups the polar groups the ionic groups will also get exposed to

the solvent .and all these are going to affect the structure of water we have discussed how the ions can affect the structure of water.

Now, let us discuss how the hydrophobic groups can affect the structure of water. Let us take a look at the next slide.

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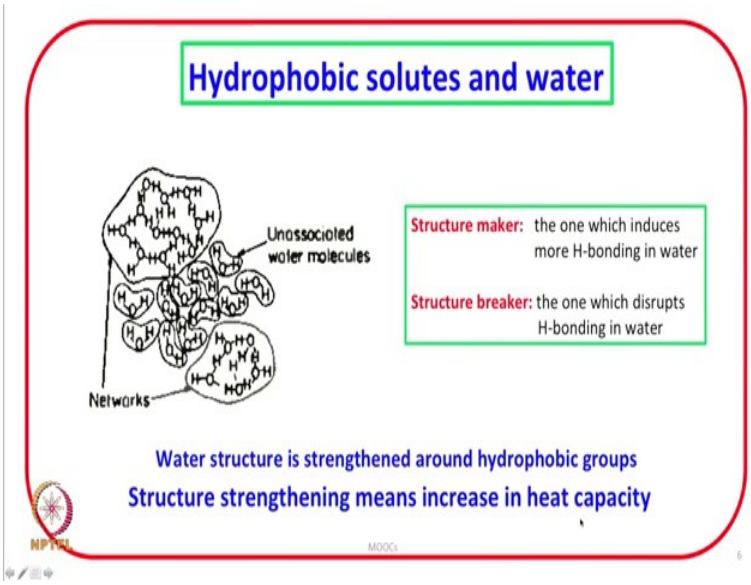

And let us once again examine the structure of water. If I add any solute which will shift the equilibrium towards the more hydrogen bonded network. Let us call that as structure maker. And any solute which shift the equilibrium towards more unassociated water molecules or the one which lead to disruption of hydrogen bonding, that will be called as a structure breaker. So, take a look at the comment structure maker the one which induces more hydrogen bonding in water means which will shift the equilibrium towards the hydrogen bonded network, and the structure breaker the one which disrupt the hydrogen bonding in water.

Now, what happens when a hydrophobic moiety is present in water. It is well known that hydrophobic substances are called as water heating or means which do not like water. And that is why you see the surfactants misalatiomisalize in aqueous solution because hydrophobic moieties will aggregate and form a misled like structure, because they do not dissolve in water. So, when a hydrophobic moiety is to added to an aqueous solution what happens is, let us take a look at this slide. Since hydrophobic molecules do not like water they feel turn to be away from water and in this process they will induce more

hydrogen bonded structure in water, because they cannot form hydrogen they cannot form hydrogen bond with water they cannot from polar interactions with water they like to be away from water and it results into more hydrogen bonded structure in water; that means, the equilibrium is shifted towards the regular hydrogen bonded tetrahedral network. Therefore, that is why this comment is written over here that water structure is strengthened around hydrophobic groups.

Now, this disruption of water structure or strengthening of water structure. Let us try to think what will be the effect of these changes in structure of water on thermodynamic quantities. Now suppose a water structure is strengthened water structure in strengthened means hydrogen bonding is strengthened in water. Hydrogen bonding is an exothermic process. And therefore, this contribution from hydrogen bonding extra hydrogen bonding will manifest will result into exothermecity. And what about the entropy, if the hydrogen bonded structure is strengthened; that means, there is more order the entropy will decrease. What about the heat capacity if the structure is strengthened; that means, the heat capacity will also increase one will require more heat to disrupt those hydrogen bonding interaction and hence to increase the temperature by one calve.

This can be extended to other thermodynamic properties also like if the structure of water is strengthened then the volume will also increase. Because hydrogen bonded structure will have in water will have more volume. And similarly one can extend it to compressibility because if there is more hydrogen bonded ice like structure then you are making the system more compressible. So, therefore, whenever a solute is added to water we are talking about the aqueous system. It will affect the structure of water and the way it affects the structure of water the thermodynamic quantities will change accordingly.

And therefore, the signs not only the magnitude the signs of the changes in thermodynamic quantities provide very important information about the role of solvent that how the structure of my water might be getting affected by the process which is under consideration. So, let us take a look at the comments further. Structure strengthening means increase in heat capacity and that is why I just discussed. So, recalling what is structure maker and what is structure breaker structure maker is the one which will shift the equilibrium towards hydrogen bonded network and structure breaker is the one which will shift the equilibrium towards monomeric water molecules.

So, what is the reason for sloping pre and post transition baselines in differential scanning calorimetric output.

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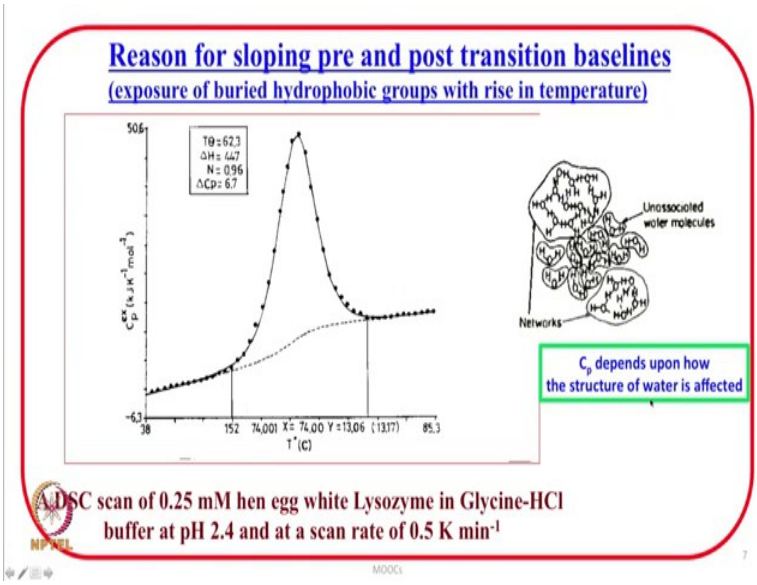

Let us take a look at the slide. We have discussed how this differential scanning calorimetric output is generated from the instrument and this particular example is for 0.25 milli molar hen egg white lysozyme in glycine HCL buffer at pH 2.4 at a scan rate of 0.5 Kelvin per minute. And let me give some more information that hen egg white lysozyme this lysozyme transitions have usually will observed to be calorimetrically reversible in nature. What I mean is if you cool it down and reheat one will again get the same transition back.

However the question that we are addressing here is let us take a look at this figure, why this pre transition baseline is sloping. And why this post transition baseline is sloping; however, there is slope if you carefully look at are different. So, let us discuss that, one conclusion that we can draw from this slope is that; obviously, here the heat capacity is dependent on temperature; obviously, heat capacity is dependent on temperature. And we have discuss this ear this earlier also by taking the examples of monatomic diatomic perfect gases. And we discuss that as the temperature increases the degrees of freedom also increase. And the contributions of those degrees of freedom to the heat capacity also increase.

A similar explanation can be offered here. Let us see what happens we consider this as the folded structure of the protein. And now when the temperature is increased; obviously, it will affect the intra molecular interactions will be weakened. And if the intra molecular interactions are weakened the structure will become relatively lose and the relative exposure of the hydrophobic groups to the solvent environment we slightly increase because one once it is very intact structure and it slightly loosens up; obviously, the degrees of freedom are increasing. And if the relative exposure of hydrophobic groups is becoming more to the aqueous environment hydrophobic groups do not like water and will lead to strengthening the structure of water. And as we just saw in the previous slide strengthening the structure of water means heat capacity will slightly go up.

Now, let us take a look at the slide. This is what I was commenting upon that if you increase the temperature there is a increasing amount of degrees of freedom and relatively larger access of the hydrophobic group towards the solvent leading to increase in heat capacity. And when there is sufficient amount of heat for the protein to unfold in a cooperative manner you see the huge transition appearing here it reflects on the cooperativity of the process, giving us another conclusion that unfolding is a cooperative process. Cooperative process is the one in which one event facilitated the next event. So, here the transition begins and the heat capacity you know at transition becomes large and once the transition is over it goes back to the original it should go back to the original baseline, but since now in the native state the protein conformation was like this and in the unfolded state it is random coiled hydrophobic groups are exposed therefore, the behavior of the heat capacity verses temperature curve is also going to be relatively different.

And that is what you see here, that the heat capacity of the unfolded protein in the aqueous solution is higher than the heat capacity of the native protein in aqueous solution. And the difference between the 2 is delta Cp, and that we have discussed in details in one of the previous lectures. So, most importantly what we should try to connect is how this process is affecting the structure of water. So, what I discussed is that when you are increasing the temperature then slowly and slowly the equilibrium is getting shifted towards more tetrahedral network strengthening the structure of water

heat capacity overall heat capacity increasing. And after the transition is over then how the unfolded protein affects the structure of water will decide it is final heat capacity.

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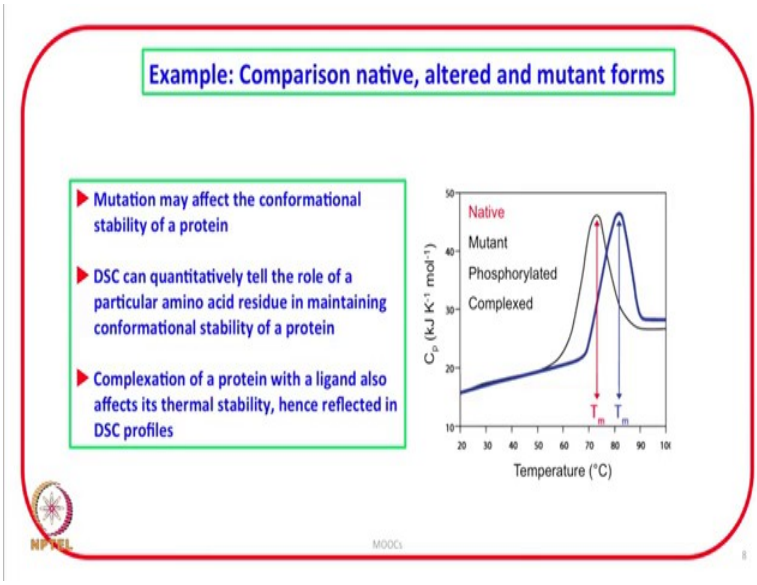

So, that is why the comment over here, Cp depends upon how the structure of water is affected. Now let us take an example a comparison of native altered and mutant forms. Differential scanning calorimetry is very important in giving information on how the conformation of protein will be affected. For example, in this figure the red line Tm corresponds to transition temperature of the protein which is in the native state. And when you carryout mutations means you alter one or more of the amino acids, and see whether the stability of the protein increases or decreases.

In this particular case if we look at the figure the mutant the mutation has led to an increase in the thermal stability of the protein. Mutations have given lot of information about the role of a particular amino acid in maintaining the conformational stability of a protein. Therefore, differential scanning calorimetry of mutated proteins has provided lot of information about the role of particular amino acids in maintaining the thermal and conformational stability of the proteins. So, let us take a look at the comments. Mutation may affect the conformational stability of a protein. DSC can quantitatively tell the role of a particular amino acid in maintaining conformational stability of a protein. And complexation of a protein with a ligand also affects the thermal stability and hence

reflected in DSC profile this comment we will discuss in more details when I talk about the ligand binding of phenomena.

So, what we have discussed is that we can get lot of thermodynamic parameter.

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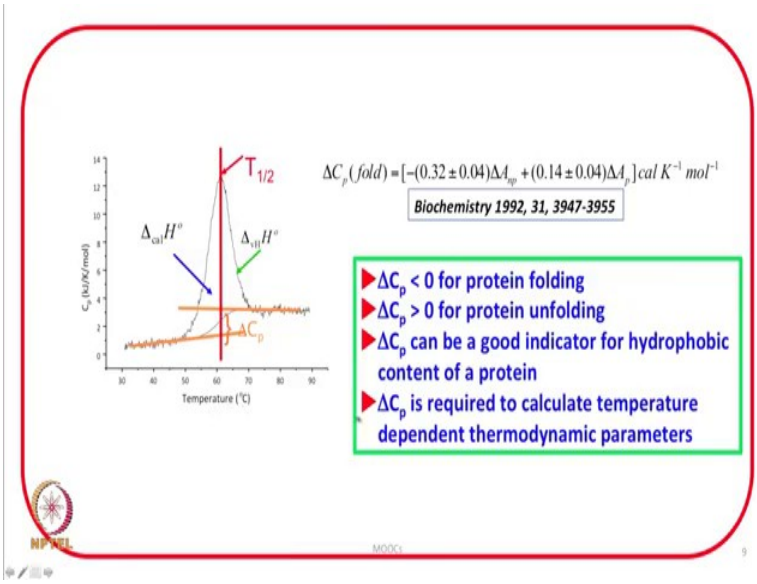

for example, if we look at this DSC profile we can get t half we can get delta h vanthoff we can get delta h calorimetry we can get delta s Cp. And delta Cp of folding or I can represent unfolding 4 folding; that means, we are going from denatured to native state it is connected with the change in accessible non polar surface area and polar surface area. So, delta Cp gives information also on how much non polar surface area is getting exposed or buried upon unfolding or folding, and also how much polar surface area is getting exposed upon unfolding or buried upon unfolding.

So, let us take a look at the comments. When protein folds the hydrophobic groups are buried inside delta Cp is negative. For unfolding delta Cp is positive because hydrophobic groups are getting exposed therefore, delta Cp can be a good indicator of hydrophobic content of a protein, what I mean is this difference if this is very large if delta Cp is very large; that means, many hydrophobic groups have been expose to the solvent and have led to increase in the structure of water to a large extent. And that is why I made this comment that delta Cp can be a good indicator of hydrophobic content of a protein. And finally, delta Cp is required to calculate the temperature dependent thermodynamic parameters as we have discussed many times.

So, what we have discussed in this lecture is that how this changes in structure of water can affect the thermodynamic parameters, and when it comes to the proteins when the protein unfolds it leads to exposure of both the apolar and polaramino acids and can affect the structure of water, and hence the thermodynamics parameters. We also discussed that the change in heat capacity can be a good indicator of hydrophobic content of a protein, we will continue our discussion on the DSC of a proteins to draw more general conclusions and those we will do in the next lecture.

Thank you very much.