Experimental Biochemistry
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Lecture - 20
Temparature Denaturation of HSA Studied By UV/VIS Absorbance

We have just seen the effect of urea on HSA Denaturation, the change in absorbance upon adding urea solution. Next thing we are going to study the effect of temperature on protein denaturation. For this what we have done we have taken a few samples of HAS, having a fixed concentration. And, we have suspended these samples taken in this apindrop as you can see here in this apindrop suspended in a water bath at different temperatures.

The temperature have taken are 25 degrees centigrade and the room temperature, 30 point degrees centigrade the physiological temperature. And the other 2 temperatures which are adjusted by using water bath were 45 degrees centigrade, 60 degrees centigrade and 80 degrees centigrade.

What basically happens is that at higher temperature the protein tends to lose it is structure. So, we are going to study how the effect of temperature determines the absorbance of protein. Now, let us see the absorbance at 25 degrees centigrade.

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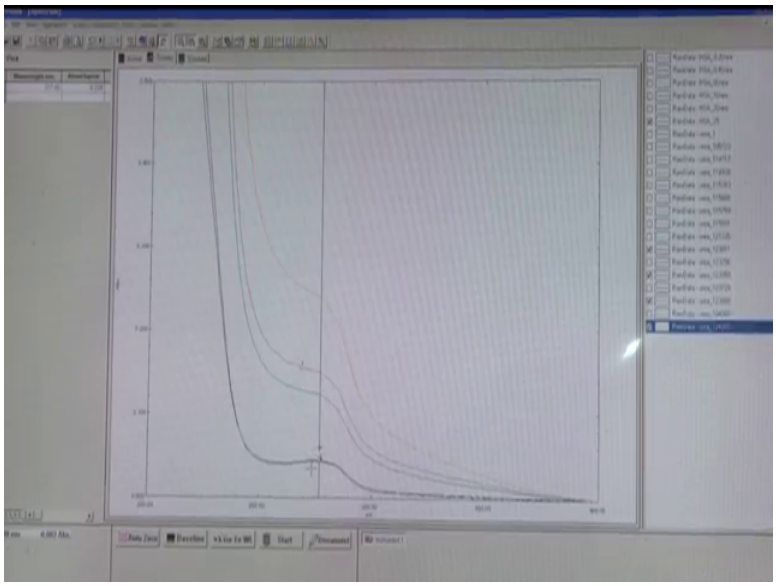

So, we have already done the baseline correction. And, now we have do the auto 0, in the reference compartment there is only double distilled water in the sample compartment we have taken HSA solution at 25 degree centigrade now. And, one thing let us see out here that we are monitoring it from 200 to 400 nanometer. Basically, what happens is that in the previous case we are monitored up to 600. So, since major peaks are not being observed observation of restricted up to this region so, it is better not to consider above 400 for the time being.

So, let us find out again it is around 278 the value is coming 0.043. Now, we can save it in a denaturation part we can make a folder having temperature, and here we can save it as has as 25 suggesting 25 degrees centigrade again save it in the text format. Now, we are going to see it is absorbance at around in the same region; we are going to side the absorbent at this region at 37 degrees centigrade. So now, we are going to take the absorbance at 37 degree centigrade for this let us zo auto 0 once again and start.

Here, we can see the protein the absorbent value is more or less equal to that of 25 degrees centigrade, we will zoom it once this is complete. Here we can see the absorbance are slightly changes were not that much out here, which signifies that to more or less the protein we tend to structure at around 37 or 25 degrees centigrade.

Now, will shift to 45 degrees centigrade and see what heat causes to protein structure. So, the auto 0 is has been done it is set to 0 and let us start at 45 degrees centigrade. I have just picked up a protein at 45 degree centigrade. We can see the absorbance increases, that is for denaturation of started occurring, again here we can see it is peak around 278. Now, one small thing to notice out here is that the short peak is not obtained here.

Because, since the protein have started in denaturating the structure basically, is not like that of the parents structure. Since, the protein have started denaturating the structure changes there by a peak is not observed out here. However, we can assume that there was a peek out here with intensity is much higher than the normal part. Now, we will go to the higher temperature it is 60 degrees centigrade.

Now, we are going to see the absorbance of protein for temperatures 60 degrees centigrade. Now, we can see a proper peak is not absorbed out here again the absorbance increases have a proper peak is not obtained here rather a shoulder for that what we need

to do we need to subtract blank. What is a blank actually basically it should be or blank or control it should be only the solvent in that current condition.

So, it again increases around 278 the absorbance again increases we can see, the last one we are going to do it is for 80 degrees centigrade. Now, we will be measuring the absorbance value for has solution which is kept at 80 degrees centigrade, we can see that absorbance is quite higher as compared to the other 2 which signifies that are very high temperature the structure completely denaturates.

So, this is for 80 degree centigrade this one is for 60, this is around 45, and this 2 are 25 and 37. So, from this part what we can basically see is that for 25 and 37 this are very close to one another and for higher part the observance increases. So, we have seen how temperature affects the structure of protein. Basically, what is happening there, when a protein is being heated it is structure gradually breaks the hydrogen bond breaks at first and then the entire structure collapses and that is why denaturation takes place.

So, hydrogen bonding we know it is not that much strong like overland bonding. So, upon the heat upon applying heat the hydrogen bond breaks and that is why we can see an increase in absorbance and why the increase in absorbance, because the same fundamental principle applies when protein is denaturated the structure breaks, it unfolds the tire tryptophan residue present within the core of the protein is being exposed within the solution, they are by more and more tryptophan is exposed in a solution and that is why the absorbance increases.

Now, while doing the experiment one thing we have to note down here, which you should follow is that in order to obtain a proper peak out here, like the peak it is shoulder is coming no proper because coming, but we have to do a control subtraction or a plan subtraction. So, what should be that? So, after you have done with this experiment you have to what you have to take the solvent, that is your double distilled water kept in that same condition and then you have to take the absorbance value.

So, what does that mean? For example, you are taking a protein solution of around say 100 micro molar protein solution and we were heating it around 40 to 45 degrees centigrade and taking the absorbance, and the solvent you are using is water or buffer whatever be the case.

Now, the control should be exactly buffer or the solvent or anything except the protein. So, if you add actually protein plus component which giving the solution then in the control you have to take only the component a without protein why, because if the component a have any sort of contribution in the absorbance may not be a peak or some sort of other contribution in the absorbance. So, it will get nullified.

So, what we have to do is that after we have taken save these files of data format and again take the temperature again take the absorbance value of the control. Now, suppose for this case for 80 degrees centigrade, we have kept taking the protein heated it and taking the absorbent value. Now, we have to similarly heat that amount of water and again take the absorbent value. Logically speaking we do not think water shall give any absorbance value, but; however, it is recommended to do it.

Because the next time you will not stick to what you will get a buffer or any other medium or what would be the case. So, it is better to take this control subtraction. So, what we have done is that, we have taken initially urea solution taken the absorbance of HSA a protein and now [FL]. So, what we have done initially we have taken HSA solution and we have applied stress in the form of denaturant or temperature. And, in both cases we can see increase in the value of absorbance and which implies that protein denaturation is taking place.

So, one small practical application of protein denaturation which we can basically relate is that why during diseases or why when we get fever, why do we feel weak? So, it might apply that when we get fever our body temperature rises physically it arises to fight with the bacteria or whatever the infection is it is a viral or virus or something like that. However, in doing so, other proteins present in our body gradually loses it is function why because the structure of protein might detoriate when the body temperature rises.

And, that is why the protein are functioning and as proteins are functioning we gradually feel weak. And that is why during fever weakness is one of the reason when protein gradually starts and that is the during fever that is the reason why we feel weak, because the protein the denaturates not at all some denaturates and as a result we start to feel weak, because the protein that will stops it is function. And, that is the reason why temperature affects the structure of protein.