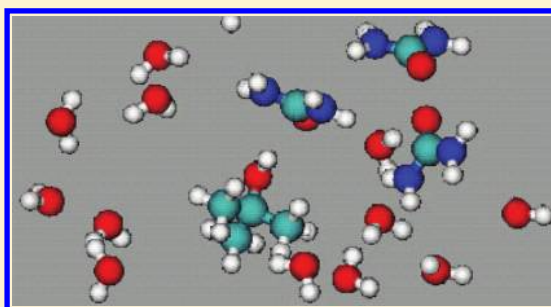


# Influence of Urea on *tert*-Butyl Alcohol Aggregation in Aqueous Solutions

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**ABSTRACT:** Ternary solutions consisting of urea, *tert*-butyl alcohol (TBA), and water are investigated employing molecular dynamics simulations. The main purpose of the present paper is to investigate the effect of urea on TBA aggregation and by extension its influence on hydrophobic interactions. The aggregation of TBA can be detected from the concentration dependence of structural properties such as first-shell TBA-water coordination numbers and TBA-TBA hydrogen-bond numbers, as well as through changes in the translational diffusion coefficients of TBA. It is found that urea acts to delay the association of TBA to concentrations greater than those required to cause TBA aggregation in binary TBA-water systems. It is shown that urea acts through a direct mechanism, whereby it preferentially binds to TBA replacing water from the first coordination shell. TBA-urea hydrogen bonds can be as strong as, or stronger than, those of TBA-water, and urea binds to both the hydrophilic and hydrophobic moieties of TBA. Our observations are qualitatively consistent with experimental results for urea-TBA-water solutions and with recent simulation studies of urea's action as a protein denaturant.



## I. INTRODUCTION

Aqueous solutions of alcohols are of long-standing interest due to their often unusual physical properties and to their practical importance as solvents for many chemical applications.<sup>1–4</sup> Because alcohols are amphiphilic molecules possessing both hydrophilic hydroxyl and hydrophobic alkyl groups, their aqueous solutions sometimes exhibit apparently anomalous behavior arising from the subtle balance between the hydrophilic and hydrophobic interactions.<sup>3–19</sup> In general, the anomalous features observed in alcohol–water mixtures can be related to some form of solute aggregation, driven mainly one would expect by hydrophobic interactions. Among the simple monoalcohols that are water-miscible in all proportions, *tert*-butyl alcohol (TBA) exhibits the largest anomalies, likely due to the large size of its hydrophobic moiety. There is a good deal of experimental,<sup>3–16</sup> computer simulation,<sup>20–24</sup> and theoretical<sup>25</sup> evidence showing that TBA molecules tend to aggregate in dilute aqueous solutions.

Aqueous urea solutions are of interest mainly because of the influence urea has on the properties of multicomponent solutions that contain other solutes in addition to urea. For example, urea can increase the solubility of hydrocarbons in water,<sup>26</sup> inhibit micelle formation,<sup>27</sup> and perhaps most importantly in relatively high concentration (~8 M) denature proteins.<sup>28,29</sup> A good deal of research effort has focused on gaining an understanding of the mechanism of urea denaturation, but the question remains a subject of active research.<sup>30</sup> Two mechanisms, which are by no means mutually exclusive, are frequently discussed. One suggestion is that urea acts indirectly by altering (breaking) the water structure such

that it is better able to “solvate” the protein, particularly the hydrophobic parts, while a second idea holds that urea stabilizes unfolded states through direct interactions with the protein. Computer simulations appear to provide at least some evidence for both mechanisms in particular systems,<sup>30–36</sup> but we note that the recent work of Berne and co-workers provides very strong evidence in favor of the direct mechanism for hydrophobic polymer chains,<sup>34</sup> as well as for a model protein.<sup>35</sup> Moreover, these authors give a very convincing argument that urea-solute dispersion interactions play a dominant role in the direct mechanism. Additional evidence in support of the direct mechanism, and of the importance of the dispersion interactions is provided by the recent free energy calculations of Kokubo et al.,<sup>36</sup> for model decaalanine solutions. Arguments in favor of the direct mechanism are further supported by the fact that simulation studies of urea-water solutions<sup>35–46</sup> show that urea has only a rather small effect on the structure of water and does not appear to act as a structure breaker. We note that at least one detailed experimental study has reached a similar conclusion.<sup>47</sup>

Although there have been many studies of binary TBA-water and urea-water systems, ternary urea-TBA-water solutions have received little attention. Exceptions are two experimental studies<sup>47,48</sup> aimed at determining the effect of urea on TBA association. Evidence from a NMR study<sup>47</sup> suggests that urea can act to enhance or reduce TBA association, depending upon

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the temperature and urea concentration, with reduced association occurring at relatively high urea concentrations consistent with observations for protein denaturation. Another investigation<sup>48</sup> based on the measurement of translational diffusion coefficients concludes that urea has essentially no effect on TBA association in aqueous solution, but only solutions with rather low urea concentrations were considered in that work. Because much evidence suggests that TBA aggregation is driven by hydrophobic interactions, and because urea is believed to weaken such interactions, it is of interest to more closely examine ternary urea-TBA-water solutions. This is the purpose of the present paper. We report a molecular dynamics study of structural and dynamical properties, and what these indicate about the influence of urea on aqueous TBA solutions.

The remainder of this paper is divided into three parts. The model and simulation details are described in Section II, results are presented and discussed in Section III, and our conclusions are summarized in Section IV.

## II. MODELS AND SIMULATION METHOD

We carry out molecular dynamics (MD) simulations of urea-TBA-water solutions at 298 K. For TBA we employ a rigid version of an all-atom model used by Kusalik et al. in studies of pure TBA<sup>19</sup> and TBA-water<sup>21</sup> systems. This model is based on earlier work of Nota et al.<sup>22</sup> and shows clear TBA association in aqueous solution, when used in conjunction with water models of the SPC type. It is worth noting that the rigid model employed here gives TBA-water radial distribution functions that are very similar to those obtained for the fully flexible version.<sup>21</sup> In particular, the TBA-water radial distribution functions associated with TBA-water hydrogen bonding (hydrogen-oxygen and oxygen-oxygen) are in very good agreement. For urea we use the all-atom model of Duffy et al.<sup>49,50</sup> (referred to as the DKJ or OPLS model). The SPC/E potential<sup>51</sup> is used for water. All models employed are rigid, and the interaction between atomic sites of different molecules is given by

$$u_{\alpha\beta}(r_{\alpha\beta}) = 4\epsilon_{\alpha\beta} \left[ \left( \frac{\sigma_{\alpha\beta}}{r_{\alpha\beta}} \right)^{12} - \left( \frac{\sigma_{\alpha\beta}}{r_{\alpha\beta}} \right)^6 \right] + \frac{q_{\alpha}q_{\beta}}{r_{\alpha\beta}} \quad (1)$$

where  $r_{\alpha\beta}$  is the distance between atomic sites  $\alpha$  and  $\beta$ , and  $q_{\alpha}$  is the charge on site  $\alpha$ . The Lennard-Jones (LJ) parameters,  $\sigma_{\alpha\beta}$  and  $\epsilon_{\alpha\beta}$ , are obtained using the combining rules  $\sigma_{\alpha\beta} = (\sigma_{\alpha} + \sigma_{\beta})/2$  and  $\epsilon_{\alpha\beta} = (\epsilon_{\alpha}\epsilon_{\beta})^{1/2}$ . The values of the potential parameters  $q_{\alpha}$ ,  $\sigma_{\alpha}$ , and  $\epsilon_{\alpha}$  for urea, TBA, and water are summarized in Table I.

We remark that a number of different model potentials have been proposed for all three components of our system (water, urea, and TBA), and that solution properties can show some variation, depending on the particular models, and/or model combinations employed. In the present context, different rigid urea models combined with SPC/E water have been compared by Sokolić et al.,<sup>40,41</sup> and they conclude that, among the models considered, the DKJ model shows the best overall agreement with experimental results for urea-water solutions. A more recent urea potential (the KBFF model) introduced by Weerasinghe and Smith<sup>52</sup> has been shown<sup>43,46</sup> to be rather similar to the DKJ model in aqueous solutions. The one difference reported is a somewhat greater tendency for urea to self-associate with the DKJ model. However, qualitatively both models are expected to give a similar picture of urea

**Table I. Lennard-Jones Parameters and Charges Used in the Models Considered<sup>a</sup>**

	atom	$\sigma$ (Å)	$\epsilon$ (kJ/mol)	charge ( $e$ )
water (SPC/E)	O	3.166	0.646	−0.8476
	H	0.0	0.0	+ 0.4238
	C	3.75	0.4365	+ 0.142
	O	2.96	0.873	− 0.390
urea (DKJ)	N	3.25	0.7067	− 0.542
	H	0.0	0.0	+ 0.333
	C (central)	3.80	0.1674	+ 0.3885
	C (of CH <sub>3</sub> )	3.80	0.1674	− 0.3510
TBA	O	3.0	0.67	− 0.5723
	H (of OH)	0.0	0.0	+ 0.3278
	H (of CH <sub>3</sub> )	2.40	0.1674	+ 0.1010

<sup>a</sup> $e$  is the elementary charge.

denaturation.<sup>43</sup> Evidence for this is provided by refs 34 and 36, which reach similar conclusions concerning the mechanism of urea denaturation, based on a flexible version of the DKJ model in the former case, and on the KBFF model in the latter.

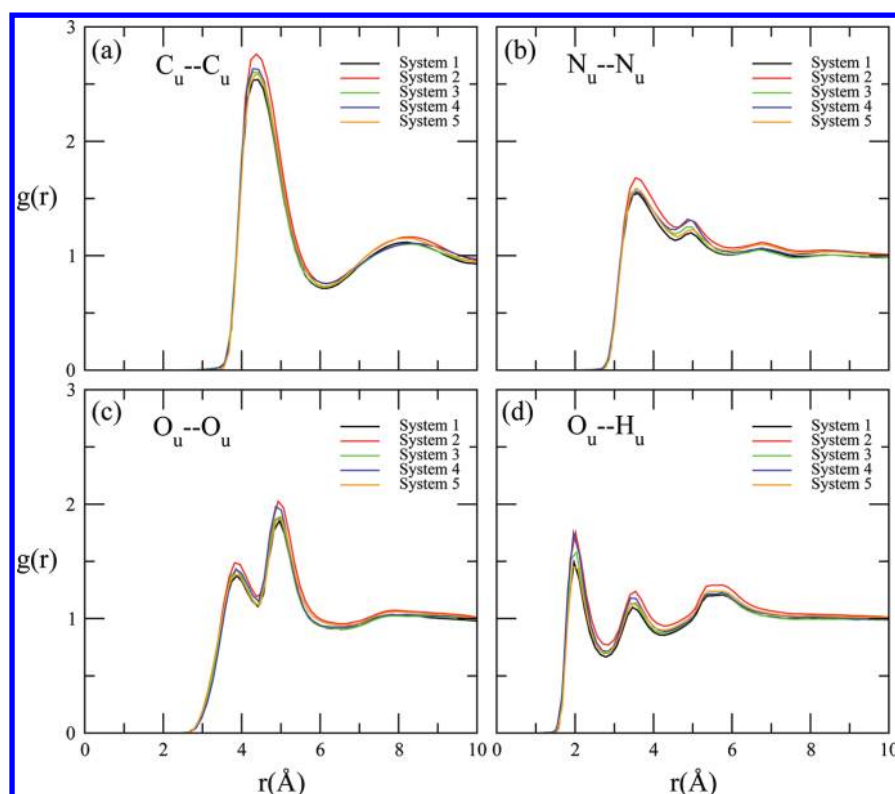
All simulations were carried out with a total of 500 molecules (water, urea, and TBA) in a central cubic cell of length  $L$ . The Ewald method<sup>53</sup> (convergence parameter  $\alpha = 6.4/L$ ) was used to take account of the long-range electrostatic interactions. The LJ interactions and the real space terms in the Ewald sum were spherically truncated at the radius  $L/2$ . The quaternion formulation<sup>53,54</sup> of the equations of rotational motion was used, and the leapfrog algorithm with a time step of  $10^{-15}$  s was employed for the time integration. In starting configurations, all molecules were located on face-centered-cubic lattice sites with random orientations. Initially, NPT MD simulations were performed to obtain the average volume consistent with a pressure of one atmosphere for each solution. Using the average cell volumes thus obtained, each system was again equilibrated for 2 ns employing the NVT ensemble. This was followed by further NVT MD runs of 3 ns, and the results reported are from these 3 ns NVT simulations. Our simulation runs are relatively long because while some properties such as the internal energies equilibrate within a few picoseconds, longer runs are required to obtain well converged structural and dynamical properties.

Five systems considered are summarized in Table II. Note that in these systems the number of urea molecules is held fixed, and TBA is added by replacing water molecules.

**Table II.  $N_{\text{urea}}$ ,  $N_{\text{TBA}}$ , and  $N_{\text{water}}$  Are the Number of Urea, TBA, and Water Molecules Used in the Simulations<sup>a</sup>**

system	$N_{\text{urea}}$	$N_{\text{TBA}}$	$N_{\text{water}}$	$L$ (Å)	density (gm/cc)	molarity of urea
1	100	10	390	27.118	1.147	8.34
2	100	20	380	27.643	1.127	7.87
3	100	30	370	28.066	1.119	7.52
4	100	50	350	28.998	1.091	6.82
5	100	0	400	26.729	1.149	8.7

<sup>a</sup>The cell lengths  $L$ , densities, and urea molarities are for solutions at 298 K and 1 atm.



**Figure 1.** Urea-urea site-site radial distribution functions.  $C_u$ ,  $N_u$ ,  $O_u$ , and  $H_u$  represent the carbon, nitrogen, oxygen, and hydrogen atoms of urea. The lines represent different systems as indicated on the figure.

**Table III.** Hydrogen-Bond Energies (kJ/mol of Solution) and Hydrogen-Bond Numbers (Given in Brackets) for the Systems Considered<sup>a</sup>

system	W–W	$W_D$ –T	$W_A$ –T	$W_D$ –U	$W_A$ –U	$U_D$ –T	$U_A$ –T	U–U	T–T
1	–19.20 (2.87)	–18.56 (0.96)	–11.52 (0.44)	–20.33 (1.49)	–15.17 (1.33)	–18.29 (0.33)	–17.21 (0.18)	–24.24 (0.75)	–16.43 (0.05)
2	–19.32 (2.81)	–18.50 (0.89)	–10.95 (0.43)	–20.56 (1.42)	–15.22 (1.29)	–19.30 (0.25)	–18.17 (0.17)	–24.89 (0.73)	–16.70 (0.05)
3	–19.43 (2.77)	–18.45 (1.0)	–11.33 (0.45)	–20.82 (1.38)	–15.38 (1.26)	–18.72 (0.29)	–17.64 (0.21)	–25.55 (0.71)	–15.90 (0.04)
4	–19.62 (2.63)	–18.64 (0.96)	–11.53 (0.51)	–20.80 (1.27)	–15.52 (1.13)	–18.75 (0.24)	–18.19 (0.18)	–25.15 (0.75)	–16.22 (0.16)
5	–19.05 (2.90)			–20.61 (1.36)	–14.93 (0.99)			–24.42 (0.86)	

<sup>a</sup>In the column headings, W, T, and U refer to water, TBA, and urea, and the subscripts D and A indicate the proton donor and acceptor, respectively. The hydrogen-bond numbers are defined with respect to the second species mentioned in the column headings (*i.e.*, per water molecule in column 2, per TBA molecule in column 3, etc.).

### III. RESULTS AND DISCUSSION

**A. Structural Properties.** A selection of site-site radial distribution functions (rdfs) that illustrate the structure of urea-TBA-water solutions are shown in Figures 1–6. Note that atoms associated with urea, TBA, and water are denoted by the subscripts u, t, and w, respectively.

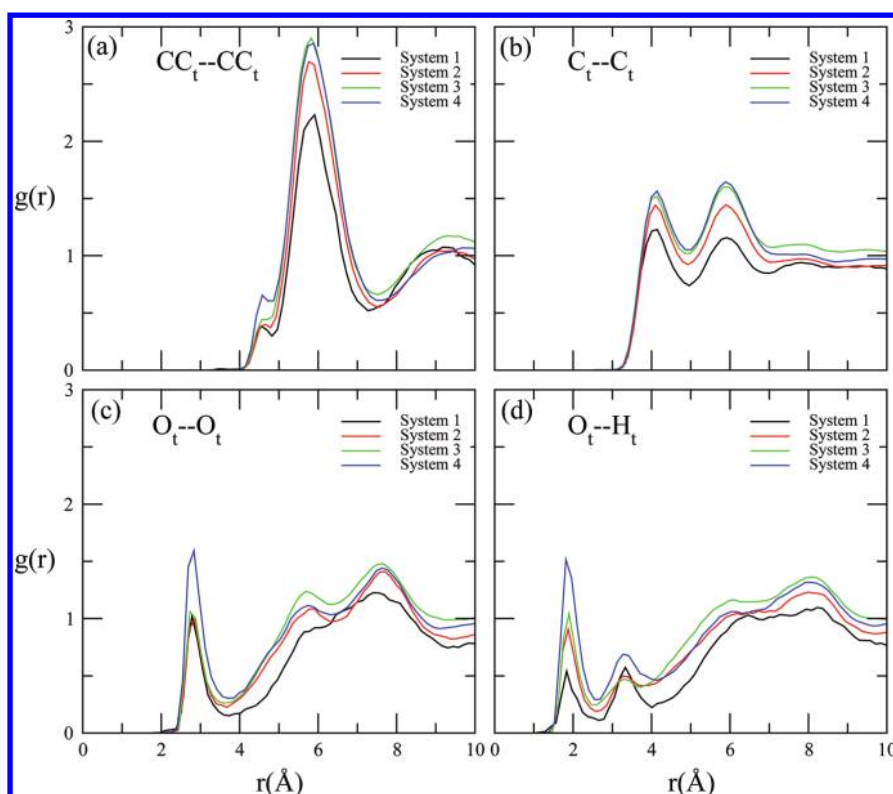
It is also useful to examine various first-shell coordination numbers defined by

$$CN = \int_0^R \rho_\beta g_{\alpha-\beta}(r) 4\pi r^2 dr \quad (2)$$

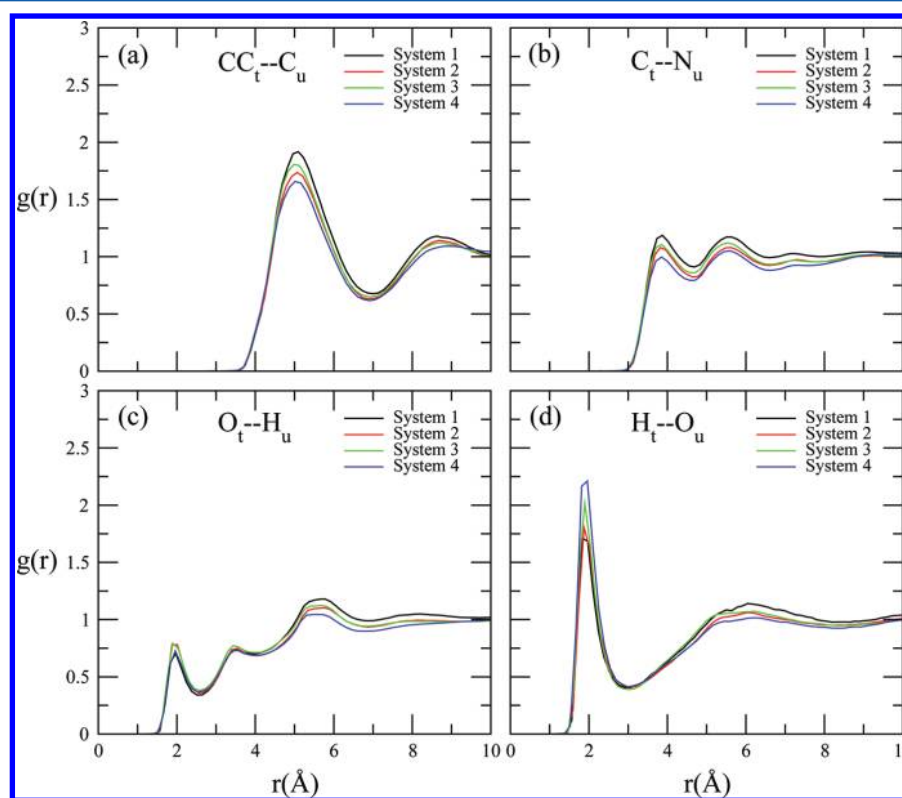
where  $\rho_\beta$  is the density of species  $\beta$ ,  $g_{\alpha-\beta}(r)$  is a rdf, and the upper limit  $R$  is taken to be the first minimum in  $g_{\alpha-\beta}(r)$ . For example, if  $\rho_w g_{C_u-C_u}(r)$  is used in eq 2, the average number of

urea carbon atoms in the first coordination shell of a urea carbon atom is obtained.

Urea-urea rdfs are plotted in Figure 1. We note that in the absence of TBA (System 5), the urea-urea structure is very similar to that observed in earlier studies.<sup>43</sup> The double peak in the  $O_u$ – $O_u$  rdf, and the three peaks in the  $O_u$ – $H_u$  rdf, indicate the presence of associated urea species (dimers and trimers) held together through  $O_u$ – $H_u$  hydrogen bonds. From Figure 1, we see that even at the mole fraction  $x_{tba} = 0.1$  (System 4), TBA has surprisingly little effect on the urea-urea structure. Some TBA influence can be seen in the  $O_u$ – $O_u$  and  $O_u$ – $H_u$  rdfs where the peaks are modestly increased in height. This reflects a small increase in the  $O_u$ – $H_u$  hydrogen-bond energy in the ternary solutions (Table III). The other two rdfs shown in Figure 1,  $N_u$ – $N_u$  and  $C_u$ – $C_u$ , show only slight awareness of the



**Figure 2.** TBA-TBA site-site radial distribution functions.  $CC_t$ ,  $C_t$ ,  $O_t$ , and  $H_t$  represent the central carbon, methyl carbon, oxygen, and hydroxyl hydrogen of TBA. The lines represent different systems as indicated on the figure.



**Figure 3.** TBA-urea site-site radial distribution functions. The symbols and line types are as in Figures 1 and 2.

presence of TBA. The insensitivity of the urea structure to TBA is also confirmed by coordination number calculations. The urea-carbon-urea-carbon ( $C_u$ - $C_u$ ) coordination numbers vary

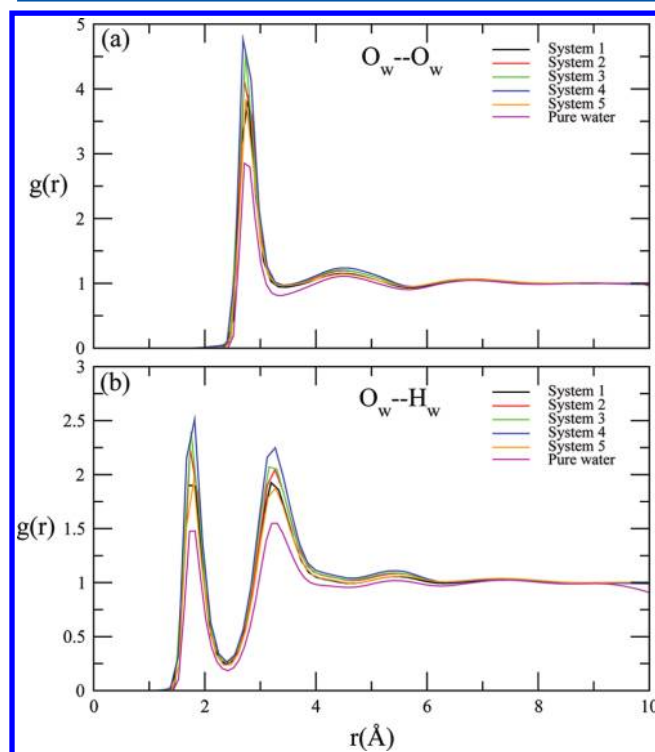
from 5.96 for the binary urea-water system to 4.65 in System 4, but this decrease is nearly completely accounted for by the decrease in the bulk density of urea (Table II).



Selected TBA-TBA rdfs are shown in Figure 2. The central and methyl carbons of TBA are designated  $CC_t$  and  $C_v$  respectively. It can be seen that the peaks in all of the rdfs increase significantly in height with increasing TBA concentration. We note in particular the strong increase in height of the first peaks in  $O_t-O_t$  and  $O_t-H_t$  at  $x_{tba} = 0.1$ , and we show below that this is associated with a sharp increase in the number of TBA-TBA hydrogen bonds at that concentration. Overall, the behavior of the TBA-TBA rdfs is qualitatively similar to what one observes (not shown) in the absence of urea and is indicative of the association tendency of TBA molecules in aqueous solutions. However, an analysis of the concentration dependence of various coordination numbers given below shows that urea acts to reduce or perhaps more accurately to “delay” TBA aggregation.

TBA-urea rdfs are plotted in Figure 3. These rdfs do not show a strong dependence on the TBA concentration, but the  $CC_t-C_u$  and  $C_t-N_u$  peaks do decrease a little in height as the TBA concentration is increased. This is perhaps related to TBA association, but clearly significant amounts of urea remain in the first coordination shell of TBA even at  $x_{tba} = 0.1$ . Moreover, we show below that in aqueous solutions TBA is strongly selectively solvated by urea. We note that the first peak in  $H_t-O_u$  is much stronger than that of  $O_t-H_w$ , although we shall see below that the associated hydrogen-bond energies are comparable (Table III).

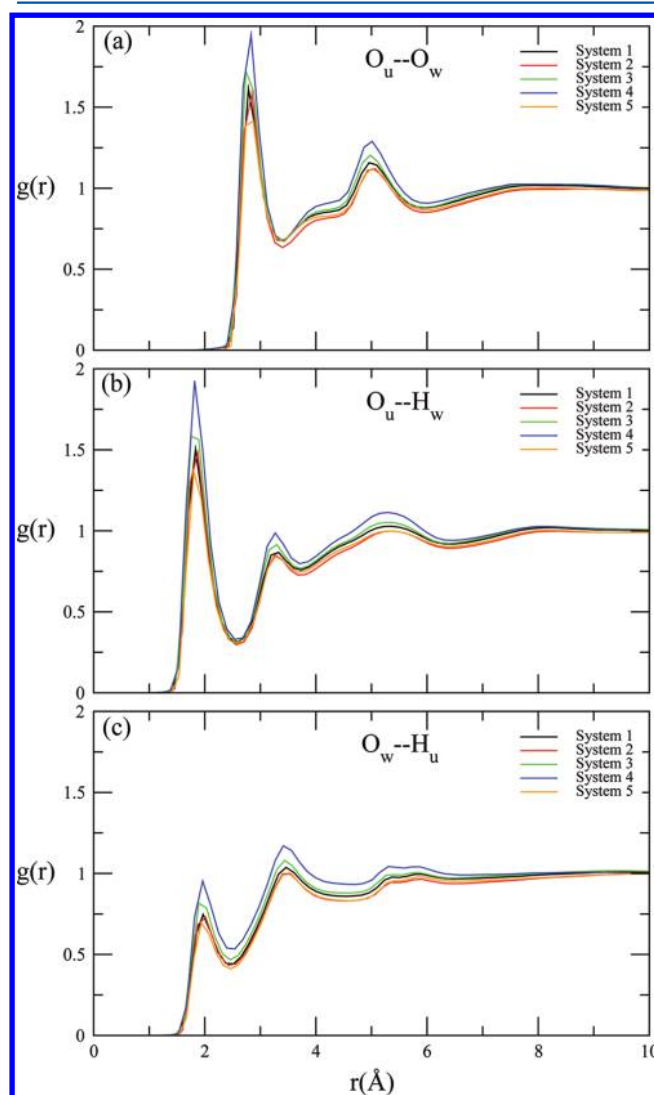
The influence of the solutes on the water-water rdfs is shown in Figure 4. Consistent with earlier work,<sup>41,43,46</sup> we note that the presence of urea alone tends to increase the peak heights in the  $O_w-O_w$  and  $O_w-H_w$  rdfs, signaling some perturbation of the water structure. The addition of TBA does nothing to decrease the solvent structural perturbation induced by urea, rather it appears to be enhanced by the TBA, with the rdf peaks further



**Figure 4.** Water-water site-site radial distribution functions. The lines represent different systems as indicated on the figure.

increasing in height. In earlier work on TBA-water solutions,<sup>19</sup> the increased peak heights have been associated with an enhancement of the local tetrahedral order in water.

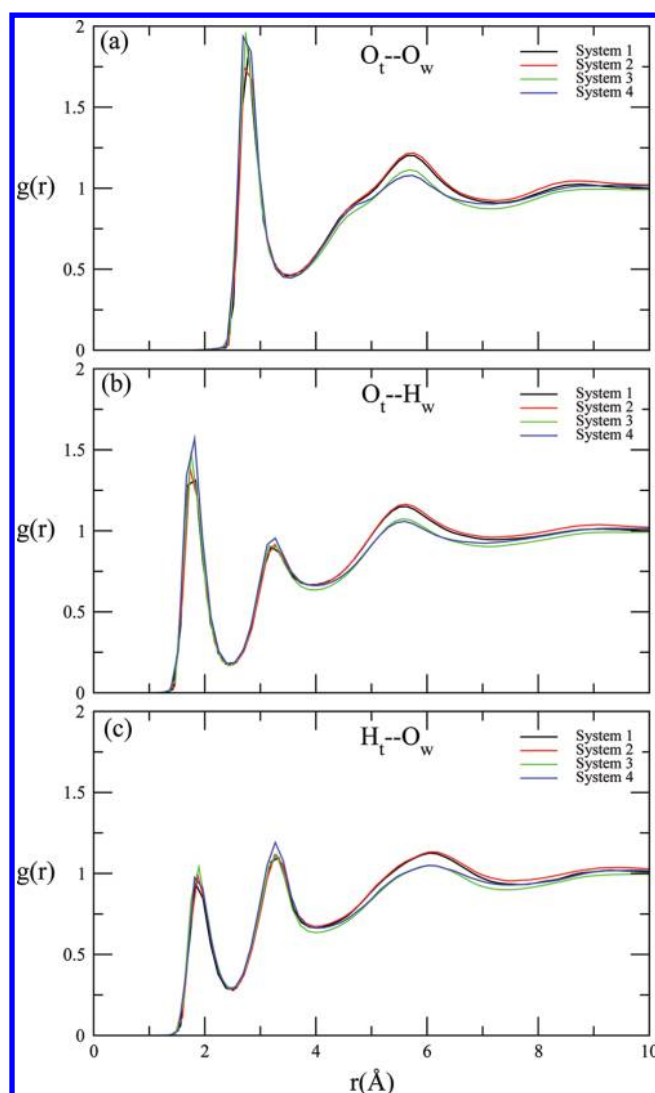
Urea-water rdfs are shown in Figure 5. We observe that on addition of TBA the first peak in the  $O_u-O_w$ ,  $H_u-O_w$ , and  $O_u-H_w$



**Figure 5.** Urea-water site-site radial distribution functions. The lines represent different systems as indicated in the figure.

rdfs increases in height indicating more structured water around urea. Additionally, the first peak in the  $O_u-H_w$  rdf is much stronger than that of the  $H_u-O_w$  rdf, indicating that water prefers to be a hydrogen donor in urea-water hydrogen bonds. This is also apparent in the hydrogen-bond energies discussed below (Table III).

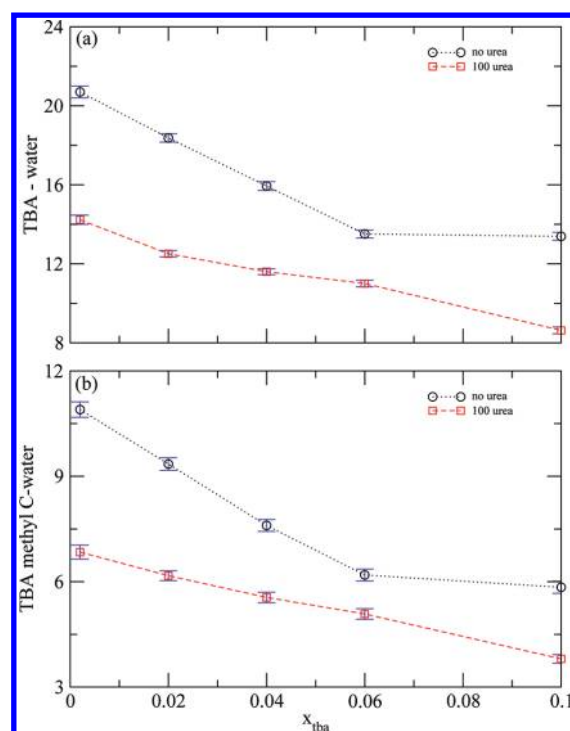
Oxygen-oxygen and oxygen-hydrogen TBA-water rdfs are shown in Figure 6. We see that the first peak of all three rdfs remains practically unchanged as the TBA concentration is increased. The  $O_t-O_w$  first-shell coordination number decreases from 2.05 to 1.75 as we move from System 1 to System 4, but this decrease is expected given the decreased bulk density of water in System 4 compared to System 1. The hydrogen bond analysis described below shows that there is little change in the average number of hydrogen bonds formed between water and a TBA molecule, as the TBA concentration is increased. The



**Figure 6.** TBA-water site-site radial distribution functions. The lines represent different systems as indicated in the figure.

rather sharp decrease in the height of the second peak of the  $O_t-O_w$  rdf indicates that there is loss of water molecules in the second shell of the TBA oxygen atom. This is confirmed by calculation of the second-shell coordination number, which decreases from 27.77 for System 1 to 18.67 for System 4. We note that the first peak in  $O_t-H_w$  is considerably stronger than that of  $O_w-H_t$ , consistent with the more favorable hydrogen-bond energy in the former case (Table III).

In earlier work,<sup>23,24</sup> it was found that first-shell coordination numbers can provide insight into the association tendency of TBA in aqueous solutions. In particular, in binary TBA-water systems, the aggregation of TBA molecules is accompanied by some “dehydration” of its hydrophobic parts, such that TBA-water coordination numbers can be rather sensitive indicators of TBA association. The average numbers of water molecules in the first coordination shell of TBA and in the first coordination shell of the TBA methyl groups are plotted as functions of TBA concentration in Figure 7. Note that these are estimated from the rdfs by first using eq 2 to calculate water oxygen coordination numbers for all atomic sites on TBA. The TBA-water “total coordination number” is then obtained by summing the coordination numbers for all atomic sites, and



**Figure 7.** The average number of water molecules in the first coordination shell of (a) TBA and (b) the methyl carbons of TBA. Results with and without urea are included. The error bars represent one standard deviation.

the contribution of the three methyl groups is found by summing only over sites associated with these groups. Results with and without urea are included. The values plotted at  $x_{tba} = 0.002$  were obtained with a single TBA molecule in the simulation cell.

Error bars representing one standard deviation are shown in Figure 7. The standard deviations were estimated by dividing 3 ns simulation runs into six blocks, each of 500 ps. Coordination numbers were obtained for each block, and standard deviations were calculated assuming that the block values provide independent estimates of the coordination numbers. We note that at all concentrations the standard deviations are much smaller than the differences in the coordination numbers found with and without urea. Thus, our results clearly have sufficient precision to detect and quantify true physical effects due to the presence of urea.

Focusing first on the binary TBA-water results, we see that the first-shell coordination numbers of both TBA and its methyl groups decrease rather sharply up to  $x_{tba} \approx 0.06$ , then flatten out, and show little further change up to  $x_{tba} = 0.1$ . These observations are consistent with earlier work for binary TBA-water solutions.<sup>23,24</sup> We note as well, that for a given TBA concentration, the number of water molecules lost from the first coordination shell of a TBA molecule is essentially equal to the number lost from the region adjacent to the methyl groups, so, not surprisingly, it is the hydrophobic part of TBA that is undergoing dehydration. It is worth mentioning that experiments<sup>3–16</sup> have revealed that many physical properties of TBA-water solutions undergo rapid change in the concentration range  $x_{tba} \lesssim 0.06$ .

It is also apparent from Figure 7, that both the TBA-water coordination numbers and their concentration dependence are significantly altered by the presence of urea. We note that in the

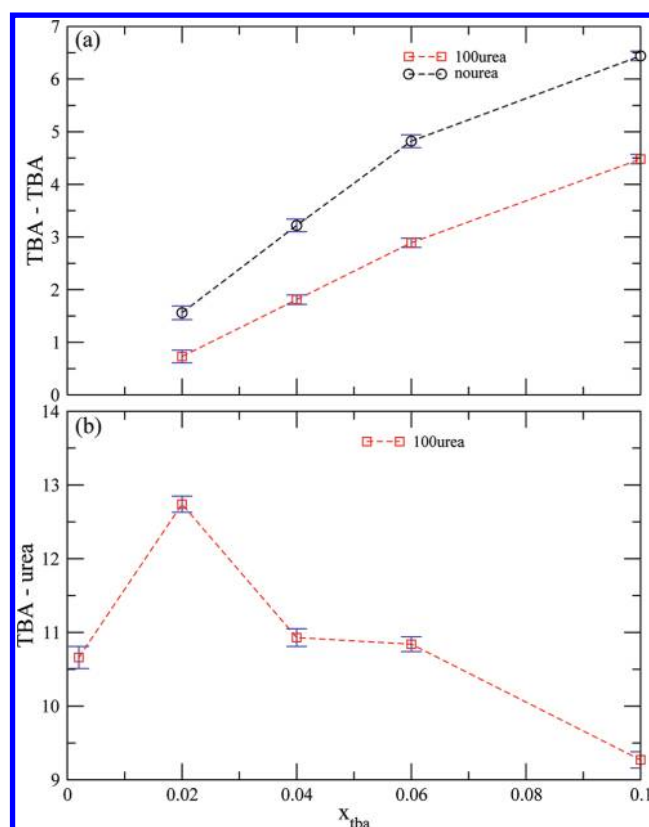
ternary system there are fewer water molecules in the first TBA coordination shell at all TBA concentrations. This is of course not unexpected because some water molecules have been replaced by urea lowering the number density of water. However, the reduction in the coordination numbers cannot be accounted for by the just the change in the density of water. For example, at  $x_{\text{tba}} = 0.002$ , the TBA-water coordination number is reduced from  $\sim 20.9$  in the binary TBA-water solution to  $\sim 14.5$  in the urea-TBA-water system, whereas the expected ternary value from the water density differences is  $\sim 16.7$ . Thus, TBA is preferentially solvated by urea, and more than the expected amount of water is displaced from the first coordination shell. This important point is addressed in more detail below. Furthermore, comparing the TBA and TBA methyl group coordination numbers, we see that only  $\sim 4$  of the  $\sim 6.4$  displaced water molecules come from this strongly hydrophobic region of TBA. Apparently, urea also displaces some water from other regions of the TBA coordination shell. This is not entirely surprising because, as discussed below, urea does hydrogen bond with the hydroxyl group of TBA and hence can also compete with water in the strongly hydrophilic part of the coordination shell.

With respect to the influence of urea on TBA aggregation, the concentration dependence of the TBA-water coordination numbers is informative. In the urea-TBA-water solutions, one does not observe the rapid change over the range  $x_{\text{tba}} \lesssim 0.06$ , followed by only a very weak variation at higher concentration, that one sees in the urea free system. Rather, the coordination numbers vary slowly over the entire concentration range considered, and there is no signal of rapid association “ending” at a particular concentration as we see in the binary TBA-water case. As a function of TBA concentration, the association of TBA appears to be significantly slowed by the presence of urea.

This impression is further enforced by the TBA-TBA and TBA-urea coordination numbers plotted in Figure 8. Note that error bars shown in Figure 8 were obtained as described above for Figure 7, and the comments made above apply equally here. The TBA-TBA plot is simply the coordination numbers obtained using the  $\text{CC}_t\text{-CC}_t$  rdf in eq 2. The TBA-urea results are the total coordination numbers obtained in a calculation analogous to that described above for TBA-water, with the urea carbon atom used in place of the water oxygen. As expected, in both the absence and presence of urea, the TBA-TBA coordination numbers increase with increasing TBA concentration. However, without urea the coordination numbers are significantly larger for all concentrations considered, and the increase is steeper up to  $x_{\text{tba}} \approx 0.06$ , where there is a distinct change to a smaller slope. In the solutions with urea, the TBA coordination numbers increase more slowly with TBA concentration, and only a small change in slope is discernible at  $x_{\text{tba}} \approx 0.06$ .

The TBA-urea coordination number shows an initial increase as  $x_{\text{tba}}$  is varied from 0.002 to 0.02, followed by a near equal decrease between 0.02 and 0.04, it then remains practically constant before decreasing again between 0.06 and 0.1. The physical significance (if any) of these behavioral details is unclear, but we do note that the net change in the TBA-urea coordination number between  $x_{\text{tba}} = 0.002$  and 0.06 is nearly zero and is  $\sim -1$  between 0.06 and 0.1. These observations are another indication that TBA is not strongly self-aggregating at concentrations below  $x_{\text{tba}} \approx 0.06$  in the urea-TBA-water system.

Given the above observations, it is of interest to more closely examine the preferential solvation of TBA in urea-TBA-water



**Figure 8.** The average number of (a) TBA and (b) urea molecules in the first coordination shell of TBA. Results with and without urea are included in (a). The error bars represent one standard deviation.

systems. Following Zangi et al.,<sup>34</sup> we calculate coefficients of the type

$$\nu_{\alpha/\beta} = \frac{n_{\alpha}}{n_{\beta}} \times \frac{N_{\beta}}{N_{\alpha}} - 1 \quad (3)$$

where  $n_{\alpha}$  is the number of  $\alpha$  molecules “bound” to TBA, and  $N_{\alpha}$  is the total number of  $\alpha$  molecules in solution. Here we take  $n_{\alpha}$  to be the number of  $\alpha$  molecules in the first coordination shell of TBA. Clearly,  $\nu_{\alpha/\beta}$  is a measure of the preferential binding of species  $\alpha$  to TBA, compared with species  $\beta$ . A positive value of  $\nu_{\alpha/\beta}$  indicates preferential binding of species  $\alpha$  over species  $\beta$ , and a negative value indicates preferential exclusion. Using coordination numbers from our simulations, at  $x_{\text{tba}} = 0.002$  (one TBA molecule) we obtain  $\nu_{\text{urea/water}} = 1.944$ , and  $\nu_{\text{water/urea}} = -0.048$  indicating strong preferential binding of urea to TBA and preferential exclusion of water. At  $x_{\text{tba}} = 0.1$ , we find  $\nu_{\text{urea/water}} = 2.268$  and  $\nu_{\text{water/urea}} = -0.073$  showing that, not unexpectedly, the preference for urea over water increases with increasing TBA concentration. At  $x_{\text{tba}} = 0.1$ , we also obtain  $\nu_{\text{urea/TBA}} = 0.027$  and  $\nu_{\text{TBA/urea}} = -0.028$ , which suggest that TBA has a slight preference for urea over itself. Given that there is some arbitrariness in defining and estimating the  $n_{\alpha}$ , one must be careful not to over interpret these small numbers. Nevertheless, they do show that TBA does not have a strong tendency to exclude urea from its first coordination shell. This is further evidence that any aggregates formed in ternary urea-TBA-water solutions likely include both urea and TBA in amounts essentially consistent with their bulk concentrations.

**B. Hydrogen Bonding.** It is also instructive to consider the strength and number of the hydrogen bonds between various



species in urea-TBA-water systems. Following earlier work, we define hydrogen bonds through a set of geometric criteria.<sup>55–59</sup> Specifically, a pair of water molecules is defined to be hydrogen bonded if their interoxygen distance is less than 3.41 Å, while simultaneously the hydrogen-oxygen distance is less than 2.38 Å, and the oxygen-oxygen-hydrogen angle is less than 45°. These oxygen-oxygen and oxygen-hydrogen distances are determined from the positions of the first minimum in the corresponding atom–atom rdfs. Similarly, for hydrogen bonds between other species (water-urea, water-TBA, urea-urea, urea-TBA, and TBA-TBA), we select cutoff distances according to the location of the first minimum in the appropriate rdf and use an angle of 45° to define all hydrogen bonds. The hydrogen-bond energy for species A and B is calculated using the interaction energy of all A-B pairs that satisfy the hydrogen-bond criteria given above. For a given configuration, the A-B hydrogen-bond energy is defined as the total interaction energy (including LJ and Coulombic contributions) of all hydrogen-bonded A-B pairs, divided by the total number of A-B hydrogen bonds. The average hydrogen-bond energies reported in this paper are obtained by averaging these values over all configurations in the usual manner.

Average hydrogen-bond energies together with average hydrogen-bond numbers (in brackets) are given in Table III. For comparison, corresponding results for binary TBA-water systems (the 100 urea molecules are replaced with water molecules) are given in Table IV. In both tables, the subscripts

**Table IV. Hydrogen-Bond Energies (kJ/mol of Solution) in the Absence of Urea<sup>a</sup>**

system	W–W	W <sub>D</sub> –T	W <sub>A</sub> –T	T–T
1	–19.08 (3.56)	–18.41 (1.42)	–11.48 (0.72)	–16.75 (0.07)
2	–19.14 (3.51)	–18.48 (1.35)	–11.23 (0.75)	–15.91 (0.07)
3	–19.29 (3.43)	–18.50 (1.30)	–11.30 (0.65)	–16.40 (0.16)
4	–19.32 (3.33)	–18.62 (1.24)	–11.55 (0.61)	–16.95 (0.17)

<sup>a</sup>Systems 1–4 are as in Table II with 100 urea molecules replaced by 100 water molecules. All else is as in Table III.

A and D indicate if a particular species is acting as a proton acceptor or as a donor, respectively. From Table III, we see that the urea-urea hydrogen bonds are the strongest in the system by significant amounts. For System 4, the urea-urea hydrogen-bond energy is ~5.5 kJ/mol more attractive than the water-water bond and ~4.4 kJ/mol more attractive than the water-urea (water donor) interaction. We note that generally the hydrogen-bond energies are not strong functions of TBA concentration. The water-water hydrogen-bond energy becomes more attractive when urea is added and shows some further decrease with increasing TBA concentration. This is similar to the situation without urea (Table IV) and is consistent with the structural changes noted above. However, this effect is not very large, the water-water hydrogen-bond energy of System 4 is just ~0.4 kJ/mol more negative than that of System 1. The average number of water-water hydrogen bonds decreases when TBA is added to the solution, both in the presence and absence of urea, but the decrease is nearly accounted for simply by the changing density of water molecules. This is also true of the numbers of water-urea

hydrogen bonds. The average numbers of urea-urea, water-TBA, and TBA-urea hydrogen bonds show only a small dependence on the TBA concentration.

The TBA-TBA hydrogen-bond numbers show an interesting feature both with and without urea. From Tables III and IV, we see that in both cases the TBA-TBA hydrogen-bond numbers are relatively small and remain practically constant as a function of TBA concentration, before increasing sharply at a particular concentration, presumably due to increasing TBA association. Qualitatively, the behavioral pattern is similar in both the binary and ternary solutions, but in the TBA-water system the “jump” occurs between  $x_{tba} = 0.04$  and 0.06, and in urea-TBA-water it occurs between  $x_{tba} = 0.06$  and 0.10. This is another indication that TBA aggregation is delayed by the presence of urea.

It is interesting to look more closely at the hydrogen-bond energies for different species. We note that for both water-TBA and water-urea hydrogen bonds, the hydrogen-bond energy is considerably more negative when water acts as a proton donor rather than as an acceptor. The differences are ~7 kJ/mol and ~5 kJ/mol for water-TBA and water-urea, respectively. These differences cannot be accounted for by corresponding differences in the partial charges associated with the relevant oxygen and hydrogen atoms (see Table I) and are most likely due to geometric constraints on the configurations possible for water-TBA and water-urea pairs. The urea-TBA hydrogen-bond energies are comparable with those of water-TBA, when both urea and water are acting as proton donors. If both urea and water act as proton acceptors, then the urea-TBA hydrogen-bond energy is ~6 kJ/mol more negative than the water-TBA case. Thus, urea hydrogen bonds with TBA at least as strongly as water does and arguably more so. As discussed below, this observation is likely of some relevance in partially explaining why urea inhibits TBA association in aqueous solutions. It is also worth noting that the TBA-TBA hydrogen-bond energies are significantly less negative (~2–3 kJ/mol) than the water-TBA and urea-TBA energies.

It is sometimes suggested<sup>30</sup> that certain solutes can significantly influence the dynamics of water-water hydrogen bonds. To examine this possibility, we consider the continuous hydrogen-bond time correlation function  $S_{HB}(t)$  defined as<sup>55–60</sup>

$$S_{HB}(t) = \langle h(0)H(t) \rangle / \langle h \rangle \quad (4)$$

In eq 4,  $h(t)$  and  $H(t)$  are hydrogen-bond population variables;  $h(t)$  is unity if a particular tagged pair of particles is hydrogen bonded (according to the definition given above) at time  $t$ , and is zero otherwise;  $H(t)$  is unity if the tagged pair of particles remains continuously hydrogen bonded from time 0 to time  $t$  and is zero otherwise. The angular brackets denote an average over all hydrogen bonds that are present at  $t = 0$ .  $S_{HB}(t)$  describes the probability that a pair of particles, which was hydrogen bonded at  $t = 0$ , remains continuously bonded up to time  $t$ . The time integral of  $S_{HB}(t)$ ,  $\tau_{HB}$ , is the average time that a hydrogen bond survives after it is selected at  $t = 0$ . Since the hydrogen bonds are chosen randomly without imposing any condition on when they were created,  $\tau_{HB}$  is the average persistence time or “life expectancy” of a randomly chosen hydrogen bond.<sup>56</sup>

Results obtained for water-water hydrogen bonds in urea-TBA-water solutions are given in Table V. The  $\tau_{HB}$  values for binary TBA-water solutions are shown in the parentheses. We see that  $\tau_{HB}$  increases from 1.38 ps in System 1 to 1.65 ps in System 4. In binary TBA-water solutions,  $\tau_{HB}$  increases from 1.22 to 1.36 ps over the same range of  $x_{tba}$ . So, the presence of



**Table V. Values of  $\tau_{HB}$  for Water-Water Hydrogen Bonds for Different Systems Together with the Corresponding Hydrogen-Bond Energies  $E_{HB}$  (kJ/mol of Solution)<sup>a</sup>**

system	$\tau_{HB}$ (ps)	$E_{HB}$ (kJ/mol)
1	1.38 (1.22)	−19.20 (−19.08)
2	1.49 (1.26)	−19.32 (−19.14)
3	1.54 (1.32)	−19.43 (−19.29)
4	1.65 (1.36)	−19.62 (−19.32)
5	1.29	−19.05

<sup>a</sup>Results for TBA-water solutions without urea are given in brackets.

TBA modestly increases the life expectancy of water-water hydrogen bonds, and urea increases it a little more. The observed increases in  $\tau_{HB}$  are consistent with the increased attraction of the hydrogen-bond energies, also included in Table V.

**C. Diffusion Coefficients.** In experimental studies of aqueous solutions of TBA and other alcohols, translational diffusion coefficients are sometimes considered as indirect measures of structure and association.<sup>48,47</sup> Therefore, it is of interest to see how the structural features of TBA aggregation discussed above are reflected in the diffusion coefficients of our model solutions. We have estimated the translational diffusion coefficients of the different solution components by integrating the velocity-velocity autocorrelation functions in the usual manner.<sup>53</sup> The results obtained for water, urea, and TBA are given Table VI. For comparison purposes, the diffusion

**Table VI. Translational Diffusion Coefficients (cm<sup>2</sup> s<sup>−1</sup>) of Water, Urea, and TBA<sup>a</sup>**

system	$D_{\text{water}} \times 10^5$	$D_{\text{urea}} \times 10^5$	$D_{\text{tba}} \times 10^5$
1	1.60 (2.08)	0.68	0.52 (0.93)
2	1.47 (1.86)	0.58	0.45 (0.78)
3	1.16 (1.77)	0.51	0.37 (0.76)
4	1.07 (1.60)	0.43	0.35 (0.71)
5	2.02	0.78	

<sup>a</sup>The values in brackets are the diffusion coefficients in absence of urea.

coefficients of binary TBA-water solutions are given in parentheses. Also note that for pure SPC/E water, we find a diffusion coefficient of  $2.65 \times 10^{-5}$  cm<sup>2</sup> s<sup>−1</sup>. The diffusion coefficients of our model solutions are in qualitative agreement with experimental values, particularly at the low TBA concentrations.<sup>47,48</sup> From Table VI, we see that addition of urea and/or TBA reduces the diffusion coefficient of water both in binary systems and in ternary urea-TBA-water solutions. In TBA-water and urea-water solutions this is in agreement with earlier simulations,<sup>24,39,46</sup> and in both binary and ternary cases it is consistent with experiment.<sup>47</sup> The fact that urea decreases rather than increases the diffusion coefficient of water has been taken as an indication that urea does not act as a “structure breaker” in aqueous solutions.<sup>47</sup> We note that urea decreases the diffusion coefficient of TBA, and TBA decreases the diffusion constant of urea, such that the smallest diffusion coefficients for all species are found for System 4, where the total solute concentration is highest.

The diffusion coefficients of TBA are particularly informative and display the same qualitative behavior both in the presence and absence of urea. Specifically, the diffusion coefficients decrease rapidly up to a certain TBA concentration, after which

any further decrease occurs at a much slower rate. In binary TBA-water solutions, the region of rapid decrease ends at  $x_{tba} \approx 0.04$ , whereas in urea-TBA-water systems it ends at  $x_{tba} \approx 0.06$ . This observation is in line with the analysis of the coordination numbers given above and is another indication that in the presence of urea TBA aggregation is delayed until higher TBA concentrations are reached. We note that in experiments the TBA diffusion coefficients show similar qualitative behavior.<sup>48</sup> However, in the solutions considered in ref 48, the urea mole fraction was  $x_u = 0.02$ , which is much lower than the urea concentration  $x_u = 0.2$  (~8 M) used in our simulations. This likely explains why the position of the sharp change in slope observed in those experiments is practically uninfluenced by the presence of urea. This is consistent with the general understanding that rather high urea concentrations (~8 M) are necessary in order to influence interactions among hydrophobic entities.

## IV. SUMMARY AND CONCLUSIONS

In this paper we employ molecular dynamics simulations in an investigation of ternary urea-TBA-water solutions. In binary TBA-water systems, TBA tends to form aggregates over the concentration range  $x_{tba} \lesssim 0.06$ , with the aggregation presumably driven by hydrophobic interactions. The main purpose of the present work is to examine the influence of urea on TBA aggregation. In all solutions considered the urea mole fraction  $x_u = 0.2$  (~8 M), comparable with the concentration necessary to denature proteins. The TBA concentrations considered vary from one TBA molecule in the simulation cell ( $x_{tba} = 0.002$ ) to  $x_{tba} = 0.1$  (~3.4 M).

It is found that urea does indeed influence the aggregation of TBA. In the presence of urea, TBA aggregation is a weaker function of TBA concentration, occurring more slowly over a wider concentration range. Evidence for this is provided by the concentration dependence of different quantities obtained from the MD simulations. These include TBA-water and TBA-TBA first-shell coordination numbers, TBA-TBA hydrogen-bond numbers, and TBA-TBA translational diffusion coefficients. Very roughly, these measures indicate that the degree of TBA aggregation in urea-TBA-water systems at  $x_{tba} = 0.1$  is comparable with binary TBA-water solutions at the lower concentration  $x_{tba} = 0.06$ . For example, the TBA-TBA first-shell coordination number is ~4.8 at  $x_{tba} = 0.06$  in TBA-water, compared with ~2.5 in urea-TBA-water at the same TBA concentration, and ~4.5 at  $x_{tba} = 0.1$ . The other measures give consistent indications of urea's tendency to delay TBA aggregation. These observations are qualitatively consistent with experiment at comparable urea concentration.<sup>47</sup>

The mechanism by which urea delays TBA aggregation appears to be simply direct. If we regard urea plus water as a mixed solvent, then TBA is preferentially solvated by urea, which displaces water from the first coordination shell of TBA in amounts that are substantially greater than expected, considering only the bulk densities of water and urea. This effect is strong at near infinite dilution and is significantly increased at  $x_{tba} = 0.1$ . Urea displaces water from both the hydrophobic and hydrophilic regions of the TBA coordination shell. If both urea and water act as proton acceptors, then the TBA-urea hydrogen-bond energy is ~6 kJ/mol more attractive than that of TBA-water. This allows for some preferential TBA-urea hydrogen bonding. Also, as noted above, Zangi et al.<sup>34</sup> have recently shown that the preferred binding of urea over water to hydrocarbon chains is due to the strength of the

dispersion interaction between urea and the methylene groups of the hydrocarbon chain. Therefore, it is likely that the similar dispersion interactions between urea and the methyl groups of TBA contribute to the preferential binding of urea to TBA. The physical picture that emerges from our simulations of urea-TBA-water solutions is in fact quite similar to that put forward by Zangi et al.<sup>34</sup> for hydrophobic polymers. Urea preferentially binds to TBA and functions as a “surfactant” inhibiting TBA aggregation, much as it inhibits folding of the hydrophobic polymer chains.

Finally, it is perhaps worth emphasizing that we find no evidence of urea acting via an indirect mechanism in urea-TBA-water systems. The simulation results appear to be inconsistent with the idea that urea acts as a water “structure breaker”. The addition of urea (and/or TBA) reduces the diffusion coefficient of water, increases the water-water hydrogen-bond lifetime, and appears to enhance the tetrahedral structure. These observations are not new, but they do lend support to similar conclusions based on simulation<sup>34</sup> and experiment.<sup>47</sup>

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### Notes

The authors declare no competing financial interest.

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