Chemical and Biological Thermodynamics: Principles to Applications

Prof. Nand Kishore
Department of Chemistry and Biochemistry
Indian Institute of Technology, Bombay

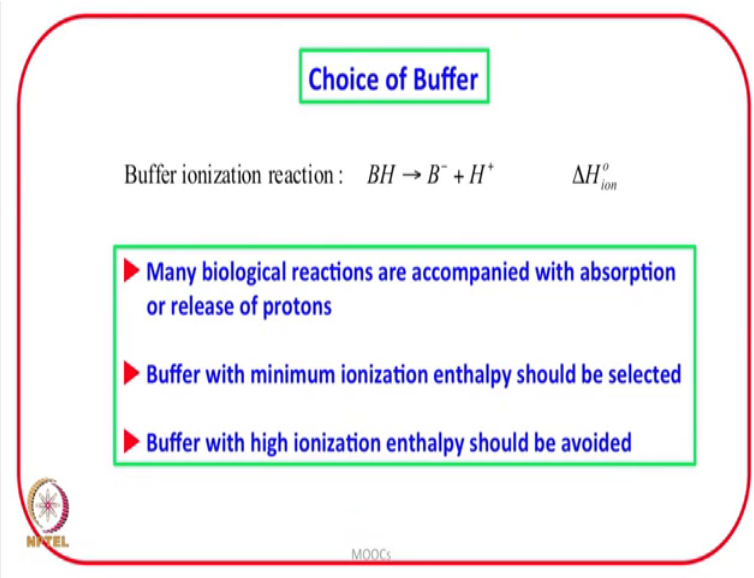
Lecture - 47
ITC Experimental Design and Isothermal Titration Calorimetry (ITC) in Drug Design

In this lecture, we will discuss the role of buffer in ITC experiments and we will also discuss isothermal titration calorimetry in drug design first of all let us talk about the role of buffer we have discussed many times earlier that when we are dealing with the biological macromolecules whether it is protein or it is DNA or other biological systems maintaining the PH becomes very important because an enzyme may have optimum activity of a certain PH and a protein or DNA may have a specific conformational stability at a certain PH therefore, the role of buffer becomes very important.

When we are doing spectroscopic experiments there we are not dealing with the amount of heat liberated or amount of heat absorbed; however, we are doing the calorimetric experiments we are dealing with the amount of heat liberated or heat absorbed several biological reactions whether it is protein folding unfolding or it is protein Ligand interactions these processes may involve either absorption or release of protons. And therefore, these absorption or these release of protons should not lead to change in PH that is why buffers are used.

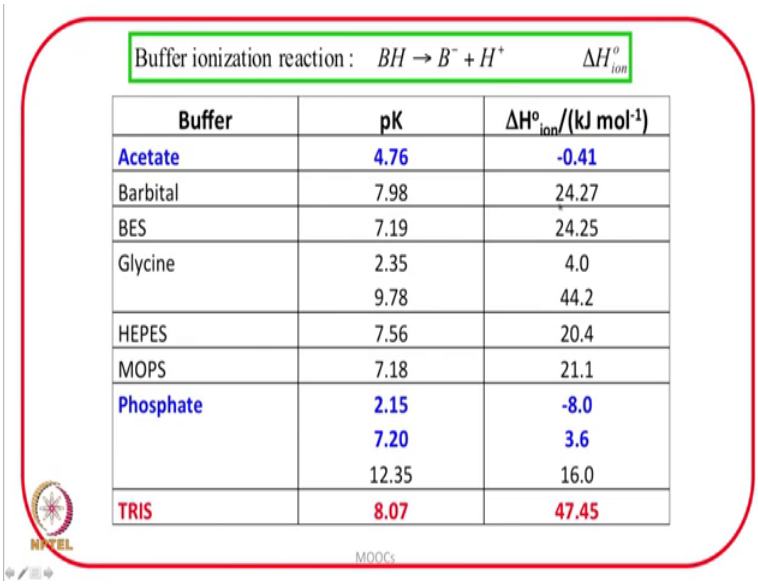
Therefore in isothermal titration calorimetric experiments where we are interested in measuring very small amount of heats we should choose a buffer in which the heat of ionization or protonation is very small because suppose if a proton is released during a biological reaction a reaction like protein folding or protein Ligand interaction that proton has to be absorbed by the buffer. And therefore, if heat of protonation of the buffer or heat of ionization of the buffer is very large that will affect or that will contribute to the actual measurement of protein Ligand interaction.

(Refer Slide Time: 03:39)



So, let us take a look at the slide in deciding the choice of buffer buffer ionization reaction means let us say buffer is b H the ionization reaction will be b minus plus H plus and the corresponding enthalpy let us call this as enthalpy of ionization of the buffer and as I discussed many biological reactions are accompanied with absorption or release of protons. And therefore, whether the absorption or release the enthalpy will be decided according to this reaction and further as we discussed buffer with minimum ionization enthalpy should be selected and the buffer with high ionization enthalpy should be avoided because the ionization high ionization enthalpy will affect the actual enthalpy of protein Ligand interaction.

(Refer Slide Time: 04:59)



So, therefore, let us take a look at ionization enthalpies of a few buffers let us take a look at this table how do we decide that which buffer we need to choose if we want to prepare a solution of PH 5 or a solution of PH 7 physiological PH is 0.74. So, therefore, may times we are interested in preparing a buffer solution of PH 7.4. So, which buffer should be selected this should be dependent upon pK we have discussed it earlier pK plus minus one that range will be very suitable for maintaining the PH of the solution let us take a look at the figure the table acetate buffer has a pK of 4.76. And the corresponding enthalpy of ionization is minus 0.41 kilo joules per mole and if we want to prepare a solution of PH 7 the other possibilities of choosing the buffer based upon the pK values are barbital because pK is 7.98 or BES; BES 7.19 MOPS 7.18 and if we look at barbital the enthalpy of ionization is large 24.27 kilo joules per mole for BES also the enthalpy of ionization is 24.25 kilo joules per mole for HEPES 20.4 another possibility is TRIS.

TRIS has been a very popular buffer in spectroscopic experiments literature is full of spectroscopic experiments in which the solutions have been prepared in TRIS buffer, but as I mentioned earlier that when we are doing spectroscopic experiments or other you know whether it is fluorescence spectroscopy or u v visible spectroscopy or any other kind of spectroscopy there we are not concerned with the amount of heat liberated or absorbed. So, therefore, there the enthalpy of ionization may not affect much in the spectral measurements, but in isothermal titration calorimetric experiment if we use TRIS the enthalpy of ionization is very high 47.45 kilo joules per mole therefore, TRIS

should be avoided in isothermal titration calorimetric experiments because if the heat of protein Ligand interaction is not very high the contribution of ionization effects of TRIS is going to be large in those measurements.

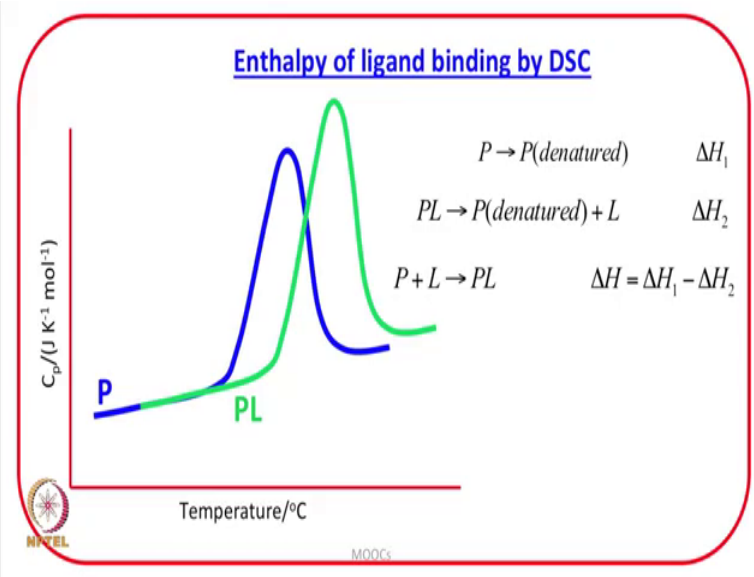
Therefore once again in isothermal titration calorimetric measurements TRIS should be avoided now let us take a look at phosphate; phosphate has three pK values 2.15; 7.2; 12.35. So, therefore, if we want to prepare a solution of PH 2 or PH 7 phosphate is an excellent buffer to be used in ITC experiments because the associated enthalpies of ionization are very small at PH 2 or between this range the enthalpy of ionization is minus 8 kilo joules per per mole and at PH 7.2 over around 7 the enthalpy of ionization is about 3.6 kilo joules per mole this are low number small numbers therefore, phosphate can be a good buffer in isothermal titration calorimetric experiments which are to be done at PH 2 or PH 7.

Glycine can also be a good buffer of choice when it comes to PH 2 see pK is 2.35. So, therefore, from 1.35 to 3.35 glycine can also be a good buffer of choice and the corresponding enthalpy of ionization is 4 kilo joules per mole. So, the conclusion of this discussion is that we should avoid choosing a buffer which has a high value of enthalpy of ionization for physiological PH phosphate can be a good buffer of choice we have been discussing isothermal titration calorimetry in extracting information on the delta G delta H and delta S associated with the protein Ligand interaction before discussing isothermal titration calorimetry we discussed differential scanning calorimetry in great details. And I made a comment here that we can also use differential scanning calorimetry in determining the value of binding constant enthalpy of binding and entropy of binding.

Isothermal titration calorimetry is excellent in determining the value of binding constant up to a certain value let us say up to value of the order of 10 rest to the power 9, but if the binding constant is very high let us say 10 rest to the power 20 10 rest to the power thirty ten rest to the power forty then the experiments kind of experiments that I discussed the ITC may not be a good choice in those cases differential scanning calorimetry offers determination of binding constant which are very very high that I will discuss later. But at least the enthalpy of interaction can be easily determined by differential scanning calorimetry if we are interested in a quick determination of delta H

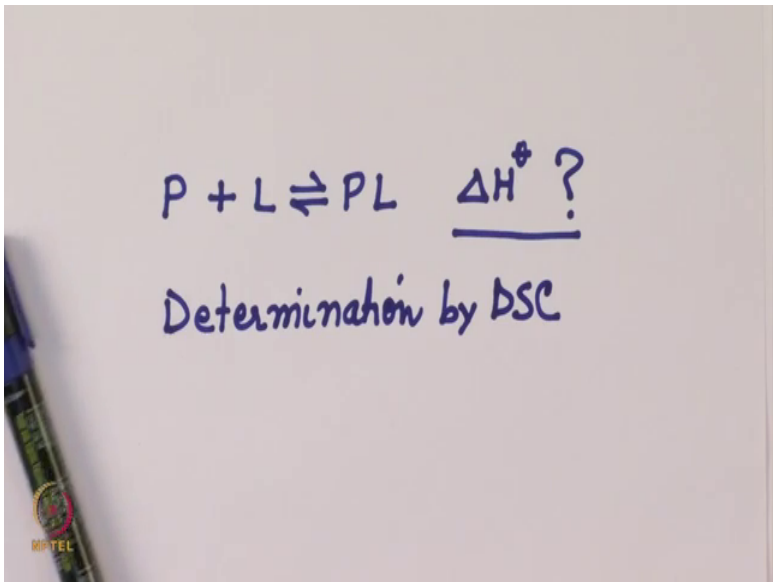
and we do not have an ITC we want to determine delta H by DSC it can be done let us discuss in the next lecture.

(Refer Slide Time: 12:29)



Determination of enthalpy of Ligand binding by differential scanning calorimetry let us say first we subject only the protein to heating in a differential scanning calorimeter the unfolding will be represented by the blue curve and when you add Ligand. And since the Ligand binds with the protein and anything which binds with the native state of a protein is expected to increase its transition temperature and that is what is seen over here let us take a look at the figure that when you form a complex of P and L P L then its transition temperature increases and the associated enthalpy of unfolding also increased.

(Refer Slide Time: 13:50)

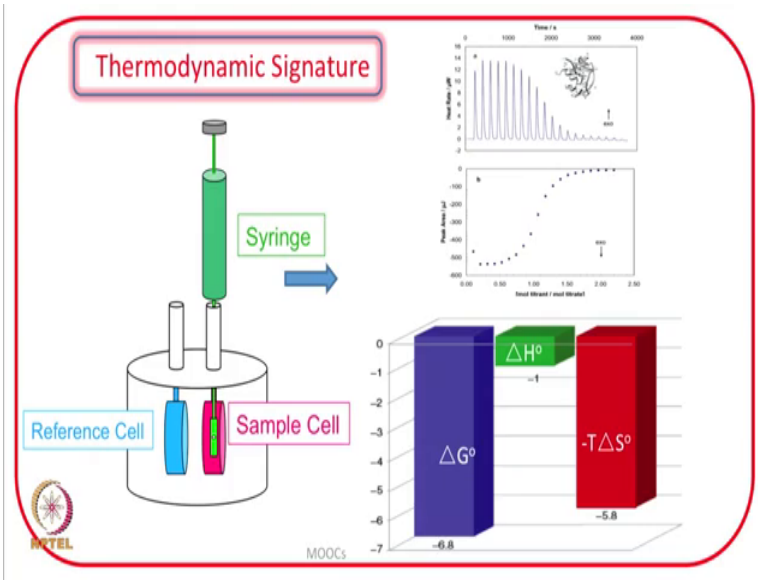


What we need to do is 2 experiments one is with the protein DSC of just the protein and the DSC of the protein Ligand complex what we are interested is in P plus L forming P L and what is the value of delta H this is the question and we want to discuss determination by DSC. Let us take a look at the figure the blue line the blue curve represents this reaction P is being denatured P going to P denatured let us say the associated enthalpy of unfolding is delta H 1.

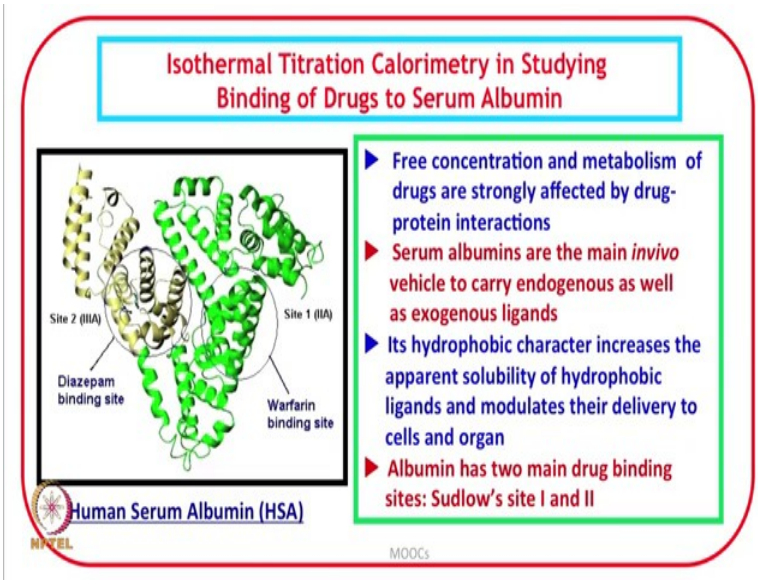
In other words I can call this as calorimetric enthalpy of unfolding which is delta H 1 and for the green line green curve you are taking P L the complex we are already forming a complex and you are heating it when the complex is heated; obviously, the denatured protein will lose its binding site and the Ligand will be dissociated from it the corresponding reaction thermal unfolding reaction is P L forming P denatured plus L and the associated enthalpy of unfolding is delta H 2. Now we have 2 reactions P native going to P denatured associated with calorimetric enthalpy of delta H 1 and P L going to P denatured plus Ligand associated with a calorimetric enthalpy of delta H 2. Now we can use the Hess's law we can subtract one minus 2 ad what we get is when you subtract 1; 1 minus 2 P denatured and P denatured will cancel what we will have P plus L is forming P L and the associated delta H is delta H 1 minus delta H 2. So, this can be used to extract information on enthalpy of binding. So, therefore, differential scanning calorimetry can also be used for determining the value of enthalpy of protein Ligand interactions.

So, now after having a knowledge of extraction of thermodynamic quantities from isothermal titration calorimetry we will start now our discussion on how to use these thermodynamic information in rational drug design.

(Refer Slide Time: 17:13)



(Refer Slide Time: 17:37)



Let us take a look at this figure we have just discussed in the previous lecture that by using isothermal titration calorimetry we can get thermodynamic signature associated with a protein Ligand interaction and that 2 in a single experiment and let us have a small discussion on what is the need of studying binding protein Ligand binding interactions

first of all let us talk about the interaction of variety of molecules with a protein like serum albumin serum albumin is a plasma protein it is a protein in the blood and its concentration in the blood can be as high as 40 gram per liter it is a major transport protein of a variety of molecules to the different parts of the body and why this variety of binding comes to a molecule like human serum albumin let us discuss a little bit about that.

There are lots of reports in literature on binding of variety of drugs with either human serum albumin or bovine serum albumin human serum albumin and bovine serum albumin are expected to have similar structure because there is a 76 percent sequent homology the crystal structure of human serum albumin is well established and. Therefore, when you look at the literature the interaction to explain the intermolecular interaction between drug and serum albumin most of time the structure of human serum albumin has been used even to explain the data with the bovine serum albumin in which getting the crystal structure has been difficult.

Now, let us take a look at the structure of human serum albumin it is a heart like structure and the different domains are shown and at least 2 sites site one and site 2 are shown here, but never the less because of the heart like structure and the kind of shape it presents variety of molecules calamine to it 2 sites have been identified site one which is Warfarin binding site and site 2 which is disappoint binding site which are the major binding sites for variety of Ligands on this protein fatty acids can also bind very easily to this protein now in view of the structure of serum albumin as we just discussed variety of molecules can bind to it and therefore, this is a good drug transport protein in the living systems in a human system.

Now this drugs have to be carried to the specific target sites and therefore, it is very important to have an optimum binding affinity of a drug with the protein because if we consider a protein to be involved in drug delivery for example, serum albumin can bind variety of molecules variety of drugs and if the drug is to be carried out or if the drug is to be carried to a particular binding site which is far away we need to have sufficient binding affinity of the drug molecule with the protein because it should not happen that the binding affinity is. So, small or the binding is. So, weak that the drug is easily lost to the surroundings and is not delivered at the required target.

It should also not happen that the binding of the drug molecule is. So, tight that it is not delivered at all and therefore, it becomes very important to have an optimum binding affinity or the binding affinity which is required for the target oriented drug delivery and you might have noticed that I am repeatedly saying the word binding affinity binding affinity is binding constant and that is where the significance of the binding constant comes in drug discovery in rational drug design we need to have an optimum binding affinity and in this lecture or in the lectures which are which I am going to discuss ahead we will thoroughly discussed how isothermal titration calorimetry has been used in rational drug design how it can give us guidelines towards rational drug design.

So, let us take a look at the comments free concentration and metabolism of drugs are strongly affected by drug protein interactions serum albumins are the main In-vivo vehicles to carry endogenous as well as exogenous Ligands its hydrophobic character increases the apparent solubility of hydrophobic Ligands and modulates their delivery to cells and organ albumin has 2 main drug binding sites which are called Sudlow site 1 and site 2. But you know if we look at the structure of serum albumin there are many other binding sites available. In fact, the various ions also have shown binding affinity towards serum albumin, but depending upon the amino acid sequences these 2 binding sites Sudlow site 1 and Sudlow site 2 have been identified where variety of drug molecules go and bind now how isothermal titration calorimetry can be useful. In fact, we are going to discuss in details, but ITC let us discuss in brief that ITC will give us the value of delta H it will give us the value of delta S and it will give us the value of delta G not.

And delta G not is expressed in terms of K whether we talk about K or we talk about delta G not it is almost the same thing and as I said that having an optimum binding affinity becomes very important when we talk about either the delivery of the drug molecules to certain target site or the action of drug molecules because many times in order for a suitable action biological action the incoming Ligand molecules or drug molecules must go and bind to the target with a suitable affinity and suitable affinity. Means I am talking about a suitable value of binding constant along with determination of binding constant if we know the information on the enthalpy of reaction and as we earlier discussed the sign of the enthalpy of interaction gives us information about the nature of intermolecular interactions nature of intermolecular interactions means are the interactions polar in nature or are the interactions non polar in nature and nature of

intermolecular interaction will depend upon the type of functional groups present on the drug and we are also depend upon the type of amino acid residues which form the binding cavity.

In other words the thermodynamic quantities determined from ITC are actually reflecting upon the nature of intermolecular interactions which we are now trying to connect with the functional groups. That means, we are now connecting the thermodynamic quantities with the types of molecules or nature of molecules nature of drug molecules therefore, it is possible to draw guidelines towards that what kind of modification should be done in a drug molecule in an existing drug molecule or what kind of molecules should be designed that is in terms of novel drug design all these things we are going to discuss in details in the future lectures.

So, in today's lecture what we have discussed is the significance importance of the thermodynamic quantities and we have started connecting it with the nature of functional group and also towards deriving guidelines for rational drug design therefore, it becomes very important to interpret the thermodynamic parameters.

In terms of intermolecular interactions and these intermolecular interactions whether it is hydrogen bonding interaction whether is it is electrostatic interaction or hydrophobic interactions or Van Der Waals interactions this will decide the overall value of delta H and as we will realize in the next lecture that it is not just the interaction of an incoming Ligand with the protein molecule the solvent also plays a major role in the rational drug design, but all these things we will discuss in details in the next lecture.

Thank you very much.