Specific Ion Binding to Non-Polar Surface Patches of Proteins

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Mounting evidence suggests that ion specific or "Hofmeister type" effects are governed by solvent mediated ion binding to molecular surfaces[1-2] but, nevertheless, the underlying physical mechanisms remain poorly understood. Hence, more than a century after the first observations[3], it is still debated why large anions (I^-, SCN^- etc.) very effectively salt out positively charged proteins such as lysozyme[4]. In this communication we present microscopic evidence that suggests that ions bind to proteins not only via specific ion-ion interactions[5], but can also exhibit a solvent assisted non-polar *attraction*. Classical continuum electrostatic models semi-quantitatively account for the direct ion-ion free energy between ions and charged surface groups and also encapsulate solvation effects at dielectric boundaries[6]. Such "reaction field" approaches imply that hydrated ions are always repelled from non-polar surfaces due to a positive desolvation free energy. However, recent experimental as well as theoretical studies[2,7] have shown that large, soft anions can be *attracted* to non-polar, hydrophobic interfaces. In particular it has been shown[8] that the binding of fluoride and iodide to a model colloid with charged and non-polar patches is governed by both direct ion-pairing interactions and hydrophobic-like interactions[9] with non-polar patches.

The remaining question is if this is also the case for real proteins with a complex arrangement of ionic and non-polar surface groups. To elucidate this issue we performed a detailed Molecular Dynamics (MD) study of lysozyme in a mixed aqueous solution of potassium chloride and iodide (0.4 M). The former anion represents a relatively small, well-hydrated ion while the latter is large, soft, and poorly solvated. The 10 ns long MD simulations were performed in the isothermal-isobaric ensemble (298 K, 1 atm.) with a single protein molecule (PDB code 1W6Z, protonated at pH 7, and described within the polarizable ff99 forcefield[10]), polarizable ions (Table 1) and roughly 7000 POL3 water molecules[11]. We employed periodic boundaries with a cutoff for non-bonded interactions of 9 Å and used the Particle Mesh Ewald summation method for long range electrostatics[12]. All simulations were carried out with the Amber 9 program[13].

Table 1. Ion and solvent interaction parameters: partial charges (q), Lennard-Jones interaction parameters (ε), diameters (σ) and polarizabilities (α).

	K^+	Cl^-	I^-	O_w	H_w
q/e	1	-1	-1	-0.73	0.365
ε/(kcal/mol)	0.10	0.10	0.10	0.156	0
σ/Å	3.74	4.87	5.78	3.596	0
α/Å^3	0.85	3.69	6.90	0.528	0.170

Ionic distributions around the fluctuating protein surface are analysed in terms of the cummulative sums, $N(r)$, of chloride and iodide, collected in non-spherical shells around specific residues[5]. In Fig. 1 we show the relative preference of chloride and iodide towards non-polar and cationic surface groups, respectively.

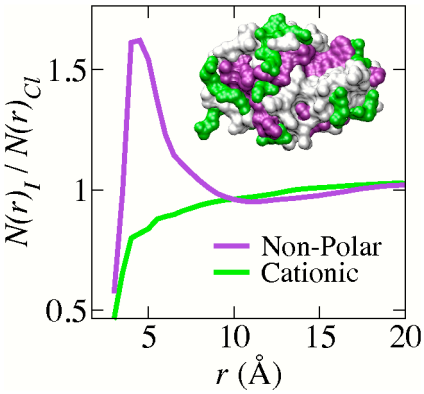

Figure 1. Relative cummulative sums of iodide vs. chloride around non-polar and cationic residues on lysozyme. The inset illustrates the location of non-polar (purple) and cationic (green) groups in lysozyme. Non-polar residues include: ALA, LEU, VAL, ILE, PRO, PHE, MET, TRP.

The emerging picture is clear: chloride is preferred at the basic (cationic) residues, while iodide is enhanced near non-polar groups. This is consistent with the notion of an ion-specific balance between ion-pairing and hydrophobic attraction. To further unravel the mechanism we differentiate $N(r)$ to obtain the distribution functions, $4\pi r^2 g(r)$, around specific residues – see Fig 2.

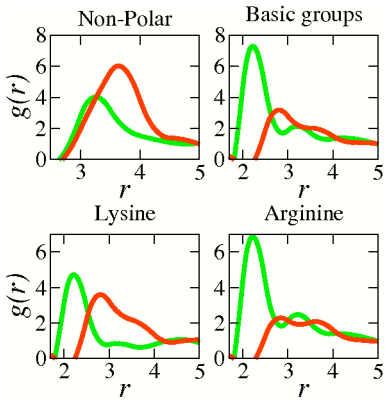

Figure 2. Distribution functions of chloride (green) and iodide (red) around various groups on lysozyme.

We note that the iodide enhancement at non-polar groups reaches ~6 times the bulk concentration. In contrast, chloride is enhanced by a similar factor in close vicinity of cationic groups; more so at arginine than at lysine. The latter segregation is consistent with previous studies of ion-pairing[14,15], indicating that chloride forms contact ion-pairs with arginine. Albeit to a lesser extent than iodide, chloride also associates with non-polar regions. Similar effect has been observed previously for the water/vapor interfaces[7] and was attributed primarily to the sizable polarizability of the chloride (and even more so iodide) anion. It is to be noted that, due to interference from neighboring groups, *g(r)* calculated in a complex molecular environment are valid only at short separations and should be regarded as qualitative measures. This could be remedied by systematical investigation of ions around isolated amino acids or model peptides[15,16], as long as pair-wise additivity can be assumed.

The reported competition between specific ionic and (effective) non-polar interactions manifests itself also in dilute bulk electrolyte solutions. As shown in Table 2, we have used experimental activity coefficient (γ) data[17,18] to estimate the excess chemical potential difference, $\Delta\mu^{ex}=kT\ln(\gamma_{NR4Cl}/\gamma_{NR4I})$, of exchanging iodide with chloride in solutions of symmetric tetraalkylammonium (TAA) salts. A clear preference of iodide for long chain length TAAs is seen, but as these are gradually shortened, the affinity is shifted towards chloride which, for the bare ammonium ion, is the preferred binding partner ($\Delta\mu^{ex}<0$). In agreement with simulation work[19], this shows a smooth transition from effective non-polar attraction in the case of iodide to direct ion-pairing in the case of the smaller chloride ion.

Table 2. Measured[17,18] excess chemical potential differences, in units of *kT*, for exchanging iodide with chloride in solutions of TAA salts, $NR_4^+X^-$, of varying chain length. *C* denotes the experimental salt molality.

C (mol/kg)	NPr_4^+	NEt_4^+	NMe_4^+	NH_4^+
0.1	0.17	0.093	0.052	-6.5e-3
0.5	0.62	0.39	-	-0.030

Our present results show that exactly the same mechanism is also operative for complex molecules such as proteins. The resulting ion-binding pattern is hence governed by the distribution and abundancy of charged and non-polar groups on the surface of a specific protein. In particular, the distributions of chloride vs. iodide anions and their effect on protein-protein association and salting out result from a subtle balance between direct pairing of small ions with positively charged amino acid residues and attraction of large soft ions to non-polar surface patches.

It is noteworthy that the investigated ion-specific effects arise due to different ionic sizes and polarizabilities and not because of dispersion interactions, often invoked for explaining ion-specificity in implicit solvent models[20]. This is not to say that dispersion interactions are not present, however, their role in ion-specific interactions seems to be of secondary importance. This notion is further supported by studies of ion binding to non-polar, planar surfaces where the net van der Waals contribution was found to be repulsive[21]. Instead, ion solvation effects – both in connection with ion pairing and ion affinity for water/non-polar interfaces, are likely the main driving forces for ion-specific phenomena.

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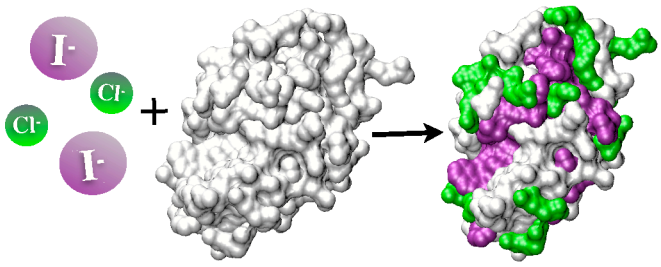
TOC Graphics



Employing detailed atomistic modeling we elucidate the mechanisms behind ion-binding to proteins and other bio-molecules and conclude that (1) small, hard ions bind via direct ion-pairing to charged surface groups and (2) large, soft ions bind to non-polar groups via a solvent assisted attraction. Our predictions are in qualitative agreement with bulk electrolyte data and may provide an important clue for the basic understanding of ion-specific effects in biological systems.