Molecular Dynamics Study on Hydrophobic Effects in Aqueous Urea Solutions

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Abstract: Hydrophobic effects in aqueous urea were analyzed by molecular dynamics simulations. The contribution of solvents to the potential of mean force between two methane molecules was calculated by using molecular dynamics simulations and was compared with the solubility data of hydrocarbons in aqueous urea. Both the simulation results and the solubility data indicated that urea stabilizes methane−methane association. The stabilization was due to increasing the solvation free energies of small hydrocarbons such as methane by addition of urea. The solvation free energies of larger hydrocarbons, on the other hand, are decreased by addition of urea. This effect of the solute size on hydrophobic free energies in aqueous urea was also analyzed by using molecular dynamics simulations by means of division of the solvation process into two parts: the cavity formation and the introduction of the solute−solvent attractive interactions. In the cavity formation, urea increased hydrophobic free energies, and in the introduction of the solute−solvent attractive interactions, urea decreased hydrophobic free energies. The influence of urea on hydrophobic free energies was determined by the balance of effects of the two parts of the solvation process.

Introduction

Urea is a well-known potent denaturant of proteins, but its mechanism of action is not well understood. Two models of urea denaturation are generally considered. One is that urea weakens hydrophobic interaction, and the other is that urea weakens the intramolecular hydrogen bonds of proteins by binding directly to the proteins themselves.

These models of urea denaturation have been investigated by evaluating the solvation of small molecules.[1−6] Roseman and Jencks reported that urea increased the solubilities of both hydrophilic molecules (uric acid) and hydrophobic molecules (naphthalene).[3] Wetlaufer et al. reported that hydrocarbons with more than two carbon atoms were more soluble in an aqueous solution of urea than in pure water.[4] Nozaki and Tanford measured the solubilities of amino acids in aqueous urea and noted that hydrophobic effects became weaker when urea was added to the solvent and also that the free energy of interaction between peptide groups and solvent became more negative.[5] Experiments on dissolution of cyclic dipeptides also revealed that the solvation free energies per methyl or methylene group and per peptide group were decreased by addition of urea.[6] These results suggest that both of the mechanisms may be operating.

Other studies investigated the effects of urea on proteins rather than on small molecules. Myers et al. observed that *m* values (rate of change of the unfolding equilibrium with increasing denaturant concentration) correlated well with the changes of the solvent-accessible surface area and the heat capacity associated with protein unfolding.[7] Separating the contributions of nonpolar and polar surfaces to *m* values, they showed that urea favorably interacted with both nonpolar and polar surfaces. Makhatadze and Privalov used calorimetry to study the thermodynamics of protein interaction with urea[8] and observed that the number of binding sites for urea on proteins correlated well with the number of exposed polar groups of those proteins.

The experimental results described above suggest that urea weakens both hydrophobic interaction in proteins and intramolecular hydrogen bonds. An efficient way to construct a molecular-level description of such effects is by using molecular simulations.[9−17] Kuharski and Rossky performed molecular dynamics simulations of a dilute aqueous urea solution[9] and of a hydrophobic solute and a urea molecule in water.[10] They observed that the water−water interactions in the solvation shell around the urea molecule were not significantly perturbed from those in bulk. They noted that the improved solvation of hydrophobic molecules in aqueous urea was due largely to each of the urea molecules displacing several water molecules from the nonpolar solvation shell. Muller[11] applied the modified hydration shell hydrogen bond model to the hydrophobic solvation in a water−cosolvent (such as urea) mixture on the assumption that the cosolvent does not alter hydrogen bonds of

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water. He succeeded in explaining the solvation free energy, enthalpy, and entropy of hydrophobic solutes in aqueous urea by introducing the displacement of water in the hydrophobic solvation shell by urea and by introducing van der Waals interactions between urea molecules and solutes. The question of whether urea alters the hydrogen bonds of water has been addressed by using molecular simulations. Tsai et al.[13] compared an aqueous urea solution with aqueous solutions of more hydrophobic analogues with the same Y-shape. They observed that urea tended to distribute evenly in solution and did not alter the water oxygen−oxygen radial distribution function significantly. Vanzi et al.,[14] however, pointed out that neither water pair interaction energies nor the distances between the oxygen atoms of two water molecules were a sensitive indicator, but hydrogen bond angles were more sensitive for structural perturbation of hydrogen bonds of water. They observed that urea altered the distribution of hydrogen bond angles of adjacent water molecules, and they succeeded in reproducing the positive hydration heat capacity of urea by using the random network model in which the effects of hydrogen bond angles are taken into account. The connection, however, between the distribution of hydrogen bond angles and the hydrophobic free energy is not clear.

Wallqvist et al.[16] recently reported the potentials of mean force (PMF) between two methane molecules in pure water and in 6 M aqueous urea. Surprisingly, urea stabilized the contact pair of methane molecules relative to the case in pure water. This result may indicate that urea enhances hydrophobic interactions. In another recent simulation, it was found that urea increased the degree of hydration on hydrophobic molecules, while guanidinium chloride decreased it.[17] The author of that report concluded that urea may prevent the denaturation of the hydrophobic core of protein molecules and that guanidinium chloride may promote its denaturation. The experimental results mentioned above, however, suggest that urea weakens hydrophobic effects.

In this study, we first addressed a question of whether the simulation result that urea stabilized the contact of two methane molecules was consistent with the experimental data, especially the solubility data. We performed the molecular dynamics simulation and extracted the contribution of solvent from the PMF, which can be compared with the solubility data. We found that the stabilization of the methane−methane association by urea, indicated by simulations, was consistent with the solubility data and was due to increasing the solvation free energy of small molecules such as methane by addition of urea. The previous hypothesis that urea weakens hydrophobic effects was based on decreasing the solvation free energy of larger hydrophobic molecules with increasing urea concentration. Thus, for complete understanding of the influence of urea on hydrophobic effects, elucidating the solute-size effect on the hydrophobic free energy in aqueous urea is important. We therefore performed another molecular dynamics simulation and evaluated the solute-size dependence of the hydrophobic solvation free energy in aqueous urea. We found that urea had different influences on the hydrophobic free energy between two parts of the solvation process: the cavity formation and the introduction of the solute− solvent attractive interactions. In the cavity formation, urea made the hydrophobic free energy larger, and in the introduction of the solute−solvent attractive interactions, urea made the hydrophobic free energy smaller. Consequently, the solute-size effect on the hydrophobic free energy in aqueous urea was determined by the balance of the effects of the two processes.

Methods

We performed molecular dynamics simulations of hydrophobic molecules in aqueous urea solutions and in pure water. The OPLS potential parameters[18] were used for urea molecules, and the length of bonds in urea molecules was fixed by RATTLE.[19] The bond angles and dihedral angles were varied, and the AMBER parameters[20] were used for the angles. To restrain urea molecules to a planar configuration, an improper dihedral angle term was introduced. The TIP3P model[21] was used for the water potential, and water molecules were treated as rigid using RATTLE. The periodic boundary condition was applied, and long-range electrostatic forces were calculated by the particle mesh Ewald method.[22] In the particle mesh Ewald method, the FFTW package was used to calculate the fast Fourier transform. The Lennard-Jones potential term was cut off at 9 Å. All simulations were performed under the NPT ensemble condition. To control the temperature, we used the Nosé−Hoover method.[23] To control the pressure, we used the Ryckaert−Ciccotti method.[24] For time integration, the velocity Verlet method was used, and the time step was set to 2 fs.

The system of the aqueous urea solution consisted of 37 urea molecules and 200 water molecules so that the concentration of urea was 7 M. We used a 7 M concentration instead of the 6 M concentration that Wallqvist et al. used because the reported experimental solubility data were obtained when the urea concentration was 7 M.[4] For equilibration, a molecular dynamics simulation was performed for 100 ps at 400 K and 0.6 g/cm³ under the constant volume and temperature condition. The system was then cooled to 298.15 K. After that, a simulation for sampling was performed under constant pressure and temperature. During the constant pressure simulation, the density was equilibrated around 1.12 g/cm³. The simulation was considered to be valid because the experimental density of 7 M urea[25] is 1.10 g/cm³. For pure water, equilibration was performed by using a similar process.

To compare the calculated PMF between two methane molecules with the solubility data, we divided the PMF ($\Delta G_{pmf}(r)$; r is the distance between two methane molecules) into the direct interaction between two methane molecules ($\Delta E(r)$), the indirect contribution of solvent ($\Delta G_{cpmf}(r)$; this term is often called the cavity potential of mean force (CPMF)), and the $P\Delta V$ term:

$$\Delta G_{pmf}(r) = \Delta E(r) + \Delta G_{cpmf}(r) + P\Delta V \tag{1}$$

The $P\Delta V$ term is negligibly small at 1 atm. It is difficult to calculate the indirect term ($\Delta G_{cpmf}(r)$) for small r by simply subtracting $\Delta E(r)$ from ΔG_{pmf}, because both terms become extremely large when r is small. The solubility data must be compared with $\Delta G_{cpmf}(r)$ for small r. To obtain $\Delta G_{cpmf}(r)$ for small r, we performed the molecular dynamics simulation for the system in which the direct interaction between two methane molecules was omitted, and then we calculated the free energy profile between two methane molecules as $\Delta G_{cpmf}(r)$.[26]

For calculation of free energy profiles, the weighted histogram analysis method (WHAM) was used. The method is a kind of umbrella sampling. From multiple simulations in which various restraint potentials are applied, the free energy profile along arbitrary reaction coordinates can be estimated with minimized statistical error. In the calculation of $\Delta G_{cpmf}(r)$, the distance between two methane molecules was assigned as a reaction coordinate. The restraint potential for the

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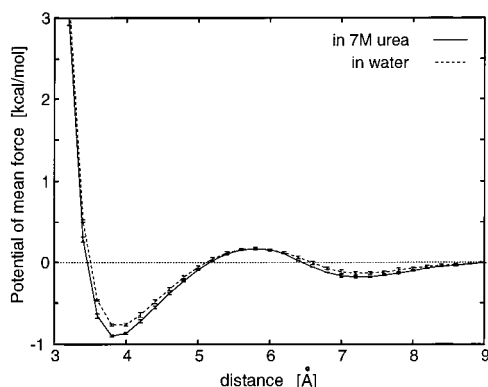
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Figure 1. Potential of mean force between two methane molecules in 7 M aqueous urea (solid line) and in pure water (dashed line). The error bars indicate minimum and maximum values obtained from divided runs.

distance E_c was

$$E_c = K_c(r - r_c)^2 \qquad (2)$$

where K_c was 2.5 kcal/mol/Å^2, and r_c was set from 1 to 4 Å at 0.5 Å intervals and from 4 to 9.5 Å at 0.1 Å. After equilibration for more than 40 ps, the sampling run for each restraint potential was performed for 600 ps. The total simulation time needed to obtain one ΔG_{cpmf} line was about 40 ns. To estimate the statistical error of the CPMF, the sampling runs were divided into two parts, and the CPMF of each part was calculated. The association constant K_a was obtained by integration[27] of the PMF to a separation distance R_{cut} which defines the geometric limit for complex formation such as

$$K_a = 4\pi \int_0^{R_{cut}} r^2 \, e^{-\Delta G_{pmf}(r)/kT} \, dr \qquad (3)$$

where k is the Boltzmann constant and T is the absolute temperature.

To investigate the solute-size effect of urea on hydrophobic hydration, we also calculated solvation free energies as the size of a hydrophobic solute was varied. In this calculation, the λ-dynamics method[28] was used. λ is a coupling parameter of potential functions. As λ is changed from 0 to 1, the parameters such as the volume of solute and the solute−solvent interaction are changed. In the λ-dynamics method, λ is also treated as a variable like a coordinate, and the free energy profile along λ can be obtained. The WHAM was used in the estimation of the free energy profile. The λ-dynamics method provides robust estimation of free energies.[28] The restraint potential E_c for λ was set as follows:

$$E_c = K_c(\lambda - \lambda_c)^2 \qquad (4)$$

where K_c was 200 kcal/mol, and λ_c was set from 0 to 1.2 at 0.1 intervals. Twelve independent simulations were performed, and one free energy profile was obtained by combining the results obtained by using the WHAM. In each simulation, a 20 ps equilibration run and a 100 ps productive run were performed. The total simulation needed to obtain one free energy profile along λ was 1.2 ns.

Results and Discussion

Potential of Mean Force between Two Methane Molecules in Aqueous Urea. The potentials of mean force between two methane molecules in pure water and 7 M urea are shown in Figure 1. The error bars in the figure indicate minimum and maximum values obtained from divided runs. The PMF indicates that urea stabilizes the methane−methane contact pair. The
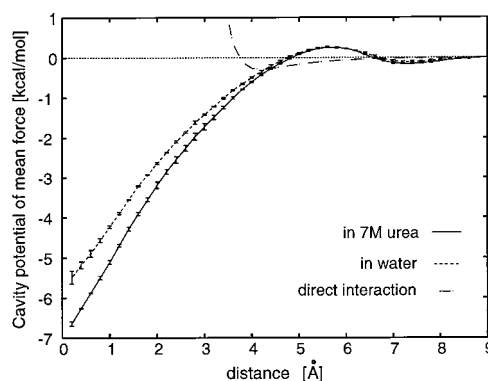
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Figure 2. Contribution of solvent to the potential of mean force between two methane molecules in 7 M aqueous urea (solid line) and in pure water (dashed line). The error bars indicate minimum and maximum values obtained from divided runs. The long-dashed line shows the direct interaction between two methane molecules. At 1.52 Å, the calculated difference in the solvent contributions is −0.71 kcal/mol, while the value estimated from solubility data is −0.298 kcal/mol.
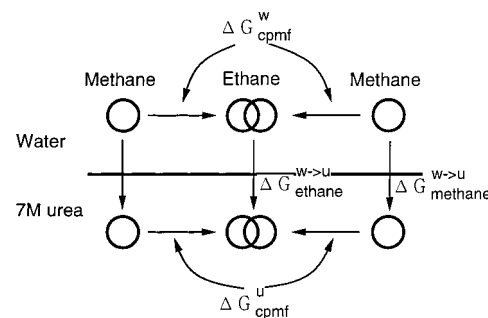

Figure 3. Schematic diagram showing how the association free energy can be estimated from experimental data. See the text for detail.

association constant K_a was 0.61 M^{-1} in 7 M urea and 0.53 M^{-1} in pure water ($R_{cut} = 5.1$ Å). These results are similar to those reported by Wallqvist et al., although we used a different water potential from the potential they used. (They used the SPC model, and we used the TIP3P model.) These results seem to contradict the hypothesis that urea weakens the hydrophobic interactions. The hypothesis is largely based on increasing solubilities of many kinds of hydrophobic molecules by addition of urea. To understand the discrepancy between the previous hypothesis and the results of our simulations, a detailed comparison between the solubility data and simulation results was made below.

As described in the Method section, to compare the calculated PMF with the solubility data, we divided the PMF into the direct interaction between two methane molecules and the cavity potential of mean force (CPMF, the indirect contribution of solvent). The CPMF indicates that the closer two methane molecules get, the more urea stabilizes the pair configuration (Figure 2).

The CPMF value can be estimated from the solubility data in the following way (Figure 3). This method is an application of the method proposed by Ben-Naim[29,30] to aqueous urea. The association state of two methane molecules is approximated as an ethane molecule, and the difference between the CPMF values at 1.52 Å (the distance between two carbon atoms of an ethane molecule) in aqueous urea and in pure water is estimated as the difference between the transfer free energy of ethane from

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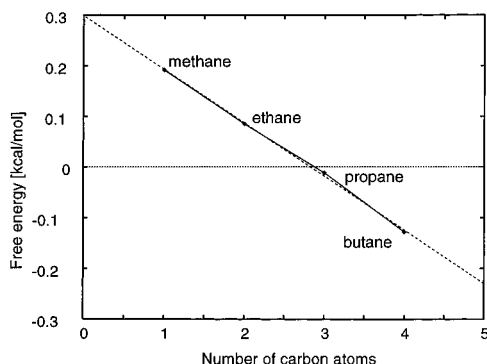(30) Ben-Naim, A. *J. Chem. Phys.* **1971**, *54*, 3696.

Figure 4. Transfer free energies of alkanes from pure water to aqueous urea. The *x* axis shows the number of carbon atoms of alkanes. Data are taken from Wetlaufer et al.[4]
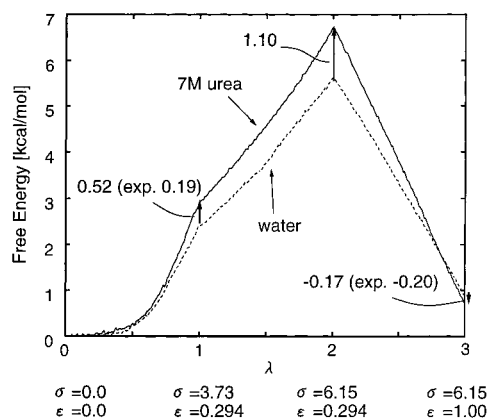

Figure 5. Solute-size dependence of the solvation free energy of a hydrophobic sphere in aqueous urea (solid line) and in pure water (dashed line). λ is a coupling parameter. When λ is 0, a solute molecule is absent. The state at $\lambda = 1$ corresponds to a methane molecule. From 1 to 2, σ increases from the value for methane to the value for neopentane, keeping ϵ constant. The state at $\lambda = 3$ approximately corresponds to a neopentane molecule. The differences between free energies in aqueous urea and in water are presented at $\lambda = 1$ and $\lambda = 3$. The unit of the free energies is kilocalories per mole. The numbers in parentheses are experimental values of the free energies.

water to aqueous urea ($\Delta G_{\text{ethane}}^{\text{w}\rightarrow\text{u}}$) and twice the transfer free energy of methane from water to aqueous urea ($2\Delta G_{\text{methane}}^{\text{w}\rightarrow\text{u}}$). If we designate the difference between these CPMF values as $\Delta\Delta G_{\text{cpmf}}(1.52\ \text{Å})$, we can write

$$\Delta\Delta G_{\text{cpmf}}(1.52\ \text{Å}) = \Delta G_{\text{cpmf}}^{\text{u}}(1.52\ \text{Å}) - \Delta G_{\text{cpmf}}^{\text{w}}(1.52\ \text{Å})$$

$$= \Delta G_{\text{ethane}}^{\text{w}\rightarrow\text{u}} - 2\Delta G_{\text{methane}}^{\text{w}\rightarrow\text{u}} \qquad (5)$$

where $\Delta G_{\text{cpmf}}^{\text{u}}(1.52\ \text{Å})$ and $\Delta G_{\text{cpmf}}^{\text{w}}(1.52\ \text{Å})$ are respectively the CPMF values at 1.52 Å in aqueous urea and in pure water. The transfer free energy of each solute, $\Delta G_{\text{solute}}^{\text{w}\rightarrow\text{u}}$, is estimated from the solubility data in aqueous urea and in pure water by using the following equation:

$$\Delta G_{\text{solute}}^{\text{w}\rightarrow\text{u}} = kT\ln\frac{\rho_{\text{solute}}^{\text{w}}}{\rho_{\text{solute}}^{\text{u}}} \qquad (6)$$

where $\rho_{\text{solute}}^{\text{u}}$ and $\rho_{\text{solute}}^{\text{w}}$ are the number densities of solute in aqueous urea and in pure water. By substituting the solubility data published by Wetlaufer el al.[4] into eqs 5 and 6, we estimate $\Delta\Delta G_{\text{cpmf}}(1.52\ \text{Å})$ to be −0.298 kcal/mol. The negative value indicates that urea stabilizes the association of two methane

molecules. Therefore, it is clarified that there is no contradiction between the simulation results and the solubility data. The value of our simulation result (−0.71 kcal/mol) is almost twice the experimental value. However, taking into account that a methane molecule and a methyl group were approximated as the same Lennard-Jones sphere, we consider that the qualitative agreement is important.

Solute-Size Effect on Hydrophobic Solvation in Aqueous Urea. The stabilization of the contact of two methane molecules by urea seems to be inconsistent with the previous hypothesis, in which urea weakens hydrophobic effects. This is because small hydrocarbons such as methane and ethane have larger solvation free energies in aqueous urea than in pure water (Figure 4). Hydrocarbons with more than two carbon atoms, however, have smaller solvation free energies in aqueous urea than in pure water. The previous hypothesis is largely based on decreasing solvation free energies of comparatively large hydrophobic molecules by addition of urea. Therefore, it is important to elucidate the solute-size dependence on solvation free energies in aqueous urea.

We therefore performed another molecular dynamics simulation to calculate the free energy profiles corresponding to the change of the solute size in 7 M aqueous urea and in pure water. The solute−solvent interaction is represented as the Lennard−Jones potential

$$u_{\text{LJ}}(r) = 4\epsilon\left\{\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right\} \qquad (7)$$

where the parameter ϵ denotes the amount of the attractive interaction and the parameter σ denotes the radius of the excluded volume. Both parameters ϵ and σ were changed in our simulation.

We first increased the parameters from 0 (solvent only) to their values for methane ($\epsilon = 0.294$ kcal/mol and $\sigma = 3.73$ Å). This is shown in Figure 5 as the change of λ from 0 to 1. The difference between free energies in aqueous urea and in pure water at $\lambda = 1$ corresponds the difference in the solvation free energies of methane. Both the simulation result and the experimental value are positive, and this indicates that methane is more soluble in pure water than in aqueous urea. Our simulation result agrees with the experimental result.

Keeping the parameter ϵ at the methane value, we increased the parameter σ from the value for methane (3.73 Å) to the value for neopentane (6.15 Å). In this process, only the excluded volume increased, while the solute−solvent attractive interaction was unchanged. The neopentane parameters published by Kuharski and Roskky[10] were used. According to solubility data,[4] neopentane is more soluble in aqueous urea than in pure water, and the solvation free energy difference for neopentane is −0.20 kcal/mol. The simulation result, however, shows that the larger the excluded volume is made, the less soluble in aqueous urea the solute is. This simulation result disagrees with the experimental result even qualitatively. Therefore, the excluded volume does not cause the stabilization of solvation of large hydrocarbons in aqueous urea. Our result does not support the hypothesis that urea lowers the free energy of cavity formation.[13]

We also performed a series of simulations in which the parameter ϵ was increased. This is shown in Figure 5 as the change of λ from 2 to 3. Although Kuharski and Roskky used $\epsilon = 0.8351$ kcal/mol for neopentane, we increased ϵ over their value to 1 kcal/mol for qualitative analysis. As ϵ increases, the difference between free energies in aqueous urea and in pure water becomes smaller, and eventually the solute becomes more
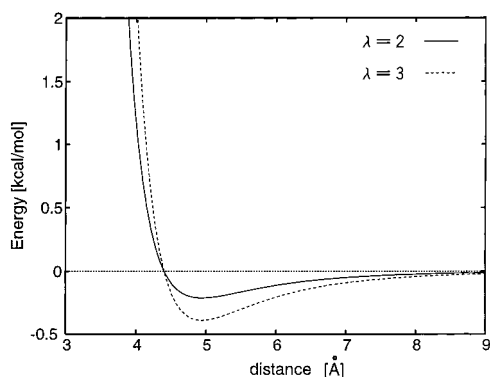
Figure 6. Solute−water potential functions $u_{LJ}(r)$ at $\lambda = 2$ and $\lambda = 3$.

soluble in aqueous urea than in pure water. This tendency agrees with the solubility data.

The solute−solvent Lennard Jones potentials $u_{LJ}(r)$ for $\lambda = 2$ and $\lambda = 3$ are displayed in Figure 6. The change of the negative part of $u_{LJ}(r)$ corresponds to the increase in the attractive interaction between solute and solvent. The positive part of $u_{LJ}(r)$ is also altered, and the change may have some influence on the effective size of the solute. To investigate the relationship between the change of $u_{LJ}(r)$ and the free energy change, we analyzed the free energy change from $\lambda = 2$ to 3 using perturbation theory.[31,32]

In the perturbation theory, the pair potential function $u(r)$ is separated into a reference part $u_0(r)$ containing the repulsive interactions and a perturbation part $u_1(r)$ containing the attractive interactions:

$$u(r) = u_0(r) + u_1(r) \tag{8}$$

Consider a coupling parameter α from $u_0(r)$ to $u(r)$. The pair potential function $u_\alpha(r)$ for the state α is

$$u_\alpha(r) = u_0(r) + \alpha u_1(r) \tag{9}$$

The difference between the free energy G for the state $\alpha = 1$ and the free energy G_0 for the reference state $\alpha = 0$ is

$$G - G_0 = \rho \int_0^1 d\alpha \int d\mathbf{r} u_1(r) g_\alpha(r) \tag{10}$$

where $g_\alpha(r)$ is the pair correlation function for the system α, and ρ is the number density of molecules. If the repulsive interaction mainly determines the structure of fluids, then $g_\alpha(r) \cong g_0(r)$, and the integration of eq 10 with respect to α is easily performed:

$$G - G_0 \cong \rho \int d\mathbf{r} u_1(r) g_0(r) \tag{11}$$

Extension of this derivation to the system of multiatomic molecules is straightforward.

The above perturbation theory was applied to the change of λ from 2 to 3. One can assign a reference part $u_0(r)$ and a perturbation part $u_1(r)$ such as

$$u_0(r) = u_{LJ}(r; \lambda = 2) \tag{12}$$

$$u_1(r) = u_{LJ}(r; \lambda = 3) - u_{LJ}(r; \lambda = 2) \tag{13}$$

where $u_{LJ}(r; \lambda = 2)$ and $u_{LJ}(r; \lambda = 3)$ are the solute−solvent Lennard-Jones potentials for $\lambda = 2$ and $\lambda = 3$, respectively.

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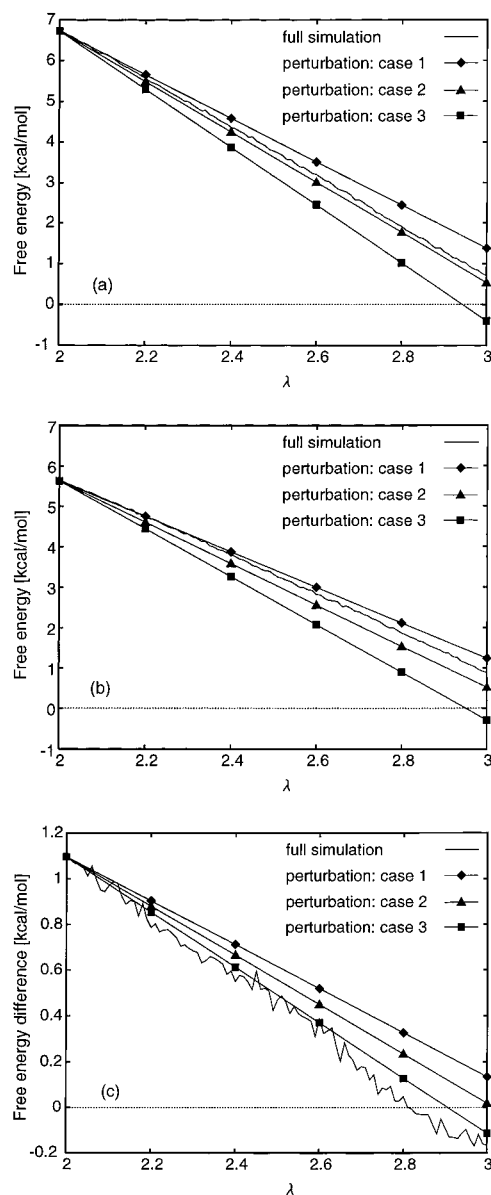(32) Andersen, H. C.; Chandler, D.; Weeks, J. D. *Adv. Chem. Phys.* **1976**, *34*, 105.

Figure 7. Solvation free energies from the perturbation theory in three cases compared with the full simulation using λ-dynamics. (a) The solvation free energies in 7 M urea. (b) The solvation free energies in pure water. (c) The difference between the solvation free energies in 7 M urea and in pure water. See text for details.

We denote this assignment as "case 1". However, in this assignment, the approximation $g_\alpha(r) \cong g_0(r)$ cannot possibly apply in the positive region of $u_{LJ}(r)$. Barker and Henderson assigned the negative part of the Lennard-Jones potential to $u_1(r)$.[31] In "case 2", we used the perturbation part $u_1^{BH}(r)$,

$$u_1^{BH}(r) = \begin{cases} u_{LJ}(r; \lambda = 3) - u_{LJ}(r; \lambda = 2) & r \geq \sigma \\ 0 & r < \sigma \end{cases} \tag{14}$$

Andersen et al. assigned the positive region of derivative of $u_{LJ}(r)$ to $u_1(r)$ in terms of attractive "force".[32] In "case 3", we used the perturbation part $u_1^{ACW}(r)$,

$$u_1^{ACW}(r) = \begin{cases} u_{LJ}(r; \lambda = 3) - u_{LJ}(r; \lambda = 2) & r \geq 2^{1/6}\sigma \\ -\epsilon(\lambda = 3) - \{-\epsilon(\lambda = 2)\} & r < 2^{1/6}\sigma \end{cases} \tag{15}$$

where $\epsilon(\lambda = 2)$ and $\epsilon(\lambda = 3)$ are the Lennard-Jones potential parameters ϵ for the systems $\lambda = 2$ and $\lambda = 3$, respectively.

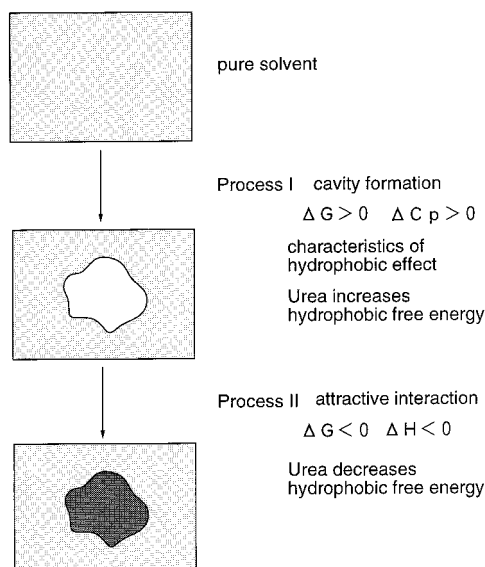682   *J. Am. Chem. Soc., Vol. 123, No. 4, 2001*

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Figure 8. Decomposition of the solvation process into two parts. Process I is the cavity formation, and process II is the introduction of the solute−solvent attractive interactions.

For the three cases, the perturbation theory was applied, and its results were compared with the full simulation using λ-dynamics. The pair correlation function $g_0(r)$ was calculated from 1 ns molecular dynamics simulations for $\lambda = 2$ in 7 M urea and in pure water. The free energies from the perturbation theory in 7 M urea and in pure water are shown in Figure 7a and 7b, respectively. Figure 7c shows the difference between the free energies in 7 M urea and in pure water. For all three cases, the difference decreased as ϵ increased. The result from the perturbation theory generally agrees with the full simulation. Because the free energy change in the perturbation theory is due to attractive interactions between solute and solvent, our result indicates that the solute−solvent attractive interaction causes the stabilization of hydrophobic solvation by urea.

Figure 8 shows how the solvation process can be divided into two parts: process I of cavity formation and process II of the introduction of the solute−solvent attractive interaction. Characteristics of hydrophobic effects such as the large positive heat capacity change and the entropy decrease at room temperature are associated with the process of cavity formation.[33] Water molecules around cavities are thought to be in the hydrophobic state such as ordering, because the water molecules cannot make hydrogen bonds in the direction to cavities. Our

results showed that urea increased the free energy of cavity formation. Thus, our results do not indicate that removing such water molecules in the hydrophobic state by urea would stabilize hydrophobic solvation. The stabilization of hydrophobic solvation by urea is due to the solute−solvent attractive interaction. For small molecules such as methane and ethane, the free energy change in process I is larger than that in process II. For large molecules which have more interaction sites, the free energy change in process II decreases, and the net free energy change becomes negative. Thus, the enhanced solvation of comparatively large hydrophobic molecules is due to the solute−solvent attractive interactions. These effects are also supported by the fact that, while the surface tension, which is the interfacial free energy between the aqueous urea and the gas phase, increases as the urea concentration increases,[34] the interfacial tension between aqueous urea and hydrocarbon liquids decreases as the urea concentration increases.[35]

Muller succeeded in explaining urea effects on the solvation free energy, entropy, and enthalpy of hydrophobic solutes by applying his modified hydration shell hydrogen bond model to an aqueous cosolvent such as urea.[11] This model introduces the attractive van der Waals interaction between solutes and urea molecules in addition to replacing water molecules in the hydrophobic state by urea. We consider that the reason for the success of his theory might be the introduction of the attractive van der Waals interaction.

How does urea denature protein? Because groups of proteins are connected by covalent bonds even in the denatured state, we consider that the model of two methane molecules is not an appropriate approximation of the exposed hydrophobic groups of proteins. The exposed hydrophobic groups might correspond to large hydrophobic molecules. Therefore, in aqueous urea, the hydrophobic interaction driving protein folding might be weakened. This possibility must be investigated using a model of the hydrophobic core that is more realistic than methane molecules.

In this paper, we studied only aliphatic hydrocarbons, although the aromatic hydrocarbons are more soluble in aqueous urea. Possible reasons for this are the dispersion force, π electrons, and electrostatic effects. The effect of urea on aromatic hydrocarbons should also be investigated in the future.

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