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Solvation Free Energies of Small Amides and Amines from Molecular Dynamics/Free Energy Perturbation Simulations Using Pairwise Additive and Many-Body Polarizable Potentials

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Molecular dynamics/free energy perturbation simulations of several small amides [acetamide (ACT), *N*-methylacetamide (NMA), and *N*,*N*-dimethylacetamide] and amines [NH_3, NH_2CH_3, $NH(CH_3)_2$, and $N(CH_3)_3$] in aqueous solution were performed to study the effects on solvation free energies arising from N-methylation. Using pairwise additive potentials, a uniform pattern of solvation effects was always obtained, indicating that successive substitution of hydrogens on the nitrogen atom by hydrophobic methyl groups leads to less favorable solvation. These results are not in agreement with experimental data, which show that the solvation free energy changes associated with N-methylation are irregular and actually favor methylation in the cases of ACT → NMA and NH_3 → NH_2CH_3. The amines were also studied using a many-body polarizable potential for both the solute and the solvent. The resulting solvation free energy differences represent improvements over the values obtained with the pairwise additive potentials, although the monotonic change in solvation free energy with increasing methylation persists. With the many-body polarizable potential, the magnitudes of the amine dipole moments in aqueous solution exhibit the same trend as the solvation free energies. This observation provides a basis for understanding the irregular behavior of the experimental amine solvation free energies.

1. Introduction

Amides and amines occupy important positions in physical and organic chemistry and in biochemistry. For example, amides provide the simplest models for the study of structural and conformational characteristics of the backbones in polypeptides and proteins, while amines may serve as prototypes for side-chain groups of proteins. The thermodynamic effects of solvation for these compounds are of widespread biological importance. Amides and amines show interesting irregular and anomalous solvation effects upon successive substitution of hydrogens on the nitrogen atom by methyl groups. A regular increase in base strength is observed in the gas phase for amines ($NH_3 < NH_2CH_3 < NH(CH_3)_2 < N(CH_3)_3$), and normal methyl substituent (inductive) effects dominate; in contrast, the base strength order for amines in aqueous solution is $NH_3 < N(CH_3)_3 < NH_2CH_3 < NH(CH_3)_2$. The order of the solvation free energies of the amines is also irregular: $NH_2CH_3 < NH_3 \approx NH(CH_3)_2 < N(CH_3)_3$; i.e., methylamine has the most favorable free energy of solvation. The intuitive expectation that a polar hydrogen attached to the electronegative nitrogen atom would be hydrophilic and better solvated than an apolar, hydrophobic methyl group is clearly at odds with the experimental results described in a paper by Jones and Arnett.[1]

A likewise anomalous order of solvation free energies exists for the amides, viz., *N*-methylacetamide (NMA) < acetamide (ACT) < *N*,*N*-dimethylacetamide (DMA).[2] NMA, in addition to being the optimally solvated molecule in the sequence, has two conformational isomers. Experiments show that the difference in solvation free energy between *trans*- and *cis*-NMA isomers is near zero,[3,4] even though the two isomers possess different permanent dipole moments. The experimentally observed irregular and seemingly counterintuitive relationships between N-methyl substitution and solvation free energy briefly outlined above have received considerable theoretical attention.

In an attempt to better understand solvation processes at the molecular level, theoretical methods including explicit or continuum solvent models have been developed and applied. In continuum models, the solute is represented as a low dielectric cavity immersed in a solvent continuum and the free energy changes are calculated according to the Poisson or Born equations; with explicit solvent models, the microscopic structure of the solvent is taken into account and the free energy differences between similar systems may be calculated from computer simulations using statistical mechanical procedures. Both continuum and explicit solvent models predict that in simple amides and amines the successive substitution of hydrogens on the nitrogen atom by methyl groups leads to less favorable solvation, results which are not in agreement with experimental data. Using explicit solvent models and a united-atom potential function (OPLS), Still *et al.*[5] showed that the amides with more hydrogens on the nitrogen atoms have more negative electrostatic solvation free energies. A very recent calculation, using the same OPLS parameters but including the cavity terms as well as the electrostatic part of the solvation free energy, produced similar results.[6] Although a study by Kollman and co-workers[7] using an all-atom model (AMBER) with explicit solvent showed approximately equal free energies of solvation for the three simple amides, in agreement with experimental findings, it was recently pointed out by this same research group that the published results were in error and up-to-date computational results maintain the initial conflict with experimental data.[8]

For the *cis*- to *trans*-NMA isomerization, two theoretical studies have attempted to elucidate the origin of the experimental result of zero differential free energy. Jorgensen and Gao,[4] using Monte Carlo free energy perturbation (FEP) methods and the OPLS united-atom model, obtained a free energy difference of 2.5 kcal/mol favoring the *trans* isomer. They found that

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different partial atomic charges were required for *cis*- and *trans*-NMA in order to reduce the free energy difference to zero. Cieplak and Kollman[9] used molecular dynamics/FEP methods with an all-atom potential function, where the atomic charges were obtained from fits to the electrostatic potentials for *cis*- and *trans*-NMA individually, to also achieve the desired result of no differential solvation energy.

The solvation free energies of amines have also been studied. Singh *et al.*[10] found that methylation of ammonia ($NH_3 \rightarrow NH_2CH_3$) did not affect the amine solvation free energy; successive methylations led to less favorable free energies of solvation. Recently, Jorgensen *et al.*[11] calculated the total solvation free energies for a number of small molecules including NH_3 and NH_2CH_3, using both all-atom explicit solvent and continuum models. Their results show that NH_3 is more favorably solvated than NH_2CH_3 by 1.2 and 1.4 kcal/mol with the use of explicit solvent and continuum models, respectively. This contrasts with experimental findings, which indicate that NH_2CH_3 is preferentially solvated by 0.3 kcal/mol.

These discrepancies between theory and experiment provide the motivation for the present study. Solvation free energy is an important thermodynamic quantity closely related to the hydrophilic and hydrophobic character of compounds. In this paper, we present systematic studies on solvation free energy differences of simple amides and amines based on united-atom or all-atom pairwise additive potential models incorporating explicit solvent molecules. Many-body polarization interactions, which describe the change of molecular charge distributions induced by the environment, are important for solvation processes. We have recently developed a polarizable potential function, which describes well the thermodynamic and dynamic properties of bulk water.[12] In an extension of that work, we shall also explore the use of a polarizable potential function for both the solute and the solvent in the prediction of solvation free energies of amines.

In section 2 we present the details of our computer simulations and summarize the methodology used to evaluate the solvation free energy. The results are presented and discussed in section 3. In section 4 we summarize the results and present our conclusions.

2. Method and Computational Details

2.1. Pairwise Additive Potential. The pairwise additive molecular potential energy functions used in this study have the following familiar form

$$U = \sum_{\text{bonds}} K_b(b - b_0)^2 + \sum_{\text{bond angles}} K_\theta(\theta - \theta_0)^2 + \sum_{\text{dihedrals}} K_\Phi[1 + \cos(n\Phi - \gamma)] + \sum_{\text{nonbond}} \left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_i q_j}{\epsilon R_{ij}}\right] \quad (1)$$

and the empirical parameters (with the possible exception of values for the partial atomic charges q_i, see below) have been taken directly from the all-atom model AMBER[13] or the united-atom model OPLS.[14]

The expression for the total free energy change going from an initial state 0 to a desired final state 1 is[15]

$$\Delta F = -RT \ln\langle \exp(-(H_1 - H_0)/RT)\rangle_0 \quad (2)$$

where H_0 and H_1 are the Hamiltonians of the states 0 and 1, respectively, R is the molar gas constant, T is the absolute temperature, $\langle\ \rangle_0$ refers to the ensemble average over the initial state 0, and final state 1 is treated as a perturbation on H_0 if the two states are very similar. However, generally the initial and final states are significantly different, and a coupling parameter λ is introduced to gradually complete the perturbation through a series of nonphysical intermediate states of $H(\lambda)$

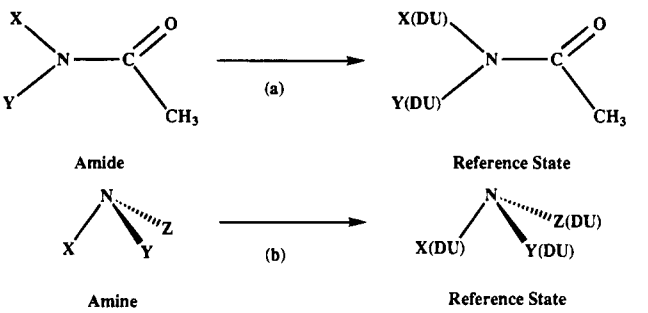

Figure 1. (a) Mutation between an amide and its corresponding reference state. The atom groups X, Y = H, CH_3 are replaced by corresponding "dummy" groups X(DU), Y(DU). The "dummy" groups carry no charge and no Lennard-Jones parameters. (b) Mutation between an amine and its corresponding reference state. The atom groups X, Y, Z = H, CH_3 are replaced by corresponding "dummy" groups X(DU), Y(DU), and Z(DU). The "dummy" groups carry no charge and no Lennard-Jones parameters.

$$H(\lambda) = (1 - \lambda)H_0 + \lambda H_1 \quad (3)$$

where $0 \le \lambda \le 1$. In the window method used in this study, the λ interval is divided into N windows and at the i-th window

$$\Delta F_i(\lambda) = -RT \ln\langle \exp(-\Delta H(\lambda_i)/RT)\rangle_i \quad (4)$$

where $\Delta H(\lambda_i) = H(\lambda_{i+1}) - H(\lambda_i)$ is the potential energy difference between the intermediate state i and the intermediate state $i + 1$. One way to calculate the quantity $\langle \exp(-\Delta H(\lambda_i)/RT)\rangle_i$ is to sample an ensemble during a molecular dynamics (MD) simulation. Following the methods outlined in ref 16 for simulations with nonpolarizable potentials, we include in H only the interaction energy between solute and solvent and exclude all intramolecular energy terms. In this approximation, the solute intramolecular energy contributions to the free energy change in the gas phase and in solution cancel. The total free energy change for the complete mutation of initial state 0 to final state 1 is the sum over all the windows:

$$\Delta F = \sum_i \Delta F_i \quad (5)$$

The statistical uncertainties for FEP simulations were calculated using the standard deviations found in independent simulations with corrections made for statistical inefficiencies[17] due to the highly correlated neighboring steps in molecular dynamics simulations.[18]

The free energy is a thermodynamic state function and solvation free energy differences must be independent of the paths chosen to connect the initial and final states; i.e., the solvation free energy difference between molecule A and molecule B may be calculated as

$$\Delta F_{A\rightarrow B} = \Delta F_{A\rightarrow ref} + \Delta F_{ref\rightarrow B} \quad (6)$$

In this study, we choose a thermodynamic cycle for calculating the free energy difference between states which avoids changes in molecular geometries. Our choices of reference states for amides and amines are defined in parts a and b of Figure 1,

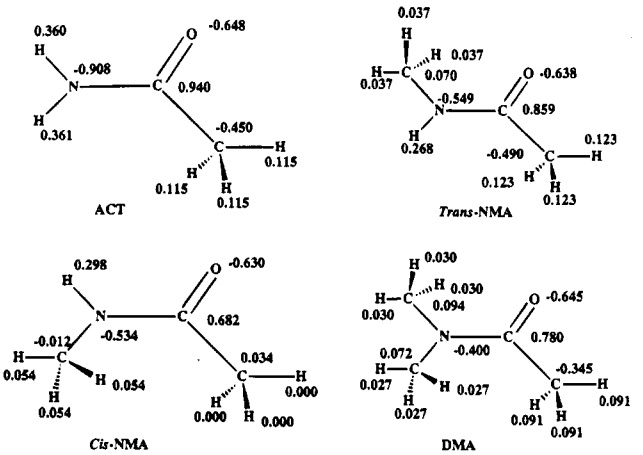

Figure 2. Electrostatic potential (HF/6-31G*//6-31G*) fitted partial charges of amides.

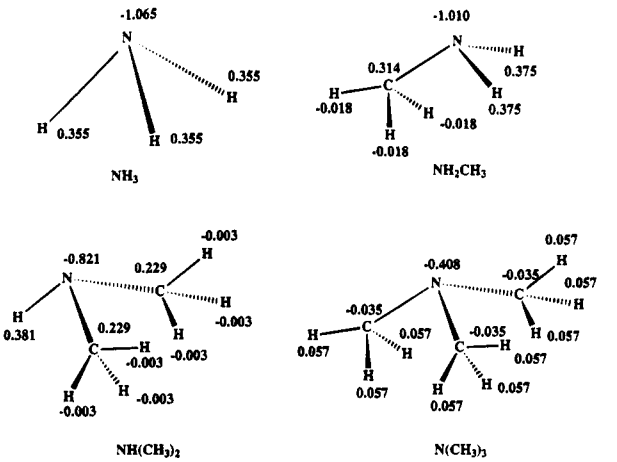

Figure 3. Electrostatic potential (HF/6-31G*//6-31G*) fitted partial charges of amines.

respectively. Computational problems associated with the direct mutation of bond lengths during thermodynamic perturbation calculations have been discussed.[19-21] In our calculations with the nonpolarizable potentials, we choose reference states which can be obtained via changes occurring solely in the nonbonded interaction parameters. For example, in order to obtain the free energy difference between NH_3 and NH_2CH_3, an uncharged nitrogen atom is the reference state and FEP simulations on both NH_3 and NH_2CH_3 are carried out with respect to this reference. The mutation of NH_3 to the reference state is achieved by discharging the entire ammonia molecule and simultaneously mutating the Lennard-Jones nonbonded interaction parameters (A_{ij} and B_{ij} in eq 1) on the three hydrogens to zero. Likewise, the mutation of NH_2CH_3 to the reference state is made by discharging the molecule and at the same time perturbing all Lennard-Jones parameters (except those on N) to zero. The solvation free energy difference between NH_3 and NH_2CH_3 can then be readily obtained from the free energy differences between the common reference state and, respectively, NH_3 and NH_2CH_3. Our approach is in the spirit of calculating the entire molecular solvation free energy except that a single cavity remains.

We calculated free energies of solvation for the amides with two sets of pairwise additive potentials. In the first set of calculations, the united-atom model with the OPLS parameters[14] was used to describe the amide solute. In the united-atom model, solute molecules have sites on each atom with the exception of the methyl group, which is treated as a single site centered on the carbon. In the second set, an all-atom model based on the AMBER[13] force field was applied to the amide solute, while the atomic partial charges were derived from fits to the molecular electrostatic potentials generated by *ab initio* HF/6-31G* molecular orbital calculations on geometries optimized at the HF/6-31G* level of theory.[22] Values of the partial atomic charges obtained with the CHELP[23] or the CHELPG model[24] are given in Figures 2 and 3.[25]

The IMPACT program[26] was used in the simulations. The solute system under study was placed in a cubic box of dimension 18.6256 Å initially containing 216 water molecules. For each solute, the appropriate number of solvent molecules was removed to maintain a density close to 1.0 g/cm^3. The TIP3P water solvent model[27] was used for all simulations with pairwise additive potentials. Periodic boundary conditions and a cutoff of 8 Å were used for the nonbonded interactions. The temperature was kept at 298.15 K using Berendsen's scaling method with a relaxation time of 0.4 ps.[28] The equations of motion were integrated using Andersen's RATTLE algorithm with a time step of 2 fs.[26,29] Intramolecular bond lengths of solute and solvent molecules were constrained to allow the use of larger time steps in the dynamics. The following procedure was used for simulations utilizing the pairwise additive potentials. Systems were first energy minimized for 500 steps and then equilibrated for 20 ps. Free energy perturbations were performed with 100 double-sampling windows for the amide mutations and 200 double-sampling windows for the amine mutations. In each window, the system was equilibrated for 1 ps (500 steps) and then data were collected for a similar amount of time. Both forward and backward perturbations were made, and the free energy was evaluated as the average of the two computed results.

2.2. Nonadditive Polarizable Potential. Polarizable models for energy calculations with either polarizable solute (quantum mechanical solute) plus nonpolarizable solvent[30,31] or polarizable solute (quantum mechanical solute) plus continuum solvent representation[32] have been described, but only a few solvation free energy studies using polarizable solutes immersed in polarizable water molecules have been published. Using a noniterative method to include first-order effects, Straatsma *et al.*[33] discussed free energy differences including polarization contributions. Calculations on monoatomic ionic solutes have been reported, where an additional three-body term was included between solute and solvent molecules and an iterative method was employed to calculate the polarization energy.[34] To our knowledge, there has been only one previous FEP study of polarizable molecular solutes in a polarizable solvent.[35] However, the accuracy employed to test convergence in the induced dipole moments (0.01 D) in the references using iterative methods[34,35] is, in our opinion, not sufficient to evaluate free energy changes (ref 36 and below).

Our model for the polarizable solute potential function is an extension of our polarizable water potential function, and we have fully described the model as well as details regarding free energy perturbation calculations using polarizable potentials elsewhere.[12,36] Briefly, an additional many-body polarization term is implemented in the nonadditive polarizable potential energy function as

$$U_{\text{pol}} = -\frac{1}{2}\sum_i \boldsymbol{\mu}_i \mathbf{E}_i^0 \qquad (7)$$

where $\mathbf{E}_i^0$ is the electrostatic field due to the permanent charges and $\boldsymbol{\mu}_i$ refers to the induced dipole moment on atom i.[37,38] The value of the induced dipole moment on site i is given by

$$\boldsymbol{\mu}_i = \boldsymbol{\alpha}_i(\mathbf{E}_i^0 - \sum_{j \neq i} \mathbf{T}_{ij}\boldsymbol{\mu}_j) \quad (8)$$

where $\boldsymbol{\alpha}_i$ is the polarizability tensor of the i-th atom and $\mathbf{T}_{ij}$ is the dipole field tensor. The calculation of the tensor includes the use of damping functions at short distances.[12,39] In evaluating the attenuated electric field, the contributions from 1−2 and 1−3 bonded interactions are not included. However, all dipole−dipole interactions, including the 1−2 and 1−3 components, are included in the evaluation of the induced dipole moments (eq 8). Inclusion of these dipole interactions is a requirement in the model from which the atomic polarizabilities (Table 2) were derived.[39] Each dipole moment responds to changes in the dipole moments on all the other sites, and the values of $\boldsymbol{\mu}$ must be obtained in a self-consistent manner using direct matrix inversion[36] or, as in the present work, iterative solution techniques.

The solvation free energy for a solute can be expressed as the difference between the free energies needed to create the solute s in solution and in the gas phase

$$\Delta F_{\text{solv}} = \Delta F(0 \rightarrow \text{s})_{\text{solution}} - \Delta F(0 \rightarrow \text{s})_{\text{gas}} \quad (9)$$

Here, ΔF_{solv} is calculated using the procedure outlined in section 2.1. Note that the annihilation term is a function of energies arising from interactions within the solute. If one neglects the solute−solute interaction energies in the calculation of both terms on the right-hand side, then the desired result can be obtained in a single set of simulations

$$\Delta F_{\text{solv}} = \Delta F(0 \rightarrow \text{s}')_{\text{solution}} \quad (10)$$

where s′ implies that solute−solute interaction energies are omitted from the calculation. Such an approach assumes that the potential can be decomposed into intramolecular plus intermolecular components, which indeed can be done with pairwise additive potentials. On the other hand, the interaction energy cannot be decomposed in this way with the many-body polarizable potential, since the induced dipole moments in eq 7 are not separable into intra- and intermolecular components. The interdependence of induced dipole moments means that each $\boldsymbol{\mu}_i$ cannot be strictly separated into intra- and intermolecular components and that the polarization energy (eq 7) cannot be so decomposed either. Therefore, two sets of simulations (eq 9) are required to obtain the solvation free energies of most polarizable solutes. Within the framework of our model, there are no intramolecular polarization interactions in small solutes such as NH_3 which only have 1−2 and 1−3 interactions, and for such solutes we can use eq 10.

Partial atomic charges used in the calculations with the many-body polarizable potential (summarized in Table 1) were determined from the requirement that they, in conjunction with the atomic polarizabilities and induced dipole moments, reproduce the experimental gas-phase molecular dipole moments. Electrostatic potential derived charges and the atomic polarizabilities defined the initial set of parameters. The atomic charges were then uniformly scaled, with intramolecular polarization taken into account for the methylated amines (in ammonia, the neglect of 1−2 and 1−3 interactions within our model implies no induced intramolecular dipole moment), in an iterative procedure until the computed gas-phase dipole moments matched the experimental values. All nonelectrostatic parameters and functional forms (Lennard-Jones, torsion, etc.) were carried over unchanged from the all-atom pairwise additive potential.

Two types of simulations were performed involving the more compute-intensive polarizable potential. The dipole moments in solution were obtained by sampling during a 5 ps molecular dynamics simulation with 2 fs time steps. Dipole moments were iteratively determined at each time step using a convergence criterion of 10^{-2} D, which generally requires five to eight iterations. FEP calculations, on the other hand, require highly accurate values for the induced dipole moments,[36] so a considerably more stringent convergence criterion (10^{-7} D), which can require as many as thirty iterations, was used in these calculations. For each solute in solution, charges and polarizabilities were first annihilated in 40 double-sampling windows of 1.5 ps duration (0.5 ps equilibration and 1 ps data collection). The Lennard-Jones parameters were then annihilated using the same number of windows and equilibration/sampling times. To properly account for intramolecular polarization effects in the thermodynamics cycle, solute molecules were subjected in the gas phase to the same annihilation sequence of charges, polarizabilities, and Lennard-Jones parameters as in solution. Each gas-phase annihilation sequence used 20 windows, with 2 ps of equilibration and 4 ps of data collection in each window.

All computations were performed on Convex C220 and HP 9000/735 computers at Rutgers University or on a Thinking Machines Corp. CM-5 parallel computer at the National Center for Supercomputing Applications.

TABLE 1: Partial Atomic Charges Used in the Polarizable Potential

molecule	atom	electronic charge (q)
water	O	−0.6690
	H	0.3345
ammonia	N	−0.8052
	H	0.2684
methylamine	N	−0.7353
	H (amine)	0.2728
	C	0.2278
	H (methyl)	−0.0127
dimethylamine	N	−0.5162
	H (amine)	0.2396
	C	0.1440
	H (methyl)	−0.0019
trimethylamine	N	−0.2796
	C	0.2231
	H (methyl)	−0.0433

TABLE 2: Atomic Polarizabilities[a]

atom	α, Å^3	atom	α, Å^3
O	0.862	N	1.105
H	0.514	C	1.405

[a] From ref 39.

3. Results and Discussion

3.1. Amide Mutations. We first discuss the results obtained for N-methylation of amides. We have calculated the solvation free energy differences between the three amides [acetamide (ACT), *N*-methylacetamide (NMA), and *N,N*-dimethylacetamide (DMA)] and their common reference state, illustrated in Figure 1a, as described in the previous section. The free energies (ΔF_{solv}) are represented in Table 3 for the OPLS-based united-atom and the AMBER-based all-atom models. The free energy differences obtained upon successive substitutions of a hydrogen atom by a methyl group are listed in Table 4. Wolfenden[2] measured the distribution coefficients for transfer of amides from aqueous solution to the vapor phase at 298 K. The published vapor/water distribution coefficients translate to solvation free energies for ACT, NMA, and DMA of $\Delta F_{\text{solv}} = -9.7$, -10.1, and -8.6 kcal/mol, respectively. Thus, ACT and NMA have almost the same solvation free energies but DMA is less favorably solvated by approximately 1.5 kcal/mol.

TABLE 3: Solvation Free Energies from United-Atom and All-Atom Force Fields

	ΔF_{solv} (kcal/mol)		
solute	united-atom[a]	all-atom[a]	exp[b]
ACT	-11.90 ± 0.17	-12.63 ± 0.21	-9.7
trans-NMA	-9.29 ± 0.21	-9.60 ± 0.24	-10.1
cis-NMA	-7.99 ± 0.18	-10.01 ± 0.23	-10.1
DMA	-7.26 ± 0.25	-8.67 ± 0.26	-8.6

[a] Free energy difference with respect to reference state illustrated in Figure 1a; see text for additional details. [b] Derived from data provided in ref 2.

TABLE 4: Computed and Experimental Solvation Free Energy Differences of Amides

	$\Delta\Delta F_{solv}$ (kcal/mol)		
mutation	united-atom	all-atom	exp
ACT → *trans*-NMA	2.61 ± 0.27	3.03 ± 0.32	-0.4
trans-NMA → DMA	2.03 ± 0.33	0.93 ± 0.33	1.5
trans-NMA → *cis*-NMA	1.30 ± 0.35	-0.41 ± 0.35	≈ 0

The relationship between N-methyl substitution and ΔF_{solv} is the same from both sets of calculations. For the all-atom potential, the solvation free energy differences are 3.0 kcal/mol for the ACT to *trans*-NMA mutation and 0.9 kcal/mol for the *trans*-NMA to DMA mutation. The calculations using the united-atom potential reveal that ACT is better solvated than *trans*-NMA by 2.6 kcal/mol and that *trans*-NMA is better solvated than DMA by 2.0 kcal/mol. Thus, we do not observe any irregular behavior in solvation free energies within the simple amide series from either computational model, and the calculations predict (incorrectly) that methyl substitution for an amide hydrogen will always lead to less favorable solvation.

Our computational results on these amides are comparable to those of Still *et al.*,[5,40] who used both explicit solvent and continuum models. They carried out Monte Carlo FEP calculations on a number of molecules, including the amides considered in the present study, using the TIP4P water model and the OPLS force field for the solutes. In addition to the slightly different water models (TIP3P vs TIP4P) and theoretical approaches (molecular dynamics vs Monte Carlo) used in their study and in ours, Still *et al.* computed only the electrostatic part of the total solvation free energy and did not take the nonbonded Lennard-Jones contributions into account. Their calculations showed differential solvation free energies of 0.7 kcal/mol between ACT and *trans*-NMA and 2.6 kcal/mol between *trans*-NMA and DMA. A recent calculation of absolute solvation free energies of amides (ACT, NMA, and DMA) by Jorgensen[6] using the parameters of Still *et al.* gave differences of 2.6 kcal/mol between ACT and *trans*-NMA and 1.3 kcal/mol between *trans*-NMA and DMA. The results from both of the above studies are consistent with our simulation results.

Published results based on continuum models are similar to those obtained from FEP calculations. The calculations by Still *et al.* showed differential solvation energies of 2.4 kcal/mol (ACT → *trans*-NMA) and 1.7 kcal/mol (*trans*-NMA → DMA). Sitkoff *et al.*[41] used a macroscopic solvent model and analyzed several sets of available parameters (AMBER, CHARMM, OPLS, etc.); they obtained 3.4 kcal/mol for the solvation free energy difference between ACT and NMA using the OPLS parameter set, which generally gave the best solvation free energy results. Sitkoff *et al.* also designed a parameter set (PARSE) for continuum calculations by explicitly parametrizing the charges and Lennard-Jones radii to reproduce solvation free energies. Their calculated solvation free energy difference between NMA and ACT is in agreement with experiment; however, it must be noted that the NMA and ACT PARSE parameters were optimized specifically to fit the solvation free energies. Using a combination of *ab initio* quantum mechanical calculations (PSGVB) on the solute and a continuum description for the solvent, Tannor *et al.*[42] obtained 2.0 kcal/mol for the solvation free energy difference between ACT and *trans*-NMA. We note that an early study by Kollman and co-workers,[7] in which charges were scaled to reproduce the experimental dipole moments, predicted solvation energies for amides in good agreement with experimental findings. However, it was subsequently found that errors in the procedure for bond mutation, rather than the modified electrostatic parameters, were responsible for the good agreement obtained between theory and experiment. Recently, Morgantini and Kollman[8] redid the calculations for the amides and obtained results which were more consistent with the findings from the other theoretical studies described above. Thus, simulations applying explicit or continuum water models all predict the same pattern for the solvation energies of simple amides (ACT, NMA, and DMA): the amides with fewer hydrogens on the amide group have less favorable solvation free energies.

There is also some disagreement between theoretical predictions and experimental results regarding the solvation free energy difference between *cis*- and *trans*-NMA. Experiments (see ref 4 and references listed therein) indicate that the differential solvation free energy between the two conformational isomers is nearly zero. The two molecular mechanics potentials used in the present study predict small but nonzero solvation free energy differences between *cis*- and *trans*-NMA (Table 4). Jorgensen *et al.*[4] obtained a free energy difference of 2.5 kcal/mol favoring the *trans* isomer when they used the original OPLS parameters in Monte Carlo FEP simulations. Modifications of the partial atomic charges were necessary in order to obtain a free energy difference of zero. Cieplak and Kollman[9] obtained a solvation free energy difference of zero using different charge sets (derived from HF/6-31G* electrostatic potentials) for the two isomers. Our result for the *cis*- vs *trans*-NMA energy difference of only 0.4 kcal/mol obtained with the all-atom model is comparable to the experimental finding.

3.2. Amine Mutations. The substituent effects on solution properties of N-methylated amines are also unusual,[1] since the solvation free energy differences between simple primary, secondary, and tertiary amines do not change regularly as they do, for example, in hydrocarbons. It is found experimentally that NH_2CH_3 is more favorably solvated than NH_3 by 0.3 kcal/mol and that $NH(CH_3)_2$ has nearly the same solvation free energy as NH_3. $N(CH_3)_3$ is solvated less favorably than NH_3 and NH_2CH_3 by approximately 1 kcal/mol. The estimates of the solvation free energies for the amines were derived by experimentally determining such classical thermodynamic properties as Henry's Law constants with proper correction for the partial ionization of the amine in aqueous solution. It seems unlikely that the irregular trend shown above was due to experimental errors. The reasons are the following. Firstly, the irregular behavior not only exists in the methyl amine series but also in other substituted amines.[1] For the ethyl substituted series, the ethylamine is more favorably solvated than ammonia by 0.3 kcal/mol, and the diethylamine and triethylamine are solvated less favorably than ammonia by 0.3 and 1.3 kcal/mol, respectively. Similarly, for propyl substituted amines, the propylamine is more favorably solvated than ammonia by 0.1 kcal/mol, and the dipropylamine is solvated less favorably than ammonia by 0.7 kcal/mol. Secondly, the "anomalous order" of substituent effects was also observed in the substitution from acetamide to *N,N*-dimethylacetamide.[2] The amide solvation free energies were obtained using radioactive labeling techniques.

TABLE 5: Solvation Free Energies of Amines

solute	ΔF_{solv} (kcal/mol)[a]	solute	ΔF_{solv} (kcal/mol)[a]
NH_3	-7.43 ± 0.11	$NH(CH_3)_2$	-3.14 ± 0.19
NH_2CH_3	-6.30 ± 0.16	$N(CH_3)_3$	-0.85 ± 0.26

[a] Free energy difference with respect to reference state illustrated in Figure 1b; see text for additional details.

TABLE 6: Computed and Experimental Solvation Free Energy Differences of Amines

	$\Delta\Delta F_{solv}$ (kcal/mol)		
mutation	all-atom	polarizable	exp[a]
$NH_3 \rightarrow NH_2CH_3$	1.13 ± 0.19	0.3 ± 0.5	-0.3
$NH_2CH_3 \rightarrow NH(CH_3)_2$	3.16 ± 0.25	2.5 ± 0.6	0.3
$NH(CH_3)_2 \rightarrow N(CH_3)_3$	2.29 ± 0.32	0.6 ± 0.6	1.1

[a] See refs 1 and 45.

Thus the observation of irregular solvation behavior involves both amines and amides measured by different groups using different methods. Furthermore, the basicities of methylamines in aqueous solution also display an irregular trend, which has been one of the classical problems of physical organic chemistry.[1] So, such irregularity seems an intrinsic property of the nitrogen-containing compounds. In the following computational study, we calculate the solvation free energy differences for the simple methyl substituted amines.

The solvation free energies with respect to the reference state illustrated in Figure 1b from the amine FEP simulations are listed in Table 5. The relative free energy differences between the amines are given in Table 6 along with experimental results. The following solvation free energy differences were obtained in the simulations using pairwise additive (AMBER + TIP3P water) potentials: $NH_3 \rightarrow NH_2CH_3$, 1.1 kcal/mol; $NH_2CH_3 \rightarrow NH(CH_3)_2$, 3.2 kcal/mol; $NH(CH_3)_2 \rightarrow N(CH_3)_3$, 2.3 kcal/mol. Thus, our results clearly predict that with increasing methylation the stability of amines in aqueous solution decreases, consistent with the picture developed above for the amides. Overall, full methylation of ammonia [$NH_3 \rightarrow N(CH_3)_3$] is computed to reduce the solvation free energy by approximately 7 kcal/mol, in sharp contrast to the experimental value of only 1 kcal/mol.

The study conducted by Singh *et al.*[10] led to energy differences approximately 1 kcal/mol less than ours for the three successive methylation steps of ammonia: -0.07, 1.93, and 1.17 kcal/mol. Their results for $NH_2CH_3 \rightarrow NH(CH_3)_2$ and $NH(CH_3)_2 \rightarrow N(CH_3)_3$ mutations thus also highlight the hydrophobic effects of methyl substitution, but for the $NH_3 \rightarrow NH_2CH_3$ mutation, their solvation free energy difference of -0.07 kcal/mol is significantly smaller than ours (1.1 kcal/mol). Furthermore, their value is smaller than that obtained recently for the same reaction by Orozco *et al.*,[11] who reported a 1.2 kcal/mol solvation free energy difference upon amine monomethylation using HF/6-31G* electrostatic potential derived charges and the same force field (AMBER + TIP3P water) as that used by Singh *et al.*[10] and by us.

It is interesting to note that all the calculations referred to above employed the AMBER potential for the solute, TIP3P for the water molecules, and atomic charges fit to electrostatic potentials from *ab initio* HF/6-31G* calculations. However, there are variations approaching 1.3 kcal/mol between the free energy differences predicted by the three sets of calculations. The atomic partial charges differ in detail in the three calculations because, even if the generated electronic wave functions were identical (same molecular geometry, etc.), the charge fitting procedure is not unique but depends strongly on the sampling scheme employed (total number of sampling points, spatial extent of sampling surfaces, etc.). In an attempt to locate the major source of the disagreement among the three calculations, we took the charges published in Orozco's paper[11] as input and used our approach to calculate the free energy difference between NH_3 and NH_2CH_3. In this way, we obtained a value of 1.3 ± 0.3 kcal/mol, which is in excellent agreement with their value of 1.2 ± 0.6 kcal/mol and our value of 1.1 ± 0.2 kcal/mol cited above. In a similar way, we tried to calculate the free energy difference using the partial charges reported by Singh *et al.*[10] The result we obtained was 2.3 ± 0.3 kcal/mol, which differs significantly from their reported value of approximately zero. The reasons for this discrepancy are not clear but may be related to the treatment of bond mutation corrections alluded to earlier.

TABLE 7: Polarizabilities and Dipole Moments of NH_3, NH_2CH_3, $NH(CH_3)_2$, and $N(CH_3)_3$

	molecular polarizability, Å^3		total dipole moments, μ, in D		
solute	exp[a]	calc	gas phase[a] (exp)	gas phase[b] (theory)	in solution[c] (polarizable)
NH_3	2.1−2.8	2.0	1.47	1.89	1.97 ± 0.15
NH_2CH_3	4.0−4.7	4.1	1.31	1.63	2.35 ± 0.38
$NH(CH_3)_2$	6.37	6.1	1.01	1.22	1.81 ± 0.35
$N(CH_3)_3$	8.15	8.1	0.61	0.84	0.83 ● 0.34

[a] Experimental values from ref 44. [b] From HF/6-31G*//6-31G* calculations. [c] From our simulations in solutions. Note: The gas-phase dipole moments in our model are scaled to the experimental gas-phase dipole moments.

Calculations with continuum models give inconsistent results for the ammonia to methylamine transformation. Sitkoff *et al.*[14] used a macroscopic solvent model specifically parametrized to predict solvation free energies and obtained relative free energy changes of -0.84 kcal/mol for $NH_3 \rightarrow NH_2CH_3$ and 0.44 kcal/mol for $NH_2CH_3 \rightarrow NH(CH_3)_2$. On the other hand, Orozco *et al.*[11] found a solvation free energy difference of 1.4 kcal/mol for the $NH_3 \rightarrow NH_2CH_3$ mutation using a continuum solvent model and charges fit to the quantum mechanical electrostatic potential. Tannor *et al.*[42] obtained 1.6 kcal/mol for the $NH_2CH_3 \rightarrow NH(CH_3)_2$ mutation and 1.5 kcal/mol for $NH(CH_3)_2 \rightarrow N(CH_3)_3$ from *ab initio* quantum mechanical calculations (PSGVB) incorporating a continuum description for the solvent. Thus, the discrepancies between theoretical and experimental solvation free energies for the methylated amine series are still very much apparent.

One possibly very important factor that has been omitted in the above FEP calculations is the explicit treatment of solute polarization in the aqueous environment. We have initiated a study of the polarization effects using our recently developed polarizable potential, and some results from these calculations will be discussed in the following section.

3.3. Polarization Effects on Amines. Will an explicit treatment of polarization effects lead to better agreement between theory and experiment for the solvation energies of simple amines and amides? The nonpolarizable models attempt to account in an "average" manner for the electronic polarization of solutes in the condensed phase through the use of partial atomic charges which generally exaggerate polarity and differ from those representative of, say, the experimentally measured dipole moments in the gas phase. For example, the partial charges used in this study from the AMBER and OPLS pairwise additive force fields are constructed to yield dipole moments for the amines and amides which are greater than the corresponding gas-phase values. *Ab initio* HF/6-31G* calculations and hence the partial atomic charges derived from HF/6-31G* electrostatic potentials typically overestimate the gas-phase dipole moment. This is illustrated in Table 7 for the amines,

where the differences between experimental and computed dipole moments are seen to be on the order 0.2−0.4 D with the computed values (HF/6-31G*//6-31G*) always being the larger. Our polarizable potential reproduces the molecular polarizabilities of the amines extremely well (Table 7).

When placed in solution, the solutes are polarized in response to the reaction field of the solvent and, as a result, the dipole moments of the solutes in our polarizable model change when the molecules are solvated. In the amine series of solutes, polar hydrophilic hydrogens are replaced by less polar but more polarizable hydrophobic methyl groups. The polarizability of the amine increases steadily as more amino hydrogen atoms are replaced by methyl groups, but methyl substitution is also accompanied by a rapidly diminishing magnitude of the gas-phase permanent dipole moment (Table 7). Both computational and experimental data show that, for each hydrogen replaced by a methyl group, the amine molecular polarizability increases by approximately 2 Å^3 but also that the dipole moment decreases by 0.2−0.4 D. The many-body potential allows development of small induced dipole moments for the amines even in the gas phase (0.28 D for NH_2CH_3, 0.19 D for $NH(CH_3)_2$, and 0.14 D for $N(CH_3)_3$), except in the case of NH_3 (since 1−2 and 1−3 polarization interactions are neglected in our model). The full magnitudes of the intra- and intermolecular induced dipoles in aqueous solution are 0.54 D for NH_3, 1.03 D for NH_2CH_3, 0.90 D for $NH(CH_3)_2$, and 0.62 D for $N(CH_3)_3$, or viewed differently, the magnitudes of the solvent intermolecular induced dipole moments are approximately 0.54 D in NH_3, 0.75 D in NH_2CH_3, 0.71 D in $NH(CH_3)_2$, and 0.48 D in $N(CH_3)_3$. The instantaneous solvent induced dipole moments have components parallel and perpendicular to the permanent dipole moment. For $N(CH_3)_3$, the average increase in the dipole moment along the direction of the permanent dipole moment is only 0.22 D whereas in NH_3 it is 0.50 D. The spread in the computed dipole moments in solution with the polarizable model (~0.3 D, Table 7) arises from the fluctuations in the solvent reaction fields, which leads to an orientational distribution of induced and hence total solute dipole moments.

The induced dipole moments in solution are not a simple function of the molecular polarizabilities—the molecule with the largest polarizability, $N(CH_3)_3$, experiences the smallest solvent induced dipole moment. The magnitudes of the solute permanent dipoles and hence partial atomic charges also play a significant role in determining the induced dipole moments. For example, a simple 50% reduction in the partial atomic charges of methylamine (i.e., effectively a reduction of the permanent dipole moment by 50%) also leads to a 50% decrease in the induced dipole moment in aqueous solution,[12,36] despite the large polarizability of this molecule. The solvent induced dipole moments in NH_3 and $N(CH_3)_3$ are similar in magnitude and the smallest in the amine series, but different factors appear to be responsible for their limited magnitudes. The NH_3 solute possesses the largest gas-phase dipole (1.47 D) and features partial atomic charges fully exposed to the polar solvent. Thus, the ordered solvent will generate a large reaction field, but the small molecular polarizability (2 Å^3) prohibits the development of a substantial induced dipole moment. A combination of steric effects, a small permanent dipole moment, and the large partial atomic charges buried in the interior of the molecule are responsible for the small induced dipole moment of the highly polarizable (α = 8 Å^3) trimethylamine molecule. Examination of Figure 3 and Table 1 shows that the charged and polarizable carbon and nitrogen atoms are shielded by the methyl hydrogens which carry little or no net charge. The small permanent dipole moment of $N(CH_3)_3$ induces a small reaction field, and this results in a small induced dipole moment, even though the polarizability of $N(CH_3)_3$ is the largest in the series. NH_2CH_3 has a permanent dipole in the gas phase comparable to that of NH_3 and, with the increasing molecular polarizability (4 Å^3) and relative openness of the molecule (two hydrogens remain bonded to the nitrogen), a substantial solvent induced dipole develops. The gas-phase dipole moment in $NH(CH_3)_2$ diminishes relative to that in NH_2CH_3, the molecule becomes less open and increases in steric bulk (just one hydrogen bonded to nitrogen), yet the molecular polarizability is larger (6 Å^3). Overall, the magnitude of the solvent induced dipole moment in $NH(CH_3)_2$ is similar to that of NH_2CH_3.

TABLE 8: Solvation Free Energies Obtained with Polarizable Potentials

molecule	ΔF_{solv} (kcal/mol) theory	exp[a]
NH_3	−4.0 ± 0.3	−4.3
NH_2CH_3	−3.7 ± 0.4	−4.6
$NH(CH_3)_2$	−1.2 ± 0.4	−4.3
$N(CH_3)_3$	−0.6 ± 0.5	−3.2

[a] From ref 1, after application of the correction specified in ref 46.

The energies obtained from the FEP simulations of the polarizable solutes in polarizable water (Table 8) show that the many-body potential yields solvation free energies which are significantly different from those obtained with the nonpolarizable potential (Table 5) and in considerably better agreement with experimental data. In particular, the solvation free energy of ammonia in water with the polarizable potential (ΔF_{solv} = −4.0 kcal/mol) is in excellent agreement with the experimental value (−4.3 kcal/mol). The difference in the solvation free energies between NH_3 and NH_2CH_3, $\Delta\Delta F_{solv}$, is now computed at only 0.3 kcal/mol, as compared to the value of 1.13 kcal/mol obtained with the pairwise additive potential (the experimental value is −0.3 kcal/mol). Likewise, $\Delta\Delta F_{solv}$ between NH_2CH_3 and $NH(CH_3)_2$ is 2.5 kcal/mol in the polarizable model, compared with the value of 3.2 kcal/mol obtained in the simulations using the nonpolarizable potential and an experimental value of 0.3 kcal/mol. Finally, $\Delta\Delta F_{solv}$ between $NH(CH_3)_2$ and $N(CH_3)_3$ is 0.6 kcal/mol with the polarizable potential, 0.8 kcal/mol with the pairwise additive potential, and 1.1 kcal/mol experimentally. The monotonic change in ΔF_{solv} with increasing methylation is still apparent, but the full amine methylation process is computed to have a $\Delta\Delta F_{solv}$ of only 3.4 kcal/mol, reduced from the 6.6 kcal/mol predicted above by the pairwise additive potentials and considerably closer to the experimental value of 1.1 kcal/mol.

For the polarizable model, the trends exhibited by the total dipole moments of the amines in solution conform to the trends in the experimental solvation free energies (Table 8). NH_2CH_3 has the largest dipole moment in solution (2.35 D), and it also possesses the most favorable solvation free energy (−4.6 kcal/mol). NH_3 and $NH(CH_3)_2$ show smaller but similar dipole moments in solution (1.97 and 1.81 D, respectively) and have identical solvation free energies (−4.3 kcal/mol), slightly reduced relative to that of NH_2CH_3. $N(CH_3)_3$, on the other hand, has a much smaller effective dipole moment in solution (0.83 D), and a notable change in solvation free energy relative to those of the other amines is observed. The computed free energy of solvation for NH_3 (−4.0 kcal/mol) matches the experimental value (−4.3 kcal/mol) extremely well. NH_2CH_3 has a measured solvation free energy slightly more negative than that of NH_3 ($\Delta\Delta F_{solv}$ = −0.3 kcal/mol), and the computed effective solution dipole moment of NH_2CH_3 is larger than that of NH_3 even though the relative magnitudes of the gas-phase dipole moments are reversed. To model solvation energies to

an accuracy of a few tenths of a kcal/mol is clearly difficult, but the present calculations do come acceptably close (−3.7 kcal/mol (comp) vs −4.6 kcal/mol (exp)). Upon further methylation, the solvation free energy does not significantly change experimentally (~0.3 kcal/mol) but the computations overestimate the change (~2.5 kcal/mol). The simulations predict that the solvation energies of $NH(CH_3)_2$ and NH_3 differ by 2.8 kcal/mol, even though the dipole moment of $NH(CH_3)_2$ in aqueous solution is only slightly less than that of NH_3 (Table 7). The magnitude of the total solvation free energy does clearly depend on more than just the magnitude of the solute dipole moment.[43]

A carefully parametrized potential energy function is necessary to fully describe the changes in solvation free energy for the substituted amine series. Our potential energy function has two components, a traditional pairwise additive function and a polarization contribution. Short of reparametrizing the entire potential function, it appears useful to take the view that most of the parameters in the pairwise additive potential function be retained and that additional work be focused on the electrostatic parameters (partial atomic charges, many-body polarizable function). We are, of course, aware that the parameters, and in particular the Lennard-Jones and partial charge parameters, may be intimately connected through the procedures used to establish the force field. Part of the residual discrepancy between the results obtained with the polarizable potential and the experimental data is likely due to the current procedure used for determining the partial charges on the atomic sites. The charges on the solute with the self-polarization included are scaled uniformly to yield the total (spatially averaged) dipole moment in the gas phase. We did not attempt to match various spatial components of the dipole moment to experimental or computed values, nor did we adjust individual charges in order to fit experimental data such as solvation free energies. Considering the results from the simulations on NH_3, the species for which there is no model induced dipole moment in the gas phase and partial atomic charge parameters are most rigorously defined, as accurate representations of the capabilities of this type of model, a plausible explanation for the discrepancy is that our polarizable model underestimates polarization in aqueous solution. Slightly larger induced dipoles in the methylated species would presumably render the computed solvation energies more negative and in even better agreement with experimental values.

4. Conclusions

The free energies of aqueous solvation for several amides (acetamide, *N*-methylacetamide, and *N,N*-dimethylacetamide) and amines (ammonia, methylamine, dimethylamine, and trimethylamine) were calculated using free energy perturbation methods. The simulations with united-atom and all-atom pairwise additive potentials produced a regular pattern of solvation free energies, wherein solvation becomes less favorable upon replacement of an N-hydrogen atom by a methyl group, fully consistent with results from other theoretical studies employing a variety of classical potentials and solvent models (explicit solvent or continuum). Experimental data, on the other hand, show an irregular pattern of solvation free energies with increasing extent of methyl substitution and indicate, in particular, that methylamine and *N*-methylacetamide are better solvated than ammonia and acetamide, respectively. The disagreement between theory and experiment points to the existence of shortcomings in current pairwise additive force fields for these molecules.

A many-body polarizable potential for both solute and solvent was also used to compute the solvation free energies for the amines. Improved absolute free energy values and differences were obtained relative to the results obtained with the pairwise additive potentials. It was found that the dipole moments in solution exhibit an irregular trend which is qualitatively similar to the trend shown by the experimental values of the solvation free energies. This is in contrast to the gas-phase dipole moments which decrease monotonically with increasing N-methylation. This observation provides the basis for understanding the anomalous behavior of the solvation free energies for the substituted amines. The solvation free energy contains a polarization induced term that depends on the interaction between the permanent charges and the induced dipoles; the coupling occurs through the solvent reaction field. For the unsubstituted NH_3, the contribution from this term is small because the polarizability is small. For the maximally substituted $N(CH_3)_3$, the contribution from this term is also small, because the permanent charges (i.e. permanent dipole) are small. The interaction between the permanent charges and the induced dipole is maximal for NH_2CH_3, which has the most favorable solvation free energy of the series. Thus the explicit treatment of polarization in these systems provides new physical insights and information concerning the solvation processes of these systems.

The gas-phase dipole moments of the simple amides considered in this study are similar 3.76 D for ACT, 3.73 D for NMA, and 3.81 D for DMA.[44] As discussed above, the solvation free energy of DMA is 0.4 kcal/mol more favorable than that of ACT; however, DMA is less favorably solvated than NMA by 1.5 kcal/mol. These free energy amide data are similar to the ammonia data: replacing one hydrogen on nitrogen by a methyl group (ACT → NMA, $NH_3 \rightarrow NH_2CH_3$) is favorable and gives a small decrease in solvation free energy, but replacing the last available hydrogen by a methyl group (NMA → DMA, $NH(CH_3)_2 \rightarrow N(CH_3)_3$) is unfavorable and increases the solvation free energy. One might speculate that the underlying physical principles for these effects in amides and amines are similar and hence that the "anomalous" behavior of the amide solvation free energies is also related to polarizability. NMA, mimicking NH_2CH_3, has a larger polarizability than ACT and still one hydrogen on the nitrogen exposed to solvent. Hence, a substantial induced dipole probably develops in NMA in aqueous solution and favors its solvation relative to ACT. In DMA, which mimicks $N(CH_3)_3$, the presence of the second methyl group (NMA → DMA) undoubtedly provides an increase in polarizability but the polar hydrogen atoms are gone and the induced reaction field is small—less favorable solvation results.

The excellent agreement obtained for the absolute free energy of solvation for NH_3 and the result that the simulations on methylated amines also lead to solvation free energies close to the experimental values do provide encouragement for further development of the polarizable potential model for modeling the thermodynamics of solvation processes. More work on polarization models and parameters therein is clearly warranted and is being actively pursued in our labs.

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