Bio-Physical Chemistry
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Lecture - 17
Thermodynamics of Protein Unfolding (Contd.)

So, we will continue our discussion on Protein denaturation.

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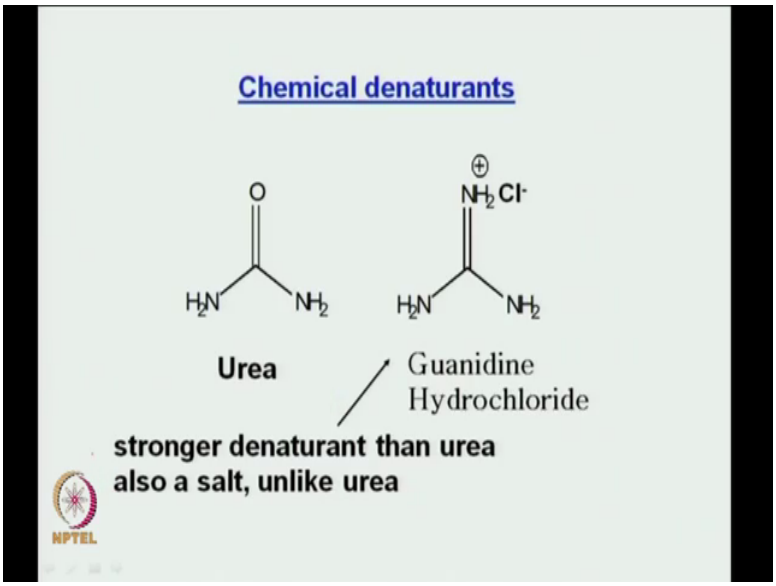

So, in last class we looked at thermal denaturation effectively. So, what we saw was that there were couple of ways of doing that, one was we started with the two state model right. And using that two state model we derive an equation and we said that any thermal transition based on any spectroscopic or any observable Y can be fit to that can be fit non-linearly to

least squares fitting of a certain equation. And you can get the corresponding thermodynamic parameters.

Then we said that the other way of doing it is taking delta h from the Van't Hoff equation. And the best way of getting delta C p was what was it? That means, you subject your protein to different P h s right. So, P h will change T m and from the plot of delta h versus T m you get what delta C p and that is the best way of getting delta Cp.

So, today we will shift gears a little bit and we will go to chemical denaturation. So, chemical denaturation is you can see on this slide. Essentially chemical denaturation is brought about by two main compounds very popularly or very commonly used very popular very commonly used. One is urea and one is guanidine hydrochloride right.

Now, as it says on the slide, guanidine hydrochloride is a stronger denaturant than urea ok. And it is proven also there is one major difference in between these two, what is that? Urea is a neutral molecule right. But what about guanidine hydrochloride? It is actually a salt. So, guanidine hydrochloride might also have a huge role in electrostatic interactions; because, it itself is a salt right it can act like a salt along with the fact that it is a chemical denaturant.

So, does this bring about any difference other than simply the fact that guanidine hydrochloride is a stronger denaturant, than urea maybe this is one of the reasons we do not yet know. But let us go forward and look what chemical denaturation's all about and how is it different than thermal denaturation.

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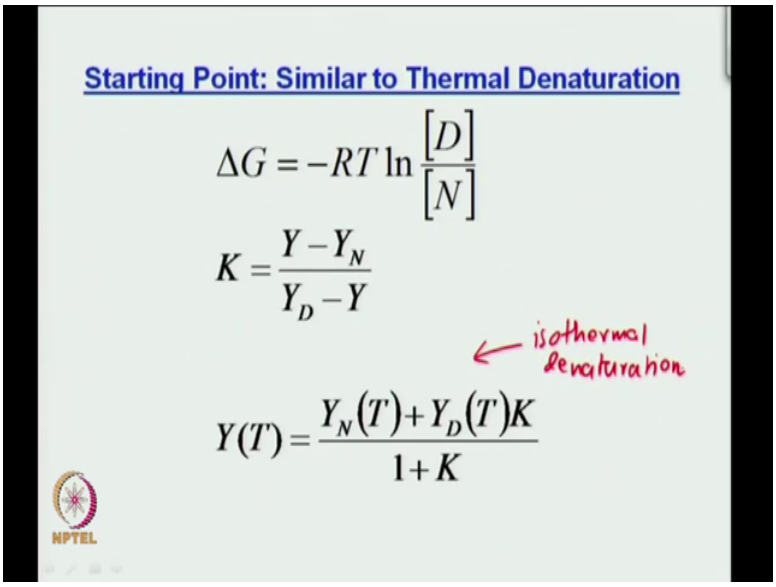

So, the starting point is very similar right, the starting point is again you take a two state; that means, you are going from N to D nothing in between. So, delta G is given by this equilibrium, constant and then you have this we have derived this yesterday in class ok.

And here Y of T is given by this expression, but remember when you are doing in a chemical denaturation, you are varying what? You are varying the concentration of the denaturant. In thermal denaturation you are varying the we are varying the temperature right everything else remaining fixed.

In chemical denaturation you are keeping temperature constant, but you are varying the concentration of the denaturation. That is why it is also referred to as isothermal denaturation

because, you are doing at the same temperature ok. So, then I can refer this one say I can refer well this one as isothermal.

Student: (Refer Time: 03:41).

Denaturation ok, it is done in the specific temperature. Now, how do we model how do we model a chemical denaturation? the best way to do it is by something known as Linear Extrapolation Method in short form LEM.

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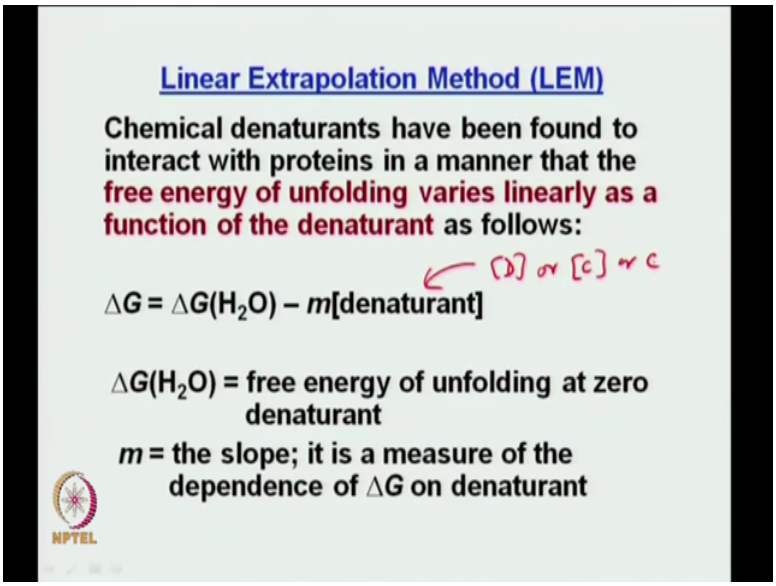

Now, this is very commonly used what does it refer to? It says that from many studies chemical denaturation's or chemical denaturants have been found to interact with proteins in a manner that the free energy of unfolding varies linearly as the function of the denaturant.

That means, if you are looking at delta G what was that delta G? That was delta G was delta D N remember delta G D hyphen N.

If you are looking at that free energy of unfolding this statement says that, it has a linear variation with the concentration of the denaturant, which means I can write down something like this. That delta G which is essentially your delta G D N or delta G unfolding is equal to delta G H 2 O minus m concentration of denaturant. So, this is a linear variation where m is the slope.

Student: Slope.

Right what is delta H 2 O? What do you think is delta G H 2 orbitals?

Student: (Refer Time: 05:05) Hydrogen and oxygen.

Yeah. Under what conditions say delta G H 2 O is your intercept right at the intercept what is the concentration of the denaturant.

Student: 0.

0.

Student: 0.

Say essentially delta G is that change in free energy, where your concentration of denaturant is.

Student: 0.

0 ok, that is what delta G H 2 O means right that is why it says H 2 O only because, we do not have any denaturant your denaturant concentration is 0 ok.

So, as I said the delta G H 2 is the free energy of unfolding at 0 denaturant, but do you think it is easy to get under normal conditions what do you think? Would it be hard to get first of all it would be based on what? See when you are saying this free energy of unfolding at 0 denaturant here there is a problem.

The problem is if your denaturation, if your denaturant concentration is 0, how would you talk about free energy of unfolding? Because, your denaturant concentration is 0 if your denaturant concentration is 0, then essentially you are not unfolding the protein right. Then; obviously, this question about finding delta G H 2 O under those conditions, will not arise why? Because essentially there is no denaturant almost all of your molecules are in the.

Student: Folded.

Folded or native state. So, how do I do that? Maybe this is where this is where this linear extrapolation method comes in you, think about this we will soon see it if you would be able to calculate delta G. If you would be able to calculate delta G, now remember when we talk about thermal transition. You know thermodynamics is very similar, is just the application which becomes different.

Student: Yes.

When we talked about thermodynamics or rather when we talked about the thermal melting what did we say? The one which you are interested in most is the transition region. Because, that is the one where the maximum change is occurring and that is the one which is giving me the most reliable delta G value right.

Student: Yes.

You know that is where I have measurable concentrations of folded and unfolded or denatured and.

Student: (Refer Time: 07:18).

Non denatured state, now the same thing is going to happen here. If I get delta G in the transition region for you know a series of denaturant values, where I have appreciable amounts of denaturant present along with the native state. And then if I am able to extrapolate it linearly to my Y axis which is essentially my delta G, then that extrapolation at 0 point essentially where x is equal to 0 should give my delta G H 2 O is not it? Because it is a linear curve. So, that is what we will do?

You have to extrapolate it because under the native conditions your delta G is very hard to get and; obviously, if your denaturant concentration is 0 what delta G are you talking about ok. So, this is the basic premise or this is the basic hypothesis on which this linear extrapolation method is based.

And as we just discussed m is the slope it is the measure of the dependence of delta G that is the change in free energy on the concentration of the denaturant ok. So, some you know some papers or some books were referred to this concentration of denaturant, as D or you can also write it as C or just C. We will mostly use C the problem is if I use D, then I have already referred to my denature state as D. So, I we just want to avoid that complication in terms of the same symbolism ok, but either of these can be used ok.

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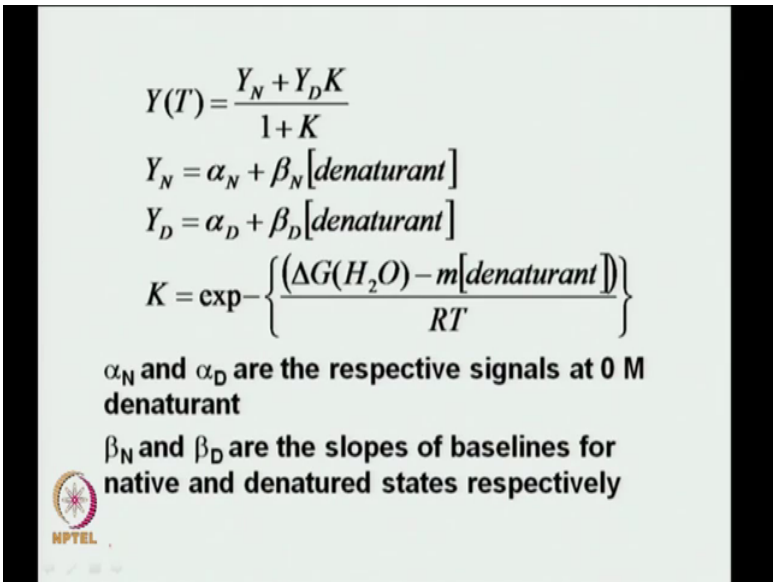

The next again we have this equation, we are going to fit I will show you the transition; the transition will be very similar to a thermal denaturation transition ok, but why at that specific temperature where you are doing the denaturation is Y N plus Y D times the K equilibrium which is equilibrium constant over 1 plus K.

Now, what is Y of N? Remember in when we were doing thermal unfolding we had seen this base line thing right. So, here also we have the same thing in that case you had what you had m N times T because, you are varying temperature; plus an intercept say a of N or b of N in this case what you are varying? You are not varying temperature you are varying.

Student: (Refer Time: 9:41).

Denaturant concentration, so that is why you see its Y N is equal to an intercept alpha N plus beta N, which is the corresponding slope of that native state as the function of denaturant. See the hypothesis or the approach is very much the same. The only thing that is changing is instead of temperature dependence you have a.

Student: Concentration.

Concentration dependence on the denaturant that is the only change you are doing. So, similar thing will happen for the unfolded state baseline or the denatured state baseline which is Y of D right. And then K can expressed like this where; obviously, in that case delta G had a huge temperature dependence remember delta G of T is was equal to delta H 1 minus T over T m and all these things, but that a the temperature dependence here we do not care we are doing at the same temperature.

So, but here we know that delta G has this linear dependence on the concentration of denaturant. So, that delta G is replaced by delta G H 2 O minus m times concentration of denaturant ok. So, if you are given a transition curve you essentially fit this, where alpha N and alpha D are the respective signals at 0 molar denaturant that you can understand Y N and Y D right. Beta N and beta D are the slopes of the baselines for native and denatured states respectively ok.

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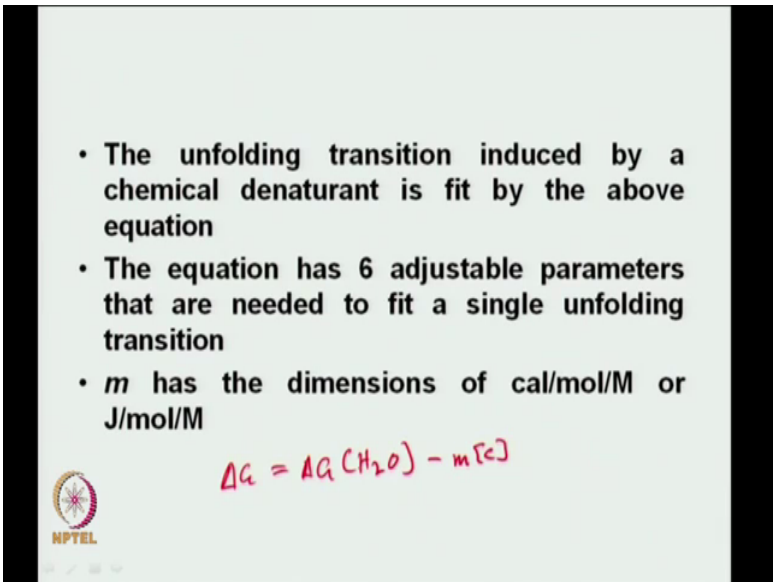

The unfolding transition is induced in I mean the unfolding transition induced by a chemical denaturation is fits by the above equation; that means, the equation we just saw. So, it is essentially fit by this equation Y of T where, the respective you know parameters of the respective stuff you have put in or the items have these expressions right, ok.

Moving on the equation has 6 adjustable parameters that are needed to fit a single unfolding transition, you will see there are 4 which come from what? m is the alpha beta alpha beta and then there are 2 in the equation itself. What are the other two? You can see here one is the delta G H 2 O and the other one is m essentially that is what you are doing everything else is constant for you T is a constant R is a constant ok. So, there are 6 adjustable parameters, you can do and again you do a non-linear fit to your observe transition.

Now, m has the dimensions of calories per mole per molar concentration of a denaturant and that is very straightforward to look at. So, what was see what was your delta G? Your delta G; your delta G observed was equal to delta G H 2 O right minus m, I can write what? Concentration right.

So, if I take the value of m the unit of m what will it be? it will be essentially delta G minus delta G H 2 O over concentration of C or over C delta G minus delta G H 2 O. I told you we are always talking about molar free energy change. So, it is calories per mole or some energy unit per mole, and then you also have C. So, it is?

Student: (Refer Time: 12:57).

Change in free energy per mole per molar concentration of denaturant is it clear? its very important ok.

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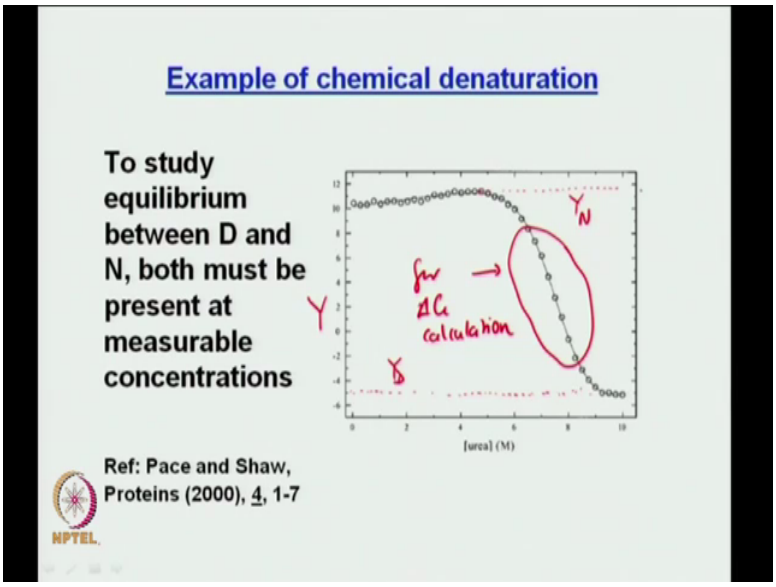

So, let us take an example of chemical denaturation. So, this is typically a certain observable Y let us see what I have here ok. I can just write Y out here, this is a certain observable Y and I am observing the change in Y as a function of denaturant concentration. So, on X axis you have the concentration of urea which is adenaturant right. So, you can understand if I do this from before this would be what? this would be Y of.

Student: (Refer Time: 13:46).

N right because this is where I am starting from see, this is Y of N because, why? At 0 concentration this is the highest this is your folded state. And then not drawn through scale though, but let us see. , if I can manage and this is Y of D ok.

Now, as the statement adjacent to the figure says and to repeat to study equilibrium between D and N both must be present at measurable concentrations. If both must be present at measurable concentrations, what transition I am looking out or what phase of this transition curve am I looking at or what region I am looking at somewhere close to this.

So, this is my transition region and this is the region which is going to give me the most reliable values of delta G right. So, this is what I am going to use for del G calculation its region. So, you can see one thing only within a very narrow region of the concentration of denaturant we have the delta G value.

Whether you are here on the top; that means, below that denaturant concentration range or above that your del G does not have much of a meaning. Because, the respective concentrations or the respective species are not present in measurable amounts as simple as that is it clear? So, now. so, if you would ever look this paper up by Pace and Shaw Proteins 2000 given it essentially I have taken the paper from there ok.

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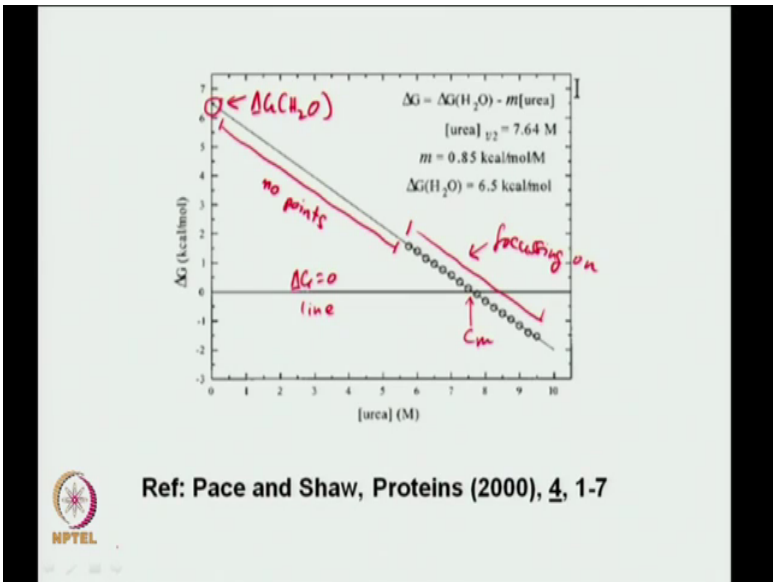

Now, what is this plot? This plot is a plot of delta G versus urea concentration. And it is not surprising that it is a linear dependence on urea concentration because, that is what we started with right; we said the del G was equal to what? Del G H 2 O minus m concentration of denaturant. And that is what you are seeing it is a negative slope. So, the thing is like this then let us see now what is this value can someone tell me this value?

Student: Delta g.

Very good. So, this is delta G H 2 O right. Now, you look at from this portion say from this portion to this portion, if you do this are there any points any measurement points? There are no points from experiment. There are absolutely no points for experiment see that and you

can see, where are we when this when we do not any points these this region corresponds to which concentration? Say 1 2 3 4 even 5 molar urea.

See we are not being able to calculate del G specially for this protein, why? Because under these concentrations your protein is not necessarily showing much of a change in its signals. Why? Because you do not have enough of the denatured state present.

So, the only region you are really focusing on the only region, you are really focusing on is this one this is what you are focusing on. And the rest you are just what you are doing you are just extrapolating.

So, now, you understand what this linear extrapolation is all about, see you get delta G over a very small range of denaturant concentrations. And then you assuming that linear dependence you extrapolate it to your Y intercept, and you get the value of delta G H 2 O which otherwise would have been very difficult for you to get. So, is the hypothesis is clear is this linear extrapolation method clear. Now, this is routinely done this is routinely done ok.

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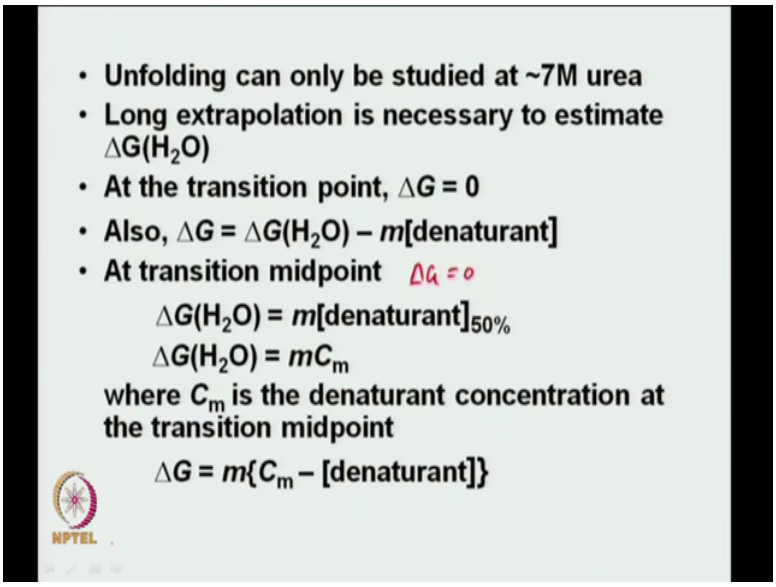

Now, let me go forward and then I will again comeback to this. So, what does this say? The first statement says that unfolding can only be studied at about at approximately closer 7 molar urea. Now, why is 7 molar urea. So, important you go back see what happens at 7 molar urea, you are very close to that line where delta G is equal to 0 and what happens at delta G is equal to 0?

Student: (Refer Time: 18:31).

In this case it is not T m it is called C m.

Student: C m.

Because temperature is not changing right so, but delta G is equal to 0 means you have equal concentrations of native and

Student: denatured.

Denatured. And that is why 7 molar is very close to that point. So, you have appreciable amounts of both ok. Next long extrapolation is necessary to estimate delta G H 2 O and that we just saw because, over that region we do have any points at all we just cannot have any points.

At the transition point delta G is equal to 0 right that because, you have equal amounts of denatured and native state. Also delta G is equal to delta G H 2 O minus m concentration of D denaturant this is the hypothesis we have started with. So, what will happen at the transition midpoint? So, at the transition midpoint this delta G would be equal to.

Student: 0.

0 ok. So, I can write as the transition midpoint delta G is equal to 0, as I have written here. And hence delta G H 2 O is equal to m concentration of denaturant. But it comes with the track a subscript which is 50 percent. What does it mean? 50 percent means I am just at the midpoint its called the transition midpoint exactly like your T of m.

So, like T of m you have a very similar thing out here its C of m right and that is what delta G H 2 O is equal to m C of m, m C of m is taking about. So, c C of m is essentially that concentration that concentration of a denaturant species which brings about 50 percent change; that means, you have 50 percent unfolded 50 percent.

Student: Folded.

Folded ok. So, that is what it says where C m is a denaturation concentration or the denaturant concentration at the transition midpoint ok. So, I can further write then that because we have

just proved that delta G H 2 O is equal to m times C of m, then I can rewrite this equation as replacing delta H 2 O by m C m and hence this is what I have ok.

So, I will just go back and I will mark the corresponding thing. So, this is your C of m is not it this is where C of m is ok, because it corresponds to the.

Student: Delta g.

Delta G is equal to.

Student: 0.

0 line. See everything is you do not have to remember anything right. You just look at the figure and everything is so, evident to you because you know what you are plotting you are plotting delta G versus urea. And then everything follows right ok.

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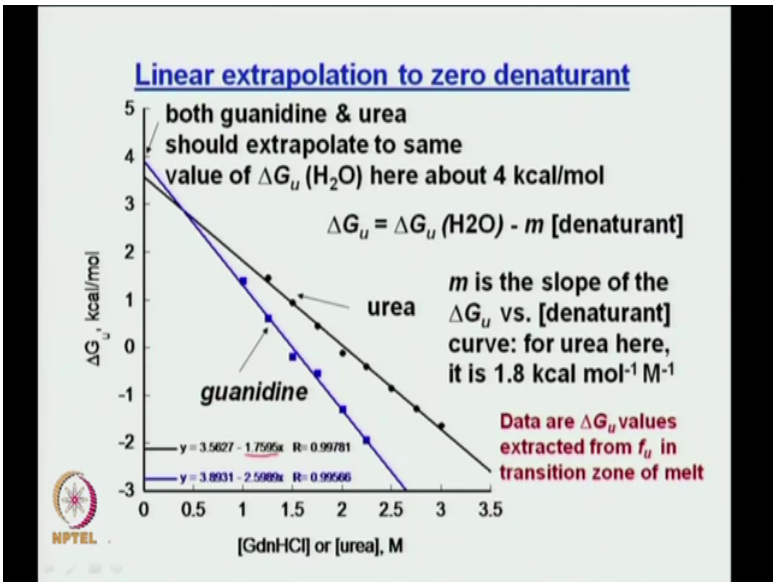

See all this time we have been talking about urea we have not mentioned guanidine hydrochloride that much right, now think about this. Suppose, I have an experiment where I do the where I do the denaturation with urea right.

Now, I take another experiment where I do the denaturation with guanidine hydrochloride see both are going to maintain the same linear variation. Now, your denaturation midpoint might be different because, the strength of this denaturants are not the same. But the linear variation would be maintained. Now, what you would expect then is delta G H 2 O should be the same what because, what was delta G H 2 orbitals?

Student: (Refer Time: 22:21).

Delta G H 2 O was that free energy of unfolding, where there was no denaturant. So, it does not matter whether you use urea or guanidine hydrochloride. If I extrapolate these things back to Y axis I should be getting back, the same value that is what you would expect based on this linear extrapolation method.

But do you see the same happening here? No, right. So, here again we are plotting this delta G so, u is essentially unfolding right like your D of D of m; m is the slope of delta G u verses denaturant and for urea. If you look at this Y is equal to you can see this intersect minus m x where x is your concentration of urea. And Y is essentially delta G. So, you can see this is urea right and then in bottom you have.

Student: (Refer Time: 23:11).

Denaturant I mean sorry guanidine hydrochloride, which one has the bigger slope?

Student: Guanidine.

Guanidine hydrochloride if it has a bigger slope; that means, your delta G has a steeper dependence on guanidine hydrochloride than urea and what does it mean? It means or it burls down to the fact, that the guanidine hydrochloride has more strength. As the chemical denaturant than urea that is simply what it is saying right , there is a the bigger change in slope right just not bother about that. So, this is the main thing.

Both guanidine which is guanidine hydrochloride and urea should extrapolate to same value of delta G H 2 O right delta G unfolding you remember H 2 O, which is about you know 4 calories kilo calories per mole approximately. But, you do not have that there must be reason behind this right.

So, people have been really bothered about this. So, people have tried to explain this and how can you try to explain this? So, what people have done is this linear extrapolation method is one model definitely, but you know think about this. If guanidine hydrochloride has some

electrostatics involved in it because, its a salt urea is the polar molecule. Then there has to be some specificity or some changes in the types of interactions, when you have guanidine hydrochloride interacting with the protein as compared to urea interacting with the protein.

The simple reason being you know proteins have charged moieties too especially on the surface. See, if you would be having a charge interacting with a charge is opposed to a polar molecule interacting with the charge; obviously, the effect would be different is this difference rising from there?

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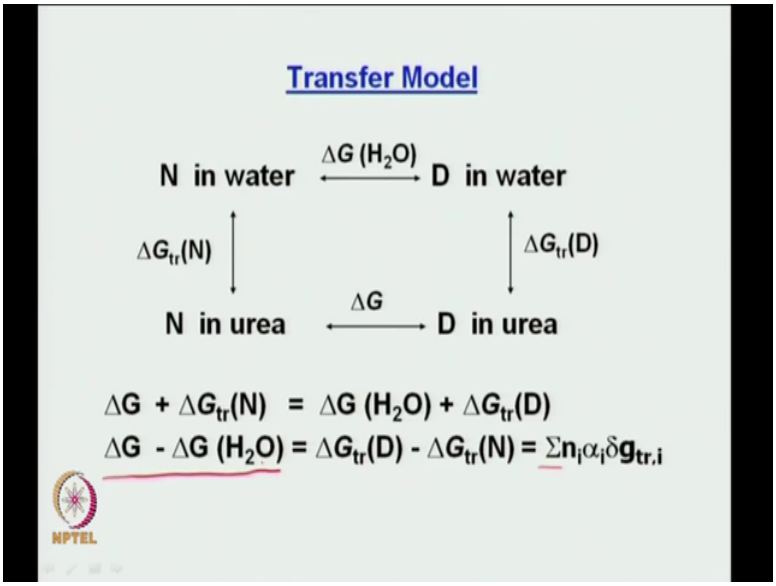

So, what people did is, they tried another model which is called the transfer model. What is this transfer model? See you look at this thermodynamic cycle, you can have the native state in water. You can transfer the native state in water to the same N in a certain concentration of urea. Now, also this native state in water can go to D in water only if N is going to if N in

water is going to D in water, then that free energy change is what delta G H 2 O because, its only in water that is the intercept you were getting remember.

Student: Yes.

Then N in water goes to N in urea that is delta G transfer of the native state that is why its called delta G transfer in brackets N. Then you go out to the right hand side of this cycle D in water goes to D in urea. So, this is this one is delta G transfer of the denatured state.

And finally, what you have is N in urea will go to D in urea and that is what you essentially follow that is what your delta G is. So, this is what the thermodynamic cycle is. The only new thing that you are putting in now is your delta G transfer. And, this transfer remember is very similar to something some type of transfer we talked about before what transfer did we talk about?

Student: Neopentane in water.

Neopentane in water that is a hydrophobicity right we talked about it is essentially the same transfer you are trying to do, but for a different reason with a different system. So, what I can write from the thermodynamic cycle is, the total change in free energy delta G plus delta G transfer of the native form, is equal to delta G H 2 O plus delta G transfer of the denatured form the thermodynamic cycle right.

Now, see how this simplifies; now, what I can write is delta G minus delta G H 2 O is equal to delta G transfer of the denatured state, minus delta G transfer of the native state, just based on the previous equation. And this is equal to; this is equal to you look at I am talking about this one. Now, its a summation of n i alpha i delta delta g transfer i I will tell you what these are; but, before I go to the next slide look at this what is this one what is delta G minus delta G H 2 O? Is not it?

Student: Minus.

Minus N times C the concentration of denaturant right times then m. So, you already have a relation between the concentration of denaturant. And this what we have under summation I am not told you what you have under summation right now.

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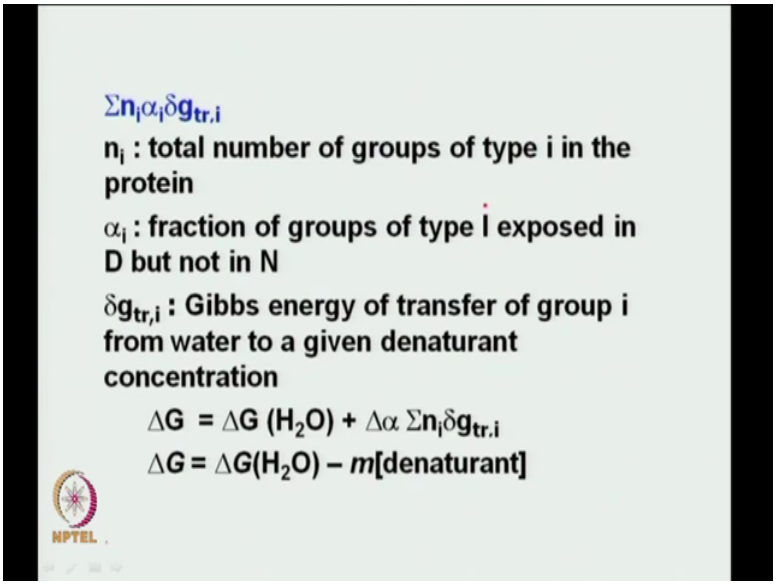

So, let us look at what this means; we were talking about this whole thing under summation. What is n i? n i is the total number of groups of type i in the protein of any type. So, any species i say in a protein and you know a protein will be having different groups.

Then what is alpha i? Alpha i is the fraction of groups of type i, i should be writing type i of type i exposed in D, but not in N ok. That means, in native state they were not exposed, but when you went to the denatured state; obviously, they got unfolded and they got exposed to the surroundings which is in our case denaturant in water.

What is delta G? Now, this is the key term this is the Gibb's energy of transfer of group i from water to a given denaturant concentration. The same thing we did for neopentane we looked at the Gibb's energy of transfer of neopentane from its neat phase to what water phase. In this case what you are doing? You are doing this Gibb's energy of transfer from now water to.

Student: Denaturant.

Denaturant that is what you are doing. So, that is what your Gibb's energy of transfer is ok. Now, you can do a simplification you can do a simplification well let us not going into that, but what we just says is delta G is equal to delta G H 2 O plus delta alpha n i delta g tr i. So, delta alpha means whatever change in exposure we have, you are saying that it is not that much depend upon the denaturant ok, that delta alpha essentially can be brought out of the summation; that means, its essentially a constant.

n i well is a number of groups summation that is fine, but the key issue is your free energy of transfer. So, if you would somehow managed to relate this free energy of transfer to the concentration of the denaturant, then possibly you can relate it to the linear extrapolation method how? If this is what I am writing I also know this delta G is equal to delta G H 2 O minus m concentration of denaturant.

See, if you compare these two if you compare these two equations, what does your delta G transfer i roughly correspond to? It corresponds to minus m times the concentration of denaturant is not. It the other one is I am saying are essentially constant it is essentially a transfer free energy, which now is going to depend upon your denaturant constant relation with a certain slope which is minus m ok. And if you are going to do that and you know this has been done by people experimental wise.

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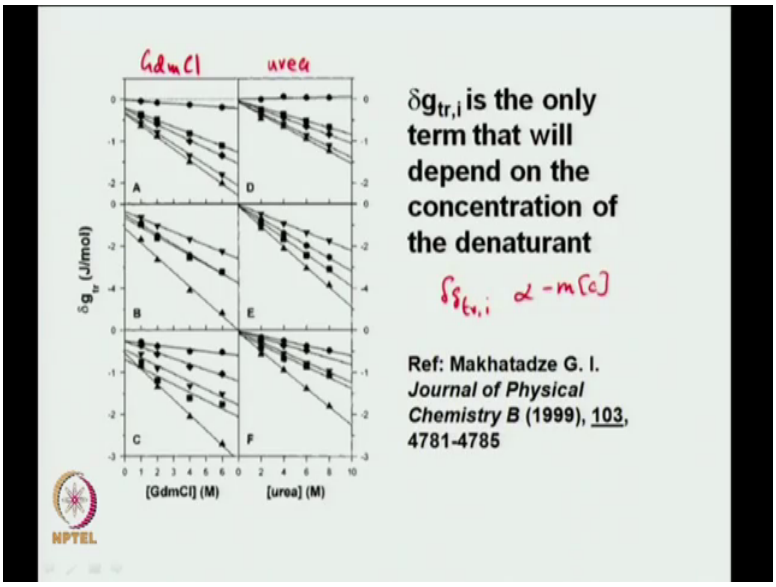

If you are going to do that now this is an ex this is curve you get. So, these are for different amino acids, but do not worry about this what is what you essentially you should worry about is on this side. The right hand side of urea which is written at the bottom. And on this side you have the guanidine hydrochloride G d m Cl is often the short form used for guanidine hydrochloride ok. Now, from the previous slide what I had said was delta g transfer should correspond to what? Minus m I can write.

Student: C.

C. Now, what does this immediately tell you? What it immediately tells you is 1; obviously, it varies linearly, but 2 is when concentration of when C is equal to 0; that means, concentration

of denaturation denaturant is equal to 0 what will happen to delta G of transfer that will also go to 0.

Student: Yes.

So, if you now this is what you have derived? Now this should be independent of what denaturant of what denaturant you are using its absolutely a universal truth. So, if this is maintained by whatever denaturant you are using, then you know that typically they are acting in a similar fashion by the same mechanism no difference.

But, look at what happens in urea, in case of urea see you are starting from here 10 molar. And you go back in case of urea, which is the right hand side. Each and every curve each and every curve goes to 0 at 0 concentration of urea is not it? All of them extrapolate to 0 at 0 concentration of urea. But is it the same for guanidine hydrochloride? Its not the same for guanidine hydrochloride right, what is it? You always get something which is.

Student: (Refer Time: 32:11).

Below that right what does it mean? You are actually over estimating it by a certain amount remember you are always below 0 right ok.

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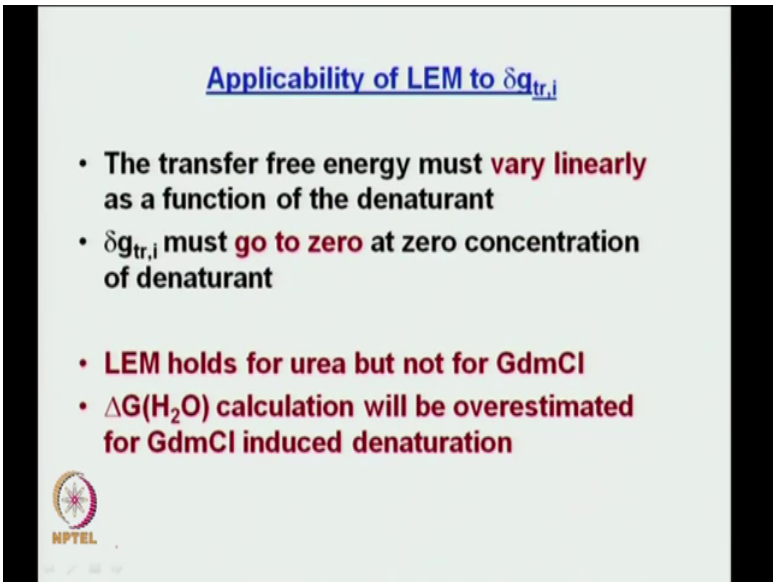

So, let me just go forward the same thing which we just discussed is going to come up on the slide now. If, you are going to apply linear extrapolation method to this transfer model, then the transfer free energy must vary linearly as a function of the denaturant, that we have seen already its applicable for both.

Now, the delta g transfer must go to zero at zero concentration of denaturant ok, but this does not happen for guanidine hydrochloride. So, then what we can say is the linear extrapolation method holds for urea, but not for guanidine hydrochloride this is a proof of that.

And next therefore, delta G H 2 O calculation will be overestimate it for guanidine hydrochloride induced denaturation, it will be overestimate it remember. Because, delta h delta g transfer should have gone to 0, but is actually not going to 0 it is giving you a finite value and that is why your delta G H 2 O is being overestimated. Do you understand, why

delta G H 2 O is being overestimated? Just go back look at equation compare this and you , you will find it out why ok.

But having said this just look back what we had, now see do not you find the same thing happening here this is a two curves the blue one is guanidine hydrochloride the black one is urea do not you think that the guanidine hydrochloride. Now is being overestimated its because of the exactly the same reason, you just saw that delta G transfer was not 0 for guanidine hydrochloride.

So, the bottom line is this the bottom line is that in guanidine hydrochloride the mere fact that it is a salt, will give rise to something which salts to what is that? Ionic strength. That ionic strength would have a huge role to play in the mechanism or the way, this guanidine hydrochloride interacts with the proteins. Because it will start disrupting or interacting with the charges much more vigorously as compared to a polar molecule like urea. And that is the reason that is precisely the reason.

The reason is essentially then electrostatic in origin right. This electrostatic in origin this reason is precisely why guanidine hydrochloride, does not follow the earlier method rigorously. Because, it is not a polar molecule it is the charged species and this charge brings about a change in the way it interacts with the corresponding protein molecule, when you compare the same with that of the interaction of urea right. So, keep this in mind.

Linear extrapolation method is very much applicable for urea, but is not so, reliable when you talk about guanidine hydrochloride and how did we know that? Because, if you are going to extrapolate to get delta G H 2 O you better have the same delta G H 2 O for both because, delta G H 2 O is the thermodynamic parameter 0 denaturant it does not change it has to be the same in both the cases, but it was not.

So, this is this you know this was one of the eye openers like, there is a very good you know there are quite a few very good papers on this denaturation chemical denaturation. Its and let

me tell you this the mechanism of denaturation is still being sought after, is still a very hot topic of research.

But even when people were talking about the thermodynamics, then people realized this fact that guanidine hydrochloride was not satisfying your LEM criteria. And people have from then on being trying to explain why and this is the closest answer they have got; obviously, it depends upon the specific protein.

For example if you take a protein which does not have very well defined charged residues, then they will be very close to each other. If you have you know too many salt bridges on the protein or other specific stuff, then you would expect the difference to be larger and larger ok.

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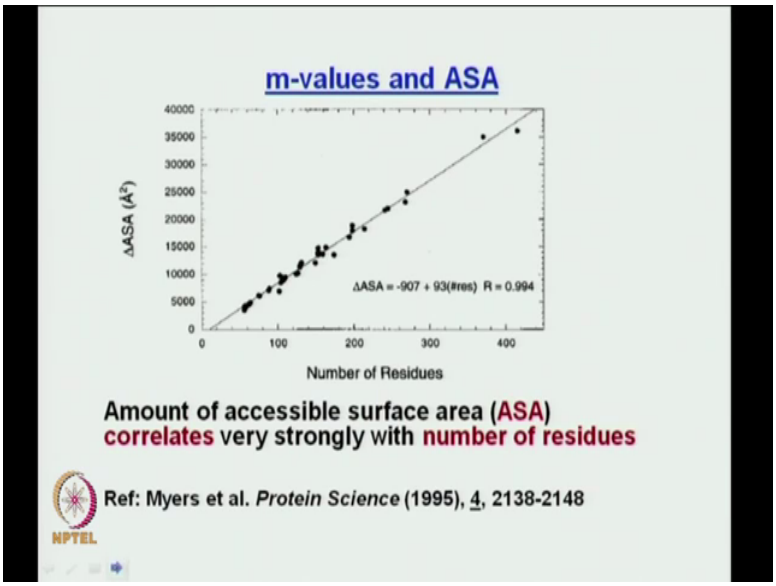

So, what do we have, next let us see. So, we talked about this we talked about the applicability right. Now, m values the dependence of m values on accessible surface area remember, this accessible surface area we talked about when did we talked about; when we talked about when we were talking about this hydrophobic effect right.

And we said that change in delta C p whatever change in delta C p or whatever change in C p you are having the delta C p was essentially because of this ordering disordering of water molecules. And remember the more hydrophobic stuff you expose that is your more accessible surface area that are exposed; that means, that surface is being accessed by your denaturant molecules.

And hence the more of delta C p effect that is coming in; that means, delta C p is also inherently related which are accessible surface area ok. Because, the more hydrophobics residue is exposed the more of water ordering you have and that is those are the water orders you have to break just increasing the delta C p.

So, look at this plot. So, this is a plot of your accessible surface area against the number of residues in a give protein and see it is very obvious right. The obvious thing is if you would increase the number of residues of a protein your accessible surface area.

Student: (Refer Time: 38:32) increase.

Would also increase and you can see almost a very very good linear correlation with a pretty high R value with a pretty high R value. So, then coming back to the question that you asked yesterday. If you would now you know the number of residues right and you can see you have this linear relation, then at least you can estimate based on the number of residues plotted accessible surface area would be the total?

And then this accessible surface area is composed of two things, one is accessible surface area coming from your non polar groups. And accessible surface area coming from your polar groups. And if the paper I will refer to has actually an in depth analysis of both, but you know

will not go into that its just that you have the idea that N value will depend upon accessible surface area why, because accessible surface area depends upon the number of residues as soon see that.

So, amount of accessible surface area correlates very strongly with the number of residues that is what is proven here. So, what they did was so, this the reference I have taken the figure from its is a very good paper I think its all of you should read it.

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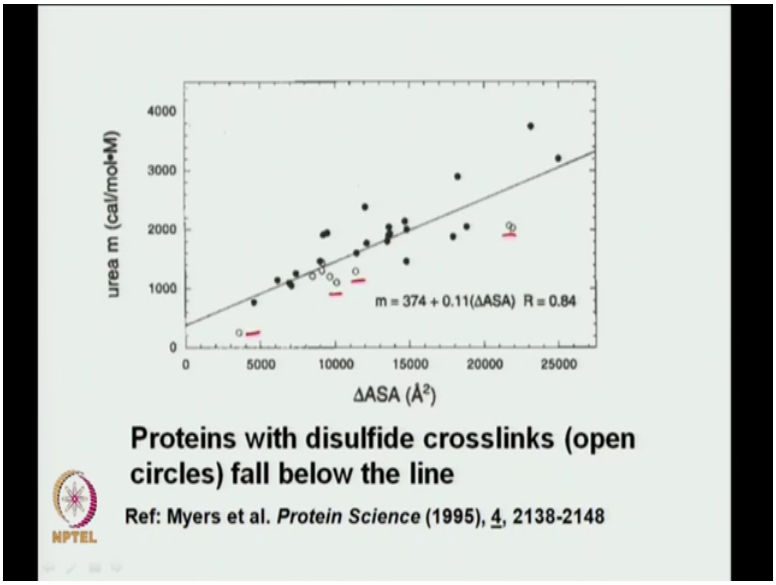

Now, you are doing are you ah plotting m against accessible surface area, what was m? Remember that the slope and in this case it is for urea. So, what you see there is some scatter, but essentially you also have a kind of not very strong as the one you saw before, but you have some well defined linear relation ok.

Only in this case your nature of fit is poor because, your R value is not close to one its it is what 0.8 so it is far off from there. And you do have a lot of scatter now why do you have these scatters; one is proteins with disulfide links or disulfide crosslinks I mean they are the open circles. So, these are these are open circles these are these are the open circles. So, these are the ones which disulfide links.

Now, is not it obvious? If we have more protein which has no disulfide bond. If you have a protein which has a disulfide bond which one would be exposed more?

Student: No sir, no disulfide.

No disulfide bond very simple; that means, the amount of surface or amount of accessible surface area, for the one which has no disulfide bond would be more than the bond which has disulfide bonds. Because the disulfide bond would do what they would?

Student: (Refer Time: 41:08).

Bring them together resist denaturation because, it is a covalent binding right itscovalent binding ok. Hence you can see that this all these circles they essentially lie below that straight line the (Refer Time: 41:25) that means, the m value is smaller than what you expect for a protein having similar number of residues, but no disulfide bond. Because, it is not getting exposed that much if you have a disulfide bond.

So, if so the same paper if you would go through the paper we are not going through that thing here, but if you would correct your model by putting a certain correction for disulfide. Then we will see that the relation are were 10 to 1; that means, if becomes much better because, then you are taking your disulfide crosslinking into consideration.

But anyway the bottom line is that m does depend upon your accessible surface area and so, does delta C p is not it. So, that m and delta C p are very closely related ok.

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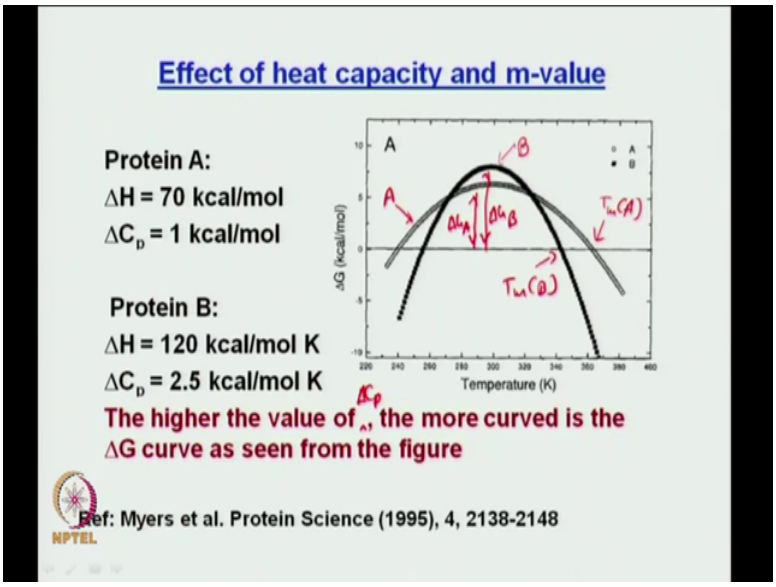

Now, let us look at this very interesting aspect. Effect of heat capacity and m value what you are doing here is here, you are plotting delta G versus T for 2 hypothetical proteins right its the model this is just a model people have modeled these delta G curves.

So, here its a thermal case where you are plotting delta G as a function of temperature you have two proteins A and B ok. So, these are the two proteins A and B. So, I think this one is the corresponding delta G curve for B. And this one is the corresponding delta G curve for protein A, and remember this was your stability curve which we talked about before right.

So, how did they actually stimulate this they say that for protein A delta H is about 70 kilo calories per mole and delta C p is 1 kilo calories per mole. For protein B, delta H is 120 kilo calories per mole and delta C p is 2.5 kilo calories per mole right. Now, the mere fact that delta C p in both the cases are different. The mere fact that delta C p in both the cases are

different, what is the immediate effect you see on the curves? If I had not given you this delta C p value, would you have been able to predict which one has more delta C p.

Student: (Refer Time: 43:56).

How?

Student: (Refer Time: 44:00) del G del is equal to del G by del (Refer Time: 44:06).

Del or.

Student: (Refer Time: 44:09).

We, there is a more direct relation we talked about something.

Student: cp square del H by del T. So, when the range is range is had come is like this. So, del H by del T is the change in unique temperature that brings the change in del H very large.

Hit the point what are you talking about you are talking about a curvature, you should not be you should not be forgetting that what is the curvature it is del 2 del G over.

Student: del (Refer Time: 44:35).

Del T squared what was it equal to?

Student: (Refer Time: 44:37).

Remember it has a relation with.

Student: delta cp.

Delta C p and that is what you are looking at, if your delta C p is high; that means, the curvature would be very high. If your delta C p is low; that means, the curvature; that means, the curvature would not be high it would be shallow, that is what are you looking at right.

So, if you are given a plot like this you should be able to figure which one has more delta C p value just based on the curvature and B has more curvature than a is not it? And that is what it figures because, for B the delta C p is 2.5 for A the delta C p is 1; you have to be able to hit the point just like that ok. You know this thermodynamic relations ok. That was one now what is the consequence? The consequence is you look at this where see which one has a higher T G or T m value now among A and B.

Student: b.

I am not talking about the cold denaturant, I am talking about the heat induced denaturation which one has a higher T m value heat induced.

Student: (Refer Time: 45:46).

How can it be where what is your delta G definition of T m or T g? It is a 0 line which one crosses it at a higher temperature?

Student: A, B.

A crosses it right. So, this is A this is so, so this is the T m for a is not it?

Student: (Refer Time: 46:04).

And this is what this is the?

Student: (Refer Time: 46:07) T m for B.

The T m for B ok. You are happy with this?

Student: (Refer Time: 46:17).

You are happy with this ok, I am also happy that you are happy, but then the problem is this

Student: Sir T g.

Well T m and T G are the same there is no problem with that, but the problem is this you look at this. Remember this delta G we are talking about the delta G which is plotted is your delta G unfolding right. And it goes to a maximum remember that point of where delta G is maximum, where is that which one has a more delta G maximum which one which one has a more delta G A or B.

Student: A (Refer Time: 46:53).

B.

Student: B.

Right. So, this is see if I do this this is delta G for B and I do not have I am just slightly displacing it and this is delta G for.

Student: A.

A and delta G for B is more than delta G for A, but this is weird is not it.

Student: No.

Is not weird good to know, but let me tell you what the weird part is.

Student: Sir.

Because I feel is weird right.

Student: Actually that delta G is (Refer Time: 47:27).

Hold on hold on I know its you are excited just hold on.

The fact that T m of A is greater than T m of B what will what does it mean to you?

Student: Delta G 0.

Delta G 0 occurs at a higher temperature for A, then for B does this have any other meaning to you?

Student: (Refer Time: 47:53) more stability (Refer Time: 47:54).

Yeah. So, he what he is saying is right you think about this what was K equilibrium K equilibrium is concentration of D by concentration of N equal to 1 at T G or T m. That means the fact that I have 50 percent of both at a higher temperature means, that to get to that 50 percent of both I would be needing a higher temperature. So, essentially I would be needing more thermal energy to denature A, but you come down to the room temperature the normal temperature the ambient temperature, what do you see which one has more conformational stability?

Student: B.

B has, B has ok. So, just let me move on so, these are written in the slide. So, one thing is the higher value of well the higher value of actually this one, should be writing delta C p the more curved is the delta G as seen from the figure ok.

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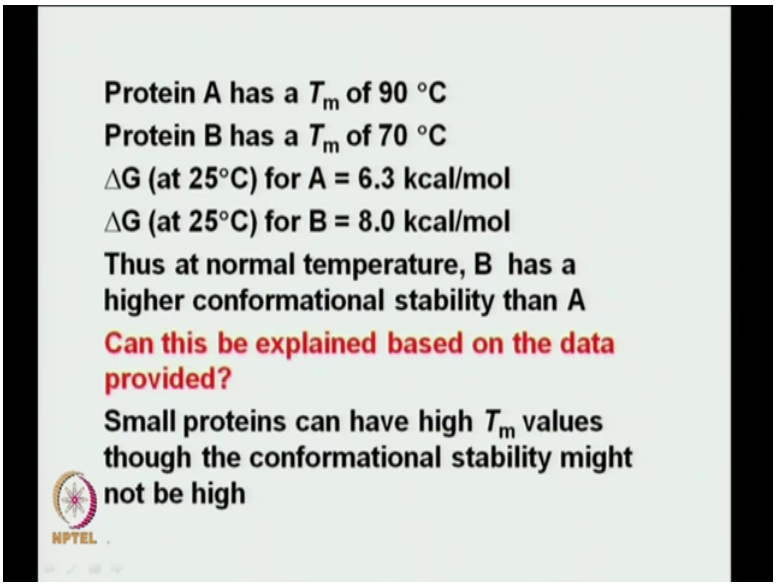

Now, let me give you with the data protein A has a T m of 90 degrees protein B has a T m of 70 degrees Celsius delta G at 25 degree Celsius, which is the ambient temperature for a is 6.3 kilo calories per mole for B, it is 8 kilo calories per mole this is what we just discussed um.

There is a normal temperature B has a higher conformational stability than A at normal temperature remember. Can this be explained based on the data provided? We were just trying to do that.

Small proteins can have high T m values though the conformational stability might not be high ok. So, again think about this the mere fact, that the mere fact that T m is higher for protein A as compared to protein B. Does not guarantee the fact that protein A is more stable than protein B that is the point I was trying to make ok.

Protein A can be a small protein which is what it is saying out here. And its conformational stability might not be that higher room temperature. Protein B say is a huge protein it has so many interactions its conformational stability might be pretty high at room temperature, but when you denature it protein B passes over denature earlier than protein A.

Now, why I am raising this point? I am raising this point because, just looking at a T m it is then very risky for you to make the statement that because, its T m is higher my protein that protein would be stable than the would be more stable than the other protein where the T m is lower.

Student: (Refer Time: 50:58).

Sure the protein which has the higher T m is possibly more stable at that temperature where protein the other protein denatures, but it is not more stable at the temperature where you are actually looking at it that is the normal ambient temperature. So, please again pay attention to this and keep this in mind this is not something I mean you come across regularly.

But see thermodynamics cannot be defined you have done just a model that is why I said there are two hypothetical protein right, there are two hypothetical proteins. And based your thermodynamic modeling the way you are trying to model, it this picture actually emerges. So, you have to redefine what T m is in terms of protein stability.

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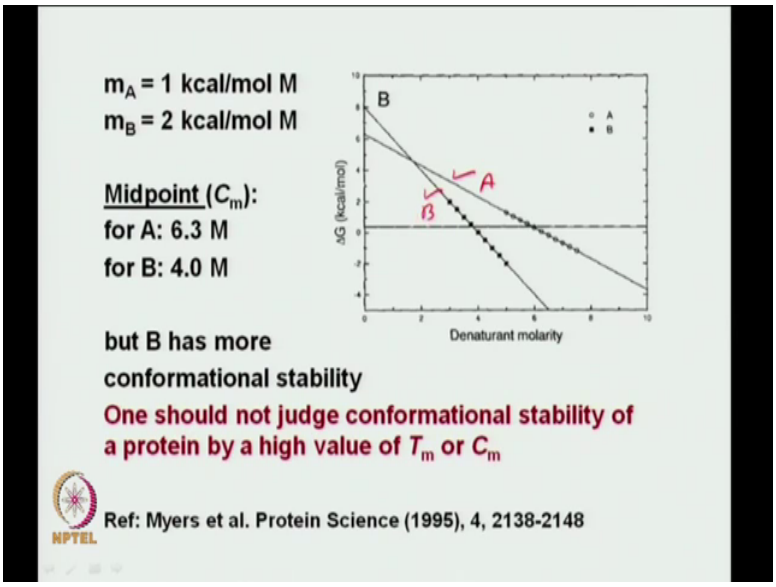

So, essentially now what Pancham was saying is look at the last statement. One should not judge conformation stability of a protein from a very high value of T m ok. If this thermodynamics of that pair of proteins gives you this information from your thermal, it should give me the same information for your chemical denaturation, because both of this follow the same thermodynamics.

Now, see what they have you look at A well its not visible you look A and look at B right. So, you can understand this is B and this is A how can I do that even without trying to look at the ligand, I will be able to do that why because, look at m which has the higher value of m A or B?

Student: B.

B has so B will be changing in a steeper fashion than A is not it. So, we immediately know which one is B which one is A good. Now, look at the midpoint of C m what happens I mean the midpoint of denaturation the C m for A is 6.3, but for B is 4 you are essentially seeing these same thing I just saw for thermal denaturation is not it?

So, again, but B has more conformational stability, B has more conformational stability, how do you know that? You look at delta G at 0 denaturant. You look at delta G at 0 denaturant which one has more stability?

Student: B.

B has ok.

So, again one should not be judging conformational stability from either a higher T m or a higher C m. And let me see whether I have the other statement here, I do not have it here. So, what it means is your T m or C m essentially just gives you this information what information does it give you? It gives you this information that it is that temperature. Where I have 50 percent denaturation it is just that temperature where I have 50 percent denaturation.

But in most cases for people who would do or who are doing research with protein or working with proteins, you would see in most cases T m would actually confirm to change to protein stability. The idea out here is it is not always the case. See, you do not always correlate T m and C m with stability of a protein. But remember this is a stringent case this is a hypothetical case you have just explained it based on your thermodynamics nothing else.

Because, protein unfolding folding is what we are concerned about mainly the thermodynamic parameters, and that is what we have taking care of there is no other assumption involved. You have already seen this and that is what we have used to do a hypothetical model ok.

So, I will well we will stop here that is essentially bringing us to end of chemical denaturation. Next class what I we will do is we will look at try to look at some mechanisms what people think that chemical denaturation, you know the mechanisms they invoke to denature proteins. And then move on to other types of denaturation's ok.