Theoretical Studies of the Interaction of Water with Compensatory and Noncompensatory Solutes for Proteins

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The interaction of compensatory and noncompensatory solutes with water is investigated and analyzed by several computational methods. For a representative set of compensatory and noncompensatory solutes, calculations are reported for isolated molecule gas-phase structures, the structures of molecules with a single water molecule, and Monte Carlo simulations on the solutes in water. There does not appear to be a clear difference in their effect on water structure between compensatory solutes and urea, but there are distinct differences with the noncompensatory solute DMSO.

1. Introduction

A relatively small number of compounds (osmolytes) are used in nature to maintain osmotic equilibria. Those that do not disturb cellular structure and function are termed compatible solutes.[1−5] By contrast, urea is an efficient protein denaturant,[4−6] a noncompatible solute. Some compatible solutes enhance the stability of biological systems. Such solutes are called compensatory[1,4] or counteracting[2,5] solutes. Examples of nondenaturing solutes include sugars and other polyols, amino acids, amino acid derivatives, and methylamines. All compensatory solutes appear to have the following properties: they are very soluble, have no net charge, and do not interact with proteins. Compensatory solutes play an important part in allowing organisms to survive extreme conditions such as high salt conditions, high temperature, freezing, desiccation, and the presence of denaturants such as urea.[5−7] When many of the most common natural compensatory solutes are accumulated intracellularly (often over 100 mM), the stability of biological macromolecules actually increases. It is the physical basis of this compensation that is of interest here.

The manner in which compensatory solutes function is not well understood. Several models have been proposed to explain the action of compensatory solutes.[9] In the preferential exclusion model,[10] compensatory solutes are excluded from the hydration sphere of proteins, resulting in a preferential hydration of protein surfaces. In a model due to P. Wiggins,[11] two types of water structure are distinguished: dense water in the hydration sphere of proteins and less dense, bulk water which is preferred by the compensatory solutes. This suggests that the noncompensatory solutes change the water structure differently from the compensatory solutes. Wiggins[12] distinguishes between urea and betaine or TMAO, with urea preferring “low density” water and betaine and TMAO preferring “high density” water. Withers et al.[13] have noted differing roles of urea and TMAO in the buoyancy of elasmobranch fishes.

To understand the interaction of compensatory (or compatible) solutes with water, several theoretical calculations have been carried out that illustrate the variety of computational chemistry methods that can be applied to understand biological systems. The calculations are as follows:

(i) calculations of gas-phase structures which give information about the charge distribution and the dipole moments of the molecules;

(ii) calculations of the interaction energy of the solute molecules with a water molecule, which when compared with the water−water interaction energy, shows whether the H-bonds in water will be broken by the solute molecule; and

(iii) Monte Carlo simulations of a solute molecule in a box of water molecules, giving information on the modified structure of water caused by the solute molecule.

As representative compensatory solutes trimethylamine oxide (TMAO) $(CH_3)_3NO$, glycine betaine $(CH_3)_3NCH_2CO_2$, and dimethylthetin $(CH_3)_2SCH_2CO_2$ were chosen. As examples of noncompensatory solutes, urea $(NH_2)_2CO$ and dimethyl sulfoxide DMSO $(CH_3)_2SO$ were chosen. In making this selection of solute molecules, the classification of urea is problematic. The ability of urea to denature proteins has been attributed both to its effect on water structure and on specific binding to proteins.[14] However, the predominant denaturing effect is the interaction of urea with the polypeptide backbone of proteins.[2,15]

2. Theoretical Details

The calculations were performed using the GAUSSIAN 98 program.[16] Optimized structures were obtained at the HF/6-31G*, MP2/6-31G*, and B3LYP/6-31G* levels of theory. The Monte Carlo simulations were performed using the BOSS program.[17] The BOSS calculations followed the model dataset *ionwater* which involves 10^6 configurations of equilibrations followed by 2×10^6 configurations of averaging. The simulated temperature was 25 °C.

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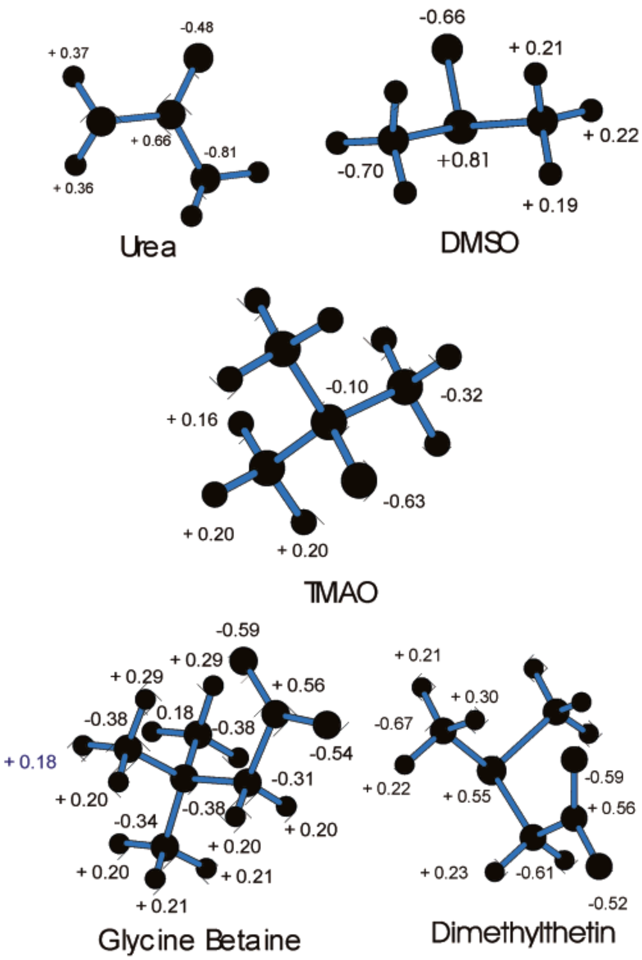

Figure 1. MP2/6-31G* structures and Mulliken charges for urea, DMSO, TMAO, glycine betaine, and dimethylthetin.

3. Results and Discussion

3.1. Gas-Phase Structures. For all five molecules chosen, calculations at various levels of theory have been reported previously. Haaland et al.[18] reported HF/6-31G(d) calculations on TMAO. Latajka and Ratajczak[19] reported B-LYP/6-31G* and HF/3-21G calculations on glycine betaine. Nyronen et al.[20] reported HF, MP2, and B3LYP calculations on glycine betaine and betaine monohydrate. In anhydrous betaine, they found three stationary states, with an intermediate state 16.7 kJ mol^{-1} above the ground state and the eclipsed transition state 40.5 kJ mol^{-1} above the ground state in MP4SDQ/6-311G(d,p)//HF/6-31+G-(d,p) calculations. Sironi et al.[21] reported HF/6-31++G(d,p) calculation on glycine betaine as part of their QM/MM molecular dynamic simulation study. Shikata[22] reported calculations at HF/6-31G** and MP2/6-31G** levels of theory for betaine and betaine monohydrate and dehydrate. Markham and Bock[23] reported HF/6-31G* structures of dimethylthetin with zero to four water molecules. Interaction energies were calculated at the MP2/aug-cc-pVDZ level of theory.

The structure of TMAO, glycine betaine, dimethylthetin, urea, and DMSO were determined at the HF/6-31G*, MP2/6-31G*, and B3LYP/6-31G* levels of theory. The calculations reported here are at the same level of theory for all molecular species. The structures are “gas-phase” structures but well approximate the structures in solution, unless, as for glycine, the solvent molecules play an important role in stabilizing a particular structure.[24,25] The structures of the five species studied are in Figure 1. The charges are Mulliken population analyses corresponding to the MP2 energy. Where comparison is possible, there are no unexpected differences between the results of our calculations and other calculations.

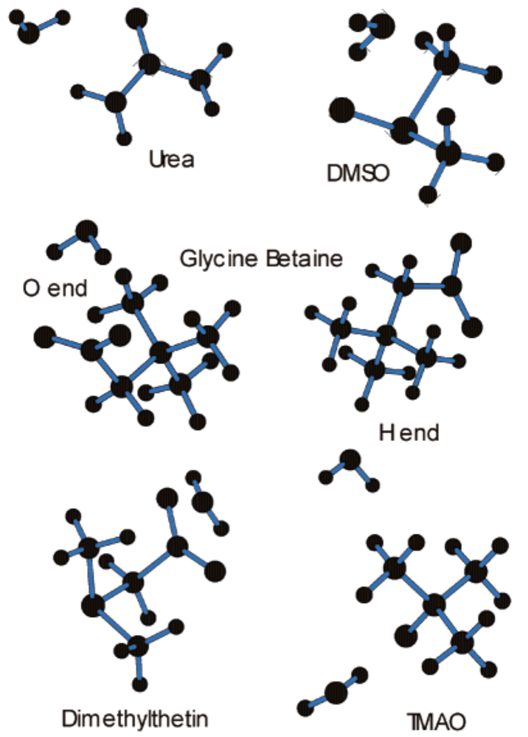

Figure 2. MP2/6-31G* solute−water structures.

TABLE 1: Calculated Dipole Moments (in Debye)

method	HF	MP2	B3LYP
TMAO	4.90	4.67	4.37
glycine betaine	11.97	11.18	10.85
dimethylthetin	10.16	9.24	8.78
urea	4.00	3.35	3.49
dimethyl sulfoxide	4.50	4.63	3.93

It is also possible to calculate molecular dipole moments from the electronic wave functions. All the compensatory solutes have the negatively charged end at the C═O/$-CO_2^-$ groups and the positively charged end on, for example, methyl groups. This is also true of DMSO and urea, but the dipole moments are smaller than most compensatory solutes. The calculated dipole moments, given in Table 1, for the nondenaturing solutes, are generally all large, about 10 D. These large dipole moments could be responsible for significant interactions with other molecules and with water molecules, perhaps altering the water structure. The dipole moment of TMAO is similar to urea and DMSO, mainly because of the size of the charge separation rather than the polarity of the molecule. The absolute values of the dipole moments are not our concern here, but rather the relative values for the two types of solute.

3.2. Interaction with a Water Molecule. While various properties of the isolated molecules, such as dipole moment or atomic charge, give a feel for the strength of interactions with water molecules, the strength of the water solute interaction must be calculated: The optimized structures of a water molecule bound to a solute molecule have been determined allowing determination of the enthalpy of association. The structures of the five solute−water structures are in Figure 2. Except for the MP2 structure for dimethylthetin−water, the structures are the same for all three methods used, with the MP2 parameters generally being between those from the HF and B3LYP calculations. A crystal structure of the glycine betaine−water

TABLE 2: Solute Water Interaction Enthalpies (in kcal mol^{-1}) at 298 K

	HF	MP2	B3LYP
TMAO	−10.66	−15.31	−13.88
glycine betaine (−CO_2)	−9.11	−13.88	−11.44
glycine betaine (−$N(CH_3)_2$)	−3.89	−5.74	−6.13
dimethylthetin	−9.77	−15.74[a]	−10.92
urea	−8.05	−11.38	−11.98
urea (−NH_2)	−9.72	−13.20	−12.96
dimethyl sulfoxide	−8.67	−11.89	−11.16
water	−4.08	−5.85	−6.13

[a] At this level of theory, in the minimum energy structure, the water is bound to two carboxyl oxygen atoms.

complex has been reported by Mak.[26] The enthalpy of association values in Table 2 show that the association energy is more exothermic than the water dimer energy, when the water molecule is bound to the carboxyl group. For glycine betaine, this does not occur for the hydrophobic methyl end; for urea, the association energy at the NH_2 end is more exothermic than both the water dimer and the C=O end. The association energies do not show the compensatory solutes to be different for the noncompensatory solutes which might be expected if there was a significantly stronger H-bond to the solute O atoms (See Figure 2 and Table 2). The enthalpies quoted were calculated without taking into account BSSE corrections. BSSE corrections are likely to be very similar in magnitude in all cases, being mainly due to contributions involving the solute O atoms and the bound water molecules.

3.3. Monte Carlo Simulations. To explore the effect of these molecules on water structure, a series of Monte Carlo statistical mechanics calculations were performed using Jorgensen's BOSS program.[17] Of interest is the way that the presence of a compensatory solute molecule perturbs the structure of water. A molecular dynamics study of the DMSO−water mixture has been reported by Vishnyakov et al.[27] Molecular dynamics studies of the TMAO−water mixture has been reported by Noto et al.[28] and Zou et al.[29] and of the glycine betaine−water mixture by Civera et al.[30] Simulations were used by Sharp et al. to study the effect of TMAO and urea on water structure.[31]

3.3.1. Parametrization. An important part of any simulation is the parametrization. The approach detailed by Duffy et al.[32] was used, in which ab initio HF/6-31G* calculations of the interaction energy between a solute molecule and a single water molecule for several representative water molecule positions/orientations around the solute molecule are calculated, and the set of interaction energies are fitted to the OPLS (optimized potentials for liquid simulations) potential by a least-squares procedure. The positions used for TMAO are in Figure 3. In the approach of Duffy et al.,[32] only one water molecule is bound to a particular site. Particularly at C=O and N=O sites, two interacting water molecules can bind. The interaction between the water molecules could alter the water−solute interaction energy by about 1 kcal mol^{-1}.[33] Parametrization calculations at higher levels of theory, such as MP2/6-31G* or B3LYP/6-31G*, could give better parameters, but for this study calculations using the level of theory used by Duffy et al.[32] were used to allow comparison with a previously published work. The OPLS interaction energy has the form:

$$\Delta E_{ab} = \sum\sum (q_i q_j e^2/r_{ij} + 4\epsilon_{ij}((\sigma_{ij}/r_{ij})^{12} - (\sigma_{ij}/r_{ij})^6)) \quad (1)$$

$$\epsilon_{ij} = (\epsilon_{ij}\,\epsilon_{ij})^{1/2},\ \sigma_{ij} = (\sigma_i\,\sigma_j)^{1/2} \quad (2)$$

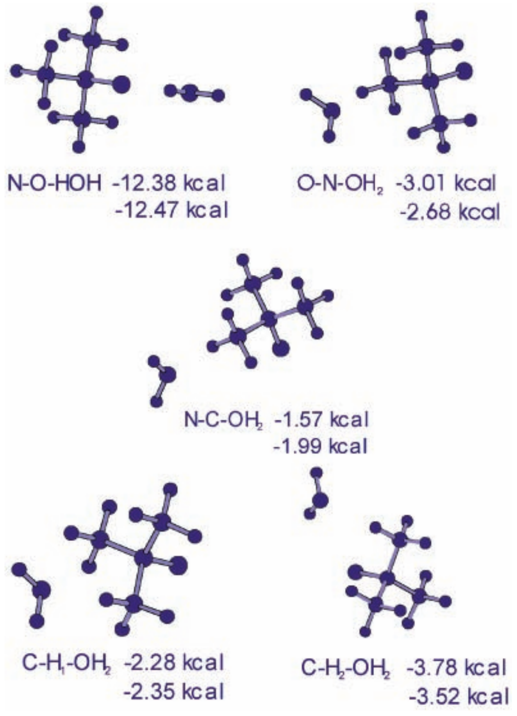

Figure 3. Positions/orientations used to determine atomic charges, and optimized empirical (upper) and ab initio (lower) interaction energies for TMAO.

The charge parameters q_I were optimized using a least-squares fit criterion while the Lennard-Jones parameters (ϵ_i, σ_i) were kept at their standard OPLS values as in the BOSS program. The potential-based charges calculated here differ from the wave function based Mulliken charges. The charges obtained from the potential-based approach are smaller in magnitude than the Mulliken charges. The greater bond polarization of the C−O bonds obtained with Mulliken charges can lead to the polarity of attached groups changing; for example, for DMSO the charge on the CH_3 group is −0.08 with the Mulliken charges but +0.165 using the Duffy et al.[32] procedure. Since the C−O bonds are not ionic, the smaller bond polarization is preferred. In Table 3 are given the ab initio and empirical solute−water interaction energies, the locations of the water molecules for each interaction calculation, and the standard deviations for the fits. The structures used for TMAO together with the ab initio and empirical solute−water interaction energies are in Figure 3. The standard deviations show that the ΔE(OPLS) calculations are not perfect but have the virtue of being based on a well understood level of theory. Other levels of theory are currently being investigated. The optimized parameters together with the Lennard-Jones parameters adopted are in Table 4.

3.3.2. Simulations. The model consists of a single solute molecule in a box of 512 water molecules. The water interactions use a TIP4P potential[34] which has been used to successfully model water properties.

To visualize the results, rather than use radial distribution functions, the nearest water molecule to an O atom or a H atom is found and the O−O distances from this water molecule to the closest 30 water molecules are calculated. The results of the five solute molecules are shown in Figure 4A. The order of closeness is plotted as a function of the distance from the closest water O atom. When there are more water molecules within a given radius, the local density of the water is greater. They indicate that for the nondenaturing solutes, except for the closest couple of water molecules, the water structure is very similar to pure water, but for the noncompensatory solutes there is a

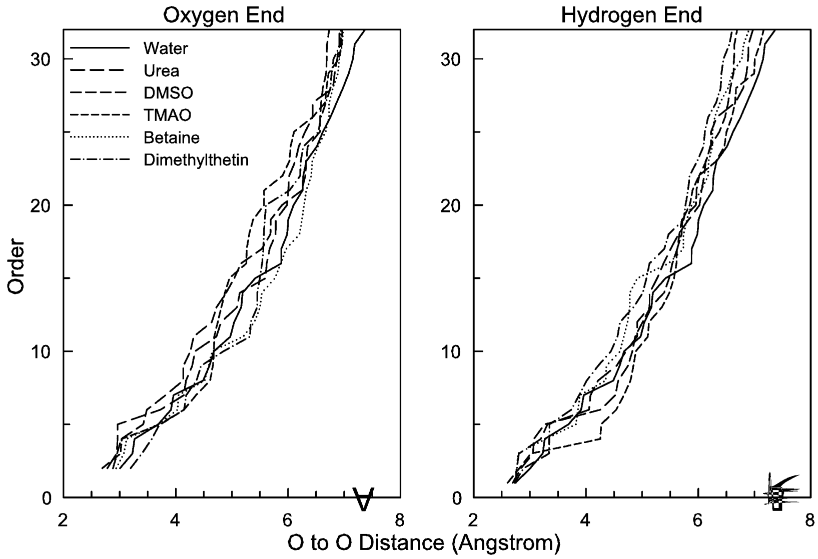

Figure 4. O−O distances (Å), in order, from oxygen atom of the water molecule closest to (A) an O atom of a solute molecule, (B) a H atom of a solute molecule.

TABLE 3: Ab Initio and Empirical Solute−Water Interaction Energies at 0 K

	position of H_2O	ΔE (HF/6-31G*)/ kcal mol^{-1}	ΔE (OPLS)/ kcal mol^{-1}	standard deviation
		TMAO		
1	$N-O-H_2O$	−12.376	−12.467	
2	$O-N-OH_2$	−3.014	−2.679	
3	$N-C-OH_2$	−1.574	−1.991	
4	$C-H_1-OH_2$	−2.279	−2.352	
5	$C-H_2-OH_2$	−3.775	−3.515	0.303
		glycine betaine		
1	$C-O-HOH$	−10.259	−10.737	
2	$C_1-C_2-H_2O$ Perp.	−7.000	−7.422	
3	$C_1-C_2-H_2O$	−11.166	−10.742	
4	C_1-H-OH_2	−5.809	−5.282	
5	$C_1H_2-OH_2$	−1.736	−2.071	
6	$C_{Me}-H_a-OH_2$	−4.536	−4.372	
7	$C_{Me}-H_b-OH_2$	−4.928	−4.317	
8	$N-C_{Me}-OH_2$	−3.738	−3.974	
9	$C-N-OH_2$	−5.021	−5.436	0.448
		dimethylthetin		
1	$C_1-C_2-H_2O$ Perp.	−10.590	−10.068	
2	$C-O-HOH$	−10.481	−10.881	
3	C_1-H-OH_2	−1.084	−2.117	
4	$C_1-H_2-OH_2$	−2.355	−1.418	
5	$C-S-OH_2$	−4.944	−4.952	
6	$S-C_{Me}-OH_2$	−2.483	−2.562	
7	$C_{Me}-H_a-OH_2$	−3.712	−3.572	
8	$C_{Me}-H_b-OH_2$	−3.734	−3.435	
9	$C_1-C_2-H_2O$	−6.559	−6.783	0.564
		urea		
1	$C-O-H_2O$	−7.329	−7.325	
2	$O-C-OH_2$	−4.792	−4.627	
3	$C-N-OH_2$	−1.984	−2.460	
4	$N-H_1-OH_2$	−5.084	−5.477	
5	$N-H_2-OH_2$	−5.217	−5.364	0.275
		dimethylsulfoxide		
1	$S-O-H_2O$	−5.689	−5.646	
2	$O-S-OH_2$	−2.779	−2.876	
3	$S-C-OH_2$	−1.296	−1.668	
4	$C-H_1-OH_2$	−2.107	−1.987	
5	$C-H_2-OH_2$	−1.738	−1.347	0.282

TABLE 4: Optimized Atomic Charges and Other OPLS Parameters

	q/e	s/Å^2	ϵ/kcal mol^{-1}
		dimethylsulfoxide	
O	−0.553	2.930	0.280
S	0.203	3.560	0.395
C	0.163	3.810	0.160
H	0.0007	2.500	0.015
		dimethylthetin	
C_1	0.175	3.750	0.210
C_2	0.467	3.500	0.066
S	0.282	3.550	0.250
H_1	−0.046	2.500	0.015
O	−0.534	2.960	0.210
C_{Me}	−0.476	3.500	0.066
H_{Me}	0.205	2.500	0.015
		glycine betaine	
C_1	0.166	3.750	0.210
C_2	0.033	3.500	0.066
N	0.363	3.250	0.170
H_1	0.042	2.500	0.015
O	−0.515	2.960	0.210
C_{Me}	−0.115	3.500	0.066
H_{Me}	0.0798	2.500	0.015
		TMAO	
O	−0.398	2.960	0.210
N	−0.797	3.250	0.170
C	−0.262	3.500	0.066
H	0.2177	2.500	0.030
		urea	
O	0.150	3.750	0.105
C	−0.396	2.960	0.210
N	−0.542	3.250	0.170
H	0.329	0.000	0.000

region where the water structure is more compact close to the O end of the solute molecule. For water, there are eight other water molecules within 4.5 Å of another water molecule. For the compensatory solutes, there are 7−8 water molecules, very similar to that for water, but for the noncompensatory solutes there are 10−11 water molecules less that 4.5 Å from the closest water molecule, an increase in density. These results are partly at variance with the predictions of the Wiggins model[11,12] which postulates a preference for bulk water structure by compensatory solute as found here, but postulates a preference for "low density" water by urea. The number of water molecules involved in these variations of density is significant, being three to four within 4.5 Å. While the bulk water density is not significantly different, as can be seen at R(O−O) = 7.0 A, the water density is significantly different close to the solute molecule for compensatory solutes, as compared with noncompensatory solutes. The results at the H end, in Figure 4B, do not distinguish two types of solutes. TMAO produces a different effect to betaine and dimethylthetin. In contrast to the O end, there are more water molecules within 4.5 Å of the nearest water molecule

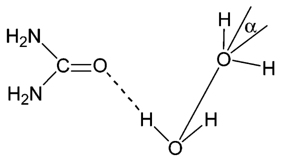

Figure 5. Angle α between an end atom of the solute and the bisector of the H−O−H angle of a water molecule.

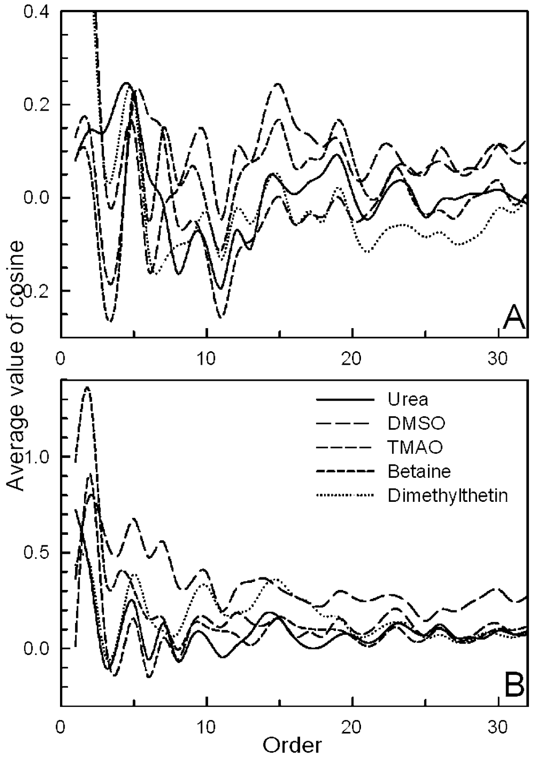

Figure 6. Cumulative average of the cosine of the angle between the position vector from the oxygen atom of the water molecule closest to (A) an O atom of a solute molecule and (B) a H atom of a solute molecule, and the bisector of H−O−H angle of water molecules, subtracted from the corresponding value for water, ordered by distance from the O atom of the closest water molecule.

adjacent to the noncompensatory solutes betaine and dimethylthetin than for water or urea and DMSO. Calculations, not reported here, with charges other than the optimized set, such as those from PM3 calculations, which are an option in the BOSS program, demonstrated that the results are very sensitive to the choice of parameters. The particular strength of the calculations reported in this paper is the use of consistent optimized parameters.

An alternative way to look at the effect of these solute molecules on the water structure is to examine the effect of the solute molecules on the orientation of the water molecules. To do this, the angle α between the oxygen atom of the water molecule closest to an end of the solute molecule (either O for the hydrophilic interactions or H for the hydrophobic interactions) and the water molecules and the bisector of the H−O−H angle of the surrounding water molecules (See Figure 5) was calculated. The average value of the cosine of this angle quickly averages to zero for pure water. The cumulative average of the cosine of this angle for the O ends, subtracted from the result for water, for clarity, is in Figure 6A. An average value of 0.2 corresponds to a deviation of about 3°.

Close to the hydrophilic (negative) end of the solute molecule, the value of cos α would be expected to be negative, with the H atoms of the water molecules closer to the solute molecule. Closer to the hydrophobic (positive) end, the value of cos α is positive with the water molecules closer to the solute molecule. This is what is observed for about the closest five water molecules. Beyond that, water molecules cannot remain both oriented and H-bonded together. The dipole field due to the H-bonded water molecules cancels the field due to the solute molecules as one moves further away from the solute molecule. For TMAO, the long-range values of cos α are positive, unlike the short-range values. The effect is more pronounced at the H end, as in Figure 6B, where all the compensatory solutes and urea quickly reach values close to the water value. The different behavior of urea compared to DMSO can be attributed to the strong bonding of water at the NH_2 end of urea. DMSO approaches the average value of zero quickly, but the cosine of the angle is consistently greater than that for water, being about 0.3 greater up to 7 Å. This different behavior is due to the hydrophilic character of the lone pair of the NH_2 groups. The shape of the hydrophobic curve of urea (Figure 6B) is closer to the shapes obtained for the hydrophilic interactions but the intensity of this curve is closer to the intensity of the hydrophobic interactions. Urea is therefore unique in its interactions with the water. Sharp et al.[31] found that TMAO gave smaller H-bond angles compared with water, unlike ionic solutes, whereas urea did not affect the H-bond angle.

4. Conclusions

The isolated molecule calculations suggest that the compensatory solute molecules, while uncharged, often have a large dipole moment, which could lead to the water structure close to the molecule being modified. However, smaller molecules such as TMAO are not much different for noncompensatory molecules such as DMSO. No distinction could be made between compensatory and noncompensatory solutes on the basis of solute−water interaction energies. Similarly, little difference was observed in Monte Carlo simulations, although over a small range of distances the noncompensatory solutes appeared to modify the water structure significantly leading to an increase in density, unlike the compensatory solutes. The orientation of the water molecules is not significantly different in the two types of solutes. Urea does not appear to act like other noncompensatory solutes such as DMSO. The results show that several different computational methods can, and should, be applied to problems such as understanding the mode of action of compensatory solutes.

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Supporting Information Available: Total energies and zero-point vibrational energies. This material is available free of charge via the Internet at http://pubs.acs.org.

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