Chemical and Biological Thermodynamics: Principles to Applications

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Lecture – 41
Further discussion on Differential Scanning Calorimetry (DSC)

In the previous lecture we discussed what kind of thermodynamic parameters can be obtained from a differential scanning calorimetry output profile. And let us continue our discussion on how to treat the data that we obtain from differential scanning calorimetry, and what kind of information we can further draw from it. We started discussing that why calorimetry that, what is the advantage of calorimetry over other methods?

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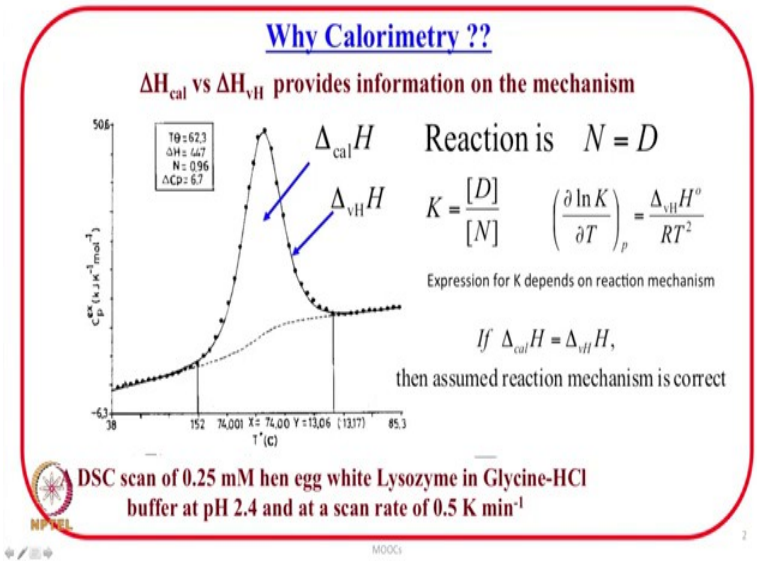

Vant hoff equation which is the variation of equilibrium constant with temperature will give you the enthalpy. And if we can get enthalpy from vant hoff equation and equilibrium constant can be obtained from any suitable method, then why? What is so special about calorimetry? One thing is as we appreciated in the previous lecture, that you are measuring true enthalpy, which is obtained from area under the curve. It is completely experimental no assumption made. So, we get calorimetry enthalpy, and then we also discuss that we can get the vant hoff enthalpy. And we started a discussion on that the comparison of calorimetry and vant hoff enthalpy provides information on the mechanism of the unfolding process.

Let us discuss more about this. We started discussing that the reaction is N going to D, where N is the native state of the protein. D is the denatured state of the protein. And the equilibrium constant can be written as the ratio of denatured state divided by the native state concentrations. An vant hoff enthalpy can be obtained from the temperature dependence of the equilibrium constant and this we discussed in the previous lecture that, how to obtain the value of equilibrium constant as a function of temperature? One thing we must again keep in mind, that the loss of equilibrium thermodynamics can be applied to reversible system. And therefore, it becomes very important to establish the reversibility of the system.

And if we recall the definition of reversibility, any process which can be reversed by an infinite decimally small modification, of one of the parameters is called reversible system; that means, if we want to establish the reversibility, we must be slow. And let us say if we want to establish the calorimetry reversibility, that is look at the this slide. This peak is associated with the unfolding of the curve, unfolding of the protein. Establishing reversibility means, that if we cool the system back we should be able to get back the transition. That is, when you heat it you get this transition and when we cool it at the same scanning rate we should be able to get back the same transition.

Now, what happens is whenever you want to use differential scanning calorimetry, and would like to establish the reversibility of the unfolding transition.

What we want, is that you heat please look at the slide, you heat up to 90 percent completion, do not go post complete transition, because many times at higher temperature because the hydrophobic groups are exposed, and therefore, there is there could be an onset of high roader associations. In order to avoid that, one should heat only up to 50 percent completion, cool it back and reheat at the same scan rate. I repeat, you heat up to 90 degree completion in the first scan, cool it back, and then reheat. And if we recover the complete transition we get the same height same area, then we will call the transition is 100 percent reversible. Otherwise if it is 90 percent 80 percent, we can quantitatively assign a number that the transition is 90 percent reverse, reversible 50 percent reversible also.

But we recover most of the transition back, then we can say that the transition is calorimetrically reversible, because we are basically using calorimetry here to establish

the reversibility of the transition. Now let us go back to our discussion, that vant hoff enthalpy can obtained from the temperature dependence of the equilibrium constant. And the expression for K depends on the reaction mechanism, what does this mean? Expression on K depends on reaction mechanism. We are talking about native state of the protein N, and it is equilibrium with denatured state which is D, I will refer to this 2 as the reaction mechanism.

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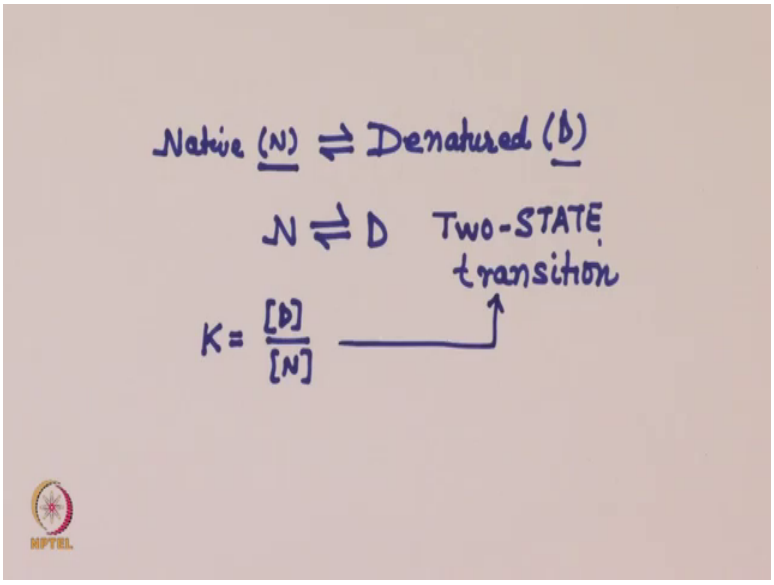

Reaction mechanism means, that I am starting with the native state and the final state is denatured state and in between there is no any other state. That is what I mean by 2 state, I can write this N going to D. And if the transition were not 2 states then there would have been appearance of other states in between, some intermediate state or So, some examples we will take later about this. But this N going to D in which either native state is populated or denatured state is populated I will call this as 2 state process. 2 state transition. And K is written as the concentration of D and the concentration of N, the way I write K it depends on the reaction mechanism.

Because the reaction mechanism were 2 N going to be D, then K would have been modified accordingly. That is what I mean that K this K expression of K is being written for a 2 state transition. Let us go back to slide, expressions for K depends on the reaction mechanism. And from the area under the curve I can get calorimetry enthalpy, from the temperature dependence of K I will get vant hoff enthalpy. And if calorimetry enthalpy is

equal to vant hoff enthalpy, then the assumed reaction mechanism is correct. Let me emphasize this that why I am stray saying this.

Calorimetry enthalpy one can be determined from the area under the curve, the vant hoff enthalpy is based upon equilibrium constant, and that equilibrium constant is written for a specific reaction or for a specific reaction mechanism. So, if I assume that this is my reaction mechanism and write an equilibrium constant for that, get the temperature dependent equilibrium and get the vant hoff enthalpy, then this vant hoff enthalpy, if it is equal to calorimetry enthalpy; that means, my assumed reaction mechanism is correct. Because the output is obviously, depending upon the reaction mechanism. So, this equivalence of calorimetry enthalpy and vant hoff enthalpy, for a reversible 2 state mechanism by this arguments establishes the mechanism of the reaction. And suppose if these 2 enthalpies do not match, then what we do is, we need to go back and correct our mechanism, and see if still there is a match between the calorimetry enthalpy and vant hoff enthalpy or not.

And if it does not match we just keep on correcting our reaction mechanism till there is a match, and this is how this comparison of calorimetry and vant hoff enthalpy establishes the reaction mechanism. And this is what I was talking about earlier, that we are not only getting the numbers we are not only getting the transition temperature. We are not only getting the enthalpy of a reaction. We are not only getting the heat capacity differences. But we have stating started getting the insides into the process. That is whether the unfolding reaction folding reaction is a 2 state reaction, and if it is not a 2 state reaction, then either it is following a difference mechanism or there is a formation of an intermediate state ok.

So, this vant hoff enthalpy which depends upon the share and the calorimetry enthalpy, which is coming from area under the curve become very, very important. Now if we take a look at the shape of the differential scanning calorimetry output profile, the heat capacity initially is having a slope. There is an increase in heat capacity when the temperature is increased, and when the transition starts; obviously, the heat capacity becomes indefinite a lot whatever temperature you know at if the transition is very sharp, then it will come like a sky, spike. Because generally at phase transitions all the heats which is supplied is used up to bring about the phase transition rather than changing the temperature. And heat capacity becomes in final.

But since here we are talking the protein system in which these transition from unfolded folded to unfolded state, is not like a solid liquid kind of phase transition. That is why it is not very sharp. So, let us discuss that why there is an increase in heat capacity with temperature, and why the heat capacity of the denatured state is higher than the heat capacity of the native state. The slopes are different. And this dotted base line that we are seeing here is actually the calculated baseline, which takes the average dependence of the C p verses T of native state and C p verses T of the denatured state you calculate the average value, and that dotted line is the heat capacity variation to be used for the calculation for area and the curve in between this transition range. Why the heat capacity should increase in temperature?

Let us recall the heat capacity of monatomic verses diatomic gases. We discussed that the value of C v or C p of diatomic gas is higher than the value of C v or C p of monatomic perfect gas, why? When you consider a monatomic gas, the only degree of freedom is translation. Now when you consider the diatomic degrees of freedom increase, there is not only a translational degree of freedom there is rotational degree of freedom. There is vibrational degree of freedom. And if the temperature becomes very high the electronic transition may also set in their contribution can come from that. So that means, with increase in temperature the degrees of freedom will increase, and accordingly the heat capacity value will increase.

When we talk about the protein and if I consider the protein is folded, and now you start increasing the temperature, with increase in temperature, the intramolecular interaction will start getting affected. It will lead to weakening of the intramolecular interactions and there will be starts there will be loosening of the structure. And the accordingly some degrees of some degrees of freedom will change. Now you see when the protein has started loosening up, the interactions intramolecular interactions are getting weaken. There will be slight exposure of whatever is the buried groups will start getting slight exposure to the outside environment. It is I am not talking yet about the complete unfolding I am talking about increase of temperature.

So, whenever there is a slight loosening of the structure, and outside what we have is water largely water because these solutions are made in buffer, buffer concentration is very small therefore, it is largely water, the structure of water is getting largely affected. Therefore, in biological systems whenever there is a change and we want to address the

thermodynamics of the process, we cannot ignore the environment, we cannot ignore the changes in the structure of water which is surrounding the protein molecules.

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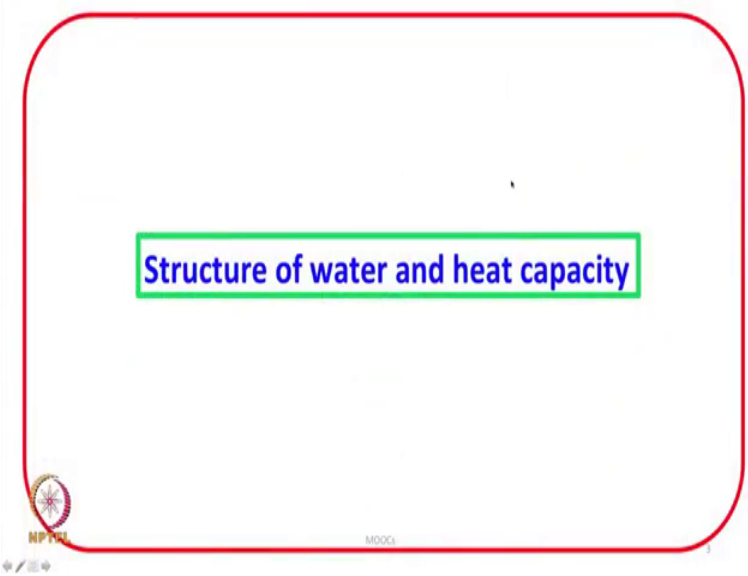

So, that is why we should understand the structure of water and how it is related to heat capacity. Let us spend some time on the discussion of structure of water.

Structure of a single water molecule if I say the gaseous water.

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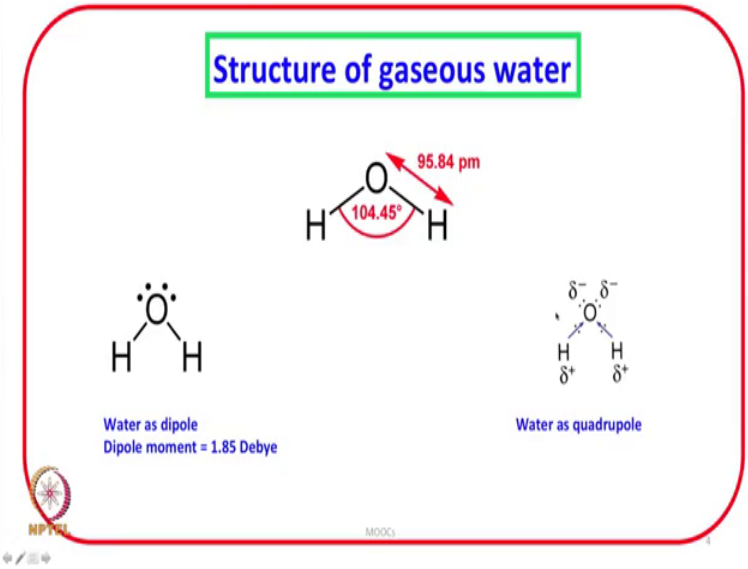

These structure is taught at class 11 or class 12 level that there are 2 covalent bonds. The bond angle HOH is about 104.5 degree. And there is about 95.84 picometre difference between oxygen and hydrogen. Knowing the properties of water is very important. Because in biological systems we have been repeatedly emphasizing on the significant of water, is water a dipole. In water we have 2 lone pairs, and we have 2 hydrogen. And if you assign a slight delta negative charge on oxygen and delta negative charge on hydrogen, water can be treated as a dipole.

And there is a permanent dipole moment of water which is 1.85 Debye, you are treating water as a dipole; however, if you closely examine this structure, closely examine this structure there are 2 lone pairs. I can assign a small delta negative charge on this small delta negative charge on this delta positive on this delta positive on this. So therefore, I can also treat this water as a quadrupole. There are 2 lone pairs each lone pair assign a delta negative, and each hydrogen you assign a delta positive. So, there is a quadrupole, water as a quadrupole, when to treat water as a diploe when to treat water as a quadrupole, whether to include the quadrupole character of water into discussion or not, it depends upon the situations.

Here we are talking about the proteins and when the protein is unfolding, how it is interacting with water? But before getting into protein discussion, let us just take an example of when you add salt to water and you consider positive ions, let us say sodium ions. How sodium ions see water molecule? Does a sodium ion see water molecule as a dipole? Or does it see view it as a quadrupole? It will depend upon how closely you are seeing. It will depend upon the distance between the ion and water.

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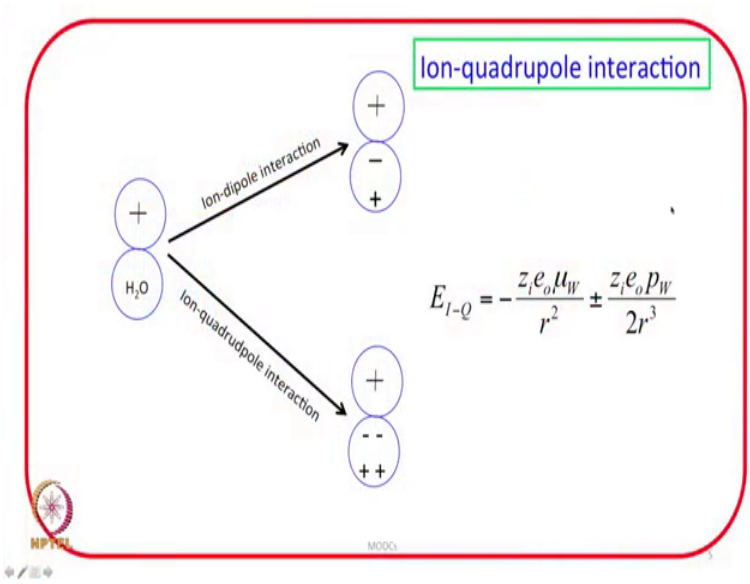

So, let us consider this ion interacting with water, and represent this as ion dipole interaction; however, when this ion this is very close when you see a situation which is very close to water, the quadrupole character of water cannot be ignored and therefore, the ion quadrupole interaction term will have contribution from permanent dipole moment mu W, and will also have contribution from quadrupole moment of water P W, with a plus minus sign depending upon whether the interaction with the positively charged end or with the negatively charged end. So, here what I have talked about the ion to make the discussion a little bit easy.

That is the interaction with the surrounding environment water molecule become very, very important. We will continue our discussion on the effects of variety solutes on structure of water, how it can affect the heat capacity.

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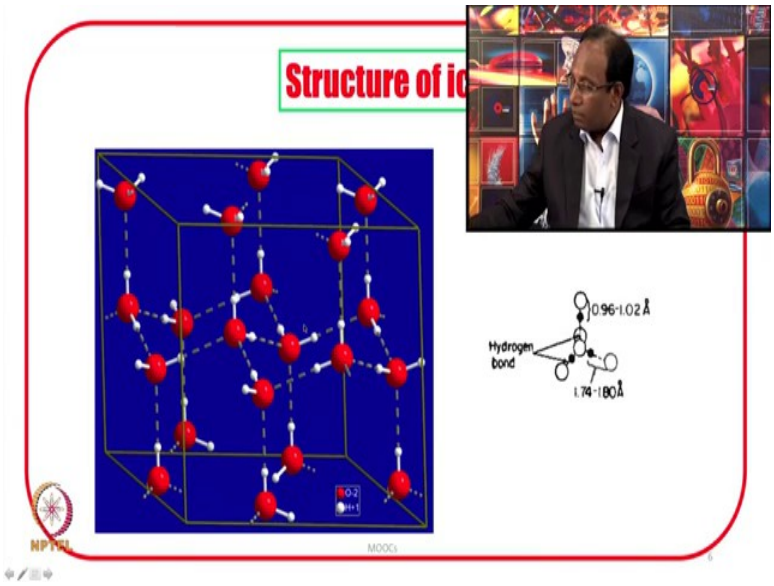

But before that let us actually try to understand, what is the structure of water? Water exists in 3 forms solid liquid and vapours. We have just seen the structure of individual water molecules that is the structure of water vapours. The structure of ice, what is the structure of ice? The structure of ice is seen in this figure. There is a regular arrangement of water molecules and if you see this red spheres, red spheres represent oxygens, and the white spheres represent the hydrogen.

If we carefully examine this structure, each oxygen is surrounded by 2 hydrogens, and these hydrogens are connected to oxygen by covalent bond. And at the same time that hydrogen is also bonding with an oxygen through. Here each red sphere is oxygen, is connected to 2 hydrogen by covalent bonds, and at the same time this hydrogen which is connected to this oxygen through covalent bond is also bonded to another oxygen through hydrogen bond. So, you see each oxygen thus is connected to 4 other oxygens, for example, if I pick up this oxygen this is one, this is second, this is third and this is fourth. Each oxygen is tetrahedrally connected to other 4 oxygen and that is why we call this arrangement is a tetrahedral network of water molecule. That is a regular tetrahedral network of water molecules and that forms the structure of ice.

And if we examine again more carefully, this is an expanded structure. And because there is a regular tetrahedral network of hydrogen bonds, and this results into the formation of voids. You see there are voids between there are spaces in between. And

this is what I was talking about, that each oxygen is surrounded by another 4 oxygens in between there are hydrogens, which are either connected through oxygen to covalent bond or through hydrogen bond. And their respective distance is are also shown over here, and since there is there are interstitial spaces there are voids, the density of ice is smaller than the density of liquid water, we will soon discuss the structure of liquid water, and if the density of ice is less than the density of liquid water that is why ice will float in water.

So, what is the structure of liquid water, what happens is some of these water molecules can break away from this hydrogen bonds, and enter the interstitial spaces. What I am saying is, some of these water molecules will break away from this regular tetrahedral network structure and enter the interstitial spaces and the structure of liquid water is going to be like this.

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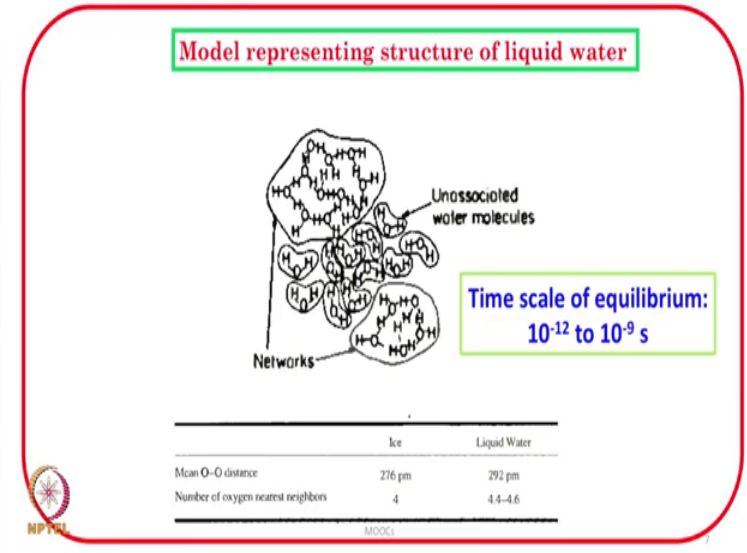

Let me represent this as the regular tetrahedral network of hydrogen bond bonded structure, this is ice like structure and these are the monomeric water molecules which are occupying the interstitial spaces. And there is an equilibrium between this monomers and the tetrahedral network. There is a fast equilibrium at any instant, one of the water molecule this water molecule may be a monomeric water molecule and at the next instant it may become a part of the regular tetrahedral network.

And the time scale of equilibrium is of the order of 10 raise to the power minus 12 to 10 raise to the power minus 9 second. This table shows the main oxygen distance in ice and liquid water, and the number of oxygen nearest neighbours. In ice we discussed it is 4 and liquid water various experiments suggests that this about 4.4 to 4.6. So therefore, when we add any solute to water. Let us say if we add salt to water, the salt will affect the structure of water. Because you dissolve sodium chloride in water sodium chloride will dissociate into sodium ions and chloride ions. And sodium ions will affect the structure of water; chloride ions will affect the structure of water. And if you add a solute which has more hydrophobic character, what do the hydrophobic groups add to the structure of water?

It may either strengthen the structure of water, any solute may either strengthen the structure of water, or it may lead to disruption of the structure of water. And if we now go back to slide and look at this equilibrium any solute which will shift the equilibrium towards the regular tetrahedral network structure will increase the structure of water. And any solute which will shift the equilibrium towards the monomeric water molecules will decrease the structure of water. This increase or decrease in structure of water is associated with the changes in thermodynamics quantities.

Like increase in structure of water more hydrogen bonding means delta it will be negative. Increase in structure of water more structure means heat capacity will increase; increase in structure of water means the entropy will decrease. So, you see now the changes in this structure of the solvent, we have started connecting with the changes in thermodynamic parameters. And these changes in thermodynamic parameters will help us in understanding that why when the protein is unfolding why the heat capacity is changing with the temperature. And this is will be this is what will be the focus of discussion in the next lecture.

Thank you very much.