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Citation for the published paper:

Friedman, R. Ions and the Protein Surface Revisited: Extensive Molecular Dynamics Simulations and Analysis of Protein Structures in Alkali-Chloride Solutions. *Journal of Physical Chemistry B*. 2011, 115 (29) 9213-9223.

Ions and the protein surface revisited: Extensive molecular dynamics simulations and analysis of protein structures in alkali-chloride solutions

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Abstract

Proteins interact with ions in various ways. The surface of proteins has an innate capability to bind ions, and is also influenced by the screening of the electrostatic potential owing to the presence of salts in the bulk solution. Alkali metal ions and chlorides interact with the protein surface, but such interactions are relatively weak and often transient. In this paper, computer simulations and analysis of protein structures are used to characterize the interactions between ions and the protein surface. The results show that the ion-binding properties of protein residues are highly variable. For example, alkali metal ions are more often associated with aspartate residues than with glutamates, whereas chlorides are most likely to be located near arginines. When comparing NaCl and KCl solutions, it was found that certain surface residues attract the anion more strongly in NaCl. This study demonstrates that protein-salt interactions should be accounted for in the planning and execution of experiments and simulations involving proteins, particularly if subtle structural details are sought after.

Keywords: Molecular dynamics; Protein surface; salt solutions; HET-S; S6 ribosomal protein; HIV protease; protein data bank

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Cl⁻ binding:
Arg > Lys > Ser ≈ His
NaCl > KCl

Na⁺ binding
Asp > Glu > Asn ≈ Ser

Introduction

The interest in interactions between salt ions and proteins dates back to the 19th century, when Hofmeister presented his accounts on the ability of some salts to precipitate protein better than others¹ (see ref.² for an English translation). Quantitative reports on the physicochemical properties of protein-containing salt solutions began to appear in the 1920s. Loeb set up experiments to study the effect of various salt on the rate of water diffusion through membranes,³ and found that the order was:

trivalent ions > divalent ions > Li^+ > Na^+ > K^+ > Rb^+

for cations and:

trivalent ions > divalent ions > I^- > Br^- > Cl^-

for anions. According to Loeb, smaller monovalent cations had a stronger influence on the rate of diffusion than larger ones, because the excess charge is located on the nucleus; whereas larger monovalent anions have a larger effect (compared to smaller ones) since the excess charge is a valence electron. Interestingly, Loeb noted that these finding could not explain the Hofmeister series. He therefore proposed that factors, other than the electrostatic properties of the solution must be involved in the interactions of ions and proteins.⁴ His successors used gelatine particles and electrostatic measurements, and showed that the concentrations of several cations (Ca^{2+} , Mg^{2+} , Zn^{2+} and K^+) inside and outside of the particles are not as expected from the Gibbs-Donnan equilibrium, concluding that the ions bind to proteins.⁵ The physical chemistry of protein solutions, and in particular interactions between serum albumin and anions, was deeply analyzed by Scatchard, who wrote a series of twelve papers on the subject during the 1940s, 1950s and 1960s. In one of the most interesting papers of this series, Scatchard and colleagues measure the binding of Cl^- to albumin and suggest that there are "... number of groups in an albumin molecule which combine with chloride ion ...".⁶ Carefully considering the experimental data available in the late 1950s, a thorough analysis of interactions between protein and ions was given by Scheraga in a review about protein structures.⁷ Scheraga noted (1) that hydrogen bonds between side-chains may affect ion binding (2) that the binding of ions should be analyzed with respect to electrostatic interactions

and hydrogen-bonding and (3) that the ions do not necessarily bind only to a single amino acid. It follows from his analysis that residues of the same type (e.g., aspartates) on the protein surface may differ in their ability to bind ions, due to hydrogen bonding or cooperative effects. Remarkably, such specific interactions are often overlooked even today.

While multivalent ions such as Fe^{3+} , Zn^{2+} , Mg^{2+} and SO_4^{2-} have a marked ability to bind proteins, the binding of halides and alkaline ions is weak and transient. However, understanding of such interactions has a tremendous importance in chemistry and biochemistry. Two pivotal examples were the activation and inhibition of pyruvate kinase by different alkali metal ions,⁸ and the binding of F^- to proteins, suggested to participate in its prophylactic action in dental caries.⁹ Some of the more recent studies concentrated on protein-salt interactions during amyloid aggregation,¹⁰ protein folding¹¹ and protein-protein recognition.¹²

Many efforts have been devoted to the adaptation of experimental methods to account for binding of ions by various proteins. Such methods include gel filtration,¹³ densitometry¹⁴ and NMR titration.^{12,15} Some data is also available from protein structures deposited in the protein data bank (PDB,¹⁶), but small ions are usually too mobile to be resolved in X-ray structures. Computer simulations, and in particular molecular dynamics (MD) have provided an atomistic view of ion-binding to proteins, see e.g.,^{17–20} Moreover, classical force-fields with fixed partial charges are constantly under ongoing development, with recent contributions dealing with calcium,²¹ alkali metal ions,^{22–25} halides,^{22,24} heavy metal ions²⁶ and lanthanide ions.²⁷

Over the recent years, several studies have aimed at an understanding of the specificity of salts to the protein surface. In a comprehensive study, Jungwirth and co-workers have used MD simulations, quantum chemistry and conductivity measurements to quantify the preference of the protein surface to Na^+ over K^+ . Their results showed that Na^+ ions bind better to the protein than K^+ , and that this is the result of interactions with carboxylates.²⁰ Supporting experimental information for this and other simulation studies was provided by K-shell x-ray absorption spectra (XAS) of cations and carboxylate anions (formate and acetate).²⁸ The XAS spectra were perturbed to a greater degree by smaller cations ($\text{Li}^+ > \text{Na}^+ > \text{K}^+$), in agreement with the early studies by

Loeb. Another XAS study showed a different order of the alkali ions, namely $\text{Na}^+ > \text{Li}^+ > \text{K}^+$.²⁹ In a later study, Dzubiella and colleagues have used a combined approach, involving liquid state theory, continuum electrostatics and MD simulations in order to explain ion specificity to interfaces (protein surfaces, air-water interface and hydrophobic surfaces).³⁰ The authors mentioned several aspects that contribute to ion specificity, namely ion hydration, pairing of oppositely charged ions, and interactions with groups on the protein surface. Netz and co-workers further studied surface-ion interactions, using idealized hydrophobic and hydrophilic surfaces, in order to provide a better understanding of the Hofmeister series.³¹ Analyzing the potential of mean force for surface-ion interactions they have shown that Na^+ ions, but not K^+ or halides, favorably interact with polar surfaces. It is not possible to separate hydrophobic and hydrophilic interactions directly at the protein surface though. An alternative approach, recently used in efforts to quantify the solubility of peptides by different sodium salts,³² is to study single amino acids and analyze their atomistic interactions with the ions.

The recent experimental and computational studies have undoubtedly led to a better understanding of protein-salt interactions at the protein-solvent interface. Several aspects, however, deserve a closer inspection. These include concentration effects and preference to specific residues over others (e.g., glu versus asp and gluXX versus aspYY where XX, YY are residue numbers). Both aspects have been analyzed to some extent in our earlier works.^{19,33} Here we analyze extensive MD simulations of the S6 ribosomal protein previously studied in the context of Na^+ and Cl^- binding at the surface.^{19,33} We use different concentrations of NaCl, KCl and LiCl solutions, namely 0.03M (ambient concentration), 0.12M (physiological concentration), and 1M (high concentration, only for NaCl and KCl). Furthermore, the distribution of ions at the protein structure is discussed for simulations of two other systems, HIV protease and an amyloid fibril. The distribution of ions at the surface of the three proteins is found to be highly non-homogeneous. Interestingly, the binding pattern of Cl^- is dependent on the conjugate cation. The patterns of ion-binding to proteins are compared to statistical analysis of protein structures from the PDB.

Methods

Molecular dynamics simulations

S6 ribosomal protein

MD simulations of the S6 ribosomal proteins were performed with the GROMACS program^{34,35} and the OPLS force field.³⁶ The X-ray structure of S6 (PDB code 1ris³⁷) was used as a starting structure. After the protonation of the protein structure (pH=7), the protein was immersed in a cubic box (d=6.5nm) containing extended simple point charge (SPC/E) model waters.³⁸ SPC/E was favored over other water models due to its dielectric constant and self diffusion coefficient which are in agreement with the experiment. Water molecules were removed from the box if the distance between any protein atom and any atom of the water was less than the sum of the van der Waals radii of both atoms. Ions were added randomly by replacing non-crystallographic water molecules to maintain salt concentrations of 0.03M, 0.12M and 1M. Cation parameters derived by Hess and van der Vegt specifically for simulations in the interface between water and proteins²³ have been used, together with the chloride parameters developed by Weerasinghe and Smith.³⁹ This combination of parameters has been developed specifically to model interactions between alkali metal ions and carboxyls. An additional set of simulations in LiCl was carried out with Li⁺ parameters derived by Åqvist.⁴⁰ Before each MD simulation, internal constraints were relaxed by energy minimization, until the maximal force on individual atoms was smaller than 100 kJ mol⁻¹ nm⁻¹. After the minimization, a constrained MD run was performed for 20 ps. During the constrained simulations, protein heavy atoms were fixed to their initial positions with a force constant of 1000 kJ mol⁻¹ nm⁻². The constraints were released, and the system was equilibrated for 5 ns before data collection for analysis. During the MD runs, the LINCS algorithm⁴¹ was used to constrain the lengths of bonds, while water molecules were kept rigid by use of the SETTLE algorithm.⁴² The time step for the simulations was 2 fs. The temperature was kept constant by use of the velocity-rescaling algorithm⁴³ ($\tau_T = 0.1$ ps). The pressure was coupled to an external bath with Berendsen's coupling algorithm⁴⁴ ($P_{ref} = 1$ bar, $\tau_P = 1$ ps) during equilibration and the

Parrinello-Rahman algorithm⁴⁵ during production ($P_{ref} = 1$ bar, $\tau_P = 1$ ps, compressibility = $4.5 \times 10^{-5} \text{ bar}^{-1}$). Van der Waals forces were truncated at 1.0nm with a plain cutoff. Coulomb interactions were calculated directly at distances shorter than 1 nm. Long-range electrostatic forces were treated using the particle mesh Ewald method.⁴⁶ Two simulations of 95ns each were carried out at each concentration. The results (e.g., binding of ions to individual amino acids) are similar for the two individual runs each pair of simulations, indicating sufficient sampling.

HIV protease and HET-s fibril

MD simulations of the HIV protease and HET-s fibril are mentioned here for comparison, but were not run predominantly to study ion-protein interactions, and different parameters and simulation set-ups were used.

HIV protease The simulations of HIV-protease were reported before.⁴⁷ Briefly, the system was prepared as above with similar parameters, differences with respect to the S6 protein simulations are stated below. The water model was TIP4P.⁴⁸ Cation parameters derived by Åqvist⁴⁰ were used for Na^+ . Parameters for Cl^- were taken from ref.⁴⁹ The salt concentration was 0.1M. The length of the restrained and equilibrium simulations were 100ps and 1ns respectively. Hydrogens were converted into virtual sites, allowing time-step of 5 fs⁵⁰ (4 fs was used in practice). One of the catalytic residues was protonated, and the system was explored for 200ns (two individual runs of 100ns each). Simulations of HIV protease were also carried out using the same ion parameter set as described for the ribosomal protein S6 in the presence of 0.1M and 1M NaCl (two 100 ns long simulations in each case). These simulations were analyzed with respect to the ability of specific residues to bind the Na^+ ions (see Results and Discussion).

HET-s fibril The fibril of HET-s pentamers (residues 218-289 and five c-terminal his-tag residues) was taken from the solid-state NMR structure.⁵¹ The NMR ensemble contains 20 structures, of which the third was used since it was estimated to be the most stable in water based on solvation-

energy calculation. The solvation energy was calculated by use of the computer-program APBS,⁵² using grid spacing of 0.045 nm and ionic strength of 0.1M. The trimer with chains B, C and D was extracted for MD simulations to improve sampling. The system was prepared as detailed above. The force field parameters were similar to those used for the HIV-protease simulations. The length of the restrained and equilibrium simulations were 100ps and 1ns respectively and the system was then explored for 100ns. The integration time step was 5 fs.⁵⁰ The structural core of the fibril was stable under this conditions, with RMSD<0.25nm for the C α atoms.

NaCl and KCl solutions Simulations of NaCl and KCl solutions were carried out using the same parameters as with S6, in a box containing 1666 water molecules and 62 ions. The set-up for the simulations included energy minimization, equilibration (1 ns, pressure coupling with Berendsen's barostat), and production (5 ns, pressure coupling with the Parrinello-Rahman barostat). The temperature was kept constant at 300K using the velocity-rescaling algorithm.

Analysis of alkali metal ions and halides in the protein data bank

Distributions of contacts between ions and protein side chains were extracted using PDBeMotif:⁵³ <http://www.ebi.ac.uk/pdbe-site/pdbemotif/> and were processed by home-written software. PDBeMotif is a search tool for the PDB that allows searches based on sequence, chemical structures or three dimensional data. Here it has been used to examine residues near alkali metal ions and halides, using the “pair bonds” option. Interactions between ions and backbone atoms were not considered, because such interactions may stem from influence of heteroatoms (non-protein ligands) or specific structures, as in ion channels⁵⁴ that are not representative of general protein folds.

Results and Discussion

In summarizing the results, we start with analysis of simulations with the S6 ribosomal protein, detailed in Table 1.

Overall structural and dynamic features

The S6 ribosomal protein is stable under the various simulation conditions, as shown in Table 1. No gross differences can be attributed to the type or concentration of added ions.

Table 1: Overall structural properties of the simulated protein. The maximal root mean square deviation (RMSD), average radius of gyration (r_{gyr}), solvent accessible surface area (sasa), and number of residues with a regular secondary structure (α -helix, β -sheet, β -bridge, turn) is shown for the simulations of S6 with different concentrations of ions. ^a Li^+ parameters from.²³ ^b Li^+ parameters from.⁴⁰

Cation	Conc.[M]	Max. RMSD [nm]	r_{gyr} [nm]	sasa [nm ²]	Number of structural residues
Na^+	0.03	0.24	1.36(01)	94.2 ± 14.5	78 ± 4
	0.12	0.27	1.37(01)	96.4 ± 13.2	78 ± 4
	1	0.21	1.36(01)	102.4 ± 15.5	78 ± 4
K^+	0.03	0.31	1.37(01)	92.9 ± 10.0	78 ± 4
	0.12	0.25	1.36(02)	102.9 ± 17.1	79 ± 4
	1	0.30	1.36(01)	90.7 ± 11.5	79 ± 4
Li^+ ^a	0.03	0.27	1.37(01)	87.0 ± 9.6	78 ± 4
	0.12	0.21	1.36(01)	96.0 ± 11.3	78 ± 5
^b	0.03	0.20	1.36(01)	94.7 ± 12.1	78 ± 5
	0.12	0.20	1.36(01)	107.3 ± 17.3	79 ± 4

Interactions of the ions with the solvent

The hydration numbers (N_w) and diffusion coefficients (D) of the ions are presented in Table 2 and compared with *ab initio* calculations and experimental measurements. A direct comparison between N_w values calculated by force-field based and *ab initio* simulations cannot be made, since

the ab initio calculations were performed with smaller systems and at higher temperatures (320K or 344K). The hydration number of sodium is smaller than that of potassium, because the sodium ion is smaller, allowing more water molecules to be present at the first hydration shell of K^+ (note that we use the atomistic definition of hydration number, i.e., the number of water molecules present at the ion's first hydration shell; the dynamic hydration number, which refers to the number of water molecules which diffuse together with the ion, behaves differently and is larger for Na^+). The similarity in K^+ and Cl^- hydration numbers in the MD simulations reported here is in better agreement (compared to the difference in the ab-initio results) with the higher tendency of K^+ to form contact pairs with Cl^- ,²³ because ions that match in size tend to form stronger contacts in water⁵⁵ (see also the theory of matching ion affinities⁵⁶). The diffusion coefficients of the ions in the simulations are slower than experimental values, most probably due to the choice of water model. Higher diffusion coefficients were recorded in SPC,¹⁹ but the diffusion coefficient of the water ($D=2.5 \pm 0.02 \cdot 10^{-5} \text{ cm}^2/\text{s}$) agrees better with the experimental value ($D \sim 2.4 \cdot 10^{-5} \text{ cm}^2/\text{s}$) using the SPC/E model. Overall, the transport properties of the ions are in agreement with experimental results or quantum-mechanical calculations. The deviations are not expected to change the qualitative picture of ion binding to proteins as presented here.

Results for interactions with Li^+ were calculated using two different sets of parameters. The first was developed by Hess and van der Vegt,²³ in conjugation with the Na^+ and K^+ parameters used above. This set of parameters aimed at studying protein-ion interactions. The second set was developed by Åqvist to reproduce the hydration free energies of cations.⁴⁰ The hydration number and diffusion coefficient of Li^+ are slightly larger when the Hess and van der Vegt parameters are used, and are smaller when compared to Na^+ and K^+ (which is in agreement with higher level calculations or experiments). However, the sets differ in the protein-binding properties (*vide infra*).

Gross interactions between the protein and the ions

The probability of finding an ion at a certain distance from the protein can be characterized by the radial distribution function (RDF, Figure 1). Na^+ , K^+ and Cl^- ions are preferentially located

Table 2: Structural and dynamic properties of ions in solution calculated from classical (force-field based) or ab-initio MD simulations and experimental data.

	Diffusion coefficient [10 ⁻⁵ cm ² /sec]			Hydration number ^a		r ₁ (max) [nm] ^b ion-protein	
	MD ^c	AI ^d	experiment ^e	MD ^c	AI ^d	MD ^b	PDB ^b
Na ⁺	0.88(1)	1.0	1.23-1.25	5.4	4.6	0.24	0.24
K ⁺	1.75(21)	-	2.03 ^f	7.4	6.5	0.30	0.28
Li ⁺ ^g	0.78(6)	-	1.02	4.0	4	no peak	0.21
^h	0.59(1)			3.5		0.21	
Cl ⁻	1.16(1) ⁱ	-	1.77 ^g	7.4	5	0.32	0.28
	1.27(6) ^j	-	1.96 ^h				

^a - The number of water molecules in the first hydration shell of the ion

^b - First maximum of the radial distribution function (calculated from the simulation) or distribution of minimal distances (calculated from the data in the PDB), see Figure 1.

^c - this work

^d - Ab initio MD, refs. ⁷⁶⁻⁷⁹

^e - Refs. ⁸⁰⁻⁸²

^f - obtained in KI

^g - Li⁺ parameters from ref. ²³

^h - Li⁺ parameters from ref. ⁴⁰

ⁱ - obtained in NaCl.

^j - obtained in KCl.

in the bulk, due to favorable entropy when not bound to the protein surface.¹⁹ However, there is some tendency of the ions to adsorb to the protein surface or to be located at the second hydration shell (i.e., be separated from the protein surface by a single water molecule). RDFs for Na⁺, K⁺ and Cl⁻ ions have maxima at 0.24, 0.30 and 0.32nm, respectively under the simulation conditions. The probability to find an ion adsorbed to the protein surface decreases with increasing concentration, due to shielding of the electrostatic interactions by the excess salt. Na⁺ ions have a higher probability to be located at the protein surface compared to K⁺ ions. Interestingly, Cl⁻ ions have a slightly higher probability to be adsorbed to the protein surface when the adjunct cation is Na⁺, especially when the ionic strength is low. This may be explained by the slightly stronger interaction between K⁺ and Cl⁻ in aqueous solutions as observed also by measurements of the activity coefficients (see e.g.,⁵⁷ and Figure 2).

When Li⁺ ions are simulated with the parameters developed by Hess and van der Vegt (as

Figure 1: Distance distribution functions of the cations (left) and anions (right) relative to the protein. (Top) RDFs calculated from the simulations. (Bottoms) Distribution of contact distances calculated for all structures in the PDB.

reported above for Na^+ and K^+), they hardly interact with the protein, as observed by the lack of structure in the RDF (Figure 3). For this reason, the simulations were also run with the parameters developed by Åqvist. In this case, Li^+ ions show marked binding to the protein, having an RDF maximum at $r=0.21\text{nm}$ (in agreement with the distribution of Li^+ ions around proteins in the PDB). Moreover, the ions have a higher probability to be located in a volume element near the protein surface than at the bulk, due to their favorable interaction with carboxyls. Experimentally, it has been shown that the interaction of Li^+ with acetates is slightly favorable ($\log K_d=-0.26$ to -0.29 , compared with $+0.11$ to $+0.18$ for Na^+ , and $+0.27$ for K^+ ^{58,59}).

There is an inherent limitation in the radial normalization of the distribution function near the protein surface.⁶⁰ Yet and , the RDF capture the interactions between ions and protein backbone and side-chains and have been used to characterize the attraction between ions and the protein surface.^{18,30,61–63} To make sure that the attraction of ions to the protein surface is not a geometrical

Figure 2: RDF of for Na^+ (black) and K^+ (blue) with respect to Cl^- . The K-Cl interactions are somewhat stronger as seen by the height of the first RDF peak.

Figure 3: Radial distribution function of Li^+ ions around the protein, calculated with two sets of parameters.

artefact, S6 was simulated in the presence of 1M NaCl, where the ions were devoid of the attractive part of the Lennard-Jones interaction potential (ion-protein $\text{C6}=0$). The protein-ion RDF have no peaks in these simulations (Figure 4).

Analysis of the X-ray coordinates of alkali metal ions and halides bound to proteins was carried out by using the PDBeMotif search tool⁵³ (Figure 5). Alkali metal ions bind preferentially to side chains of glu and asp residues, and less often to polar residues such as ser, asn or thr. Halides bind predominantly to the guanido moiety of the arg side chain. Analysis of the minimal distance between the ions and corresponding protein side chains (carboxyl for alkali metal ions, amine or guanido for halides), reveals preference to ion-protein distances that are similar to those obtained from the calculated RDFs (see Figure 1 and Table 2).

Figure 4: Radial distribution function of Na^+ and Cl^- ions around the protein, calculated from a simulation ($C=0.12\text{M}$) where the attractive part of the Lennard-Jones potential was nullified.

Specific interactions between protein surface residues and ions

The distribution of the ions on the protein surface is far from being homogeneous. Oppositely charged amino acids and ions tend to attract, but even these electrostatic effects are quite specific, with some residues much more attractive to the ions than others. The RDFs of Na^+ and K^+ with respect to negatively charged amino acids and the N-terminus (calculated in physiological salt concentration) are given in Figure 6. Interestingly, some residues (glu22, asp55, glu69) have no ability at all to attract oppositely charged ions, as indicated by the lack of a maximum in the RDF up to 1 nm. Other charged residues do attract oppositely charged ions, but not to the same degree. Na^+ ions adsorb to the protein surface more than K^+ ions. The RDFs typically have a bi-modal structure, with the first peak being dominant if its value is greater than two, indicating a preference towards a *closed sphere complex* (also called *inner sphere complex*), i.e. an ion which is bound to the surface directly, with no intervening water molecules. Differences between the ions, and between the heights of the first and second peaks are less evident when the concentration is higher ($C=1\text{M}$, Figure 7). Results for simulations with Li^+ are not reported, because Li^+ bind strongly to almost all carboxylates when simulated with the Åqvist parameters, whereas very weak binding is observed with the parameters developed by Hess and van der Vegt.

An uneven distribution of the ions on the protein surface is also evident for the Cl^- anion

Figure 5: Distribution of the binding of ions to side chain in protein X-ray structures. Cations are most often associated with asp, followed by glu. Cl^- and I^- ions bind most frequently to arg residues, but the halides are larger and more hydrophobic than alkali metal ions and have a lower affinity towards the charged side chains. The number of structures from which this distribution is calculated is 1660 (762 different proteins) for Na^+ , 453 (225) for K^+ , 27 (17) for Li^+ , 28 (11) for Rb^+ , 48 (23) for Cs^+ , 6980 (1835) for Cl^- , 947 (120) for Br^- and 1321 (163) for I^- .

(Figure 8). Four positive residues are virtually unable to attract chlorides at physiological concentrations, whereas chlorides are about 10 times more likely to be found at a volume element near arg54, arg87 and lys92 than in the bulk. Interestingly, the tendency of chloride ions to be located near some surface residues is influenced by the adjunct cation. For example, the N-terminus attracts Cl^- anions more strongly in KCl solution, whereas the opposite is true for arg77. These features are evident, though less pronounced, when the salt concentration is 1M (not shown).

The reason for the difference between residues in their ability to attract oppositely charged ions lies in their immediate environment. Under low ionic strength, the electrostatic potential that surrounds the protein is able to detain ions at the vicinity of attractor residues and channel them between the residues.³³ This potential, however, is much weaker under physiological or higher ionic strength,⁶⁴ indicating that geometrical features must also play a role. Here, two of the three strongest Na^+ attractors, asp74 and glu78 are able to share contact with a single ion: the same sodium ion is effectively shared between asp74 and glu78 in about 57% of the simulation time during which an ion is bound to one of them. Binding to a pair of residues is even more pronounced for Cl^- ions for the pairs arg2/lys92 and arg71/arg77 (in the simulations with NaCl, Figure 9). In fact, in 95% of the time when arg71 binds a Cl^- ion, the ion is shared with arg77. Interestingly, sharing of Cl^- ions between two residues is much rarer for chlorides in KCl solutions. On the

Figure 6: Radial distributions of sodium (black) and potassium (blue) ions relative to O δ of glutamate or O γ of aspartate residues at physiological concentration. Note that the y-axis range is different.

Figure 7: Radial distribution functions of the anions and cations relative to residues glu41 and glu78 at physiological (0.12M) and standard (1M) ionic concentrations.

other hand, K^+ ions are able to bind to pairs of residues, predominantly asp74/glu78 as Na^+ . The distance between residues arg2 and lys92 is smaller when the concentration of Cl^- ions is higher in solution (Table 3). For asp74/glu78, this is not the case for the 1M solution, because the residues are able to bind two sodium ions (one per carboxyl). In other words, the distance between the pair of carboxylates is long when few ions are present in solution, due to electrostatic repulsion. It becomes smaller if the two residues bind to a single ion (at physiological concentration). At higher concentration (1M) the two carboxyls bind two Na^+ ions and the distance between them grows again. These examples show that although the structure of the protein is maintained regardless of the ionic concentration, distances between like-charged residues depend on their ability to bind oppositely charged ions.

Table 3: Distance between pairs of like-charged residues in simulations of the ribosomal protein S6 in difference concentrations of NaCl. The distances are calculated between asp C γ and glu C δ or arg N η and lys N ζ . Arg-lys distance is calculated as $\min(d_{argN\eta1 - lysN\zeta}, d_{argN\eta2 - lysN\zeta})$. Statistical analysis reveals that the difference between the mean distances is significant in all cases ($p < 0.001$).

C[M]	$d_{arg2-lys92}$	$d_{asp74-glu78}$
0.03	0.78 ± 0.09	0.73 ± 0.02
0.12	0.76 ± 0.06	0.65 ± 0.04
1	0.70 ± 0.04	0.74 ± 0.01

Ions at the surface of enzymes and amyloid fibrils

The S6 protein is part of the bacterial ribosome and does not possess any enzymatic activity. We have previously carried out extensive simulations of enzymes, predominantly aspartic proteases.^{47,65} Here, we analyze these simulations with respect to the distribution of ions near oppositely charged residues in HIV protease. In addition, the distribution of ions was also studied around a trimer of HET-s, forming a fibril. The results show that the heterogeneity in the ability of similar residues to attract ions is not unique to the ribosomal protein S6.

HIV protease

The HIV-protease dimer is positively charged (+7) under physiological conditions. As a result, only two residues can significantly attract cations, glu21 and glu34 (Figure 10). The protein attracts anions much better, with 6 of 11 positive residues of each monomer having an ability to significantly attract ions (first maximum in the rdf > 5). Yet, the average density of Cl⁻ ions is higher in the bulk, despite the overall charge of the protein. Interestingly, no Na⁺ ions were associated with the catalytic residues in the simulations. Note, however, that although many of the known inhibitors of HIV-protease are positively charged, their binding to the catalytic site is mainly due to favorable vdW interactions.⁶⁶

Figure 8: Radial distributions of Cl^- relative to $\text{N}\epsilon$ (arginine), $\text{N}\zeta$ (lysine) or amino terminus nitrogen. The salt used in the MD runs was NaCl (black) or KCl (blue).

Figure 9: Ions bound by pairs of residues at the surface of the S6 ribosomal proteins. A sodium ion (silver) is displayed as it is bound simultaneously to residues asp74 and glu78 while a chloride ion (green) is bound to arg2 and lys92. The binding of ions by multiple residues modifies the distance between the pairs of residues, as a function of salt concentration.

In a recent paper,⁶⁷ Jungwirth, Vondrášek and colleagues studied the catalytic activity of HIV-protease under different concentrations of NaCl and KCl. The catalysis of cleavage of a positively charged peptide substrate was faster (increased k_{cat} and V_0) with increasing concentrations of the salt, and this effect was more pronounced at KCl. MD simulations of the protein in the presence of 0.25M NaCl, 0.25M KCl or a mixed solution of 0.25M NaCl and 0.25M KCl were also reported in the same publication, and revealed that Na^+ ions have a marked ability to bind to the protein (and some ability to bind to the catalytic residues). Here, we found no evidence of binding of sodium ions at the catalytic aspartates under physiological conditions (and for a mono-protonated catalytic site), and the average number of ions at the protein surface was <1 during the simulations. This suggests that asp and glu residues, located at the protein surface and not directly at the active

site may be involved in attracting the substrate and then steering it towards the active site. Our simulations were rerun with an additional set of ion-parameters as used also for the simulation of S6 and in ref.²³ but the results were very similar and no contacts have been observed between any of the Na⁺ ions and the catalytic residues. Although this does not rule out that differences with respect to the previously reported HIV-protease simulations are due to the force-field parameters of the ions, it is more likely that the variations should be attributed to the concentration of the ions. The difference in the PDB structures used in the simulations (2PC0 in⁴⁷ and here, 3HVP in⁶⁷) may also play a role. To verify that the difference between our results and those of Jungwirth, Vondrášek and colleagues can be at least partially attributed to the ion concentration, a third set of simulations has been run with HIV in 1M NaCl solution. Binding of Na⁺ to the catalytic residues has been observed in this case, pointing out to the importance of concentration effects, as discussed above for the ribosomal protein S6.

Figure 10: A cartoon model of HIV-protease, showing anion attractors in blue, other lysine and arginine residues in cyan, cation attractors in red and other asp and glu residues in pink. The catalytic residues, which do not bind to the ions, are shown in a stick representation, colored by atom type (carbon in cyan, oxygen in red). The protein is viewed from the front (left) and top (right).

HET-s amyloid fibril

The structure of an amyloid fibril from the prion-forming domain (residues 218 to 289) of the HET-s protein from the filamentous fungus *Podospora anserina* has been solved by solid-state NMR.⁵¹ The structure is a pentamer of peptides identical in sequence. The NMR study reveals several salt bridges along the ribbon of the fibril. Ten positive and nine negative amino acids in each

monomer make the fibril positively charged in physiological pH. Here, a substructure made of three monomers is studied by MD simulations. The distribution of residues that bind Na^+ and Cl^- ions on the fibril's surface is shown in Figure 11. Interestingly, the distribution of ions is nonhomogeneous, with some regions along the surface (e.g., c-termini of adjacent units) having a higher density of ions around them. Most of these residues are located along the structural core of the fibril. However, positive residues that are part of the more flexible loops are more exposed to the solvent and have a marked ability to attract chlorides (see e.g., the lower left of Figure 11).

Amorphous aggregation is influenced by the presence of high concentrations of salt due to the Hofmeister effect. Interactions with NaCl have already been shown to influence the aggregation and fibrillation rates of human serum albumin, even at ambient concentration (<50mM).¹⁰ It remains to verify whether such interactions also take part in the aggregation of other proteins, and how. Formation of local structures such as observed by two residues binding the same ion (Figure 9) may drive or prevent aggregation of specific proteins. The S6 protein itself undergoes significant structural changes and starts to aggregate in the presence of sub-micellar concentrations of sodium dodecyl sulfate (SDS), where it is estimated that 12 SDS molecules bind to each S6 monomer⁶⁸ indicating a possible role for electrostatic interactions with surface residues.

Figure 11: Stereoview of a fibril made by three Het-S monomers in cartoon representation, with anion attractors in blue, other lysine and arginine residues in cyan, cation attractors in red and other asp and glu residues in pink. Both ions are attracted to the structural core of the peptide while there is a preference to chloride ions on the loops.

Conclusions

The distribution of alkali metal ions and halides on the surface of proteins was studied by analysis of multiple MD simulations and protein structures. It has been shown that the binding of alkali metal ions was different between glu and asp residues in the PDB (Figure 1) and even between similar residues of the same protein. Halides were found to bind to arg side chains more often than to lys (note that glu is more prevalent than asp and lys than arg⁶⁹). The binding of ions to the protein surface did not change the overall structure of the protein (Table 1) but could make the distances between side chains of like-charged residues smaller due to binding of multiple residues to a single ion (Table 3, Figure 9). Interestingly, binding of chlorides to protein residues was influenced by the adjunct cation. This can be explained by the ability of Na⁺ ions to bind better to the protein surface than K⁺, modifying the electrostatic field which surrounds the protein.

The strength of specific interactions of ions with the protein surface depends on the concentration of the salt. The first peak in the RDF of ions relative to the protein surface becomes smaller with increasing concentration and the same is true for interactions with specific residues on the protein surface. However, binding of the ions to residues at the protein surface at a physiological salt concentration may be important for catalysis and protein-protein recognition, not to mention the activity of ion channels. The concentration and nature of the salt should therefore be carefully considered in experiments and simulations involving proteins.

Addendum: ion force field parameters

Different ion force-field parameters suffer from various limitations. Some examples include formation of salt clusters at high concentrations,^{70,71} incorrect number of ions in the hydration shell of macromolecules⁷¹ and insufficient description of the coordination number when compared with *ab initio* calculations.⁷² Recent studies on ion force-field development reveal the importance of several factors, namely (1) correct modeling of the ion-solvent interactions⁷³ (2) ion-pair solvation properties,^{22,24} and (3) the balance between solution and interface behavior.^{21,23} On the other

hand, this study, as well as comparative studies of various force fields^{74,75} show that the behavior of macromolecules at physiological concentrations can be modeled correctly by different parameters (this, however, does not pertain for highly concentrated solutions i.e., above 1M).

This study discusses analysis of several independent MD simulation trajectories. Simulations made for the sake of analysis of protein-ion interactions alone were conducted with the parameters used by Hess and van der Vegt, because these were tested specifically for dealing with binding of ions to the protein surface. In other studies and for the description of Li^+ , we have used the cation parameters by Åqvist and the chloride parameters of the OPLS-AA force-field.⁴⁹ The ability of certain residues to attract oppositely charged ions better than others is shown in all studies, and the agreement between the parameters used here and other calculations or experiments is shown in Table 2.

Acknowledgement

The author would like to thank both referees for their comments on the manuscript and Prof. Amedeo Caflisch for interesting discussions. This work was supported by a research grant from the Faculty of Sciences and Engineering, Linnæus University, Sweden, for the project “Metal ions in life”. Computer calculations were carried out on resources that belong to The Lund University Center for Scientific and Technical Computing (LUNARC) project SNIC 001/10-186.

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