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Interactions between Hydrophobic and Ionic Solutes in Aqueous Guanidinium Chloride and Urea Solutions: Lessons for Protein Denaturation Mechanism

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Abstract: In order to clarify the mechanism of denaturant-induced unfolding of proteins we have calculated the interactions between hydrophobic and ionic species in aqueous guanidinium chloride and urea solutions using molecular dynamics simulations. Hydrophobic association is not significantly changed in urea or guanidinium chloride solutions. The strength of interaction between ion pairs is greatly diminished by the guanidinium ion. Although the changes in electrostatic interactions in urea are small, examination of structures, using appropriate pair functions, of urea and water around the solutes show strong hydrogen bonding between urea's carbonyl oxygen and the positively charged solute. Our results strongly suggest protein denaturation occurs by the direct interaction model according to which the most commonly used denaturants unfold proteins by altering electrostatic interactions either by solvating the charged residues or by engaging in hydrogen bonds with the protein backbone. To further validate the direct interaction model we show that, in urea and guanidinium chloride solutions, unfolding of an unusually stable helix (H1) from mouse PrP^C (residues 144−153) occurs by hydrogen bonding of denaturants to charged side chains and backbone carbonyl groups.

1. Introduction

Denaturants, such as guanidinium chloride (GdmCl) and urea, destabilize globular proteins.[1,2] Despite extensive studies the destabilization mechanism is not fully understood largely because of the paucity of protein structures in the presence of denaturants. Moreover, the free energies of interaction between denaturant molecules and the peptide backbone and amino acid side chains are small[3] which makes it difficult to use experimental data to infer plausible denaturation mechanisms. Much of our understanding of the interactions of denaturants with polypeptide chains comes from transfer experiments that measure solubilities of peptide units and amino acid side chains in water, apolar solvents, and aqueous urea or GdmCl solutions.[3,4] The changes in the Gibbs free energy upon transfer of most amino acid side chains from water to aqueous GdmCl or urea solutions is <1 kcal/mol.[5,6] On the basis of such measurements and related experiments,[7] simulations of small peptides,[8] and model systems,[9] two distinct models for the mechanism of denaturation have been proposed. In the direct interaction model, whose origin can be found in the pioneering experiments of Robinson and Jencks,[10] urea and GdmCl interact with the polar side chains and the peptide backbone by forming hydrogen bonds. On the other hand, Tanford[2,3] suggested on the basis of transfer experiments that protein denaturation in aqueous urea and GdmCl occurs by alterations in the hydrophobic interaction. Molecular dynamics simulations on proteins[11−13] in aqueous urea have been interpreted in terms of both the direct interaction model and mechanisms that rely on the changes in the hydrophobic energies.

The small free energy changes in the solvation free energies (ΔG) of solutes upon transfer from water to denaturant solutions make it difficult to estimate the contributions hydrophobic and electrostatic interactions separately make to ΔG. In the absence of experiments that provide denaturant-induced changes in protein or water structures, computer simulations have been used

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to rationalize the denaturation process.[11−15] Simulations of small peptides and model compounds, for which converged simulations can be carried out, have shown that denaturation by urea occurs largely by the direct interaction model.[8,16] Much less work has been done to probe interactions of apolar and charged solutes in GdmCl solution which is more efficient in denaturing proteins.

The study of denaturant-induced changes in hydrophobic and ionic interactions is also important in other physical situations. Salts (e.g., GdmCl) and polar molecules (e.g., urea) can either increase or decrease the strength of the hydrophobic interactions, depending on the extent to which the water structure is altered.[17] Salts, known as chaotropes, increase the strength of hydrophobic interactions by disrupting water structure,[14] whereas kosmotropes decrease the strength of hydrophobic interactions by ordering water structure. Ion pairs, which in proteins often form salt bridges, are also affected by cosolutes. Elucidating the factors that affect these interactions is important in understanding the molecular basis of the Hofmeister series[17,18] in which ions and other cosolutes are arranged in order of their ability to precipitate proteins.

Previous studies have elucidated the nature of interactions between solutes in water and in the presence of cosolutes by using detailed simulations of linear alkane chains[16] and small hydrophobic and charged species.[9,19,20] Here, we explore the effect of denaturants on hydrophobic and ionic interactions between small solutes using extensive molecular dynamics (MD) simulations. As a measure of interaction between solutes we compute the potentials of mean force (PMFs) between small spherical hydrophobic and ionic species. It is well-known that the PMF between methane molecules has two minima, namely a contact minimum (CM) and a solvent separated minimum (SSM). The minima are separated by a desolvation barrier.[21−26] To illustrate the effect of denaturants on interactions involving hydrophobic and ionic solutes we calculate the PMFs between methanes and oppositely charged ions at varying concentration of denaturants. From the PMFs we calculate the free energy of association of a pair of methane molecules as a function of denaturant concentration. In order to probe the role of ionic interactions we also consider methane molecules that are decorated with opposite charges. Even at the highest denaturant concentrations the PMF between methane molecules in urea shows insignificant change relative to that of pure water. In agreement with experiments on globular proteins,[27] urea also does not alter the energetics of ionic interactions. The mechanism of urea-induced denaturation is most directly linked to its ability to form hydrogen bonds readily with charged cosolutes[9] and presumably with the peptide backbone.[8] Just as in aqueous urea solutions, GdmCl has negligible effect on the PMFs between hydrophobic methane molecules. In contrast, both the contact minimum and the solvent-separated minimum in the charged systems are greatly destabilized in aqueous GdmCl solution.

In order to test the proposal that direct interaction between denaturants and polypeptide chains is the predominant unfolding mechanism, we have carried out simulations of a stable helix H1 from mouse PrP^C protein. The analysis of the dynamics of unfolding shows, both in urea and guanidinium chloride, that the denaturant molecules directly engage in hydrogen-bond formation with charged groups and backbone carbonyl groups. Thus, the results for the model systems and helix unfolding demonstrate that the most commonly used denaturants unfold proteins by direct interactions with polypeptide chains, either through efficient hydrogen-bond formation or through changes in the ionic interactions.

Table 1. Force-Field Parameters for Urea, Guanidinium, Chloride, Me, M^+, and M^-

atomic center	urea			guanidinium			chloride		
	σ^a	ϵ^b	q^c	σ	ϵ	q	σ	ϵ	q
C	3.564	−0.110	0.51	3.564	−0.110	0.64	–	–	–
N	3.296	−0.200	−0.62	3.296	−0.200	−0.80	–	–	–
H	0.400	−0.046	0.31	0.400	−0.046	0.46	–	–	–
O	3.029	−0.120	−0.51	–	–	–	–	–	–
Cl	–	–	–	–	–	–	4.050	−0.15	−1.00

atomic center	Me			M^+			M^-		
	σ	ϵ	q	σ	ϵ	q	σ	ϵ	q
C	3.677	−0.080	−0.36	3.671	−0.080	0.64	3.671	−0.080	−1.36
H	2.352	−0.022	0.09	2.352	−0.022	0.09	2.352	−0.022	0.09

[a] The closest distance of approach between the atomic centers in units of Å. [b] The well depth in units of kcal/mol. [c] The partial charge on the atomic center in units of *e*.

2. Computational Methods

Models. We simulate the interactions between methane molecules in aqueous urea and guanidinium (Gdm^+) chloride (Cl^-) solutions using all-atom representations of all the chemical species. The molecules interact with each other through pairwise potentials that are composed of electrostatic and van der Waals interactions between the atomic centers. The electrostatic interactions arise from partial charges on each atomic center, whereas van der Waals interactions are modeled using the Lennard-Jones potential,

$$V_{LJ}(r) = 4\epsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] \quad (1)$$

where r and σ are the distances and diameter between the two atomic centers, respectively, and ϵ is the well depth. The values of the partial charges, and the Lennard-Jones parameters for each chemical component are listed in Table 1 and are taken from the CHARMM22 force field.[28] The urea parameters were taken from ref 11. The parameters for interactions between two different types of atomic centers are computed using the Lorentz−Berthelot mixing rules.[29] A sigmoidal switch function, which smoothly brings the Lennard-Jones interactions to zero at 12 Å, is applied to the Lennard-Jones interactions starting at 8 Å and ending at 12 Å. Electrostatic interactions are calculated using the particle mesh Ewald method[30] with a grid spacing of ∼1 Å. In addition to the neutral methane systems, we also consider interactions

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between charged solutes using methane molecules with either a positive (designated M^+) or negative (designated M^-) charge placed at the carbon center of the methane (see Table 1).

Simulation Details. We use the NAMD software package[31] to perform unrestrained molecular dynamics (MD) simulations. The systems simulated consisted of between 8 and 10 neutral or M^+ and M^- molecules in a periodically replicated cubic box.[32] In the case of ionic solutes there are five M^+ and five M^- molecules in the primary cell. The concentrations of the denaturants were varied by changing the number of urea or GdmCl molecules in the periodic box. We performed simulations in the canonical ensemble (NVT) at 300 K with a 1 fs integration time step and full electrostatic updates at each time step. The temperature is maintained using a Langevin thermostat with a 5 ps^{-1} friction coefficient on non-hydrogen atoms. The SHAKE/RATTLE algorithm[33] is used to restrain the covalent bonds between hydrogen atoms and heavy atoms at their equilibrium distances. We generated between four and six independent trajectories at each denaturant concentration. Each trajectory is between 25 and 40 ns in length. Additional details of the simulations are given in Tables S.I and S.II in the Supporting Information.

The systems are prepared by initially solvating between 8 and 10 methanes or M^+ and M^- molecules in TIP3P water[28,34] in a cubic box ~32 Å per side. Urea, guanidinium, and chloride molecules are inserted randomly in the box. We retained the inserted molecules that do not overlap with the solutes and removed water molecules that are within 2 Å of any heavy atoms of the inserted molecules. The systems are then minimized with a conjugate gradient method iterated for 5000 steps and equilibrated for 30 ps at 1 atm pressure and 300 K in the NPT ensemble to achieve a proper water density. Finally, using the final box size from the previous NPT equilibration step, the system is equilibrated for 3 ns in the NVT ensemble at 300 K. Production runs are then started from the equilibrated configurations.

Potential of Mean Force. We calculated the PMF between the solutes using

$$W_{\alpha\beta}(r) = -k_B T \ln(g(r)) \tag{2}$$

where $W_{\alpha\beta}(r)$ ($\alpha = \beta = Me$, or $\alpha = M^+$ and $\beta = M^-$) is the PMF, k_B is the Boltzmann constant, T is the simulation temperature, and $g(r)$ is the radial distribution function between the solutes.[35] In order to determine the changes in the interaction between solutes we compute the free energy of association relative to a dissociated state. The solute molecules are associated if the distance between them is less than the position of the desolvation barrier, whose location is obtained from the PMF. The probability of being associated or dissociated at a given given denaturant concentration [D], which we respectively denote $P_A[D]$ and $P_D[D]$, are computed from the probability distribution, $P(r,[D])$, of finding two solute molecules at a distance r, using

$$P_A[D] = \int_0^{r_{B_1}} P(r,[D])\mathrm{d}r \tag{3}$$

and

$$P_D[D] = \int_{r_{B_1}}^{l_b/2} P(r,[D])\mathrm{d}r \tag{4}$$

where r_{B_1} is the position of the barrier separating the CM and the SSM in the PMF, and l_b is the length of the cubic box (l_b values are listed in Tables S.I and S.II). For methanes $r_{B_1} = 5.8$ Å, and for M^+ and M^- $r_{B_1} = 4.3$ Å. The change in the stability of the associated state at the denaturant concentration [D], with respect to [D] = 0, is

$$\Delta\Delta G_{AD}[D] = -k_B T \ln\left(\frac{P_A[D]}{P_D[D]}\right) - \Delta G_{AD}[0] \tag{5}$$

where $\Delta G_{AD}[0] = -k_B T \ln(P_A[0]/P_D[0])$.

We define the solvent-separated region to be between the desolvation barrier (r_{B_1}) and the secondary barrier (r_{B_2}). For methanes, $r_{B_2} \approx 8.8$ Å and is independent of urea concentration, whereas at high GdmCl concentrations $r_{B_2} \approx 9.2$ Å. In the case of M^+ and M^-, $r_{B_2} \approx 6.8$ Å and is largely independent of urea and GdmCl concentration. From the probability of having two solute molecules in the basin of attraction corresponding to the SSM,

$$P_{SSM}[D] = \int_{r_{B_1}}^{r_{B_2}} P(r,[D])\,\mathrm{d}r$$

we compute the change in the stability of the SSM relative to pure water using

$$\Delta\Delta G_{ASSM}[D] = -k_B T \ln\left(\frac{P_A[D]}{P_{SSM}[D]}\right) - \Delta G_{ASSM}[0] \tag{6}$$

Structural Probes. In order to clarify the nature of interactions between the denaturants and the solutes we have examined the structures of the denaturant molecules that are in the vicinity of the solutes. As a probe of the effect of denaturants on the solute and water we computed a number of pair functions that describe the effects of cosolutes and solvation. In addition, we devised local probes to monitor if denaturant molecules displace water around the solutes especially around the SSM. At zero denaturant concentration the SSM has a discrete water molecule that is juxtaposed between the methane molecules.[21] The region of the SSM is taken to be $r_{SSM} \pm 0.25$ Å where r_{SSM} (see Tables S.III and S.IV in the Supporting Information for the [D]-dependent values of r_{SSM}) is the location of the second minimum in the methane−methane PMF. We consider a urea or guanidinium molecule to be intercalated between a pair of solutes if the carbon atom in the denaturant is within a distance of $r_{SSM} - 0.25$ Å of both carbon atoms on methane molecules. Using this definition we compute the average number of denaturant molecules (denoted $\langle N_D \rangle$) intercalated between solvent-separated methanes.

Effect on an α-Helix (H1) from mPrP^C. In order to asses the generality of our findings we also simulated the effects of these denaturants on a helical polypeptide. We studied a small, highly stable helical peptide fragment H1 from the mouse prion protein (mPrP^C with Protein Data Bank (PDB) code 1AG2[36]). The sequence of H1, that spans residues 144−153 in mPrP^C is NH_3^+−DWEDRYYREN−CO_2^-. Experiments[37,38] and simulations[39] have shown that H1 is exceptionally stable at room temperature.

The initial conformation of the H1 peptide is taken from the PDB NMR structure.[36] The system is solvated in TIP3P water molecules, and an appropriate number of urea or guanidinium chloride molecules are inserted to achieve the desired concentration. The preparation procedure is the same as for the methane systems. We used constant volume simulations to generate trajectories at 300 K in aqueous urea (3.94 M) and GdmCl (3.05 M). In the GdmCl simulations there are

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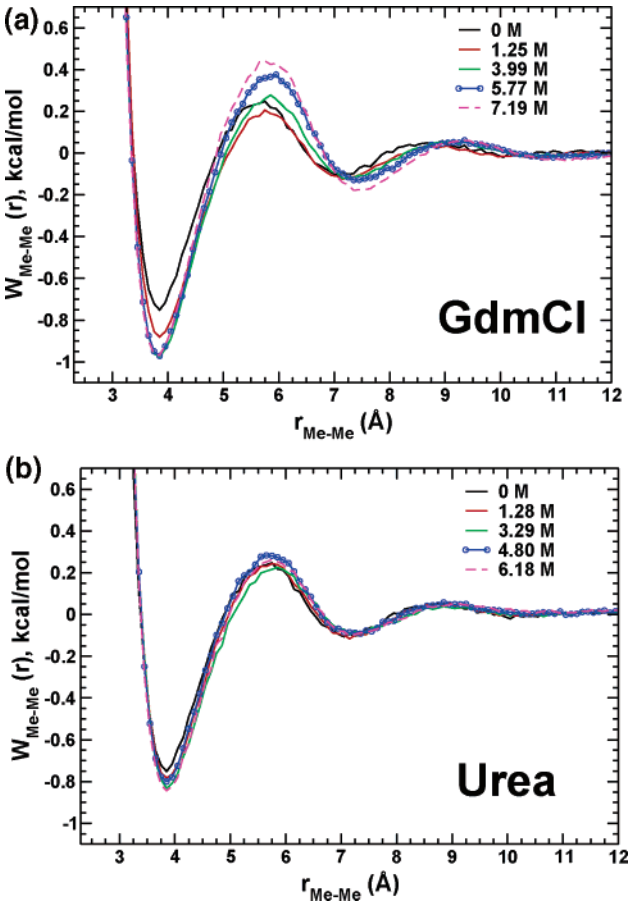

Figure 1. Potentials of mean force (PMFs) between two methane molecules in aqueous denaturant solutions. (a) The PMFs at various concentrations of GdmCl show changes in the contact minimum and desolvation barrier. (b) Same as (a) except for urea. The denaturant concentrations, given in molarity, are explicitly shown.

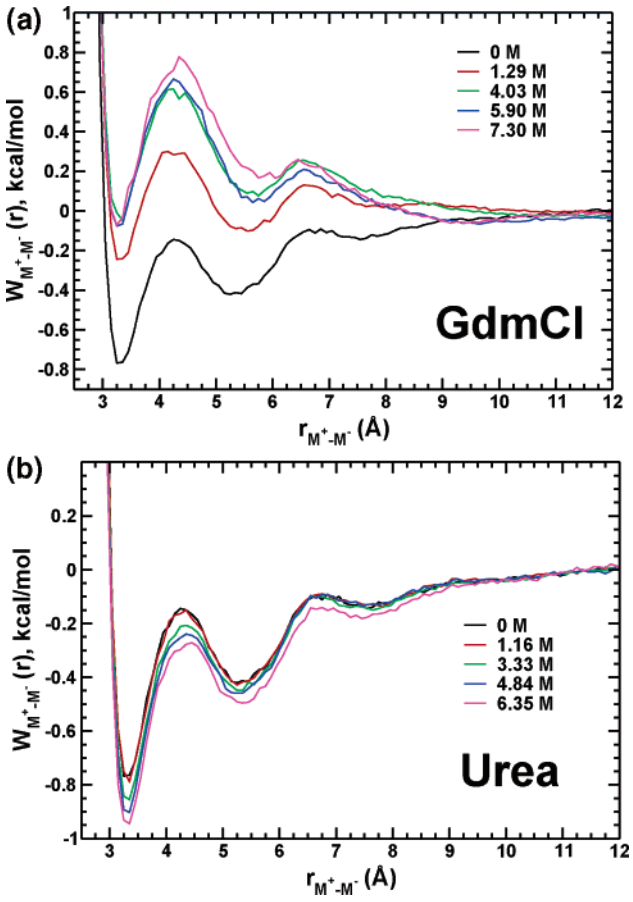

Figure 2. (a) PMFs between M^+ and M^- as a function of GdmCl concentration. (b) Same as (a) except the PMFs are in aqueous urea.

102 Gdm^+ molecules, 100 Cl^- ions, and 1441 water molecules. There are 102 urea molecules, 1155 water molecules, and two sodium ions in the urea simulations. The CHARMM22 force field is used to model the protein.[28]

The extent of unfolding was monitored using the helical content in H1 in pure water and denaturant solutions. The helical content is computed as the fraction of residues with backbone dihedral angles (Φ, Ψ) in the helical region of the Ramachandran map. The helical region of the Ramachandran map is defined by a polygon with (Φ, Ψ) vertices at (−90, 0), (−90, −54), (−72, −54), (−72, −72), (−36, −72), (−36, −18), (−54, −18), and (−54, 0).[40]

3. Results and Discussion

Differences in PMFs between Ionic and Hydrophobic Solutes in Water Are Significant. In pure water the PMFs between methane pairs and M^+ and M^- pairs are similar in that they both have a clearly defined CM, barrier, and SSM (Figures 1 and 2). However, the details differ significantly. Compared to the PMFs between methane molecules the locations of the CM and SSM between M^+ and M^- are shifted by as much as 2 Å. In addition, the desolvation barrier for the charged species relative to the hydrophobic solutes is smaller by ∼0.4 kcal/mol. For example, comparison of the PMFs between methanes and the ionic species shows that the SSM shifts from 7.3 to 5.3 Å, the CM shifts shifts from 3.9 to 3.3 Å, and the desolvation barrier shifts from 5.6 to 4.3 Å (Figures 1 and 2). Surprisingly, the values of the PMF at the CM for the ionic and hydrophobic solutes (Figures 1 and 2) are similar. However, the stability of the contact pairs, as assessed by $\Delta G_{AD}[0]$ in eq 5, is different. The free energy of the associated state, $\Delta G_{AD}[0]$ (eq 5), between the M^+ and M^- is 4.1 kcal/mol, whereas for the neutral pair the free energy is 3.2 kcal/mol. In this sense, the hydrophobic interaction is stronger than ionic interactions. The differences in $\Delta G_{AD}[0]$ between hydrophobic solutes and charged species should diminish as the ion charge density decreases.[41]

Aqueous GdmCl and Urea Solutions Have Negligible Effect on the PMFs between Small Hydrophobic Solutes. The PMFs between methanes in aqueous GdmCl and urea solutions show that the positions of the CM ($r \approx 3.8$ Å) and the first barrier ($r \approx 5.8$ Å) are approximately the same at all denaturant concentrations (Figure 1). Figure 1a shows that the depth of the contact minimum (CM) increases as GdmCl increases. At higher GdmCl concentrations the desolvation barrier also increases. In contrast, the PMFs in urea are much less affected (Figure 1b). The mild increase in the well depth of the CM in urea is in accord with previous studies.[9,19] These observations are also reflected in the free energies of association ($\Delta\Delta G_{AD}$, data not shown). In GdmCl the contact pair is stabilized by about $0.2k_BT$, relative to pure water, whereas urea has negligible effect on the hydrophobic interaction.

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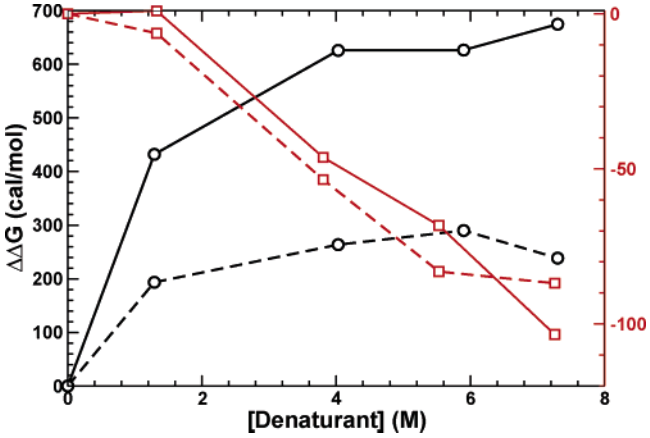

Figure 3. Change in stability (eqs 3−6) of the associated M^+-M^- pair relative to the dissociated state (solid lines, $\Delta\Delta G_{AD}$) or relative to the SSM (dashed lines, $\Delta\Delta G_{ASSM}$), as a function of denaturant concentration in GdmCl (black lines) and urea (red lines). The free energy scale for urea (in cal/mol) is shown on the right.

Interactions between Ion Pairs in Aqueous GdmCl Are Greatly Destabilized. In aqueous GdmCl solution the CM and SSM between M^+ and M^- are extensively destabilized. Figure 2a shows the PMF at five concentrations of GdmCl ranging from 0 to 7.3 M. As the concentration of GdmCl increases, the CM and SSM are destabilized (Figure 2a). The free energy of the associated state increases by as much as 0.7 kcal/mol (Figure 3) which implies that GdmCl perturbs electrostatic interactions much more than hydrophobic interactions.

In contrast to GdmCl solutions, the PMFs between M^+ and M^- do not change significantly in aqueous urea (Figure 2b). At the highest urea concentration the M^+ and M^- pair is stabilized by about 0.2 kcal/mol relative to pure water. Thus, the strength of hydrophobic and electrostatic interactions between these small solutes remains largely unchanged by urea. These observations are consistent with molecular dynamics studies on dipeptides[8] which also showed that the urea-induced differences in the PMF, in terms of a coordinate that probes conformational changes, are negligible. The small free energy changes make it difficult to infer the urea-induced denaturation mechanism from energetic considerations alone.

The dependence of the CM and SSM on the urea concentration is roughly linear (Figure 3) which is in accord with experiments.[5,42] The relationship between $\Delta\Delta G$[D] inferred from the PMF and the transfer free energy of the solutes from water to urea solution is unclear.[43] Nevertheless, the computed stabilization (∼100 cal/mol) in aqueous urea solution (Figure 3) is in rough accord with the transfer free energies associated with small amino acid side chains.[3,42] The changes in $\Delta\Delta G$ with [D] for CM and SSM are completely different in GdmCl solution (Figure 3). The destabilization of the ion pairs is largely driven by strong electrostatic interactions that manifest themselves by effective solvation of charged species by Gdm^+ (see below).

Electrostatic Interactions Determine Denaturant Efficacy. The two striking observations, namely inhibition of the association between M^+ and M^- in aqueous GdmCl solution and the lack of significant changes in ionic and hydrophobic interactions in aqueous urea, are linked to local structural preferences of water and denaturants for the solutes. From a chemical perspective urea can form eight hydrogen bonds, with the amide hydrogens being donors and the nitrogen and oxygen serving as acceptors. However, we showed in an earlier MD study of a urea−water mixture,[16] using different models for water and urea, that excluded volume of urea prevents it from satisfying all the possible hydrogen bonds. The carbonyl oxygen can most readily form hydrogen bonds with water or solvate M^+. Similarly, the amide hydrogens can solvate M^- or engage in hydrogen bonding with water. If electrostatic interactions, involving carbonyl oxygen or amide hydrogens, are the principle mechanism of solvation by urea, they should be revealed in the various pair functions. The height of the first peak in $g_{H_U}C_{M^-}(r)$ at $r \approx 2.2$ Å is less than unity which shows depletion of amide hydrogens in the vicinity of M^- (Figure 4a). In contrast, water forms a strong hydrogen bond (Figure 4a) with M^-. The results in Figure 4a show that the negatively charged M^- is mostly hydrated and urea has negligible effect on M^-. In contrast, the carbonyl oxygen and oxygen atom in water solvate M^+ as evidenced by the sharp first peaks in $g_{O_U}C_{M^+}(r)$ and $g_{O_W}C_{M^+}(r)$ at $r \approx 3$ Å (Figures 4b and S.1).

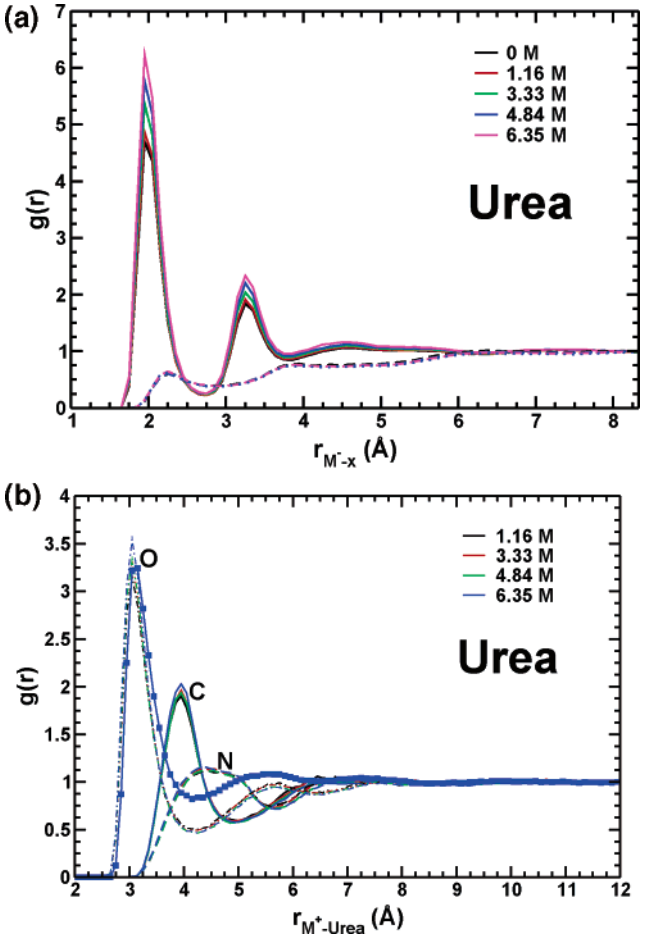

Figure 4. Radial distribution functions, as a function of urea concentration, between different atoms in the urea molecules and M^+ and M^-. The plot in (a) corresponds to M^-, and the plot in (b) is for M^+. Because of the weak interaction between M^- and the urea, only $g_{H_U}C_{M^-}$ at two urea concentrations are shown (dashed lines). For comparison, $g_{H_W}C_{M^-}$ (solid lines), the pair function between the water hydrogen atoms and the carbon on M^- is shown. (b) Pair functions, at various urea concentrations, between the carbon atom on M^+ and the atoms of urea ($g_{C_U}C_{M^+}$: solid lines. $g_{N_U}C_{M^+}$: dashed lines. $g_{O_U}C_{M^+}$: dashed/dotted lines). For comparison we also show $g_{O_W}C_{M^+}$ at 6.35 M (blue squares).

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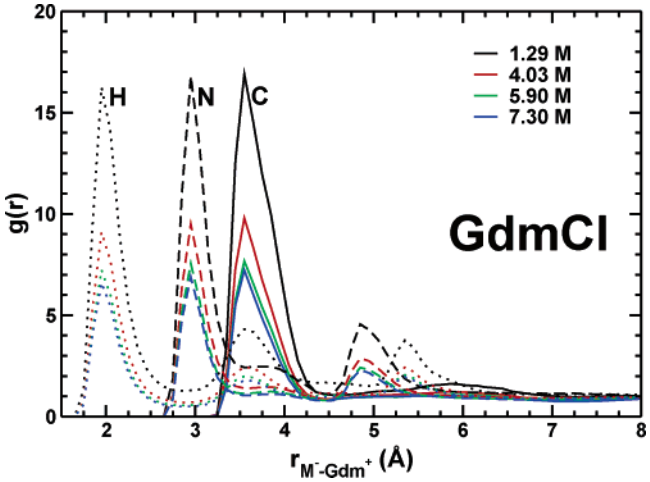

Figure 5. Radial distribution functions between the carbon M^- and the nitrogen and the carbon atoms on the Gdm^+ molecules, at various concentrations of GdmCl. ($g_{C_{Gdm}{}^+C_M{}^-}$: solid lines. $g_{N_{Gdm}{}^+C_M{}^-}$: dashed lines. $g_{H_{Gdm}{}^+C_M{}^-}$: dotted lines.) The dramatic shifts in the solvation of charged species as Gdm^+ concentration increases attests to the importance of electrostatic interactions in aqueous GdmCl solution.

The solvation mechanism in aqueous GdmCl is clearer because Gdm^+, a positively charged ion, can only interact favorably with M^-. This is reflected in the pair functions between the carbon atom on M^- and the atoms on Gdm^+ (Figure 5). The effective interaction between Gdm^+ and M^- at the CM is about 2.3 times more attractive than the interaction between urea and M^+ (comparing Figures S.2 and S.3). This suggests that Gdm^+ is more effective in destabilizing ion pairs because it hydrogen bonds more strongly than urea to the charged solutes. The results in Figures 4 and 5 readily explain the enhanced efficiency of GdmCl in denaturing proteins compared to that of urea. Such a conclusion can only be reached using local structural probes (especially for urea) because most of the interactions are relatively small (<1 kcal/mol).

Intercalation of Denaturant Molecules between Solutes in the SSM is Rare. Recently, Lee et al.[44] argued that urea's denaturation mechanism includes denaturation through stabilization of solvent-separated hydrophobic groups. They found that urea molecules "wet" hydrophobic pairs of neopentanes that result in a metastable structure in which a urea molecule is sandwiched between the two hydrophobic groups. To probe such a possibility for the smaller solutes considered here, we computed the average number of denaturant molecules, $\langle N_D \rangle$ (see Computational Methods) between methane pairs that are in the solvent-separated minimum region. At high denaturant concentrations there is a possibility of finding a denaturant molecule between methanes (Table S.III). In aqueous urea $\langle N_D \rangle \approx \langle N_{D,0} \rangle$ where $\langle N_{D,0} \rangle$ is the number of juxtaposed denaturant molecules that arise due to density fluctuations (see Supporting Information for details). Furthermore, $\langle N_D \rangle < 1$. These two results show that intercalation of the denaturant between neutral methanes does not lead to a net favorable interaction that stabilizes the structures in the SSM basin. Compared to urea, Gdm^+ molecules are more likely to be found between two methane molecules at high concentrations of GdmCl (Table S.IV). However, for the M^+ and M^- ion pair guanidinium intercalation does not result in a favorable interaction, that is

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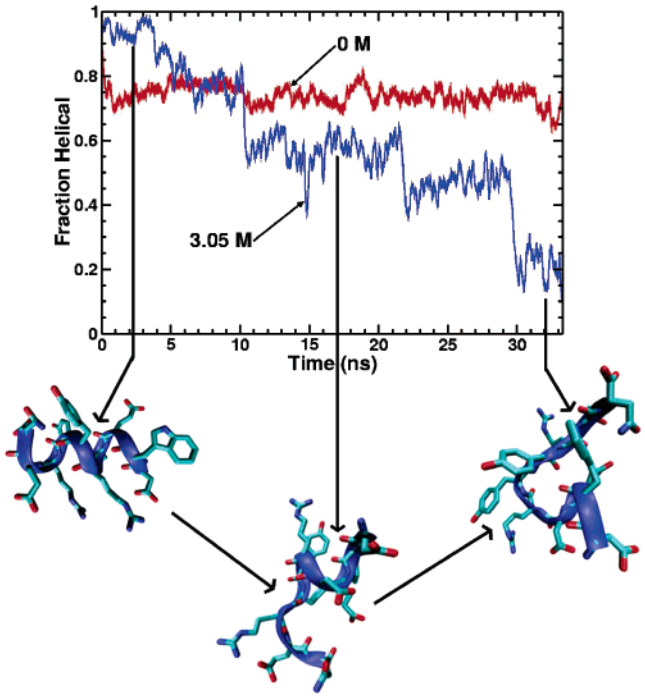

Figure 6. Time-dependent helix content of H1 from mPrP^C at 0 M (red line) and 3.05 M GdmCl (blue line). The 0 M time-trace is averaged over four independent trajectories. In all of these trajectories the helix fraction exceeds 0.5. The 3.05 M GdmCl time-trace is for a single trajectory that is smoothed with a running average window of 10 ps. Denaturation occurs in steps along this trajectory. H1 structures shown correspond to conformations found during this unfolding trajectory.

$\langle N_D \rangle \approx \langle N_{D,0} \rangle$. We conclude that juxtaposition of denaturant molecules between small hydrophobic solutes is not a significant factor in the mechanism of protein denaturation.

Direct Interaction Model Explains the Denaturation of α-Helix (H1) from mPrP^C. In order to test the applicability of the denaturation mechanism inferred from detailed study of the model systems we have probed the interaction of guanidinium and urea molecules with a highly charged helix-forming peptide from the prion protein. The stability of the 10-residue H1 helix arises largely due to the presence of three salt bridges. We have shown previously, using two different force fields, that H1 remains helical. In the absence of denaturants we find that H1 is kinetically stable (Figure 6). The transition from helix to random coil occurs in one of the trajectories in 3.05 GdmCl solution (Figure 6). The interactions that drive the transition can be inferred from the radial distribution functions involving Gdm^+ and the helical peptide (Figure 7a−c). The strong interactions between Gdm^+ and the charged side chains is vividly illustrated in the pair function between the oxygen atoms on the side chains and the hydrogen, nitrogen, and carbon on Gdm^+ (Figure 7a). The highly structured first peak and the presence of a second peak attest to the solvation of charged side chains by Gdm^+. Similarly, we find that the carbon, nitrogen, and hydrogen atoms on urea also solvate the charged groups (Figure 7b). However, the strength of interaction is greatly diminished in urea compared to that in Gdm^+ (see Figure 7a,b).

In addition to direct electrostatically dominated interactions with the charged side chains of H1, urea and Gdm^+ form hydrogen bonds with the carbonyl groups of the peptide backbone (Figure 7c). In this instance, the extent of interactions

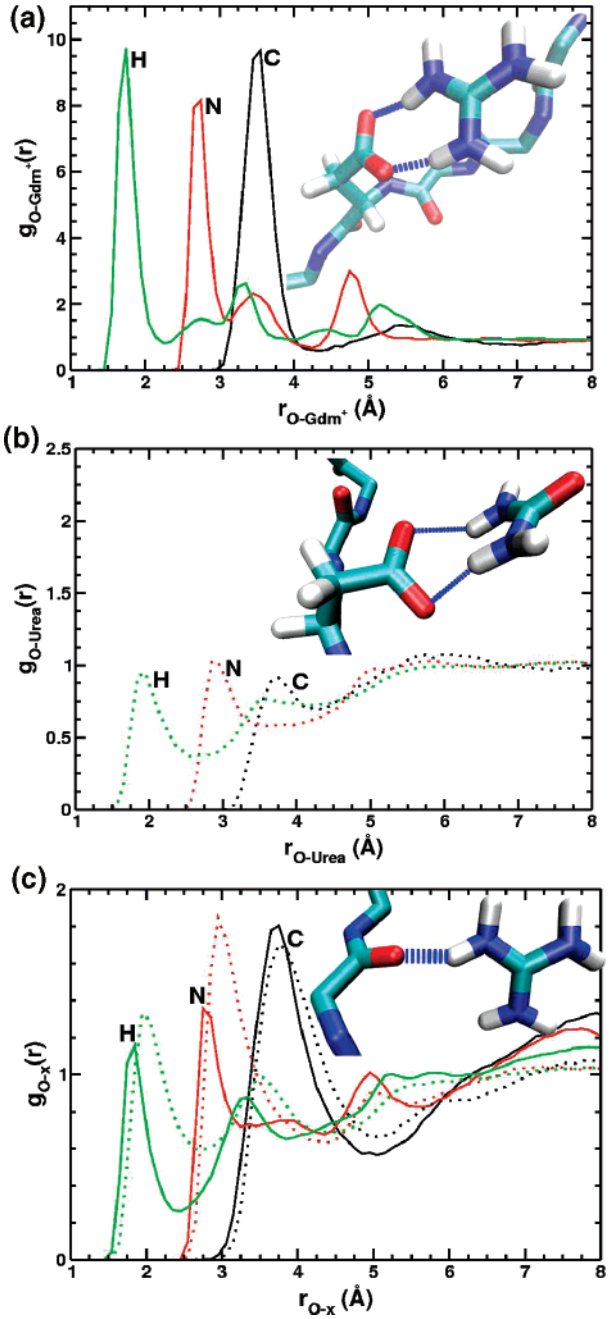

Figure 7. (a) Radial distribution functions between Gdm^+ molecules and oxygen on the negatively charged side chains of the H1 peptide (solid lines) at 3.05 M GdmCl. (b) Urea molecules and oxygen on the negatively charged side chains of the H1 peptide (dotted lines) at 3.94 M urea. Pair functions between the carbon, nitrogen, and hydrogen on Gdm^+ or urea, and oxygen on the negatively charged side chains are shown in black, red, and green, respectively. (c) Radial distribution functions between Gdm^+ or urea molecules, at the same concentrations as in (a) and (b), and oxygens that are part of peptide backbone carbonyl groups are shown as solid lines for Gdm^+ and dotted lines for urea. Pair distribution colors are the same as in (a). These radial distribution functions are computed when the peptide has a fraction helical content of zero.

with carbonyl groups of the peptide is similar for both Gdm^+ and urea when the peptide is in the random coil ensemble. Hydrogen bonding is the likely cause of the experimentally observed favorable free energy change upon transferring a peptide unit from water to aqueous denaturant solution.[5,6] This finding supports the direct binding mechanism of denaturation, where the hydrogen bonds between denaturant molecules and the peptide are thought to stabilize the denatured state and lead to protein denaturation.

4. Conclusions

Motivated by the need to understand the structural basis of denaturant-induced destabilization of proteins we have investigated the alterations in the hydrophobic and ionic interactions between small solutes in aqueous urea and GdmCl solutions. The PMFs between methane molecules show that urea has negligible effect on the depth of the contact minimum and on the solvent-separated minimum. Surprisingly, the strength of the ionic interactions between small solutes is largely unaffected in aqueous urea solutions even at elevated concentrations. These findings are in accord with previous studies involving small solutes,[9] dipeptides,[8] and globular proteins.[27] Thus, it is difficult to infer the mechanism of urea-induced denaturation of proteins using free energetic considerations alone. Urea, a polar nonelectrolyte, can efficiently form hydrogen bonds with water molecules as well as with other species such as the peptide backbone or charged species provided there are no restrictions due to excluded volume interactions. The ability of urea, which is about twice as large as a water molecule, to efficiently form hydrogen bonds is the primary reason that the water structure is unperturbed even in high denaturant concentration.[9,23,45,46] Urea also can form hydrogen bonds with the peptide backbone as well as charged residues in much the same way that it does with water.[8] In principle, urea which mimics the peptide backbone, can form eight hydrogen bonds. The present simulations show that the interaction of M^+ with urea occurs predominantly through direct interaction with the carbonyl oxygen (see Figure 4). Our previous study on urea interaction with peptides,[8] which also showed minor changes in the potential of mean force, revealed that urea interacts with the peptide backbone. From this work and other studies[7−9] it is clear that, despite the absence of clear energetic changes, urea indeed interacts directly with the peptide backbone and charged residues by engaging in hydrogen bonding. The subtle nature of the interaction has made it difficult to experimentally ascertain the energetic basis of protein denaturation by urea. However, a preponderance of experimental work and simulations shows that urea denaturation is due to the direct interaction mechanism as envisioned by Robinson and Jencks.[10]

The present work strongly suggests that electrostatic interaction between Gdm^+ and the charged residues as well as the peptide backbone is the dominant mechanism by which proteins are destabilized in aqueous GdmCl solution. Even at modest concentrations of GdmCl the interactions between M^+ and M^- are greatly destabilized. The destabilization process is reflected in the local structures of Gdm^+ around the negatively charged M^-. The strong direct interaction of Gdm^+ with charged species compared to that of urea also explains the enhanced efficiency of denaturation of proteins by GdmCl compared to urea. In general, the concentration of GdmCl needed to reach the midpoint of the unfolding transition of protein is less than that in urea. The present simulations show that this is due to the stronger solvation of charged residues and backbone by Gdm^+ than by urea. Our work also shows that the free energy changes

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of ionic and hydrophobic interactions as a function of [D] are nonlinear in GdmCl solution. Indeed, it is often found that the destabilization of proteins as [D] is changed is not linear in aqueous GdmCl solution. Thus, the straightforward interpretation of the movements of the folded state or the transition states in GdmCl solution may not reveal the underlying folding mechanism.

The systematic investigation of the changes in hydrophobic and charged interactions between small solutes in aqueous urea and GdmCl suggests that the primary mechanism of denaturation involves direct electrostatic interaction between the denaturant molecules and proteins. While such a proposal is physically reasonable for the charged Gdm^+, the situation involving urea is less clear because of the weak urea-induced perturbation of the solutes. As a result the results can depend on the models used for urea. Examination of the various urea models shows that the values of the partial charges vary greatly.[23] For example, the partial charge on the carbonyl oxygen, which is involved in efficient hydrogen-bond formation, varies from $-0.39e$[47] to about $-0.675e$,[48] whereas the value used here is $-0.51e$. Despite such variations, the ability of urea to form hydrogen bonds is largely determined by its excluded volume.[1,46] Thus, we expect the conclusions of our work to be fairly robust. Finally, the denaturation mechanism, based on electrostatic interactions, is dependent on the charge density (ζ) of the solutes.[41,49] Increases in ζ will diminish the strength of ionic interactions, which leads to the prediction that the efficiency of the denaturation mechanism can be increased by increasing ζ.

Our work also explains why small metal ions (Na^+ or K^+) do not denature proteins at low concentrations. The small ions have high ζ and hence are fully hydrated. Direct interaction of small ions with charged groups require desolvation which is enthalpically unfavorable. In contrast, the low ζ of the Gdm^+ ion interacts efficiently with charged groups of amino acids and the peptide backbone, thus leading to protein denaturation.

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Supporting Information Available: Additional information on the procedure for computing $\langle N_{D,0} \rangle$; PMFs between water and the charged solutes, urea and the charged solutes, and Gdm^+ and the charged solutes, and a complete citation for reference 28. This material is available free of charge via the Internet at http://pubs.acs.org.

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