Communication

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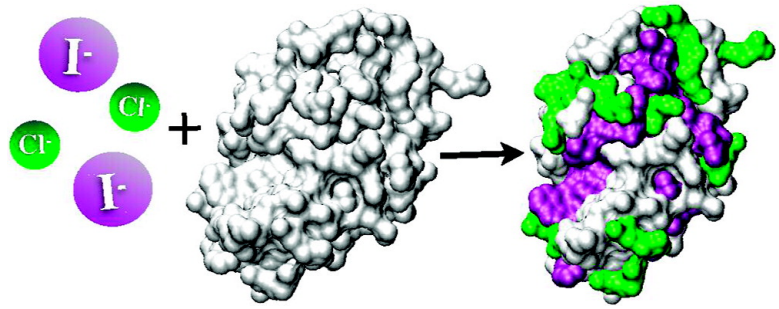

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Specific Ion Binding to Nonpolar Surface Patches of Proteins

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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 induce attractive interactions between positively charged proteins such as lysozyme.[4−6]

In this communication we present microscopic evidence that suggests that ions bind to proteins via not only specific ion−ion interactions[7] but also solvent assisted *attraction* to nonpolar surface groups. Classical continuum electrostatic models semiquantitatively account for the direct ion−ion free energy between ions and charged surface groups and also encapsulate solvation effects at dielectric boundaries.[8] At the same time the latter reaction field approach implies that hydrated ions are repelled from nonpolar surfaces due to a positive desolvation free energy.

However, recent experimental as well as theoretical studies[2,9,10] have shown that large, soft anions can be attracted to nonpolar interfaces. In particular it has been shown[10] that the binding of fluoride and iodide to a model colloid with charged and nonpolar patches is governed by both direct ion-pairing interactions and solvent-induced interactions with nonpolar patches. As for the latter mechanism, moving a large ion closer to a nonpolar region brings about several contributions to the effective intermolecular interaction: (1) Loss of ion−dipole energy, (2) reduction of the unfavorable water network around the large ion and the nonpolar interface,[11] and (3) attractive induced-dipole interactions with the polarizable ion and the electrostatic potential set up by oriented interfacial water molecules.[12] Finally, (4) solvent−solute, solute−solute, and solvent−solvent dispersion interactions contribute.[13,14] For small ions the first term (1) dominates and the behavior resembles the above mentioned reaction field.[8] Due to the central role played by water in (1) through (4) we mark the combined, effective interaction as *solvent assisted*.

The remaining question is if the polar and nonpolar ion segregation is also present in real proteins with a complex arrangement of ionic and nonpolar 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 force field[15]), polarizable ions[16,17] (Table 1), and roughly 7000 POL3 water molecules.[18] We employed periodic boundaries with a cutoff for nonbonded interactions of 9 Å and used the Particle Mesh Ewald summation method for long-range electrostatics[19]. All simulations were carried out with the Amber 9 program.[20]

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Table 1. Ion and Solvent Interaction Parameters[a]

	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)	3.33	4.34	5.15	3.20	0
α (Å^3)	0.85	3.69	6.90	0.528	0.170

[a] Partial charges (q), Lennard−Jones interaction parameters (ϵ), diameters (σ), and polarizabilities (α).

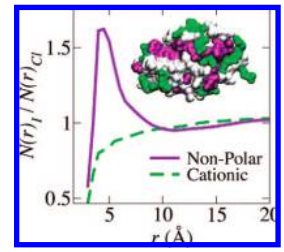

Figure 1. Relative cumulative sums of iodide vs chloride around nonpolar and cationic residues on lysozyme. The inset illustrates the location of nonpolar (purple) and cationic (green) groups in lysozyme. Nonpolar residues include ALA, LEU, VAL, ILE, PRO, PHE, MET, and TRP.

Ionic distributions around the fluctuating protein surface are analyzed in terms of the cumulative sums $N(r)$, of chloride and iodide, collected in nonspherical shells around cationic and nonpolar residues.[7] For each configuration we use the *shortest* distance between the ion and any of the atoms of the selected protein residue for the evaluation of the sums. In Figure 1 we show the relative preference of chloride and iodide toward nonpolar and cationic surface groups, respectively.

The emerging picture is clear: chloride is preferred at the basic (cationic) residues, while iodide is enhanced near nonpolar groups. This is consistent with the notion of an ion-specific balance between ion pairing and nonpolar 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 Figure 2).

We note that the iodide enhancement at nonpolar 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,[21,22] indicating that chloride forms contact ion pairs with arginine. Albeit to a lesser extent than iodide, chloride also associates with nonpolar regions. A similar effect has been observed previously for the water/vapor interfaces[9] and was attributed primarily to the sizable polarizability of the chloride (and even more so iodide) anion.

As expected, we also observe higher water densities close to charged groups than at nonpolar groups (see Figure 3). This solvent structuring provides a molecular basis for hydrophobic assembly[11,23] in this context between poorly solvated ions and nonpolar patches. It is to be noted that, due to interference from neighboring groups, distribution functions calculated in a complex molecular environment are valid only at short separations and should be regarded as qualitative mea-

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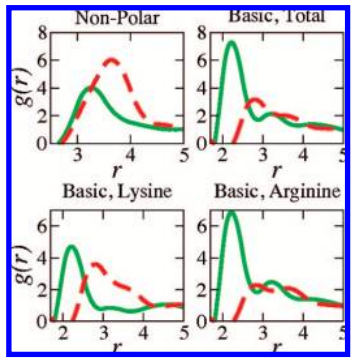

Figure 2. Distribution functions of chloride (green) and iodide (red, dashed) around various groups on lysozyme. All $g(r)$'s are normalized to unity at a 5 Å separation, approximately the Debye length of the system.

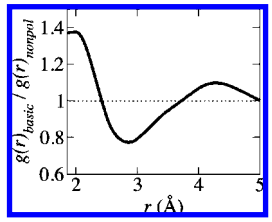

Figure 3. Ratio between water densities around basic and nonpolar surface groups.

Table 2. Measured[26,27] Excess Chemical Potential Differences for Exchanging Iodide with Chloride in Solutions of TAA Salts, $NR_4^+X^-$, of Varying Chain Length[a]

	NPr_4^+	NEt_4^+	NMe_4^+	NH_4^+
$\partial\Delta\mu^{ex}/\partial m$ (kT·kg·mol^{-1})	1.11	0.697	0.285	−0.0564

[a] In the experimental concentration range (0.1−0.5 mol/kg), $\Delta\mu^{ex}$ is linear ($\langle r^2\rangle = 0.996$) with respect to the solute molality, m, and we therefore present the slopes, 176 $\partial\Delta\mu^{ex}/\partial m$.

sures. To some extent, this could be remedied by systematic investigation of ions around isolated amino acids or model peptides,[22,24,25] as long as pairwise additivity can be assumed.

The reported competition between specific ionic and (effective) nonpolar interactions manifests itself also in dilute bulk electrolyte solutions. As shown in Table 2, we have used experimental activity coefficient (γ) data[26,27] to estimate the excess chemical potential difference, $\Delta\mu^{ex} = kT\ln(\gamma_{NR_4Cl}/\gamma_{NR_4I})$, of exchanging iodide with chloride in solutions of symmetric tetraalkylammonium (TAA) salts. A clear preference for iodide in long chain length TAAs is seen, but as these are gradually shortened, the affinity is shifted toward chloride which, for the bare ammonium ion, is the preferred binding partner ($\Delta\mu^{ex} < 0$). In agreement with simulation work,[28] this shows a smooth transition from effective nonpolar attraction in the case of iodide to direct ion pairing in the case of the smaller chloride ion.

Our present results show that the same mechanism is also operative for complex biomolecules such as proteins. The resulting ion-binding pattern is hence governed by the distribution and abundance of charged and nonpolar groups on the surface of a specific protein. In particular, the protein affinities of chloride vs iodide anions and the effect on protein−protein association and salting out[29] result from a subtle balance between direct pairing of small ions with positively charged amino acid residues and solvent assisted attraction of large, soft ions to nonpolar surface patches. In support of this notion, NMR experiments[30] have shown that the binding of anions to proteins is not limited to direct interactions with cationic surface groups and that other sites can attract larger ions. Although not discussed here, backbone amide N–H groups may be another hotspot for specific anion binding. The fact that the combined solvent mediated salt−protein interaction plays an important role for ion specific phenomena has also been underpinned in a recent statistical mechanical study where experimental data were rationalized using the Kirkwood−Buff framework.[1]

Previous studies[10,12] suggest that the ion-specific mechanisms presented here arise mainly due to different ionic sizes and polarizabilities. In an initial study[10] of iodide and fluoride binding to a nonpolar nanosphere with charged patches, we investigated the relative contributions from Coulomb and Lennard−Jones interactions. The former contributed with a repulsive energy due to loss of hydration water when moving an ion closer to a nonpolar surface. The Lennard−Jones interactions contributed chiefly to exchange repulsion and, to a lesser extent, dispersion. This picture differs from that often employed in continuum electrostatics, where surface averaged dispersion interactions are invoked to account for ion-specific phenomena.[13,14] This is not to say that dispersion interactions are not present; however, findings from classical water and ionic force fields suggest that other mechanisms dominate. In particular, solvation effects, in connection with both ion pairing[21] and ion affinity for water/nonpolar interfaces, are likely the foremost driving forces for ion-specific surface phenomena.

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