Ions at Biological Interfaces

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The effects of salt ions on the behavior of biomolecules in solutions, such as salting-out of proteins, has been traditionally ascribed to ion–water interactions in the aqueous bulk.[1] The ion-specific behavior, expressed, e.g., in the famous Hofmeister series,[2,3] has been then rationalized by classifying ions as either kosmotropes ("structure makers") or chaotropes ("structure breakers") according to their ability to structure water molecules around themselves.[4] According to this picture, cosmotropes, but not chaotropes, organize layers of water molecules around themselves, thus effectively removing the solvent from proteins, which leads to salting-out. There is, however, mounting experimental evidence that this picture is incomplete at best and that ions (at least monovalent ones) are not able to strongly affect more water molecules than their immediate hydration shells.[5-7] Alternative or additional explanations of salt action are, therefore, being searched for with the prime suspect (indeed, the elephant in the closet) is the interface between the biomolecule and the surrounding salt solution.

Ion-protein interactions

As a simplest possible model, the picture of a protein as homogeneous sphere of a low dielectric constant has been repeatedly invoked in the literature .[8,9] Within this picture the interface between a globular protein and an aqueous solution should resemble that between air or oil and water. However, when comparing the behavior of ions at the protein/water and air(oil)/water interface, there are striking differences. For example, alkali cations such as sodium or potassium, and divalent ions like calcium or sulfate are repelled from the water surface, but they exhibit affinities for the protein/water interface.[10] Calculations and experiments also show that interactions of ions with the protein surface are mostly of a local nature. E.g., the alkali cations are primarily attracted by negatively charged moieties such as the carboxylic groups in the side chains of glutamate and aspartate and, to a lesser extent, the amide oxygens of the backbone, whereas sulfate exhibits an affinity for the positively charged groups in the side chains of lysine and arginine.[11] The dielectric similarity between the air/water and protein/water interface is thus of little use here because the ion-protein behavior is dominated by local interactions with charge and polar groups at the protein surface in the presence of explicit water molecules rather than by the average dielectric properties of the protein. An exception is the case of large polarizable monovalent ions which exhibit qualitatively comparable affinities for the air/water interface and for hydrophobic parts of the protein surface, primarily due to cavitation and polarization effects. The situation is, however, more complicated and subtle in the latter case, where not not only the hydrophobicity of the non-polar groups but also their proximity to electron withdrawing atoms (particularly nitrogen and oxygen in the peptide bond) enhances their interaction with soft anions.

Since the local ion-protein interactions are of prime importance for ion segregation at surfaces of hydrated proteins, one can invoke reductionism as a reasonable first step. Thus, if we understand how different ions interact with individual (terminated) amino acids or even with their side-chain groups and with the amide group in water, we can extrapolate to a rough picture of their segregation at a protein surface.[12] For these cases, the empirical rule of matching water affinities, stating that an ion pairs most efficiently with an oppositely charged ion of comparable hydration enthalpy (i.e., surface charge density, within the simple Born model[13]), can be applied as a first estimate.[14,15] Ion specific interactions with amino acid residues, as well as complex proteins, are thus governed by two main mechanisms that target distinct surface groups on the macromolecule - ion-pairing with charged side chain groups, as well as the backbone amide moiety, and weak interactions with non-polar groups.

In summary, solubilities and stabilities of proteins in solutions are governed not only by the macromolecular net charge, salt concentration, and valency, but also by the chemical nature of the dissolved ions.[1] For common anions, the Hofmeister ordering in the ability to precipitate egg white proteins is as follows: $F^- > CH_3COO^- > Cl^- > NO_3^- > Br^- > I^- > SCN^-$, while the effect of (alkali) cations is usually less pronounced.[2,3] The Hofmeister ordering is, however, dependent also on the particular counterion, solution pH, and the protein iso-electric point, pI. It is well know that several proteins such as lysozyme follow the reverse Hofmeister series at low and intermediate pHs, when they are positively charged, which has been rationalized in terms of a fine interplay between the above ion-specific interaction.[16,17] It should be noted that dispersion interaction are also present and ion-specific,[18] however, except for special cases involving (quasi)aromatic residues (such as the "Coulomb-defying" pairing between like-charge guanidinium groups[19,20]) they are of a secondary importance.

Ion-membrane and ion-nucleic acid interactions

Despite the main focus has always been on ion channels, the influence of physiologically most relevant ions (such as Na^+, K^+, Cl^-, Ca^{++}, or Mg^{++}) on model lipid membranes was also studied.[21-24] Additionally, other ions, such as Li^+, Cs^+, NH_4^+, Ba^{++}, La^{+++}, F^-, Br^-, I^-, NO_3^-, and SCN^- were also investigated[24-26] in order to elucidate the factors influencing the specific ionic effects observed. This specificity has been known from measurements to be more pronounced for anions than for cations, consequently, more experimental data are available for the former ions.[26-29] Computer simulations are, however, typically more focused on cations, since a proper description of the effects of larger anions often requires the use of resource-consuming polarizable force field,[24] while cationic interactions with model membranes have been satisfactorily described using non-polarizable potentials.[23,30-32] The strongest cationic effects have been observed both in simulations and experiments for multivalent cations (Mg^{++} and Ca^{++}) and monovalent cations with large charge density (Li^+),[26,33] which interact appreciably with lipid bilayers,[25,34] rigidifying them[35] and stabilizing their gel phase.[35-37] Association of larger monovalent cations (Na^+, K^+, Rb^+, and Cs^+) with neutral (zwitterionic) lipid bilayer is much weaker; it is, therefore, difficult to measure directly their ion-specific effects.[26,29] Fluorescence measurements using solvent relaxation techniques have shown weak dehydration and hindered mobility at the glycerol level of DOPC membrane upon addition of 150 mM NaCl[23] and molecular dynamics simulations showed that Na^+ (in contrast to K^+ or Cs^+) exhibits affinity to the headgroups of DOPC membrane.[23,38] The binding site of Na^+ was found to be the phosphodiester and/or the carbonyl groups of phospholipids, depending on the force field emplyed.[21,23,38,39] The cationic effects are strongly amplified in negatively charged membranes (e.g., phosphatidylserine bilayers) or in those composed from a mixture of zwitterionic and negatively charged phospholipids.[40]

Nucleic acids represent another type of biological systems whether specific interactions with ions are of a crucial importance. As a highly charged polyelectrolyte, the negative charges on the phosphate groups need to be compensated by counter-cations. Among monovalent cations, specific interactions of Na^+ and K^+ have been investigated in considerable detail. Molecular dynamics and quantum chemical calculations[41-47]point to an interesting observation, namely that while an isolated monovalent phosphate group exhibits only a weak and roughly comparable affinity to these two alkali cations, in DNA the polyelectrolyte effect enhances both ion binding and ion specificity which goes in favor of Na^+ over K^+. These observations are in general supported by measurements, although the experimental evidence is somewhat internally conflicting.[48-50] Divalent cations interact even more strongly with nucleic acids leading to counter-ion condensation or even charge reversal in the vicinity of aqueous DNA.[45]

Future Directions

Within the last decade significant progress has been made in understanding of the molecular origins of the ion specific Hofmeister effects. The attention has clearly moved from ion properties in homogeneous aqueous solutions to their behaviour at the biomolecule/solution interface. Researchers got a lot of millage

from the reductionist approach assuming a local character and additivity in ion-biomolecule interactions. Within this picture the key components responsible for the specific ion-biomolecule interaction are pairing of ions from the solution with charged and highly polar groups at the biomolecular surface, together with the ability of large soft ions to segregate at the interface between the solution and hydrophobic surface groups. In this context, the effects of aromatic groups, as well as that of neighboring electron-withdrawing atoms (nitrogen and oxygen in particular) deserve a closer scrutiny.

Future will likely see the third step of the Hegelian triad of *thesis* (i.e., the bulk origin of ion-specific effects), negated by *antithesis* (i.e., the interfacial origin of ion-specific effects), to be finally replaced by *synthesis* which will interpret ion specific Hofmeister effects in terms of both bulk and interfacial behaviour of ions. This synthesis will also likely lead us to the understanding that cationic and anionic effects cannot be always divided into separate Hofmeister series for cations and anions and that the local reductionist picture can in many cases (particularly if polyvalent ions are involved) serve only as a first approximation and non-local collective effects may come into play. This synthetic view is likely to prove useful also when further investigating the specific spatial and chemical arrangements nature has engineered to manipulate ions in trans-membrane ion channels and pumps.

Cross-References

Specific Ion Effects, Evidences; Specific Ion Effects, Theory

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