Investigation of the Anomalous Solvation Free Energies of Amides and Amines: FEP Calculations in Cyclohexane and PS-GVB Calculations on Amide−Water Complexes

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Free energy perturbation (FEP) calculations of acetamide, *N*-methylacetamide, *N*,*N*-dimethylacetamide, ammonia, and methylamine in cyclohexane were performed to help rationalize their counterintuitive experimental free energies of solvation in water (i.e., *N*-methylacetamide is more favorably solvated than acetamide, and methylamine is more favorably solvated than ammonia). Analogously to the aqueous FEP calculations in water carried out previously, the calculations in cyclohexane find an approximately additive methyl substitution effect for both the amides and amines, with, in this case, the most highly methylated molecule most favorably solvated in cyclohexane. PS-GVB calculations at the LMP2/cc-pVDZ level were also performed on the amides and suggest that their anomalous experimental aqueous solvation free energies could be due to differences in their hydrated gas-phase structures.

Introduction

Over the last 15 years accurate force field methods have been developed to determine the absolute and relative free energies of solvation of small organic and biological molecules.[1,2] Specifically, using free energy perturbation (FEP) methods, chemists have primarily focused on examining the aqueous solvation of polar solutes as models for biological systems. For example, the aqueous solvation of *N*-methylacetamide has been studied as a model for peptide bonds.[3] More recently, the difficult task of accurately simulating the solvation free energies of hydrocarbons in water has been accomplished.[4] Such simulations of nonpolar solutes increase our understanding of molar volume effects on the free energy of transfer between solvents and model the aqueous solvation of alanine and valine in peptides and proteins.

Additionally, the computational determination of the free energies of solvation of small solutes in nonaqueous solvents have been used to examine the uniqueness of water solvation. For example, Rao and Singh's FEP studies of ionic and neutral species in methanol, dimethyl sulfoxide, hydrazine, and carbon tetrachloride points to a "special structural effect", rather than the small size of water, as the primary reason for its unique mode of solvation.[5] The development of nonaqueous solvent systems for FEP calculations is also important for direct comparison with experimentally determined nonaqueous free energies of solvation and molar distribution constants of solutes in aqueous/nonaqueous systems. Specifically, the distribution constants of solutes in aqueous/alkane systems have been used to conveniently determine the absolute free energies of solvation of biologically relevant solutes in water.[6,7] Here, on the basis of the assumption that alkanes do not interact significantly with solute, determination of the free energy of transfer of a solute from alkane into water is presumed equivalent to the absolute free energy of solvation of that solute in water. Alkane solvents have also been used to experimentally model the interaction between potential pharmaceuticals and lipid bilayers and to examine the hydrophobic interactions of proteins.[8,9] In this context, Wolfenden's examination of the cyclohexane/water partition coefficient of acetamide as a model for asparagine's R-group hydrophobicity is particularly notable.[10]

In total, the results of the experimental determinations of the solvation free energies of biologically relevant amides finds that the aqueous free energies of solvation of acetamide (ACT), *N*-methylacetamide (NMA), and *N*,*N*-dimethylacetamide (NND-MA) are nonadditive.[11] Thus, in contrast to their expected relative hydrophobicities, *N*-methylacetamide is more soluble in water than either acetamide or *N*,*N*-dimethylacetamide. Intriguingly, on the basis of their aqueous/alkane distribution constants, the aqueous free energy of solvation of methylamine was also found to be greater than those of ammonia and dimethylamine.[6,7] Because amines and amides are both nitrogen, containing functional groups, it is tempting to attribute their coincident anomalous aqueous solvation energies to analogous effects of methyl substitution on their ability to hydrogen bond with solvent water. In actuality, however, amines hydrogen bond to water via their nitrogen lone pair electrons, while for amides the principle hydrogen bonds to water occur at the carbonyl oxygen and/or their nitrogen hydrogens. These differential modes of amine and amide hydrogen bonding imply that the effect of substituting a methyl for a hydrogen on nitrogen would alter their hydrogen bonding, and thus their solvation in water, via distinctly different structural mechanisms. Indeed, to date the anomalous orderings for the amines have been attributed to the inductive effects of the alkyl groups on the nitrogen lone pair,[7,12] while for the amides substitution of a methyl for a hydrogen alters the number of hydrogen-bonding sites.

The importance of amides and amines in biochemical systems and the challenge of understanding the counterintuitive solvation orderings described above have lead to numerous calculations of the free energies of solvation of amines and amides in water.[12−15] Provocatively, none of these computational studies

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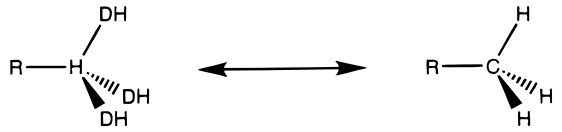

1 **2**

Figure 1. R−H to R−CH_3 perturbation. In structure **1** the hydrogen is connected to three dummy hydrogen atoms (DH) to maintain the same topology throughout the simulation. The H and DH's are slowly mutated into a CH_3 group to yield structure **2**.

have reproduced the experimental trends for either the amines or amides. Most recently, force field calculations including polarization[16,17] and dynamic fluctuating charges,[18] which more accurately describe solvent/solute interactions, were used in an attempt to replicate the experimental nonadditivity. These modified force fields come closer to reproducing the experimental free energies of solvation of amides and amines, yet still do not predict the observed anomalous order for the free energies of solvation.

In this paper, on the basis of the important role that alkanes have played in the experimental determination of aqueous amine and amide solvation, we have extended the examination of the anomalous ordering of the amide and amine aqueous solvation energies (i.e., acetamide, *trans-N*-methylacetamide, *N*,*N*-dimethylacetamide, ammonia, and *N*-methylamine) by computing their solvation free energies in a periodic box of an all-atom model of cyclohexane created with AMBER 4.1[19] for this study. Although little experimental data for the free energies of solvation of these species in alkanes is available, we found, in keeping with earlier aqueous solvation calculations, an additive methyl substitution effect for both the amides and amines in cyclohexane.

To further probe the counterintuitive experimentally observed aqueous solvation ordering for the amides, we also computed water−amide binding energies for acetamide, *cis-N*-methylacetamide, and *trans-N*-methylacetamide using PS-GVB at the LMP2/cc-pVDZ level.[20] These calculations suggest that acetamide's ability to form a tightly bound-bridged hydrogen bond to a single water molecule in the gas phase, as compared to *trans-N*-methylacetamide's significantly less stable gas-phase monohydrate structure, could account for the anomalous aqueous solvation orderings found experimentally.

Computational Methods

All molecular dynamics (MD) and free energy perturbation (FEP) calculations were performed using the AMBER 4.1 suite of programs.[19] Molecular mechanics calculations were carried out using either the SANDER module of AMBER 4.1 or BIOGRAF using the DREIDING force field.[21] Quantum mechanical calculations were carried out using Gaussian 92[22] or PS-GVB.[20] A HP-735 or Silicon Graphics Indy workstation was used for all of the calculations.

The AMBER 4.1 force field[23] and FEP thermodynamics windows (window growth) methods used in this work to determined the free energies of solvation have been described in detail previously.[24] The FEP method was implemented in the GIBBS module of AMBER 4.1. In this study the perturbations performed to determine the relative free energies of solvation of the amines and amides in cyclohexane involved mutation of a hydrogen with three dummy hydrogens into a methyl group or the reverse transformation (Figure 1). Absolute solvation free energies in cyclohexane were obtained by mutating a complete molecule into nothing (i.e., complete dummy atoms).

The geometry, charges, and force field parameters of all of the solutes employed in this study were reported earlier by Morgantini and Kollman.[12] The starting geometries for the cyclohexane solvent molecules where obtained via quantum mechanical optimization at the 6-31G* basis set level. The RESP charges were determined to be C = 0.016 and H = −0.008 and are in agreement with the charges used by Jorgensen for his all-atom nonpolarizable OPLS cyclohexane.[25] Using the EDIT and PARM modules of AMBER 4.1 a ∼44.8 × 44.7 × 42.6 Å periodic box of 216 cyclohexane molecules was created. The RESP charged solvent box was minimized at constant volume using 100 cycles of the steepest descent and then 400 cycles of the conjugate gradient method. This system was then equilibrated at constant volume at 298 K with a 1 fs time step for 3 ps and at constant pressure for 150 ps to yield a RESP charged cyclohexane box, with a density of 0.75 g/mL. For the minimization/equilibration of the cyclohexane box the nonbonded pair list was updated every 10 fs (NSNB = 10 fs) with SHAKE[26] on bonds to hydrogen (NTC = 2), a nonbonded cutoff of 12.0 Å (CUT = 12), and Berendsen temperature coupling[27] (NTT = 1). For the constant pressure equilibrations isotropic position (NTP = 1), molecule (NPSCAL = 1) scaling and a compressibility of 114 × 10^{-6} bar (COMP = 114) was applied. The enthalpy of vaporization of the cyclohexane solvent box was determined by performing a 10 ps simulation on this system with a nonbonded cutoff of 0.01 Å and nonbonded pair list update of 100 ps to determine the average intramolecular interactions. This yielded an enthalpy of vaporization ($\Delta H_{vap} = (E_{total} - E_{intra} + nRT)$/no. of molecules) of 7.85 kcal/mol at 298 K (exptl = 7.83 kcal/mol).

For all of the amide and amine FEP calculations in cyclohexane, the solute was placed in the center of a cubic MD equilibrated RESP charged cyclohexane box. These solute/solvent systems (amide/cyclohexane = 212 molecules; amine/cyclohexane = 215 molecules) were minimized for 100 cycles of steepest descent and then 400 cycles of conjugate gradient and then equilibrated in GIBBS at constant volume for 10 ps at 100 K, 10 ps at 200 K, and 10 ps at 298 K with a 2 fs time step and at constant pressure for 100 ps at 298 K with a 1 fs time step. NSNB = 10, NSB = 2, CUT = 12, NTT = 1 was applied throughout the equilibration procedure. The constant pressure equilibrations were performed with NTP = 1 and NPSCAL = 1.

All of the FEP calculations were carried out using the standard window growth methods with 101 windows ($\Delta\lambda = 0.01$).[24] For each window 1000 equilibration steps and 1000 collection steps were performed and a CUT = 12, NSNB = 10, NTC = 2, and bond-PMF correction[28] (NCORC = 1) was applied. All of the absolute free energy calculations (i.e., solute disappeared into nothing) were performed with electrostatic decoupling. For these decoupled simulations, the electrostatic run was performed with a 2 fs time step in only one direction and the error for the electrostatics was estimated from double wide sampling; the nonbond (VDW's) runs were performed with a 1 fs time step forward ($\lambda = 1$ to 0) and backward ($\lambda = 0$ to 1), and the errors correspond to the observed hysteresis. For all of the relative free energy calculations (i.e., solute A into solute B), the FEP calculations were performed as regular runs with a 2 fs time step without electrostatic decoupling. All of the regular runs were performed forward ($\lambda = 1$ to 0) and backward ($\lambda = 0$ to 1), and the error corresponds to the observed hysteresis.

For the gas-phase quantum mechanical calculations, aceta-

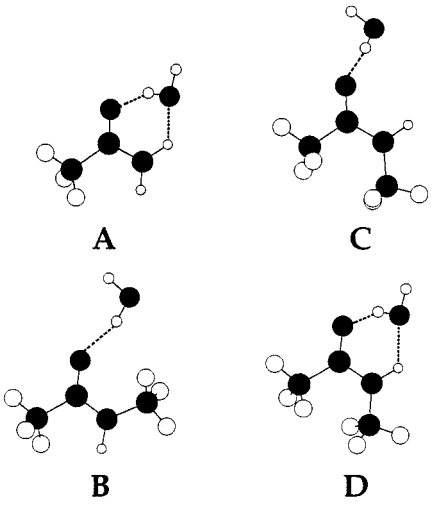

Figure 2. Hydrogen-bonded structures of doubly bound acetamide hydrate (**A**), singly bound *trans*-*N*-methylacetamide hydrate (**B**), singly bound *cis*-*N*-methylacetamide hydrate (**C**), and doubly bound *cis*-*N*-methylacetamide hydrate (**D**).

mide, *cis*-*N*-methylacetamide, and *trans*-*N*-methylacetamide and their water complexes (Figure 2) were model built using BIOGRAF and minimized using the DRIEDING force field.[21] Using PS-GVB all of the systems were fully geometry optimized at the HF/6-31G* basis set level.[20] To determine the water−amide binding energies single-point LMP2/cc-pVDZ with only the valance electrons correlated and counterpoise corrections were done. The binding energies are reported in terms of $\Delta E_{\text{electronic}}$ at 0 K.

Results and Discussion

As described in the computational methods, a periodic box of cyclohexane with a density = 0.75 g/mL (0.7781 g/mL exptl) and $\Delta H_{\text{vap}} = 7.85$ kcal/mol at 298 K (7.83 kcal/mol exptl) was created using RESP charges and the standard van der Waals parameters for carbon and hydrogen.[4] The accuracies of the computed density and enthalpy of this cyclohexane box are evident from comparison with the experimental values and with the OPLS-AA cyclohexane model.[25] Such accuracy is not surprising since the AMBER 4.1 hydrocarbon force field parameters used for the cyclohexane system were developed to reproduce liquid methane, propane, and butane.[4] In addition, as found for the previously developed liquid hydrocarbon models, there is a minimal electrostatic contribution and relatively large van der Waals contribution to the intra- and intermolecular interaction energies of the cyclohexane model developed for this work.

With this cyclohexane model, we determined the relative free energies of solvation of the homologous series of amides: acetamide (ACT), *trans*-*N*-methylacetamide (NMA), and *N*,*N*-dimethylacetamide (NNDMA). We also calculated the absolute free energy of solvation of NMA in cyclohexane. The results are presented in Tables 1 and 2. Using the same amide partial charges as Morgantini and Kollman,[12] we found that the relative free energies of solvation in cyclohexane follow the expected trends with acetamide the least and NNDMA the most favorably solvated. The addition of each methyl group increases the dispersion attraction in the cyclohexane liquid and, thus, increases its free energy of vaporization. On the other hand, aqueous solvation FEP studies on the same series of amides found a consistent decrease in free energy of vaporization because of an increase in hydrophobicity with successive methylations.

TABLE 1: Relative Free Energies of Solvation of Amides and Amines (kcal/mol)

perturbation	$\Delta\Delta G_{\text{cyc}}$	$\Delta\Delta G_{\text{aq}}$[a]	exptl$_{\text{aq}}$	exptl$_{\text{benz}}$
ACT to NMA	-1.34 ± 0.13	2.09 ± 0.11	-0.40[b]	
NMA to NNDMA	-1.29 ± 0.03	1.05 ± 0.02	1.53[b]	
NH_3 to $MeNH_2$	-1.68 ± 0.14	0.62 ± 0.05	-0.26[c]	
$MeNH_2$ to Me_2NH		1.62 ± 0.01	0.27[c]	-0.70[c]
Me_2NH to Me_3N		2.34 ± 0.02	1.06[c]	-0.98[c]

[a] Aqueous data from ref 12. [b] Experimental data in water from ref 11. [c] Experimental data in water and benzene from refs 6 and 7.

TABLE 2: Absolute Free Energies of Solvation of Amides and Amines (kcal/mol)

perturbation	electrostatic[a]	van der Waals	ΔG_{solv}	ΔG_{exptl}
		Cyclohexane		
ACT to nothing			4.06[b]	3.04[c]
NMA to nothing	0.004 ± 0.001	5.4 ± 0.49	5.40 ± 0.49	
NNDMA to nothing			6.69[b]	
NH3 to nothing	0.002 ± 0.000	0.10 ± 0.38	0.10 ± 0.38	0.33[d]
MeNH2 to nothing			1.78[b]	2.45[e]
		Aqueous		
ACT to nothing			11.79[b]	
NMA to nothing			9.6 ± 0.2	10.1[f]
NNDMA to nothing			8.55[b]	
NH_3 to nothing	6.14 ± 0.02	-1.98 ± 0.09	4.16 ± 0.11	4.31[f]
MeNH2 to nothing			3.57	4.57[f]

[a] Calculated in only one direction with error estimated from double wide sampling. [b] Determined from relative and absolute solvation free energies. [c] Experimental data from ref 10. [d] Experimental data determined in hexadecane from ref 37. [e] Experimental data in benzene from ref 7. [f] Experimental values from refs 6 and 11.

Unfortunately, to date there is only limited data for direct comparison of our results with experiment. For the amides, only the free energy for the solvation of acetamide in cyclohexane is available.[10] As reported by Wolfenden, the ΔG of transfer of acetamide from vapor phase to water was determined directly from partial pressures[11] and the ΔG of transfer from cyclohexane to water was determined from the distribution constant of acetamide between the two phases using the expression $\Delta Gt = -RT \ln K_{\text{d}}$.[10] With these data Radzicka et al. determined the free energy of solvation of acetamide in cyclohexane to be -3.04 kcal/mol.[10] In comparison, on the basis of our FEP calculation for NMA to nothing (5.40 ± 0.40 kcal/mol) and for ACT to NMA (-1.34 ± 0.13 kcal/mol), we find an absolute free energy of solvation of ACT in cyclohexane of -4.06 kcal/mol, while the Sitkoff et al. continuum solvation model yielded a value of -3.25 kcal/mol.[29]

The results from the decoupled calculation for the absolute solvation free energy of NMA in cyclohexane are presented in Table 2. Here the van der Waals contribution is much larger than the electrostatic contribution to the ΔG of solvation. This contrasts with the continuum solvation results of Sitkoff et al., which find comparable electrostatic and nonpolar contributions to the overall free energy of solvation of acetamide in cyclohexane.[29] In addition, this larger VDW contribution to the absolute free energy of solvation of NMA in cyclohexane is opposite to the analogous perturbation in water,[30] where the ΔG for the perturbation of NMA with ESP charges into nothing (10.4 ± 0.2 kcal/mol) is dominated by the electrostatic contribution of 11.23 ± 0.01 kcal/mol. To our knowledge the experimental free energies of solvation of *N*-methylacetamide and *N*,*N*-dimethylacetamide in cyclohexane are not available. Nevertheless, our free energies are analogous to the calculated solvation free energies for the same species in water, which found that substitution of a hydrogen for a methyl leads to a more hydrophobic product.[12]

TABLE 3: Electronic Association Energies $\Delta E°$(kcal/mol) for Amide−Water Complexes

	LMP2/cc-pVDZ			B3LYP/6-311++G(d,p)[d]		MP2/aug-cc-pVDZ[e]	
complex	CPC[a]	BSSE[b]	HF/6-31g*[c]	CPC[a]	BSSE[b]	CPC[a]	BSSE[b]
ACET-H20 (**A**)	−9.8	−12.6					
t-NMA-H20 (**B**)	−6.2	−8.7	−7.3 (−9.8)	−7.2 (−6.5)	−7.7 (−7.0)	−7.0	−8.2
c-NMA-H20 (**C**)	−6.6	−9.0		−7.5 (−6.8)	−7.8 (−7.2)	−7.0	−8.0
c-NMA-H20 (**D**)	−8.7	−11.8		−9.7 (−9.1)	−10.2 (−9.6)	−8.9	−9.9

[a] Interaction energy with HF counterpoise correction. [b] Interaction energy with basis set superposition energy. [c] Reference 34, number in parentheses is from HF/6-31g calculation. [d] Reference 32, numbers in parentheses are from BLYP/6-311++G(d,p) calculation. [e] Reference 31.

To further probe Wolfenden's[11] anomalous experimental ordering for the aqueous free energy of solvation of acetamide and *trans*-*N*-methylacetamide, we examined their gas-phase structures. Specifically, under Wolfenden's experimental conditions,[11] we hypothesized that acetamide and *trans*-NMA might exist as hydrates rather than as monomeric species. On the basis of this assumption, the solvation free energies of acetamide and *trans*-NMA in water could ultimately be accounted for by the difference in their gas-phase monohydrate binding energies. Thus, if the monohydrate of *trans*-NMA is significantly destabilized in the gas phase relative to the monohydrate of acetamide, the observed free energy of solvation of NMA relative to acetamide in water could appear to be more favorable. Previous quantum mechanical calculations have found that, as shown in Figure 2, *cis*-*N*-methylacetamide's lowest energy monohydrate (**D**) has a strongly bound water bridge between its carbonyl oxygen and amino hydrogen, while *trans*-NMA's lowest energy hydrate is the singly hydrogen-bound structure **B**.[31−34] On the basis of these previously calculated structures, we determined at the LMP2/cc-pVDZ level that the binding energies of the monohydrates of acetamide (**A**) and *trans*-NMA (**B**) are −9.8 and −6.2 kcal/mol, respectively (Table 3). We also found that the amide hydrates of acetamide and NMA form significantly more stable hydrates than water dimer ($\Delta E° = -4.9$ kcal/mol), again supporting the possibility that the amide hydrates, rather than the uncomplexed amides, exist in the gas phase under Wolfenden's[11] experimental conditions. On the basis of our calculations, the binding energy of acetamide hydrate is 3.6 kcal/mol stronger than the binding energy of *trans*-NMA hydrate, suggesting that the experimental free energies of solvation of acetamide and *trans*-NMA may be based on a determination of the relative solvation free energy of acetamide hydrate and *trans*-NMA hydrate in water and not the uncomplexed amides.

Assuming that the amide hydrate of acetamide, rather than the uncomplexed amide, exists under Wolfenden's experimental conditions, we calculated the vapor pressure of the complex over a 0.1 M aqueous solution from $\Delta G = -RT \ln Kd$, where $K_d = M(\text{vap})/M(\text{aq})$ at 298 K.[11] Thus, using the NMODE module of AMBER 4.1, the ΔG of formation at 298 K of the complex in the gas phase is −1.3 kcal/mol ($\Delta H_{298} = -10.8$ kcal/mol, based on $\Delta E° = -9.8$ kcal/mol, thermal energy correction = 3.4 kcal/mol, and ZPE correction of 3.0 kcal/mol; $T\Delta S = -9.5$ kcal/mol). On the basis of this ΔG, the vapor-phase pressure of the complex over a 0.1 M solution of acetamide in water at 298 K is 4.2×10^{-8} atm (22% complex) if we assume Wolfenden's[11] experimental K_d of 7.6×10^{-8} and ideal conditions. We also investigated the impact of the accuracy of our binding energies on our vapor pressure calculations. Here if we assume a substantial underestimation in our calculated free energy of amide−water binding a vapor pressure of 1.6×10^{-7} atm (assuming $\Delta G = -3$ kcal/mol) or 1.9×10^{-7} atm (assuming $\Delta G = -5$ kcal/mol) is found. Here the percentage of complex in the vapor phase would be 84% and 100% respectively. In total, these calculations yield vapor pressures for the amide−water complex consistent with the experimental vapor pressure reported by Wolfenden for what is assumed to be acetamide monomer.[11] From these calculations it is apparent that these vapor-phase experiments are not suitable for differentiating which vapor phase species (acetamide or acetamide−water) is present. Further, these results suggest that a spectroscopic study to determine the structure of acetamide over water could lead to a greater understanding of the aqueous solvation of acetamide.

TABLE 4: AMBER 4.1 and ab Initio Amide−Water Association Energies $\Delta E°$ (kcal/mol)

complex	AMBER 4.1	ab initio
trans-NMA (**B**) (CO···H_2O)	−7.7	−6.2[a] (−7.3)[b]
trans-NMA (NH···OH_2)	−6.6	−4.9[a] (−5.4)[b]
ACET (CO··· H_2O)	−8.0	
ACET (NH···OH_2)	−6.9	−5.4[a]
ACET (**A**) (CO···H_2O and NH···OH_2)	−10.6	−9.8[a]

[a] LMP2/cc-pVDZ interaction energy with HF counterpoise correction. [b] Ab initio data from ref 3.

To determine whether the inconsistencies between the experimental and calculated relative free energies of solvation of the amides was due to inaccurate representation of amide−water hydrogen-bonding energies by the Cornell et al. force field,[23] a comparison between the ab initio and AMBER 4.1 binding energies of acetamide and *trans*-NMA was made (Table 4). Here, as has been shown previously for *trans*-NMA,[35] we found that although the Cornell et al. force field does overestimate the binding energies of all of the possible amide monohydrate structures by approximately 1 kcal/mol, the relative stabilities of the possible ACET and *trans*-NMA hydrogen bonds are consistent with ab initio calculations (Table 4). Thus, the bridged hydrogen bond of ACET−water is 2.5 kcal/mol more stable than the CO···H_2O hydrogen bond and 3.7 kcal/mol more stable that the NH···OH_2 hydrogen bond. On the basis of these relative binding energies, we also believe that more accurate representation of nitrogen (polarization) in the Cornell et al. force field would not enhance our understanding of the anomalous order for aqueous amide solvation. This contrasts with the results of a similar study by Marten et al., which concluded that overestimation of NH···OH_2 binding by the force field might lead to the discrepancy between the experimental and AMBER calculated amine solvation free energies.[36]

To probe the experimental nonadditivity observed for the aqueous solvation free energies of ammonia and methylamine, we calculated the relative free energy of solvation of ammonia and methylamine in cyclohexane. In addition, we determined the absolute free energy of solvation of ammonia in cyclohexane. The results are presented in Tables 1 and 2. As for the amide series, the substitution of an ammonia hydrogen with a methyl group leads to significant increase in the solubility of the molecule in cyclohexane. In addition, because the perturbations in cyclohexane for the amides and amines are dominated by van der Waals interactions, the relative FEP's of acetamide into *N*-methylacetamide and ammonia into *N*-methylamine in cy-

clohexane are similar (−1.34 vs −1.68 kcal/mol). Not surprisingly, this contrasts with the aqueous study, where owing to their differential modes of hydrogen bonding, the relative free energies associated with substitution of a hydrogen for a methyl group on acetamide as compared to ammonia are quite different (2.09 vs 0.62 kcal/mol).[12]

As for the amides, there is only limited data for comparison of our amine solvation free energies with experiment. Jones and Arnett reported the free energies of transfer of methylamine, dimethylamine, and trimethylamine from benzene, toluene, and xylene to water.[7] These free energies of transfer were determined indirectly from the partition coefficients of these solutes between the aromatic solvent and water and have been corrected for partial ionization effects but not for amine hydrate formation in the hydrocarbon phase. These data, along with Ben-Naim and Marcus'[6] aqueous absolute solvation free energy for methylamine, yield an absolute free energy of solvation of methylamine in benzene of −2.45 kcal/mol. This free energy presumably overestimates the actual free energy of solvation of methylamine in benzene but does reveal qualitatively that methylamine is favorably solvated by aromatic hydrocarbons.

Similarly, the experimental relative free energies of solvation of (a) methylamine and dimethylamine and (b) dimethylamine and trimethylamine in benzene can be derived using this same uncorrected data.[6,7] Here we find that dimethylamine is more favorably solvated than methylamine by −0.7 kcal/mol and trimethylamine is more favorably solvated than dimethylamine by −0.98 kcal/mol. Again this relative order of solubility in benzene indicates an increase in hydrophobicity with each methyl addition. It should be noted, however, that in contrast to the aqueous solvation energies,[12] the difference in energy between addition of a methyl to methylamine and addition of a methyl to dimethylamine (0.27 vs 1.06 kcal/mol aqueous) is quite small (−0.7 vs. −0.98 kcal/mol). Thus, unlike in water, each successive methylation in benzene appears to stabilize the solvation by approximately the same amount.

This same trend was found for the successive methylation of the amides in cyclohexane computationally (Table 1) and indicates that the first and second methyl additions are stabilized by equivalent van der Waals interactions. This contrasts with the aqueous solvation of the amides[12] where addition of the second methyl substituent is presumed to occur in a solvent that has already been disordered by the first methylation. Specifically, the first methylation disrupts both the hydrogen bonding of the solute with water and the organized structure of the water molecules around the solute, while, as indicated by the smaller $\Delta\Delta G$, the second methylation does not disrupt the solvent as much. In cyclohexane the nonelectrostatic nature of the alkane leads to a solvent model that is dominated by van der Waals rather than electrostatic hydrogen-bonding effects. Thus, addition of each methyl substituent has similar van der Waals stabilization.

Recently, the calculated PM3-SM4 free energy of solvation of ammonia in cyclohexane was reported to be −1.07 kcal/mol[37] in comparison with our calculated value of -0.1 ± 0.38 kcal/mol (exptl (hexadecane) = −0.33).[38−40] Unlike our relatively simple all-atom force field model for cyclohexane, the PM3-SM4 model[37] includes explicit terms for solute polarization and surface tension terms to account for first-solvation-shell and solvent-ordering effects. Our value was determined from a electrostatically decoupled FEP calculation without explicit polarization of the solute. Presumably, as indicated by Jorgensen's[25] results on the free energy of solvation of water in cyclohexane, polarization would have brought our results further in line with the experimental free energy of solvation of ammonia in alkane solvent. As shown in Table 2 the van der Waals contribution to the overall ΔG of ammonia in cyclohexane is dominant; this contrasts with the dominant electrostatic effects for the same perturbation in TIP3P water.[12]

TABLE 5: Relative and Absolute Log *P* Values of Amides and Amines in Cyclohexane/Water

solute(s)	$\Delta\log P^a$	$\log P^b$	$\log P_{\text{exptl}}$
ACT/NMA	2.5		
NMA/NNDMA	1.7		
ACT		−5.7 (−6.7)	−4.88[c]
NMA		−3.1 (−3.8)	
NNDMA		−1.4 (−1.9)	
$NH_3/MeNH_2$	1.7		
NH3		−3.0 (−3.3)	−2.88[d]
$MeNH_2$		−1.3 (−1.5)	−2.04[e]

[a] Determined from $\Delta\log P = \log P_B - \log P_A = [\Delta G_{AB(H_2O)} - \Delta G_{AB(cyc)}]/2.3RT$. [b] Determined from ΔG of $A = \Delta G_{(H_2O)} - \Delta G_{(cyc)} = -2.3RT \log P$. The polarization corrected values shown in parentheses were determined from the 0.86 kcal/mol polarization correction reported in ref 25 adjusted by μ^2/R^3, where μ = solute dipole moment (ref 43) and R = cavity radius approximated from determination of solute molar volume (ref 44). [c] From ref 10. [d] From ref 38 in hexadecane. [e] From ref 39 in hexadecane.

We have also determined the relative free energy of solvation of ammonia and methylamine without electrostatic decoupling. From our FEP calculations for ammonia to nothing and for ammonia to methylamine the absolute ΔG for methylamine was determined to be −1.78 kcal/mol as compared to the −2.45 kcal/mol determined experimentally for the absolute free energy of solvation of methylamine in benzene[6,7] and in hexadecane (exptl hexadecane $\Delta G = -2.8$ kcal/mol).[38]

Finally, as shown in Table 5, using our computed amide and amine free energies of solvation and Morgantini and Kollman's aqueous FEP calculations, the relative and absolute partition coefficients of these solutes between cyclohexane and water can be determined for direct comparison with experimentally determined partition coefficients.[41] As shown in Table 5 some of these partition coefficients can be compared directly with experimental values and appear to be quite accurate. For the amines the experimental values are determined in hexadecane, rather than cyclohexane, and as noted by Abraham this can lead to "small but significant differences between water−alkane and water−cyclohexane partition coefficients" owing to the greater packing ability of cycloalkanes as compared to *n*-alkanes as indicated by their relative densities.[39,40] In addition, it is apparent that, on the basis of Jorgensen et al.'s determination that solvent polarization should be taken into account when the free energy of solvation of polar solutes in apolar solvents are calculated and the enhanced accuracy reported by Meng et al. when amine polarization was included in their aqueous FEP study,[16] it is apparent that polarization corrections to our amine log *P* values should be included. Thus, on the basis of Jorgensen et al.'s 0.86 kcal/mol simple solvent polarization correction for the solvation free energy of water in cyclohexane,[25] and assuming that this correction is proportional to μ^2/R^3 (μ = dipole moment, R = cavity radius), we determined corrected log *P* values for all of the solutes (Table 5). As shown in Table 5 this polarization correction slightly improves the agreement with experiment for ammonia and slightly worsens it for methylamine but significantly worsens it for acetamide.

Conclusion

In this study the counterintuitive experimental finding that *trans*-*N*-methylacetamide is more favorably solvated in water

than acetamide, and methylamine more favorably solvated than ammonia, was examined computationally. In particular this study sought to investigate the following previously overlooked aspects of the experimental methods: first, the impact of the alkane solvent on the hydrocarbon/water partition coefficient used experimentally to determine the aqueous solvation free energies of the amines and, second, the possibility that determination of the absolute free energies of the amides through careful measurement of their gas-phase concentrations over aqueous solutions was based on determination of the concentration of stable amide−water complexes, rather than the uncomplexed amides.

Specifically, we have presented computational evidence to show that the methyl substituent effect (N−H to N−CH_3) leads to a uniform increase in solubility in cyclohexane consistent with one's expectation of an increased dispersion attraction and with available experimental data in nonpolar solvents. This nearly additive methyl effect in cyclohexane is in contrast to the conflict between theory and experiment for the N−H to N−CH_3 solvation in aqueous solution, where theory finds a decrease in solubility upon methyl substitution and experiment finds an irregular trend.

As is apparent from the FEP calculations, the calculated free energies of solvation of the amides in cyclohexane do not help to explain their anomalous experimental solvation orderings in water. However, theoretical examination of gas-phase amide−water complexes of acetamide and *trans*-*N*-methylacetamide, which might have gone undetected under the experimental conditions, did lead to computational results allied with the counterintuitive ordering of the amides solvation energies in water. Specifically, we have carried out quantum mechanical calculations on gas-phase amide hydrates shown in Figure 2. These calculations support our hypothesis that, in the case of the relative solvation of acetamide and *N*-methylacetamide, *N*-methylacetamide (NMA) appears more soluble in water because it is not as "stabilized" in the gas phase (in its more favorable trans-conformation) as acetamide. We suggest that this is because acetamide (and *cis*-NMA) can form a double (bridged) hydrogen bond, while *trans*-NMA cannot.

In addition, it is clear that acetamide could form a doubly bridged hydrogen-bonded dimer, whereas *trans*-NMA cannot. Formation of this dimer could also explain the discrepancy between calculation and experiment, since the dimer, like the water complex, would lead to a stabilization of acetamide over *trans*-NMA in the gas phase and, thus, change the relative gas phase/aqueous partition coefficient for acetamide and *trans*-NMA. However, at present we do not have a satisfactory explanation for why this discrepancy is not seen in analogous calculations of the relative solvation free energies of acetic acid and methyl acetate. Specifically, Carlson et al. calculated aqueous solvation free energies for acetic acid and methyl acetate of −8.5 and −5.3, respectively.[2] These values, unlike those found for acetamide and *trans*-NMA, are shifted, but in good relative agreement with the experiment (−6.7 and −3.3).[42] Thus, as we found for the analogous amide structures, Carlson et al. found acetic acid's dimer or gas-phase hydrate to be much more stable than methyl acetate's hydrogen-bonded complexes. We also determined the hydrogen-bond energy of the doubly bound hydrate of the more stable *cis*-conformation of acetic acid (analogous to Figure 2a) to be −9.2 kcal/mol (CPC, LMP2/cc-pVDZ), which is similar to the −9.8 kcal/mol found for acetamide.

At this point we do not understand why acetic acid/methyl acetate give consistent theoretical and experimental results while acetamide and *trans*-NMA do not. Perhaps this difference lies in variances in the experimental protocols leading to more dimer/hydrate formation for the amides. Alternatively, differences in the force fields and/or simulation protocols might account for the discrepancy between the acid/ester and amides. However, on the basis of the fact that the calculated free energy for N−H to N−CH_3 of 2.2 kcal/mol is,[12] as one would expect, smaller than that for O−H to O−CH_3 of 3.2 kcal/mol,[2] it does not seem likely that such variances in simulation methods could account for the discrepancy. The large difference between the calculated and experimental acetamide partition coefficients is also notable and could possibly be explained by hydrate formation or dimerization of acetamide in cyclohexane. On the basis of this hypothesis, and the fact that dimerization/hydrate formation in cyclohexane is less likely for *trans*-NMA and NNDMA, our calculated free energies for NMA and NNDMA would be expected to be closer to their experimentally measured solvation energies in cyclohexane than acetamide.

In contrast to the amides, where, as noted, our hypothesis is that the interpretation of the experimental gas-phase data may have been incomplete, the amines are probably an example where theoretical molecular mechanical models, even with polarization included, are too limited. Recently, Marten et al. have presented high-level ab initio calculations on amine−water complexes that show a nonadditive effect, and, on the basis of their basicity, one would expect proton-accepting amines to include a larger contribution from charge transfer than any other neutral hydrogen-bonding functional group.[36] To correctly describe this charge-transfer contribution is a challenge for future modeling efforts.

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