Solvation of Hydrophobic Species in Aqueous Urea Solution: A Molecular Dynamics Study

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Abstract: A molecular dynamics study of a ternary system consisting of 200 water molecules, 1 urea molecule, and a Lennard-Jones sphere has been carried out in order to study the solvation of an apolar solute by both urea and water. The presence of urea in the solvation region of the apolar sphere is found to weaken the water–water interactions in this region, although they are still stronger than in the bulk solvent. Urea–water interactions are enhanced in the solvation region as compared to aqueous urea. The implications of the results for the experimentally known improved solvation of apolar molecules in aqueous urea are discussed. The phenomenon appears likely to be dominated by the fact that each urea molecule displaces several water molecules from the apolar solvation shell.

I. Introduction

There has been a great deal of discussion on the mechanism of urea-induced protein denaturation.[1-16] Much of this has focused on the apparent ability of urea to weaken hydrophobic interactions.[5-11] This focus, which we pursue here as well, is not meant to imply that other interactions, with polar groups, are not important. The evidence, in fact, suggests that an effective denaturing cosolvent must possess the ability to solvate polar groups.[13-16] However, the ability to solubilize nonpolar species appears to be a critical characteristic.

Wetlaufer et al.[5] found that hydrocarbons of sufficient size (larger than ethane) were more soluble in aqueous urea than water at 25 °C and proposed two mechanisms for this increased solubility: (1) an indirect mechanism, where urea alters the "structure" of water in a way that facilitates the solvation of a hydrocarbon by water, and (2) a direct mechanism, where the hydrocarbons are solvated by both urea and water molecules.

The indirect mechanism has received the majority of attention in the literature.[5-11,17,18] Frank and Franks[17] proposed that the water around urea was less hydrogen bonded than bulk water, and many experimental results have been cited as showing that urea acts as a water "structure breaker". Nevertheless, there have also been objections raised to the indirect mechanism, stemming from other experiments which indicate no significant effect of urea on water structure and, perhaps of more importance, a lack of correlation between the effect of a given solute on water structure and its ability to solubilize hydrocarbons.[4,12,14] In a simulation of an aqueous urea solution that we have carried out,[19] hereafter referred to as paper 1, no evidence for significant urea-induced changes in water structure were found, although very small structural differences which appear consistent with available experimental data were noted. These results have provided rather direct evidence against the indirect mechanism.

The direct mechanism has received much less attention, although there are good reasons to consider it. In 7 m urea, frequently used for denaturation, the ratio of water molecules to urea molecules is only about 6 to 1, owing to the larger size of urea, and this represents a volume fraction of urea of about 0.3. Under these conditions the inclusion of urea in the solvation of a hydrocarbon appears unavoidable. Nozaki and Tanford[13] suggested that, since both water and urea form crystalline clathrates with hydrocarbons, mixed "clathrate-like" structures composed of urea and water molecules may be formed around hydrocarbons in aqueous urea. It was suggested that the increased solubility would then result from a greater structural adaptability when two types of solvent molecules were available to form these structures. We note, however, that the structure of such a "mixed clathrate" is unlikely to resemble those formed by pure urea, based on the very different nature of those structures[20] as compared to aqueous clathrates.[21]

Jencks and co-workers[14,15] have proposed that the increased solubility of hydrocarbons in aqueous urea results primarily from a smaller free energy of cavity formation in the mixed solvent, resulting from the replacement of water by the larger urea molecule in the solvation region. They note that the water–hydrocarbon interfacial tension is reduced by addition of urea, although the surface tension of aqueous urea is slightly greater than that of pure water. The importance of the size of urea is supported by the impressive correlation which they have demonstrated between the parachor (approximate measure of molecular volume) of a cosolvent and the free energy of transfer of naphthalene from water to water–cosolvent mixtures for a large number of cosolvents.

In the present work, we examine how the presence of a urea molecule in the aqueous solvation shell of an apolar molecule influences the molecular description of apolar solvation. To accomplish this we employ the molecular dynamics computer simulation technique on a system composed primarily of water molecules but including also one urea molecule and a nonpolar sphere. The relevance of a system which includes only a single urea molecule to the enhanced solvation of apolar groups is clear from experiment.[5] The solubility of nonpolar solutes in aqueous urea solution is linear in the concentration of the urea cosolvent even at moderate concentrations. There is no evidence that urea–urea interactions play any essential role in the phenomenon.

The apolar solute is chosen to be representative of the quasi-spherical neopentane molecule. The choice of a relatively large solute was dictated by the fact, mentioned above, that for small

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hydrocarbons urea does not enhance solubility at room temperature. Although the origin of this behavior is not clear, a comparable size for solute and cosolvent could easily place special requirements on solvation structure. It was desired to avoid such potential additional complicating factors in the analysis here.

In section II, we describe the model solution and the procedure for the simulation. An analysis of the structure of the solution is presented in section III. We first discuss the orientation of the urea molecule with respect to the apolar sphere. We then analyze the energetic properties of water and of urea in the solvation region of the apolar sphere and compare the properties of water molecules in the solvation region which are close to urea with those which are not. Further we compare the energetic behavior of urea when it is near the apolar sphere to that with no sphere present, available from our earlier study. In section IV we discuss the implications of the observations for the mechanism of the increased solubility of hydrocarbons in aqueous urea.

II. Model Solution

The urea–water and water–water interaction potentials are the same as were used in the previous aqueous urea simulation[19] (paper 1). The urea model is a rigid body planar arrangement of atoms which are represented by point charges and Lennard-Jones spheres. The water model is that of Rahman and Stillinger,[22] the ST2 model.

The model hydrophobic solute is a Lennard-Jones sphere with parameters $\sigma = 6.15$ Å and $\epsilon = 0.8351$ kcal/mol. This choice was made to correspond roughly to neopentane.[23] As noted above, we use a solute large enough to ensure that the potentially peculiar factors that lead to the decreased solubility of methane and ethane in aqueous urea at 25 °C are not present. Neopentane's solubility is increased in aqueous urea solution over a wide temperature range.

The initial configuration for the simulation was obtained by placing the Lennard-Jones sphere into the final configuration of the aqueous urea simulation[19] and removing the ten water molecules which were found to be in close contact with the sphere. The simulation sample then contains 1 Lennard-Jones sphere, 1 urea molecule, and 200 water molecules. The initial placement of the sphere was chosen with the plane of the urea molecule tangential to the spherical surface and with the carbon atom of urea at the Lennard-Jones contact distance (4.7 Å).

Such a placement is, of course, not unbiased. The choice is made based on two considerations. First, we explicitly wish to examine the molecular description of hydrophobic hydration with incorporation of urea in the solvation layer. An initial placement of the urea and apolar sphere in contact is therefore a reasonable configuration. The choice of urea orientation is more subtle. The choice made, with the plane of urea perpendicular to the surface normal of the sphere, is based on the fact that a tangential orientation of this type maximizes the exposure of hydrogen-bonding solute groups to the aqueous solvent. Such exposure would be expected to strongly influence the preferred orientation in the solvation layer. As described in more detail below, the urea–apolar sphere pair maintains a relative geometry of this general type throughout the simulation, which is suggestive evidence that this configuration is a relatively stable one, in accord with expectations.

The edge length of the cubic volume containing the solution molecules was set to 18.438 Å, corresponding to apparent molar volumes of 122.1 mL/mol,[24] 18.053 mL/mol,[25] and 42.241 mL/mol[25] for neopentane, water, and urea, respectively. The procedure for the molecular dynamics calculation is the same as described in paper 1. We employ periodic boundary conditions and a spherical cutoff of 8.0 Å for all interactions. A time step of 1×10^{-15} s was used for the integration of the equations of motion.

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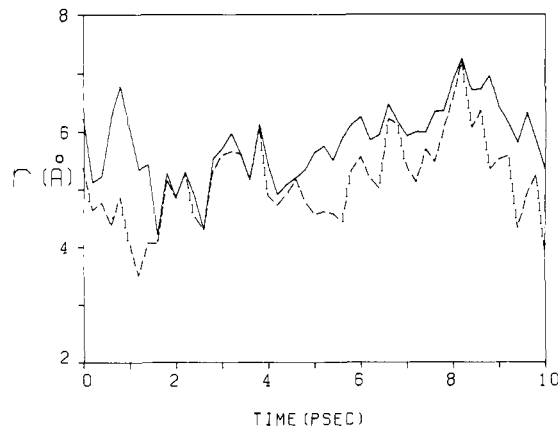

Figure 1. History of the distances between the center of the apolar sphere and the urea carbon atom (—) and the closest urea atom (---).

The system described above was equilibrated for a period of 10 000 steps (10 ps) with no constraints on relative molecular geometries. During the equilibration period, the velocities were periodically replaced from a Boltzmann distribution (298 K) to assist thermal equilibration in the sample. Following the equilibration, an additional 10 000-step (10 ps) simulation was run for analysis. The average temperature of the water molecules in the simulation was found to be 288 K (translational temperature of 286 K and rotational temperature of 289 K). The average temperature of the water molecules whose centers of mass were within 6 Å of the apolar sphere was 287 K and the average temperature of those within 6 Å of the center of charge of urea was 292 K, while the average temperatures of the single urea molecule was 298 K. The solvent molecule temperatures indicated that good thermal equilibrium was achieved. The small residual temperature differences have little effect on the structural quantities of interest here.[26]

III. Structural Analysis

The purpose of this study is to obtain a molecular description of the solvation of the hydrophobic sphere simultaneously by both urea and water. To carry out a meaningful analysis, we compare this description with that obtained for the two binary systems, the first, the solvation of apolar entities by water alone, and the second, for the aqueous urea solution. We will consider several features which may play important roles in producing the increased solubility of apolar molecules in aqueous urea. These include the influence of urea and of the apolar solute on the interactions among water molecules as well as an analysis of urea–water interactions within the apolar solvation region.

Before describing this solvation structure in detail, we briefly describe the variations in urea–sphere relative geometry which occur during the simulation and thus specify the solvation geometry which is characteristic of the present realization of the three-component system.

A. Sphere–Urea Relative Geometry. Although in the initial configuration created for this simulation the sphere was positioned at the Lennard-Jones contact distance from the urea carbon, no external constraint was used to keep them close together. However, the current analysis would be of limited value if the relative position of urea with respect to the apolar solute varied qualitatively in various segments of the simulation. As indicated earlier, the present study is characterized by a single idealized structure in which the urea is present in the first solvation layer of the sphere, with the molecular plane tangential to the apolar surface.

To show this we give, in Figures 1 and 2, characteristics of separation and relative orientation for the sphere–urea pair. In Figure 1 we show a time history of the distances between the sphere and the urea carbon atom and the distances between the sphere and the closest urea atom. There is nearly always a urea atom within 6 Å of the sphere center; it is clear that water does

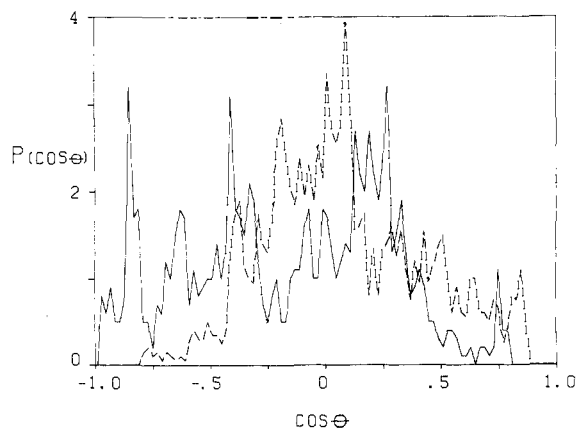(26) Mezei, M.; Beveridge, D. L. *J. Chem. Phys.* **1981**, *74*, 622.

Figure 2. Distributions of cosines of the angles between the vector from the urea carbon to the center of the apolar sphere and the vectors along the C–O (—) and C–N (- - -) bonds in urea.
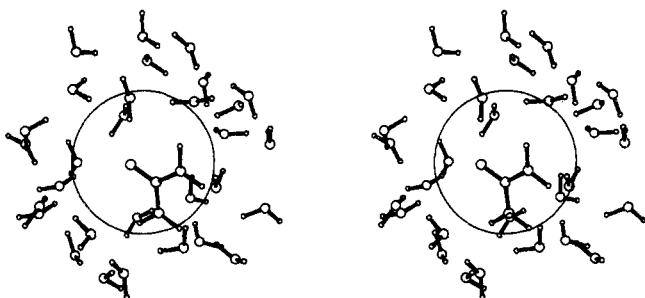

Figure 3. Stereographic view of the solvation region of the apolar sphere.

not penetrate between urea and the apolar solute.

As argued above, it is reasonable to expect that since urea, like water, is a relatively small molecule with several hydrogen bonding sites, it will exhibit similar orientational preferences, namely the tendency to avoid having a hydrogen-bonding site directed toward the sphere.[27] We have calculated the angle between the vector joining the urea carbon atom and the sphere center and each of the vectors along the C–O and C–N bonds in urea. The distributions of the cosines of these angles are shown in Figure 2; a negative cosine corresponds to the outward pointing direction. The distributions are relatively noisy due to the limited data available. Nevertheless, a substantial tendency to retain the tangential orientation is evident, although a variety of orientations is sampled. There is apparently a slight tendency for the C–O to be directed into the bulk solvent, while for C–N the trend is the opposite.

We must emphasize that the results cited above do not demonstrate that the structures observed are the most favorable for solvation of a nonpolar solute by urea. They are only consistent with this view and the reasonable arguments which led us originally to choose this structure. We cannot rule out the possibility that the spatial distribution observed is dominated by our initial choice of configuration. The analysis below must be viewed in any case as a description of the solvation structure for a particular narrow range of urea–apolar solute relative geometries.

The structure characteristic of the present simulation is easily seen in the stereographic picture shown in Figure 3. In the figure we include the sphere, urea molecule, and the 30 water molecules with oxygen atoms nearest the sphere center. The configuration was chosen at random from the middle of the simulation. The urea molecule is seen to be oriented so that it can hydrogen bond with water molecules in the region, and there are several of these hydrogen bonds in this configuration.

B. Definition of Apolar Solvation Regions. In Figure 4, we show the apolar sphere–water oxygen radial distribution function, $g_{AO}(r)$. For present purposes, we will consider water molecules as being in the solvation shell of the sphere if their oxygen atoms are within
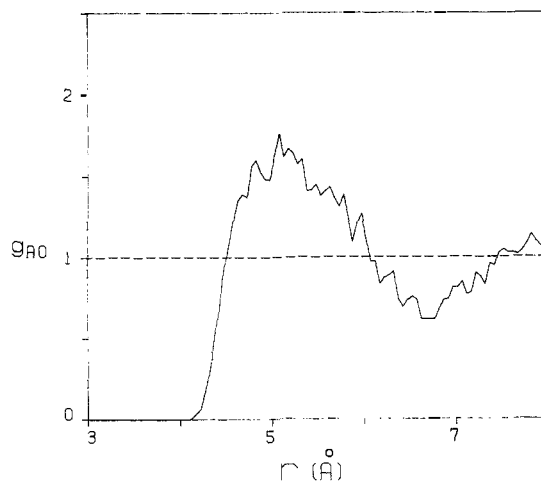
(27) Geiger, A.; Rahman, A.; Stillinger, F. H. *J. Chem. Phys.* **1979**, *70*, 263.

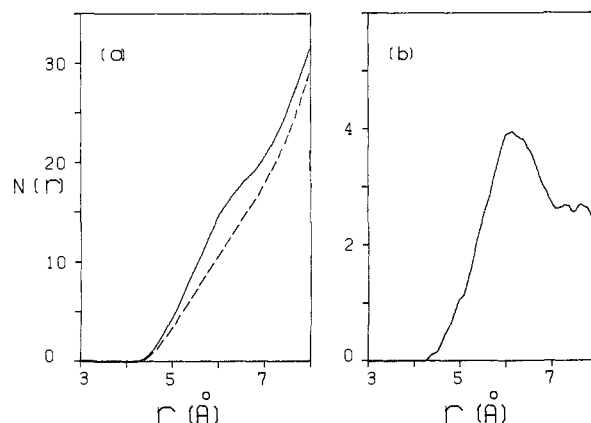**Figure 4.** Apolar sphere–water oxygen pair correlation function.

Figure 5. (a) Running coordination numbers ($N(r)$) for water molecule oxygen atoms with respect to the center of the apolar sphere for the w side (—) and the u side (- - -). (b) Difference in $N(r)$: w side – u side.

6 Å of the center of the sphere. Such a shell has frequently been defined as extending to the first minimum in the distribution function, or about 6.7 Å for this case. However, here there are two criteria which the shell must meet. First, we wish to include only water molecules that are near neighbors of the sphere, and second, we must include enough water molecules to get reliable statistics for the shell region. Our definition of the sphere shell satisfies these conditions.

We further divide the apolar solvation shell into two distinct regions as follows. For each water molecule in the designated sphere shell, we determine the angle between the sphere–water oxygen atom vector and the sphere–urea carbon atom vector. If this angle is less than 90°, the water molecule is designated as being on the "urea side" of the sphere shell, hereafter denoted as u side. Otherwise, it is on the opposite side of the sphere shell; this opposite, purely aqueous, side will be denoted as the w side.

We also define a urea solvation shell in the identical manner as in the aqueous urea simulation of paper 1; that is, it contains water molecules whose oxygen atoms are within 2.75 Å of a urea hydrogen, 3.75 Å of the urea oxygen, or 4.75 Å of the urea carbon. Water molecules which are in neither the sphere nor urea shell regions are classified as "bulk". In the analysis, the classification of a water molecule into these four groups is based on its instantaneous position in each configuration. This results in an average of 10.5 molecules on the u side, 14.4 on the w side, 11.9 in the urea shell region, and 166.7 in the bulk region. On the average, there are only 3.5 water molecules which are simultaneously in the urea solvation shell and on the u side of the sphere and 8.4 which are in the urea solvation shell but not in the immediate solvation layer of the sphere.

On the average there are 3.9 more water molecules on the w side of the defined sphere shell than on the u side. This difference

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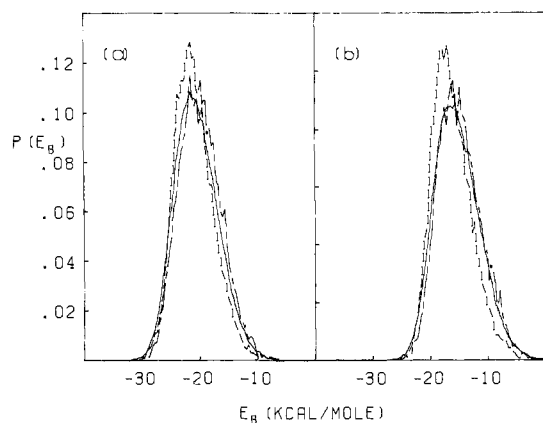J. Am. Chem. Soc., Vol. 106, No. 20, 1984 5797

Figure 6. Probability distributions for binding energy; total (a), near neighbor (b), bulk (—), w side (---), u side (— — —).

Table I. Energetic Characteristics of Water Molecules

| | binding energies[a] | | $\langle n_{HB} \rangle$ | |
	total	near neighbor	$\epsilon = -3$ kcal/mol	$\epsilon = -4$ kcal/mol
bulk	−20.56	−15.32	3.06	2.11
w side	−20.91	−16.17	3.21	2.32
u side	−19.91	−14.92	2.99	2.02

[a] Energies in kcal/mol.

is considerably larger than the ratio of the apparent molar volumes of urea and water[25] which is 2.4. In order to clarify this, we show, in Figure 5, the running coordination numbers ($N(r)$) for water molecule oxygen atoms with respect to the sphere center for the u and w sides of the sphere.

The $N(r)$ difference plot (Figure 5b) has a peak of about 4 around 6 Å and then drops off to the value of 2.4 for larger distances. One should not be surprised that the presence of a cosolvent (here urea) can cause a radial redistribution of water molecules around the sphere. This can result, for example, from packing considerations related to solute geometry or changes in the hydrogen bonding network needed for solute accommodation. The shift we observe corresponds to the presence of somewhat fewer water molecules very close to the sphere than one would have predicted simply from the volume of urea.

C. Energetic Properties of Aqueous Solvent. Here, we present and compare the binding energies and hydrogen bonding characteristics of the water molecules which are on the u or w side of the solvation shell or in the bulk. These energetic calculations utilize the same potential cutoffs as in the simulation unless indicated otherwise. One must expect that any significant effect that urea may have on the water structure in the sphere shell will be limited to the u side, so that the opposite, w side, of the sphere shell can be taken as representative of the solvation region of the sphere in the absence of urea. This is readily verified by comparison with the results of previous studies of hydrophobic hydration.[27-31] We then compare the properties of the two sides of the sphere shell to determine if urea has a significant effect on those water molecules which are close to both urea and the sphere or, alternatively, if the properties of these water molecules are essentially unchanged, as was observed for the water molecules in the shell of urea in bulk aqueous urea solution (paper 1).

1. Binding Energies. In Figure 6 we show the distributions of total and near-neighbor binding energies for the three classifications of water molecules, bulk, u side, and w side. For the "near-neighbors" binding energies, water–water interactions are included for pairs of water molecules whose oxygen atoms are separated by no more than 3.5 Å and for water–urea and
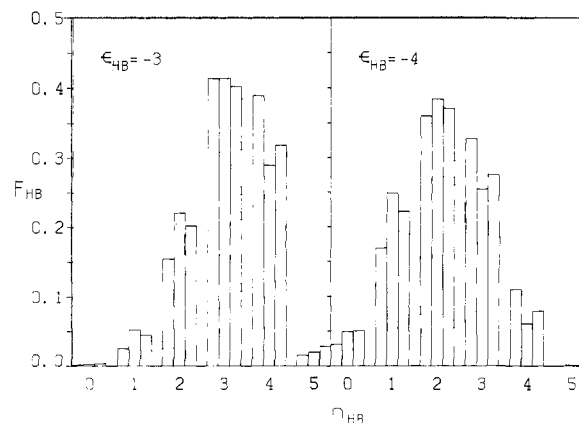
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Figure 7. Fraction of water molecules participating in n_{HB} hydrogen bonds for two values of the energetic cutoff, ϵ_{HB}. W side is on the left, bulk is the center, and u side is on the right, for each value of n_{HB}.
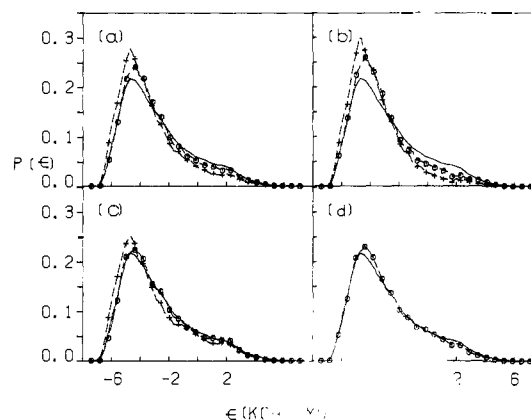

Figure 8. Normalized pair interaction energy distributions for near-neighbor molecules. For the w and u sides, only interactions among pairs of water molecules which are both in the sphere shell are included in (b), and only interactions among pairs where only one is in the sphere shell are included (c). Urea–water interactions are included in the u-side distribution in (d). Bulk (—), w side (---), u side (— — —).

water–sphere interactions only for water molecules which are in the corresponding shell regions. The average binding energies from the distributions in Figure 6 are given in Table I.

The binding energy distributions for the w side of the sphere shell are narrower than those of the bulk, and the average binding energies are more negative. Such changes correspond closely to those observed in earlier studies of hydrophobic hydration[27-31] and indicate the consistency of our assumption that urea would have no noticeable effect on the solvation on the w side of the sphere.

In contrast, the binding energy distributions for the u side of the sphere shell have about the same widths as those of the bulk, and the average binding energies are more positive. The presence of urea has resulted in the binding energy distributions for the water molecules on the u side of the sphere shell being wider and shifted to more positive energies than on the w side.

2. Hydrogen Bonding. We employ an energetic definition of a hydrogen bond; two molecules are considered as hydrogen bonded if their interaction energies are less than or equal to the energetic cutoff employed. In Figure 7 we show histograms of the average fraction of water molecules (F_{HB}) in each region participating in n_{HB} hydrogen bonds, evaluated for two different values of the energetic cutoff. The average number of hydrogen bonds per water molecule, $\langle n_{HB} \rangle$, calculated from the histograms are contained in Table I. Both water–water and water–urea hydrogen bonds are included in these calculations. The values of $\langle n_{HB} \rangle$ for the bulk and the u side of the sphere shell are close to the same, while there is an increase in $\langle n_{HB} \rangle$ for the opposite side of the sphere shell.

The histograms in Figure 7 show that a larger fraction of the water molecules on the w side have three or four hydrogen bonds

Table II. Numbers of Urea–Water Hydrogen Bonds[a,b]

ε, kcal/mol	total		proton acceptor		proton donor	
	A	B	A	B	A	B
−2	3.18	2.64	1.55	1.58	1.63	1.06
−3	1.88	1.56	1.13	1.13	0.75	0.43
−4	1.04	0.73	0.68	0.62	0.36	0.11

[a] A = present simultation; B = aqueous urea simulation (paper 1). [b] The total urea binding energy is −16.7 kcal/mol here and −15.8 kcal/mol in the previous aqueous urea simulation.

and a smaller fraction have only one or two compared to the other solvent regions, in accord with the results of earlier studies of hydrophobic solvation.[27,31] The water molecules on the u side of the sphere shell *do not* manifest an increased tendency to be four bonded or to form more hydrogen bonds than bulk water.

In Figure 8 we show pair interaction energy distributions for near-neighbor water molecules in each of the three solvation regions. For these distributions water–water interactions are included only for pairs of water molecules with an oxygen–oxygen separation less than 3.5 Å. In Figure 8a–c urea–water interactions are excluded while in Figure 8d they are included, but consistently, only for water molecules simultaneously in both the sphere and urea solvation shells. Figure 8a includes all water–water pairs, while Figure 8b includes only those interactions between pairs of water molecules which are both in the sphere shell. Figure 8c includes all pairs where only one molecule is in the sphere shell. The corresponding bulk distribution is included in each case for comparison. The curves are all integrably normalized to unity, so that the height is proportional to the fraction of near-neighbor molecules with the indicated interaction energy.

A comparison of the distributions for the w side of the sphere shell with that of the bulk again shows expected behavior.[26,27,29] That is, the distributions indicate a shift to more negative interactions, with the shift being larger for pairs of water molecules which are both in the sphere shell (Figure 8b) than for pairs with only one in the sphere shell (Figure 8c).

The distribution of water–water interactions on the u side of the sphere shell (see Figure 8a) also shows a shift to more negative energies, but the shift is not as pronounced as for the w side. Further, for the u side, this region of enhanced probability does not extend into the very strong negative interaction region (ε < −5.5 kcal/mol), in contrast to the w-side distribution. On the u side, the interactions among pairs of water molecules which are both in the shell (Figure 8b) are entirely responsible for the shift, as there is little difference between the bulk and u-side distributions with only one water molecule in the shell (Figure 8c).

When urea–water interactions are also included (Figure 8d), the u-side distribution is very similar to that of the bulk. Correspondingly, it is clear that the interactions between urea and water molecules within the solvation shell of the sphere must be weaker than are near-neighbor water interactions. This will be evident from the discussion of urea–water interactions presented below.

In summary, a comparison of the energetic properties between the u and w sides of the sphere shell shows that, in contrast to the aqueous urea simulation, urea has a substantial effect on the interaction among water molecules; the presence of urea induces a weakening within the solvation layer, compared to those in the absence of urea. It is then apparent that the water molecules in the sphere shell have *not* incorporated urea into the clathrate-like structure around the apolar solute without weakening the near-neighbor interactions among shell water molecules (see Figure 8b). Nevertheless, even on the u side the water–water pair interactions are typically somewhat stronger than in the bulk.

D. Energetics of Urea–Water Interactions. The differences between the energetic behavior of water molecules in the three solvation regions of the apolar solute solution are clearly shown in the comparisons above. To perform an analogous comparison for urea, we can use the present simulation for the properties of urea in the solvation region of the apolar sphere and the previous, aqueous urea, simulation (paper 1) for the properties of urea in the absence of the sphere. There is a small temperature difference
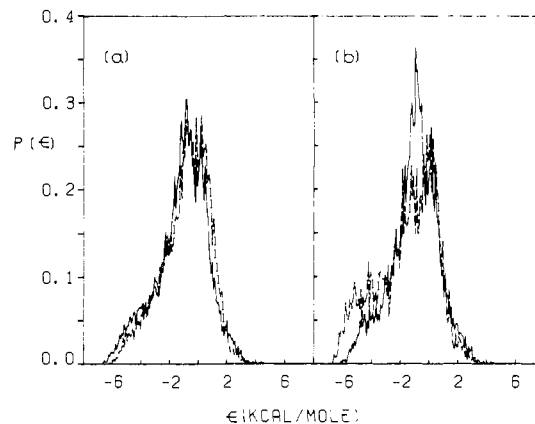

Figure 9. Normalized distributions of near-neighbor urea–water interactions. (a) This simulation (—), aqueous urea simulation (paper 1) (---); (b) this simulation, including only water molecules not in the sphere shell (—), only water molecules in both the urea and sphere shells (---).

between the two simulations (here the mean temperature in the urea solvation shell is 6 K lower). However, we know that for pure water, the quantities of interest are not very sensitive to temperature differences of this magnitude.[26] Hence, any large changes observed in urea's energetic environment can be reasonably attributed to the presence of the apolar sphere.

1. Urea–Water Hydrogen Bonding. In Table II we show the average numbers of hydrogen bonds between urea and water in the two simulations for three values of the energetic cutoff and further divide these into classes according to whether urea acts as a proton acceptor or as a proton donor. These results show that urea forms more and stronger hydrogen bonds with water when it is near the sphere than it does in the absence of the sphere and that the majority of this difference is at the urea proton donor sites.

For the −3 kcal/mol cutoff, the number of urea proton acceptor bonds is about the same in the two simulations. For the −4 kcal/mol cutoff there is only a slight increase in the number of such bonds when urea is near the sphere. There is, however, a significant increase in the number of proton donor bonds in all cases; compared to the results of the present simulation, the aqueous urea simulation manifests only 57% and 30% of the bonding for criteria of −3 kcal/mol and −4 kcal/mol, respectively. This behavior is in contrast to that of water molecules, which have only a small increase in the number of hydrogen bonds when in the solvation region of an apolar solute.

It should be noted that even with this increase the number of urea proton donor bonds is still substantially smaller than the maximum of about three or four[32] and the fraction of bonds per site remains smaller than for water. The increase here reflects more directly the relative lack of urea proton donor bonds in the purely aqueous environment observed in paper 1.

2. Urea–Water Pair Interaction Energy Distributions. In Figure 9, we show the distributions of pair interaction energies between urea and those water molecules within the defined solvation shell of urea. The distributions obtained from the present simulation and those from aqueous urea simulation are given in Figure 9a. In Figure 9b, we show separately the distributions for interactions with water which are simultaneously in both the urea and apolar

(32) Orita, Y.; Pullman, A. *Theor. Chim. Acta* 1977, *45*, 257.

sphere shells and for those which are in the urea shell but not that of the sphere.

The curves in Figure 9a show that when urea is in the vicinity of the sphere there is an increased probability of strong negative ($\epsilon \leq -4$ kcal/mol) interactions and a decreased probability of positive interactions with near-neighbor water molecules. Comparing these curves to the analogous distributions for water (w side and bulk in Figure 8a) we note a similarity in the behavior of urea and water in the vicinity of the sphere. The shift in probability toward negative interactions evident in Figure 9a is indicative of the tendency for stronger (straighter) hydrogen bonds between urea and water when both occupy apolar solvation positions. As noted in paper 1, the relatively large peak around 0 kcal/mol in the distributions is due to the fact that the majority of water molecules adjacent to urea must have weak interactions with urea. That is, there are on the average 11.9 water molecules counted as near neighbors for urea in the present simulation while urea can form only about 5 simultaneous strong hydrogen bonds with water.[32] For comparison these numbers for bulk water are 5.46 near neighbors and 4 simultaneous strong hydrogen bonds.

The distribution of pair interaction energies between urea and water molecules simultaneously in both the urea and sphere shells (Figure 9b) shows clearly that a significantly larger fraction of these water molecules have strong negative interactions with the urea molecule than is the case for urea solvation in water alone.

In light of the results above, the smaller magnitude of the water binding energy on the u side of the solvation sphere, as compared to other solution regions, can be interpreted as arising from a balance between two contributions. First, the water–water interactions are somewhat stronger than in bulk, although this enhancement is less significant than on the w side of the sphere. However, the number of near neighbors, and thus hydrogen bonding interactions, is reduced, in comparison to water molecules in the bulk, in all regions of the apolar solvation layer. Nevertheless, on the w side, the balance between these two effects leads to an enhanced binding energy compared to bulk water, as is well-known.[27-31] However, on the u side, despite the somewhat enhanced urea–water bonding, urea remains a relatively weaker bonding partner than is another water molecule. It appears that it is the presence of this weaker partner which leads to the net energetic destabilization of water on the u side.

IV. Discussion and Conclusions

In this section we consider the implications of the present observations for the mechanism of improved apolar solvation in aqueous urea. We must emphasize at the outset that no attempt has been made to obtain free energy information in the present study. Hence, our goal here is to incorporate the observations made into a reasonable molecular-level description of the origin of the experimentally observed phenomenon.

Correspondingly, it is an important implicit assumption that the simulated system would manifest the same behavior as that evident in experiment. The primary questions in this regard are first whether the urea–apolar solute association geometry studied here is that typical of the experimental situation (since the arguments in favor of this geometry are strong, this seems to us to be a relatively minor concern) and second, and of more importance, whether we should use a model for urea which is derived from quite limited information, as discussed in paper 1. We cannot rule out the possibility that the results obtained with a different model with, for example, stronger hydrogen-bond interactions would show some alternative behavior. Therefore, we do not place heavy emphasis on the quantitative results obtained, but rather focus on the very distinct trends evident in the present study. We believe that the model gives a correct characterization of urea and therefore that it should manifest such qualitative trends faithfully. It is clear that a demonstration of this expectation will require a further study, corresponding to that presented here but using a different urea model.

The results presented in earlier sections show that inclusion of urea in the solvation region of an apolar solute has significant consequences for the solvation structure. In the vicinity of the urea molecule, the hydrogen-bonding interactions between water molecules and between urea and water are somewhat stronger than in bulk aqueous urea solution. However, the enhancement of interactions is significantly larger for aqueous solvation of the apolar solute in the absence of urea. Since, at least in the present model, the urea–water hydrogen bonds are found to be typically weaker than water–water interactions, the observed distribution of hydrogen bond energies involving water is essentially identical in the region around urea and in the bulk solution. Since the number of such interactions is smaller than in bulk for any molecule in the solvation shell of the apolar solute,[25-29] the binding energy of the water molecules in the simultaneous vicinity of the urea and apolar solute is actually significantly smaller in magnitude than for solvent in the bulk. Although the urea molecule has a somewhat more negative binding energy in the apolar solvation shell than in the bulk solution (by about 1 kcal/mol), this does not compensate for the loss in water binding energy.

Probably of equal importance to the above, but not at all surprising, is the fact that urea displaces water from the solvation layer of the apolar solute. In the present calculation, the urea molecule replaces approximately four water molecules in the immediate neighborhood of the apolar surface.

These results show that urea is not incorporated into the clathrate-like structure around an apolar solute without significant distortion of this structure, and thus a literal interpretation of the type of mechanism envisioned by Nozaki and Tanford[13] at least requires some modification. It is well-known[5] that the transfer of apolar solutes from water to aqueous urea solution is substantially endothermic. Our simulation results indicate that the water molecules on the urea side of the apolar sphere will contribute to such a positive enthalpy of transfer. One must expect some compensation, so that a positive contribution to the entropy is also anticipated. Nevertheless, we have no basis to argue that any associated entropy increase would be of sufficient magnitude to offset the positive enthalpy change and yield a negative contribution to the free energy for the transfer.

The fact that the incorporation of urea into the solvation region of the apolar solute produces a negative contribution to the enthalpy through enhanced urea–water interactions at the expense of orientational restrictions on the urea is analogous to the behavior of water. For water, this is in net an unfavorable process.

One is then led to focus on the fact that there are fewer water molecules in the solvation region due to their replacement by urea. For the transfer of an apolar solute from water to aqueous urea, this "release" of several water molecules per urea from the solvation region to the bulk will have both positive enthalpic and entropic contributions to the transfer. A significant negative contribution to the free energy of transfer is certainly expected as it is precisely the restriction of the configurational freedom of water which characterizes the unfavorable hydrophobic hydration phenomenon.

From the present data, it is not possible to determine the relative importance of the various contributions listed above. However, the fact that the results in paper 1 showed at most a very small effect on water in an aqueous urea solution is strong evidence against the indirect mechanism. Hence, it appears that the (favorable) displacement of a number of water molecules by a single urea is the critical element in the solvation process. In this context, it should be recalled from our discussion that the urea *is* incorporated into the solvation shell with the maintenance of bonding comparable to the bulk. It is only in comparison to the enhanced, clathrate-like, structure associated with purely aqueous solvation that distortion occurs.

The molecular interpretation above appears to be in general accord with the discussion and experimental results of Roseman and Jencks.[14] Figure 4 of ref 14 shows that for urea and the methylureas there is a linear relationship between the free energy of transfer of naphthalene from water to the aqueous solution containing cosolvent and the molecular volume of the cosolvent. Since in the series from urea to tetramethylurea the hydrogen bonding character changes substantially, the potential for replacement of water molecules in the solvation region implicit in their size appears to be a uniting factor.

An element which clouds this interpretation is the possibility for preferential solvation of the apolar species by the larger, but also relatively less polar, cosolvent molecules considered. We have not invoked any need for preferential solvation by cosolvent in discussing the solvation mechanism, but it is reasonable to anticipate important quantitative contributions to the effectiveness of the cosolvent from this origin. For example, the data presented in the figure cited above also shows that urea is not unusually effective in solubilizing naphthalene. In fact, the smaller ethanol molecule is slightly more effective.

In conclusion, we believe that the present study, in combination with that presented in paper 1, is most consistent with the view that the critical feature of urea which leads to enhanced solubilization of apolar species is the simple displacement of a number of water molecules from the apolar solvation sphere. The significant influence of urea on the hydrogen bonding structure in the apolar region must, however, make an important quantitative contribution. In regard to the protein denaturing ability of urea, it is most reasonable to regard this as a result of the unusual *combination* of abilities including both the improved solvation of hydrophobic side chains and the ability to solvate polar groups, as suggested earlier.[13,16]

Registry No. Water, 7732-18-5; urea, 57-13-6.

# Dissociation Dynamics of Photochemically Activated 3,5-Cycloheptadienone in the Gas Phase

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Abstract: Energy partitioning in the photoactivated fragmentation of 3,5-cycloheptadienone has been studied by using time resolved laser absorption spectroscopy. We have directly measured the CO product vibrational energy distribution and find that for excitation at 193 nm, $N_0:N_1:N_2 = 1.00:0.33:0.09$. Excitation at 249 nm yields $N_0:N_1:N_2 = 1.00:0.24:0.04$, and excitation at 308 nm yields $N_0:N_1:N_2 = 1.00:0.14:0.02$. The data at each excitation wavelength are consistent with a statistical model for energy disposal if the full reaction exoergicity is available to be randomly distributed among all the developing products' degrees of freedom. This suggests the products are strongly coupled well into the exit channel. These observations are contrasted with results on energy disposal for an apparently similar reaction, the dissociation of 3-cyclopentenone, where it was found that the full reaction exoergicity was not available to the products' vibrational modes. A mechanistically based model is suggested to account for the observed differences in energy disposal dynamics for these two ketones.

The exoergicity of a chemical reaction can be distributed in various ways among the products' vibrational, rotational, and translational degrees of freedom. The manner in which this energy is partitioned is determined by factors such as the transition-state geometry and the extent to which the internal and external degrees of freedom of the developing products are coupled with one another (i.e., exit channel effects). Measurements of energy distributions in the nascent products, prior to collisional relaxation, constitute a probe of reaction dynamics in the exit channel. In the case of thermally or chemically activated unimolecular dissociations, experiments typically yield a measure of the rate of conversion of activated reactant to transition state. This corresponds to information regarding the reaction dynamics on the bound side of the critical surface. Of course, this is precisely the problem encompassed by statistical unimolecular rate theories, such as RRKM theory.[1] In its conventional formulation, statistical rate theory cannot provide a model for dynamics in the exit channel. Although a variety of statistical[2,3] and nonstatistical[4] models for energy disposal have been proposed, none has proven to be of *general* use in predicting or rationalizing product energy distributions. The need for further experimental work along these lines is thus evident. This is particularly the case for larger polyatomic reactants where, until quite recently, little information has been available on energy disposal associated even with processes as "simple" as fragmentation reactions. In this article we discuss some results on energy partitioning in the photochemically activated dissociation of 3,5-cycloheptadienone (CHD), eq 1.
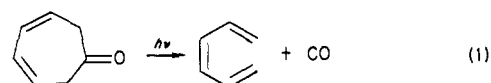
Our approach in this case is similar to that adopted in our studies of the dissociation dynamics of 3-cyclopentenone,[5,6] eq 2.
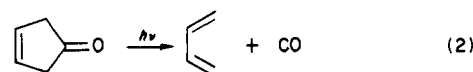
Specifically, nascent CO product vibrational energy distributions are determined by time-resolved laser absorption spectroscopy for several choices of photoactivation wavelength. The results are compared with distributions computed on the basis of a statistical model for energy partitioning. In this way, some insight can be obtained regarding the point in the exit channel where product internal energy distributions are established.

Reactions 1 and 2 can be characterized as cheletropic fragmentations.[7] This suggests that electronic or orbital symmetry conservation rules may provide a basis for predicting the facility

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