Specific Ion-Binding to Macromolecules: Effects of Hydrophobicity and Ion Pairing

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November 1, 2007

Abstract

Using molecular dynamics simulations in an explicit aqueous solvent we examine the binding of fluoride vs. iodide to a spherical macromolecule with both hydrophobic and positively charged patches. Rationalizing our observations we divide the ion association interaction into two mechanisms: (1) poorly solvated iodide ions are attracted to hydrophobic surface patches, while (2) the strongly solvated fluoride and to a minor extent also iodide binds via cation-anion interactions. Quantitatively, the binding affinities vary significantly with the accessibility of the charged groups as well as the surface potential, therefore, we expect the ion-macromolecule association to be modulated by local surface characteristics of the (bio-)macromolecule. The observed cation-anion pairing preference is in excellent agreement with experimental data.

Introduction

The physicochemical properties of a range of biological and colloidal systems such as macromolecular association, protein activity, denaturation etc. [1, 2, 3, 4] are affected by the presence of salt ions in the surrounding aqueous solution. Not only does this influence depend on the salt concentration and valency, but also on more subtle characteristics of the solvated ions, commonly bundled under empirical terms such as "Hofmeister effects" and "electro-sensitivity". Part of this loose terminology stems from the lack of a clear molecular understanding of the abundant number of macroscopic observations [4]. In a recent review [5] it was pointed out that the origins of many ion-specific effects are to be found in ion-binding to the macromolecule [6, 7], rather than in water structuring effects. A thorough investigation of the possible ion-macromolecular association mechanisms is therefore desirable.

So how do ions interact with macromolecules and what is the origin of ion specificity? These are the central questions of this work and we now give a brief overview of the molecular driving forces that may (or may not) be at play:

1. Coulomb interactions lead to a neutralizing counter-ion distribution outside charged molecular surfaces [8, 9]. For highly charged systems, multivalent counter-ions may even – via ion-ion correlations, overcompensate for the molecular charge resulting in a *charge reversal* [10, 11].

2. Short range dispersion or London type interactions are effective for all types of atoms, polar or not, and are screened by salt only to a mild extent [12]. Since the high frequency polarizability is roughly proportional to the volume of the electron cloud of a given species, ion specific effects will arise. A number of workers have pursued this idea by combining dispersion interactions with continuum electrostatics, investigating colloidal as well as biological systems [13, 14, 15].

3. Similar to the air/water interface [16, 17], poorly solvated surface groups may lead to a hydrophobic-like attraction of large, polarizable ions such as iodide and thiocyanate. In contrast, small highly solvated ions will experience a repulsion as described within classical electrostatics by a reaction field or image charge repulsion [18].

4. Ion-pairing, a term coined in the first quarter of the twentieth century [19], implies that the interaction between a cation and an anion is sufficiently strong to form a temporary neutral species. The tendency to form ion pairs is ion specific [20] and we can expect different charged surface groups and free ions to pair with varying strength. As a recent example, a study of ion-binding to horse radish peroxidase [21] showed that the segregation of sodium versus potassium to the protein surface is caused mainly by preferential binding to carboxylic groups.

The next question to address is how ion-binding is coupled with macromolecular assembly. In traditional double-layer theory [8] two charged surfaces start to interact when their counter-ion distributions overlap. Hence, any modification of these distributions – via salt screening, ion-binding etc. – will modulate the intermolecular interaction. For example, iodide and thiocyanate have been found to effectively induce attractive interactions between positively charged proteins [22, 23, 24, 25] indicating that the ions accumulate at the surface so as to lower the overall charge or, rather, decrease the range of the neutralizing counter-ion distribution. Ion-binding will further affect the solvation free energy of the macromolecule and this combined with the inter-molecular potential will govern the salting in and out properties.

From the above discussion it is clear that the study of specific ion-binding and the ensuing implications for solution stability are highly non-trivial. In this study we use atomistic Molecular Dynamics (MD) simulations to investigate the microscopic distribution of ions around a *single* charged macromolecule. Due to the multitude of interactions taking place at the interface of complex molecules a clear identification of the responsible driving forces may prove difficult. Hence, more direct physical insight can be gained by mimicking the system using a coarse grained representation that encompasses major features such as dispersion, hydrophobic, and ionic interactions. This is accomplished by invoking a model where the macromolecular core is treated as

a large, hydrophobic sphere with charges distributed on the surface as shown in Figure 1. A similar model has previously been employed for the study of hydrophobic interactions between protein-like nano-spheres, albeit with no focus on ion specific effects [26]. As for the anions, we investigate the two extremes from the halide group, fluoride and iodide; the former represents a small highly solvated species, while the latter exemplifies a large, poorly hydrated ion.

Model and simulation details

The configurational space is sampled in the NPT-ensemble using molecular dynamics (MD) simulations in a cubic box with periodic boundaries. Energies and forces are based on the following pair potential function comprised of Coulomb and Lennard-Jones (LJ) interactions,

$$u_{ij} = \frac{e^2 q_i q_j}{4\pi\epsilon_0 r_{ij}} + 4\epsilon_{ij} \left[(\sigma_{ij}/r_{ij})^{12} - (\sigma_{ij}/r_{ij})^6 \right] \tag{1}$$

where q represents the particle charges, r the distance between them, σ and ϵ their combined LJ parameters, e the electron unit charge and ϵ_0 the permittivity of vacuum. Detailed interaction parameters are given in Table 1. Long ranged electrostatic corrections are accounted for by the particle mesh Ewald summation method [27] and we use a spherical cut-off of 1.35 nm for both electrostatic and Lennard-Jones pair interactions. The diameter of the nano-sphere is 1.6 nm and its LJ parameter, ϵ, is chosen such that a water oxygen experience a net repulsion of ~ 1 kT when penetrating the surface by one Ångström ($r_{ij} = \sigma_{ij} - 1$ Å). Charges are placed either one Ångström above the sphere or one Ångström below the spherical surface as shown in Figure 1. This is to mimic two situations of solvent accessibility of the charged surface groups. The angular distribution of the surface charges is such that their mutual electrostatic energy is at a minimum. For comparison we also simulate a charged sphere of a smaller size ($\sigma = 0.56$ nm) as well as a non-charged sphere of the original size. All simulations contain a background sodium ion concentration of 160 mM and an evenly mixed number of neutralizing flouride and iodide anions. A typical simulation cell (1 atm, 298 K) contains around 4000 SPC/E water molecules [28], one fixed nano-sphere, counter ions and a background salt concentration corresponding to that of physiological

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Table 1: Charges and Lennard-Jones parameters using the combination rules $\sigma_{ij} = (\sigma_i + \sigma_j)/2$ and $\epsilon_{ij} = \sqrt{\epsilon_i \epsilon_j}$.

	σ/nm	ϵ/kJ·mol^{-1}	q/e
Na	0.24	0.54	+1
Cs	0.38	0.42	+1
F	0.31	0.84	-1
I	0.51	0.42	-1
HC	1.6	0.053	0
H_w	0	0	0.4238
O_w	0.32	0.65	-0.8476
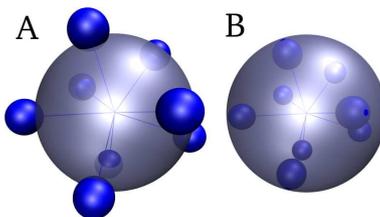
Figure 1: Two models of a macromolecule with a hydrophobic core as well as charged groups that can be either solvent exposed (A) or buried beneath the surface (B).

conditions. Simulations are equilibrated for 400 ps and sampled for 30–60 ns with a time step of 2 fs which is sufficient to converge the potential of mean force between ions and the macro-sphere to within a few tenths of a kT. All MD results are generated using the GROMACS [29] simulation package version 3.1.1.

To extract the overall ion-binding to the macro-sphere we integrate the radial distribution functions, $g(r)$. This yields approximate thermodynamic binding constants [30],

$$K_{\text{sphere-ion}} \approx \int_{\sim 0}^{\infty} \left(g(r) - 1 \right) r^2 dr. \tag{2}$$

where we note that long-ranged, attractive interactions will dominate due to the r-squared weighting. The fraction K_I/K_F is used as a measure of the relative binding of iodide versus fluoride.

Results

Interaction with an uncharged sphere

Large ions with a low surface charge density – iodide, thiocyanate etc. – are in general poorly solvated and due to the large cohesiveness of water, these solutes may effectively be expelled to other low solvated regions such as non-polar molecular interfaces [6]. This is indeed the case for iodide as shown in Figure 2 where we observe a small but distinct free energy minimum ($g(r)$ maximum) in the interaction with a neutral nano-sphere. Conversely, fluoride being strongly solvated is repelled from the nano-sphere. We also observe a slight water structuring, typical for relatively hard surfaces; in contrast, no ordering is seen at the air/water interface [16] which may be regarded as "infinitely" soft.
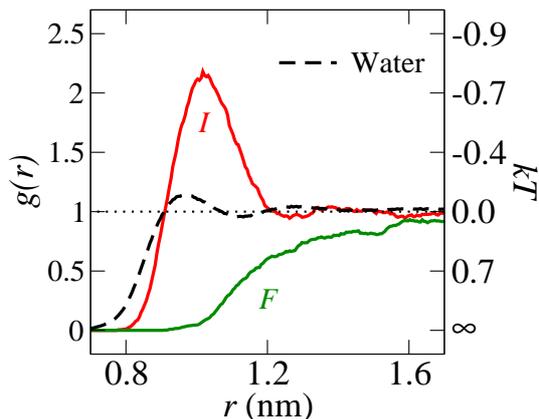

Figure 2: The radial distribution of iodide, fluoride and water outside a neutral LJ-sphere of diameter 1.6 nm. Note the non-linear scale of the right-hand axis, showing the potential of mean force (PMF).

To determine the relative importance of dispersion interactions we calculate the statistical mechanical average of the total Lennard-Jones and Coulomb energy for a single anion, j, as a function of the distance, R to the macro-sphere,

$$u_j(R) = \left\langle \sum_{i \neq j}^{N} u_{ij}(R) \right\rangle \tag{3}$$

This energy decomposition is shown for iodide in Figure 3. As for the LJ energy, noise makes it difficult to decipher the distance dependent behavior, nonetheless it is weak and hardly sufficient

to account for the 1 kT minimum observed in the potential of mean force. Further, the Coulomb energy is strongly repulsive near the sphere, manifesting the loss of ion-dipole interactions when moving iodide closer to the hydrophobic surface. Thus, iodide's affinity to the surface has the character of a hydrophobic attraction, driven mainly by the solvent. Increasing the temperature by 25 degrees we observe a decrease in the ion-binding free energy indicative of an entropic repulsion which at the $g(r)$ maximum amounts to,

$$-TS = T\frac{dG}{dT} = -\frac{T d\ln g(r_{\max})}{dT} \approx 4\ kT \tag{4}$$

at 298 K. Similarly, small-angle X-ray scattering experiments of protein-protein interactions [24] show evidence of a short range, ion-specific attraction that is also weakened at elevated temperatures, compatible with the idea that specific ion-binding is a central driving force for Hofmeister effects on macromolecular assembly.
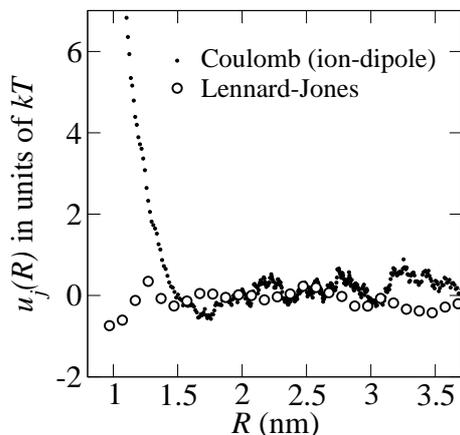

Figure 3: The average Lennard-Jones and Coulomb self-energy of a single iodide ion as a function of its distance from the neutral macro-sphere.

The molecular solvent simulations performed here suggest that dispersion interactions may contribute less to the ion specificity than hitherto thought and that a major contribution stems rather from solvation effects. It is to be noted that treating the hydrophobic core as a LJ particle somewhat overestimates the distance dependent decay of the dispersion interactions [9]. A straight forward improvement would be to construct the nano-sphere from a number of smaller spheres and work is in progress to implement this with proteins at the amino acid level [31]. The presented

results are obtained from simulations with a non-polarizable force field. For iodide at the air/water interface inclusion of polarizable atoms results in an enhanced surface affinity [16] and a similar effect may present itself at molecular interfaces. Preliminary results obtained by calculations with explicit polarizabilities of both solvent molecules and ions indeed intensify the hydrophobic attraction, yet the qualitative picture is maintained.

Charged Groups

We now attach eight explicit unit charges to the spherical core, each one having the same size as a sodium ion. Let us first examine the situation where the ions are situated one Ångström *above* the spherical core (see Figure 1, left), corresponding to solvent exposed groups as found in micelles, peptides and other macromolecules. Figure 4 shows that both halides are attracted to the nano-particle and that the larger iodide anion penetrates the sphere significantly more than fluoride ($K_I/K_F \approx 2$). Furthermore, the iodide peak is wide, whereas fluoride exhibits a more narrow spike. To elucidate the structural differences between iodide and fluoride we resort to spatial isodensity plots [32] of both ions outside the nano-molecule. Figure 5 demonstrates the striking difference between the two halides: fluoride binds highly specifically to the solvent exposed charged groups while iodide tends to occupy the surface area in between these groups in a more de-localized fashion. The fact that fluoride indeed has a preference for the charged groups is further illustrated in Figure 4 (bottom) where we have shifted the charges one Ångstrom *below* the surface of the nano-sphere as sketched in Figure 1. The charges are thus less accessible for the strongly solvated fluoride anion and we see a decreased binding of this species ($K_I/K_F \approx 3$). By contrast, iodide no longer needs to compete with fluoride for surface sites and we observe a slight increase in its binding to the macromolecule.

In summary, while both halide anions are attracted to the nano-sphere they do so in fundamentally different manners. Fluoride binds with strong, specific charge-charge interactions while iodide associates via de-localized interactions to the lesser solvated surface *between* the charged groups due to a combination of hydrophobic and electrostatic interactions.

Finally, the inset of Fig. 4 shows the effect of moving all eight charges to the *middle* of the
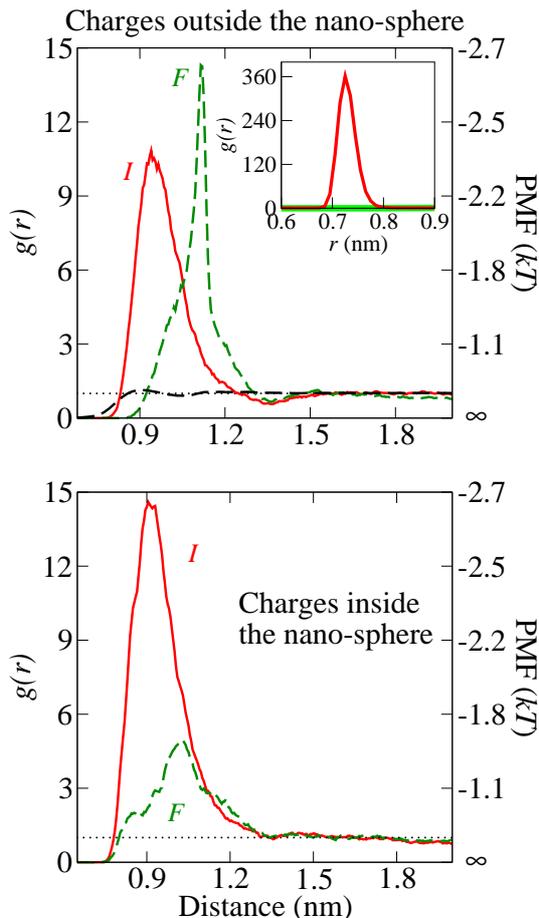
Figure 4: Radial distribution of fluoride and iodide outside a nano-sphere with eight attached charges that are either one Ångström above the surface (top) or one Ångström below the surface (bottom). The inset shows the case where all charges are placed in the center of the sphere.

macromolecule, which corresponds to the key approximation in classic DLVO theory [33, 34, 9]. The iodide peak now corresponds to a free energy minimum of $\sim 6 \; kT$ or two to three times more than when the charges are placed outside the sphere. Thus, while the central charge approximation may be appropriate in the continuum model, it has a larger impact in an explicit solvent description, in accord with other studies [26].

Effect of charge density

Based on experimental data for a wide range of systems, an empirical rule of "matching water affinities" has been proposed [35, 36] to explain ion-specific behavior. Note that within the Born solvation model [37] this rule translates into "matching ion sizes". That is, cationic and anionic groups with similar sizes – or surface charge densities – tend to bind to each other. Our
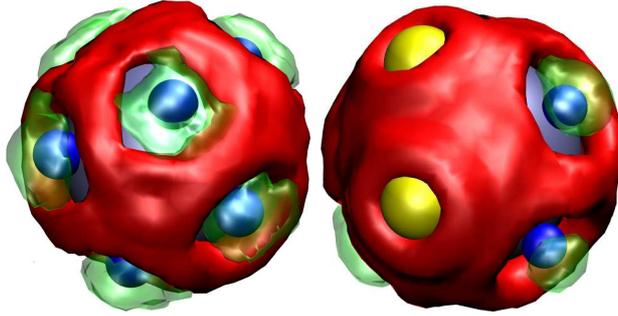
Figure 5: LEFT: spatial iso-density plot of iodide (red, $c = 4.3 \cdot 10^{-3}$ 1/Å³) and fluoride (green, $c = 2.8 \cdot 10^{-2}$ 1/Å³) outside the nano-sphere with eight solvent exposed positively charged groups (dark blue). RIGHT: Same as on the left, but with half of the (sodium-sized) surface charges inflated to the size of *cesium* (yellow).

findings support this idea in that fluoride binds to the small charged patches (here the size of sodium), while iodide stays in regions of lower surface potentials. This ion-pairing preference is also manifested experimentally in, for example, activity coefficients, γ, for pure alkali fluoride and iodide solutions [38, 39] where the excess chemical potential difference,

$$\Delta\mu_{I \to F}^{ex} = kT \ln(\gamma_{MF}/\gamma_{MI}) \tag{5}$$

reveals that fluoride is the preferred halide anion for pairing with sodium, cf. Figure 6. In contrast, larger cations such as rubidium and cesium, iodide is the most favorable binding partner. Following this experimental rationale, replacing the sodium sized, charged patches with *cesium* should lead a substantial decrease in fluoride binding. As shown on the right-hand side of Figure 5 this is indeed the case: exchanging half of the sodium ions with cesium results in an increased binding of iodide at the expense of fluoride. It is interesting to note that fluoride binding is sustained only at the remaining sodium sized patches, emphasizing the local nature of the interaction.

To further demonstrate the important balance between hydrophobicity and surface charge, we now increase the surface charge density by decreasing the size of the spherical nano-core ($\sigma = 1.6 \to 0.56$ nm). The positions of the eight sodium-sized charges are also re-scaled so that they remain one Ångström above the surface of the nano-sphere. As seen in Figure 7 the picture is now *reversed* and fluoride binds more strongly than iodide. We see a pronounced fluoride peak stemming from direct pairing with the charged patches and as these are now closer together, the
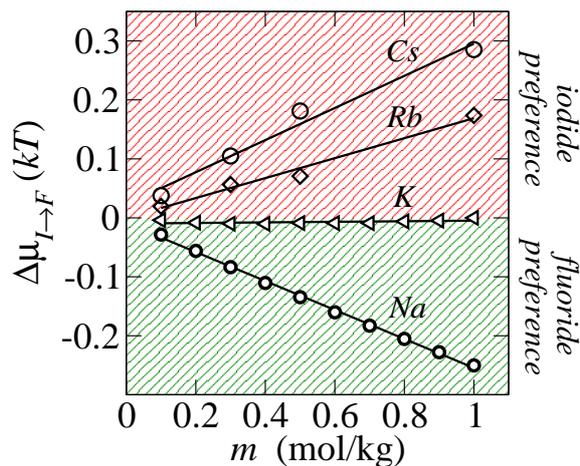
Figure 6: Experimental excess chemical potential differences for exchanging iodide with fluoride in aqueous alkali halide salt solutions.

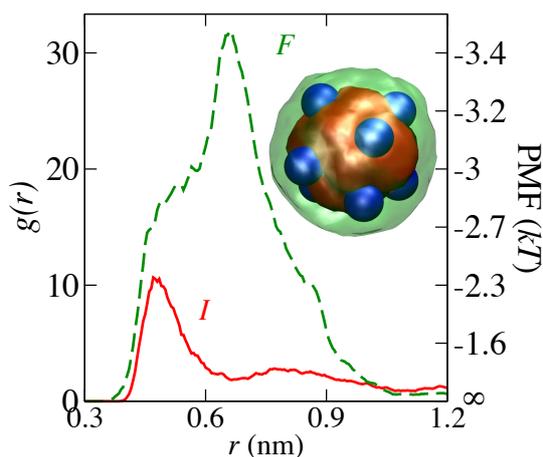distribution function gets broader.

Figure 7: Radial distribution of fluoride and iodide outside a smaller (σ=5.6 Å) sphere with eight surface exposed, charged groups. The graphical inset shows the iso-dentity plot of iodide (red) and fluoride (green).

Thus, the size and availability of the charged surface groups are expected to play an important role for ion-binding and may explain how weakly hydrated ions can also interact via ion-ion interactions as previously suggested [40]. In a recent MD study of ion-binding to a small flexible tri-peptide [41] it was shown that fluoride and iodide behave in much the same way as presented here. This hints that the proposed physical mechanisms are valid over a range of molecular categories, compatible with the notion that the specific ion-binding is determined by *local* surface properties [5].

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Conclusions

Using explicit solvent molecular dynamics we have investigated the distribution of fluoride and iodide anions around a spherical macromolecule both with and without discrete charged patches. In the case of an uncharged macromolecule we find that fluoride, being strongly hydrated, is repelled while the less solvated iodide is weakly attracted to the surface. This study suggests that the main contribution to specific ion binding originates from hydrophilic and hydrophobic interactions while we observe little or no effect from dispersion interactions. After charging up the nano-sphere, both anions are attracted, albeit in two distinct manners:

1. Fluoride, as a small ion, binds by specific anion-cation interactions that overcome the repulsion with the hydrophobic core.

2. Iodide, as a large ion, binds by ion-pairing combined with a more de-localized, hydrophobic attraction.

Burying the positively charged groups below the surface of the core, iodide binding is strongly favored. If we increase the surface charge density fluoride dominates the binding. Thus, the specific anion-binding is governed by *local* interactions that may be approximately divided into the two molecular mechanisms proposed above.

While our generalized coarse grained approach is in qualitative agreement with both bulk electrolyte data and MD simulations of small peptides [38, 39, 41], the distinct nature of the "real" (bio-)macromolecule is expected to modulate the quantitative binding of small ions.

Acknowledgments

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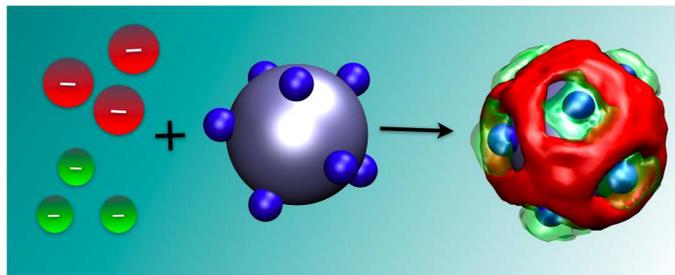[41] Fedorov, M. V., Goodman, J. M., Schumm, S. (2007) *Phys. Chem. Chem. Phys. 9,* 5423–5435.

Figure 8: TOC graphics