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On the Hofmeister Effect: Fluctuations at the Protein–Water Interface and the Surface Tension

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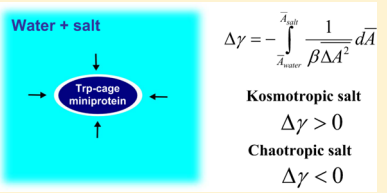
Supporting Information

ABSTRACT: We performed molecular dynamics simulations on the tryptophane-cage miniprotein using a nonpolarizable force field, in order to model the effect of concentrated water solutions of neutral salts on protein conformation, which is a manifestation of Hofmeister effects. From the equilibrium values and the fluctuations of the solvent accessible surface area of the miniprotein, the salt-induced changes of the mean value of protein–water interfacial tension were determined. At 300 K, the chaotropic ClO_4^- and NO_3^- decreased the interfacial tension according to their position in the Hofmeister series (by approximately 5 and 2.7 mN/m, respectively), while the kosmotropic F^- increased it (by 1 mN/m). These values were compared to those obtained from the Gibbs equation using the excess surface adsorption calculated from the probability distribution of the water molecules and ions around the miniprotein, and the two sets were found to be very close to each other. Our results present a direct evidence for the central role of interfacial tension and fluctuations at the protein–water interface in Hofmeister phenomena, and provide a computational method for the determination of the protein–water interfacial tension, establishing a link between the phenomenological and microscopic description of protein–water interfaces.



INTRODUCTION

Proteins perform their sophisticated function in watery environments. Water is a medium that provides proteins with stability and flexibility at the same time,[1,2] both inevitably necessary for their optimal performance.[3] It has been experimentally demonstrated that dominant conformational motions of proteins are "slaved" by the hydration shell and the bulk solvent;[4,5] consequently, structural changes of ambient water should inevitably affect protein structure and function as well. Neutral salts are ideally suited to investigate such effects, since they are known to alter the dynamical, H-bonded matrix structure of water molecules. Salts that increase H-bond strength are called water structure makers or "kosmotropes", while those that weaken H-bonds are called water structure breakers or "chaotropes".[6] As expected from the above arguments, neutral salts do have pronounced effects on protein conformation and aggregation, that are termed Hofmeister effects (HE) after their first systematic investigator, Franz Hofmeister.[7] As early as 1888, he established that the effects are dominated by anions rather than cations, and ordered the anions according to their ability to precipitate globular proteins from water: SO_4^- > F^- > CH_3COO^- > Cl^- > Br^- > I^- > NO_3^- > ClO_4^-. Kosmotropes standing on the left-hand side increase aggregation ("salting out"), as opposed to chaotropes, on the right-hand side, that increase solubility ("salting in"). The same series was found for protein conformation and activity, too: normally, kosmotropes stabilize conformation and increase activity, whereas chaotropes destabilize conformation and decrease activity.[6,8]

The Hofmeister or specific ion effects are not restricted to the proteins; they also appear in the case of polysaccharides, nucleic acids, and phospholipids that constitute the building blocks for biomembranes and self-assembled structures and whose functionalities regulate the vital processes of living organisms. Recently, Lo Nostro et al. published an extended overview of several biological aspects of HE.[9]

Despite their widespread occurrence and the extensive research efforts focused on them, all attempts aiming to assign a single physical parameter as an approximate measure of HE have failed for more than a century.

In a recent work, we have demonstrated that a unified, phenomenological formalism based on solute–water interfacial tension is able to qualitatively account for the entire spectrum of HE.[10] The most important conclusion from the theory is that the addition of kosmotropic or chaotropic salts to the solvent is expected to increase or decrease solute–water interfacial tension, respectively, giving rise to a corresponding change of ΔG. It was also suggested by Neagu and Der[11] that

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the relation between interfacial tension and protein structural stability is linked to protein conformational fluctuations, providing a keystone for the microscopic interpretation of HE. Despite the success of the theory in describing the diversity of the Hofmeister phenomena, a direct evidence for salt-induced changes of protein−water interfacial tension correlated with the HE has still been missing, due to technical difficulties.

In the framework of the present study, we addressed this issue with a molecular dynamics (MD) approach applied to a model protein. First, we point out that Hofmeister-active salts modify conformational fluctuations of the protein, which is accompanied by fluctuations of the solvent accessible surface area (SASA), too. We show that, from the SASA and its fluctuations, the salt-induced alterations of the protein−water interfacial tension can be calculated and the results are in accordance with the qualitative predictions of the theory in ref 10. To support the validity of the results obtained from our simple model, we compare to those obtained using the Gibbs' equation. This method was recently formulated by Chen and Smith[12] and applied to the air−water interface[13] and peptide chains by Horinek et al.[14] In order to reveal the possible microphysical reasons behind the salt-induced changes of the interfacial tension, in another set of simulations, we also determine the ion distribution and the reorientation dynamics of water molecules in various regions of the protein−water interface.

To this end, MD simulations were performed on the tryptophane-cage (Trp-cage) miniprotein (it is also called TC5B), using explicit water molecules to model the surroundings of the protein. Trp-cage is a 20-residue-long peptide with the sequence NLYIQ WLKDG GPSSG RPPPS, which is often called miniprotein, since it is one of the smallest polypeptides that possesses a well-defined secondary structure.[15] The three-dimensional structural features of this peptide are well characterized by several experimental methods (e.g., the NMR structure is downloadable from Protein Data Bank: 1L2Y) and molecular modeling techniques.[16−19] The most important structure-stabilizing factors of Trp-cage are the hydrophobic stacking of the aromatic rings of Tyr3 and Trp6 amino acids and the salt bridge formed between the Asp9 and Arg16 residues. Both of them are expected to be influenced by structural changes of water induced by Hofmeister salts. Its small size, temperature-sensitive structure, and the simultaneous appearance of two important stabilizing interactions make this system an ideal protein model for MD studies. Garcia and his co-workers carried out a microsecond-long replica exchange MD (REMD) calculation to study the folding/unfolding thermodynamics of Trp-cage miniprotein,[20] and also investigated the influence of the application of two popular water models (TIP3P and TIP4P-EW) on the stability of this miniprotein.[21] The detailed molecular mechanism of the urea and guanidinium induced denaturation process of the miniprotein was unravelled by Heyda et al.[22] using experimental techniques as well as large scale MD simulations. Applying extended REMD simulations, Canchi et al.[23] investigated also the urea induced denaturation of Trp-cage miniprotein focusing on the thermodynamical aspects of the process.

As it is claimed in several studies, the accurate calculation of physical quantities related to ion solvation require polarizable force fields (see, e.g., papers by Carignano et al.[24] and Caleman et al.[25]). At the same time, Kalcher et al.[26] could derive halide ion (Cl, Br, I) induced surface tension changes at the water−vacuum interface from MD simulations using nonpolarizable force fields. The values they obtained followed the experimental trend, namely, the larger the anion the smaller the surface tension increment. Sun et al.[27] also used nonpolarizable force fields for the ions and water molecules (semiflexible SPC/E) in their simulations and calculated the surface tension alterations induced by sodium halides at the vacuum−water interface. Their results were in good accordance with the experimental data obtaining 3.1, 2.6, and 1.5 mN/m increase of the surface tension in the presence of NaF, NaCl, and NaI in 1.2 M concentration in the solution.

During the last years, it was also pointed out in a series of papers by Dzubiella and his co-workers that the effects of Hofmeister-active alkali halide salts (in high concentration) on the stability of simple salt bridge forming[26,28] and charged[29] alanine-based peptides with high helical propensity can be simulated using nonpolarizable force fields. Asciutto et al.[30] pointed out that the sodium perchlorate in 0.2 M concentration stabilizes the helical structure of $AAAAA(AAARA)_3A$ peptide.

Utilizing their ideas, we carried out similar calculations on the more protein-like Trp-cage peptide. Four anions were selected with different Hofmeister activity (F^-, Cl^-, NO_3^-, and ClO_4^-) for our calculations in the form of their Na salts. We used nonpolarizable force fields, as is described in detail in the section Methods.

METHODS

Molecular Dynamics. Two types of simulations were performed on our test system using the GROMACS[31] molecular dynamics package. In both types of calculations, we carried out simulations in pure water and in water with the selected Hofmeister salts, each of 1 M final concentration.

In one type of calculations, the peptide geometry was fixed (using a harmonic restraint with a force constant of 10^3 kJ mol^{-1} nm^{-2}) at the middle of a cubic box with a side length of 4.18 nm containing around 2200 water molecules. As a first step, an energy minimization of the solvent molecules and the ions was applied using 10 000 steps of the steepest descent method. Starting from the final structure, 100 ns long NPT MD simulations were performed at 300 K using a stochastic dynamics integrator with a time step of 1 fs. The temperature was controlled using velocity rescaling with a stochastic term,[32] and the pressure was regulated with the Parrinello−Rahman[33] method. Covalent bonds involving hydrogen atoms were constrained with the LINCS algorithm.[34]

In the other type of calculations, we used 600 ns long replica exchange MD (REMD) simulations in pure water and in water with the selected Hofmeister salts at 32 temperatures between 300 and 450 K for the investigated systems. The relevant MD parameters were identical to the first set of simulations. Replica exchanges were attempted every 2000 integration steps (2 ps). Only the last 300 ns of the simulations and the conformational ensembles obtained for temperatures between 300 and 360 K were used to calculate the physical properties presented here.

The Amber ff99SB-ILDN[35] force field and the TIP3P[36] water model were used in our MD simulations. For the Na^+, F^-, and Cl^- ions, the parametrization of Joung et al.[37] was applied, and for the ClO_4^- and NO_3^- ions, the parameters from Baaden et al.[38] were applied.

Interfacial Tension and Area Fluctuations. Probably the simplest protein solvation theory is the droplet model which assumes that the free energy of a protein in a solvent depends linearly on the solvent accessible molecular surface[10,39]

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$$G = G_0 + \gamma A \tag{1}$$

Here G_0 is constant, A is the SASA of our system, and γ is the effective protein−water interfacial tension.

Some authors call attention to the problem of using such a definition for a quantitative description of protein free energies (see, e.g., ref 40). On the other hand, others argue (see, e.g., refs 41 and 42) that the interfacial tension between the solute and water and the accessible surface area of the solute are the basic factors contributing to creating a cavity in water, although the macroscopic surface tension must be corrected by a factor for the radius of curvature of the microscopic cavity (see also Chandler[43]). Without entering into this discussion in detail, it seems plausible to assume that a molecular-sized surface should respond in a manner qualitatively similar to that of a macroscopic surface of the same kind to changes in the thermodynamic activity and hydrogen-bonding properties of bulk water (see also ref 44). Consequently, the concept of interfacial tension should give a useful guide to what is happening at molecular interfaces. Still, such factors as, e.g., charged amino acids might cause some complications. However, such groups are typically exposed to the protein surface, and buried charged groups represent rare and atypical exceptions. Hence, the number of charged groups exposed to the water interface is unlikely to change significantly during conformational changes (or fluctuations) accompanying ordinary protein functions, we intend to address by our approach. We, therefore, consider an average surface-specific free energy (or interfacial tension) for interfaces that become temporarily exposed or hidden during protein conformational changes, and we assume that the interfacial tension does not change significantly during conformational fluctuations.

In this case, the textbook expression of statistical mechanics can be applied, that connects γ and the fluctuations of A:[45]

$$\frac{\partial \bar{A}}{\partial \gamma} = -\beta \overline{\Delta A^2} \tag{2}$$

Here $\bar{A}$ and $\overline{\Delta A^2}$ are the average SASA and its variance or mean square fluctuation, respectively. Further, $\beta = (k_B T)^{-1}$, where k_B is the Boltzmann constant and T is the absolute temperature. From this, we can obtain the following expression for the salt-induced change of interfacial tension:

$$\Delta\gamma^{\mathrm{SASA}}_{\mathrm{salt-water}} = -\int_{\bar{A}_{\mathrm{water}}}^{\bar{A}_{\mathrm{salt}}} \frac{1}{\beta \overline{\Delta A^2}}\, \mathrm{d}\bar{A} \tag{3}$$

Ion Distribution and Interfacial Tension. The surface tension alterations of mixed solute−solvent systems can be derived from the traditional theory of surface adsorption. In a recent paper, Chen and Smith[12] reformulated this theory in a form that is easily applicable for the analysis of computer simulation data. Their theory uses the probability distributions of the system components for the description of interfacial region and the radial distribution function based formulation of the Kirkwood−Buff theory (see refs 46 and 47) for the characterization of bulk properties. For a binary system (e.g., water and a solute), the surface tension (γ) derivative with respect to the solute molecular number density in the bulk (ρ_S) can be expressed as

$$\left(\frac{\partial \gamma}{\partial \rho_S}\right)_{T,P} = -\beta^{-1} a_{SS} \frac{\Gamma_{SW}}{\rho_S} \tag{4}$$

where

$$\Gamma_{SW} = \rho_S \int_0^\infty [g_S(r) - g_W(r)]\, \mathrm{d}r \tag{5}$$

is the excess surface adsorption (ESA) per unit surface area and $g_i(r)$'s (i = W, S) are the normalized local molecular number densities at a distance r measured from the surface. a_{SS} in eq 4 describes the solute activity change with respect to the solute concentration, and can be calculated from Kirkwood−Buff theory (for the details, see ref 12).

Recently, Horinek and Netz[14] published an extended study on peptide transfer free energies from water to urea solutions. As a part of their work, the above-described method was used for the calculation of urea-induced surface tension changes at the interface of water and several linear peptide chains, as well. In their study, they used the ideal solution approximation (a_{SS} = 1) that was also applied in our present study. In the calculation presented here, supposing the linear concentration dependence of γ for the concentration used, we obtain

$$\Delta\gamma^{\mathrm{ESA}}_{\mathrm{salt-water}} = -\beta^{-1}\Gamma_{SW} \tag{6}$$

In our case, as it was already mentioned, sodium salts were added to the water to influence the interfacial tension at the interface of the miniprotein. For such a system, the excess surface adsorption (eq 5), using the indistinguishable ion approximation, can be written as[12]

$$\Gamma_{SW} = \rho_S^+ \int_0^\infty [g_S^+(r) - g_W(r)]\, \mathrm{d}r + \rho_S^- \int_0^\infty [g_S^-(r) - g_W(r)]\, \mathrm{d}r \tag{7}$$

Here, ρ_S^+/ρ_S^- are cation and anion concentrations in the bulk and $g_S^+(r)/g_S^-(r)$ are the corresponding distribution functions. These quantities are calculated from the unnormalized radial distribution function of water and ions around the center of mass of the miniprotein. For the determination of the bulk densities, we used their average values over 1.8 nm.

Orientation Time Correlation Function of Water Molecules. The orientation time correlation functions for a single water molecule are defined as

$$C_n(t) = \lim_{T\to\infty} \frac{1}{T} \int_0^T P_n(\mathbf{u}(\tau)\mathbf{u}(\tau + t))\, \mathrm{d}\tau \tag{8}$$

where P_n is the nth order Legendre polynomial and $\mathbf{u}$ is a molecular body-fixed unit vector such as a normal vector of the plane of a water molecule. The calculated values are averaged for the molecules in a selected region. For decreasing the numerical noise, we also averaged 500 independent time correlation functions using different, equally distributed, 20 ps long parts of the trajectory. For the sake of comparability with the experiments, we used the time correlation function $C_2(t)$, as it is accessible, for example, from time-resolved ultrafast IR spectroscopy.

RESULTS AND DISCUSSION

Interfacial Tension and Area Fluctuations. In the first set of calculations, we used 600 ns long REMD simulations in pure water, and in water with the selected Hofmeister salts, each of 1 M final concentration, at 32 temperatures between 300 and 450 K for the investigated systems. As the Hofmeister-active ions influence the hydrophobic interactions, which is the main stabilizing effect of the structure of globular proteins, we

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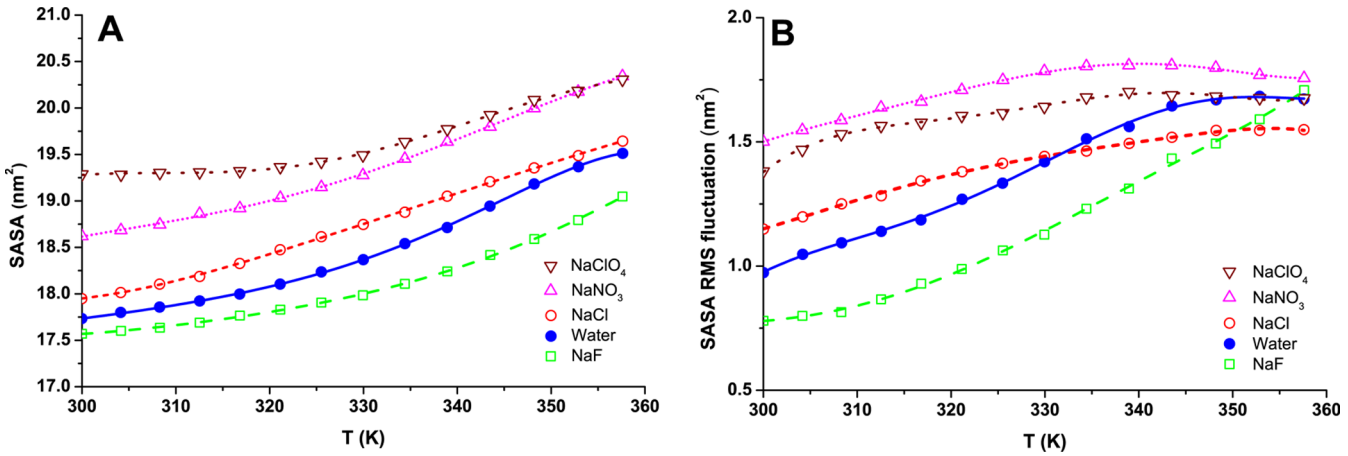

Figure 1. SASA (A) and its RMS fluctuations (B) obtained from REMD simulation of TC5B miniprotein for pure water and for the cosoluted salts.

selected the average SASA value (calculated by the g_sas utility of the GROMACS package[31,48]) as the quantity characterizing the compactness of our peptide. As we can see in Figure 1A, the SASA values follow the tendency deducible from the position of the anions in the Hofmeister series. It is considerably higher for the ClO_4^- and NO_3^- ions (~1.5 and ~1 nm^2, respectively) than for water, while for the kosmotropic F^- its value is lower (~0.2 nm^2) than that obtained for water. The addition of NaCl somewhat increased the SASA, as well. Root mean square (RMS) fluctuations of SASA (Figure 1B) show a similar tendency in a wide temperature range (300−345 K): fluctuations are higher for the chaotropic and smaller for the kosmotropic anions than the value calculated for pure water.

Note that a similar "loosening" of the protein structure was observed experimentally for the prototypical ion pump bacteriorhodopsin when suspended in a chaotropic solution.[49] In a recent study, Tadeo et al.[50] investigated the effect of Hofmeister anions on protein stability using the midpoint denaturation temperature, derived from CD and fluorescent spectroscopy, as a reporter of the changes in stability of the immunoglobulin G binding domain of protein L from *Streptoccocus magnus* as a model system. This system shows several similarities to the Trp-cage miniprotein, although it is somewhat larger. It contains 62 amino acids with stable secondary and tertiary structures, built by an α helix and β structures. The hydrophobic interaction plays an important role in its stabilization as well. Tadeo et al. used lysine to glutamine mutations to modify the apolar surface area of the protein. For all investigated mutants, they detected structure stabilization using the kosmotropic F^- and structure destabilization in the case of chaotropic NO_3^- and ClO_4^- ions for the investigated concentrations (0.1−1 M). These results strongly support the validity of our MD-based results.

From the results presented in Figure 1, using simple numerical integration, we obtain $\Delta\gamma_{salt-water}$ values for the investigated salts (see Figure 2). At 300 K, the chaotropic NO_3^- and ClO_4^- ions decreased the interfacial tension by approximately ~2.7 and ~5 mN/m, respectively. The Cl^- ion decreased while the kosmotropic F^- increased γ by 1 mN/m. With increasing temperature, the deviations from the pure water−protein interfacial tension decrease, as at higher temperatures the structure of TC5B is destabilized, and the influence of surface-induced fluctuations becomes relatively smaller compared to the temperature-induced ones.

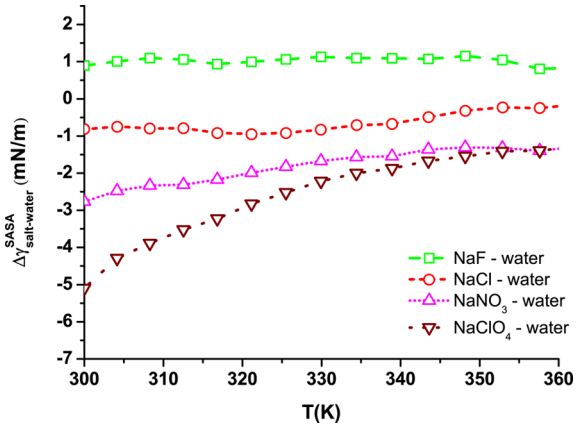

Figure 2. Surface tension alterations calculated for the TC5B−water interface with cosoluted salts NaF, NaCl, $NaClO_4$ and $NaNO_3$ using the surface fluctuations.

These are the main results of this paper, presenting the first direct evidence for the main conclusion of the theory in refs 10 and 11 pointing at the central role of solute−water interfacial tension and the related conformational fluctuations in the description of protein-related HE.

The above treatment, nevertheless, does not bring forward detailed information about the particular microphysical mechanism(s) responsible for the salt-induced change of the interfacial tension. Hence, in the following section, we concentrate on the possible microscopic interpretation of HE, which is a matter of long-standing debate. Earlier interpretations were based on the idea that the structure-maker ions strengthen while structure-breaker ones weaken the H-bonding network of the water at a large distance.[51] Several recent experimental results, however, have seemed to contradict with this idea. Measuring the orientation correlation time of water molecules in $Mg(ClO_4)_2$, $NaClO_4$, and Na_2SO_4 solutions by means of femtosecond pump−probe spectroscopy, Omta et al.[52] pointed out that ions influence only their first solvation shell considerably. The same effect was investigated by polarizable force field MD simulations by Stirnemann et al.[53] who gave also an explanation for why all concentrated salt solutions (above ca. 1 M concentration) slow down water dynamics. A retardation of water reorientation dynamics was also found to appear at the lysosyme−water interface, both experimentally[54,55] and computationally.[56] Recently, several

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experimental and computational studies have pointed out direct interactions of proteins and chaotropic anions accumulated at the protein−water interface.[57,58] Theoretically, both ion distribution differences and structural changes of water at the interface may influence protein−water interfacial tension.[10,13,14,46,47]

Ion Distribution and Interfacial Tension. In order to investigate how these phenomena manifest themselves in our system, another type of calculations was carried out, where, instead of the peptide itself, we concentrated rather on the dynamics of ions and water molecules at the interface. In this case, the peptide was fixed (using a harmonic restraint) in 100 ns long MD simulations in pure water, and in water with the selected salts, each of 1 M final concentration.

The average surface preference (radial distribution function around the fixed peptide surface) of the anions is presented in Figure 3A. The overall result is that chaotropic ClO_4^- and

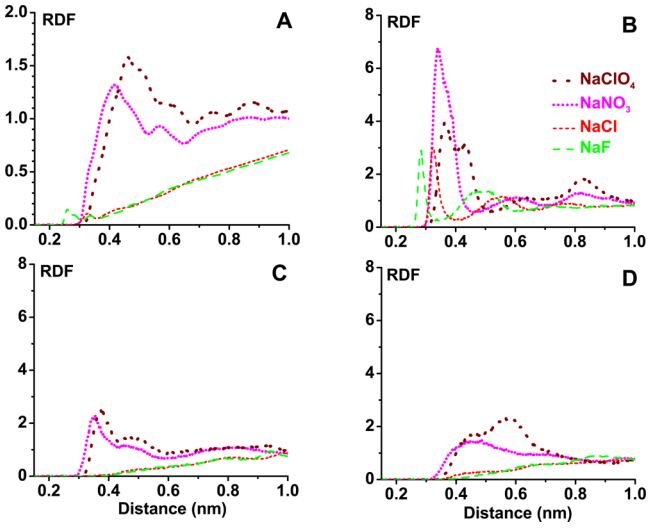

Figure 3. Radial distribution function (RDF) of the center of cosoluted anions around the heavy atoms of the whole peptide (A), the N atom of the charged Lys8 side chain (B), the H-bond donor N atom of Gly15 (C), and a C atom of the apolar ring of Pro12 (D) for the TC5B miniprotein.

NO_3^- anions accumulate near the protein−water interface, while F^- and Cl^- anions are depleted from there. These data were obtained from a constant pressure simulation of TC5B at 300 K. As we can see, the local concentration of the chaotropic ions (ClO_4^- and NO_3^-) is considerably higher already in the closest interfacial region (between 0.3 and 0.5 nm) than the same quantity for F^- and Cl^- ions. The local Na^+ concentration is also influenced by the surface charge and anion distributions (see Figure S1 in the Supporting Information).

The influence of ions on the interfacial region, besides their radial distribution function, can be characterized by the average number of ions and water molecules in the close vicinity of the miniprotein. Table 1 shows the average number of water molecules having their oxygen atoms, and ions having at least one of their atoms, within 0.35 nm from the closest atom of the miniprotein. In accordance with the previous results, ClO_4^- ions are accumulated in the highest degree at the protein surface, followed by the NO_3^-; on average, 8.02 and 6.42 of them were found in this border region, respectively. The corresponding average numbers of F^- and Cl^- ions are considerably lower, both having a value of ∼1.3. The number of

Table 1. Average Number of Ions and Water Molecules within 0.35 nm of the Trp-Cage Miniprotein Surface in Pure Water and in Water with Salts

	water	$NaClO_4$	$NaNO_3$	NaCl	NaF
H_2O	131.5	115.3	123.2	129.8	129.8
Na^+		2.3	2.7	1.7	1.8
anions		8.0	6.4	1.3	1.3

Na^+ ions is 2.31 and 2.72 for the chaotropic salts $NaClO_4$ and $NaNO_3$, and somewhat smaller (1.33 and 1.83) for NaCl and NaF, respectively. The average number of water molecules in this region follows an opposite tendency as compared to the anions: the larger the number of anions, the smaller the number of water molecules found in this region.

We also investigated the protein surface regions of different surface charges, separately. Figure 3B−D shows the radial distribution function of anions at the characteristic regions having different surface charges: (B) the headgroup of positively charged Lys8, (C) the NH group of the Gly15 backbone, and (D) a carbon atom of the apolar ring of Pro12 (see arrows in Figure S2, Supporting Information). The F^- and Cl^- accumulates only at region B having the largest surface charge and expelled from the other regions with smaller positive surface charge. The chaotropic ClO_4^- and NO_3^- ions accumulate at all three regions, and local density seems to correlate with the magnitude of the surface charge density. The electrostatic potential at the miniprotein surface, as well as the graphical representation of anion distributions is presented in Figures S2 and S3 of the Supporting Information. Accumulation of chaotropic ions at the protein surface has been observed also experimentally (as a review, see ref 8), and is supposed to decrease the protein−water interfacial tension, in accordance with our results in the previous section, and with the prediction of ref 10. In addition to the surface charge, geometrical features of the protein surface (like concavity) may also influence the local ion density, as it is described by Gibb et al.[59]

Knowing the distribution of ions and water molecules in the vicinity of the surface allows us to calculate their contribution to the salt-induced interfacial tension changes from the excess surface adsorption using eq 6. We carried out these calculations for the fixed protein and for the conformational ensembles obtained from REMD simulations, as well. The former calculation is analogous to the peptide chain calculation of Horinek et al.,[14] but instead of their cylindrical averaging, we used a spherical one to obtain the distribution function $g(r)$ in eq 7. However, in the latter case, an additional averaging is included for the conformations appearing in the ensemble obtained from the REMD simulation at a given temperature. Furthermore, the calculated $\Delta\gamma(T)$ values correspond to the same conformational ensemble (obtained with ∼1 M salt concentration at temperature T) immersed in pure water and salt solution. That is, the difference due to the surface change is not included, unlike in the SASA-based calculation.

In Table 2, the interfacial tension differences between 1 M and zero salt concentration are presented at 300 K. As we can see, the surface tension changes obtained from excess surface adsorption follow the same tendency as in the SASA-based calculation. However, for the fixed miniprotein structure, the magnitude of the calculated values is considerably smaller than in the SASA case for $NaClO_4$ and $NaNO_3$, while somewhat larger for NaF. Surprisingly, the interfacial tension change for

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Table 2. Surface Tension Alterations Calculated for the TC5B−Water Interface with Cosoluted Salts Using the Surface Fluctuations ($\Delta\gamma^{SASA,REMD}_{salt-water}$) and the Excess Surface Adsorption for REMD Conformational Ensembles ($\Delta\gamma^{ESA,REMD}_{salt-water}$) and for Fixed Miniprotein Surface ($\Delta\gamma^{ESA,fixed}_{salt-water}$)

	$NaClO_4$	$NaNO_3$	NaCl	NaF
$\Delta\gamma^{SASA,REMD}_{salt-water}$	−5.10	−2.77	−0.82	0.90
$\Delta\gamma^{ESA,REMD}_{salt-water}$	−4.31	−2.78	0.96	1.08
$\Delta\gamma^{ESA,fixed}_{salt-water}$	−1.64	−0.87	1.05	1.19

NaCl is positive (contrary to the SASA-based results), which indicates a slight kosmotropic character.

Figure 4 presents the average $\Delta\gamma$ values obtained from the excess surface adsorption for the REMD conformational

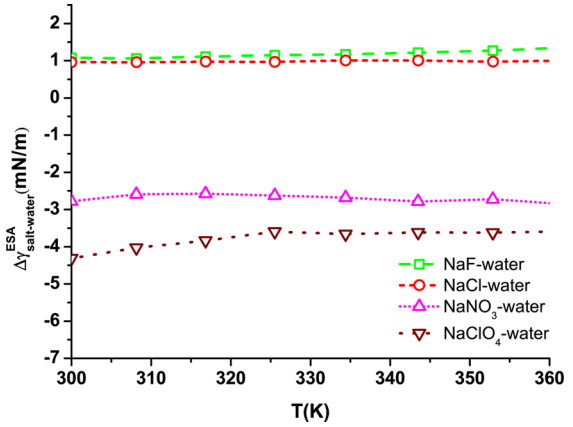

Figure 4. Surface tension alterations calculated for the TC5B−water interface with cosoluted salts NaF, NaCl, $NaClO_4$, and $NaNO_3$, using the excess surface adsorptions.

ensembles at different temperatures. These values are similar to the SASA-based ones, although the exceptional behavior of NaCl remained unchanged. This may stem from the unique interaction of Cl^- ions with the miniprotein. The calculated values show moderate temperature dependence in the whole investigated temperature range. The variances between 300 and 360 K for $NaClO_4$, $NaNO_3$, and NaF are ∼0.7, ∼0.3, and ∼0.2 mN/m, respectively, while the surface tension remains almost constant for NaCl.

Altogether, the average $\Delta\gamma$ values obtained from excess surface absorption show a high similarity to those calculated from the SASA fluctuations, especially near 300 K. (The only exception is the NaCl, where even the direction of the alteration was also changed.)

Orientation Time Correlation of Water Molecules. As it was mentioned earlier, both the presence of anions and protein induces a retardation of water reorientation dynamics in their first solvation shell in most of the cases. In the following section, we investigate the reorientation dynamics of the water molecules in the first solvation shell of the miniprotein, in concentrated solutions of the investigated salts. In this region, both effects are expected to appear simultaneously, especially at those places where anions are accumulated.

As a benchmark, in Figure 5, we present first the average orientation time correlation function of water molecules in a 0.05 nm wide shell at 0.7 nm distance from the miniprotein surface, which can be considered as bulk water. The curves obtained for the investigated salts are all above the one

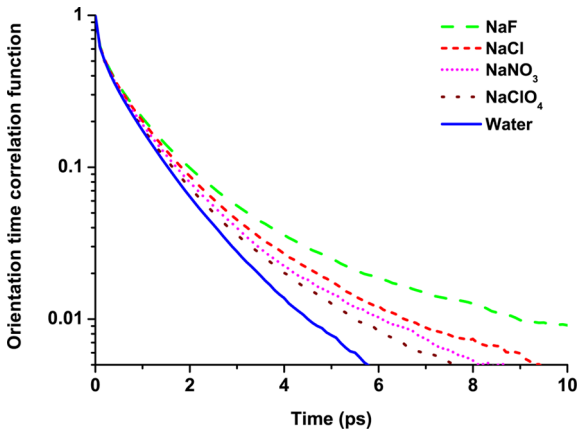

Figure 5. Orientation time correlation function of water molecules in a 0.05 nm wide shell of the bulk water at 0.7 nm distance from the TC5B surface for pure water and for the cosoluted salts.

obtained for pure water, and their sequence correlates with the position of the ions in the Hofmeister series. Smaller increments belong to chaotropic ions, and larger ones, to kosmotropic ions. Our results follow the tendency obtained by Stirnemann et al.[60] in their MD simulations for the first solvation shell of anions.

The first solvation shell can be identified with the help of the radial distribution function of water oxygen atoms around the surface of the miniprotein (see Figure 6). The first peak of the

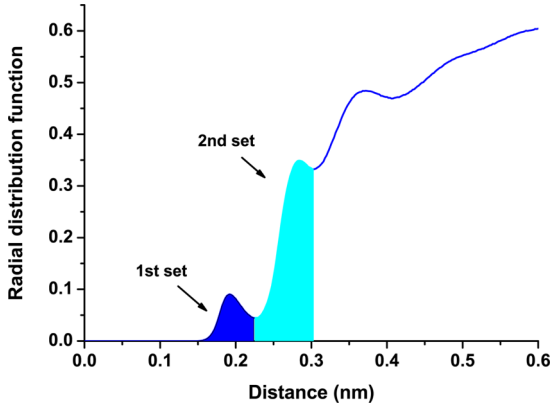

Figure 6. Radial distribution function of water oxygen atoms around the miniprotein surface.

curve has its maximum at ∼0.19 nm, while the second one, at ∼0.28 nm. These two peaks form the first solvation shell, and belong to the water molecules turning their OH bonds away from and toward the surface, acting as acceptors and donors of H-bonds with different strengths between water and the miniprotein, respectively. The water molecules in the first set are most probably close to positively charged regions, where chaotropic anions also accumulate. The members of the second set are placed dominantly around the remaining part of the miniprotein surface.

The orientation time correlation functions of water molecules belonging to the first and second peaks of the radial distribution function (having their oxygen atoms closer than 0.23 nm and between 0.23 and 0.31 nm to the miniprotein surface, respectively), calculated for pure water and the investigated salts, are shown in Figure 7.

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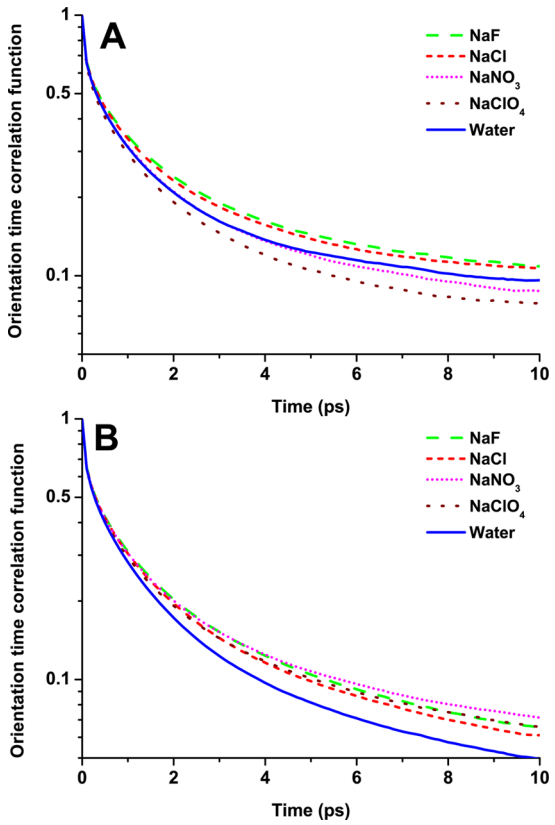

Figure 7. Orientation time correlation function of water molecules having their O atoms at a distance (A) <0.23 nm (first set) and (B) between 0.23 and 0.31 nm (second set) from the fixed TC5B surface for pure water and for the cosoluted salts.

The first important observation is that the decay of these curves, and this way the average speed of reorientation dynamics, is much slower for both sets of water molecules in the investigated systems than that for water molecules in the bulk (see Figure 5). The retardation of the reorientation dynamics for water molecules acting at the surface as H-bond acceptors (first set) is larger than the same quantity for H-bond donors (second set). Retardation of the interfacial water dynamics was also proved experimentally using terahertz time-domain spectroscopy by Aoki et al.[55] for aqueous solution of lysozyme as a test system. MD simulation of Sterpone et al.[56] on the same protein provided a slowdown factor of larger than 2 for most of these water molecules.

The other important finding is that the investigated salts influence the orientation time correlation function of the water molecules in the two sets in a different way. The chaotropic anions (ClO_4^- and NO_3^-) increase, while Cl^- and F^- ions decrease the average orientation flexibility of water molecules having the closest O atoms to the protein surface, as compared to the pure water case. However, the reorientation dynamics of water molecules in the second set is retarded by all of the investigated salts and shows only small differences.

Since the effect of investigated salts on the reorientation dynamics of water molecules at the positively charged surface regions of the miniprotein follows the Hofmeister series, one may infer that, besides ion distribution, changes of the water dynamics in the first hydration shell are indicative of structural changes in the H-bonded water network, as has been observed experimentally in the case of water solutions of Hofmeister salts.[60,61] Most probably, the orientation time correlation function is interrelated with the water density fluctuations in the vicinity of protein interfaces proposed by Garde et al.,[62,63] which are expected to have a(n entropic) contribution to the interfacial tension changes, but this remains to be shown. Similarly, the fine details of the interfacial effects, revealing the role of polarization in Hofmeister phenomena,[58,64] are expected to be explored by future investigations.

CONCLUSIONS

On the whole, our simulations demonstrated that the Trp-cage miniprotein with explicit water molecules and nonpolarizable force fields is an appropriate model system to study the basic features of HE on proteins. From the average value of the solvent accessible surface area of the protein and its fluctuations, the alterations of protein−water interfacial tension ($\Delta\gamma$) induced by Hofmeister-active salts could be determined. It was found that γ increased if kosmotropic and decreased if chaotropic salts were added to the simulation volume. Looking for the possible microphysical reasons of the interfacial tension change, we identified characteristic differences in the distribution of anions as a function of their position in the Hofmeister series, as well as in the corresponding reorientation dynamics of interfacial water molecules, which are both expected to influence the compactness of the TC5B miniprotein (described by the SASA). Using these data, we calculated the contribution of the ion and water distributions to the interfacial tension, based on the Gibbs equation. The values are fairly close to the ones found by our general method, giving nice support for the latter. The results establish a link between the phenomenological and microscopic interpretations of Hofmeister effects, and emphasize the determining role of interfacial properties in protein structure and dynamics, in general.

ASSOCIATED CONTENT

Supporting Information

Additional figures of the radial distribution function of Na^+ ions, equidensity plots of anion distributions around the miniprotein, as well as the graphical representation of electrostatic potential at the miniprotein surface. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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