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The hydrogen bond network structure within the hydration shell around simple osmolytes: Urea, tetramethylurea, and trimethylamine-*N*-oxide, investigated using both a fixed charge and a polarizable water model

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Despite numerous experimental and computer simulation studies, a controversy still exists regarding the effect of osmolytes on the structure of surrounding water. There is a question, to what extent some of the contradictory results may arise from differences in potential models used to simulate the system or parameters employed to describe physical properties of the mixture and interpretation of the results. Bearing this in mind, we determine two main aims of this work as follows: description of the water-water hydrogen bond network structure within the solvation layer around solute molecules (urea, trimethylamine-*N*-oxide, and tetramethylurea), and also comparison of rigid simple point charges (SPC) and polarizable (POL3) models of water. The following quantities have been examined: radial distribution functions of water molecules around the investigated solutes, both local and overall characteristics of the hydrogen bond network structure (using recently elaborated method), along with estimation of the mean energy of a single hydrogen bond, and also the probability distributions which describe the orientation of a single water particle plane relatively to the center of mass of the solute molecule. As an independent method for the evaluation of the degree of changes in local structural ordering, a harmonic approximation has been adopted to estimate the absolute entropy of water. It was found that within the solvation shell of the investigated solutes, the structure of hydrogen bond network changes only slightly comparing to bulk water. Therefore, we conclude that the investigated osmolyte molecules do not disturb significantly the structure of surrounding water. This conclusion was also confirmed by calculations of the absolute entropy of water using a harmonic approximation. In the immediate vicinity of the solutes, we observe that the water-water hydrogen bonds are slightly more stable; they are slightly less distorted and a little shorter than in bulk water. Nevertheless, although this local water structure is more stable and stiffer, our results do not indicate that it is more ordered compared to bulk. Finally, the comparison of both used models of water, the fixed charge and the polarizable, leads to unambiguous conclusion that rigid (SPC) water model may be successfully used in simulations instead of polarizable (POL3), as no significant differences between these two models have been observed. © *2010 American Institute of Physics*. [doi:10.1063/1.3464768]

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I. INTRODUCTION

Osmolytes are small organic molecules, the addition of which to solution dramatically affects the stability of dissolved proteins. There are two classes of osmolytes: urea, for example, is the well-known protein denaturant, which similarly acts tetramethylurea (TMU). On the contrary, trimethylamine-*N*-oxide (TMAO) is known to be a chemical chaperone and is able to correct protein-folding defects; it also counteracts the denaturant effect of urea. Mechanisms of both of these processes—denaturation and stabilization—are not fully recognized. In general, two possibilities are taken into account. One supposition is that osmolytes interact directly[1–4] by hydrogen bonding with the protein. Another possibility is that they act indirectly by altering the solvent (water) structure.[5–7] It should be noted, however, that there is no evidence for excluding any of these possibilities: certain

results emphasize direct mechanism, while other studies suggest the indirect one. It seems that both these explanations are not mutually exclusive and it is likely that at various stages of protein folding/unfolding process, various mechanisms are active. Therefore, investigation of the structure of water within the solvation layer is an important task. This fact has motivated our work.

The local properties of water within solvation shell of osmolytes, especially around urea molecule, were investigated extensively, using both experimental[8–11] and molecular dynamics methods.[6,7,11–16] However, in spite of many efforts, structure of water within this layer still arouses some controversies, and it is the case of both: experiments and molecular simulations.

To illustrate the problem, let us choose a few examples from recent works devoted to molecular simulations of urea. Stumpe and Grubmüller[17] stated that urea enhance water structure. Hua *et al.*[4] concluded that urea has negligible ef-

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fect on water local structure and dynamics. Lately, Idrissi et al.[15] reported that urea induces a distortion of the tetrahedral arrangement of water molecules.

It is not clear to what extent we can ascribe some contradictory statements found in the literature to differences in potential models applied to simulate the systems or to parameters used to describe physical properties of the mixtures and interpretation of the results. This is especially important when we deal with quite concentrated solutions of osmolytes, and that is the case of numerous simulations. Appropriate simulations of mixtures require more attention than simulations of simple liquids. For example Sokolić et al.[18] studied four different urea models combined with one water model and found that changing the parameters may lead to different results.

Herein, we focus our attention on the solvent and use two different water models while keeping parametrization of all three solutes the same. Furthermore, we analyze systems which can be called infinitely diluted solutions (containing only one solute molecule in each simulation box). It means that all possible interactions between solute molecules are deliberately not taken into account.

What is more is that the usefulness of some of the parameters frequently employed to describe the structure of solvation layer sometimes raises some doubts. As an example, we can mention radial distribution functions and popular order parameter for tetrahedral configurations introduced by Chau and Hardwick.[19] Athawale et al.[20] performed molecular dynamics simulations of TMAO in water and concluded that calculated radial distribution functions indicated enhanced structuring of water, while analysis of orientational parameter (slightly modified Chau and Hardwick's parameter) indicated something opposite. A bit similar conclusion concerning solution of urea was drawn by Mountain and Thirumalai.[21] They stated that examination of water pair functions showed that the structure of water was not significantly perturbed, and nevertheless could indicate that hydrogen bond network was a bit more structured than in pure water. Although calculations of distribution of cos θ (θ was defined as an angle between atom of water molecule connected with urea by hydrogen bond and two neighbor water molecules hydrogen bonded to it) pointed out, according to them, that there were great distortions in the hydrogen bond network of water near the urea molecule.

In our opinion, the structure of water may be, to some extent, identified with the whole structure of hydrogen bond network created among the water molecules. Therefore, in this paper we wish to report the results of investigations of such structure, applying recently elaborated method, used by us in a previous paper for different systems.[22]

II. METHOD

A. Molecular dynamics simulations

The simulations were carried out using the AMBER10 (Ref. 23) molecular dynamics package. The cubic box contained one solute molecule and 1000 of rigid simple point charge (SPC) or polarizable POL3 (Ref. 24) water molecules; the minimal distance of the solute molecule from the

box walls exceeded 1.25 nm. Approximate size of the boxes was $2.8 \times 3.4 \times 3.2$ nm^3 and varied only slightly between the systems. Besides the solute-water systems, systems containing only 1000 SPC or POL3 water molecules have been investigated; they have served as reference systems to determine the physical properties of both water models. The simulations of all systems were carried out using *NPT* conditions; temperature ($T = 298$ K) was kept constant by the weak coupling to an external bath ($\tau_T = 0.2$ ps); pressure ($p = 1$ bar) was kept constant, using the weak coupling method ($\tau_p = 0.5$ ps). Periodic boundary conditions were adopted. Every system was initially equilibrated for at least 2 ns; then the trajectory was written to a file for further analysis. The trajectory was written after each 8 fs, and the total simulation time equals to (6–8) ns for each system.

Parameters for solute molecules are collected in Tables I–IV. Parameters for urea had been developed for force field consistent with SPC model of water and the ones used in this paper were derived from Ref. 25. Parameters for TMU were taken from Ref. 26 (Lennard-Jones parameters and partial charges), Ref. 25, and using force field (43a1) of GROMOS.[27] Parameters for TMAO were adapted from Ref. 28.

TABLE I. Lennard-Jones parameters (σ, ε) and partial charges (q) of the atom types.

Compound	Atom type	σ (Å)	ε (kcal mol^{-1})	q (e)
Urea	C	3.7500	0.1050	0.1420
	O	2.9600	0.2100	−0.3900
	N	3.2500	0.1700	−0.5420
	H	0.0000	0.0000	0.3330
TMU	C	3.7500	0.1050	0.7954
	O	2.9650	0.1770	−0.6437
	N	3.2500	0.1270	−0.2961
	CH$_3$	3.7538	0.1280	0.1101
TMAO	N	2.9260	0.2000	0.4400
	O	3.2660	0.1526	−0.6500
	C	3.0410	0.0676	−0.2600
	H	1.7750	0.0185	0.1100

TABLE II. Harmonic bond stretching parameters: equilibrium distances (b_0) and force constants (K_b).

Compound	Bond	b_0 (Å)	K_b (kcal mol^{-1} Å^{-2})
Urea	C–O	1.265	501.0
	C–N	1.350	448.7
	N–H	1.000	446.9
TMU	C–O	1.265	501.0
	C–N	1.350	448.7
	N–CH$_3$	1.470	449.8
TMAO	N–O	1.407	171.290
	N–C	1.506	128.085
	C–H	1.082	295.480

TABLE III. Harmonic angle bending parameters: equilibrium angles (θ_0) and force constants (K_θ).

Compound	Angle	θ_0 (deg)	K_θ (kcal mol^{-1} deg^{-2})
Urea	O–C–N	121.4	60.00
	N–C–N	117.2	60.04
	C–N–H	120.0	34.88
	H–N–H	120.0	39.81
TMU	O–C–N	121.4	60.00
	N–C–N	117.2	60.04
	C–N–CH$_3$	120.0	34.88
	CH$_3$–N–CH$_3$	120.0	39.81
TMAO[a]	O–N–C	109.99	30.470
	C–N–C	108.16	68.850
	N–C–H	108.07	24.970
	H–C–H	108.25	27.435

[a]For additional Urey–Bradley parameters, see the Ref. [28].

Whenever it was necessary, force constants were recalculated according to instructions in Ref. [27] to fit in potential of AMBER, where the force field has the following form:[29]

$$U = \sum_{b_{ij}} K_{b_{ij}}(b_{ij} - b_{ij}^0)^2 + \sum_{\theta_{ijk}} K_{\theta_{ijk}}(\theta_{ijk} - \theta_{ijk}^0)^2$$
$$+ \sum_{\varphi_{ijkl}} \frac{V_n}{2}[1 + \cos(n\varphi_{ijkl} - \gamma_n)]$$
$$+ \sum_{i<j}^N \left\{ 4\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^6 \right] + \frac{q_i q_j}{r_{ij}} \right\}. \quad (1)$$

In the expression presented above, r is the distance between atoms, b, b_0, θ, θ_0, and φ are bond length, bond angle (actual and equilibrium, respectively), and dihedral angle. K_b, K_θ, and V_n are the force constants for the bond, bond angle, and dihedral angle. Phase angle γ takes values of either 0° or 180°. As long as the nonbonded part of the potential is concerned, Lennard-Jones parameters are represented by σ and ε, and partial charges by q.

The equation, in case of simulation with POL3 water model, is extended with term describing the polarization energy[23,24]

$$U_{\text{pot}} = -\frac{1}{2}\sum_i (\vec{\mu}_i \vec{E}_i^0), \quad (2)$$

where $\vec{\mu}_i$ is the induced dipole moment of atom i and $\vec{E}_i^0$ is the local electrostatic field due to charges. Molecule of water

TABLE IV. Torsional parameters: force constants ($V_n/2$), phase angles (γ), and multiplicity (n). Parameters for torsional dihedral angles and improper dihedral angles for urea and TMU are standard parameters present in applied AMBER force field (ff02pol.r1).

Compound	Dihedral	$V_n/2$ (kcal mol^{-1})	γ (deg)	n
TMAO	O–N–C–H	0.27	0	3
	C–N–C–H	0.27	0	3

POL3 is regarded as rigid, with geometry of SPC water and polarizabilities α_i placed at the nuclei of the atoms.

The induced dipole moments $\vec{\mu}_i$ are determined by the local electrostatic field $\vec{E}_i^0$, which is a sum of two terms: local electrostatic field due to permanent charges and local electrostatic field due to induced dipoles. The following equation is fulfilled:

$$\vec{\mu}_i = \alpha_i \vec{E}_i = \alpha_i\left[\vec{E}_i^0 + \sum_{j\neq i} T_{ij}\vec{\mu}_i \right], \quad (3)$$

where T_{ij} is the dipole tensor. To find the induced dipole moments, we solve the equation iteratively at each dynamics step.

B. Hydrogen bond analysis and structure of hydrogen bond network

An important factor which determines structural ordering in liquid water is, of course, the hydrogen bond network. Thus, we take into account both the geometry of the water-water hydrogen bond network and the mean geometry of a single bond.

In the computer simulation studies two definitions of hydrogen bond are commonly applied: the energetic[30,31] one and the geometric[31,32] one. Moreover, recently Wernet *et al.*[33] proposed a relatively generous "cone" criterion for defining such bond. Although various criteria are used in the literature, it should be noted here that selection of definition of hydrogen bond is not of primary importance, as long as it is physically reasonable. As it was shown by Luzar,[34] the functional form of probability distribution of hydrogen bond lifetimes is independent on the definition of hydrogen bond used for calculations. This means that although absolute values of various parameters (such as time constants) are, of course, model dependent, relations between these parameters do not depend on the model used.

In this work we adopt the Wernet *et al.*[33] definition because it agrees with the x-ray measurements of local water structure. According to this definition, the hydrogen bond between two water molecules is formed if the oxygen-oxygen distance fulfills the relation

$$R_{\text{OO}} \leq -0.000\,044\beta_{\text{OOH}}^2 + 0.33 \;[\text{nm}], \quad (4)$$

where β_{OOH} is the O–O–H angle (in degrees).

For SPC model of water, we also calculate the total interaction energy between two "hydrogen-bonded" water molecules. This quantity has been calculated as the sum of both Lennard-Jones and electrostatic terms between two molecules for which relation (4) is fulfilled; its absolute value, averaged over all H-bonded molecules, was assumed to be the mean H-bond energy of the system. For polarizable (POL3) water model, however, the calculations of an electrostatic term were not conducted because in this case the dipole moment of water molecule is not constant. Therefore, for this model the mean interaction energy has not been calculated.

To analyze the properties of a single hydrogen bond, we take into account the probability distributions of the β_{OOH}

angle and the oxygen-oxygen distance R_{OO}. All of these properties have been determined within the first solvation layer, and also for pure water.

To describe the overall hydrogen bond network structure, we wish to analyze the tetrahedral arrangement of such bonds, and to this purpose, we have modified Chau and Hardwick's[19] idea. To describe local water ordering these authors have introduced the well-known "tetrahedral ordering parameter:" after selection of four nearest oxygen atoms which surround a tagged "central" water molecule, they calculate six angles between the vectors which link the selected atoms with the central water molecule oxygen atom. In our present and previous[22] work, this idea is modified as follows. Investigating a single snapshot of trajectory file, we take into account—for a tagged central water molecule—all the water molecules which are bonded (by the hydrogen bond) with the central one; let us notice that the number of such partners, of course, is not fixed, unlike in Chau and Hardwick's approach. Next, we calculate—similarly as it has been proposed by Chau and Hardwick—all the angles between the vectors linking the oxygen atom of the central water molecule with the oxygen atoms of all its partners. Adopting this procedure to all water molecules, and repeating it over the whole trajectory file, we build histogram describing the probability distribution for such angles. The obtained histogram has been normalized, assuming a total area enclosed by the graph equals to 100. This histogram reflects (geometrical) structure of the hydrogen bond network. The reason for changing the original definition by Chau and Hardwick's was that it may not prove useful when applied to water in hydration layer. In the nearest neighborhood of the solute, four water molecules closest to the central one practically must be arranged differently than in bulk. This difference is not necessary caused by the change of the structure of water itself, but because of the fact that some part of the space is occupied by the solute molecule and therefore is inaccessible for water or because of hydrogen bonding of water to solute molecule.

III. RESULTS AND DISCUSSION

There are two main aims of this work: description of the water-water hydrogen network structure within the solvation layer around solute molecules, and also comparison of rigid (SPC) and polarizable (POL3) models of water. Thus, all the obtained results are presented for both these models.

At the beginning, we calculated the radial distribution function of water molecules (as their center of mass) around the center of mass of the solute. It allowed us to determine the radius of the solvation layer around this particle. Results of these calculations are presented in Fig. 1. Shapes of the curves are determined by the fact that shapes of solute molecules are not spherical and different from each other. According to the presented graph, the radius of solvation layer, R_{sol}, is estimated to be equal to 0.60 nm for urea, 0.70 nm for TMU, and 0.65 nm for TMAO. We have examined properties of water beyond this limit and found that various physical quantities differ only negligibly from the ones determined for pure water. Thus, we take into account only the water mol-
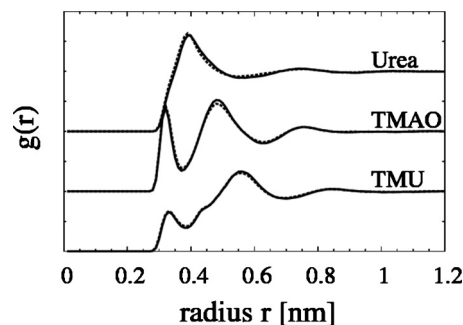

FIG. 1. (a) The radial distribution functions, describing distribution of water molecules (as the center of mass) around the center of mass of the osmolyte molecule. The dashed line represents the SPC water model, while the solid one represents the polarizable (POL3) model of water.

ecules present within the sphere of radius R_{sol} around the solutes, and the properties of hydrogen bonds created by these molecules are analyzed.

The average geometry of a single hydrogen bond is characterized by mean values of the angle β_{OOH} and the oxygen-oxygen distance R_{OO}, and they are collected in Table V. For comparison purposes, the values calculated for bulk (pure) water are included in this table, too. As it can be seen, the mean values calculated within solvation shell deviate from the bulk ones only slightly. A similar behavior of water in the solvation shell has been observed previously for almost the same[11] and some other[22,35] systems. It suggests that structure of hydrogen bond network within the solvation layer remains almost unperturbed compared to bulk water. Analysis of Figs. 2 and 3, showing the deviations in probability distributions of hydrogen bond angles and oxygen-oxygen distances between the first solvation shell and bulk water, also supports the same supposition. The presented deviations are small; they do not exceed a few per cent of the histogram values. It can be noticed, though, that the direction of these changes is the same in every case: for all investigated systems and both water models. Hydrogen bonds in solvation shells are slightly shorter and more linear, what is accompanied with a little higher average energy of the bond.

To describe overall properties of hydrogen bond network, we adopt the procedure described in Sec. II. Results of our calculations are presented in Fig. 4, in the form of differences between the probability distribution of tetrahedral angles for water within solvation layer and the bulk one. For comparison purposes, the distribution for bulk water is presented in this figure, too. Note that this histogram confirms roughly tetrahedral structure of hydrogen bond network in pure water, as it was reported previously—see Fig. 3 in Ref. 36—with the angle between adjacent bonds equal to 109° approximately. Two important deductions can be outlined from this figure. First, the most visible fact is that the angle distribution for hydration water remains nearly unaltered comparing to bulk water. Some small differences do not exceed a few percent. It confirms our above expressed supposition that structure of "solvation" water around investigated molecules (TMAO, TMU, and urea) differs only slightly from the bulk one. In other words, geometry of the water-water hydrogen bond network within solvation layer is very similar to the geometry of this network in the bulk water.

TABLE V. Average structural properties of water-water hydrogen bonds within the solvation layer, determined around various osmolyte molecules. $\overline{E_{HB}}$, $\overline{n_{HB}}$, $\overline{R_{OO}}$, and $\overline{\beta_{OOH}}$ represent the mean energy of the water-water hydrogen bond, the mean number of hydrogen bonds per water molecule, the mean oxygen-oxygen distance and the mean O–O–H angle [Eq. (1)], respectively. R_{sol} symbolizes radius of solvation shell, determined from the radial distribution function (Fig. 1).

Model system		$\overline{E_{HB}}$ (kJ/mol)	$\overline{n_{HB}}$	$\overline{R_{OO}}$ (nm)	$\overline{\beta_{OOH}}$ (deg)
TMAO (R_{sol}=0.65 nm)	SPC	18.413	2.986	0.2827	12.61
	POL3	$(\cdots)$ [a]	3.073	0.2802	11.61
TMU (R_{sol}=0.7 nm)	SPC	18.452	2.985	0.2828	12.60
	POL3	$(\cdots)$ [a]	3.072	0.2802	11.61
Urea (R_{sol}=0.6 nm)	SPC	18.312	2.912	0.2827	12.74
	POL3	$(\cdots)$ [a]	3.059	0.2802	11.67
Bulk water	SPC	18.269	3.058	0.2829	12.77
	POL3	$(\cdots)$ [a]	3.152	0.2804	11.73

[a]For POL3 water model the energies $\overline{E_{HB}}$ have not been calculated—see Sec. II.

However, we observe some systematic deviations on the graphs representing the differences between the calculated probability distributions for water within solvation layer, P_{solv}, and the ones calculated for bulk water, P_{bulk}. Second, although observed changes are, in terms of quality, very similar, some quantitative differences between investigated osmolytes are visible: urea disturbs the hydrogen bond network less than TMU and TMAO. Note that a glance on Fig. 2, and searching the last column of Table V, also allows us to discern the same tendency. The fact that all differences are similar when it comes to their direction is inconsistent with one of the recent papers by Wei *et al.*[16] They concluded that urea practically does not affect the structure of the hydrogen bond network, TMAO seems to be a "structure maker," while TMU can be classified as a structure breaker. It should be added, however, that they used quite concentrated solutions of osmolytes, while we analyzed infinitely diluted solutions.

As the last analyzed quantity, we take into account the probability distribution of the angle α between two vectors:
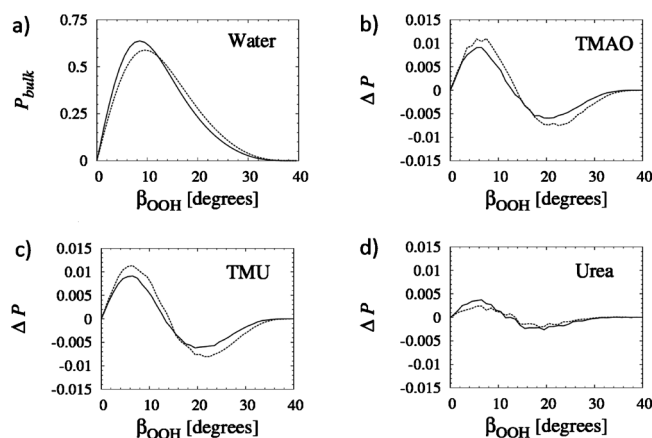
the one linking the center of mass of a tagged water molecule and the center of mass of the solute, and the second one, which is normal to the H–O–H plane of this water particle. This distribution describes the orientation of the water particle plane relatively to the center of mass of the solute molecule. Calculated histograms are presented in Fig. 5. Looking at this figure, we again conclude that urea disturbs the structure of surrounding water the least in comparison with other osmolytes.

In the case of urea, our results agree with some of the literature data. It is said that urea "fits" well into the water structure because it has the right size to be able to substitute for water dimer in the hydrogen bond network.[37] This supposition has been supported using both molecular dynamics[17] and experimental methods.[9] Urea amine and carbonyl groups can serve as donors and acceptors of hydrogen bonds and there might occur specific "water bridges" between the carbonyl oxygen and the *cis* hydrogen of the urea NH$_2$ group.[9] Similarly as it was reported previously,[17] we

FIG. 2. (a) The probability distribution P_{bulk} of the hydrogen bond angle β_{OOH} in bulk water. [(b)–(d)] The differences ($\Delta P = P_{solv} - P_{bulk}$) in probability distributions of the hydrogen bond angle β_{OOH} between the first solvation shell and bulk water. The dashed line represents the SPC water model, while the solid one represents the polarizable (POL3) model of water.
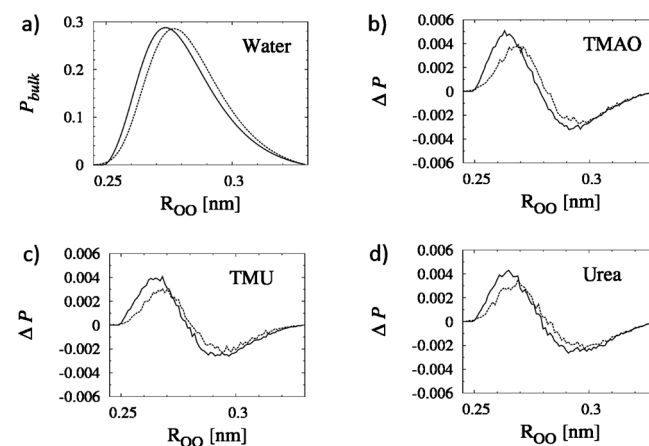

FIG. 3. (a) The probability distribution P_{bulk} of the oxygen-oxygen distance R_{OO} in bulk water. [(b)–(d)] The differences ($\Delta P = P_{solv} - P_{bulk}$) in probability distributions of the oxygen-oxygen distance R_{OO} between the first solvation shell and bulk water. The dashed line represents the SPC water model, while the solid one represents the polarizable (POL3) model of water.
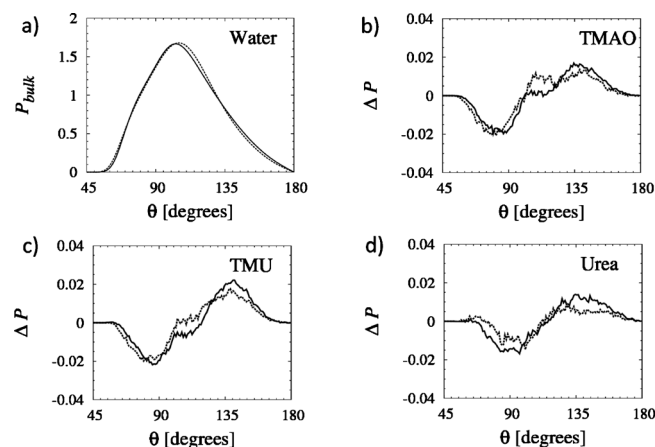
FIG. 4. (a) The probability distribution P_{bulk} of tetrahedral angles θ (see text) in bulk water. [(b)–(d)] Presentation of the differences ($\Delta P = P_{solv} - P_{bulk}$) between the probability distribution of such angles within the solvation layer, P_{solv}, and bulk water. The dashed line represents the SPC water model, while the solid one represents the polarizable (POL3) model of water.

have also found a slight increase of the water-water hydrogen bond energy (for the bonds within the solvation shell of urea molecule). Stumpe and Grubmüller[17] concluded that urea strengthens water structure; a similar conclusion has also been derived earlier by Idrissi et al.,[13] from analysis of the velocity autocorrelation function. However, as it has been pointed out previously, this is not a universal point of view.

It should be noted here that such effects—slight increase of the water-water hydrogen bond energy, and increased stiffness of local water structure around the solute molecule—have been observed not only around other osmolytes (see Table I), but also around some large solutes.[22,35,38] Thus, in our opinion, the increased rigidity of local water structure within the hydration shell of solutes is rather some general property, which arises as a consequence of both the orientational effects, and the slowing down of water dynamics within this layer.[22]

Both TMAO and TMU molecules disturb the water-water hydrogen bond structure in a very similar manner: the water-water hydrogen bonds within solvation shell are slightly less distorted (see Fig. 2), and O–O distance becomes shorter (Fig. 3). We also observe similar increase
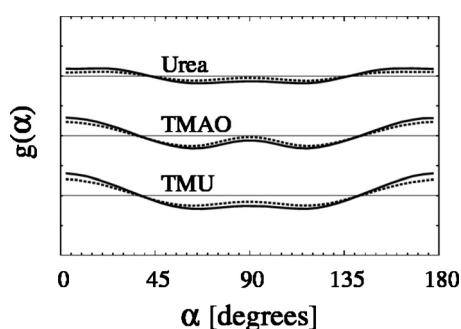

FIG. 5. The probability distribution of angle α, describing orientation of H–O–H plane of water molecules within the solvation shell relative to the center of mass of the solute molecule (see text). Similarly as in other figures, the dashed line represents the SPC water model, while the solid one represents the polarizable (POL3) model.

(comparing to bulk water) of the hydrogen bond energy: ~0.15 kJ/mol for TMAO and ~0.18 kJ/mol for TMU, while for urea this difference equals to ~0.04 kJ/mol only. The experimental and computational studies of other authors also indicate that TMAO enhances local water structure.[6,7] This special arrangement of water around TMAO is sometimes ascribed to its semispherical symmetry with four methyl groups and only one oxygen, which can serve as an acceptor for quite long-lived hydrogen bonds.[6,11] However, to make the picture more complicated, Rezus and Bakker[8] speculated that polar NO group of TMAO may locally increases the density of network defects, like, for example, five-coordinated water molecules. The reason for this may be that oxygen in TMAO molecule contains three lone pairs, which can accept more than two hydrogen bonds and in this way disrupt local tetrahedral hydrogen bond network. Rezus and Bakker[8,10] in their studies showed that around both TMAO and TMU particles, two kinds of water molecules are present: some of them displays bulklike dynamics, while others are "immobilized," display much slower orientational dynamics, and the immobilization of water molecules arises around hydrophobic CH_3 groups because of a steric effect.[10] A detailed explanation of this phenomenon is given in the paper of Laage et al.,[14] where authors, invoking into the excluded volume concept, adopt their jump model describing the hydrogen bonds exchange process. On the other hand, both these molecules (TMAO and TMU) show dual, hydrophobic and hydrophilic, character. Therefore, their solvation picture is determined by the presence of hydrophobic group (CH_3), balanced by the hydrophilic character of the oxygen atom.[20] This is why the local water structure is so similar in both cases. Moreover, slowing down of the rotational motions of water molecules around hydrophobic groups causes the strengthening of their positions, which is clearly visible in Fig. 5.

IV. CONCLUDING REMARKS

As it was mentioned in Sec. I, it seems that the water structure may be, at least to some extent, identified with the whole structure of hydrogen bond network created among the water molecules. We were led to this conclusion by our previous[36] results, concerning the entropy calculations of pure water. We keep in mind that entropy is the most appropriate measure of overall structural ordering. In the cited work[36] the interesting, almost linear correlation between the mean number of the hydrogen bonds (per water molecule) and two-particle contribution to entropy has been found.[36] Moreover, our other (still unpublished) results also strongly suggest the existence of such correlation for water within solvation shell around some peptides. Also analyzing the structural properties of "solvation" water around simple polypeptides, it was found that small structural changes of such water correspond with small changes in structural properties of the hydrogen bond network.[22] If so, describing the structure of such hydrogen bond network for water within the solvation layer, and comparing it with the one for bulk water, seems to be a rational approach to the problem of structural changes in the solvent present in the vicinity of the

TABLE VI. Calculated (for the investigated systems) differences in the "harmonic" entropy, S_H, (determined using Henchman's method (Refs. 40 and 41), and recalculated per one water molecule) between solvation water and the bulk one: $\Delta S_H = (S_H)_{\text{bulk}} - (S_H)_{\text{solv}}$. Presented values represent averages over simulation run of 2.5 ns. long. All the values are given in [J/mol K] units.

	Water model	SPC	POL3
TMAO	$\Delta S_H=$	0.37 ± 0.10	0.30 ± 0.10
TMU	$\Delta S_H=$	0.01 ± 0.10	-0.11 ± 0.10
Urea	$\Delta S_H=$	-0.13 ± 0.10	-0.25 ± 0.10
Bulk water entropy,	$S_H=$	70.54 ± 0.03	66.86 ± 0.03

solute. Our results indicate that observed differences are relatively small. Thus, it can be outlined that structure of solvation water around all the investigated osmolytes differs only slightly from the bulk one. However, such a strong-minded conclusion seems to be controversial. Therefore, any independent method of the estimation of local water structural ordering (within solvation shell) is highly desired, and such a method is undoubtedly the calculation of the entropy. Recently Henchman[39,40] proposed an elegant and simple method for estimation of the absolute entropy of pure water, using the harmonic approximation. This method bases on the calculation of the mean force (or torque) acting on a single water molecule, and therefore it is also sensitive to water structure. After a slight modification of this procedure,[41] we adopt it to the estimation of the absolute entropy of water within the solvation shell, and the results of our calculations are included in Table VI. It should be noted, however, that using this method may rise some doubts. For the water molecules within the solvation layer (near the peptide surface), their environment is anisotropic, and therefore the results of the entropy calculations using Henchman's method may not be fully correct. However, in spite of doubts expressed above, results of entropy calculations also indicate that structure of water within solvation layer remains nearly unchanged, and it amazingly agrees well with all our previous conclusions. Therefore, in our opinion, such calculations confirm all our previous deductions. This indicates that investigated osmolyte molecules fit very well into the structure of surrounding water. In the immediate vicinity of the solutes we observe that the water-water hydrogen bonds are slightly more stable; they are less distorted and a little shorter than in bulk water. Note again that although this local water structure is more stable and stiffer, our results do not indicate that it is more ordered comparing to bulk.

The second aim of the paper is a comparison of two water models. It seemed to be obvious that the hydrogen bond network structure within solvation layer should be sensitive to the choice of parameters describing the water molecule (model). However, as it can be seen from all of the figures presented in this paper, there are no very significant differences between both the fixed charge and the polarizable models of water. What is more is that when there are small differences between bulk water and solvation layer, the direction of these changes is the same for both models. Therefore, our second conclusion is that SPC water model, one of the most popular rigid ones, may be successfully used in

simulation of aqueous solutions of small osmolyte molecules (TMAO, TMU, and urea) instead of polarizable POL3 model, at least for description of structural properties of hydration layer. This is an important observation because replacing the polarizable model with the rigid one allows us to shorten the time of simulation significantly (using a polarizable model consumes roughly six times more of computer time). However, this conclusion concerns only two investigated models: SPC and POL3, and we do not know, of course, how more sophisticated polarizable models would work.

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