Chemical and Biological Thermodynamics: Principles to Applications

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Lecture - 54
Identifying sites for Drug-Protein Interactions by ITC

In this lecture, we will discuss how to design suitable experiments to identify the nature of protein ligand interactions by isothermal titration calorimetry. We have at length discussed that how to choose protein concentration, how to choose ligand concentration, we have also discussed that how to obtain the thermodynamic parameters from an isothermal titration calorimetric profile and then we further discussed that how to get the values of K, delta H, delta S and hence delta G naught and in what way these should be interpreted.

Obviously the next question is that if we look at the overall value of delta H naught, it could be either positive or it could be negative a positive value of delta H naught of binding does not necessarily mean that the binding is only hydrophobic in nature or an overall negative value of delta H naught does not necessarily mean that the protein ligand interaction involves only ionic or polar interactions. Whenever a ligand binds to a target site, the target site the binding site involves amino acid residue and those amino acid residues will have some polar character.

It will have some hydrophobic character depending upon the type of amino acids which constitute the binding site and therefore, when the incoming ligand comes and fits there, it is possible that there could be a combination of the polar interactions and the non polar interaction and whatever predominate the sign of delta H will reflect on that and that is why when the value of delta H naught is overall positive or the value of delta H naught is overall negative, we say that the binding is predominantly polar in nature or the binding is predominantly hydrophobic in nature.

Now, can we design further experiments which can support our claim more authentically about the contribution or establishing the contribution of polar interaction; whether these are hydrogen bonds electrostatic interactions or how to identify the involvement of hydrophobic interactions?

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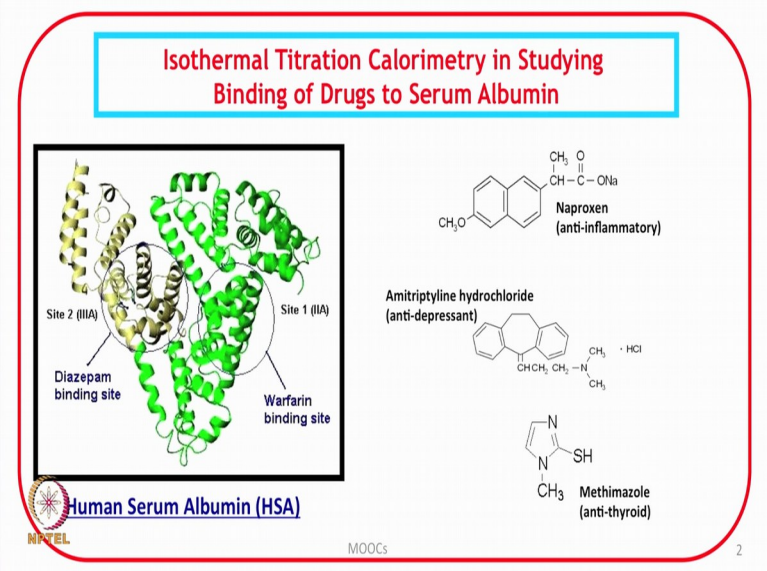

Let us discuss those issues in this lecture. Let us take a look at again the same slide which I discussed in the previous lecture on the binding of anti inflammatory drugs naproxen with serum albumin amitriptyline which is antidepressant and methimazole with serum albumin.

Let us see what kind of experiments can be design. Now suppose that the ligand is able to establish ionic interactions at the binding site, what kind of experiments can be designed. So, that this ionic interaction between the incoming ligand or drug molecule and the protein binding site can be reduced and this can be reduced, if we introduce in the solution something which can interfere in this ionic interactions; that means, if we introduced some other ions into the solution which will interfere in the ionic interactions between the ligand and protein, then it will affect the value of binding constant.

It will also affect the values of delta H naught and delta S naught. So, let us think of what kind of additives can be added which can reduce the ionic interactions one electrolyte that immediately comes to mind is sodium chloride. If I increase the concentration of sodium chloride in solution, the concentration of sodium chloride many times, we also talk in terms of ionic strength. If we look at the literature people also when they increase the consent when they introduce electrolyte into the solution, they also talk about ionic strength.

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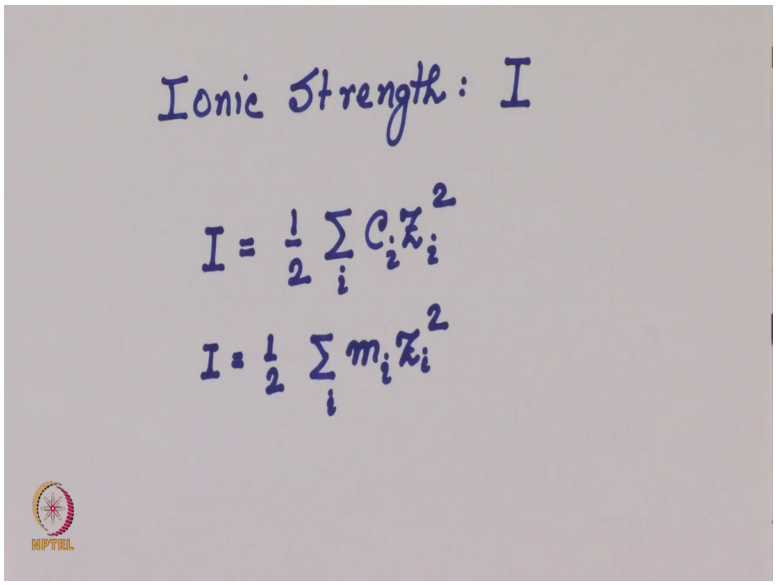

Let us take a look at what is ionic strength. Ionic strength represented by the symbol I and what is this I defined this I is defined as half summation i C i Z i square where the C is concentration Z is charge and if one wants to express in terms of molality, then I can also write I equal to half summation i m i Z is square where m is molality, C is concentration, what I have been discussing is that if I add sodium chloride in solution, I can say that the concentration of sodium chloride is this or I can also express in terms of ionic strength, but you know a word of caution over here, when you talk about ionic strength and let us take a look at the definition of ionic strength given over here, it does not say that in calculation of ionic strength of the solution you only consider sodium ions and chloride ions.

Ionic strength of a solution will be calculated by including all possible ions present in the solution, right. Now suppose if you have only sodium chloride in solution.

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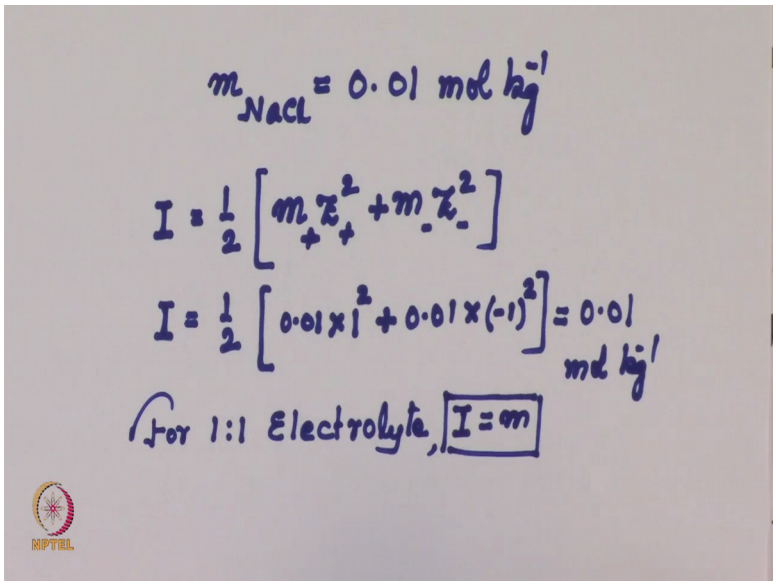

Let us see if I have 0 point, let us say if I have molality of sodium chloride as 0.01 mole per kg then ionic strength will be equal to half m plus Z plus square. Z is the positively charged cation m i minus Z minus square where Z minus is an ion i is equal to half m plus is 0.01 into charge on sodium ionic is 1 plus concentration of chloride ion is also same 1 is to 1 electrolyte 0 1 and minus 1 square is also 1. So, this is 0.01 plus 0.01 divided by 2. This is equal to 0.01 mole per kg; that means, I can immediately write for 1 is to 1 electrolyte, i is equal to m, but this calculation I discussed over here only to make the things clearer that if you have only sodium chloride in solution sodium chloride is 1 is to 1 electrolyte in that case, i is equal to m.

However, if there are more than 2 ions present in solution then the according to the definition of ionic strength we need to consider all possible ions in solution. Therefore, this a word of caution that when we are reporting the ionic strength in a solution, we must include contributions due to all possible ions present in the solution. Now let us discuss further as we were talking about introducing something into the solution which we can interfere in the ionic interaction; one thing that immediately comes to mind is sodium chloride when you add sodium chloride in solution.

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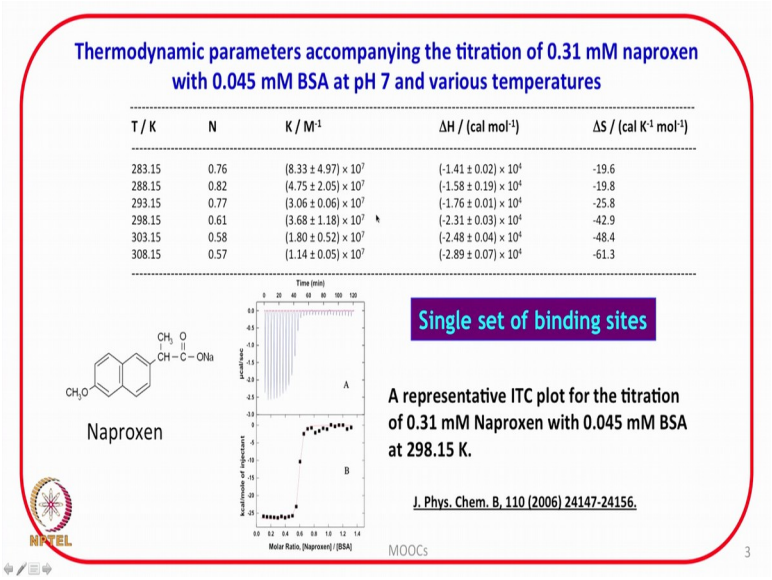

Let us see how it affects the binding parameter. Let us take a look at the table, this table is the same table that we discussed earlier expressing that the binding constant of naproxen with serum albumin is of the order of 10 raise to the power 10, let us keep in mind and for amitriptyline the binding constant is of the order of 10 raise to the power 4.

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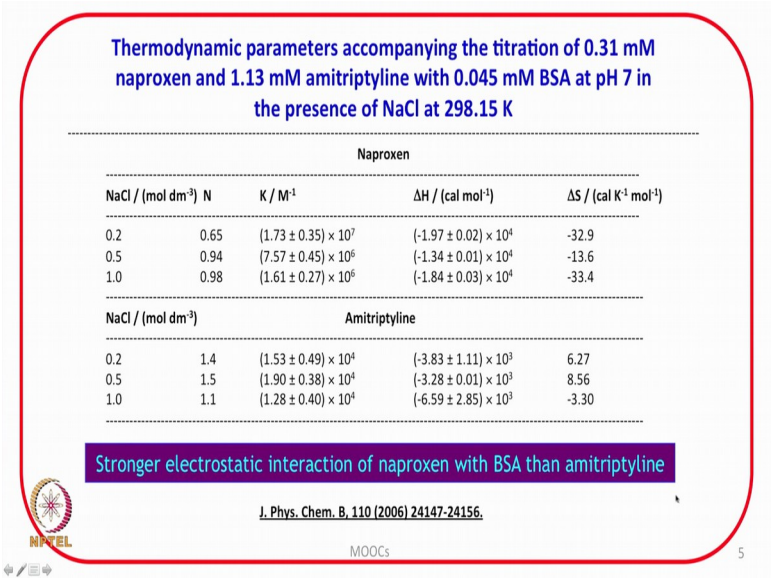

Now, let us take a look at how the data the binding data gets affected when the concentration of sodium chloride is increased in solution, let us first take a look at the binding of naproxen as the concentration of sodium chloride is increased in solution from

0.2 molar to 0.5 to 1. The order of the binding affinity decreases, it decreases from 10 raise to the power 10 to 10 raise to the power 6 and further increase in concentration of sodium chloride, you see further leads to decrease in the value of binding constant and this is what I was referring to that if an additive interferes in the interaction between 2 molecules that will affect the value of K.

Now what is happening here is since naproxen is having ionic character because of COO molecule u COO end sodium chloride is able to make some electrostatic bonding with naproxen and in turn, it affects it binding at the binding site that is why; what we have observed, what we see in this table is that the addition of sodium chloride at a higher concentration decreases the value of binding affinity.

That means sodium chloride is able to interfere in the electrostatic interaction. So, therefore, it establishes that the electrostatic interactions are playing a dominant role in the binding of naproxen with serum albumin because sodium chloride is able to interfere in this electrostatic interaction. Now let us take a look at the table again for the data with amitriptyline increase in the concentration of sodium chloride from 0.2 molar to 0.5 molar to 1 molar. It did not change the order of binding constant clearly suggesting that the sodium chloride is not able to affect the binding interactions of amitriptyline with serum albumin.

So, this suggests that the electrostatic interactions are not playing a significant role or a dominant role in the binding of amitriptyline with serum albumin. So, this is one kind of example where in this report the use of sodium chloride in studying the binding affinity suggested that the electrostatic interactions are playing a significant role in the binding of naproxen with serum albumin, but these do not play a significant role in the binding of amitriptyline with with serum albumin.

Now, if we are interested in understanding hydrogen bonding interactions, similar type of experiments can be thought of and suppose if the ligand; if the drug molecule has several functional groups which can establish hydrogen bond, when it enters the binding site and before it binds, if we introduced some other additives into the solution which have several hydroxyl groups or several hydrogen bond acceptors or donors then it is possible that that additive may make hydrogen bond with the drug molecule and hence affect its availability; free availability for binding to the protein molecule, some examples which

one can think of such additive are glucose sucrose amino acids because they can also from hydrogen bonds or some other molecules which are able to either donate or which are or the other molecules which are either accepter or donors you know to establish hydrogen bonds.

Now, if one is interested in unraveling in understanding whether hydrophobic effect plays a role in the binding of protein ligand interactions then what kind of experiments can be designed; obviously, the effort should be to choose the additives which can engage in hydrophobic effect with the solute molecule with the drug molecule and hence affect its availability for interaction with the protein let us take a look at some example with sodium chloride in this particular case it got established that there are stronger electrostatic interaction of naproxen with BSA than amitriptyline then in this report surfactants were chosen as additives surfactants.

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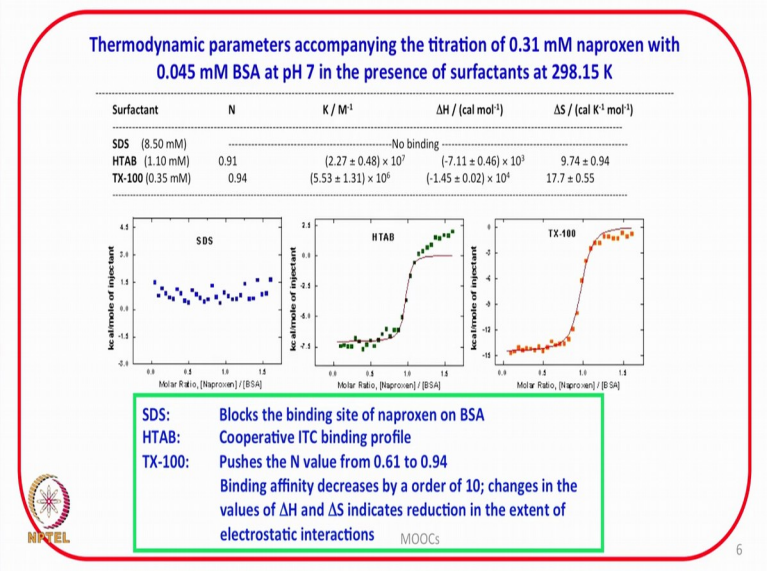

Surfactant molecules will have a long hydrophobic tail and a polar head group or ionic head group the hydrophobic content of surfactant is large, but if one chooses surfactant molecules as additive one must be careful. Here is a word of caution, it has been observed that the surfactant at higher concentration can lead to denaturation of the protein because the surfactants have higher hydrophobic group and the hydrophobic because of that hydrophobic content, it can interact more with the denatured state of the protein and hence shift the native to denatured equilibrium towards, right.

Surfactants have been observed to denature the protein above a certain concentration therefore, if we want to choose surfactants as additives in the protein ligand binding studies, we should take an appropriate concentration of surfactant which does not affect the integrity of the binding site and that should be established by independent methods we will discuss about those later. Let us first take a look at this figure this table; the table gives the thermodynamic parameters associated with the binding of naproxen with serum albumin in presence of SDS H tab H tab is a cationic surfactant SDM sodium dodecyl sulfate is an ionic surfactant TX 100; Triton X 100 is an nonionic surfactant, anionic surfactant, cationic surfactant and Triton X 100 which is a neutral surfactant nonionic surfactant.

In presence of sodium dodecyl sulfate the concentration of sodium dodecyl sulfate in this experiment in this report was taken as 8.5 millimolar, it was observed that the binding is completely lost as long as SDS at this concentration 8.5 millimolar does not lead to the denaturation of the protein, it establishes that the ionic interactions are significant because it has sulphates negatively charged end and you see it gets a support to this conclusion because the nonionic surfactant is not interfering in the binding to a large extant it has only reduced the binding affinity from 10 raise to the power 10 to 10 raise to the power 6. Similarly experiments can be designed with amitriptyline to understand that what kinds of interactions are predominant in the binding of the drug with the protein.

In short buy a suitable choice of the additives and doing the isothermal titration calorimetric experiments the comparison of the data suggest in further establishing the relative contribution of individual type of interactions, in the overall binding process. Now it is possible that the different drugs may go and bind at the same binding site, you know sometimes the drugs are given in combination and if one is interested in understanding that whether the individual drugs go and bind at the different binding sites or these drugs go and bind at the same binding site can isothermal titration calorimetry help in understanding this the answer is yes.

So, how does one proceed amitriptyline which is an antidepressant and naproxen which is anti inflammatory drug are sometimes given in combination and therefore, it will be an it will be of interest to understand whether these drugs go and bind at the same binding site or they bind at the different binding sites.

How we can design the experiment to understand this; what one can do is you form a complex of serum albumin with naproxen and titrate with the amitriptyline and vice versa and then one can compare the value of binding affinity. Now suppose if the binding affinity is not affected at all what I mean is you form a complex of the drug with serum albumin and titrate with another drug and if the binding affinity is not affected at all; that means, the incoming drug is going and binding at some different binding site and if the binding site binding is affected, if the binding affinity is reduced; that means, there is an overlap of binding site, let us take a look at the data in this report.

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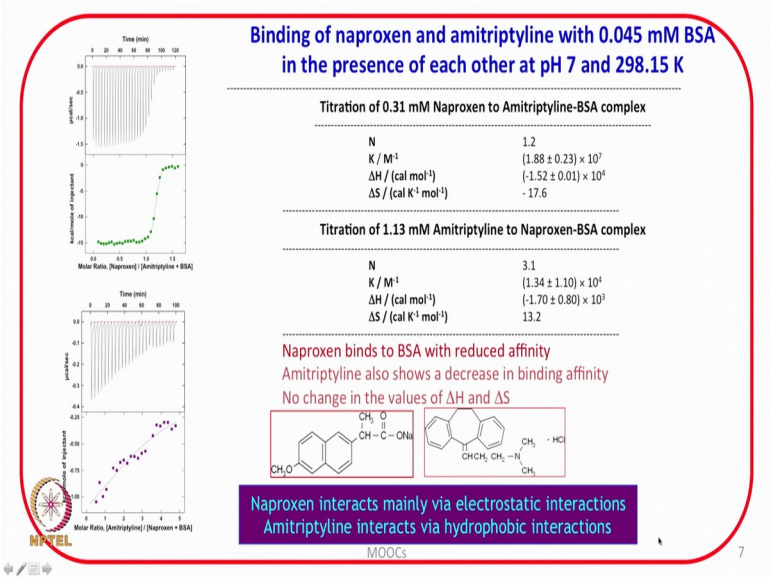

What was done is the binding of naproxen and amitriptyline with serum albumin was studied in presence of each other. So, that is amitriptyline BSA complex was formed and it was titrated with naproxen and the resulting isothermal titration calorimetric profile yielded the data presented in this table where you see the value of K remain of the order of 10 raise to the power 10 although the absolute number decrease to a small extent.

Whereas for amitriptyline when it was titrated to naproxen BSA complex, it remain of the order of 10 raise to the power 4, one thing is there that if a site is occupied by a ligand which is weakly bond and another ligand comes which has a strong affinity at the same binding site that will displace this molecule.

Now look at this table here naproxen is titrative with amitriptyline BSA complex the value of K remains of the order of 10 raise to the power 10 although, there is a slight

change in the absolute value, but when amitriptyline is added to naproxen BSA complex the value of K is remaining nearly same. So, what conclusions can be drawn is naproxen binds to BSA with reduced affinity and amitriptyline also shows a slightly decrease binding affinity a slightly decrease only a slight decrease which is there in the absolute value.

So, based upon such kind of experiments conclusions can be drawn whether the drug bind at the same site or at the different site, as I mentioned if the drugs bind at different site, then the complexation of the protein with one drug molecule will not affect the binding affinity, when it is interacted with the other drug molecule and when the bind at the same site then the value of the binding affinity will be affected and the values of the binding thermodynamic parameters will be effect.

So, therefore, isothermal titration calorimetry can also assist in establishing whether the different drugs bind at the same site or the different drugs binds at the separate site different site, different sites and in this case, in case of naproxen and amitriptyline, this forms a nice example of making a study in this direction and getting some useful results the main conclusions from this report was that naproxen interacts mainly via electrostatic interactions and amitriptyline interacts me via hydrophobic interaction.

So, what we discussed in today's lecture that isothermal titration calorimetry not only helps in giving us the quantitative numbers on the binding thermodynamic parameters, it also helps us in identifying the nature of intermolecular interactions one is that identifying the nature of intermolecular interactions, based on the overall values of delta H naught or delta S naught and second is to get further insights into the relative contribution of individual type of interactions suitable experiments can be designed by using variety of additives so that we can try to understand that what type of interactions, whether the hydrogen bonding interaction and electrostatic interactions hydrophobic effects or some other type of interactions possible to play it dominant role in the binding process or not.

Isothermal titration calorimetry can also be used to establish the binding site of course, the binding sites where is the binding site the nature of binding site, actually is well established by X-Ray, but if we know that a given protein has more than one binding sites from incoming ligands isothermal titration calorimetry can help in establishing that

the incoming ligand is binding at site 1 or side 2 or if there are more binding sites, then it goes to which binding site and that is by using the suitable site markets suitable experiments need to be designed in this direction and how to design those experiments those issues we will discuss in the next lecture.

Also we will discuss that how the binding of a ligand molecule to a drug molecule affects the differential scanning calorimetric profile and how to interpret those differential scanning calorimetric profiles to get more information about the binding process those we will discuss in the next lecture.

Thank you very much.