Specificity of Ion-Protein Interactions: Complementary and Competitive Effects of Tetrapropylammonium, Guanidinium, Sulfate, and Chloride Ions

by

Philip E. Mason,[§*] Christopher E. Dempsey,[†] Luboš Vrbka,[‡#] Jan Heyda,[‡] John W. Brady,[§] and Pavel Jungwirth[‡*]

[§]*Department of Food Science, Cornell University, Ithaca, N. Y. 14853 USA*
[†]*Department of Biochemistry, Bristol University, Bristol BS8 1TD, U.K.*
[‡]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic and Center for Biomolecules and Complex Molecular Systems, Flemingovo nám. 2, 16610 Prague 6, Czech Republic*
[#]*Institute of Physical and Theoretical Chemistry, University of Regensburg, 93050 Regensburg, Germany*

[*]*Corresponding authors: pem27@cornell.edu (P.E.M.) and pavel.jungwirth@uochb.cas.cz (P.J.).*

Abstract

The interactions of ions with a model peptide (a single melittin α-helix) in solutions of tetrapropylammonium sulfate or guanidinium chloride were examined by molecular dynamics simulations. The tetrapropylammonium cation shares the geometrical property of essentially flat faces with the previously examined guanidinium cation, and it was found that that this geometry leads to a strong preference for tetrapropylammonium to interact in a similar stacking type fashion with flat non-polar groups such as the indole side chain of tryptophan. In contrast to guanidinium, however, tetrapropylammonium does not exhibit strong ion pairing or clustering with sulfate counterions in the solution. Sulfate was found to interact almost exclusively and strongly with the cationic groups of the peptide, such that already in a 0.1m solution of tetrapropylammonium sulfate the 6+ charge of the peptide is effectively locally neutralized. In combination with previous simulations, neutron scattering, and biochemical experiments on the conformational stability of model peptides, the present results suggest that the Hofmeister series may be explained in higher detail by splitting ions according to the effect they have on hydrogen bonding, salt bridges, and hydrophobic interactions in the protein and how these effects are altered by the counter-ion.

Introduction

Over 100 years ago Hofmeister empirically ordered a series of cations and anions according to their ability to salt proteins out of a solution .[1] Essentially the same rankings in reverse order was subsequently found to affect the stability of protein conformations toward denaturation (Gdm^+~TPA^+>K^+~NH_4^+> Na^+>Li^+ for the cations and I^->Cl^->SO_4^{2-} for the anions). In recent years the origin of this series has been the subject of renewed interest.[2-6] Indeed, the level of biochemical knowledge has advanced to the point where the ambiguity about interactions of ions with proteins and the effects thereof represent a potential barrier to obtaining a higher understanding of biological systems.[7-9] There are two general schools of thought on the subject. One holds that the origin of the effect lies within the generalized alteration of the water structure.[10-13] The other school contends that it is the direct interaction of the ions with the protein that is the primary factor in determining the effect of electrolytes on the protein.[7,14-21]

The combination of molecular dynamics (MD) simulations and experimental structural techniques has yielded new insight into these interactions in recent years,[22] and has facilitated detailed analysis of complex ions beyond the scope of methodologies applicable to the study of simple spherical ions. For example, the guanidinium cation ($C(NH_2)_3^+$, denoted as Gdm^+), which is also incorporated within the charged side chain group of the amino acid arginine (Arg), has been shown to have a peculiar hydration pattern, with hydrogen bonds to water in the molecular plane, but lacking ordered waters above the faces of the ion.[23] This facilitates face-to-face Gdm^+ interactions that resemble hydrophobic stacking,[23-25] and indicates that part of the strong denaturant activity of Gdm^+ is due to Gdm^+ pairing with planar non-polar groups in proteins (particularly the aromatic amino acid side chains).[23] Further MD and neutron scattering studies showed that in aqueous Gdm_2SO_4 solution at denaturant level concentrations, the hetero-ion pairing is so strong that essentially all of the ions form clusters, and that this clustering destroys the Gdm^+- Gdm^+ stacking.[16] The strong ion pairing between Gdm^+ and SO_4^{2-} has also been demonstrated by measurement of osmotic coefficients,[26] and similar ion clustering has been observed directly by small angle neutron scattering in solutions of

Gdm_2CO_3.[27] These observations led to the experimentally testable prediction that the difference in the potency of the powerful denaturant GdmCl and the weak denaturant Gdm_2SO_4 was primarily due to the differing level of ion pairing in these two electrolytes, and *not* due to the additive effect of the water structuring by the kosmotrope SO_4^{2-} and the destructuring by the chaotrope Gdm^+. This prediction was found to be consistent with experimental observations of small tryptophan-zipper (trpzip) peptides stabilized largely by indole-indole interactions.[15] It was found that trpzip peptides are strongly destabilized by GdmCl, while Na_2SO_4 had no effect on conformational stability. The absence of trpzip peptide denaturant activity in Gdm_2SO_4 indicates that SO_4^{2-} is effective in "reversing" the strong denaturant activity of Gdm^+ without itself being a stabilizer of trpzip peptides, entirely consistent with expectations from the ion pairing scenario.[15] These results tie in with previous MD and neutron scattering studies on the ion-ion structure in electrolytes such as Gdm_2SO_4, Gdm_2CO_3, GdmSCN, and GdmCl,[16,23,27] and indicate that Gdm^+-SO_4^{2-} hetero-ion pairing in solution can be strong enough to attenuate specific Gdm^+- protein interactions that contribute to denaturation. Recent MD simulations of a single α-helix of the peptide melittin in GdmCl solution demonstrated that the Gdm^+ ion indeed interacts in a face-on fashion with the specific groups of the peptide (especially the planar aromatic π - bonded systems of the tryptophan and arginine side chains, but also more diffusely with exposed non-polar surfaces).[16,23] The resulting weak accumulation of Gdm^+ at the polypeptide surface is consistent with experimental characterization of the enhanced local concentration of Gdm^+ at the surface of folded proteins.[28]

The present study uses MD simulations of ion-protein interactions in aqueous sulfate solutions of a very different complex cation, tetrapropylammonium (TPA^+), to further examine ionic association and its role in the Hofmeister series. TPA^+ has a roughly tetrahedral shape (Figure 1) and a very low charge density since the formal positive charge is spread over many atoms. The sides of this tetrahedron, at a broad-brush level, resemble a flat face, a geometric feature that TPA^+ shares with the Gdm^+ ion (Figure 1). Salts containing tetraalkylammonium cations are widely used in host guest chemistry,[29-31] as surfactants,[32,33] phase transfer catalysts,[34,35] ionic liquids,[36-38] etc. The quaternary ammonium salt choline is found in several biochemical contexts, playing a

central role in some methyl group transfer reactions,[39] as a component of the neurotransmitter acetylcholine[40,41] and the zwitterionic headgroups of phosphocholine lipids.[42] Quaternary ammonium salts are useful in the study of aqueous electrolytes, since they can be highly symmetric, are available in a range of sizes, and are generally very soluble. These features allow experimental insight into the physical properties of such solutions both at the microscopic[43-46] and macroscopic level.[47-52] Here, the interaction of tetrapropylammonium sulfate (TPA_2SO_4) with the melittin α-helix was investigated. The ion-ion and ion-peptide interactions were examined and compared with other electrolytes, in particular with those containing Gdm^+.

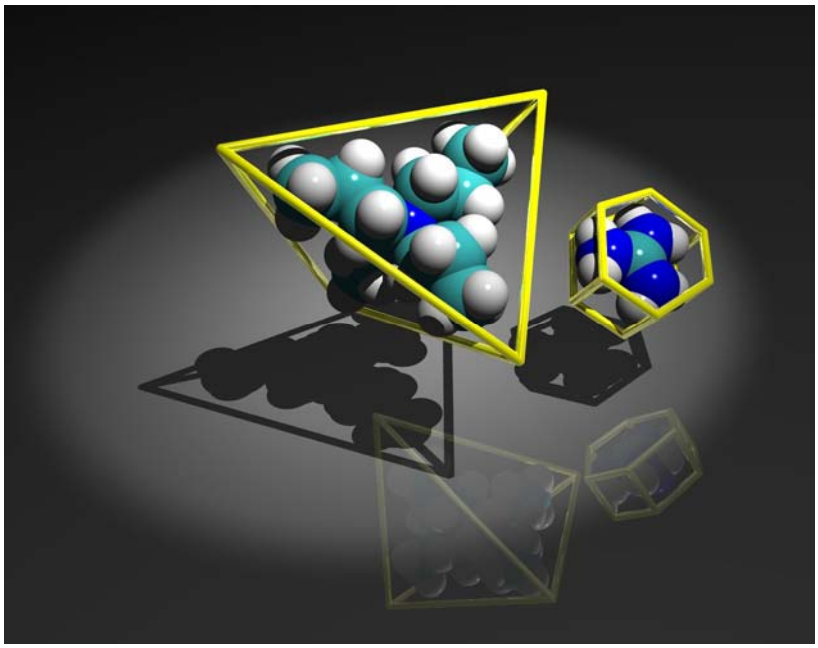

Figure 1. Left, the geometry of the TPA^+, highlighting its tetrahedral shape and quasi-flat faces. Note that the TPA^+ ion is considerably larger and has larger flat faces than the Gdm^+ ion (right), which is also shown for comparison. Graphics produced by VMD[56] (University of Illinois) and POV (freeware ray-tracer).

The 26 amino acid, α-helical (with a slight bend at Pro14) peptide melittin[57] was chosen as the model system for this study. Melittin is a highly positively-charged (6+), membrane-active peptide found in bee venom. Four individual melittin chains bind together non-covalently into a tetramer when in the "native" α-helical conformation, but

as monomers they exist as random coils.[58] The α-helical monomer peptide has a useful mix of amino acid side chains, including a significant number of surface non-polar residues not represented in folded polypeptides,[14] which mediate the helix associations in the tetramer. For this reason, a single α-helical monomer was selected to study the interactions with TPA ions, even though it is not a probable conformation in solution. In addition, melittin was the peptide previously selected for the study of guanidinium ions with peptide side chains, and thus facilitates comparison.[14]

Computational Details

A single melittin chain taken from the Protein Data Bank structure (2MLT) was protonated consistent with a pH 7 (methyl amide C-terminus; protonated N-terminus, +6 overall charge: Nter, K7, K21, R22, K23, R24). It was then introduced into a 3D periodic simulation cell with approximate dimensions of 75x75x75 $Å^3$, together with 40 TPA^+ and 23 SO_4^{2-} ions (the excess of 3 sulfates serving as a charge compensation for the total 6+ charge of the peptide), and 10304 SPC/E type water molecules. As a result, a mixture with an approximate TPA_2SO_4 concentration of 0.1m was created. The concentration of TPA_2SO_4 in this study was chosen to reflect biological concentrations. As will be shown below, the behavior of this solute presents a contrast in both ion-ion and ion-protein interactions to that of the previously examined GdmCl solutions at denaturant concentrations. Since it was previously observed that concentration does not have a large effect on the form of ion-ion interactions,[16] the different concentrations of this study are not a significant problem in determining the general qualitative factors that determine ion-ion and ion-protein interactions.

The simulation protocol was kept consistent with previous studies.[7,21] After initial energy minimization (which removed potential close contacts introduced during the construction of the simulation cell) with total volume and peptide geometry constrained, a 20 ps constant volume heating phase and a 20 ps constant pressure pre-equilibration followed. Then, after a 0.5 ns equilibration period, the dynamics of the system was

monitored for an additional 10 ns. For comparison, a simulation without the peptide was also performed for 0.5m TPA_2SO_4 in a periodic box of approximately 39x39x39 $Å^3$ containing 1660 SPC/E water molecules, 30 TPA^+, and 15 SO_4^{2-} ions. The molecular dynamics program package AMBER (version 8) was used for all the calculations, employing the parm99 parameter set.[53] Interaction parameters for ions were taken as a non-polarizable force field from our previous studies.[45,21]

Classical Newton equations of motion were solved numerically with a timestep of 1 fs. A cutoff distance of 12 Å was used for van der Waals and electrostatic interactions. The long range electrostatic interactions were accounted for using the particle mesh Ewald scheme.[54] The Berendsen temperature (at 300 K) and pressure (at 1 atm) couplings were used and all bonds involving hydrogen atoms were frozen using the SHAKE algorithm.[55] The resulting trajectories were analyzed in terms of ion-ion radial distribution functions, distributions of ions in the vicinity of the peptide residues, 3D density distribution maps, and via a visual inspection using the VMD program.[56]

Results

Figure 2 displays the radial distribution function characterizing the structure of the pure TPA_2SO_4 solution without the peptide as calculated from the MD simulations, and compares it to the structure of 3 m GdmCl as calculated from a previous study.[23] We show these results before discussing the ion-peptide interactions, since it is instructive to first compare the behavior of TPA^+ vs Gdm^+-containing electrolytes. The cation-cation, cation-anion, and anion-anion radial distribution functions from both simulations are shown. The Gdm^+-Cl^- hetero-ion pairing can be seen from the sharp peak at 4 Å in $g_{CCl}(r)$. The homo-ion pairing in the Gdm^+ simulation is seen as a weak peak in $g_{CC}(r)$ at *ca* 4 Å. The only Cl-Cl pairing is solvent or cation mediated. In contrast, the ion pairing behavior in the present TPA_2SO_4 solution is significantly different. There is no clear homo-ion pairing of either TPA^+ or SO_4^{2-} and neither is there any direct TPA^+-SO_4^{2-} pairing. The behavior of TPA^+ thus displays significant differences to that of Gdm^+,

which pairs directly with Cl^-, and even more strongly (even at lower concentrations) with divalent anions such as SO_4^{2-} or CO_3^{2-}.[16,23,27]

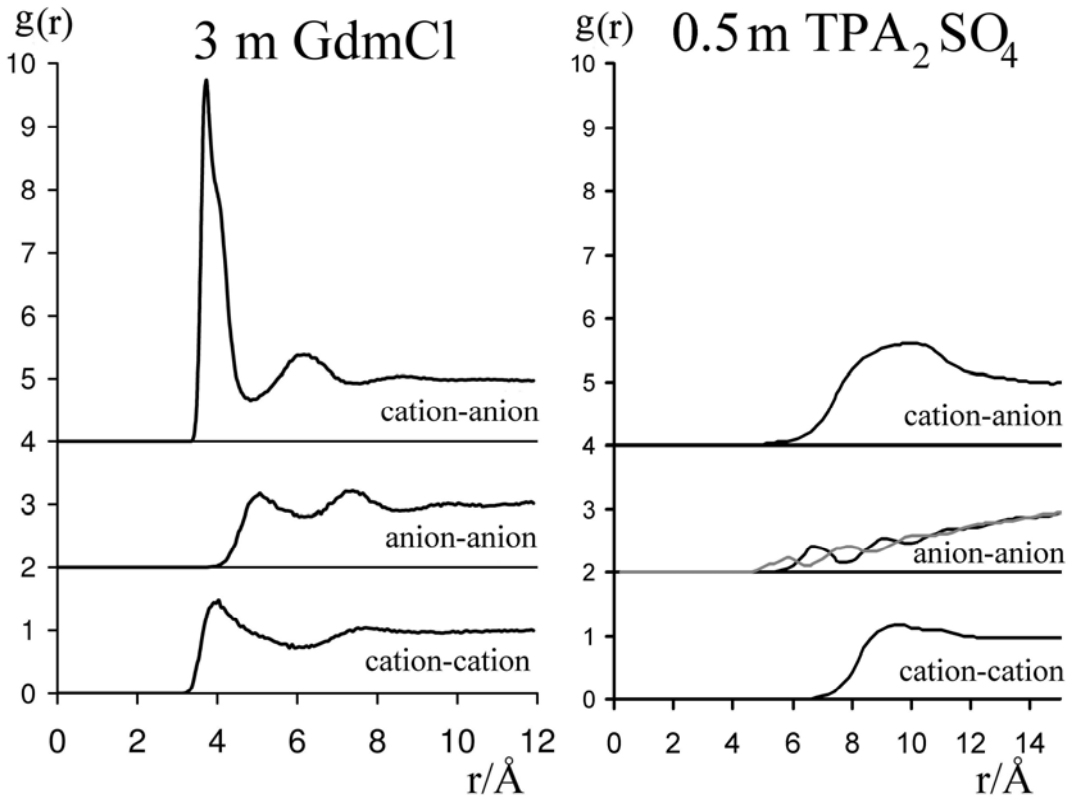

Figure 2, left, the ion-ion radial distribution functions (g(r)) for a 3m GdmCl solution (in each case for the Gdm^+ ion the central carbon is taken as the position of the Gdm^+ ion).[23] Right, the current simulation of 0.5m TPA_2SO_4. Bottom: g(r) for the central N to the N of TPA^+. Middle: g(r) for the central S of SO_4^{2-} to S (black) and g(r) from the central S to the oxygen of SO_4^{2-} (grey; the intramolecular S-O correlation have been removed for clarity). Top: g(r) from S on SO_4^{2-} to N on TPA^+.

The difference in the homo-ion pairing behavior for Gdm^+ versus TPA^+ can be explored further by comparison of the three dimensional density maps of these cations. Figure 3 displays the ordering of TPA^+ around TPA^+ in TPA_2SO_4 solution and compares it to the density of Gdm^+ around Gdm^+ in GdmCl.[23] The density contours in these figures are presented in a fashion which factors out concentration differences. As can be seen,

there is virtually no ordering of TPA^+ around TPA^+, in strong contrast to the significant guanidinium stacking found in the previous study.[23]

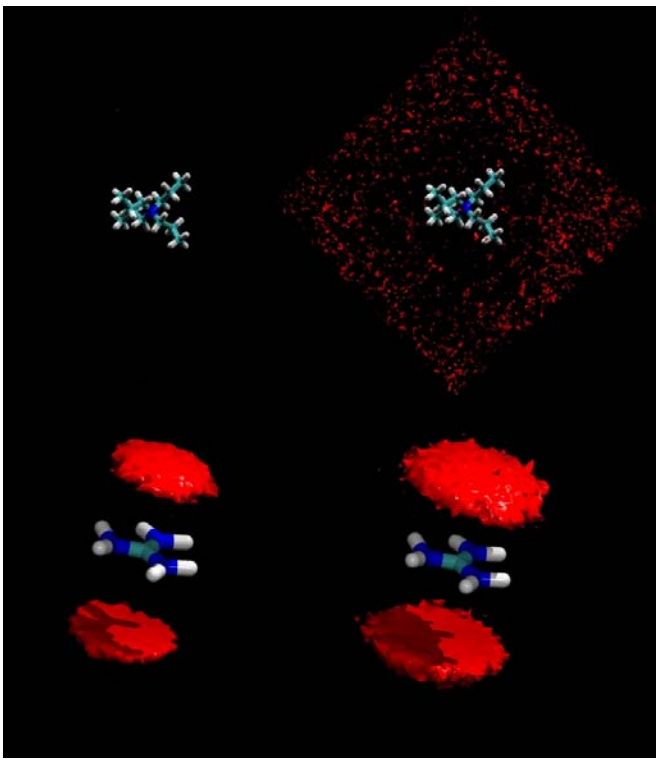

Figure 3, top, the density of C_{TPA} around TPA^+ at (left) 5x and (right) 3x the average number density of C_{TPA}, bottom, the density of C_{Gdm} around Gdm^+ at (left) 5x and (right) 3x the average number density of C_{Gdm}.[23]

The ion-ion radial distribution functions g(r) for the 0.1 molal TPA_2SO_4 with a single melittin chain are essentially indistinguishable from those for a pure 0.5 molal TPA_2SO_4 with one notable exception: the g(r) for SO_4^{2-}- SO_4^{2-} interactions (Figure 4). In the simulations the only sulfate ions that come close enough to each other to contribute to the peak in $g_{SS}(r)$ at 6 Å are those interacting directly with the cationic side chains (Arg or Lys) of the peptide. This can be demonstrated if the sulfates are split into two populations, those in contact with the peptide (within 4.5 Å of the peptide) and those that are not. This allows the homo-anion g(r) to be split into three components, S_pS_p (sulfate in contact with peptide to sulfate in contact with peptide), S_pS_f (sulfate in contact with protein to sulfate 'free' in solution) and S_fS_f (correlations between sulfates not in contact

with the protein). As is clear from Figure 4 the S_pS_p correlation accounts for the majority of the peak at 6 Å in $g_{SS}(r)$, while the g(r) for S_fS_f component resembles the SO_4^{2-} -SO_4^{2-} g(r) for the 0.5 molal TPA_2SO_4 solution. It is evident from this data that the sulfates, with the exception of when the interactions are mediated by a cationic peptide group, show no significant pairing in TPA_2SO_4 solution, and, furthermore repel each other over long length scales; there is a depletion of sulfates around sulfate even as far away as 20 Å. In contrast, in the case of Cl^- in the GdmCl solution, there is essentially no homo-ion correlation beyond 8 Å. The repulsion of the sulfates observed in these simulations is only turned into an 'attraction' when the interaction is mediated by a positively charged cationic group of the peptide, in analogy to the Gdm^+ mediated structure found in Gdm_2SO_4 solution.[16]

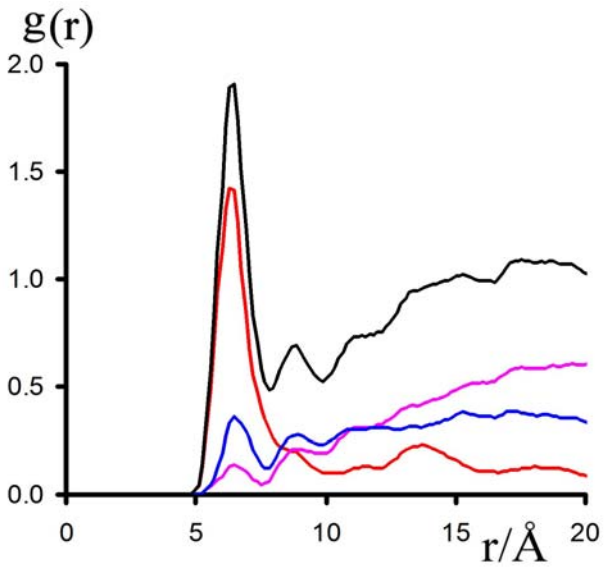

Figure 4, shown in black is $g_{SS}(r)$ for SO_4^{2-} in 0.1m TPA_2SO_4 solution containing melittin. Shown in red is the component that is due to SO_4^{2-} ions within 4.5 Å of any atom on the peptide (S_pS_p), shown in purple is the component due to SO_4^{2-} ions that are not within 4.5 Å of any atom on the peptide (S_fS_f), and shown in blue is the contribution to $g_{SS}(r)$ where one ion is within 4.5 Å of the peptide, and one is not (S_pS_f). The peak at 6 Å is mostly due to two sulfates bridged by a cationic Lys or Arg group of the peptide.

It was also found that the TPA^+-peptide interactions are considerably different from the Gdm^+-peptide interactions, which was apparent when comparing the number of ions in contact with the peptide. The definition used here for an ion-peptide contact was that a heavy atom of the ion must be within 4.5 Å of a heavy atom of the peptide. This definition yielded a molecular coordination number for the peptide of 11.8 Gdm^+ ions. (Note that in the previous study the more stringent criterion that the central carbon must be within 4.5 Å of the peptide was used and gave a value of *ca* 7.5 Gdm^+ ions for the same data.[14]) In contrast, the molecular coordination number for TPA^+ next to the protein was calculated in the present study to be about only three.

In the simulation of TPA_2SO_4 with a melittin chain, it was found by direct inspection of the trajectory that TPA^+ interacts in a face-to-face manner with the indole sidechain of Trp19 (Figure 6). In the simulation, once a TPA^+ diffused to the indole side chain it had a remarkably long residence time of 1 ns or even longer. Several times during the contact with Trp the TPA^+ ion briefly left (i.e., lost the face-to-face contact), rotated, and returned, often with a different face of the TPA^+ tetrahedron in contact with the indole. TPA^+ also exhibits a similar face-to-face interaction with other non-aromatic hydrophobic groups at the melittin surface, although these contacts are more transient and weaker compared to the TPA^+-indole interaction.

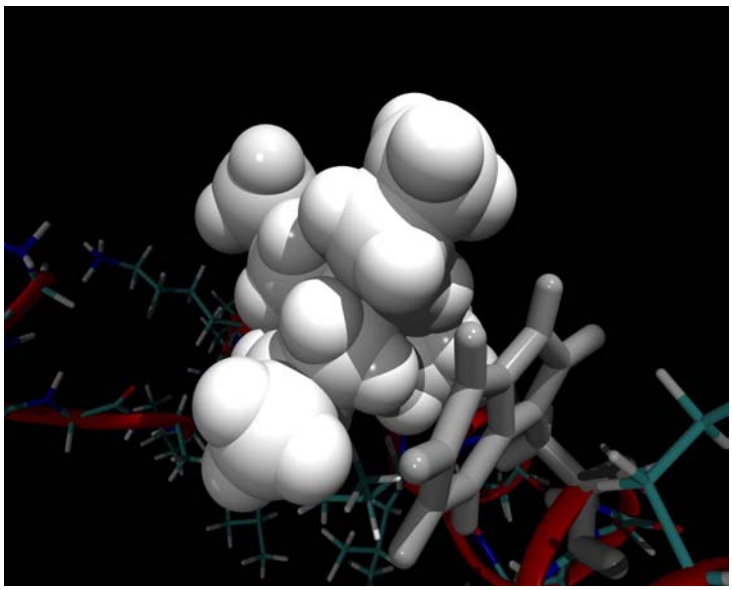

Figure 6, The interaction of a flat face of the TPA^+ ion with the flat face of the indole side chain of Trp19.

These results suggest that TPA^+ would be particularly good at attenuating hydrophobic interactions in polypeptides, especially those involving geometrically flat aromatic groups. In contrast, this cation would be relatively ineffective at competing for H-bonds that stabilize peptides. For comparison, Gdm^+ was found to interact in a rather generic fashion with hydrophobic groups,[14] but it also had a significant propensity to stack against the guanidine moiety in the sidechain of Arg22 and Arg24 of mellitin. This behavior is different from that of TPA^+, for which in the present simulation no similar strong interaction was observed between TPA^+ and the Arg side chains.

In the simulations the interactions of the sulfate anion with the peptide were very strong and relatively long-lived (several peptide-SO_4^{2-} interactions were observed to last longer than 2 ns). The SO_4^{2-} ions bound almost exclusively to the cationic residues (Arg and Lys). This point is highlighted by splitting the molecular coordination number of sulfate around the peptide into various categories, in particular those within 4.5 Å of a cationic residue, and those within 4.5 Å of a hydrophobic group. It was found that about 90% of the SO_4^{2-} ions close to the peptide were in contact with cationic groups. This illustrates the propensity of sulfate to neutralize the charge on the peptide (as summarized in Table 1). At 0.1 m the strong binding of sulfate (sulfate coordination number of 4.6 corresponding to charge -9) and the weaker binding of TPA^+ (TPA^+ coordination number of 3, corresponding to a charge of +3) effectively neutralized the 6+ charge of the peptide. This situation can be contrasted to that in 3m GdmCl, where on average there were about 12 Gdm^+ and 9 Cl^- ions in contact with the peptide, increasing its effective charge from +6 to +9.[14] It should also be noted that TPA^+ is the first counter-ion of SO_4^{2-} that we are aware of which does not display strong hetero-ion pairing, as suggested by previous calorimetric studies.[62] The lack of hetero-ion pairing found here contrasts significantly with previous observations of solutions of Na_2SO_4, $(NH_4)_2SO_4$ and Gdm_2SO_4.[21,60,61] The practical significance of this is that TPA_2SO_4 is likely to be particularly efficient at neutralizing the cationic charge of peptides (with implications for

salting out). Sulfate in this salt is effectively a 'lone player', which does not bring charge neutralizing cations with it.

Near the end of the simulation, a sulfate ion was observed to 'snap' into a very stable conformation with the frayed C-terminus of the peptide where it remained for about 2 ns (Figure 7) which evidently stabilized the helix from further uncoiling. It should be noted that the C-terminus usually has oxygens exposed, while the N-terminus has NH groups available. It might thus be expected that SO_4^{2-} would bind at the N-terminus rather than the C-terminus, as also found in the x-ray crystal structure of melittin. In this x-ray crystal structure it was also observed that the SO_4^{2-} ion interacts with Arg24 (albeit on an adjacent melittin strand in the crystal structure). In the present case the strong SO_4^{2-} binding happens at the C-terminus due to the partial uncoiling. While anecdotal in itself this is in a noteworthy observation in that this may present a mechanism by which SO_4^{2-} may stabilize an α-helix.

			Coordination number		
Electrolyte	Concentration	Ion	**All**	Charged	Hydrophobic
TPA_2SO_4	0.1M	**TPA^+**	**3.07**	0.86	2.37
		SO_4^{2-}	**4.60**	4.26	0.66
GdmCl	3M	**Gdm^+**	**11.88**	3.46	6.49
		Cl^-	**8.97**	5.73	2.20

Table 1, the molecular coordination number of various ions around melittin in 0.1m TPA_2SO_4 and 3m GdmCl solution. In the current study the following definition is used. If any heavy atom of the ion is within 4.5 Å of a heavy atom of the peptide, then it is counted as being coordinated (note that this is somewhat different from the definition used in previous studies).[14] In each case the molecular coordination number is broken down into those within 4.5 Å of a charged group (Nter, Lys7, Lys21, Arg22, Lys23, Arg24) or a hydrophobic group (Ile2, Ala4, Val5, Leu6, Val8, Leu13, Ala15, Leu16, Ile17, Trp19, Ile20); these groups are not mutually exclusive (e.g. an ion can simultaneously be within 4.5 Å of both a charged and a hydrophobic group). It is also noteworthy that SO_4^{2-} at

0.1m has a larger outcome on the effective charge of the peptide than 3m Cl^- (i.e., at about 30x the SO_4^{2-} concentration).

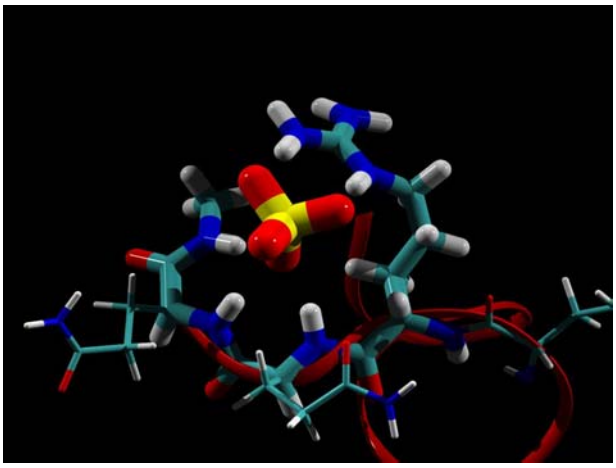

Figure 7, Strong SO_4^{2-} binding toward the sidechain of Arg24, the backbone NH groups of Gln25, Gln26, and the amidated group of the C terminus observed for the last 2 ns of the TPA_2SO_4-melittin simulation. This picture suggests that the double H-bond between the Gdm^+ side chain moiety of Arg and the SO_4^{2-} (similar to those previously seen in Gdm_2SO_4 solution) [16] and the three backbone NH H-bonds are the primary interactions that stabilize this sulfate-peptide binding. The strong association of sulfate with both Lys and Arg [21], as well as with ammonium ions[60,61], has also been observed in previous simulations.

Discussion

Ion pairing

The present results reinforce the previous earlier finding that internal charge distribution and ion geometry are significant factors in determining the position of molecular ions in the Hofmeister series. In previous simulations the Gdm^+ ion was found to display unexpected interactions, including face-to-face homo-ion stacking resembling hydrophobic pairing.[23] Unlike Gdm^+, however, TPA^+ does not have the ability to form

strong hydrogen bonds. Even though TPA^+ and Gdm^+ share the properties of being cationic molecular ions with flat faces, and are next to each other in the Hofmeister series as strong denaturants,[52] the ion-ion interactions in TPA^+ salts are completely different from those found in Gdm^+ salts.

Solutions of Gdm_2SO_4 show strong hetero-ion pairing leading to nanometer sized ion clusters,[16] while in the present MD simulations of TPA_2SO_4 no such clusters were observed. This clustering of guanidinium ions is primarily driven by the ability of the Gdm^+ ion to form strong double hydrogen bonds to the SO_4^{2-} ion, whereas there is a lack of a comparably strong interaction between TPA^+ and SO_4^{2-}. Ion pairing and clustering is a significant factor in the reversal of the denaturant potency of Gdm^+ by SO_4^{2-},[15] so that it would be expected that there would be no such reversal of the denaturant potency of TPA^+ by SO_4^{2-}.

Homo-ion pairing, such as that observed for Gdm^+ is weak at best due to the Coulomb repulsion, but it can be suggestive of how such ions can interact with other moieties. In contrast to the guanidinium case, there is a lack of homo-cation pairing in TPA^+ ions in TPA_2SO_4 (Figure 2). The TPA^+-TPA^+ g(r) both with melittin at 0.1m (not shown) and without at 0.5m (Figure 2)) are similar to those found in Monte Carlo simulations refined to neutron scattering results on a solution of TPABr at ~1.4m.[59]

Thus, there are two key differences between the Gdm^+ and TPA^+ ions. The first is that Gdm^+ (and also the guanidinium moiety in the side chain of arginine) has a higher charge density and more inhomogeneous charge distribution than TPA^+. The second difference is that the Gdm^+ ion possesses hydrogen bonding capacity, while TPA^+ does not. These factors explain why previous MD studies of Gdm_2SO_4, Na_2SO_4 and $(NH_4)_2SO_4$ have all shown strong ion-ion ordering of SO_4^{2-} mediated through the cation.[21,60,61] Similar effects have also been found for the CO_3^{2-} ion (for both Cs^+ and Gdm^+),[22,27] and it seems reasonable to assume that all divalent oxy-anions with relatively high charge density cations would display a similar behavior. By contrast, the present results show that the charge density of TPA^+ is so low that it cannot effectively bridge two sulfates, nor is it capable of bridging two sulfates by a hydrogen bonding.

Ion-protein interactions and protein stability

The degree to which the ion-ion interactions affect the properties of the electrolyte with respect to salting in/out or denaturation is rarely considered within Hofmeister series, where it is assumed that the cations and anions essentially act independently and additively.[63] Yet, ion-ion interactions in several salts (especially Gdm_2SO_4) have been observed experimentally to influence the stability of a peptide, which are either stabilized though cross strand indole-indole interactions (trpzip peptides) or backbone helical hydrogen bonds (alanine-based helical peptides).[15] Thus, the strong denaturant activity of GdmCl on each of these classes of peptides is either completely (trpzip peptides) or significantly (~50% for the helical peptides), attenuated in the Gdm_2SO_4 salt as a result of the strong hetero-ion (Gdm^+-SO_4^{2-}) interaction in solution, which tie up the Gdm^+ ions and make them unavailable for binding to peptide groups.[23]

The current study finds that TPA_2SO_4 is at the other end of the scale, where there is an absence of ion-ion interactions such that both ions interact independently with the polypeptide surface. In this case, the effects of the ions are largely additive, which is in the spirit of the Hofmeister series. We can, therefore, predict that not only TPACl but also TPA_2SO_4 should be a strong destabilizer of trpzip peptides, since sulfate should not suppress (via hetero-ion interactions as it does for Gdm^+) the denaturating activity of TPA^+ that is predicted from the strong TPA^+-indole interaction observed here.[15] Likewise TPA^+ is unlikely to compete for peptide backbone hydrogen bonds, and we predict that TPA^+ should not significantly destabilize alanine-based helical peptides. Thus, even though Gdm^+ and TPA^+ are next to each other in the Hofmeister series,[52] they are expected to have qualitatively different effects on the conformational stability of peptides chosen such as to dissect specific contributions (indole-indole interactions or hydrogen bonds) to non-covalent conformational stability in proteins. For clarity, the existing

experimental results and predictions based on the current simulations are summarized in Tables 2 and 3. Experiments to verify these predictions are in progress.

Peptide/primary stabilization	GdmCl	Gdm_2SO_4	Na_2SO_4
Trpzip/cross-strand indole interactions	Strong denaturant	No effect	No effect
Alahel/H-bond stabilized α-helix	Strong denaturant	50% of Gdm^+ activity retained	Weak non-linear stabilization

Table 2, The experimentally observed effects of various salts on the stability of a peptide (trpzip) mostly stabilized by cross strand indole (hydrophobic) interactions and a peptide mostly stabilized by hydrogen bonding (alahel).

Peptide/primary stabilization	TPACl	TPA_2SO_4
Trpzip/cross-strand indole interactions	Strong denaturant	Strong denaturant
Alahel/H-bond stabilized α-helix	Weak denaturant/stabilizer	Weak denaturant/stabilizer

Table 3, Predictions of the current study for the effects of various TPA^+ salts on the stability of the same peptides. This study predicts that TPA^+ and Gdm^+, which are near each other in the Hofmeister series,[52] will have strikingly different properties depending upon the counter anion, and whether the peptide involved is mostly stabilized by cross strand indole (hydrophobic) or H-bonding interactions.

The present results indicate that the ability of an ion to specifically compete with hydrogen bonding or hydrophobic interactions that stabilize proteins is a significant factor in determining the position of the ion in the Hofmeister series. At the same time,

interactions with counter-ions can be of significant importance. These results together with our previous calculations and experiments can be summarized to the following three points:

1. Ion pairing can be responsible for partially or totally reversing the denaturant potency of an ion. These experimental observations (Table 2) and testable predictions (Table 3) serve as a measure of the veracity of the hypothesis that ion-ion interactions play a significant role in the ordering of ions in the Hofmeister series. If these predictions are experimentally confirmed, then this has far-reaching implications for the concept of Hofmeister series. Namely, a series of ions (cations or anions) loses some of its meaning if the order of these ions changes depending on the counterion.

2. The effectivity of an ion to denaturate a protein and its position in the Hofmeister series is a complex result of the ability of the ion to disrupt H-bonding, non-polar interactions, and electrostatic effects that contribute to protein stability. This position may change depending on the extent that each category of interactions stabilizes a particular protein. While some ions such as Gdm^+ possess the ability to attenuate many or all of these interactions, the current study indicates that TPA^+ competes almost exclusively for hydrophobic interactions and is likely to be ineffective at influencing H-bonding. If confirmed experimentally, this suggest that the Hofmeister series could be assessed in more detail as the order in which the ions appear in the series will depend on the type of interaction stabilizing the protein in question.[64]

3. *The degree of ion association with the protein or peptide has also consequences for protein functionalities and salting out since the electrolyte perturbs the effective charge of the protein.* In GdmCl it was found that the melittin monomer had its effective charge increased from +6 to +9, while in a 0.1 M solution of TPA_2SO_4 the effective charge of the peptide was reduced from +6 to 0. The specific locations at which the ions interact with the protein will affect the charge density profile of the protein. For instance in TPA_2SO_4, an exposed Trp group is effectively changed from a hydrophobic group into a

cationic group, while a cationic Arg or Lys group is effectively changed into an anionic group due to the strong SO_4^{2-} association.

The above results and predictions are schematically depicted in Table 4, which provides a semiquantitative summary of this section.

Salt	GdmCl	Gdm_2SO_4	TPACl	TPA_2SO_4
Homo-cation pairing	○○○●●	Greatly reduced from GdmCl. Gdm^+-Gdm^+ ordering due to mediating SO_4^{2-} ions	○○○○○	○○○○○
Homo-anion pairing	○○○○○	○○○○○	○○○○○	○○○○○
Hetero-ion pairing	○○●●●	●●●●●	○○○○○	○○○○○
Interaction of cation with cationic groups (Lys, Arg)	○○○●●	○○○○●	○○○○○	○○○○○
Interaction of anion with cationic groups (Lys, Arg)	○○●●●	●●●●●	○○●●●	●●●●●
Interaction of cation with anionic groups	●●●●●	○●●●●	○○○○○	○○○○○
Interaction of anion with anionic groups	○○○○○	○○○○○	○○○○○	○○○○○
Interaction of cation with hydrophobic groups	○○○●●	○○○○●	○●●●● (particularly with Trp)	○●●●● (particularly with Trp)
Interaction of anion with hydrophobic groups	○○○○●	○○○○○	○○○○●	○○○○○

Table 4, A summary of the ion-ion and ion-protein interactions. The number of full circles represents the strength of the particular interaction for a given salt. Those shown in black have either been observed or implied from our previous studies, while those in blue are predictions based on the present simulations.

Conclusions

The results from the present MD simulations of TPA_2SO_4 solution with the model peptide melittin, when considered in a combination with our previous neutron scattering and molecular dynamics studies on various Gdm^+ electrolytes[16,23,27], MD studies of the

interactions of these salts with peptides,[14] and biochemical experiments on the effect of various Gdm^+ and SO_4^{2-} salts on the stability of model peptides,[15,64,65] have far reaching implications. In particular, these results suggest limitations of the classification of ions within the Hofmeister series due to the effect of counterions. In particular:

1) Hetero-ion pairing can be responsible for totally or partially inhibiting the denaturant power of a strongly denaturing ion such as Gdm^+,[15] while the lack of ion-pairing for TPA^+ preserves its denaturating ability.
2) The nature of the ion affects the mechanism by which it acts as a denaturant. For instance TPA^+ interacts strongly with indole groups and is predicted to be good at attenuating indole-indole/ hydrophobic interactions, but is ineffective at attenuating hydrogen bonding, while Gdm^+ is effective at attenuating both.
3) The degree to which ions bind to the protein surface changes the effective charge of the protein and the charge profile of the protein, with implications for protein functionality and salting out. For instance in the present study SO_4^{2-} sticks so effectively to the cationic peptides that even at 0.1m it effectively neutralizes the 6+ charge of the peptide.

Acknowledgment

We thank Prof. Kim Collins for valuable discussions. Support from Czech Ministry of Education (grant LC512) and the Czech Science Foundation (grant 203/08/1114) is gratefully acknowledged. The access to the METACentrum supercomputing facilities provided under the research intent MSM6383917201 is highly appreciated. J. H. thanks the Czech Science Foundation (grant 203/05/H001) and L.V. the Alexander von Humboldt Foundation for support. Part of the work in Prague was supported via Project Z40550506. This project was supported by grant GM63018 from the National Institutes of Health.

References

(1) Hofmeister, F. *Arch. Exp. Pathol. Pharmakol.* **1888**, *24*, 247-260.

(2) Kunz, W.; Lo Nostro, P.; Ninham, B. W. *Current Opinion in Colloid & Interface Science* **2004**, *9*, 1-18.

(3) Collins, K. D.; Washabaugh, M. W. *Quarterly Reviews of Biophysics* **1985**, *18*, 323-422.

(4) Cacace, M. G.; Landau, E. M.; Ramsden, J. J. *Quarterly Reviews of Biophysics* **1997**, *30*, 241-277.

(5) Baldwin, R. L. *Biophys. J.* **1996**, *71*, 2056-2063.

(6) Ninham, B. W.; Yaminsky, V. *Langmuir* **1997**, *13*, 2097-2108.

(7) Vrbka, L.; Vondrasek, J.; Jagoda-Cwiklik, B.; Vacha, R.; Jungwirth, P. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15440-15444.

(8) Ramos, C. H. I.; Baldwin, R. L. *Protein Science* **2002**, *11*, 1771–1778.

(9) Sakurai, K.; Oobatake, M.; Goto, Y. *Protein Science* **2001**, *10*, 2325-2335.

(10) Sharp, K. A.; Madan, B.; Manas, E.; Vanderkooi, J. M. *J. Chem. Phys.* **2001**, *114*.

(11) Soper, A. K.; Castner, E. W.; Luzar, A. *Biophysical Chemistry* **2003**, *105*, 649-666.

(12) Dixit, S.; Soper, A. K.; Finney, J. L.; Crain, J. *Europhysics Letters* **2002**, *59*, 377-383.

(13) Rezus, Y. L. A.; Bakker, H. J. *Physical Review Letters* **2007**, *99*.

(14) Mason, P. E.; Brady, J. W.; Neilson, G. W.; Dempsey, C. E. *Biophys. J.* **2007**, *93*, L4-L6.

(15) Dempsey, C. E.; Mason, P. E.; Brady, J. W.; Neilson, G. W. *J. Am. Chem. Soc.* **2007**, *129*, 15895-15902.

(16) Mason, P. E.; Dempsey, C. E.; Neilson, G. W.; Brady, J. W. *J. Phys. Chem. B.* **2005**, *109*, 24185-24196.

(17) Zhang, Y.; Furyk, S.; Bergbreiter, D. E.; Cremer, P. S. *J. Am. Chem. Soc.* **2005**, *127*, 14505 -14510.

(18) Zhang, Y.; Cremer, P. S. *Current Opinion in Chemical Biology* **2006**, *10*, 658–663.

(19) Pegram, L. M.; Jr, M. T. R. *J. Phys. Chem. B.* **2007**, *119*, 5411 -5417.

(20) Makhatadze, G. I.; Lopez, M. M.; III, J. M. R.; Thomas, S. T. *Protein Science* **1998**, 689-697.

(21) Vrbka, L.; Jungwirth, P.; Bauduin, P.; Touraud, D.; Kunz, W. *J. Phys. Chem. B.* **2006**, *110*, 7036-7043.

(22) Mason, P. E.; Neilson, G. W.; Dempsey, C. E.; Brady, J. W. *J. Am. Chem. Soc.* **2006**, *128*, 15136-15144.

(23) Mason, P. E.; Neilson, G. W.; Enderby, J. E.; Saboungi, M. L.; Dempsey, C. E.; MacKerell, A. D.; Brady, J. W. *J. Am. Chem. Soc.* **2004**, *126*, 11462-11470.

(24) Flocco, M. M.; Mowbray, S. L. *J. Mol. Biol.* **1994**, *235*, 709-717.

(25) Soetens, J. C.; Millot, C.; Chipot, C.; Jansen, G.; Angyan, J. G.; Maigret, B. *J. Phys. Chem. B.* **1997**, *101*, 10910-10917.

(26) Kumar, A. *Fluid Phase Equilibria* **2001**, *180*, 195-204.

(27) Mason, P. E.; Neilson, G. W.; Kline, S. R.; Dempsey, C. E.; Brady, J. W. *J. Phys. Chem. B.* **2006**, *110*, 13477-13483.
(28) Courtenay, E. S.; Capp, M. W.; Jr, T. M. R. *Protein Science* **2001**, *10*, 2485–2497.
(29) Biggin, P. C.; Sansom, M. S. P. *Biophys. J.* **2002**, *83*, 1867-1876.
(30) Kearney, P. C.; Mizoue, L. S.; Kumpf, R. A.; Forman, J. E.; McCurdy, A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 9907-9919.
(31) Arduini, A.; Pochini, A.; Secchi, A. *European Journal of Organic Chemistry* **2000**, 2325-2334.
(32) Benrraou, M.; Bales, B. L.; Zana, R. *J. Phys. Chem. B.* **2003**, *107*, 13432-13440.
(33) Osman, M. A.; Ploetze, M.; Skrabal, P. *J. Phys. Chem. B.* **2004**, *108*, 2580-2588.
(34) Jeffery, T. *Tetrahedron* **1996**, *52*, 10113-10130.
(35) Tsanov, T.; Vassilev, K.; Stamenova, R.; Tsvetanov, C. *Journal of Polymer Science Part a-Polymer Chemistry* **1995**, *33*, 2623-2628.
(36) Jiang, Y. Y.; Wang, G. N.; Zhou, Z.; Wu, Y. T.; Geng, J.; Zhang, Z. B. *Chemical Communications* **2008**, 505-507.
(37) Zhang, Z.; Yang, L.; Luo, S.; Tian, M.; Tachibana, K.; Kamijima, K. *Journal of Power Sources* **2007**, *167*, 217-222.
(38) Galinski, M.; Lewandowski, A.; Stepniak, I. *Electrochimica Acta* **2006**, *51*, 5567-5580.
(39) Zeisel, S. H.; Mar, M. H.; Howe, J. C.; Holden, J. M. *Journal of Nutrition* **2003**, *133*, 1302-1307.
(40) Furchgott, R. F.; Zawadzki, J. V. *Nature* **1980**, *288*, 373-376.
(41) Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872-879.
(42) Bremer, J.; Figard, P. H.; Greenberg, D. M. *Biochimica Et Biophysica Acta* **1960**, *43*, 477-488.
(43) Turner, J.; Soper, A. K. *J. Chem. Phys.* **1994**, *101*, 6116-6125.
(44) Turner, J. Z.; Soper, A. K.; Finney, J. L. *J. Chem. Phys.* **1995**, *102*, 5438-5443.
(45) Hrobarik, T.; Vrbka, L.; Jungwirth, P. *Biophysical Chemistry* **2006**, *124*, 238-242.
(46) Slusher, J. T.; Cummings, P. T. *J. Phys. Chem. B.* **1997**, *101*, 3818-3826.
(47) Liegl, B.; Bradl, S.; Schatz, T.; Lang, E. W. *J. Phys. Chem.* **1996**, *100*, 897-904.
(48) Kanno, H.; Ohnishi, A.; Tomikawa, K.; Yoshimura, Y. *Journal of Raman Spectroscopy* **1999**, *30*, 705-713.
(49) Buchner, R.; Holzl, C.; Stauber, J.; Barthel, J. *Physical Chemistry Chemical Physics* **2002**, *4*, 2169-2179.
(50) Manin, N. G.; Kustov, A. V.; Korolev, V. P. *Russian Journal of Inorganic Chemistry* **2004**, *49*, 115-120.
(51) Pieniazek, P. A.; Stangret, J. *Vibrational Spectroscopy* **2005**, *39*, 81-87.
(52) von Hippel, P. H.; Wong, K. Y. *Journal of Biological Chemistry* **1965**, *240*, 3909-&.

(53) Wang, J. M.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. *J. Comput. Chem.* **2004**, *25*, 1157-1174.
(54) Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. *J. Chem. Phys.* **1995**, *103*, 8577-8593.
(55) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *Journal of Computational Physics* **1977**, *23*, 327-341.
(56) Humphrey, W.; Dalke, A.; Schulten, K. *Journal of Molecular Graphics* **1996**, *14*, 33-&.
(57) Terwilligert, T. C.; Eisenbergg, D. *The Journal of Biological Chemistry* **1982**, *257*, 6016-6022.
(58) Brown, L. R.; Lauterwein, J.; Wüthrich, K. *Biochimica et Biophysica Acta* **1980**, *622*, 231-244.
(59) Polydorou, N. G.; Wicks, J. D.; Turner, J. Z. *J. Chem. Phys.* **1997**, *107*, 197-204.
(60) Smith, P. E. *J. Phys. Chem. B.* **1999**, *103*, 525-534.
(61) Gopalakrishnan, S.; Jungwirth, P.; Tobias, D. J.; Allen, H. C. *J. Phys. Chem. B.* **2005**, *109*, 8861-8872.
(62) Izatt, R. M.; Eatough, D.; Christen.Jj; Bartholo.Ch *Journal of the Chemical Society a -Inorganic Physical Theoretical* **1969**, 45-&.
(63) Celinski, S. A.; Scholtz, J. M. *Protein Science* **2002**, *11*, 2048-2051.
(64) Dempsey, C. E.; Piggot, T. J.; Mason, P. E. *Biochemistry* **2005**, *44*, 775-781.
(65) Dempsey, C. E.; Mason, P. E. *J. Am. Chem. Soc.* **2006**, *128*, 2762-2763.