Ion Specific Protein Assembly and Hydrophobic Surface Forces

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Large anions are attracted to hydrophobic surfaces while smaller, well solvated ions are repelled. Using a combination of explicit solvent and continuum model simulations we show that this leads to significant ion specific protein-protein interactions due to hydrophobic patches on the protein surfaces. In solutions of NaI and NaCl we calculate the potentials of mean force and find that the resulting second virial coefficients for lysozyme corresponds well with experiment. We argue that ionic interactions with non-polar surface groups may play an important role for bio-molecular assembly and Hofmeister type effects.

Molecular Dynamics (MD) simulations [1–5] have shown that large anions are attracted to hydrophobic interfaces while smaller anions are repelled. These findings are supported by a number of experimental studies [6–11]. Measurements on bulk electrolytes can also indicate that non-polar surfaces interact more strongly with ions of larger size. For example, excess chemical potential differences, $\Delta\mu^{\mathrm{ex}}$ for salts with identical cations measure the change in free energy associated with anion exchange. Exchanging iodide with chloride in the presence of a common cation, M, one obtains $\beta\Delta\mu^{\mathrm{ex}}_{\mathrm{I}\to\mathrm{Cl}} = \ln(\gamma_{\mathrm{MCl}}/\gamma_{\mathrm{MI}})$, where γ are the mean activity coefficients and β is the inverse thermal energy. Experimental measurements for this quantity are shown in Figure 1, for a series of increasingly hydrophobic alkyl ammonium cations. The results indicate that chloride is the favored anion for the small ammonium ion ($\Delta\mu^{\mathrm{ex}} < 0$) while increasing the alkyl chain-length of the cation causes a shift in preference towards iodide ($\Delta\mu^{\mathrm{ex}} > 0$).

Similar ion-specific surface effects may also play an important role in protein solutions. While the interface of water soluble, globular proteins are usually mostly polar, solvent exposed patches of hydrophobic surface groups do indeed occur [14]. In recent simulation work [5] we showed that iodide ions showed a preference to hydrophobic regions of a simple, non-specific, protein model. This being the case, it is then likely that ion adsorption at hydrophobic surfaces will also affect the important phenomenon of protein aggregation. Tellingly, light scattering experiments [15] and crystallization studies [16] show that the *self-association* of the lysozyme protein is assisted by large anions such as iodide and thiocyanate, while the influence of chloride is less pronounced. Despite this evidence, specific ion binding to non-polar surface groups, has not yet been explicitly included in any theoretical studies on protein aggregation. In previous work [17–19] ion-specific association has instead been attributed to attractive dispersion interactions between ions and charged macro-spheres. However, simulations using explicit solvent models of ions at surfaces [4, 5, 20] suggest that surface modified ion solvation plays a major role in the ion-surface interaction and that, surprisingly, the water mediated dispersion component of the interaction may even be repulsive! [4] The aim of the work, reported in this letter, is to provide a first step toward including a solvent induced ion-specific surface interaction in a simulation study of protein aggregation.

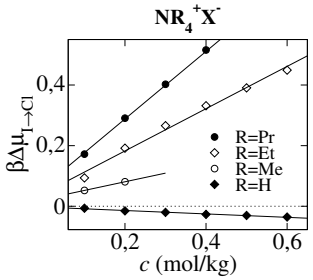

FIG. 1: Experimental excess chemical potential difference, $\Delta\beta\mu^{\mathrm{ex}}_{\mathrm{I}\to\mathrm{Cl}}$, between aqueous solutions of symmetrical tetraalkylammonium halides of increasing chain lengths [12, 13].

Here, we study the interaction between two mesoscopic lysozyme molecules in an aqueous salt solution using Monte Carlo simulations; the model is depicted in Figure 2. Each amino acid in the two proteins is represented by a neutral sphere, centered at the residue center of mass according the the X-ray structure [21]. The protonation sites of all titratable groups are explicitly included at their original positions and their charge state is set according to pH=4.7. The rigid protein molecules are allowed to randomly translate and rotate [22] while mobile salt and counter ions are explicitly included as soft spheres. All species are encapsulated in a spherical simulation cell [23]. The solvent is treated as a dielec-

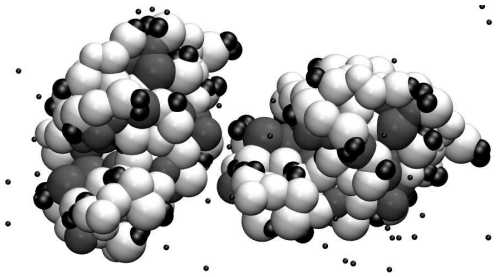

FIG. 2: Monte Carlo simulation snapshot of two lysozyme molecules in a salt solution. The spheres on the proteins represent neutral residues (white), hydrophobic residues (grey) and charged sites (black). For clarity the size of the salt particles (small, black) has been reduced.

tric continuum, which precludes our ability to explicitly model solvent structural effects. These will be *implicitly* included, as will be described below. During the simulation, coordinate space is sampled using the traditional Metropolis algorithm [24], based on the following energy function, $\beta U = \sum_{ij}^{\prime N} \beta V_{sp}(r_{ij}) + \sum_{ij}^{N} \frac{l_B z_i z_j}{r_{ij}} + 4\epsilon_{\mathrm{LJ}}[(\sigma_{ij}/r_{ij})^{12} - (\sigma_{ij}/r_{ij})^{6}]$ where l_B is the Bjerrum length, r_{ij} is the distance between particle i and j, z their charge numbers and ϵ_{LJ} and σ_{ij} are the Lennard-Jones parameters. Details of the model parameters are summarized in Table I. The potential term $V_{sp}(r_{ij})$, represents the interactions between ions and the protein surfaces, due to solvent structure. The prime on the summation means that we only consider ion-protein pairs. As argued elsewhere [4], no rigorous theory as yet exists for the this interaction. In this work, we take a pragmatic approach and use an empirical potential expression, fitted [4] to results obtained from MD simulations of ions in the presence of an air/water interface. In these simulations, an explicit solvent with a polarizable force field was used [1]. The expression has the form, $\beta V_{sp}(z) = A\{[e^{-B(z-z')} + (-1)^n]^2 - 1\} + C_1(z - C_2)e^{-C_3(z-C_2)^2} + D_1 e^{-D_3(z-D_2)^2}$ where z is the distance between the ion and the hydrophobic surface and the coefficients are given in reference [4]. The coefficients are different for iodide and chloride ions, making hydrophobic surfaces attractive to the former and repulsive to the latter. In order to adapt this expression to our simulations, mobile ions were assumed to interact, via $V_{sp}(z)$, with the *nearest* hydrophobic residue (ALA, LEU, VAL, ILE, PRO, PHE, MET, TRP). The value z was chosen as the closest distance between the ion and the surface of that hydrophobic residue. This approximation neglects effects of local surface curvature on the proteins and may potentially overestimate the ion-surface interaction. However, we note that there are other consequences of our modelling that may counter this. For example, our proteins are *rigid* and hence no side chain rearrangements are allowed to accommodate ions near the (mostly) buried hydrophobic groups.

TABLE I: Monte Carlo simulation details and Lennard-Jones (LJ) parameters. Note that the ionic radii are *effective* and appropriate for continuum model electrostatics [26].

Ions	Na^+	Cl^-	I^-	Residues
Radius/Å	1.8	1.7	2.0	2.8-4.1
$\epsilon_{\mathrm{LJ}}/kT$		0.075		
Proteins	Residues	Hydrophobic	Net charge	PDB
Lysozyme	129	45	$+9e$ (pH 4.7)	4LZT
Cell	Diameter	Temperature	Ensemble	l_B
	180 Å	298 K	NVT	7.1 Å

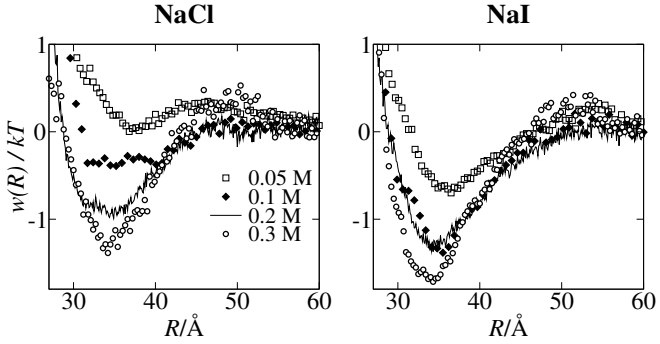

FIG. 3: Potential of mean force, $w(R)$, between two lysozyme molecules in various solutions of sodium chloride and sodium iodide, respectively. Obtained from Monte Carlo simulations using explicit salt particles and a mesoscopic description of the proteins [22] – R is the protein mass-center separation.

All Monte Carlo simulations were performed using the Faunus coding framework [25].

Our initial investigation considered the influence of salt concentration on the angular averaged free energy of interaction, $w(R)$, between two lysozyme molecules in sodium chloride and iodide solution, respectively. As shown in Fig. 3, the electrostatic repulsion between the proteins is effectively screened and becomes attractive with increasing salt concentrations. We note that in all cases iodide leads to more attractive protein interactions than chloride as is also observed experimentally [15, 16]. At intermediate salt concentrations, iodide has a much more dramatic effect, with a significant attractive well in $w(R)$ already apparent at 50 mM salt, far lower than predicted by the DLVO theory. The distribution of ions, cylindrically averaged along the line connecting the protein mass centers (Fig. 4) reveals that the iodide density in the mid-plane between the proteins is roughly twice that of chloride, for a 0.1 M salt solution. This is due to the fact that, through $V_{sp}(z)$, iodide is attracted to hydrophobic groups while chloride and sodium are repelled. Hence, in addition to neutralizing the protein charge, iodide may also act to bridge non-polar surface groups on the two proteins, leading to attractive protein-protein interactions.

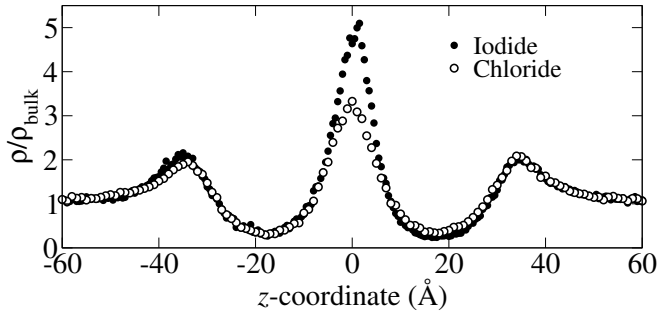

FIG. 4: Cylindrical average of the anion concentration along the axis, z, connecting the two (fixed) mass centers of the rotating proteins at a 35 Ångström separation. The cylinder radius is 16 Å – approximately the size of lysozyme – and the salt concentration is 0.1 M.

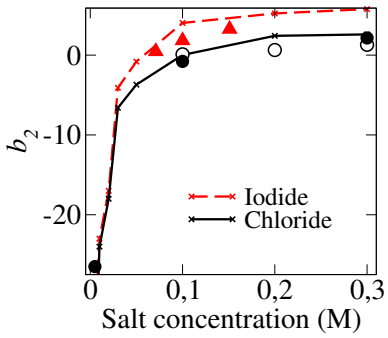

FIG. 5: Experimental [15, 27] (symbols) and simulated (lines) virial coefficients for lysozyme at pH 4.7 with chloride (full drawn/circles) and iodide (dashed/triangles) anions, respectively. The experimental data are – as the calculations – obtained at pH 4.7 and with potassium cations instead of sodium (this has no effect on the virial coefficients [15]). The closed circles represent sodium chloride at pH 4.5 [27]. Note that $b_2 = (B_2^{\rm hs} - B_2)/B_2^{\rm hs} = 1 - 3B_2/2\pi\sigma_{hs}^3$.

Using the potential of mean force, $w(R)$, we are able to calculate the protein-protein second virial coefficient, B_2, as detailed in the appendix. At very low salt concentrations the ion-specific effect is minimal since B_2 is dominated by the electrostatic repulsion between the two $+9e$ charged proteins – see Fig. 5. Under these conditions, the behavior is dominated by entropy of the mobile ions, and the protein-protein interaction is more like the DLVO potential. As the concentration is increased the difference between iodide and chloride becomes increasingly larger and we note that our calculations show good agreement with available data obtained from scattering experiments [15, 27]. Note that the calculated B_2 represents an explicit average over all microscopic salt positions, protein orientations as well as separations and we have made no effort to adjust input parameters to match the experimental data. Since $V_{sp}(z)$ is strictly only valid for infinitely dilute salt solutions our approach is expected to become less accurate at higher salt concentrations.

The present results provide one of the keys to the molecular understanding of Hofmeister ordering of ions. It has been suggested, that this ion ordering is due to a multitude of different effects rather than due to a single one [28, 29]. Indeed, in the present case we clearly observe at least two such effects. Namely, it is the direct interaction of aqueous anions with positively charged amino acid residues and the affinity of these anions for hydrophobic patches at protein surfaces. While the former interactions are stronger for chloride than for iodide, the opposite is true for the latter effect. The protein-protein interaction in a specific salt solution is then a result of a subtle balance between these (and also other [30]) forces. In the present case, the hydrophobic effect of iodide wins over the ion-pairing effect of chloride, which results in a stronger lysozyme-lysozyme association in aqueous NaI than NaCl.

APPENDIX: SECOND VIRIAL CALCULATION

The osmotic second virial coefficient is given by $B_2 = 2\pi[\sigma_{hs}^3/3 - \int_{\sigma_{hs}}^{\infty}(e^{-\beta w(R)} - 1)R^2 dR]$ where R is the inter-protein mass center separation and σ_{hs} is the "hard sphere" diameter here taken as the smallest observed mass center separation (∼26 Å). To avoid the increasing numerical noise when integrating at large protein separations we replace any long-ranged tail of $w(R)$ with a simple salt-screened Debye-Hückel potential, $\beta w_{\rm tail}(R) = l_B Z^2 e^{-\kappa R}/R$, where Z is the protein net-charge and κ is fitted to the simulated $w(R)$.

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[1] L. X. Dang, J. Phys. Chem B. **106**, 10388 (2002).
[2] P. Jungwirth and D. J. Tobias, Chem. Rev. **106**, 1259 (2006).
[3] D. Huang, C. Cottin-Bizonne, C. Ybert, and L. Bocquet, Phys. Rev. Lett. **98**, 177801 (2007).
[4] D. Horinek and R. R. Netz, Phys. Rev. Lett. **99** (2007).
[5] M. Lund, R. Vacha, and P. Jungwirth, Langmuir p. In press. (2008).
[6] D. F. Liu, G. Ma, L. M. Levering, and H. C. Allen, J. Phys. Chem. B **108**, 2252 (2004).
[7] E. A. Raymond and G. L. Richmond, J. Phys. Chem. B **108**, 5051 (2004).
[8] P. B. Petersen and R. J. Saykally, Chem. Phys. Lett. **397**, 51 (2004).
[9] R. Weber, B. Winter, P. M. Schmidt, W. Widdra, I. V. Hertel, M. Dittmar, and M. Faubel, J. Phys. Chem. B

108, 4729 (2004).
[10] S. Ghosal, J. C. Hemminger, H. Bluhm, B. S. Mun, E. D. Hebenstreit, G. Ketteler, D. F. Ogletree, F. G. Requejo, and M. Salmeron, Science **307**, 563 (2005).
[11] P. B. Petersen, J. C. Johnson, K. P. Knutsen, and R. J. Saykally, Chem. Phys. Lett. **397**, 46 (2004).
[12] R. A. Robinson and R. H. Stokes, *Electrolyte Solutions* (Butterworths Scientific Publications, London, 1959).
[13] S. Lindenbaum and G. E. Boyd, J. Phys. Chem. **68**, 911 (1964).
[14] J. Dzubiella and J.-P. Hansen, J. Chem. Phys. **121**, 5514 (2004).
[15] R. Piazza and M. Pierno, J. Phys.: Condens. Matter **12**, A443 (2000).
[16] M. M. Ries-Kautt and A. F. Ducruix, J. Biol. Chem. **264**, 745 (1989).
[17] F. Tavares, D. Bratko, and J. Prausnitz, Current Opinion in Colloid and Interface Science **9**, 81 (2004).
[18] M. Boström, F. W. Tavares, S. Finet, F. Skouri-Panet, A. Tardieu, and B. W. Ninham, Biophys Chem. **117**, 217 (2005).
[19] M. Boström, F. W. Tavares, D. Bratko, and B. W. Ninham, J. Phys. Chem. B **109**, 24489 (2005).
[20] M. V. Fedorov, J. M. Goodman, and S. Schumm, Phys. Chem. Chem. Phys. **9**, 5423 (2007).
[21] M. Ramanadham, L. C. Sieker, and L. H. Jensen, Acta Cryst. **B46**, 63 (1990).
[22] M. Lund and B. Jönsson, Biophys J **85**, 2940 (2003).
[23] R. A. Marcus, J. Phys. Chem. **58**, 621 (1954).
[24] N. A. Metropolis, A. W. Rosenbluth, M. N. Rosenbluth, A. Teller, and E. Teller, J. Chem. Phys. **21**, 1087 (1953).
[25] M. Lund, M. Trulsson, and B. Persson, Source Code Biol. Med. **3**, 1 (2008).
[26] Z. Abbas, E. Ahlberg, and S. Nordholm, Fluid Phase Equilibria **260**, 233 (2007).
[27] O. D. Velev, E. W. Kaler, and A. M. Lenhoff, Biophys. J. **75**, 2682 (1998).
[28] L. Vrbka, P. Jungwirth, P. Bauduin, D. Touraud, and W. Kunz, J Phys Chem B **110**, 7036 (2006).
[29] Y. Zhang and P. Cremer, Curr. Op. Chem. Biol. **10**, 658 (2006).
[30] A. S. Thomas and A. H. Elcock, J. Am. Chem. Soc. **129**, 14887 (2007), URL `http://dx.doi.org/10.1021\%2Fja073097z`.