Chemical and Biological Thermodynamics: Principles to Applications

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Lecture - 43
Application of DSC in thermal unfolding of proteins and protein-solvent interactions

Let us continue our discussion on applications of differential scanning calorimetry in thermal unfolding of proteins and protein solvent interactions. We have taken some examples of hen egg white lysozyme. Whatever protein we take the conformational stability of a protein is decided by the solvent environment also and one way you can alter the conformational stability of a protein is by changing the pH what happens when you change the pH? When you change the pH when you increase the pH or decrease the pH tremendously it will affect the conformation of the protein, if you lower the pH to a large extent then many amino acid residues you know will have positive charges protonated and excess positive charge will lead to repulsion and lead to the denaturation of the protein.

And similar thing may happened at the other extreme of p H. So, definitely pH may affect or will affect the thermal unfolding of protein that mean it will affect the conformational stability of protein. Let us take a look at the slide what is the effect of pH on thermal stability of a protein.

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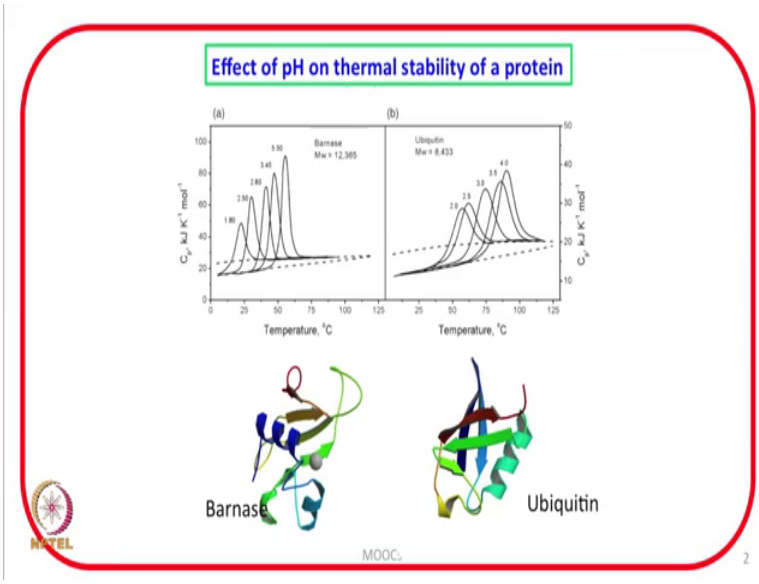

I am showing here two differential scanning calorimetric profiles one is for the protein barnase this one of the left hand panel and the other is for ubiquitin this is on the right hand panel. And you can clearly see that when pH is changed from 1.8 to 5.5 or higher there is a continuous increase in the thermal stability of the protein. Every protein will have its optimum activity under certain conditions at a certain combination of pH temperature etcetera.

And similarly if we look at the transitions for ubiquitin when you change the temperature from 2 to 4, we see a consistent increase in the thermal stability and actually if you see in the both the panels the increase in thermal stability is also associated with area under the curve. In other words the increase in thermal stability is also leading to an increase in the calorimetric enthalpy of unfolding; obviously, if the conformation stability of the protein is strengthened, the calorimetric enthalpy will also be more because the process will become more endothermic. You will require more heat to overcome those additional interactions.

What else we can derived from this we will discuss it a bit later, but let us see from the differential scanning calorimetric profile what are the various thermodynamic parameters we can get more than those what we get at the transition temperature.

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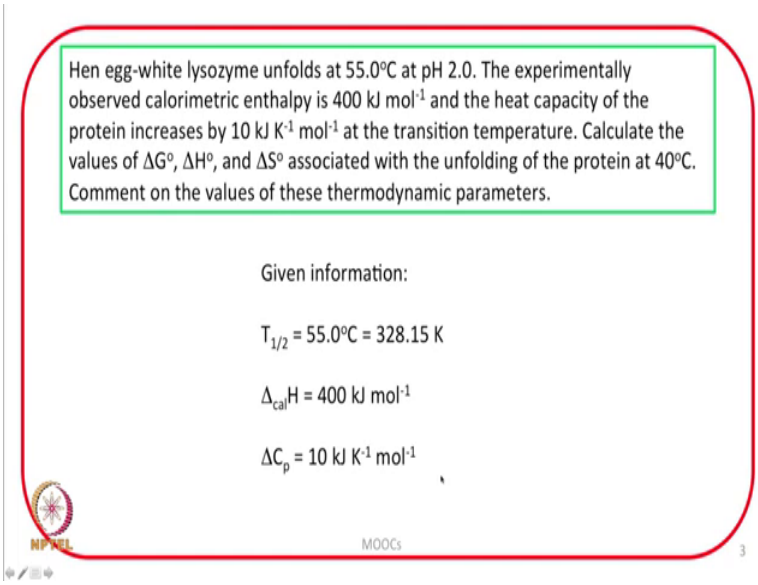

Let us look at this question which is hen egg white lysozyme unfolds at 55 degree centigrade at pH 2. The experimentally observed calorimetric enthalpy is 400 kilojoules per mol and the heat capacity of the protein increases by 10 kilo joules per Kelvin per mol at the transition temperature.

Calculate the values of delta G naught naught delta H naught naught and delta S naught naught associated with the unfolding of the protein at 40 degree centigrade comment on the values of these thermodynamics parameters. In the question we have been given information at 55 degree centigrade and we have been asked to calculate the thermodynamics parameters at 40 degree centigrade though; obviously, since the temperature is changing 55 to 40 therefore, it immediately reminds us that what is the thermodynamic parameter which connects a thermodynamic property from one temperature to another temperature and that is heat capacity. Therefore, we will definitely need the value of delta C p the value of change in heat capacity to calculate the required thermodynamic parameters and that information is given the given information we look at this slide T half is given 55 degree centigrade that is the temperature where transition is half complete, which is 328.15 Kelvin.

Calorimetric enthalpy is given as 400 kilo joules per mol and delta C p is given as 10 kilo joules per Kelvin per point let us keep this data in mind and look at the differential scanning calorimetric profile let us take a look at this figure.

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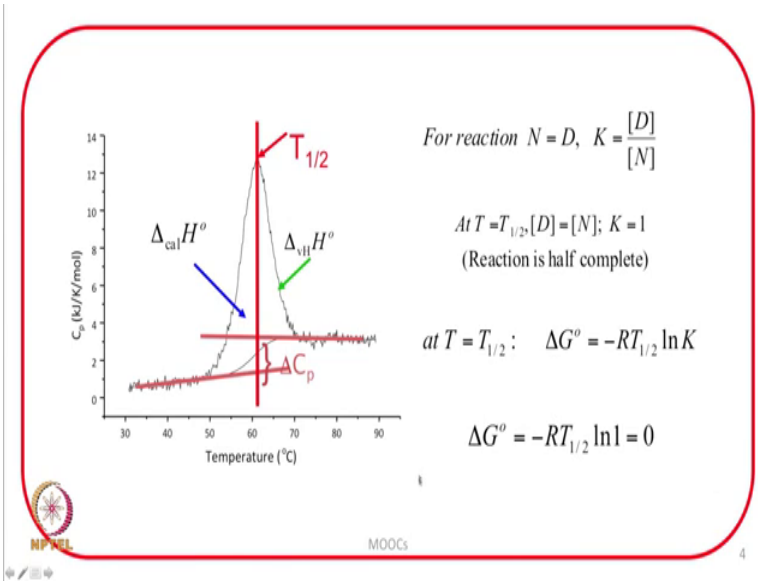

We are given the information on T half we are given the value of calorimetric enthalpy and we are given the value of delta C p. Now let us do some derivation.

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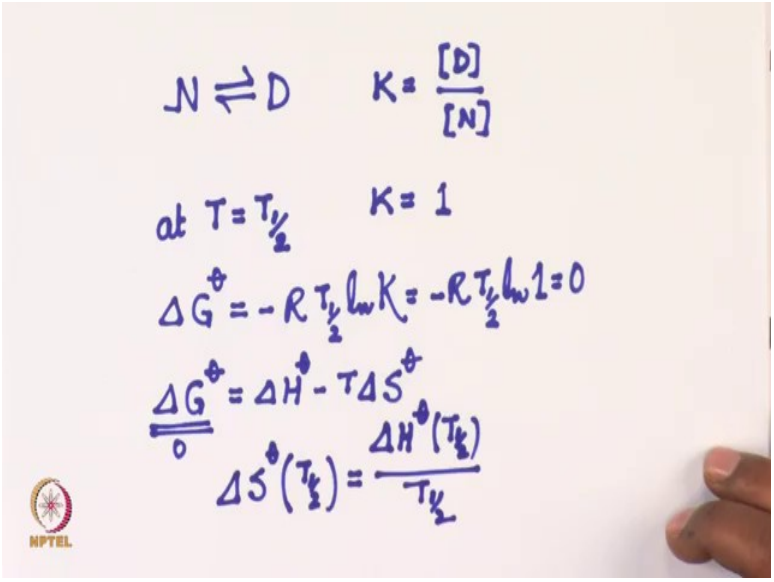

The process under consideration is native state going to denatured state and for this I can write equilibrium constant as concentration of denatured state divided by the concentration of native state.

I am assuming the activities to be same as concentration in other words I am assuming that the solution is very dilute. Now at a temperature where half of the reaction is over k

will be equal to if half the reaction is over; that means, both the concentrations are same once again T half is the temperature where half of the transition is done; that means, the concentration of denatured state and concentration of native state when half the reaction has taken place is one. So, therefore, delta G naught is equal to minus R T half log k will be equal to minus R T half log 1 and this is 0. So, we must keep in mind that when the transition is two states; two state means either native state is populated or denatured state is significantly populated and there are no intermediate states.

And when the reaction is half complete k is equal to 1; that means, at the transition temperature the standard reaction gives energy that change is 0, but only for two state process and if I now use this expression delta G naught is equal to delta H naught minus T delta S naught and since this is 0 I can write delta S naught at T half will be equal to delta H naught at T half divided by T half. This equation I am rewriting because delta G naught is 0 therefore, delta S naught at T half is delta H naught.

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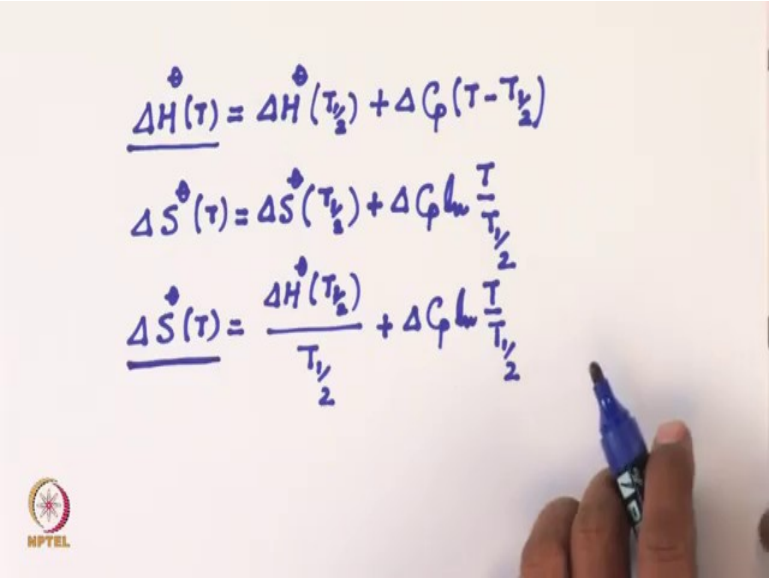

At T half divided by T half with these now I can write temperature dependence delta H naught at any general temperature will be equal to delta H naught at T half plus delta C p into T minus T half this is Kirchoffs law. That is if I know the enthalpy of unfolding at transition temperature if T half I am calling as transition temperature and then since I know delta C p, I can calculate delta h at any other temperature then let us recall delta S naught at any temperature will be equal to delta S naught at T half plus delta C p log T by

T half. This equation we have discussed many time that entropy at any temperature is entropy at a reference temperature plus C p log T by T reference temperature and I am introducing deltas in that equation.

I can further write this as delta S naught at a general temperature T, instead of delta S naught T half as I just derived delta S naught at T half is equal to delta H naught at T half divided by T half, plus delta C p log T by T half and this equation allows me to calculate entropy change at a given temperature from the knowledge of enthalpy of transition at transition temperature and the changes in delta C p. Now you see I have variation of enthalpy with temperature I have an expression of variation of entropy with temperature and now I can calculate delta G naught, because G naught will be equal to delta H naught minus T delta s naught. So, I will substitute these two equations and get the value of delta G naught that is let us now do it on a next sheet.

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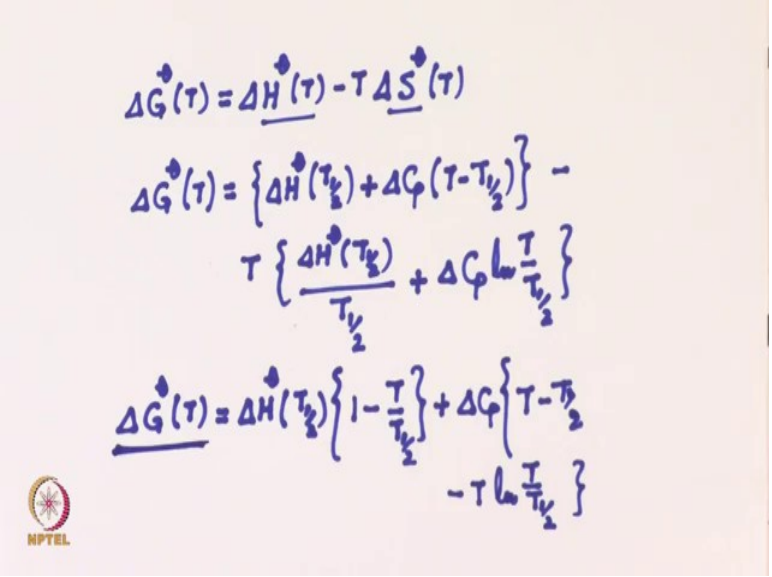

So, delta G naught at any temperature T will be equal to delta H naught at that temperature minus T delta S naught at that temperature. Now I will substitute for delta h and delta S. So, delta G naught at any temperature will be equal to delta H naught that will be delta H naught at T half plus delta C p into T minus T half this is delta H naught minus T times delta S naught. I will write for delta S naught delta S naught was delta H naught at T half divided by T half plus delta C p log T by T half. Once I solve this I get delta G naught at any temperature is equal to delta H naught at T half into 1 minus T by

T half plus then I have delta C p and inside I have T minus T half T minus T half minus T into log T by half delta H naught into 1 minus T by T half plus delta C p T minus T half will come here and then minus T log T by T half will come from this. So, I have an expression for temperature dependence of standard reaction gives energy.

Now, let us go to the slide what I discussed was for this expression we just derived or discuss that k will be equal to one and then delta G naught which is minus R T half log k delta G naught is 0. So, for such a reaction which is two state we can immediately say that at the temperature where transition is half complete delta G naught is 0 and then we discussed.

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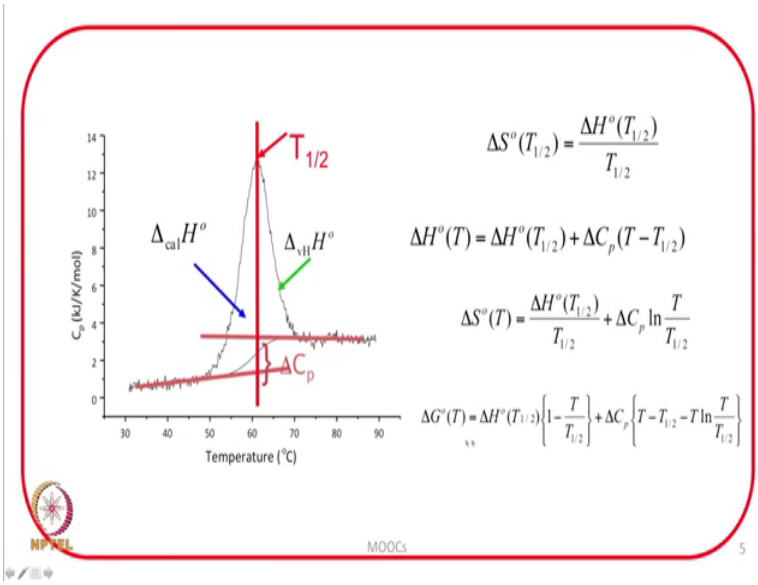

How to get delta S naught and how to get delta H naught; delta S naught at any temperature an eventually we came up with this expression delta G naught at any temperature in terms of delta H naught and delta C p now you see these three equations this equation this equation and this equation will give you a temperature dependence of enthalpy, entropy change and free energy change. And these three equations are very important when you talk about folding unfolding transitions in proteins; that means, if you know the data associated with the transition of the protein at a given temperature; at a certain temperature let us say transition temperature.

You can draw delta G naught versus temperature or delta H naught versus temperature or delta S naught versus temperature profile for a long range of temperature and discuss the

conformational stability of the protein as a function of temperature without doing the experiments at that temperature. Now coming back to the question which was being asked was.

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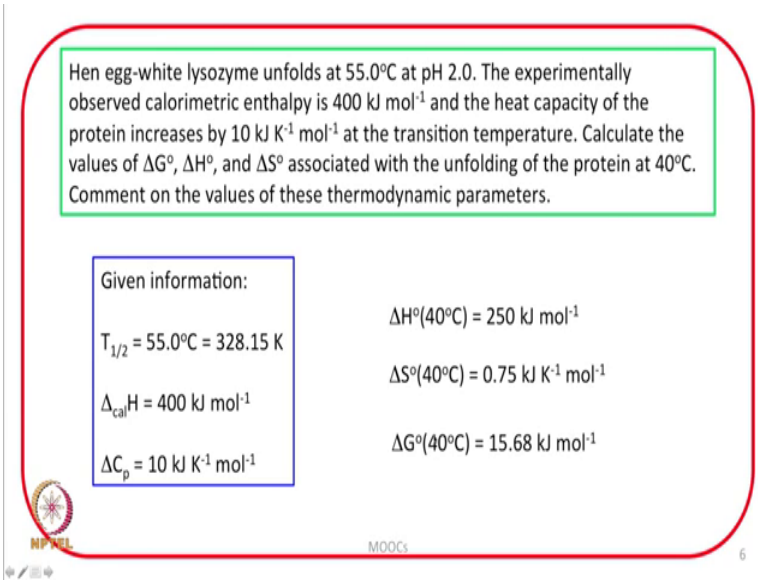

With the given information because that is all we needed the transition temperature we needed calorimetric enthalpy, and we needed the change in heat capacity when you substitute this data. In those three derived equation we get the enthalpy of unfolding 40 degree centigrade as 200 kilo joules per mol entropy of unfolding at 40 degree centigrade as 0.75 kilo joules per kelvin per mol and delta G naught at 40 degree centigrade as plus 15.68 kilo joules per mol.

A comment can be made over here delta G naught at 40 degree centigrade is coming out to be positive, it is plus 15.68 kilo joules per mol, a positive change in gives energy means the reaction is not spontaneous; that means, at 40 degree centigrade the protein will not unfold and that is in line with the given information that hen egg white lysozyme unfolds at 55 degree centigrade; that means, if the temperature is below 55 degree centigrade the reaction will not be unfolding reaction will not be spontaneous and that is what you see here is the delta G naught is positive at 40 degree centigrade.

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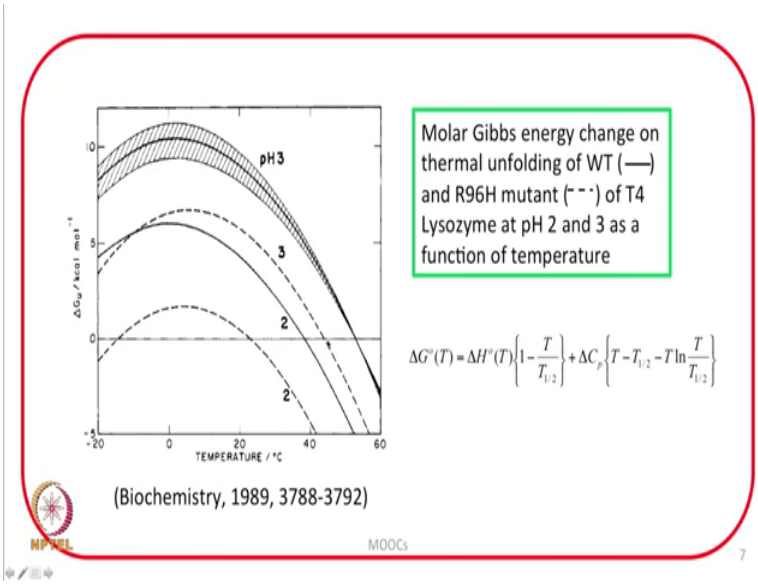

In fact if we look at this figure this figure is drawn for the thermal unfolding of wild type that is the solid lines are for wild type, and the dash lines are for R96 H mutant of T 4 lysozymes at pH 2 and 3.

This delta G naught versus temperature curve you see has been generated from minus 20 degree celsius to 60 degree celsius by using this equation delta G naught is equal to delta H naught actually read this as T half delta G naught at T is equal to delta H naught at T half into 1 minus T by T half plus delta C p and the remaining terms as we discussed earlier. So, by using this expression the complete delta G naught versus temperature curves have been drawn as a function of temperature from minus 20 degree celsius to 60 degree celsius and this one line up and the shaded area is actually representing the standard deviations in delta G naught when you incorporate the standard deviations of delta H naught T half and delta C p.

According to this equation or in general based upon our previous discussion. The value of delta G naught should be 0 at transition temperature for a two state transition and you see you draw a line at 0, wherever this line crosses the delta G naught curve that should be the transition temperature. So, at pH 2 for the mutant the transition temperature is this for the wild type T for lysozymes at pH 2 transition temperature is this at pH 3 for the mutant transition temperature is this and for the wild type. At pH 3 the transition temperature is this at pH 2 you know sometimes one may see two transition temperature

and these two transition temperatures respectively corresponding to this is the thermal unfolding and this is corresponded to cold denaturation because denaturation can be carried about or brought about by heat as well as by reducing the temperature.

So, in this way we can not only generate the free energy versus temperature curve, we can also generate the enthalpy and entropy versus temperature curve and draw lot of conclusions from such plots. Now let us discuss some more features from the differential scanning calorimetric output.

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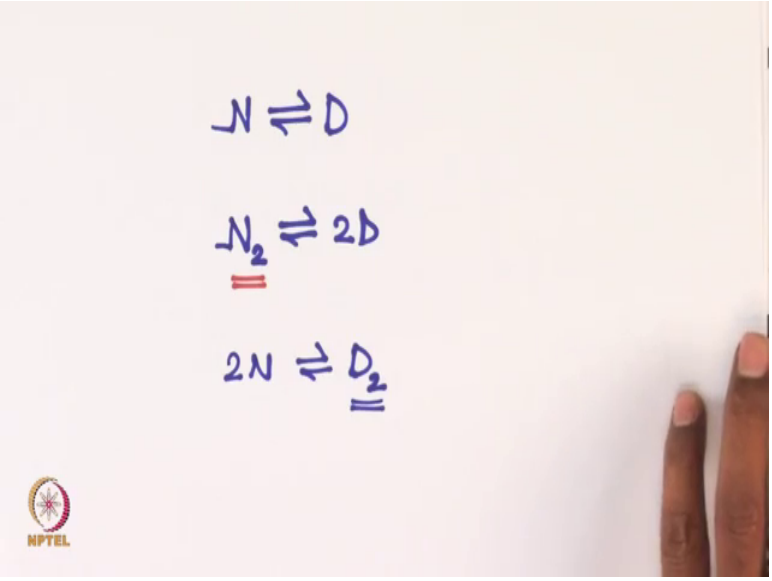

Let us consider this equilibrium N going towards D where N I am writing for the native state and D I am writing for the denatured state. Now if you increase the concentration of the protein will it affect the transition temperature. If we look at this stoichiometry of the reaction it is N going to D one mol of native state producing one mol of denatured state. According to le chatelier le chatelier principle if you increase the concentration of the protein to begin with concentration of protein increasing mean you are increasing the concentration of the native state it should not affect the transition temperature at all because it is not going to affect the equilibrium composition.

Now, suppose if my process is N 2 going to 2D and now if I increase the concentration of the protein. Once again increasing the concentration of protein means we are increasing the concentration of N 2 according to Le-Chatelier principle the equilibrium should be shifted in favor of N 2. An increase in concentration; that means, equilibrium is in favor

of N2; that means, when you thermally unfold the extant of oligomerization decreases during the denaturation because the reaction is towards denatured state. So, if an increase in concentration of the protein leads to an increase in the transition temperature; that means, the extant of oligomerization is decreasing. Now if I consider 2N going to D2. Now what happens if you increase the concentration if you increase the concentration now the shift is the Le-Chatelier principle says that this should be favor; that means, the equilibrium will be shifted towards D2.

So, in that case increasing the temperature will lead to decease sorry in that case increasing the concentration of the protein will lead to decrease in the transition temperature; that means, the extant of oligomerization is then increasing during the transition. So, this is what is commented now here if the value.

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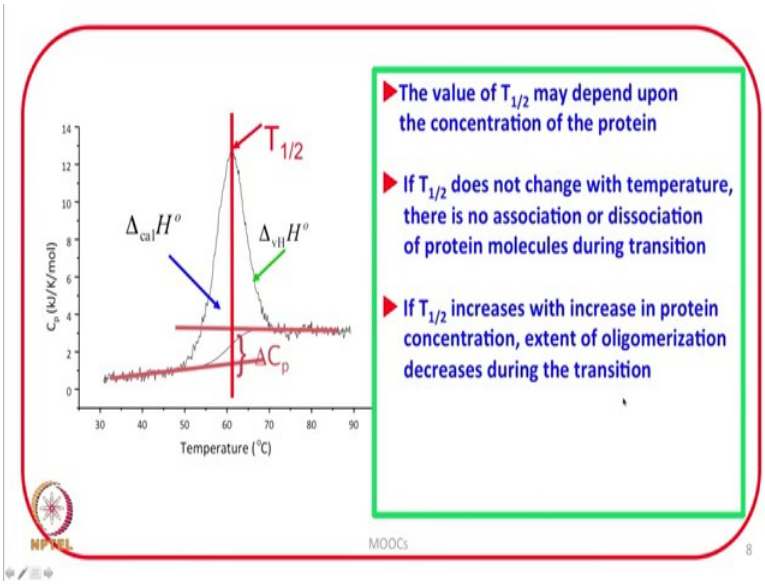

Of T half depends on temperature then some conclusion can be drawn for example, the value of T half may depend upon the concentration of the protein. If T half does not change with temperature there is no association or dissociation of protein molecules during transition. If T half increases with increase in protein concentration extant of oligomerization decreases during the transition that is what I was trying to explain over here that if you increase the concentration of the protein, the equilibrium is more towards N2; that means, the transition temperature will increased with increase in concentration.

But what is the net result that the extant of oligomerization is decreasing during the transition. And conversely if T half decreases with increase in protein concentration the extant of oligomerization increases during the transition.

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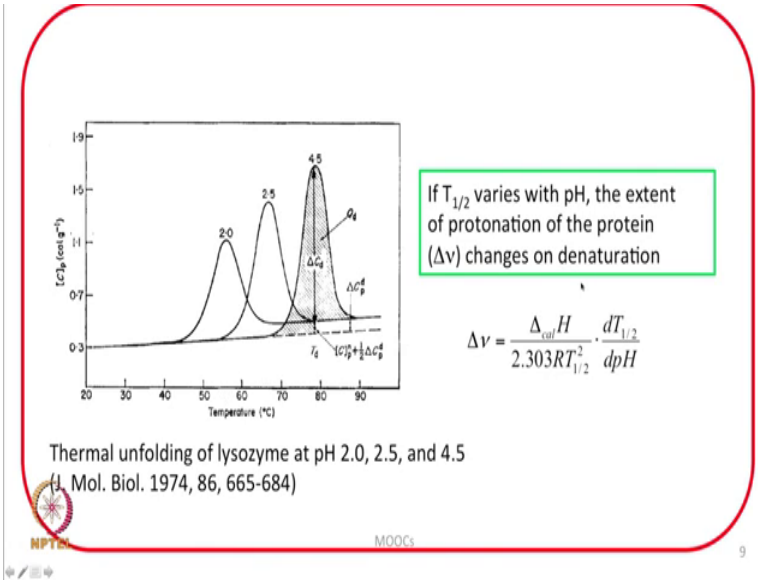

In addition to commenting upon the extant of oligomerization increasing or decreasing with increase in or decrease in concentration of the protein, let us see what else we can get when we change the pH. We just discussed in the beginning of this lecture the pH may affect the conformational stability of a protein and when you increase the pH and that is associated with the change in transition temperature and calorimetric enthalpy that data can be used to calculate the extant of protonation of the protein when it undergoes denaturation.

For further details one can refer to this general of molecular biology the reference which is listed over here. So, therefore, if the transition temperature either increases or decreases with change in pH, we should be able to calculate another property another quantity that is how many protons are either being excepted or how many proteins are being loss, when this folding unfolding transition takes place. In other words the extant of protonation or deprotonation of the protein when it undergoes denaturation or folding as a function of pH if we have the data that property can be calculated.

So, a in this lecture we discussed further applications of differential scanning calorimetry, and treatment of the data which we obtain at transition temperature that is

how the data obtained at transition temperature can help us in calculating the thermodynamic properties over and extended range of temperature. And if we can know these thermodynamic properties over an extended range of temperature then we can talk about the behavior of the biological macro molecules in that extended range of temperature. We will further take more examples on how this differential scanning calorimetry gives more information on proteins solvent interactions and protein ligand binding and these things we will discuss in further lectures.

Thank you very much.