Experimental Biotechnology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology – Guwahati

Lecture – 22
Gel filtration chromatography - part-3

Hello everybody, this is Dr. Vishal Trivedi from department of biosciences and bioengineering IIT Guwahati. And what we were discussing about the gel filtration chromatography and in the previous lecture we discussed about the basic principle of gel filtration chromatography, how to pack the columns and then what are the different aspects you have to consider why you are running the gel filtration column or when you are maintaining a gel filtration packed column.

So, now, in todays lecture, we are going to discuss about how you can be able to exploit the gel filtration chromatography to answer some of the questions or scientific problems. So, in this series, let us discuss the first problem. So, research problem, but we are planning to discuss related to gel filtration chromatography is where, you know it could be for the scientist or it could be for the PhD students.

The basic idea of discussing these issues problem is that we would like to emphasize that the gel filtration chromatography is a very robust tool to answer many of the complicated questions and performing a gel filtration chromatography is easy. So, that is why if you would be interested you could be able to utilize the gel filtration chromatography is as one of the tools to answer these questions. So, let us start with the first question.

(Refer Slide Time: 02:42)

Research Problems 1

A PhD student wants to determine the structure of a protein X from *Mycobacterium tuberculosis H37Rv* with the help of X-ray crystallography. He has cloned the protein in E.coli expression system and setup the crystal but ended up in getting precipitate instead of crystals.

Now, he want to design few experiments to check the quality of protein purified from E.coli expression system.

Molecular weight

Native conformation with oligomeric status

So, the first question is about a PhD student who wants to determine the structure of a protein X from the mycobacterium tuberculosis H37Rv is the causative agent for TB or the tuberculosis with the help of the x-ray crystallography. And what he has done, he has cloned the protein in equally efficient system and then he has purified the protein and set up the crystals, but what he could get is he was getting the precipitate instead of getting the crystals.

So, in a typical x-ray crystallography related structural determinations, what you are supposed to do is you have to first isolate the proteins in a large quantity in a purified form. So, what you are going to do is, first you are going to get the purified protein then, what you have to do you have to concentrate these proteins, so, that it should reach to a concentration where it will start showing the crystallization and then you are incubate this in a in a different conditions or different crystallization conditions.

And at the end in any of these conditions, the protein is going to get precipitate in a regulated fashions and as a result, the protein is going to crystallize and give you the crystals, but with this a student who has actually be interested to solve the structure of the protein X using the x-ray crystallography, he has cloned the protein so, one of the easiest way of getting the purified protein is that you clone the protein in any of the overexpression systems.

Such as e coli or bacilli or mammalian expression system depending on the yield and the protein of the choice and then he has performed and then he incubated the protein, the concentrated

protein in a different conditions and what he could found is that he instead of getting the crystal, he was getting the precipitate which means the precipitation of the protein was very fast compared to the very slow so that he should get the crystal.

Now, what the first thing he want to ask is whether the protein what he has purified from the e coli is good enough or it is in a good native conditions or if the protein the quality of the protein what he has isolated from the e coli is good, so that he could actually be able to verify the other parameters. So, what he wants to design the few experiments to check the quality of the protein purified from the e coli system.

So, what do you mean by the quality of the protein is that he would like to determine the molecular weight of the protein and then he wants to also ask whether the protein is present in the native conformation and what are the different oligomeric status of this particular protein. So, he wants to ask 3 questions, whether the protein is properly folded, means here is the protein is maintaining 3 dimensional confirmations.

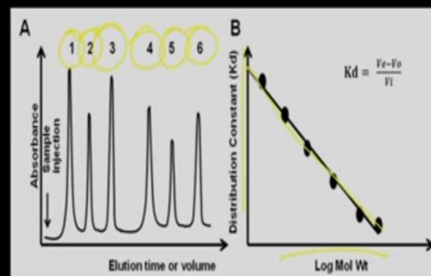
Number 2, what is the molecular weight of the protein. And, number 3, what are the different conformational status of this particular protein, you know, the protein is being produced as a monomer. But these monomers comes together to give you dimer or tetramer or even the oligomers and there are many proteins which are actually been present either as a monomer, dimer or the higher molecule oligomers for to answer these questions, he has to design an experiment related to gel filtration.

(Refer Slide Time: 06:55)

But based on the basic principle that the distribution coefficient is direct is equivalent to the V e - V o by the V i, which means the if you would like to utilize the gel filtration to determine the molecular weight, the molecular weight should be in proportion to the radius of gyrations. So, the molecular weight and the size of the protein is related to the shape of the molecule and the relationship between the molecular weight and the radius of gyration is R g is directly proportional to the M a.

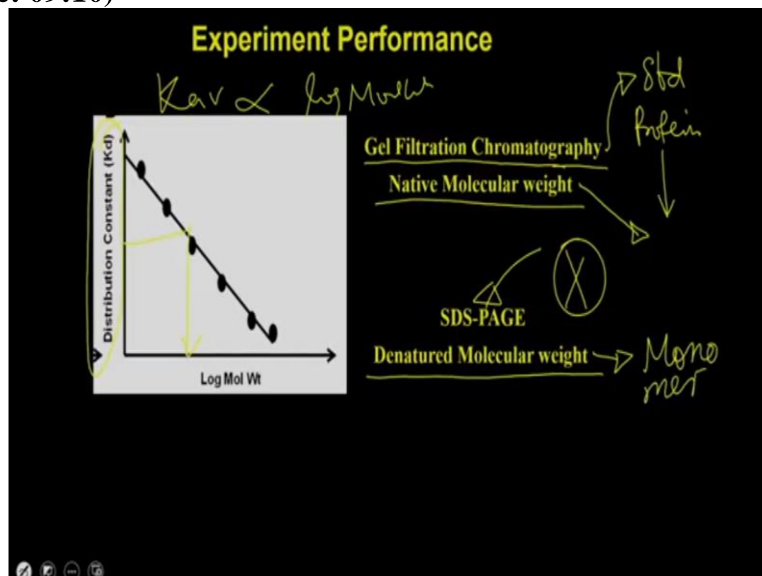
Where a, is a constant and it depends on the shape of the molecule a is 1 for the rod, which means the R g is directly proportional to the molecular weight, a is 0.5 for coils and a is 0.33 for the spherical molecules. So, this means that the molecular weight is directly proportional to the radius of gyration. And because of this relationship, you can be able to utilize the gel filtration to determine the molecular weight of this particular protein and also you can be able to determine the oligomeric status as well as the whether the protein is present in native conformation.

(Refer Slide Time: 08:20)



So, what is the experimental design, for the experimental design the first thing what you have to do is you have to run the multiple proteins of the different molecular weight. So, in this case, for example, we are running the different types of molecular weight proteins and the then you have to draw a calibration curve between the kav versus the log molecular weight. And that is how it is that calibration curve can be used to determine the molecular weight of the unknown protein X as well as the oligomeric status as well as to whether the protein is present in native conformation or not.

(Refer Slide Time: 09:10)



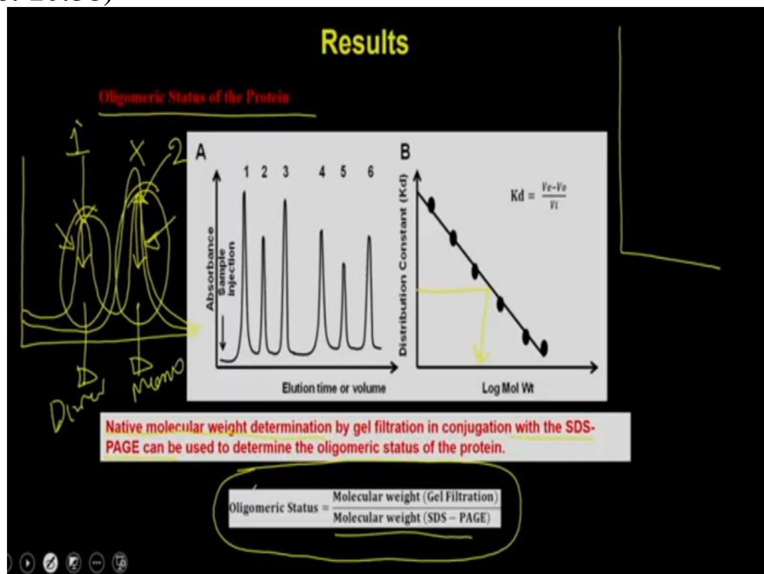
To perform these experiments, what you are supposed to do, you are supposed to do a gel filtration chromatography, where you are going to first run the standard proteins to draw a calibration curve, which means, you have to determine the relationship between the kav versus

the log molecular weight. And once you know that relationship, you can be able to run your native proteins or the protein of the protein X.

So, suppose the protein X is giving you the kav value of this that actually can be used with the help of the calibration curve to determine the molecular weight. Apart from that, you can also run the protein X into the SDS page and that actually will give you the denatured molecular weight, whereas the gel filtration chromatography is going to give you the native molecular weight, which means, what is mean by native molecular weight means is the if the protein is a dimer, it is actually going to give you the dimeric molecular weight.

Whereas, the denatured molecular weight means, it is actually going to give you the molecular weight for the monomer. Now, utilizing these 2 tools, the gel filtration chromatography and the SDS page, you can be able to determine the oligomeric status.

(Refer Slide Time: 10:38)



So, how to determine the oligomeric status of the protein first you have calibrated the column then you have drawn the calibration curve now, you have the native molecular weight, which you are going to determine with the help of the calibration curve and then you are going to determine the denatured molecular weight with the help of the SDS page. And that you if you put it into the formula that is the oligomeric status is equivalent to the molecular weight you are going to get from the gel filtration divided by the molecular weight which is going to be get by the SDS page.

Apart from that the quality testing can be also done, when you are going to analyze the protein X on to the gel filtration column, what you are going to get is you are going to get the pattern of protein X in that particular buffer conditions. So, you can imagine that if have the protein X either giving you single peak or you can have the protein X or in a different conditions you can imagine that the protein x might be giving you 2 peaks.

So, then what you can do is you can determine the molecular weight of the peak number 1, you can determine the molecular weight of protein number 2, and if the peak number 1 is of the double the size or higher molecular weight size that means, that the protein X is probably be present in the 2 molecular 2 oligomeric status, which means it either it could be present as the dimer or the monomer or the mixture of both and that is very common in many of the proteins.

That the proteins are present in a dimeric conditions as well as the monomeric conditions and when you have such situations and you are interested to study this protein it is always important and always recommended that you actually isolate these individual peaks, so, that you will be able to study the dimer versus monomers separately and that any of the either of these molecules or either of these oligomeric status of the proteins might give you the crystals or might precipitate very slowly and it will give you the crystal.

So, that is also one of the strategies, what people also plan and try and that actually gives you the successful crystallization of protein and it actually eventually give you the structure of that particular protein.

(Refer Slide Time: 13:29)

Now, let us move on to the next problem. And the next problem is that the protein X is present in 3 oligomeric status. So, here you already know that the protein X is present in 3 oligomeric status one is monomer, dimer, and tetramer which means the protein is present in 3 oligomeric status, this is the tetramer, this is the dimer and this is the monomer. So, it is already been set that the protein X, but you have synthesized or what you have produced having the 3 oligomeric status.

And now, the scientists want to study the stability of this particular protein. So, before get into the detail of the stability of the protein, because a stability is a very weak term and you can actually have the stability of a protein against multiple parameters. For example, you can have the stability of a protein against the protease statement, you can have the stability of a protein against the denaturation conditions such as the urea, GDMCL and all other conditions and you can have the stability of a protein against the thermal denaturation.

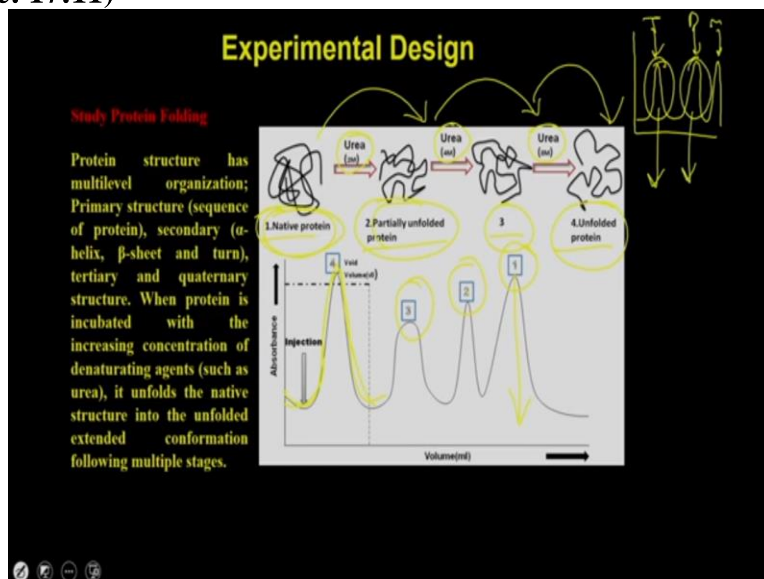
For example, you can incubate the protein at different temperatures, irrespective of the conditions except the protease if you are treating a protein with the denaturing agents or if you are treating a protein with a physical parameters such as the temperature or other kinds of parameters, it is actually going through our different discrete steps in which the protein which is 3 dimensional confirmations is going to denature and going to adopt extended confirmations.

So, in that process the protein is going to be giving you the multiple confirmations for example, you start with the native conformation which is actually 3 dimensionally folded proteins. So, that is actually a compact structure it will be you know if we add the protein is going to actually arrange all its amino acids around center, but as soon as you apply the small amount of urea the protein is going to be partially folded and as you can see, the diameter of this protein is now increasing because you are breaking the interaction between the side chains and even the main chain also.

And because of that, it is actually adopting those extended confirmations and the structure is now opening. So, that is the diameter of the molecule is also increasing. Now, if you further denature with the help of slightly more concentration of urea, what will happen is it will further going to break more and more interactions and ultimately it is going to increase its diameter and ultimately, when you have a very high consideration of urea such as the 8 molar urea, the protein is going to be in a extended conformation.

And that is how it is actually going to acquire the very high molecule high hydrodynamic surface area or the diameter. Now, if you would like to follow this kind of changes, and you would like to use that as a way to answer whether the protein is stable or not, what you can do is you can take this protein X and you can perform a gel filtration chromatography to answer these questions.

(Refer Slide Time: 17:11)



So, in the experimental design, what you are going to see is, if you are having all these confirmations, and if you are going to analyze them on to the gel filtration chroman what you will happen is this is the largest size, this is the middle size, this is even smaller than this and this is the smallest among them. So, if you follow this, what you will see is that the unfolded protein which is completely unfolded protein is going to be present in the void volume.

Whereas, the other 3 confirmations like the 3, 2 and 1, so 1 is going to be loot as per its native confirmation for the native positions, the 2 and 3 are going to give you the intermediate positions and the 4 which is actually the unfolded protein is going to be presenting extended confirmations. So, that will be completely excluded from the column and it will be present in the void volume. So, that is the way you can be able to design the experiment.

And can be asked to answer that whether the protein is more stable or less stable, because the amount of urea what you are going to use in this experiment is going to determine how much urea is going to require to bring the 1 position to 2 position or 2 to 3 position or from 3 to 4 positions, the amount of urea what you are going to use is actually going to indirectly tell you the how much stable the native conformation is, and that question can be even asked even for the different oligomers.

Because what you can do is if you run this protein on a gel filtration column, you are going to get 3 peaks, which is actually the tetrameric peak, dimeric peak and the monomeric peak, what you can do is you can isolate these peaks individually and then you can be able to perform the similar experiments along with the individual oligomeric status and that actually will tell you which oligomer is more stable and which oligomer is less stable.

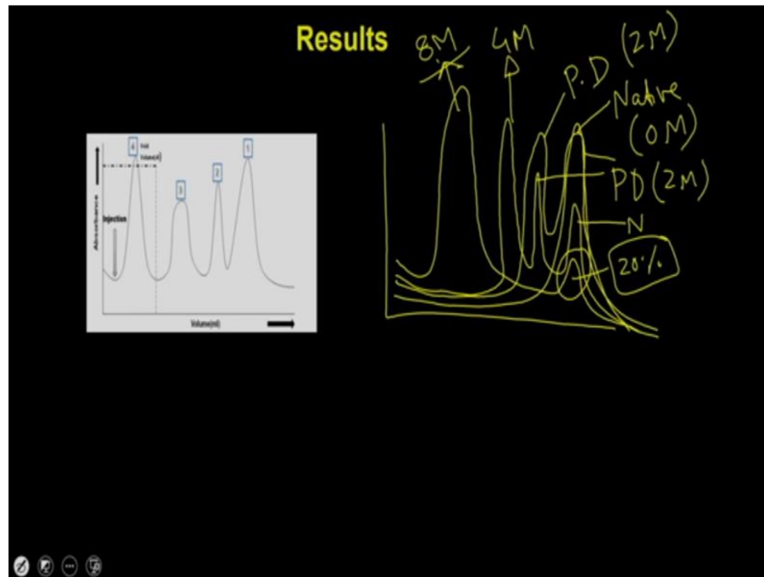
(Refer Slide Time: 19:29)

So, in the experimental performance, what you are supposed to do, the protein is incubated with different concentration urea which means from 0 to 8 molar urea for 8 to 10 hour at 37 degrees Celsius and in parallel you have to run a gel filtration column which is equilibrated with a buffer containing urea same as an incubation buffer which means if you are running the 1 molar urea and you incubated the protein in 1 molar urea, that buffer also should contain 1 molar urea.

So that while the protein is also running through the column, it should not experience any kind of reversible folding, because you know that if you will provide the non-denaturing conditions and if you provide the non-denaturing conditions, the protein will tend to fold. So, that is how it is actually not going to give you the real picture. So, what you are supposed to do is you have to use the same concentration of urea for equilibration of the buffer and the same urea in which you are going to incubate the protein.

And then you analyze the results as the in consideration of the denaturing agent is increasing the protein will unfold with the increase in hydrodynamic surface area. As a result, the protein will peak will shift towards left at highest consideration or denaturant, the protein unfolds completely and mostly appear in the void volume. So, what you are supposed to do, you are going to do a gel filtration chromatography with the buffer containing urea and you also have to incubate your protein with the different consideration of urea which means from 0 to 8 molar urea, here also 0 to 8 molar urea.

(Refer Slide Time: 21:21)



Now, how the results will come, the results will come like this where you are actually initially going to see a peak of the native confirmation. Now, this is at 0 molar urea 1, once you increase the urea concentration, what will happen, it is actually going to shift and going to start going to give you the 2 peaks. One peak which is for the native protein, the second peak, which is for the partially denatured protein.

Now, as you increase, so, it suppose this is at 2 molar urea. Now, if you increase the further what will happen, you are going to see one more peak, you are going to see one more peak and you are going to see that the native protein is now reducing in concentrations and you are getting the partially denatured protein which is of 2 molar urea and you are getting another peak which is actually corresponding to the 4 molar urea.

And now, if you further increase you are going to get very high peak and probably native peak if there is still the protein which present in the native conditions Otherwise, the all the protein is going to be get converted into the denatured protein. So, if you can see this movement, you can be able to conclude that the protein is showing you a denaturation curve, and what will be the stability of the protein, because the mutual proportion of these confirmations in different peaks is going to give you an idea how much the protein is stable because even at 8 molar.

Suppose you have the 20% protein still left in the native conformation, which means this protein is very stable, and probably it tastes can withstand the very harsh degenerating conditions.

(Refer Slide Time: 23:25)

Research Problems 3

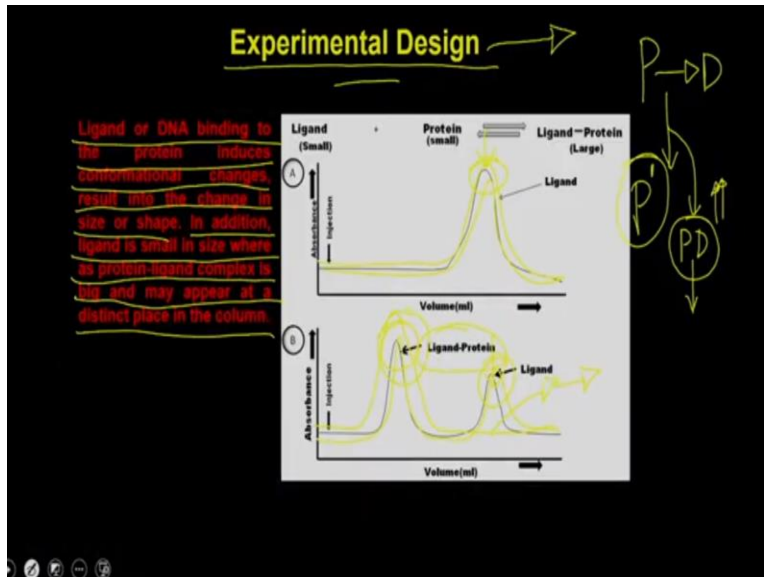
A scientist has isolated a unique protein responsible for induction of apoptosis in the cell. He suspects that the protein might be interacting with DNA and disturb its replication. Now we needs to design experiment to study interaction of DNA with protein.

Now let us move on to the third problem. And the third problem is that a scientist has isolated a unique protein responsible for induction of apoptosis in the cell. He suspect that the protein might be interacting with DNA and disturb its replication. Now, he needs to design an experiment to study the interaction of DNA with the protein. So what he wants he has isolated a protein p which is actually interacting with DNA.

And that is how it is actually interfering or disturbing the replication of the organism. And once you are interfering the replication of the organism, the genomic DNA of that particular organism cannot be synthesized. So, as a result, the cell will have no option, but to go for the apoptosis pathway, where the cell is going to stop its growth and it will go through the death pathway. So, ultimately, it will go for the apoptosis.

Now, if I have to design an experiment, and it would like to answer these questions, if you remember, when we were discussing about the ion exchange chromatography, that time also, we have discussed how you can be able to study the interaction of the DNA with the protein, where we have taken an example of a transcription factor and the promoter region present on the on the gene. So, in this case also, you can be able to answer the similar questions or you can be able to explore the similar questions also with the help of the gel filtration chromatography.

(Refer Slide Time: 25:25)



Now, what you are supposed to do in your experimental design, what you are, so, what is the experimental design where you are actually in going to incubate the DNA? So, when the ligand or the DNA binds to the protein, it induces the conformational changes into the protein and also it increases the molecular weight? So, when the protein is going to interact with DNA, it is actually going to affect the first the size of the protein.

The second the protein DNA conformation is going to be of higher molecular weight, which means, you are actually going to study this simply by 2 ways one, if the conformation of the protein is going to be changed, it is actually going to change its position on the gel filtration curve. So, that is what you see, if you are injecting the native protein it will actually going to be present at a different position.

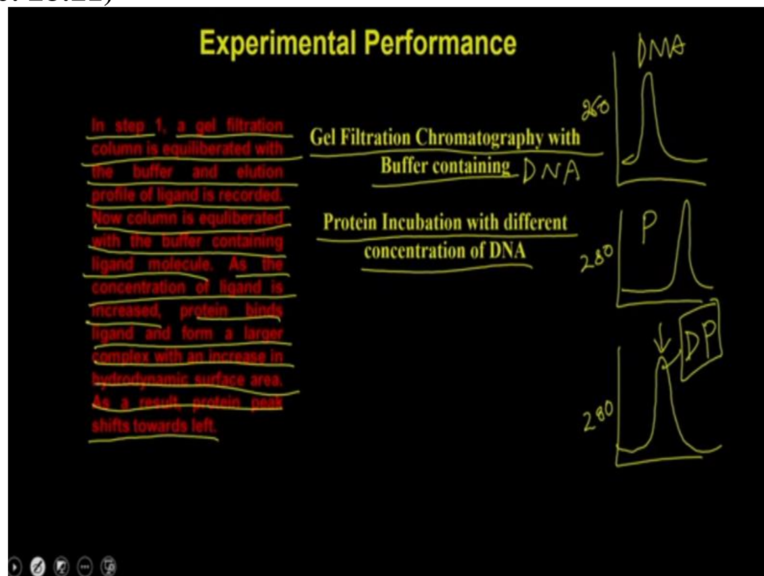
But if the like the ligand and the protein is present, it is actually going to give you the high molecular weight and that is how it is actually going to show you the separate peaks. So, results into the change in the shape size or the shape in addition ligand is small in size whereas, the protein ligand complex is big and may appear at a distinct place in the column. So, this experiment we are showing you only for the DNA.

But, you can be able to answer even the interaction of the small ligands such as the substrate with the protein also utilizing the gel filtration chromatography, because the substrates are very small, they are going to be run as separate peak and when they are in a complex with the protein

they are actually going to run very separately from the ligand peak and that is how you can be able to understand the interaction of the ligand with the protein.

Also the amount of the ligand present in the in the condition B versus the amount of the ligand present in the condition A can be used to ask how much ligand is being consumed to give you the ligand protein complexes. So, if you follow that the ultimately what will happen is you are only going to get the ligand protein complexes, but there will be no ligand present and that is the status where all the ligand is been consumed to form the protein ligand complex and that is the concentration can be used event to calculate dissociation constant and all other parameters.

(Refer Slide Time: 28:21)



How to perform these experiments you perform this experiment and what you require you need a gel filtration chromatography, where you are going to incubate the gel filtration buffers containing the DNA and you have to incubate the protein with different concentration of DNA. So, what do you have to do in the step one, a gel filtration column is equilibrated with the buffer and the elution profile of the ligand is recorded.

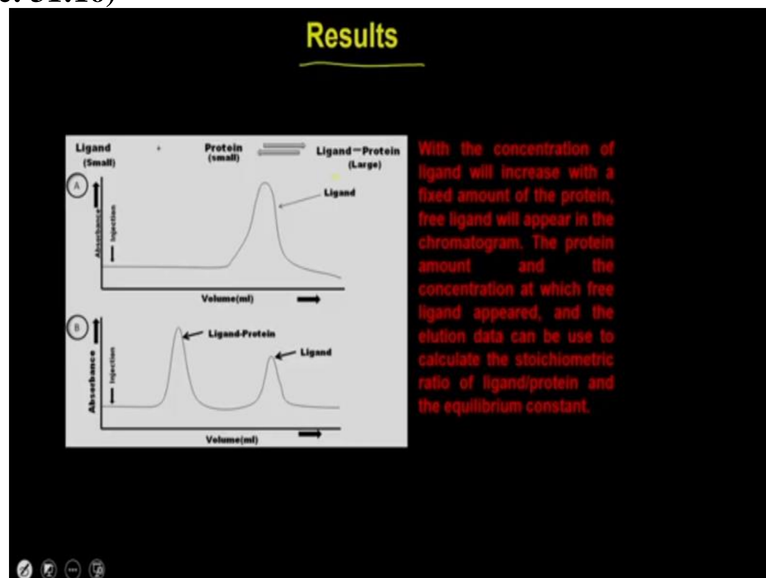
Now, the column is equilibrated with the buffer, containing the ligand molecule, or if the like if the ligand molecule is big, for example, the DNA then you do not need to do equilibration of the column with the ligands then you can directly equilibrate the column with the buffer and you can run the DNA separately, you can run the protein separately and then you can put them together and see where they are actually eluting in these conditions.

So, as the concentration of the ligand is increased, the protein binds the ligand and form a larger complex with an increase in hydrodynamic surface area. As a result, the protein shoots towards the left. So, in this case, what you are supposed to do is you are going to run the 3 gel filtration column first for DNA. So, DNA is very large, it will going to give you a peak, the second the protein so, protein is also going to give you a peak. So, this is for protein and then for the DNA proteins

So, in this case, you are going to get an intermediate peak, which is because here you are monitoring the peak at 260, here you are monitoring at 280, and here also you are going to monitor the protein not the DNA. So, as a result you are going to get our intermediate peak and that actually is going to be corresponding to the DNA protein complex. Now, the question is if you get a change in the peak of the protein that could be specific that could be nonspecific.

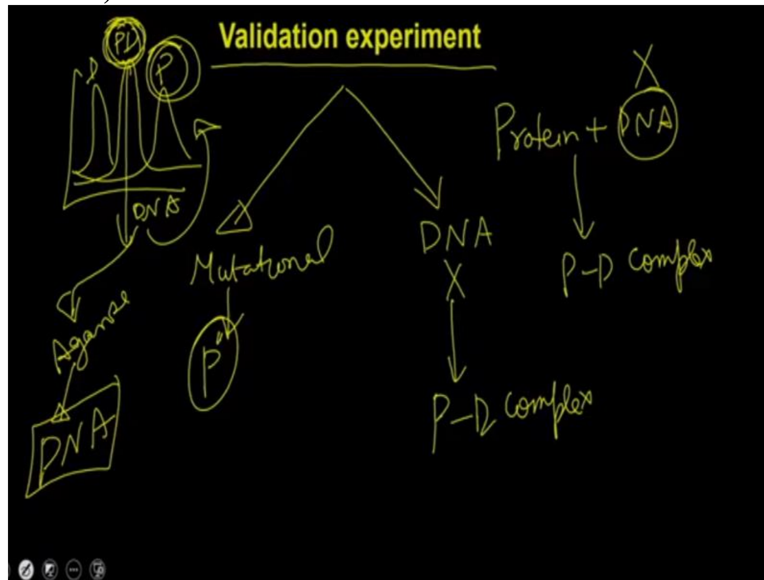
Because the protein that for example, especially in the case of DNA which is actually molecule with the very you know diversified charges and it has a basis so, it actually provides a lot of positive and negative charges it provides the hydrophobic cores, so, there are possibility that the protein might interact with the DNA nonspecific as well. So, to confirm this, what you have to do is you have to do a validation experiments to confirm this.

(Refer Slide Time: 31:16)



Now, what is the results you are going to get and how to validate that is that in the absence of the in the presence in the absence of protein the ligand is going to run separate and when you are putting a protein the ligand is going to form a complex with protein and going to run a separate peak, but how to verify whether the protein DNA complex is a real complex or to nonspecific complex you have to do our verification experiments.

(Refer Slide Time: 31:51)



Now, how to do a validation experiments now, validation experiment can be done in 2 ways. One where you can just remove the DNA because the protein is forming a complex plus DNA to give you the proteins DNA complex. Now, if I remove this so, if I remove this I should not get the P D complex which means, if I am seeing a peak, which is intermediate peak because this is the DNA peak, this is the protein peak.

So, this is the DNA peak, this is the protein DNA complex peak and this is the protein peak a shift in the peak could be because the DNA is interacting with the protein or shift could be because some kind of artifacts while you are running the second time. So, to understand that what you do is you take this complex, isolate it and then you degrade the DNA and run it again. If you run it again it should give you a peek at the protein positions if that happens that actually indirectly will verify that the shift in the peak is because of the DNA.

The second thing is because you have isolated the complex, you can also analyze this complex on to our agarose gel and that actually will give you a band for the DNA which you have used

for the interaction studies. This is the one approach. The second approach is that because the DNA protein interaction is whether it is in specific or nonspecific to answer this, what you can do is you can simply do mutational studies.

So, what you can do is you can just generate, you know, the mutated protein, and that mutated protein should lost the binding of the protein to give you the complex and it should also run at you know the native conformations. So, this is all about the designing an experiment to study the protein DNA interactions. We have also discussed a similar type of experiment when we were doing the ion exchange chromatography and with this let us move on to the next experiments.

(Refer Slide Time: 34:30)

Research Problems 3

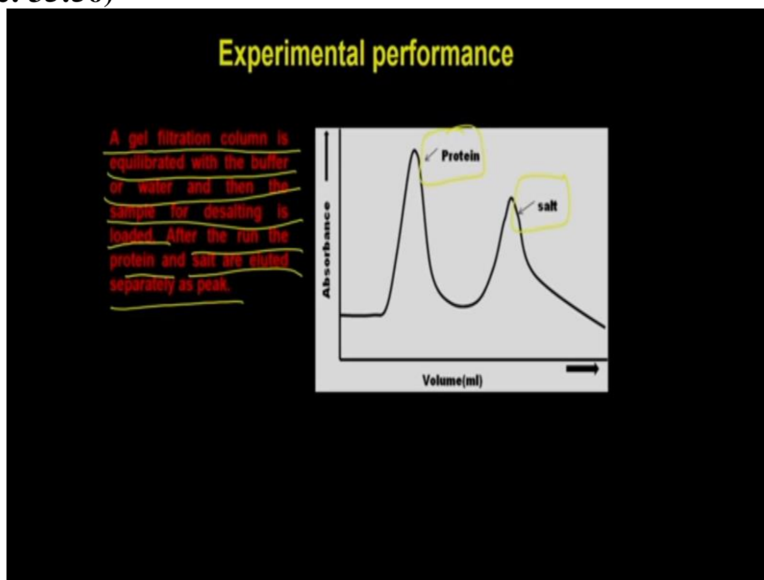
A scientist wants to record CD spectrum in water but protein is been purified with the differential precipitation of ammonium sulfate and has large amount of ammonium sulfate. The protein is sensitive and degrads during long hour dialysis so he has to design a experiment to remove ammonium sulfate from protein.

The next experiment is or the problem third is that the scientist wants to record a CD spectra in water, but the protein is been purified with that differential precipitation of ammonium sulphate and has a large amount of ammonium sulphate, the protein is sensitive and degrades during long hour dialysis. So, he has to design an experiment to remove the ammonium sulphate from the protein. So, what is mean by that you have a cell lysate they have incubated with ammonium sulphate?

So, what happened is that has actually has given multiple peaks, and out of these multiple peaks one of the peak was containing the protein of your interest, but the issue is and this for this protein, he wants to collect our Circular Dichroism spectrum, but this protein contains large quantity of ammonium sulphate. So, if you contain or if the protein contains the large quantity of

ammonium sulphate, it actually is going to interfere with the further analysis and getting the proper CD spectrum.

(Refer Slide Time: 35:56)



So, what you have to do is you have to do a desalting or the removal of the small molecule from the protein is important for activity assay and other downstream processing. So, what you do is, you load this protein which is a having a complex with the ammonium sulphate and if you run it on the gel filtration, the protein is going to be run separate and the salt is going to be run separate. And you can be able to do that simply by running by performing the chromatography in a very small column.

So, one of the columns which is called as the NAP-10 column. So, these are the commercially available NAP-10 column which you can use to desalt the protein you can remove any ligand which is of a smaller size. So, when you load the desalting column onto the NAP-10 so, you can get the protein separate and the salts separate because what happens is a gel filtration column is equilibrated with the buffer of water.

And then the sample for dissolving is loaded after the run the protein and the salts are eluted separately as a peak, we have prepared a small movie clip with the NAP-10 desalting column. And with that, with the help of this in the movie or the demo, you will be able to understand more nicely how to perform the salting of a proteins sample, whether it contains ammonium sulphate or other kinds of small molecules. And how you can get rid of this?

(Video Starts: 37:33)

Today we are going to give you a demo about how to desalt the protein using the NAP-10 gel filtration column. So, this is a typical NAP-10 gel protection column what you see here is the packed column, this is a prepack column what we have purchased from the company and it is been preserved in the buffer containing 0.2% azide because the NAP-10 columns are mostly sensitive for the organic solvents, so, that is why it is recommended that to preserve them in a buffer containing the azide.

So, now, first we have to do is what we have to do we have to equilibrate this column with the solvent in which you are interested to do the buffer exchange. So, in this case, we are actually trying to desalt the proteins. So, what we are going to do is we are going to equilibrate this column with the water and then we are going to load the protein with the help of the buffer itself. And then we are going to load the protein into this column.

And then we are going to start collecting diffractions, and then that is how it is actually going to remove the salt from the protein in case you are interested to keep your protein in some buffer condition for example, if it is not very stable in water or non-buffer conditions, then you can incubate the column with a particular buffer. So let us start the Nap-10 column mediated dissolving.

So what you have to do is first you have to fix your column into the stand then you have to remove the cap from the top and then you remove the lower end and then you let the column to run and you see this level this level should come to the level of disk and then you can start pouring the buffer of your choice. So this time we are running the column with the help of only the manual mode. If you are interested, you can also connect it machine this column to a purification machine also.

But since this only takes 5 to 10 minutes, people normally do not prefer to add the connector column to the verification system. So what you see is now the buffer is reached to the disk and now what we can do is we can simply add the buffer or the water and fill it up to the top. So,

while you are adding you to ensure that there is a smooth flow of buffer from the lower end of the column, and also there is no particulate matter present on this top filter.

Because that top filter is being kept simply to avoid you know the exposure of the column material to the particulate matter or the aggregated proteins. So, that is why it actually protects the column from getting any kind of damages. So, let us first do the equilibration and then we are going to load the sample and then we are going to tell you the further downstream procedures. So, now what we have done.

We have equilibrated the column with the 4 to 5 column volume of the water or the buffer in which you are going to do the buffer exchange of your protein. So, what you see is now the level is going to the disk and in between what you have to do is you have to prepare these samples. So, in this case, what we have is we have the 0.5ml of the protein. So, whatever the protein solution you have, you have to bring the protein solution to 1.5ml.

So, what you can do is just simply add the water or the buffer whatever you are interested, so add it so that it is going to be 1.5ml and then you can just load that so now, you can see the level is reached to the disk. And now what we can do is you can simply make the solution first and then you load it onto the protein onto the column, and you have to wait for this protein solution to get into the column.

And you can if you are interested you can be able to preserve some amount of samples so that you can verify what will be your recovery. And once it will go down to the level of the column we are going to add the buffer or the water, so now it did not stick to this. And now what you can do is just simply fill the buffer with the column with your water and you have to fill this up to the top and then you start collecting the fractions.

And you what you can do is you can just simply collect the 0.5ml fraction and your protein will come out into this 0.5ml fashion. So, what you can do is you can simply collect the 6.5ml fractions and that actually is going to so, what you can do is you can collect the 6.5ml fraction.

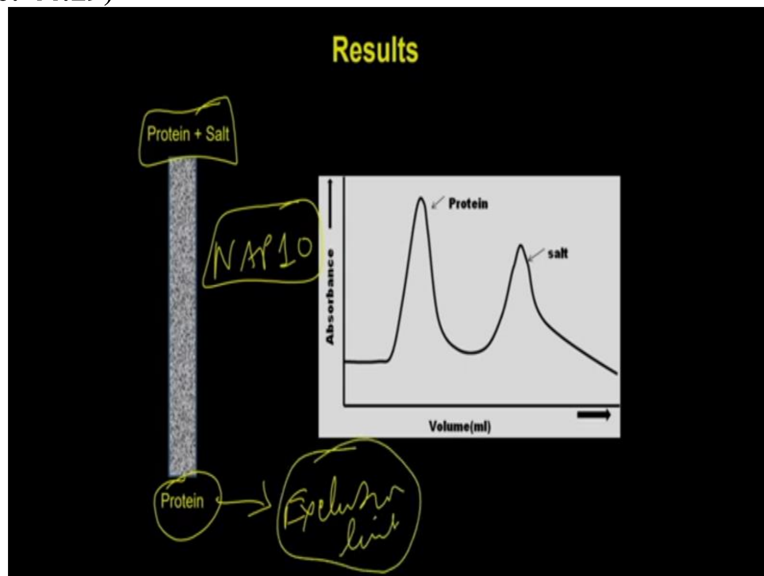
So, I have selected the faction number 2 and the similar way you can collect another 4 fractions and because, the protein is going to be present into the exclusion limit.

Because the protein what you are adding is going to be very big compared to the salt what has been present in this particular protein. So, what will happen is the protein is going to be in the exclusion limit of the column. So, the protein will come out first and the if you collect these fractions and analyze on to the SDS page, what you will see is that initial 3 fractions are going to have the protein of your interest.

Or the maximum amounts were in which the fraction number 2 is going to have the maximum amount of protein and fraction 1 and 3 are going to have a lower amount of protein and the from fraction 4, 5 and 6, you are going to get the salt. So, this is all about the desalting of the proteins with the help of the NAP-10 column.

(Video Ends: 44:28)

(Refer Slide Time: 44:29)



So, ideally what we are doing we are just loading a protein salt complex onto our NAP-10 column. NAP-10 column is a G 25 column and ultimately you are going to get a protein because the protein is present in the exclusion limit of that column. And because it is very big, so, it is actually not going to fractionate the protein and it will come into the exclusion limit and as a result the protein will come first and you can collect the proteins.

So, with this we have discussed about the gel filtration chromatography and how you can be able to utilize the gel filtration chromatography for addressing the different types of scientific problems. And now subsequently in the next lectures we are going to discuss about the affinity chromatography and how you can be able to utilize the affinity chromatography for performing the different types of experiments or solving the biological problems. With this I would like to conclude our lecture here. Thank you.