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Do Denaturants Interact with Aromatic Hydrocarbons in Water?

Erin M. Duffy,[†] Paul J. Kowalczyk,[‡] and William L. Jorgensen*,[†]

Contribution from the Department of Chemistry, Yale University, New Haven, Connecticut 06511, and Sterling Winthrop Inc., Collegeville, Pennsylvania 19426

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Abstract: The possibility of direct interactions between protein denaturants and aromatic hydrocarbons in water has been investigated through the computation of free energy profiles for the approach of urea and guanidinium ion (Gdm$^+$) to benzene and of urea to naphthalene in water. The calculations feature Monte Carlo statistical mechanics simulations at 25 °C with explicit representation of the solutes and solvent via OPLS potential functions and the TIP4P water model. The reaction coordinate (r_c) was defined as the distance between the center of mass of the arene and the chaotrope's central atom, and statistical perturbation theory (SPT) afforded the free energy profiles over the 3–8-Å range. In all cases, single minima were obtained with well depths of 2–3.5 kcal/mol near 4 Å. This supports the existence of direct chaotrope–arene interactions. The structures of the complexes in water are discussed and show differences from the optimal gas-phase structures. In view of the interest in cation-π interactions in host–guest complexes, computations were also carried out for tetramethylammonium ion (TMA) with benzene in water and yielded a similar free energy profile.

Introduction

Although small molecules like urea and guanidinium chloride (GdmCl) are routinely used to unfold proteins, the mechanism of denaturation via these chaotropes is not well-established. Efforts to understand this mechanism have generated a body of interesting physicochemical data,[1-14] and from these data, two hypotheses emerged.

The first hypothesis involves an indirect mechanism in which the chaotropes serve as water "structure-breakers"; this is basically a bulk solvent effect. Nuclear magnetic resonance (NMR) studies of dilute aqueous urea solutions have shown upfield proton shifts in water resonances and modest increases in the observed rotational correlation times of water molecules due to the addition of urea.[7] This is consistent with an overall disruption of long-range order in pure water but is claimed to preclude explanation of this behavior in terms of urea–water structures with substantial longevity. However, the large positive molal heat capacity change (20 cal/mol·K) for urea in a dilute aqueous solution is consistent with structure-making solutes.[9] Furthermore, a molecular dynamics (MD) study of urea in water at low concentrations attributed the modest differences between water in the locality of urea and in the bulk as resulting from direct water–urea interaction rather than wholesale urea-induced perturbation of water–water association.[11]

The second hypothesis advances a direct mechanism, characterized by specific interactions between the denaturants and protein. An important observation is that the surface tension of water increases upon addition of urea or GdmCl.[12] This suggests that the enhanced solubility results from direct chaotrope–solute interactions, since cavity formation in solution is impeded by the increased surface tension. The possibility of the direct interactions is also featured in other experimental reports. Comprehensive study of the enhanced solubilities of small molecules such as toluene, naphthalene, uric acid, and the polypeptide acetyltetraglycine ethyl ester (ATGEE) in aqueous solutions of urea or GdmCl has indicated the importance of the following interactions: dispersion, dipole-induced dipole, cation-π, and hydrogen bonding.[13,15] Furthermore, a recent calorimetric investigation of interactions of proteins with urea and GdmCl found a sizeable negative enthalpic contribution to denaturant–protein binding, which is closely correlated with hydrogen bonding to the exposed polar groups in the protein.[14]

Clearly, interesting data have been obtained, though direct probes of chaotrope–solute interactions have been lacking. With this in mind, we set out to explore computationally whether or not urea and guanidinium ion (Gdm$^+$) form complexes with protein constituents in water. To begin, interactions between chaotropes and aromatic hydrocarbons have been considered. Extensions to polar solutes and polypeptides can be envisioned. The issue of complexation is addressed by computing free energy profiles (potentials of mean force) for the approach of the chaotropes to the solutes in water. Formation of complexes requires minima to exist in the profiles. Specifically, in the present study, the interactions of urea with benzene and naphthalene and

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[†] Yale University.

[‡] Sterling Winthrop Inc.

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of Gdm$^+$ with benzene have been investigated. Furthermore, in view of the current interest in cation-π interactions,[16] the association of tetramethylammonium ion (TMA) with benzene has also been considered.

Monte Carlo Simulations

Methodology. Monte Carlo (MC) methods are used here to compute equilibrium structural and thermodynamic features of the aqueous systems via statistical mechanical averages.[17] The systems consist of an aromatic hydrocarbon and the chaotrope in a rectangular box containing 740 water molecules. In order to simulate a bulk liquid using a finite number of molecules, periodic boundary conditions, whereby the box is completely surrounded by images of itself, are employed. The interaction between one molecule and the nearest image of another is then evaluated within a spherical cutoff radius. The averages are obtained from instantaneous geometrical configurations selected by the Metropolis algorithm,[18] which is augmented by preferential sampling.[19] The latter focuses the sampling on the solute and nearby solvent molecules by moving them two to three times more frequently than solvent molecules in the bulk.

Imperative to the success of the MC simulations is the accurate representation of the intramolecular and intermolecular interactions among all components of the system. The OPLS potential functions developed in this laboratory feature a Coulomb plus Lennard-Jones format (eq 1) in which the interaction energy between molecules *a* and *b* is given

$$\Delta E = \sum_{i}^{a}\sum_{j}^{a}(q_iq_je^2/r_{ij} + A_{ij}/r_{ij}^{12} - C_{ij}/r_{ij}^{6}) \qquad (1)$$

by a summation over the individual interactions between all pairs of atomic sites (*i* and *j*) in those molecules. The parameters *A* and *C* are related to the more familiar Lennard-Jones σ's and ϵ's by $A_{ii} = 4\epsilon_i\sigma_i^{12}$ and $C_{ii} = 4\epsilon_i\sigma_i^{6}$. The combining rules, further, are expressed as geometric means, such that $A_{ij} = (A_{ii}A_{jj})^{1/2}$ and $C_{ij} = (C_{ii}C_{jj})^{1/2}$. For the most part, the OPLS parameters have been optimized to reproduce experimental thermodynamic (e.g., heats of vaporization, densities, free energies of hydration, and relative partition coefficients) and structural (e.g., radial distribution functions) results for organic systems including more than forty pure liquids[20] as well as water.[21] Otherwise, parameter development is based upon reproduction of high-level ab initio molecular orbital calculations for interaction energies and geometries of solute–water complexes. This procedure has been effective for ions,[20,22] amides,[23] and nucleoside bases.[24] It should be noted that the partial charges are held fixed such that instantaneous polarization is not explicitly treated; however, such effects are reflected in the original parametrization in an average sense.

Table I. OPLS Parameters for the Solutes

monomer	atom	q (e)	σ (Å)	ϵ (kcal/mol)	ref
urea	C	+0.142	3.750	0.105	31
	O	–0.390	2.960	0.210	
	N	–0.542	3.250	0.170	
	H	+0.333	0.000	0.000	
Gdm$^+$	C	+0.640	2.250	0.050	20
	N	–0.800	3.250	0.170	
	H	+0.460	0.000	0.000	
TMA	C	+0.250	3.960	0.145	22c
	N	0.000	3.250	0.170	
benzene	C	–0.115	3.550	0.070	34
	H	+0.115	2.420	0.030	
naphthalene	C	–0.115	3.550	0.070	34
	C_{fusion}	0.000	3.550	0.070	
	H	+0.115	2.420	0.030	

Intramolecular torsional motion is normally included in the simulations and requires torsional potentials, $V(\phi)$, for the dihedral angles ϕ, which are represented by Fourier series (eq 2). The coefficients are determined

$$V(\phi) = V_0 + {}^1/_2V_1(1 + \cos\phi) + {}^1/_2V_2(1 - \cos 2\phi) + {}^1/_2V_3(1 + \cos 3\phi) \qquad (2)$$

from gas-phase data on the energy differences between conformers. Bond stretching and angle bending may also be sampled; however, these terms have not been included in the present calculations. The emphasis here is on computation of free energy profiles, $\omega(r_c)$, for the approach of two solutes as a function of an intermolecular distance, r_c. The free energy change (ΔG) between two points *i* and *j* on the profile or potential of mean force (pmf) is calculated from statistical perturbation theory (SPT) according to eq 3.[25] Thus, the ΔG values are averages of a function of

$$\Delta G = G_j - G_i = -k_BT \ln \langle \exp[-(E_j - E_i)/k_BT] \rangle_i \qquad (3)$$

the total energy difference between points *i* and *j* with the sampling based on position *i*. In practice, this is implemented with "double-wide sampling"[26] such that two incremental ΔG values are evaluated at each step by perturbing to smaller and larger values of r_c simultaneously. Combination of these results then produces the complete free energy profile. Subsequently, integration of $\omega(r_c)$ to a cutoff distance *c*, which defines the geometric limit for complex formation, yields the association constant, K_a (eq 4).[27]

$$K_a = 4\pi \int_0^c r^2 \exp[-\omega(r)/k_BT]\, dr \qquad (4)$$

Computational Details. Free energy profiles for the approach of urea and Gdm$^+$ to benzene, urea to naphthalene, and TMA to benzene were computed. OPLS potential functions were used for the solutes (Table I) along with the TIP4P model of water.[21] This corresponds to all-atom representations except for the methyl groups in TMA, which are taken as single sites centered on the carbons. Initially, series of low-temperature, gas-phase MC searches were performed to assess the optimal strength of each solute-pair association. These gas-phase minima were confirmed by Fletcher–Powell optimizations, where the convergence criterion was specified as 1×10^{-6} kcal/mol. The simulations in water were then carried out and involved gradual perturbation of the distance between the central points in the solutes, namely, the carbon atom in urea and Gdm$^+$, the nitrogen in TMA, the ring center in benzene, and the midpoint of the C9–C10 fusion bond in naphthalene. The perturbation steps were set at 0.125 Å (each center moved in and out by 0.0625 Å), and separations from 3 to 8 Å were covered. The uncertainties in the incremental ΔG values ($\pm 1\sigma$), as obtained by the batch means procedure,[17] were maintained at or below 0.1 kcal/mol. Moreover, since SPT generates only relative free energies, the pmf's were anchored to zero at 8 Å.

For the solute monomers, geometries were based upon experimental data (urea,[28] benzene,[29] naphthalene[30]) and ab initio 6–31G(d) geometry optimizations (Gdm$^+$,[22a] TMA[22c]). The torsional motions about the C–N bonds in urea and Gdm$^+$ were included; attempted variations in the characteristic dihedral angles were made within ±5°. Rigid rotation was performed with barrier heights determined from 6–31G(d)//6–31G(d) gas-phase computations. This required only the twofold term in eq 2 with V_2 values of 17.7 and 15.7 kcal/mol for urea and Gdm$^+$, respectively. Finally, the solutes were allowed to move independently except for

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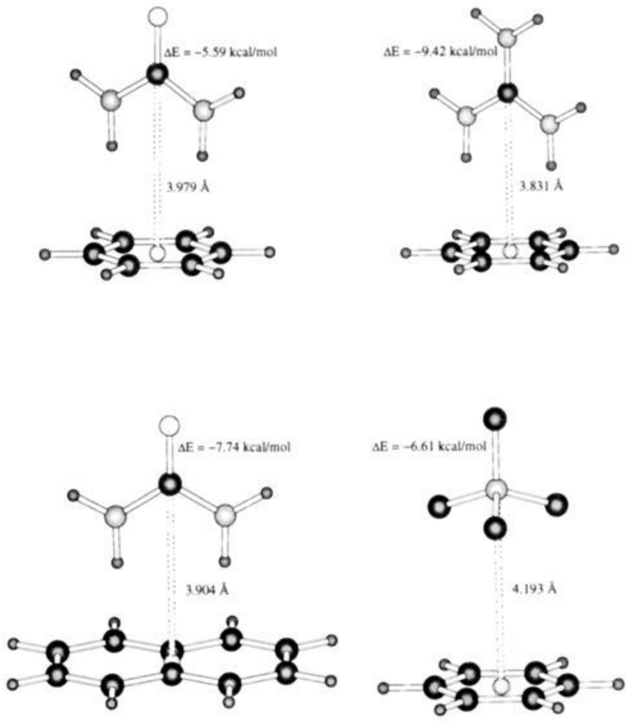

Figure 1. Gas-phase energy minima for urea–benzene, Gdm$^+$–benzene, urea–naphthalene, and TMA–benzene.

maintenance of the r_c value, and the ranges for their translations and rotations were chosen to yield new configurations with a ca. 40% acceptance rate.

The systems consisting of the two solutes and the 740 TIP4P water molecules occupied periodic cells with dimensions ca. 25 × 25 × 37.5 Å. In all cases, the solvent–solvent and solute–solvent interactions were truncated at 9 and 12 Å based on the central atom separations and were quadratically feathered via a switching function to zero over the last 0.5 Å. Initially, the vector corresponding to r_c was aligned with the long axis of the periodic cell.

Standard procedures were employed in the isothermal–isobaric (NPT) ensemble at 25 °C and 1 atm. Each perturbation step involved 2×10^6 configurations of equilibration followed by averaging over 4×10^6 configurations. A total of twenty perturbation steps was necessary to cover the range of r_c from 3 to 8 Å. Subsequent hydrogen-bonding analyses were performed for coordinates saved every 5×10^4 configurations during the simulations. The present definition of a hydrogen bond involves a geometric constraint, N···H or O···H ≤ 2.5 Å, augmented by an energetic criterion, $\Delta E \leq -2.25$ kcal/mol. These restrictions have been used previously[23b,31,32] and derive from the position of the first minimum in radial distribution functions involving hydrogen-bonding hydrogens and from the range of the low energy band in solute–solvent energy pair distributions for such systems. All calculations were performed with the BOSS program on Silicon Graphics 4D/35 and R/4000 computers as well as an IBM RS/6000 workstation in our laboratories.[33]

Results and Discussion

Gas-Phase Structures and Interaction Energies. The global minima located for the four complexes of interest in the gas phase are shown in Figure 1. Notably, the optimal interactions feature Coulombically sensible T-structures; analogous structures for benzene interacting with a single water molecule have been reported.[34] In particular, the three chaotrope–arene complexes display C_{2v} symmetry with the hydrogens pointing into the center of the benzene and naphthalene rings, and the TMA–benzene pair has C_{3v} symmetry with the three coplanar united-atom methyl groups directed toward the ring center. The distance between the molecular centers in each complex is near 4 Å, and the interactions are attractive by 5–10 kcal/mol. It is interesting to note that, with the exception of Gdm$^+$–benzene, the components of the total interaction energy of each complex show the van der Waals attractive term ($1/r^6$) is actually somewhat greater than the Coulombic attraction (Table II). The Gdm$^+$–benzene pair, however, is clearly dominated by the electrostatic contribution.

Table II. Breakdown of Optimal Gas-Phase Interaction Energies (kcal/mol) for Each Complex into Coulombic and van der Waals Contributions

complex	ΔE	$1/r$	$1/r^6$	$1/r^{12}$
urea–benzene	−5.59	−3.91	−4.96	+3.28
Gdm$^+$–benzene	−9.63	−8.90	−4.92	+4.19
urea–naphthalene	−7.75	−5.24	−6.99	+4.49
TMA–benzene	−6.61	−4.35	−5.73	+3.46

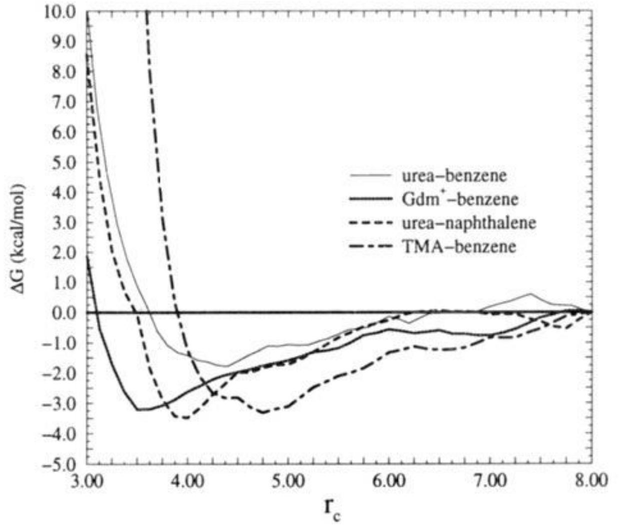

Figure 2. Computed free energy profiles for the separation of prototypical chaotrope–arene complexes in solution. The reaction coordinate is the distance in Å between the central atom of the denaturant molecule and the center of mass of the arene.

Though the potential functions for TMA[22c] have not been as well-tested as those for urea,[31] the computed optimal energy for the complex of TMA with water, −9.5 kcal/mol, agrees well with an experimental interaction enthalpy of −9.0 kcal/mol from high-pressure mass spectroscopy.[35] The computed optimal interaction energy for benzene with water, −2.3 kcal/mol, is also essentially correct.[34] Consequently, it is surprising that the computed value of −6.6 kcal/mol for the TMA–benzene complex (Figure 1) differs significantly from the interaction enthalpy of −9.4 kcal/mol reported for this pairing in the same mass spectroscopic study.[35] An explanation is not evident, while it may also be noted that recent AM1 calculations by Gao et al. yielded an interaction energy of −4.1 kcal/mol for TMA–benzene in the optimal C_{3v} geometry at 4.08-Å separation.[36]

Chaotrope–Arene Associations in Water. The computed free energy profiles for the separation of the model complexes in aqueous solution are shown in Figure 2. The key finding for each pmf is a unique minimum corresponding to a contact pair in the

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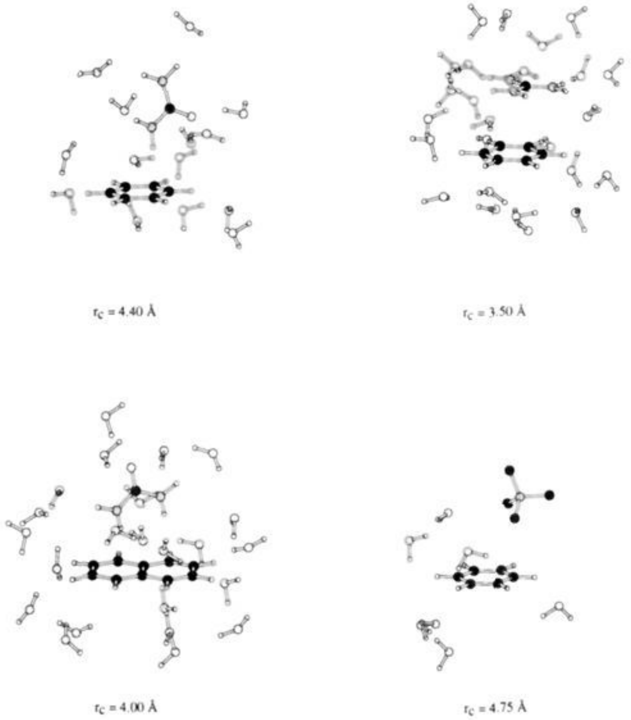

$r_c = 4.40$ Å

$r_c = 3.50$ Å

$r_c = 4.00$ Å

$r_c = 4.75$ Å

Figure 3. Random configurations of the four complexes in water near their contact minima. Only those water molecules with an atom within 3 Å of a solute atom are included.

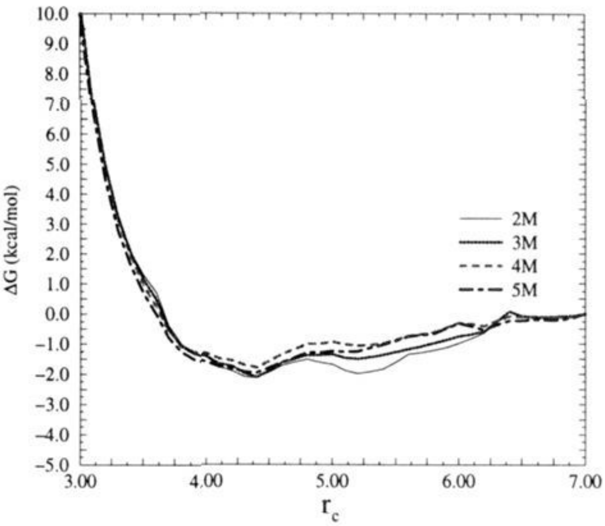

Figure 4. Evolution of the potential of mean force for the interaction of ureawith benzene in water. The four curves correspond to averaging for 2–5 million configurations in each

Table III. Results of Hydrogen-Bonding Analyses for the Complexes with Urea in Water[a]

denaturant atom	urea–benzene, skewed T		urea–benzene, C_{2v}		urea–naphthalene	
	#hb	ΔE	#hb	ΔE	#hb	ΔE
O	2.11	−4.69	2.03	−5.12	1.65	−5.51
N1	0.01	−4.81	0.01	−4.01	0.06	−3.90
N2	0.03	−2.65	0.01	−4.25	0.02	−2.87
$H1_{syn}$	0.01	−4.14	0.74	−4.14	0.50	−3.45
$H1_{anti}$	0.60	−3.78	0.05	−3.49	0.21	−3.42
$H2_{syn}$	0.72	−3.86	0.57	−3.49	0.37	−3.16
$H2_{anti}$	0.75	−3.96	0.17	−3.31	0.03	−3.54

[a] All values represent averages over 80 random configurations. #hb is the average number of hydrogen bonds with the average interaction energy ΔE (kcal/mol).

neighborhood of the optimal separation in the gas phase. Not surprisingly, solvent effects as well as entropic factors associated with the thermal, configurational averaging considerably diminish the well depths from those for the gas-phase minima. Nevertheless, association is indicated in all cases. Illustrations of random configurations near these minima are given in Figure 3; the pictures represent the last configurations from the simulations and contain only those water molecules with an atom within 3 Å of a solute atom. Before the results are discussed in detail, a few technical points deserve mention. A source of concern in simulations of this type is precision. As previously mentioned, the pmf's were zeroed at 8 Å. The pmf's appear to be flattening out well in this vicinity, though there is some residual uncertainty in the zeroing. Additionally, because the sole constraint on each system was the maintenance of the reaction coordinate value, the sampling should reflect full angular averaging. Convergence of the pmf's was investigated in detail for urea–benzene. As shown in Figure 4, extending the averaging period from 2 to 5 million configurations had little effect on the results. Moreover, preliminary results for a Gdm^+–benzene pmf extended to 9-Å separation with an increased solvent–solvent cutoff (12 Å) indicate that the profile closely resembles the initial Gdm^+–benzene pmf in Figure 2.

In the specific case of association between urea and benzene, the most favorable separation is computed to occur at 4.4-Å separation with a well depth of −1.8 kcal/mol in the pmf. Display of several random configurations of the contact pair at this point demonstrates a preference for the structure shown in Figure 3 in which the urea hydrogen has only one syn hydrogen pointing into the ring (a skewed T), the other hydrogens being available to the solvent for possible hydrogen bonding. Further inspection of configurations generated during that perturbation step revealed a balance between sampling this structure and ones similar to the gas-phase minimum (Figure 1). This behavior is apparent across the broad minimum in the profile from 4 to 5 Å. Attempts to clarify the nature of this interplay yielded logical results. First, gas-phase optimizations found the skewed T as a viable minimum with a contact distance of 4.29 Å and an interaction energy of −4.50 kcal/mol. Second, hydrogen bond analyses for the alternative structures indicated that the skewed T had, on average, more hydrogen bonds (4.6 vs 4.0) than the C_{2v}-like complex, as summarized in Table III. Most obviously, the former structure displayed a fairly equal distribution of hydrogen bonds to three of the hydrogens (≈0.7) and practically no hydrogen bonds (≈0.01) to a syn hydrogen pointing toward the ring, while the C_{2v}-like structure showed shielding of both anti hydrogens and similar hydrogen-bonding probabilities for the syn hydrogens. In both cases, the urea oxygen participated in two strong hydrogen bonds with water molecules. If one totals the energetic contributions from the hydrogen bonds in Table III, the skewed T structure is found to benefit by 1.8 kcal/mol more than the C_{2v}-like structure. Thus, the skewed T complex, which is approximately 1 kcal/mol less favored in the gas phase, becomes competitive in water due to the enhanced hydrogen bonding.

The association constants were also computed from the pmf's according to eq 4. The choice of the cutoff limit c provides some uncertainty, though the results are not too sensitive for reasonable choices beyond the minimum. Namely, the computed K_a's for urea–benzene with c = 6, 7, and 8 Å are 0.11, 0.38, and 0.65 M^{-1}. Direct comparison with experiment is not possible due to the unavailability of data on the urea–benzene system; nevertheless, the results can be compared with data from solubility measurements for toluene in urea–water solutions. Assuming that the observed increases in toluene solubility arise solely from 1:1 toluene–urea complex formation, the procedure outlined by Robinson and Jencks yields a K_a of 0.16 M^{-1} for toluene with urea in a 3 M aqueous urea solution.[15]

The Gdm^+–benzene contact minimum in water was found to occur at 3.50 Å with a well depth of 3.20 kcal/mol in the pmf. This separation is considerably shorter than that for the gas-

Table IV. Results of Hydrogen-Bonding Analyses for the Guanidinium Ion–Benzene Complex in Water[a]

denaturant atom	Gdm^+–benzene		denaturant atom	Gdm^+–benzene	
	#hb	ΔE		#hb	ΔE
N1	0.0	0.0	$H2_{syn}$	0.98	−10.35
N2	0.0	0.0	$H2_{anti}$	0.97	−8.27
N3	0.0	0.0	$H3_{syn}$	0.95	−10.08
$H1_{syn}$	1.00	−11.79	$H3_{anti}$	1.05	−11.25
$H1_{anti}$	0.87	−11.40			

[a] Details as in Table III.

phase optimal interaction (Figure 1), and it reflects a shift to a parallel-planes structure in water (Figure 3). Subsequent gas-phase optimizations, constrained to have the solutes in parallel planes, produced such a minimum at 3.59 Å with an interaction energy of −4.72 kcal/mol. Naturally, the large difference in gas-phase interaction energies between the parallel-planes and the C_{2v} complexes (4.70 kcal/mol) is compensated by better hydrogen bonding for the former case. A hydrogen-bonding analysis near the pmf minimum reveals that all six Gdm^+ hydrogens function as strong hydrogen-bond donors with an average interaction energy of −10.5 kcal/mol (see Table IV for the full analysis). Moreover, as in the urea–benzene case, the competition between the solute–solute intrinsic interaction and the hydrogen bonding is consistent with the broad minimum observed; there is a considerable teetering between the parallel-planes and C_{2v}-like vicinities.

Association constants for Gdm^+–benzene in water were evaluated as 17.2, 18.2, and 18.9 M^{-1} out to 6, 7, and 8 Å. The increase in binding follows from the deeper, broader minimum in this pmf compared with the urea–benzene case. The results clearly corroborate Breslow's view, based on surface tension measurements, that direct chaotrope–benzene interactions occur for this system in water.[12] The computed K_a's, though not wildly excessive, seem large in view of the results of Robinson and Jencks on the solubilization of toluene in 3 M GdmCl solution.[15] The accuracy of the computed K_a's may suffer from inadequacies in the pmf zeroing, the potential functions, the treatment of long-range forces, and the sampling. However, the confluence of the present results makes the qualitative predictions of association for these systems secure.

The optimal interaction between urea and naphthalene in water yielded a minimum at 4.00-Å separation with a depth of −3.48 kcal/mol in the pmf. A survey of configurations in that vicinity uncovered predominantly C_{2v}-like structures, e.g., in which one anti urea hydrogen was freed for hydrogen bonding. Once again, hydrogen-bonding analyses at the minimum supported the noted interactions. Specifically, an average of two strong (≈−5.5 kcal/mol) hydrogen bonds to the urea oxygen as well as fairly equal hydrogen-bond probabilities with the syn hydrogens and the emergence of some hydrogen bonding for the anti hydrogens is found, as summarized in Table III. The association constants evaluated for this system are 20.3, 20.6, and 21.3 M^{-1} with c = 6, 7, and 8 Å. The enhanced complexation for urea with naphthalene compared to benzene is consistent with the enhanced gas-phase interaction (Figure 1) and with the data of Roseman and Jencks which show that urea has a greater solubilizing effect on naphthalene than on toluene in aqueous solutions.[13] However, the K_a suggested from their data is significantly smaller at 0.2–0.3 M^{-1} and may again reflect inadequacies in the computations, as noted above.

Finally, for TMA with benzene the minimum for the pmf in Figure 2 has a depth of −3.31 kcal/mol at a separation of 4.75 Å. Structural analyses in this neighborhood reveal a variety of complexes shifted from the C_{3v} structure, such as in Figure 3. The pmf results indicate that the cation-π interaction is substantial over a wide range of separations, e.g., 4–6 Å. Consequently, the computed association constants are larger: 33.8, 36.1, and 37.1 M^{-1} with c = 6, 7, and 8 Å. This translates to a binding free energy of ca. −2 kcal/mol via $\Delta G = -RT \ln K_a$. Gao and coworkers have recently reported a pmf for the same system.[36a] They used a combined AM1/Monte Carlo approach with TIP3P water and a constraint that the TMA approaches along the sixfold axis of benzene. In spite of these differences, they also obtained a profile reflecting weak association. However, their pmf shows both a contact minimum near 5-Å separation, as found here, and an additional solvent-separated minimum at 7–8 Å. The shallower wells in their pmf are consistent with the weaker gas-phase attraction for TMA and benzene from the AM1 calculations, as noted above.

Such cation-π interactions involving ammonium and guanidinium fragments with aromatic rings of amino acid side chains and ligands are receiving heightened attention.[16] In many of these systems, the contribution to the free energy of binding from a single interaction of this type has been approximated to be near or in excess of −1 kcal/mol,[16c,d,h] which is not out of line with the present results. Nevertheless, concomitant interactions like π-stacking and ion pairing make a clear separation of the contribution from the cation-π interactions elusive.

Conclusion

The present computational results provide structural and energetic information espousing direct binding between the well-known denaturants urea and guanidinium ion and aromatic molecules in water. It seems likely that such interactions are relevant to the mechanism of protein denaturation promoted by the chaotropes. Presumably, greater exposure of aromatic side chains in the unfolded rather than folded state provides enhanced opportunities for favorable interactions with the chaotropes. Clearly, it is now desirable to consider interactions of the chaotropes with models for the peptide backbone and even with complete proteins to further explore the mechanism of protein denaturation induced by these molecules.

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