Importance of Excluded Volume on the Solvation of Urea in Water†

Raymond D. Mountain*

Physical and Chemical Properties Division, National Institutes of Standards and Technology, Gaithersburg, Maryland 20899-8380

D. Thirumalai

Institute for Physical Science and Technology, University of Maryland, College Park, Maryland 20742

Received: November 26, 2003; In Final Form: February 3, 2004

We have used molecular dynamics simulations to probe the concentration-dependent solvation of urea in water. Two models of urea are considered: one is the OPLS potential, and the other is the recently introduced KBFF model whose parameters were obtained to reproduce the experimental values of the Kirkwood-Buff integrals. Although the partial charges on the urea atoms in the models are dramatically different, the concentration-dependent structural characteristics of water are similar. The largest difference between the two models is in the prediction of the tendency of urea to self-associate. The OPLS model leads to stronger urea−urea association than the KBFF model. Surprisingly, this difference is traced to the variations in the Lennard-Jones parameters rather than the charge distributions. These results suggest that solvation of urea depends not only on its ability to form hydrogen bonds with water but also its excluded volume. More generally, we propose that the denaturation efficiency of polar nonelectrolytes, such as urea, is determined by the ability of the cosolute to form hydrogen bonds with the polypeptide chain which, in turn, depends on its size.

1. Introduction

Urea, a polar nonelectrolyte, is commonly used to denature proteins. Despite extensive studies spanning nearly fifty years,[1] the mechanism by which urea denatures proteins is not fully understood. An interplay of a number of factors in the ternary system consisting of protein, water, and urea makes it difficult to obtain a molecular explanation for urea denaturation. The binding constants for the association of urea with peptide bonds or the side chains are small. Similarly, it appears that the energetics of urea−urea interactions are also on the order of only a few k_BT (k_B is the Boltzmann constant, and T is the temperature). Thus, the urea denaturation mechanism is determined by several weak associations with no dominating interaction. Historically the interaction of urea with water has been described largely in terms of hydrogen bond formation.[2−4] The present simulations show that solvation of urea is also determined by excluded volume interactions. The volume of urea is nearly three times that of water. Thus excluded volume has to be taken into account to explain the solubilities of polar and nonpolar species in aqueous urea solutions. Urea can, in principle, engage in eight hydrogen bonds either with water or the protein. However, the steric constraints due to the size of urea prevents it from forming all these hydrogen bonds.

Because of the complexities of urea interactions with water and proteins, a satisfactory explanation of the mechanism of urea denaturation has been difficult to obtain. There are two explanations for the mechanism of urea denaturation. (1) On the basis of the observation that the solubilities of hydrophobic side chains of peptides increase in aqueous urea solution it was proposed that protein denaturation is due to the alterations in the hydrophobic effect.[5] In this picture the effective solvation of hydrophobic species is thought to be the cause of protein denaturation. (2) The electrostatic mechanism posits that urea engages directly in hydrogen bonds with the peptide backbone, thus decreasing the overall stability of the protein.[6] At a critical value, the number of hydrogen bonds between the urea molecules and the protein the unfolded state becomes more stable. Recent all atom molecular dynamics simulations seem to suggest that the second mechanism is more dominant in the denaturation process.[7]

The enhanced solubility of amino acid side chains in aqueous urea solution can be explained in terms of the excluded volume of the side chains themselves *without regard* to the volume of the cosolute urea. However, according to the electrostatic mechanism (also referred to as the direct interaction mechanism) the denaturation efficiency is attributed to the number of hydrogen bonds the solute can form with the backbone. The strength of the hydrogen bond is directly related to the partial charges on the atoms engaged in hydrogen bonding. This would suggest that urea models that have larger partial charges on the urea oxygen or nitrogen might be more efficient in denaturing proteins. By considering two very different models for urea, we show that besides the partial charges on the urea atoms the overall excluded volume of urea also must play a crucial role in the denaturation process.

As a step toward a molecular understanding of urea denaturation of proteins we have, in this paper, undertaken a series of molecular dynamics simulations to probe the dependence of aqueous solvation of urea as a function of its concentration. Begining with the pioneering studies by Rossky and coworkers,[4,8] there have been numerous computer simulation[9−12] studies that have examined urea solvation in water. To address the efficiency of urea denaturation, there have also been simulations of aqueous urea solution in the presence of a third component.[7,13−15] Previous studies, that have simulated urea−water interactions at various urea concentrations, show that there

* Corresponding author. E-mail: RMountain@nist.gov.
† Part of the special issue "Hans C. Andersen Festschrift".

10.1021/jp037610g CCC: $27.50
Published on Web 04/03/2004

TABLE 1: The Potential Parameters for the KBFF and OPLS-AA Urea Models Are Listed for the Atom Types. The Unlike Pair Parameters Are Obtained Using the Geometric Mean Mixing Rule for Both the ϵ and σ Parameters

model	atom	ϵ/k_B, K	σ, nm	q, ($\|e\|$)
KBFF	C	50.2	0.3770	0.921
	O	67.4	0.3100	−0.675
	N	60.2	0.3110	−0.693
	H	10.6	0.1580	0.285
OPLS	C	52.9	0.3751	0.142
	O	105.8	0.2960	−0.390
	N	85.7	0.3850	−0.542
	H	0.0		0.333

is uncertainity in the interpretation of the nature of urea solvation in water. Part of the problem is because urea solvation is determined by an interplay of a number of factors (see above). In addition, there are large variations in the models of urea used which also obscure the physics of urea−water interactions. Although we currently do not have accurate models for urea−water and urea−urea interactions, qualitatively meaningful conclusions may be drawn by a careful study of different models. In this spirit following earlier studies, we have used molecular dynamics simulations to probe the variations in the structural characterstics of urea and water as the concentration of urea is altered. We consider two models for urea. One of them is based on the OPLS force field[16] which has been recently used to decipher denaturation mechanisms.[7,13,17]

The second potential, referred to as the KBFF model, used in our simulations was introduced by Weerasinghe and Smith[18] who parametrized the charge distribution on urea to reproduce the experimental Kirkwood−Buff integrals.[19] Such a parametrization ensures that the thermodynamics of the urea−water mixture is reproduced accurately. On the other hand, the predictions of the OPLS model are not consistent with the constraints imposed by the Kirkwood−Buff integrals.

The partial charges, especially on the oxygen atom, in the two models are drastically different (see Table 1). Recently, molecular dynamics simulations have shown that the dominant mechanism of urea denaturation is largely due to electrostatic interaction between urea and the peptide backbone or the exposed polar and charged side chains of proteins.[7,13,20] Thus, it is logical to conclude that the solvation of urea using the two models can be qualitatively different. Our main purpose in this article is to highlight the structural changes in water as the urea concentration is increased. Surprisingly, we find that despite the large differences in the charge distribution the overall mechanism of urea solvation in water is qualitatively similar for the two models. However, there are substantial differences in the packing of urea at high concentrations. This is especially evident in the increased tendency of the OPLS model to predict greater self-association. The differences in the two models arise because of substantial variations in the Lennard-Jones parameters, in particular, $\epsilon_H = 0$ for the OPLS model. Our results suggest that excluded volume effects must play a big role in the efficiency of urea denaturation of proteins and nucleic acids.

2. Methods

Models. For water we used the standard SPC/E model[21] in which the hydrogen atoms are 0.1 nm from oxygen and the HOH angle is 109.47°. The charges on oxygen and hydrogen are $q_O = -0.8476e$ and $q_H = 0.4238e$, respectively. The Lennard-Jones (LJ) parameters for the oxygen sites are $\epsilon_O = 0.0370$ kJ/mol and $\sigma_O = 0.3166$ nm. The distances are measured in units of σ_O. The parameters for urea for the KBFF model and the OPLS potential are given in Table 1. Note that there substantial differences in the values of partial charges as well as in the Lennard-Jones parameters. Comparison of the four models (see Table 1 of ref 11) and the KBFF model (Table 1 of the present paper) shows that the details of the models vary to a great extent. For example, the charge on the carbonyl oxygen varies from $-0.39e$ to $-0.675e$. Similarly, the value for ϵ/k_B for hydrogen varies from 0 to about 10.

Simulation Details. Both the water and urea molecules are treated as rigid objects, and the orientation of the molecules is described using quaternions.[22−24] The equations of motion are integrated with a time step of 1 fs using an integrated form of the Beeman algorithm.[25,26] The long-range part of the Couloumb interactions is evaluated using the Ewald summation method with a convergence factor of 5.6.[27] The temperature of the system is maintained at 297 K using separate Nosé-Hoover thermostats for the translational and orientational degrees of freedom.[28] The volume of each system is adjusted so that the calculated pressure is close to 0.1 MPa. With the volume and number of molecules fixed, the sampling is from the Canonical Ensemble.

Because of the size of the urea molecule and the spatial correlations that are developed when urea is placed in water, it was necessary to examine a large system consisting of a total (urea and water) of 1000 molecules. We simulated three concentrations of urea under ambient conditions. The urea concentrations are 0.05 M(1), 6.06 M(130), and 8.99 M(200). The numbers in paranthesis are the total number of urea molecules at the three concentrations and are 1000 times the mole fraction of urea in solution. The two largest concentrations are typical of the values used to denature proteins. The saturation level for urea in water at 297 K is about 20 M,[29] so these mixtures are not close to being saturated.

Since the KBFF model does not lead to extensive local association of water with water and urea with urea, a 100 ps duration stabilization run followed by a 100 ps duration sampling run is sufficient to obtain adequate pair function samples. Significantly longer stabilization runs are needed with models that lead to local association of urea as is the case for the OPLS potential. The adequacy of the sampling is verified by obtaining stable results from sequential runs of 100 ps duration.

3. Results

To probe the structural aspects of aqueous urea solution we have calculated all the site−site pair functions for the urea−water mixture. Here we show only those pair functions for water and for urea that are most relevant for urea solvation. Most of the results are for the highest urea concentration (8.99 M). All pair functions shown in this section are normalized to approach unity for large separations. Subscripts in the pair functions indicate the atom sites.

Pair Functions for Water. It has been previously noted that despite the much larger size of urea compared to water (the volume of a urea molecule is nearly three times greater than a water molecule), urea readily dissolves in water.[10,13,14,30] This implies that the ability to engage in hydrogen bonds with water is not diminished if water is replaced by urea. From this observation we expect that there should not be significant changes in the local water structure, at least at low urea concentrations. There are quantitative changes in the water pair functions (Figure 1). In particular, the O_WO_W pair function $g_{O_WO_W}(r)$ between the oxygen atoms of the water molecules changes as the urea concentration is increased. The height of the first peak gets larger, for both the OPLS and the KBFF

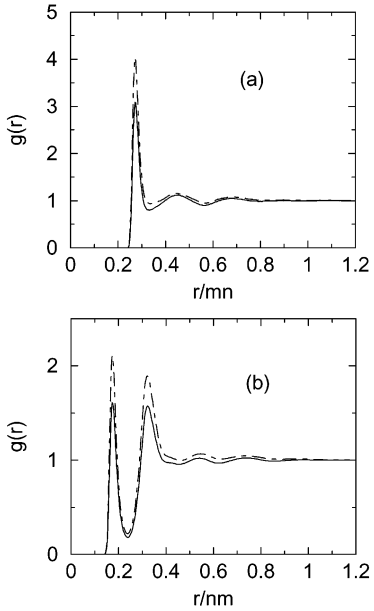

Figure 1. (a) The oxygen−oxygen pair functions between water molecules are shown for two urea compositions for the OPLS model. The dashed curve is for 0 M urea (pure water) and the solid curve is for 8.99 M. (b) Radial distribution functions for oxygen−hydrogen between water molecules. The line codes are the same as in (a). The corresponding curves for the KBFF model are similar except that the differences in the pure water and 8.99 M cases are negligible for $r >$ 0.4 nm.

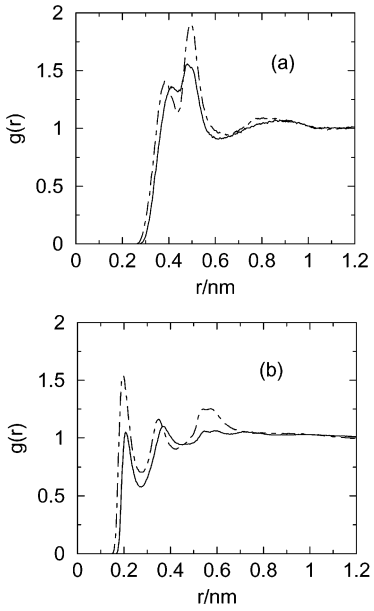

Figure 2. (a) Radial distribution functions between urea oxygen atoms. The dashed curve is obtained using the OPLS potential, and the solid curve corresponds to the KBFF model. (b) Same as (a) except the curves are pair functions for oxygen−hydrogen atoms belonging to urea. The concentration of urea in both figures is 8.99 M (urea mole fraction is 0.2).

models, at urea concentration exceeding 6 M. Neither the position nor the peak heights the second peak changes much, even at elevated urea concentrations. This observation, which is in accord with the findings of Tsai et al.,[10] is in contrast to the recent report by Soper et al., for the OPLS potental, in which they noted that the second peak in $g_{O_WO_W}(r)$ is considerably diminished at 8.55 M urea concentration. The discrepancy between the present results and those reported by Soper et al.[30] may be due to the smaller system size used in their simulations.

Radial Distribution Function for Urea. In contrast to the radial distributions for water molecules, we find that there are substantial differences in the urea pair functions between the two force fields (Figure 2). In particular, $g_{O_UO_U}(r)$ for the two potentials are qualitatively different. For the OPLS potential we find that there are two distinct peaks in $g_{O_UO_U}(r)$ at $r = 0.4$ nm and $r = 0.51$ nm, respectively. On the other hand, for the KBFF potential the peak at $r = 0.51$ nm survives, while the peak at the smaller value of r appears less pronounced. More importantly, the peak heights in the $g_{O_UO_U}(r)$ using the KBFF potential are less than for the OPLS potential (see Figure 2a). These observations suggest that the urea molecules are more tightly packed with larger structure for the OPLS model than for the KBFF potential. According to the OPLS model there is a greater tendency for urea to self-associate than predicted by the KBFF force field. These trends are also found in the pair functions involving oxygen and nitrogen.

Urea Hydrogen Bonding and Self-Association. The pair function between the oxygen and hydrogen atoms $g_{O_UH_U}(r)$ on urea gives insights into the tendency of inter-urea hydrogen bonding. The differing tendencies for self-association of urea between the two models is most easily rationalized in terms of urea−urea hydrogen bonding. For the KBFF potential we find (Figure 2b) that there is only a minor tendency of urea to form a strong hydrogen bond with the neighboring urea molecules. The peak at $r \approx 0.18$ nm is hardly above 1. In contrast, for the OPLS potential, there is a relatively sharp peak at $r \approx 0.18$ nm, which suggests that urea molecules are strongly hydrogen bonded with each other. In addition there are strong peaks at $r \approx 0.36$ nm and $r \approx 0.53$ nm, which implies that the urea molecules in the OPLS model are strongly associated. To ascertain if the tendency for the OPLS urea to form strongly associated clusters is a consequence of initial conditions we performed the following simulation. We started the simulation with a well-equilibrated configuration produced using the KBFF potential. With this initial condition, we generated a trajectory for the OPLS potential. We found that within about 500 ps the OPLS urea showed a tendency to self-associate strongly. From these observations we conclude that the OPLS model of urea predicts a greater tendency for urea to self-associate than the KBFF model. As the urea concentration decreases, the tendency of urea to self-associate become less pronounced.

The major differences in the models used in the urea models simulated in the literature lie in the prediction for the extent to which urea self-associates. Urea self-association is not the same as microphase separation or stable cluster formation. The absence of a distinct long-lived interface or major structural perturbation of water (at concentrations far from saturation values) rules out microphase separation. The self-associated urea molecules undergo dynamic fluctuations and hence are transient. However, if we adopt the convention that a neighbor of a neighbor belongs to a cluster, all the models predict self-association.

Water−Urea Pair Functions. The solubility of urea in water is largely dictated by its ability to form hydrogen bonds with water. The nearly planar urea molecule can, in principle, form up to 8 hydrogen bonds. Urea oxygen and nitrogen are acceptors, whereas the amide hydrogens are donors. Due to excluded volume, the nitrogens on urea are unable to form hydrogen bonds. This suggests that the charge on the nitrogen molecule is not directly an important factor in urea solvation. If we exclude nitrogen, the maximum number of hydrogen bonds that urea can form is 6. The amide hydrogen forms hydrogen bonds

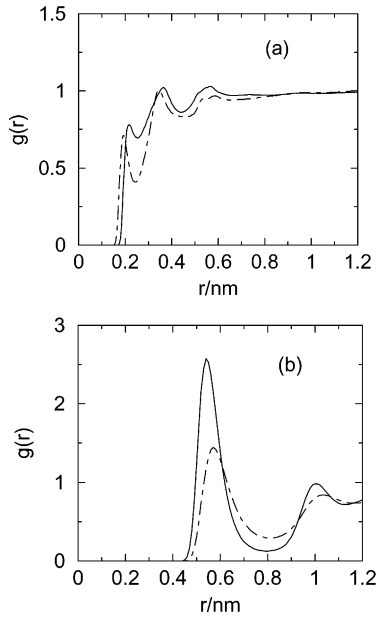

Figure 3. Pair functions describing hydrogen bond formation between urea and water with urea concentration being 8.99 M. (a) Pair function between water oxygen atom and amide hydrogen. The dashed line is obtained using the OPLS potential and the solid curve is for the KBFF model. (b) Radial distribution function between carbonyl oxygen and water hydrogen atoms. The line codes have the same meaning as in (a).

TABLE 2: The Number of Hydrogen Bonds for the 8.99 M Urea Concentration. The Number of Bonds Is Estimated by Integrating the Composition Weighted Site−Site Pair Function to the Position of the Miniumum Following the Maximum in the Pair Function Identified with a Hydrogen Bond

sites	N_{KBFF}	N_{OPLS}
H_WO_W	2.36	2.41
O_WH_U	0.50	0.37
O_UH_U	0.19	0.30
O_uH_W	0.67	0.62

with the water oxygen atoms, and the water hydrogen can engage in hydrogen bonds with the carbonyl oxygen of urea.

In Figures 3a and 3b we show the pair functions, $g_{O_WH_U}(r)$ and $g_{O_U\ H_W}(r)$ as a function of r for the OPLS and KBFF potential for 8.99 M urea concentration. It is striking that, for both models, the first peak in $g_{O_WH_U}(r)$ at $r \approx 0.196$ nm is relatively small. The first peak that is a hydrogen bond signature is located at $r \approx 0.196$ nm, indicating that this is a longer bond and probably energetically weaker. This suggests that the amide hydrogen of urea does not contribute significantly to the solvation of urea. On the other hand, the carbonyl oxygen forms a tight hydrogen bond with water hydrogens. The first peak in both the models is at $r \approx 0.180$ nm. The peak height is considerably larger for the KBFF potential than for the OPLS force field. This is not surprising because the charge on the urea oxygen in the KBFF potential is about 1.7 times larger than in the OPLS potential. Note that the differences in the hydrogen charge between the two models is less (see Table 1). The composition weighted number of hydrogen bonds that urea forms with water (mole fraction of urea = 0.2) is 1.17 and 0.97 for the KBFF and OPLS potentials, respectively (Table 2). This is surprising given that there are great variations in the partial charges on urea between the two models. The explanation lies in the observation that the excluded volume interactions in both models are treated reasonably accurately. Thus, the larger charge in the KBFF model does not facilitate the formation of all the possible hydrogen bonds.

The qualitative conclusion from the study of both models is that the solubility of urea in water is directly related to the ability of carbonyl to form strong hydrogen bonds with water. Despite the larger charge on the carbonyl oxygen in the KBFF potential compared to OPLS force field, the number of hydrogen bonds that it forms with water is nearly the same in the two models. In the KBFF model the ratio, $N_{O_WH_W}/N_{O_WH_U}$ where $N_{O_WH_W}$ is the number of hydrogen bonds water forms with hydrogen atoms and $N_{O_WH_U}$ is the number it forms with amide hydrogen is, in proportion to the stoichiometry. For the OPLS potential, this ratio is somewhat higher. Nevertheless, these results suggest that urea is not a structure breaker. Instead, urea can fit into the water structure without greatly disturbing the overall hydrogen-bond network of water. However, there is a large perturbation of the tetrahedral network of water in the vicinity of urea (see below). The distortion is most prominent at high urea concentrations.

Importance of Excluded Volume of Urea. Given that from nearly all structural perspectives the results between the OPLS model and the KBFF potential are qualitatively similar, why does the OPLS predict greater tendency for urea to self-associate than the KBFF model? The answer is that the number of hydrogen bonds that urea oxygen forms with the amide hydrogen (inter-urea interaction) is greater in the OPLS model than in the KBFF potential. For the urea mole fraction of 0.2, the OPLS carbonyl oxygen forms twice as many hydrogen bonds with amide hydrogen as the carbonyl oxygen in the KBFF potential. This finding *cannot be predicted on the basis of partial charges alone*! In fact, in the KBFF model the strength of a hydrogen bond between oxygen and hydrogen of urea, according to partial charges, is 1.5 times larger than in the OPLS potential. The larger number of oxygen−hydrogen bonds in the OPLS model is related to the values of Lennard-Jones parameters (see Table 1). In the OPLS model the value of ϵ_H is zero, which implies that urea molecules can be more tightly packed, which in turn enables formation of a larger number of hydrogen bonds. More generally, we assert that the *excluded volume of the cosolvent can greatly impede or enhance the formation of hydrogen bonds*. The ability of water to engage in hydrogen bonds effectively is directly related to its size. Similarly, the inability of urea to form as many hydrogen bonds as theoretically possible is also related to its larger size. The tighter packing of urea in the OPLS model follows from the treatment of the Lennard-Jones parameters, especially the one associated with amide hydrogen.

Water Hydrogen Bond Network near Urea Is Greatly Perturbed. Examination of the water pair functions, even at the highest urea concentration, shows that there are only relatively minor variations. The peak positions in all of the radial distribution functions are in the same position as in the bulk water. Only the heights of the first peak in $g_{O_WO_W}(r)$ and $g_{H_WO_W}(r)$ are enhanced. This suggests that the overall hydrogen bond network is more structured than in pure water. To probe if there are larger structural perturbations of the water hydrogen bond network in the proximity of urea water molecules, we have looked at the distribution of cos θ where θ is the angle between three water oxygen atoms that are in the neighborhood of urea molecules. To compute cos θ, we first identify and label the water molecule that is hydrogen bonded to the oxygen site of urea. For the labeled O_W, that forms a hydrogen bond with urea, we identify two neighbor oxygen atoms hydrogen bonded to the first water molecule. The angle between the three oxygen

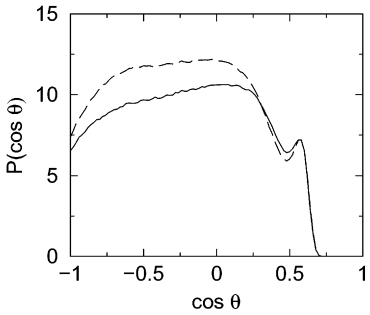

Figure 4. Distribution of cos θ, with θ being the angle between three water oxygen atoms. One of the water hydrogen atoms from the water molecule at the vertex of the triangle formed by the three water molecules is hydrogen bonded to the carbonyl oxygen. Note the peak at cos $\theta \approx -1/3$ characteristic of the water tetrahedral network is absent. This implies that there are substantial alterations in the hydrogen bond network near the urea molecules. The concentration of urea is 8.99 M. The solid curve is for the OPLS potential and the dashed curve is for the KBFF potential. These distributions are averaged over configurations separated by 0.1 ps.

atoms, with the one forming the hydrogen bond with urea at the vertex, is θ. Because oxygen atoms in pure water are arranged in a tetrahedral network, we expect that the most probable value of cos $\theta \approx -1/3$. In Figure 4 we show, for the two force fields, $P(\cos\theta)$ for water molecules in the neighborhood of urea. The two curves are qualitatively similar but are dramatically different from the distribution in the bulk. The expected sharp peak at cos $\theta \approx -1/3$ is extremely broad suggesting a great compromise in the tetrahedral arrangement of water oxygen atoms. There is a sharper peak at cos $\theta \approx 0.6$, which shows a much larger distortion of the tetrahedral network. Thus, regardless of the urea model used, it appears that there are qualitative changes in the arrangement of water in the vicinity of urea. The ability of urea to engage in a hydrogen bond with water even at the expense of distortion of water structure has implications for the urea-denaturation mechanism.

Hydrogen Bond Lifetimes The plasticity of hydrogen bond network of water implies that the lifetime of hydrogen bonds should not be significantly altered by urea. Recently, it has been shown that the hydrogen bond structure is only negligibly altered even when large concentration of ions are solvated in water.[31] Because the urea−water interaction is considerably weaker than ion−water interactions, we expect the lifetimes of water hydrogen bonds to be nearly the same as in the bulk. To measure the lifetime of hydrogen bonds we calculate the auto-correlation function

$$C(t) = \langle \Theta(t)\Theta(0) \rangle \qquad (1)$$

where Θ is the probability of forming a hydrogen bond and t is time. The correlation function $C(t)$ measures the probability that a hydrogen bond formed at $t = 0$ remains at a later time. The lifetime of hydrogen bonds can be inferred from the time depenence of $C(t)$. In Figure 5, we show $C(t)$ as a function of t for O_uH_W hydrogen bonds. This is for the KBFF potential at two urea concentrations. The lifetime for these concentrations is comparable to the H_WO_W bond lifetimes in the bulk.[32] There is no significant change in the lifetime of hydrogen bonds involving water with the addition of urea. This supports the picture that, despite local distortions of the hydrogen bond network, there are no significant changes in the overall hydrogen bond network of liquid water. In an earlier study, Tsai et al.[10] calculated hydrogen bond lifetimes of water in aqueous urea solution using two different models for urea. In the urea concentration range (0.23−6.71)M they found that the lifetime

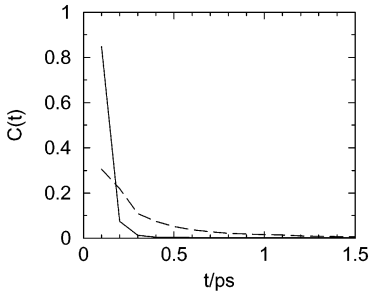

Figure 5. Time dependence of the autocorrelation function that measures the lifetime of hydrogen bonds between water and urea molecules (H_WO_U) for the KBFF potential. The dashed curve is 6.06 M urea and the solid curve is for 8.99 M urea. The hydrogen bond lifetime is not significantly altered, even at elevated urea concentration.

of water hydrogen bonds changed by at most a factor of 1.6. Their findings, using very different models for urea and water, are in accord with the present simulations. This observation along with the predictions based on structural characteristics show that globally urea, even at relatively high concentration, does not significantly perturb the properties of bulk water.

4. Conclusions

We have found that, despite large differences in the charge distribution in the two models of urea examined, most of the structural characteristics of water are qualitatively the same at all urea concentrations. Both models predict that urea can easily form hydrogen bonds with water molecules which readily explains the solvation of urea even at high concentrations. In accord with previous studies,[10,13] we find that urea does not greatly perturb the water structure. However, there are great distortions in the hydrogen bond network of water near the urea molecules.

The largest difference between the two models is in the predictions for self-association of urea. It has been noted in an earlier study that the differences in various models arise in the prediction of the tendency for urea−urea interaction. We have shown that these differences in the two models arise not only because of dramatic variations in the charge distributions but also because of the variations in the Lennard-Jones parameters. The tendency for the OPLS urea to self-associate more than the KBFF urea is attributed not to the differences in the strength of the hydrogen bonds but due to the differences in the number of hydrogen bonds between urea molecules. The OPLS model predicts formation of a larger number of inter-urea hydrogen bonds than the KBFF model due to the reduction in the hydrogen Lennard-Jones parameters. This finding emphasizes the importance of treating excluded volume on the solvation of urea in water, and more importantly in the denaturation of proteins.

It should be noted that several other models for urea interactions have been developed. The ordering exhibited by four such models that includes the ones introduced by ref 10 has been examined.[11,12] All models exhibit some degree of aggregation of urea at the molecular level. However, from the perspective of the denaturation mechanism we believe that the denaturation efficiency is determined largely by the ability of urea to engage in hydrogen bonds with the polypeptide chain. Hydrogen bond formation is determined by both charge distributions as well excluded volume. Models that capture both features, even if they vary in detail, would be expected to give qualitatively similar mechanism of urea denaturation.

If urea denaturation is dominated by the electrostatic mechanism then the ability of the denaturant to engage in as many hydrogen bonds with the peptide backbone as possible should

be the key determinant of efficiency of denaturation. Comparison of the two models suggests that the KBFF model would predict a stronger hydrogen bond between the carbonyl urea oxygen and the amide hydrogen of the peptide. However, the somewhat smaller size of the OPLS urea might enable formation of many hydrogen bonds with the peptide backbone. These arguments, which suggest that urea denaturation is a subtle combination of hydrogen bond forming ability and its excluded volume, imply that the qualitative mechanism of denaturation might be similar. In other words both models could reflect the actual denaturation mechanism.

Our results, that emphasize the importance of excluded volume on urea solvation, have implications for urea denaturation of proteins. Denaturation efficiency is determined by the ability of the cosolvent to interact favorably with the peptide backbone and the side chains. Recent simulations have argued that urea denaturation occurs by an electrostatic mechanism in which the ability to form hydrogen bonds with proteins promotes unfolding. The present results suggest that the efficiency of denaturation also depends on the excluded volume of the denaturant. The inability of urea to form as many hydrogen bonds (nearly 6) as allowed is linked to its large size. The excluded volume of urea demands that protein unfolding can occur only at elevated (greater than about (4−5)M) urea concentration.

Acknowledgment. D.T. acknowledges the National Science Foundation (CHE-02-9340) for partial support for this research.

References and Notes

(1) Schellman, J. A. *Biophys. Chem.* **2002**, *96*, 91.
(2) Kreschek, G. C.; Scheraga, H. A. *J. Phys. Chem.* **1965**, *69*, 1704.
(3) Muller, N. *J. Phys. Chem.* **1990**, *94*, 5794.
(4) Kuharski, R. A.; Rossky, P. J. *J. Am. Chem. Soc.* **1984**, *106*, 5786.
(5) Nozaki, Y.; Tanford, C. *J. Biol Chem.* **1963**, *238*, 4074.
(6) Robinson, D. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 2462.
(7) Mountain, R. D.; Thirumalai, D. *J. Am. Chem. Soc.* **2003**, *125*, 1950.
(8) Kuharski, R. A.; Rossky, P. J. *J. Am. Chem. Soc.* **1984**, *106*, 5794.
(9) Hernandez-Cobos, J.; Ortega-Blake, I.; Bonila-Marin, M.; Morenno-Bello, M. *J. Chem. Phys.* **1993**, *99*, 9122.
(10) Tsai, J.; Gerstein, M.; Levitt, M. *J. Chem. Phys.* **1996**, *104*, 9417.
(11) Sokolić, F.; Idrissi, A.; Perera, A. *J. Mol. Liquids* **2002**, *101*, 81.
(12) Sokolić, F.; Idrissi, A.; Perera, A. *J. Chem. Phys.* **2002**, *116*, 1636.
(13) Wallqvist, A.; Covell, D. G.; Thirumalai, D. *J. Am. Chem. Soc.* **1998**, *120*, 427.
(14) Sharp, K. A.; Madan, B.; Manas, E.; Vanderkooi, J. M. *J. Chem. Phys.* **2001**, *114*, 1791.
(15) Ikeguchi, M.; Nakamura, S.; Shimizu, K. *J. Am. Chem. Soc*. **2001**, *123*, 677.
(16) Duffy, E. M.; Severance, D. L.; Jorgensen, W. L. *Isr. J. Chem.* **1993**, *33*, 323.
(17) Tirado-Rives, J.; Orozo, M.; Jorgensen, W. L. *Biochemistry* **1997**, *36*, 7313.
(18) Weerasinghe, S.; Smith, P. E. *J. Phys. Chem.* **2003**, *107*, 3891.
(19) Kirkwood, J. G.; Buff, F. P. *J. Chem. Phys.* **1951**, *19*, 774.
(20) Tobi, D.; Elber, R.; Thirumalai, D. *Biopolymers* **2003**, *68*, 359.
(21) Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P. *J. Phys. Chem.* **1987**, *91*, 6269.
(22) Evans, D. J.; Murad, S. *Mol. Phys.* **1977**, *34*, 327.
(23) Sonnenschein, R. *J. Comput. Phys.* **1985**, *59*, 347.
(24) Rapaport, D. C. *J. Comput. Phys.* **1985**, *60*, 306.
(25) Schofield, P. *Comput. Phys. Commun.* **1973**, *5*, 17.
(26) Mountain, R. D.; Brown, A. C. *J. Chem. Phys.* **1985**, *82*, 4236.
(27) Sangster, M. J. L.; Dixon, M. *Adv. Phys.* **1976**, *25*, 247.
(28) Martyna, G. J.; Klein, M. L.; Tuckerman, M. *J. Chem. Phys.* **1992**, *97*, 2635.
(29) Trypuć, M.; Mazurek, K.; Białowicz, K. *Fluid Phase Equilib.* **2002**, *203*, 285.
(30) Soper, A. K.; Castner, E. W.; Luzar, A. *Biophys. Chem.* **2003**, *105*, 649.
(31) Omta, A. W.; Kropman, M. F.; Woutersen, S.; Bakker, H. J. *Science* **2003**, *301*, 347.
(32) Mountain, R. D.; Thirumalai, D. *Comput. Phys. Commun.* **1991**, *62*, 352.