Fundamentals and Applications of Supramolecular Chemistry
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Week 8
Lecture 39

W8L39_Anion binding in Proteins and Transport processes in biology

Hello, everybody. So, now let us continue our discussion with respect to the binding of anions and what we observed in the last few lectures: the factors responsible for anion binding, and we discussed different kinds of examples.

We also talked about guanidinium-based receptors, which actually capture phosphate anions very effectively, and we also looked at how we can modify the host so that it can efficiently bind with the anions, that is, the guest, and induce high magnitudes of affinity in different kinds of systems.

So, it is very important that when you have anion binding electrostatics, we realize it plays a very important role, and it is preferable to have the host molecule that is going to capture the anions, which are the guests, to have good electrostatic complementarity. And the host should preferably be highly charged species, and we also realized that pH plays a very important role.

But in addition to that of cationic host, we can also have neutral molecules that have the necessary hydrogen bond donors which can interact with the anions and bind anions very efficiently.

Towards the end of the last lecture, we looked at the hydride sponge, which has a boron center that can sequester the hydride ions, equivalent to that of a proton sponge, where we had the NMe2 group, in which the lone pair of electrons from both sides can capture the proton as well.

So, we looked at different kinds of systems that function as binding sites for the anions. Now, let us take up some more examples just to complete the discussion. So, we saw that we have different types of hosts that are going to bind with the guests.

To start with, we can look at charged hosts. And here we also looked at organic cations, at [NH4]+, and we can have anions as guests as well. So, we can also have a charged species, which can actually function as the host. To start with, we can have species like the ammonium cation.

Then we can have this species as a charged one; we can have the protonated pyridine, and we also looked at guanidinium-based species as well.

We can now have a neutral host. So, we see that all the species mentioned here can function as a good host for binding with anions, and if they are present separately, these are organic cations, which can also bind with the neutral host. So, we can also have this separately as cations; they can bind with the neutral host.

Now we can also look at other neutral hosts, which can bind with anions. For example, we can look at this kind of system, where X is equal to oxygen, sulfur, selenium, and this can hydrogen bond with chloride or any halide.

We can have the pyrrole, the pyrrole-based system, which also has a donor and can interact with a halide. We can have indoles, which can again bind with a chloride, and we can have urea-based systems, where we can have oxygen, sulfur, or selenium. Then we can have the hydrogen that can form N-H...Cl- hydrogen bonds.

So, this functions as a bifurcated acceptor. And we can extend these further to squaramides. For example, we can have this kind of system. These can function as donors; you can also have oxygen or sulfur here.

So, we can have this squaramide, and we can also have the thiosquaramide. We can have simple amines as well, and we can have semicarbazides. So, we can have this as a donor, this as a donor, and this as a donor.

So, this is a semicarbazide if this is oxygen, but if this is sulfur, then this is called thiosemicarbazide. So, you can see there is a lot of diversity when it comes to the different functional groups that can now sense anions.

We can also have carbazoles. Carbazoles are also present, and these have a donor that can now interact with your halide, and we also have the triazoles. Okay, and we see that this is an acidic donor. We can also use boric acid, which has these donors, and we can use activated fluorinated systems. So, these hydrogens are all at the top, and we have all the fluorines.

So, we have hexafluoro 1, 2, 3, 4, 5, 6. So this is the all-cis hexafluoro cyclohexane. Now, you see these are the highly acidic hydrogens because of the presence of the strong electron-withdrawing -I effect of fluorine. Now these hydrogens become acidic, and therefore they can function as a good host for the binding of anions.

And we can further activate the substrate, where we put a metal that can activate the formation of hydrogen bonds.

For example, we can take a system where we have a metal. There is a metal now, and we have N-H here. Now, because of the presence of the metal, which withdraws the electron density, from the nitrogen and, in turn, withdraws the electron density from this nitrogen, that enhances the acidity of this N-H.

So, the presence of this delocalization increases the electron density on this nitrogen, which in turn pushes more electron density onto the metal in a particular oxidation state, thereby enhancing the acidity of these hydrogens, which can now function as donor atoms and interact with different kinds of anions. Similarly, we can have metal 3-aminopyridine-based systems.

For example, let us consider the pyridine; the nitrogen lone pair is donated to the metal. We have the hydrogen here, and then we have the three aminopyridines. So, because of this donation of electrons to the metal center, the C-H bonds become acidic, and therefore they can now participate in anion binding. We also looked at some of the Lewis acids. For example, we examined the boron center.

So, the boron center is now electron deficient, allowing it to bind with anions. This has a lot of applications in the field of sensing different kinds of anions. For example, water can have different kinds of anions, and boron-based reagents can be used as sensors for sensing these kinds of anions. Mercury, a divalent species, can also be accompanied by tin, which is organotin, and we can have organomercury.

And to conclude this overall discussion about a different kind of substrate that we have, we can also utilize halogen bonding to bring about efficient anion capture.

For example, if you have electron-withdrawing groups, then that will enhance the sigma hole, allowing it to bind with a halide or negatively charged species. This can bind with a halide and negatively charged species.

For example, we can have species such as di-iodotetrafluorobenzene, or we can have this; we can also have this because this is the sp carbon. So, that enhances the electrophilicity or the sigma hole on this iodine, which can now interact with X minus.

We can also have the iodo-triazole, and here we see that this can function as a halogen bond donor, which can interact with an anion.

And we can now activate this further by taking the methyl substituted iodo-triazolium. So, the iodo triazolium compound, can now also function as a halogen bond donor. This is even more activated and then can accept anions.

So, we have looked at the vast spectrum of this particular anion host, which is now present in the systems, and keeping this in mind, we would now like to appreciate further the application of anion sensing and cation sensing in biological systems. You would also now like to look at biological anion receptors.

So, here's what happens: we know that in the case of a biological system, we have an enzyme, and the enzyme is the host. Now, we can have different kinds of anions that will come and bind to this particular host. Now, it is very important that in the case of protein structure, the structure need not be very rigid.

It can be a flexible structure, which can undergo conformational changes so that it can accommodate anion binding. So, the prerequisite of pre-organization, which was very important in the case of cation-binding hosts, is not a very strict requirement here.

And many times, in protein structures, it is the change, the conformational change, in the tertiary structure of the protein that is very important to accommodate the anion binding. Another very important aspect of biological receptors is that they play a crucial role in enzymatic catalysis.

So, here you see that if an anion has been captured, there is complexation, and then there is this anion that has to be transported to a particular place, and then it has to be released at the site of action. So, this is a process of complexation transport, and decomplexation plays a very important role. So, if the anions are initially solvated, you have desolvation.

The anion is captured by the biological protein, which we call an enzyme; then it is transported to the site of action, where it has to undergo decomplexation. So, kinetics play a very important role; kinetic selectivity plays a very important role. Thermodynamics is also important for efficiently binding the anion, but this binding should not be too strong; otherwise, the anion will not be released at the site of action.

So, this kinetic selectivity is very important. Therefore, proteins in this case need to be more thread-like flexible structures that can have conformational changes to accommodate the anions.

And when an anion binds with a protein, it is primarily dictated by enthalpic considerations, where a large number of hydrogen bonds and ionic interactions are present that stabilize the anion at the point of binding with the protein.

And also, we would like to keep in mind that a protein structure is a case where you have the primary structure, the secondary structure, the tertiary structure, and the quaternary structure, and it is a very specific mode of self-assembly of the protein.

This word, self-assembly, is something we will explore more next week and how it is different from molecular recognition. In the case of self-assembly, each step is very precise, and the information coded or contained in the system is also very specific. Such that if the primary structure is not formed first, then the secondary structure will not occur, and the tertiary and quaternary states will not be reached.

So, the primary structure is essentially the sequence of amino acids, which will form the peptide bond, and depending on how many such amino acids are formed, a large number of such amino acids will give you the primary sequence of the protein, which can effectively be considered a long thread-like arrangement of amino acids that gives you the primary structure.

Now, this chain of amino acids or this long-chain peptide, which gives you the protein, can now start getting coiled up in different ways, and this can give rise to the formation of beta helices and alpha sheets.

So, the formation of these alpha sheets is what gives the primary structure, and beta helices, which give rise to the secondary structure. That means the formation of these beta helices and alpha sheets, and the manner in which these alpha sheets and beta helices further fold the conformation of the protein, takes it into a particular conformation.

And in most cases, it is the globular conformation or the globular shape of the proteins that is very common, and this compact conformation, the globular shape or the globularity of the protein, essentially arises from the different types of folds that are undertaken by these alpha sheets and beta helices to give you the tertiary structure.

And it is here at this time, when you have the formation of the alpha sheets and the beta helices, that intramolecular hydrogen bonds and then dispersive interactions become important. Once you have a particular protein, how it interacts with the other protein is, again, through these hydrogen bonds.

But now they are intermolecular hydrogen bonds. So, how one protein interacts with another protein is important. For example, in a crystal structure, you have one protein interacting with another protein that gives rise to the quaternary structure of the protein.

So, in this regard, proteins are very important molecules, and understanding the processes of formation is relevant. An extensive study of anion-binding proteins has been observed, and in this regard, sulfate-binding proteins have been recognized. We call it SBP. And here you can see that the active site is P=O, -OH, -O-, -O-, where this functions as an acceptor of hydrogen bonds. So, it can accept hydrogen bonds from, say, water molecules, which are present in the protein structure.

So, protein structure has got large number of water molecules. So, these anions will get extensively solvated by the water molecules, and in turn these hydrogen bonds, the O-H, these are the donors, they can now interact with the acceptors at different protein sites.

Similarly, this is an acceptor, it can now interact with different donors, via hydrogen bonds. So, we can have this [HPO4]-, we can also have P=O, -OH, -OH, -O-. So, we can also have H2PO4 minus we can have HPO4 2 minus.

So, this kind of species is present. And what has been observed in the case of sulphate binding proteins, there is a specific case, where it has been showed, that in a particular protein, at the active site, the phosphate is surrounded by 12 hydrogen bonding interactions. And these hydrogen bonding interactions are such that you can have N-H...O or you can have O-H...O hydrogen bonds, these are between 2.6 to 2.9 angstrom. And we have, 7 such hydrogen bonds, which involve the arginine side chain or the protein main chain.

So, the protein mentioned is the CONH-CONH sequence and the arginine is one of the amino acids, which has a NH donor, which can interact with the phosphate unit, and 7 such hydrogen bonds are there. There are 4 such hydrogen bonds, where 2 are coming from the serine O-H, and two are coming from the threonine O-H and one which is coming from the oxygen atom of the carboxylate ion.

And this kind of very specific hydrogen bonding pattern, between an anion with an active site of the protein, gives the protein its selectivity. And the fact, that proteins function in a certain way, is because of this distinct hydrogen bonding arrangements which are of extreme relevance. So, this is with respect to sulfate binding proteins. There is another important class of proteins which are called phosphate binding proteins. Now we will go to the sulfate binding protein. So, in the case of sulfate binding protein we have now the sulphate anion and this can now interact at the active site with different kind of amino acids and again you can have different kind of hydrogen bonds which are present here. And there are 7 such hydrogen bonds which are formed. These come from the backbone NH, serine OH and tryptophan NH.

These are all the hydrogen bond donors. And this is what gives this particular protein its selectivity, and what has been observed is that this function is very specific to this atomic arrangement, because if you replace the O-H of serine with S-H, or you replace it with CH3, or you replace it with glycine, in this case it is alanine.

Okay, so when you do this simple replacement of one of the atoms say OH with SH, CH3 or glycine then it reduces the affinity for sulfate binding. So that reduces the affinity of the protein for sulphate binding, and it drops by a factor of 3200, here 100 and here 15.

So, this is also very important. It is slight modification of the electronic environment, we replace this OH, with sulphur, SH, dramatically or drastically reduces the affinity of the same protein. Now for the sulfate, because you have now modified the donor when you have replaced oxygen with sulfur, it now becomes a much much weaker donor compared to OH, which is a much stronger donor and also it forms much stronger hydrogen bonds compared to that of SH.

So, this plays a very important role in anion recognition in case of sulfate binding proteins. So, what we have learnt now is that in case of proteins particularly we do not need pre-organization and we see that essentially these are stabilized by the formation of large number of hydrogen bonds, enthalpy considerations are very very important and more is the extensive hydrogen bonding and even we have got van der Waals interactions between the hydrophobic parts of the protein., then these will tend to be formed and favored and that will actually stabilize the aggregation.

Now, the next thing we would like to further discuss here is we would like to take up the topic of transmembrane anion transport or cation transport. Now this is again very important because you know we have not looked at anions as a whole.

We have looked at cations as a whole and this anion transport and cation transport is very important in biological cells. And in a biological cell what essentially happens is that which is we have got an aqueous interior which is separated from the exterior, by a phospholipid bilayer cell membrane which is around 5 to 6 nanometer in width.

So, outside the cell, it is an aqueous environment and inside is an aqueous environment and in between we have got this phospholipid bilayer cell membrane. And it is through the cell membrane, that the communication takes place.

Different kinds of active ingredients have to come from outside to inside or they have to go from inside to the outside via these bilayers. So, the formation of this bilayer is extremely important. And the phospholipid bilayer is chemically containing a phosphate linkage.

So, this is a phospholipid bilayer and this we can represent it as a negatively charged species which represents the phosphate anion and then we have this corresponding hydrophobic backbone. For the case of simplicity I will also write it as follows. So, now we have this bilayer arrangement where we have these.

This is just for the sake of simplicity because we know that there is a hydrocarbon chain here. So, we have the aqueous region here, we also have the aqueous region here, and in between we have got the bilayer.

And we can actually have the transport, for example of a cation, say potassium, or anion, say chloride and it transports, from say the outside to the inside of the cell. For example, this is referred to as the uniport mechanism, a way of transport, such that you have the cation, and you have the anion, and they both are transported, simultaneously through this particular bilayer. And this is referred, to as the process of a symport, because they are being transported in the same direction.

On the other hand, if they are being transported in the opposite direction, say the cation moves in this direction, and the anion moves in the opposite direction, that is referred to as an antiport mechanism.

And overall, this symport and antiport, is referred to as co-transport.

So, this symport and antiport mechanism, where the anions are going or moving or being transported from one side to the other, across this bilayer, is a very important process in biology, and in maintaining the equilibrium of the system. And so, we will look at how does this, for example, the cation move from the outside to the inside of the cell.

And we will look at these mechanisms in the next lecture. Thank you.