

Enthalpy–Entropy Compensation in the Effects of Urea on Hydrophobic Interactions

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By comparison of neopentane pair potentials of mean force (PMFs) in room temperature water and 6.9 molar aqueous urea, it was recently shown that urea molecules affect the PMF minima in an unexpected way (Lee, M.-E.; van der Vegt, N. F. A. *J. Am. Chem. Soc.* **2006**, *128*, 4948). While the first PMF minimum in urea solution has an identical shape and depth to those of the corresponding minimum in water, the second minimum in urea solution is broader, deeper, and shifted out to a slightly larger distance. Here, we present a study of the enthalpic and entropic contributions to these PMFs. Its significance for understanding the driving forces responsible for thermodynamically favorable neopentane contact and solvent-separated distances in urea solution is discussed. We propose that the *solute–solvent entropy* and *solute–solvent enthalpy* changes should be analyzed for obtaining an unambiguous molecular-scale picture. In urea solution, enthalpy–entropy compensation effects associated with structural solvent reorganization processes are large, causing changes of the system's enthalpy and entropy with hydrophobic pair separation to be very different from the solute–solvent enthalpy and entropy changes. The entropies are discussed in terms of the molecular-scale solvent reorganization processes.

Introduction

In water, nonpolar solute molecules associate with each other. This so-called hydrophobic interaction is one of the major driving forces in protein folding. In the simplest, long-standing view, pairing up offers the molecules the advantage of reducing the solvent-exposed surface area, reducing the number of first shell water molecules, and thus reducing the unfavorable entropy at 298 K.¹ This picture has been confirmed by computer simulation studies of the potential of mean force (PMF) of bringing together two nonpolar molecules in water, which revealed that the contact pair is stabilized by the entropy while the solvent-separated pair is stabilized by the enthalpy (energy).^{2–4}

A decomposition of free energies in enthalpic and entropic components provides useful insights in atomic-scale solvation mechanisms. For example, recently, Özal and Van der Vegt⁵ showed that the entropy change of methane transfer from water to aqueous solutions of dimethyl sulfoxide is positive because dimethyl sulfoxide molecules, in preferentially solvating the nonpolar solute, release some of their hydration waters to the bulk. The same authors however also showed that this solvent reorganization process is strongly enthalpy–entropy compensating in the free energy; therefore, the entropy change cannot be invoked to explain the preferential interaction of methane with dimethyl sulfoxide molecules.

In this short report, we discuss *neo*-pentane pair PMFs in an aqueous urea solution with particular emphasis on the contributions of the enthalpy and entropy. A comparison is made with

the same system in pure water, and the observed urea-induced changes of the hydrophobic interaction are discussed in terms of the changes in these two quantities. In particular, effects of solvent reorganization that contribute to the enthalpies and entropies but compensate (cancel) in the PMF are singled out. In aqueous urea, these contributions are large and it will be shown that, therefore, the enthalpic and entropic components of the PMF cannot unambiguously be used to argue that minima of the PMF are stabilized by enthalpy- or entropy-driven molecular-scale processes. The molecular-solvation mechanisms responsible for the large enthalpy–entropy compensation phenomenon occurring in the PMF as well as the mechanisms underlying the changes of the entropy will be discussed and quantified separately.

Simulation Details

All simulations were performed using the SPC/E water model⁶ and the KBFF urea model⁷ and were conducted under constant pressure (1 atm) and temperature conditions. The system size and simulation details were chosen to be identical to those in ref 8. PMFs were calculated by simulating 80 independently equilibrated systems, in each applying a distance constraint fixing the distance r between the mass centers of the two solutes at a preset value in the range $0.40 \text{ nm} \leq r \leq 1.2 \text{ nm}$ (using equidistant spacings of $\Delta r = 0.01 \text{ nm}$), and integrating the average constraint force accumulated in each 10–20 ns of simulation from 1.2 nm downward. These long runs, which in total require more than $1 \mu\text{s}$ sampling time per PMF curve, are required to reduce the statistical uncertainty of the first PMF minimum to 0.2 kJ/mol. The PMF was corrected with the term $2k_B T \log(r)$ to ensure that trivial entropy contributions,

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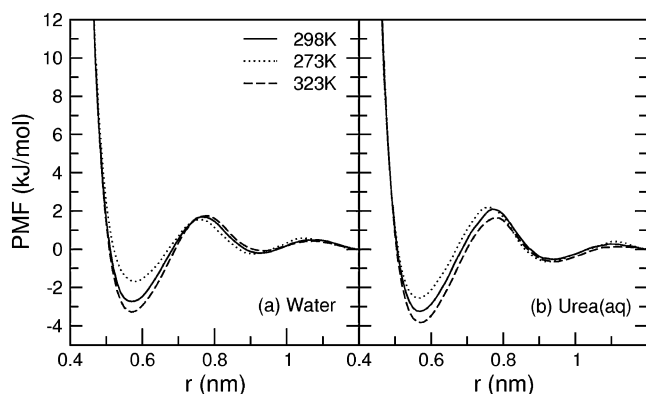


Figure 1. (a) *neo*-Pentane pair PMF in water at 273 K (dotted), 298 K (full), and 323 K (dashed). (b) *neo*-Pentane pair PMF in 6.9 mol/L urea solution at 273 K (dotted), 298 K (full), and 323 K (dashed). The statistical uncertainty in the first minima is 0.2 kJ/mol.

related to a larger volume element ($\sim r^2$) sampled by the constrained but freely rotating solute pair at larger distance r , do not enter the PMF. To obtain the entropy $\Delta S(r)$ and enthalpy $\Delta H(r)$ as a function of the solute–solute distance r , the PMFs ($\Delta G(r)$ from now on) were calculated at three temperatures ($T = 273, 298$, and 323 K) and the entropy was obtained by taking the numerical finite difference $\Delta S(r) = -[\Delta G(r; T + \Delta T) - \Delta G(r; T - \Delta T)]/2\Delta T$, with $T = 298$ K and $\Delta T = 25$ K. The enthalpy was finally obtained from $\Delta H(r) = \Delta G(r) + T\Delta S(r)$.

Results

Figure 1 shows the *neo*-pentane pair PMF in water (a) and 6.9 M urea solution (b) at 273, 298, and 323 K. The first PMF minimum, further on in this paper referred to as the contact minimum (CM), becomes deeper with increasing temperature in water as well as in urea solution, indicating that *neo*-pentane association is entropic in both systems. A comparison of the PMFs in water and aqueous urea, in relation to aspects of protein denaturation, has recently been discussed in detail elsewhere.⁹ In parts a and b of Figure 2, the PMFs in, respectively, water and urea solution at 298 K are shown together with the enthalpic and entropic contributions obtained from the data in Figure 1. In water, the entropy stabilizes the CM, while the enthalpy destabilizes it. Note that these contributions have the same sign and therefore largely cancel each other out in the PMF. Southall and Dill,⁴ based on calculations with the Mercedes-Benz model of water,^{10,11} showed that the oscillations of the enthalpy and entropy correlate with water hydrogen bonding. The second PMF minimum in Figure 2a, further on in the paper referred to as the solvent-separated minimum (SSM), is enthalpically favorable while being entropically unfavorable. Note that the location of the minimum in the $\Delta H(r)$ and $T\Delta S(r)$ curves does not coincide with the SSM of the PMF. We show later on (Figure 2c) that this shift is caused by the water–water (hydrogen-bonding) interactions being stronger at a slightly smaller distance. Rick^{3,12} observed a similar shift of the entropy minimum relative to the SSM in his study of methane–methane interactions in water using a polarizable, fluctuating charge (FQ) model for water.³ In urea solution (Figure 2b), approach of the CM ($r < 0.7$ nm) causes an increase of the entropy and a decrease of the enthalpy; hence, the enthalpy and entropy changes are reinforcing in the PMF and the enthalpy (in contrast to what is observed in water; Figure 2a) contributes favorably to the stability of the CM.

The tendency of the enthalpy (ΔH) and entropy ($T\Delta S$) curves to oscillate in phase with comparable amplitudes means that

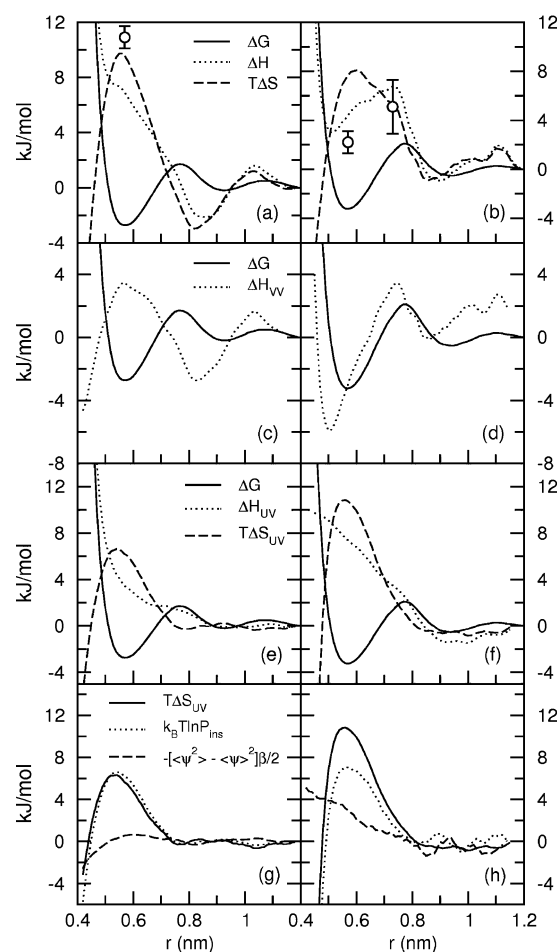


Figure 2. (a) *neo*-Pentane pair PMF (full line) and the enthalpy (dotted) and entropy (dashed) contributions in water and (b) 6.9 mol/L urea solution. (c) Solvent reorganization enthalpy (dotted line) in water and (d) 6.9 mol/L urea solution (the PMFs (full lines) are included for comparison). (e) Solute–solvent enthalpy (dotted line), solute–solvent entropy (dashed), and the PMF (full) in water and (f) 6.9 mol/L urea solution. (g) Solute–solvent entropy (full line) and contributions (cf. eq 3) of the insertion probability (dotted) and solute–solvent energy fluctuations (dashed) in water and (h) 6.9 mol/L urea solution. Solute–solvent energy fluctuations were obtained by extending the MD simulations at each distance r to 50 ns. All data were obtained at 298 K and $p = 1$ atm. The data points in parts a and b indicated with a circle were obtained by subtracting the average enthalpy of a box with a *neo*-pentane–*neo*-pentane constraint distance of 0.57 nm (and 0.73 nm in urea solution) from the average enthalpy of a box with a *neo*-pentane–*neo*-pentane constraint distance of 1.2 nm. The average enthalpies were obtained from 100 ns NPT simulations at $T = 298$ K except for the point at $r = 0.73$ nm in part b where the simulation run was 50 ns.

significant parts will cancel in the PMF ($=\Delta H - T\Delta S$). Statistical–mechanical analyses of solvation processes have previously shown that the solvent reorganization enthalpy, defined as the change in the enthalpy of solvent–solvent interactions, is exactly enthalpy–entropy compensating.^{13–15} That means that only those enthalpy and entropy contributions deriving directly from the solute–solvent coupling determine the free energy change. Contributions deriving from solute-induced changes of cohesive solvent–solvent interactions do not affect the change of free energy. In urea solution, we expect that compensating solvent–solvent contributions are large. Early experiments by Wetlaufer et al.¹⁶ have shown that enthalpies and entropies of *neo*-pentane transfer from water to aqueous urea solution are positive while the transfer free energy is negative. Neglecting (the obvious) enthalpy–entropy compensa-

tion of this process, one would conclude the transfer to be entropically driven. Computer simulations⁸ and theoretical predictions based on scaled-particle theory¹⁷ however have indicated that the transfer is driven by favorable *neo*-pentane–urea van der Waals interactions, not by the entropy. The combined experimental and theoretical work thus indicates that the contributions of (compensating) solvent reorganization processes to the solvation enthalpy and entropy in aqueous urea are significantly larger than those in water and affect the solute transfer thermodynamics in a way that these processes blur signatures of the true molecular driving force.

For the present case of a pairwise hydrophobic interaction, the solute pair (with the separation distance r fixed) is considered as “one solute” and a decomposition scheme based on solute–solvent and solvent–solvent contributions—as it has been used in solvation studies of a single solute^{5,8,18,19}—is applied to the enthalpy and entropy:

$$\begin{aligned}\Delta H(r) &= \Delta H_{UV}(r) + \Delta H_{VV}(r) \\ \Delta S(r) &= \Delta S_{UV}(r) + \Delta H_{VV}(r)/T\end{aligned}\quad (1)$$

The solute–solvent enthalpy change $\Delta H_{UV}(r)$, solvent–solvent enthalpy change $\Delta H_{VV}(r)$ and solute–solvent entropy change $\Delta S_{UV}(r)$ are functions of the constrained distance r within the “solute”. On the basis of eq 1, the PMF becomes

$$\begin{aligned}\Delta G(r) &= \Delta H(r) - T\Delta S(r) \\ &= \Delta H_{UV}(r) - T\Delta S_{UV}(r)\end{aligned}\quad (2)$$

where $\Delta H_{VV}(r)$ has canceled out (statistical–mechanical enthalpy–entropy compensation). $\Delta H_{UV}(r)$ is obtained directly from the simulation by separating the solute–solvent potential energy from the total potential energy.²⁰ The *neo*-pentane–*neo*-pentane van der Waals interactions are included in $\Delta H_{UV}(r)$. The solvent reorganization enthalpy $\Delta H_{VV}(r)$ is obtained by taking the difference between $\Delta H(r)$ and $\Delta H_{UV}(r)$, and the solute–solvent entropy change $\Delta S_{UV}(r)$ is obtained from the difference between $\Delta H_{UV}(r)/T$ and $\Delta G(r)/T$. We note that all changes in water–water, urea–water, or urea–urea hydrogen-bonding and van der Waals interactions with changing solute separation r will contribute to the solvent reorganization enthalpy.

The solvent reorganization enthalpies $\Delta H_{VV}(r)$ in water and urea solution are shown in parts c and d of Figure 2, respectively, together with the PMFs. In water, the reorganization enthalpy oscillates. This is consistent with the observation of Southall and Dill⁴ who showed that the enthalpy and entropy oscillations correlate with water–water hydrogen bonding. In the CM, hydrogen bonds are broken, causing an increase of the enthalpy and entropy (eq 1). In the SSM, hydrogen bonds are stabilized causing a decrease of, both, enthalpy and entropy. In urea solution, an oscillatory trend is also observed; however, a rather deep minimum arises at the CM. Urea preferentially solvates the two nonpolar solutes (see Figure 2 in ref 9), and in bringing them into contact, urea molecules are expelled from the gap between the two solutes. The “released” urea molecules form hydrogen bonds with water and/or urea molecules in the bulk solution, causing a large, and negative, solvent reorganization enthalpy, as observed in Figure 2d. The strengthening of solvent–solvent cohesive interactions in the bulk contributes negatively to the entropy (eq 1). Because the entropy of the CM in aqueous urea (Figure 2b) is only marginally smaller than the entropy of the CM in water, an additional process must occur in urea solution that increases the solute–solvent entropy ΔS_{UV}

and compensates for the negative solvent reorganization entropy ($\Delta H_{VV}/T$). This process will be addressed below. In water (Figure 2c), not only the trend of ΔH_{VV} but beyond $r = 0.7$ nm also the magnitude matches the entropy $T\Delta S$ in Figure 2a. The entropy change in water at distances of $r > 0.7$ nm therefore is completely determined by the reorganization of water–water hydrogen bonds.

Parts e and f of Figure 2 show the contributions of the solute–solvent enthalpy ΔH_{UV} and solute–solvent entropy $T\Delta S_{UV}$ to the PMF in water and 6.9 M urea solution, respectively. In water, the CM is stabilized by the solute–solvent entropy but destabilized by the solute–solvent enthalpy. The latter happens because the contact pair has a smaller solvent accessible area than the solvent-separated pair. In the CM, both, ΔH_{UV} and $T\Delta S_{UV}$ are positive but smaller in magnitude than ΔH and $T\Delta S$ (Figure 2a), because the former quantities do not include the positive enthalpy and entropy contributions of hydrogen-bond reorganization ($\sim +3.5$ kJ/mol; Figure 2c). The SSM (0.92 nm) is stabilized by the solute–solvent entropy. In urea solution (Figure 2f), the CM is stabilized by the solute–solvent entropy and destabilized by the solute–solvent enthalpy. In the CM, both ΔH_{UV} and $T\Delta S_{UV}$ are positive and larger in magnitude than ΔH and $T\Delta S$ (Figure 2b) because the solvent reorganization enthalpy, contributing to the latter two quantities, is negative (Figure 2d). The SSM is stabilized by the solute–solvent enthalpy.

Solute–solvent enthalpic stabilization of the SSM in aqueous urea solution is caused by preferential interactions between urea and the aliphatic solutes. This preferential solvation phenomenon is driven by urea–hydrocarbon van der Waals interactions⁸ and stabilizes the SSM because solute-intermediate urea molecules interact with two solutes at the same time (see Figure 2 in ref 9). The solute–solvent entropic stabilization of the CM can be understood better after considering the statistical mechanical expression for the solute–solvent entropy upon solute solvation^{5,22}

$$\Delta S_{UV} = k_B \ln P_{\text{ins}} - k_B [(\langle \psi^2 \rangle - \langle \psi \rangle^2) \beta^2 / 2 + \dots]_a \quad (3)$$

In eq 3, k_B is the Boltzmann constant and $\beta = 1/k_B T$. The quantity P_{ins} is the probability that a randomly inserted solute molecule (or a solute pair separated at a fixed distance r) into a system of only solvent molecules will experience an attractive or zero interaction energy ($\psi \leq 0$). Thus, $k_B \ln P_{\text{ins}}$ may be viewed as a solute–solvent excluded volume contribution to the entropy. The second term on the right-hand side of eq 3 describes an additional entropy penalty related to how strongly attractive solute–solvent interactions ($\psi < 0$; denoted by subscript a) bias the solvation shell composition.⁵ In the (hypothetical) case that *neo*-pentane–urea dispersion interactions are of the same magnitude as *neo*-pentane–water dispersion interactions, the fluctuations ($\langle \psi^2 \rangle - \langle \psi \rangle^2$) of the solute–solvent interaction energy ψ are small and solute–solvent attractive interactions put little bias on the solvation shell composition. Favorable *neo*-pentane–urea attractive interactions do however cause a strong bias on sampling configurations with excess local urea concentrations (see Figure 2 in ref 9). This biasing process leads to a selected, nonrandom solvation shell structure and a lowering of entropy which is stronger in the SSM than the CM. The solute–solvent entropy (Figure 2f) therefore stabilizes the CM relative to the SSM as well as larger solute–solute separations. On approaching the CM, the solute–solvent enthalpy increases (Figure 2f) because favorable *neo*-pentane–urea van der Waals interactions are lost. Parts g and h of Figure 2 show the contributions of the two terms on the right-hand

side of eq 3 to the solute–solvent entropy in water and urea solution, respectively. The contribution $k_B T \ln P_{\text{ins}}$ is very similar in both systems, whereas the second contribution indeed contributes positively to the solute–solvent entropy in passing from the SSM to the CM in aqueous urea solution.

Conclusion

In summary, we have shown that a solute–solvent and solvent–solvent decomposition of enthalpies and entropies of solute association in solution provides (i) an improved atomic-scale picture of the contributions of solute–solvent interactions and solvent reorganization processes to the observed enthalpies and entropies and (ii) an improved atomic-scale picture of molecular-scale driving forces for solute–solute association. The formalism expressed in eqs 1 and 2 has been used in this letter to provide an explanation for our recent observation that neopentane pairs associate more strongly in concentrated urea solution than in water at room temperature.⁹ More generally, we anticipate that the formalism will be of benefit also in studies of, for example, the binding of a ligand to a receptor as well as protein aggregation and folding.

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