Hydrophobic Interactions in Aqueous Urea Solutions with Implications for the Mechanism of Protein Denaturation

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It is well-known that globular proteins unfold upon addition of excess amounts of denaturants such as urea or guanidine hydrochloride. Numerous experimental studies[1−4] have attempted to explain the mechanism of unfolding of proteins in aqueous urea solutions. Most of these phenomenological studies are based on untested assumptions. Using a number of assumptions and experimental data on solubilities of individual amino acids, some early theoretical studies made estimates of the free energy changes in the presence of urea. While such estimates have proven useful, the underlying assumptions have not been critically examined. In particular, there has been no test of the often-used notion that urea-induced denaturation proceeds by the breakup of water structure.[5] Inspired by the need to understand the microscopic basis of the mechanism of protein denaturation, we have performed molecular dynamics simulations to assess the effects of urea on the hydrophobic interaction between two methane molecules. Our simulations suggest a mechanism of urea-induced unfolding that is drastically different from those reported in the literature. Recently, there have been several simulation studies investigating other aspects of aqueous solutions of urea.[6−10] Molecular interactions in the ternary methane−water−urea system are based on a standard pairwise additive potentials of interaction between molecules. These pair potentials include Lennard−Jones and interactions between partial charges located on atoms. We adopt the usual three-center, nonpolarizable SPC[11] (simple point charge) model for water. The parameters for interactions involving urea are derived using the OPLS[12] procedure. Jorgensen and co-workers[13] have used such a parametrization to examine hydration properties of urea. The methane molecule (M) is modeled as a single-site Lennard−Jones solvent with a suitable van der Waals radius. The interactions between methane molecules and other atomic centers described by Lennard−Jones potentials, with the interaction coefficients being determined by standard combination rules.[12] We designate the binary system of water and methane as "A".

The charged (hydrophilic) residues of proteins in their compact conformation typically reside on the surface. To examine the effect of charges on the effective hydrophobic interaction, we also consider a model solute in which a set of partial charges of opposite sign is added to the center of each methane molecule, designated M^+, M^-. The resulting binary system of water with charged methanes is labeled "B". The magnitude of the charge on the methane is chosen to be similar to that of the partial charges on water and urea. The details of the potentials will be published elsewhere.

Molecular dynamics simulations at room temperature in the (*N*,*V*,*T*) ensemble were performed using 214 water molecules with systems A and B at zero urea concentration. When the concentration of urea was increased to 6 M, the number of SPC waters in our simulations was 166 and the number of urea molecules was 25. The length of the cubic box is 18.96 Å. The equations of motion were integrated using the Rattle version of the velocity Verlet algorithm to maintain the internal bond lengths and angles of urea and water. All interactions were spherically truncated at 9.3 Å. The effect of urea on the hydrophobic interaction is monitored by computing the potential of mean force (POMF) between the solutes. To probe the microscopic changes in water structure in the aqueous urea solution, we also calculated a number of quantities pertaining to the structure of the hydration shell, both in the presence and in the absence of the denaturant. These correlation functions provide a detailed picture of the action of urea on the hydrophobic interaction between the solute atoms.

To assess the role of urea as a structure breaker,[5] which in turn is supposed to lead to denaturation, we have examined the hydration of urea in water. We find that the aqueous urea solution is in a single phase and that the urea molecules are spread homogeneously throughout the sample. Typical hydrogen-bonding configurations are seen throughout the cell with both urea−urea hydrogen bonds and water−urea hydrogen bonds. Either carbonyl oxygen and the amide hydrogen on urea provides an acceptable site for water acceptor or donor bonds. The ability of large amounts of urea to dissolve in water is a consequence of the minimum disruption of the overall hydrogen bonding of the aqueous solution. The structural aspects of the mixed system are shown in Figure 1, in which the various radial distribution functions are plotted. The two primary hydrogen-bond classes between urea and water are composed of urea amide hydrogens and water oxygen, or urea oxygen and water hydrogens. These bonds are remarkably similar to the hydrogen bonds between water molecules as indicated by the first peak in the pair distribution function located at 1.8 Å (see Figure 1). As a result of the similarity of hydrogen bonding, the oxygens in urea are distributed locally in a pattern similar to the water oxygens in bulk water. These observations are independent of the two solute models considered. The implication of these findings is that *urea does not function as structure breaker* as has been previously supposed[5] to explain the denaturation mechanism.

Another possible explanation for denaturation is that urea preferentially solvates the hydrophobic residues[14−16] and leads to an effective reduction of the hydrophobic interaction, which in turn destabilizes the native state. The validity of this argument is examined by calculating the POMF for system A, representing

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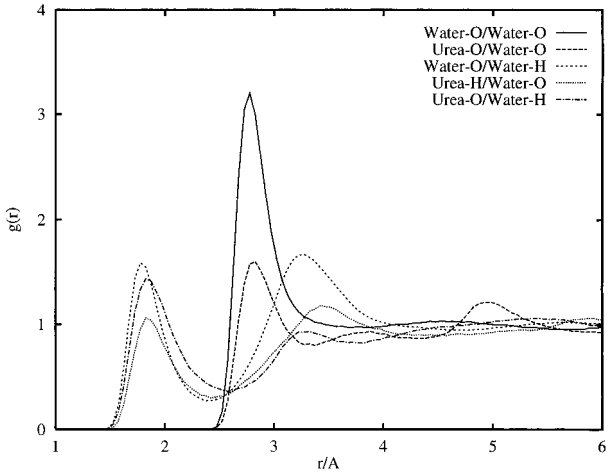

Figure 1. Various hydrogen-bond (oxygen−hydrogen) correlations for between urea and water as compared to water−water hydrogen bonding. The distributions are derived from the urea containing systems with solutes in contact, $r_{MM} = 3.75$ Å. The ability of urea to partly mimic liquid water is also indicated by the positional agreement of the first peak in the oxygen−oxygen distribution between urea−water and water−water.

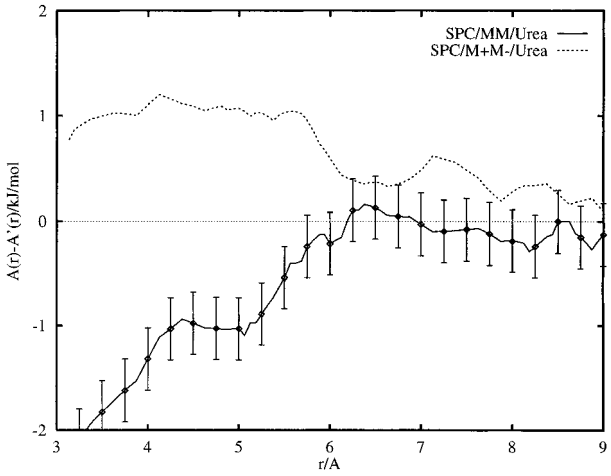

Figure 2. Difference in potential of mean force between the systems with and without denaturant using SPC waters. A positive difference indicates a denaturing effect on the stability of methane−methane contact, a negative difference indicates a renaturing effect. The typical error in these curve is ±0.3 kJ/mol.

a spherical hydrophobic species, with and without 6 M urea. The POMF does not change qualitatively in aqueous urea solution, and thus, it suffices for use in the consideration of the difference in POMF, $\Delta\Delta A(r)$, with r being the intersolute distance, in the presence and in the absence of urea. A plot of $\Delta\Delta A(r)$ for the two solute models shown in Figure 2 indicates that the effect of urea is largely confined to the region where the solutes are in contact. This figure dramatically shows that when the interaction between methane and urea is weak, i.e., for the uncharged methane, urea actually *stabilizes* the contact pair! Thus for these model systems urea appears to enhance the hydrophobic interaction and acts as a *renaturant*. Our calculations contradict the hypothesis that urea preferentially solvates the hydrophobic residues.[14−16]

It is interesting to consider $\Delta\Delta A(r)$ for the solute consisting of charged methanes. For this case, Figure 2 shows that the free energy of the contact pair increases when an excess of urea is added. Urea acts thus as a denaturant in this ternary system by destabilizing the "hydrophobic" bond between the solutes. The destabilization leads to an increase in the probability of finding separated solutes. Systems A and B only differ in the interaction between the solute and urea. For the charged solutes, there is a

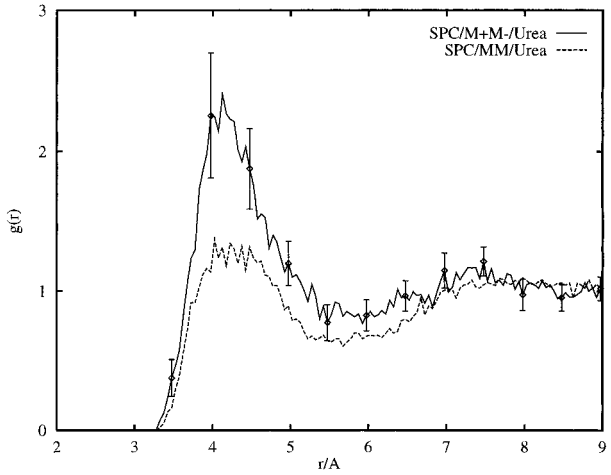

Figure 3. Pair distribution of methane and urea C atoms, $g(r)_{MC}$, for the contact pair in the urea containing systems, i.e., SPC/MM/Urea, SPC/M^+ M^-/Urea. Enhanced correlation of urea molecules around the methane pair indicates preferential attraction of the denaturant to the solutes.

strong attraction between the solute pair and the urea molecules. This interaction difference does not lead to a preferential change in the solubility of urea itself. In fact, we find that the urea−urea radial distribution functions are relatively unchanged for both solutes.[17] However, as shown in Figure 3, the atom pair distribution function between solute molecules and the carbon atom of the urea molecule (in essence the center of mass of urea) indicates that, compared with the uncharged solute, urea is preferentially adsorbed by the charged solute pair. It is this adsorption or solvation of the strongly interacting solute by urea that destabilizes the contacts between the solutes. A similar picture appears to be implied in the unfolding simulations of barnase in the presence of urea.[18]

Our simulations suggest a novel mechanism of chemical denaturation of globular proteins. The urea molecules preferentially adsorb onto the charged hydrophilic residues on the surface. This adsorption leads to a repulsion between the residues on the surface of proteins and gives rise to a swelling of the protein, which exposes the hydrophobic residues. The onset of water into the interior leads to a destabilization of the native state resulting in denaturation. The "outside−in" action of urea in denaturation also suggests that, in the presence of large amount of denaturants, the effective driving force for compact structure formation in proteins is decreased, as is the hydrophobic interaction. The driving force has been argued to be a subtle balance between hydrophobic interactions and interfacial free energies[19] both being altered by urea. It also follows from this work that, because urea readily dissolves in water without disruption of the water structure, one requires an excess amount of urea (typically 6−8 M) before adsorption onto the surface residues of proteins becomes effective.

The "outside−in" action proposed here has some experimental support. Studies of the effect of urea on peptides[20] suggest that by electrostatic binding to the peptide groups denaturants can effectively unfold a protein. The microscopic picture provided here further clarifies the nature of such urea-adsorbed interactions.

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