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Does Urea Denature Hydrophobic Interactions?

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Hydrophobic interactions play an important role in folding of proteins, formation of vesicles, membranes, etc. Because this interaction is mediated through the aqueous solvent, the stability of the above structures is particularly sensitive to external thermodynamic conditions. Hence, temperature, pressure, ionic strength, or the activities of cosolvents affect the magnitude of hydrophobic interactions and the stability of biomolecules. It is generally believed that hydrophobic contacts are dissolved in the presence of urea, thereby contributing to the denaturing process of globular proteins in concentrated urea solutions.[1−11] In this communication, we propose that hydrophobic clusters do not dissolve entirely in aqueous urea, but instead, urea acts as a "glue" bridging between hydrophobic pairs holding them together. The implications of this finding for urea-induced protein denaturation will be discussed.

As a model system for the influence of urea on pair interactions of hydrophobic moieties, we investigate the potentials of mean force (PMFs) of neopentane in water and in an aqueous solution of 6.9 M urea by molecular dynamics (MD) simulations. MD simulations were performed based on two nonpolarizable urea models (OPLS[12] and KBFF[13]) and three rigid, nonpolarizable water models (SPC,[14] SPC/E,[15] and TIP4P[16]). In previous MD studies of urea−water mixtures, the KBFF/SPC/E force field has been shown to provide an improved description of the solution structure and activity derivatives, whereas the OPLS/SPC and OPLS/TIP4P force fields produced too large urea−urea and water−water aggregation.[13] We nonetheless examined PMFs with the different water and urea force fields to get a better idea of model dependencies. All systems contained two neopentane solutes, 1694 water and 306 urea molecules (2000 waters in case of pure water) in a cubic box with an edge length of 3.9−4.2 nm. PMFs were calculated by simulating a series of 100 independently equilibrated systems, each applying a rigid constraint fixing the distance r between the mass centers of the two solutes at a preset value, $0.4 < r < 1.6$ nm, and integrating the average constraint force accumulated in each 10−20 ns simulation from 1.6 nm downward. At 1.6 nm, all PMFs converge to zero. PMFs were corrected with a term $2k_BT\ln(r)$ to ensure that (trivial) entropy contributions related to a larger volume element ($\sim r^2$) sampled by the constrained but freely rotating solute pair at larger distance r do not enter the PMF. All simulations were conducted at constant pressure (1 atm) and temperature (298 K). All simulation settings were identical to those in ref 17.

Figure 1 shows the neopentane pair PMFs in pure water (solid lines) and in 6.9 M aqueous urea (dashed lines) for different combinations of water and urea models. In addition to the five-site neopentane model[18] used in Figure 1a−c, we also included in Figure 1d the neopentane pair PMF, based on a single-site Lennard-Jones (LJ) model for neopentane,[8] previously studied by Shimizu and Chan.[10] Comparison of the well depths in the PMFs for water and aqueous urea in Figure 1a−c shows no evidence for urea-induced destabilization of the neopentane pair interaction. The first minimum of the PMF in aqueous urea in Figure 1d is slightly shifted upward relative to the PMF in water.

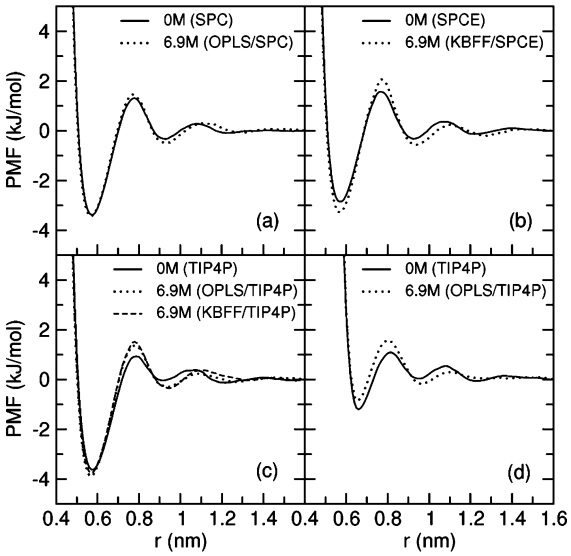

Figure 1. PMFs for neopentane pair interactions in pure water (solid lines; water model in parentheses) and 6.9 M aqueous urea (dashed lines; urea/water models in parentheses). In a−c, neopentane is modeled with the GROMOS,[18] five-site, united atom model in which CH_3 groups are modeled with an effective interaction site. In d, neopentane is modeled with a spherical Lennard-Jones potential whose parameters were taken from Kuharski and Rossky.[8] The statistical error, obtained by integrating the error in the mean constraint force from 1.6 nm downward, varies between 0.1 kJ/mol in the second minimum (solvent-separated pair) and 0.2 kJ/mol in the first minimum (contact pair).

Note, however, that urea-induced changes of this so-called contact pair (CP) are model dependent and fall just outside the error bar of the calculations. For all models, urea *stabilizes* the solvent separated pair (SSP) relative to the CP due to two corroborative effects: (1) the free energy basin of the SSP in aqueous urea is broader and is shifted to larger distance than in pure water, and (2) the free energy difference between the SSP and CP in urea solution decreases relative to that in pure water (except for the KBFF/SPCE system in Figure 1b). The second effect evidently causes the CP ↔ SSP equilibrium in aqueous urea to shift in favor of the SSP in comparison to the equilibrium in pure water. The first effect, however, shifts the equilibrium toward the SSP even further because it results in a larger volume available to the SSP. Both effects together cause a significant stabilization of the SSP relative to the CP, the extent of which we quantified by computing the equilibrium constant K_{eq} defined as

$$K_{eq} = \frac{[\mathrm{SSP}]}{[\mathrm{CP}]} = \frac{\int_{R_1}^{R_2} r^2 \exp[-w(r)/k_BT]\mathrm{d}r}{\int_0^{R_1} r^2 \exp[-w(r)/k_BT]\mathrm{d}r}$$

where [SSP] and [CP] denote the concentrations of SSPs and CPs, respectively; $R_1 = 7.8$ Å, $R_2 = 10.8$ Å (water), $R_2 = 11.1$ Å (aqueous urea), $w(r)$ is the PMF, k_B the Boltzmann constant, and

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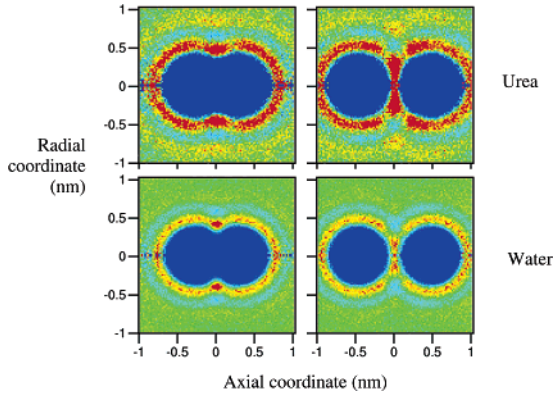

Figure 2. Urea and water number density maps for the neopentane CP (left) and SSP (right) based on the KBFF/SPC/E model. Blue, zero density; green, average bulk solution density; red, twice the average bulk density. Notice that urea preferentially bridges between the hydrophobic solutes in the solvent-separated state (top right panel).

$T = 298$ K is the temperature. With the models studied in Figure 1a−d, K_{eq} in 6.9 M urea increases with 19% (OPLS/SPC), 6% (KBFF/SPC/E), 38% (KBFF/TIP4P), 36% (OPLS/TIP4P), and 45% (OPLS/TIP4P; LJ-neopentane) relative to pure water. Previous simulation studies have reported urea-induced stabilization of methane−methane association.[20,22] In contrast to these small solutes, on the basis of the study in ref 10, where only the (urea-destabilized) CP was investigated and the SSP was neglected, hydrophobic interactions of relatively large nonpolar solutes are believed to be destabilized.[10]

Our observations can be explained on the basis of preferential urea−solute interactions. Figure 2 shows number density maps of water and urea molecules for the neopentane CP and SSP. Urea preferentially interacts with the nonpolar solutes. This observation agrees with previous simulation studies on hydrophobic solvation of aliphatic hydrocarbons[17] and aromatic hydrocarbons[19] in urea solution. The SSP preferably contains urea molecules interstitial to the solute pair. The free energy minimum corresponding to the urea-separated neopentane pair (Figure 1) shifts out to larger distances because urea molecules have a larger excluded volume than water molecules. Trzesniak et al.[17] argued that preferential urea−hydrocarbon interactions are driven by dispersion energy. The urea-separated pair is stabilized (the free energy minimum is deeper than in water) because the interstitial urea molecules interact through dispersion forces with two nonpolar solutes at the same time.

As model systems for the influence of urea on pair interactions of nonpolar aromatic amino acid residues, we also investigated PMFs of toluene and 3-methylindole in water and 6.9 M urea solution. The PMFs, presented in the Supporting Information, indicate a stronger aromatic−aromatic association in aqueous urea as compared to water due to stabilization of the SSP.

The above results shed new light on mechanisms of protein denaturation. Direct and indirect mechanisms have been discussed in the literature.[10,11,20−24] The direct mechanism involves urea H-bonding to the peptide backbone, thereby favoring the denatured state. The indirect mechanism usually adopts a chaotrope argument: urea perturbs the water structure so that hydrophobic groups are more easily solvated. Despite recent indications in favor of a direct mechanism,[20,23,24] protein denaturing via both the direct and indirect mechanism has been emphasized as well.[11] Our results lend credit to the suggestion that, upon solvent exposure of the protein core, urea denaturing proceeds by swelling the protein through formation of urea-separated nonpolar contacts. Urea H-bonding with peptide groups likely favors open denatured states; however, thermodynamically stable contact- and urea-separated pairs of nonpolar residues prevent this state of being reached. Hence, relatively compact denatured states with residual hydrophobic clustering may form, resulting from the equilibrium between these competing forces. In this study, we have not addressed urea-modulated polar and charged interaction types important for protein folding. Also, we limited our attention to pairwise interactions. Thermodynamic behavior of proteins may well arise from many-body effects and a cooperative interplay of several interactions. Notwithstanding these obvious limitations of the present study, we believe our simulation results may provide a physical reasoning for experimental observations in which the urea-denatured states of several proteins have been found to be relatively compact and to contain residual hydrophobic clustering[25,26] or even elements of native-like topology.[27,28]

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Supporting Information Available: Pair potentials of mean force for toluene−toluene interactions and 3-methylindole−3-methylindole interactions in water and 6.9 M urea solution. This material is available free of charge via the Internet at http://pubs.acs.org.

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