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Solvation Free Energies of Amides and Amines: Disagreement between Free Energy Calculations and Experiment

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Abstract: We present molecular dynamics/free energy calculations on the molecules acetamide, *N*-methylacetamide, *N,N*-dimethylacetamide, ammonia, methylamine, dimethylamine, and trimethylamine. Unlike the experimental data, which suggest a very non-additive solvation free energy (*N*-methylacetamide and methylamine having the most negative free energy of solvation), the calculations all find that the free energy of solvation monotonically increases as a function of methyl addition. The disagreement with experiment is surprising, given the very good agreement (within 0.5 kcal/mol) with experiment for calculation of the solvation free energy of methane, ethane, propane, water, methanol, and dimethyl ether.

Introduction

One of the most important recent developments in computational chemistry is the ability to calculate solvation free energies of molecules,[1] both per se and as part of the calculation of host−guest binding free energies. The very first application of free energy calculation to solvation was the study of the relative solvation free energies of methanol and ethane.[2] Subsequent studies of the relative and absolute solvation free energies of methane, ethane, propane,[3] water,[4] methanol,[5] and dimethyl ether[6] have shown that appropriately developed models can lead to absolute solvation free energies within ±0.5 kcal/mol of experiment and relative solvation energies within ±0.3 kcal/mol of experiment.

In this context, "non-additive" solvation effects, as emphasized by Wolfenden,[7] have been of particular interest. Wolfenden has studied the solvation of highly polar amides using radioactive isotopes to analyze the vapor pressures and has determined that *N*-methylacetamide is more water soluble than acetamide or *N,N*-dimethylacetamide. Wolfenden cites data on the amines that neutral methylamine is more water soluble than ammonia and dimethylamine to suggest that such non-additive solvation of N−CH$_3$ groups is more general than found in the amides.

In fact, the earlier calculations of Bash *et al.*[8] did find acetamide and *N*-methylacetamide to have nearly identical solvation free energy, whereas the addition of the second methyl group to form *N,N*-dimethylacetamide clearly increased the solvation free energy. This result was at least qualitatively consistent with Wolfenden's experimental data, but it suffered from the fact that these earlier free energy calculations did not properly include the bond PMF correction[9] for bond shrinking/growing. Such a correction is required for an accurate representation of solvation free energies.

In this work, we redo the calculations of the three acetamides with our latest force field methodologies, employing simulations as long as 800 ps. Given the apparent non-additive solvation free energies in the amines, we also calculated the relative solvation free energies in NH$_3$, MeNH$_2$, Me$_2$NH, and Me$_3$N. In both amides and amines, we find a "regular" methyl substituent effect, in significant disagreement with experiment.

Methods and Computational Details

In this work, all the molecular mechanics (MM), molecular dynamics (MD), and free energy perturbation (FEP) calculations have been performed using the AMBER 4.0 package.[10] Quantum mechanics (QM) calculations have been carried out using either the Gaussian 90[11] or the Gaussian 92[12] programs. An HP-735 cluster, a Cray C90 computer, or a Silicon Graphic Indy workstation were used to do all the computations.

The force field[5,13] is based on the empirical potential energy function given in eq 1.

$$V_{\text{total}}(x) = \sum_{\text{bonds}} K_r(r - r_{\text{eq}})^2 + \sum_{\text{angles}} K_\theta(\theta - \theta_{\text{eq}})^2 + \sum_{\text{dihedrals}} \frac{V_n}{2}[1 + \cos(n\phi - \gamma)] + \sum_{i<j} \left\{ \left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} \right] + \frac{q_i q_j}{\epsilon R_{ij}} \right\} \quad (1)$$

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$$\begin{tikzcd}
M(g) \arrow[r, "\Delta G_{MN(g)}"] \arrow[d, "\Delta G_{solv}(M)"] & N(g) \arrow[d, "\Delta G_{solv}(N)"] \\
M(aq) \arrow[r, "\Delta G_{MN(aq)}"] & N(aq)
\end{tikzcd}$$

Figure 1. The thermodynamic cycle used to determine the relative free energy of solvation ($\Delta\Delta G_{\mathrm{solv}}$) of the two molecules M and N.

$V_{\mathrm{total}}(x)$ is the potential energy of the system for the set of atomic coordinates x; K_r and r_{eq} are the bond-stretching constant and the equilibrium bond distance; K_θ and θ_{eq} are the bond angle stretching constant and the equilibrium bond angle; V_n, n, and γ are the torsional force constant, the periodicity of the torsional term, and the phase angle; A_{ij} and B_{ij} are the nonbond repulsion and attraction coefficients; R_{ij} is the interatomic distance between atoms i and j; q_i and q_j are the atomic charges on atoms i and j; and ϵ is the effective dielectric constant.

Resulting from statistical mechanics theory,[14] eq 2 can be used to calculate the free energy difference ΔG between two very similar states of a system, A and B.

$$\Delta G = G_{\mathrm{B}} - G_{\mathrm{A}} = -RT\ln\langle e^{-[V_{\mathrm{B}}(x)-V_{\mathrm{A}}(x)]/RT}\rangle_{\mathrm{A}} \qquad (2)$$

In this equation, $V_{\mathrm{B}}(x) - V_{\mathrm{A}}(x)$ is the potential energy difference (ΔV) between states A and B, R is the molar gas constant, T is the absolute temperature, and the symbol $\langle\,\rangle_{\mathrm{A}}$ indicates an ensemble average generated using V_{A}. In practice, states A and B differ significantly and ΔV can be many orders of magnitude bigger than RT making eq 2 inapplicable. To overcome this problem, a coupling parameter λ is introduced in the potential V is such a way that $V_{\lambda=0} = V_{\mathrm{A}}$ and $V_{\lambda=1} = V_{\mathrm{B}}$, and many different methods[15] have been developed to calculate ΔG. In the thermodynamic windows (window growth) method, the calculation of the free energy difference is broken into $N - 1$ windows. Equation 2 can be rewritten to describe the free energy difference ΔG_{λ_i} between the two similar non-physical intermediate states defined by $V_{\lambda_{i+1}}$ and V_{λ_i}.

$$\Delta G_{\lambda_i} = -RT\ln\langle e^{-[V_{\lambda_{i+1}}-V_{\lambda_i}]/RT}\rangle_{\lambda_i} \qquad (3)$$

By taking $\lambda_0 = 0$ and $\lambda_N = 1$, the summation over all the windows (eq 4) leads to the free energy difference between the two states A and B.

$$\Delta G = G_{\mathrm{B}} - G_{\mathrm{A}} = \sum_{i=0}^{N-1}\Delta G_{\lambda_i} \qquad (4)$$

In the slow growth approach, the coupling parameter λ is defined as a function of time. By adding, at each MD time step, a $\Delta\lambda$ small enough to allow the system to remain in equilibrium, the ensemble average can be replaced by a single value and eq 4 becomes

$$\Delta G = \sum_{\lambda} V_{\lambda'}(x) - V_{\lambda}(x) \qquad (5)$$

where $V_{\lambda'}(x)$ is the perturbed potential. These two FEP methods[16] as well as the dynamically modified windows method[17] are implemented in the GIBBS module of AMBER 4.0 and use the empirical potential V_{total} shown in eq 1.

Calculation of relative solvation free energy $\Delta\Delta G_{\mathrm{solv}}$ of two molecules, M and N, is based on the thermodynamic cycle shown in Figure 1. Since the free energy is a state function, $\Delta\Delta G_{\mathrm{solv}}$ is given by

$$\Delta\Delta G_{\mathrm{solv}} = \Delta G_{\mathrm{solv}}(\mathrm{N}) - \Delta G_{\mathrm{solv}}(\mathrm{M}) = \Delta G_{\mathrm{MN(aq)}} - \Delta G_{\mathrm{MN(g)}} \qquad (6)$$

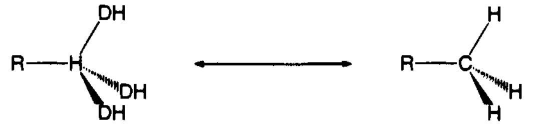

Figure 2. The R–H → R–CH$_3$ perturbation. In the "R–H" state (left), the hydrogen atom is connected to three dummy hydrogens (DH), which are required to maintain the same topology throughout the run. They are slowly mutated into a carbon atom and three "real" hydrogen atoms, respectively, during the perturbation to give the "R–CH$_3$" state (right).

The solvation free energies, $\Delta G_{\mathrm{solv}}(\mathrm{M})$ and $\Delta G_{\mathrm{solv}}(\mathrm{N})$, are experimentally measurable but are difficult to calculate. In contrast, $\Delta G_{\mathrm{MN(g)}}$ and $\Delta G_{\mathrm{MN(aq)}}$, which correspond to the non-physical mutation of M into N in the gas phase and in aqueous solution, respectively, could be obtained by computer simulations. Assuming comparable internal contributions to the free energy in the gas phase and in solution,[18] $\Delta G_{\mathrm{MN(g)}}$ can be neglected and the relative solvation free energy $\Delta\Delta G_{\mathrm{solv}}$ can be determined by a FEP calculation of $\Delta G_{\mathrm{MN(aq)}}$.

In this study, most of the perturbations performed consist of the mutation of one hydrogen to a methyl group, as shown in Figure 2, or of the reverse transformation. In the "R–H" state (Figure 2, left), the H atom is connected to three dummy hydrogens (DH) with zero van der Waals radii and well depth, and no charge. This H atom and the three DH dummies are slowly mutated to a carbon atom and three H atoms, respectively, via the coupling parameter λ, to lead to the "R–CH$_3$" state (Figure 2, right). Absolute solvation free energies can be obtained by mutating a complete molecule into Nothing (i.e., into dummy atoms) in explicit solvent. Although less accurate results are generally produced by this type of perturbation, satisfactory values could be obtained in the case of a small dipolar molecule by using longer simulation time and very small window width.

Geometries and Charges. The starting geometries of all the amides and amines were obtained by QM optimization at the 6-31G* basis set level. Charges were derived from the 6-31G* electrostatic potential using a two-stage fitting process. In the first stage, all the charges were optimized with weak hyperbolic restraints (wk) of 0.0005 au on non-hydrogen atoms. In the second stage, only the charges on methyl groups were reoptimized using strong hyperbolic restraints (st) of 0.001 au on non-hydrogen atoms, the charges on all other atoms being frozen. The methyl hydrogens were free (fr) during the first stage and constrained to be equivalent (eq) in the second stage. This restrained electrostatic potential (RESP) fit charge model has been described in detail in another paper.[5] The conformations chosen and the two-stage RESP (wk fr/st eq) charges (in electrons) obtained for the amides and for the amines are shown in Charts 1 and 2, respectively. The dipole moments (in debye) calculated from the RESP charges and the experimental values[19] are also displayed on these two charts.

For the amides, two other sets of charges were calculated. First, standard ESP (un ap) charges[5] were obtained from 6-31G* electrostatic potentials according to the method described by Singh and Kollman.[20] In this case, charges were unrestrained (un) and methyl hydrogens were averaged a posteriori (ap) to the fit. The second set of charges was calculated for AMBER geometries. For each amide, an *in vacuo* geometry optimization has been performed, using the MINMD module of the AMBER 4.0 package and the RESP charges previously obtained. From these geometries, new 6-31G* electrostatic potentials have been calculated and new RESP (wk fr/st eq) charges have been derived.

For all perturbations involving a non-solvent water molecule, TIP3P[21] geometry and charges were used.

Bonded and van der Waals Parameters. The atom types used to describe amides and amines are shown in Charts 1 and 2. For the

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Chart 1

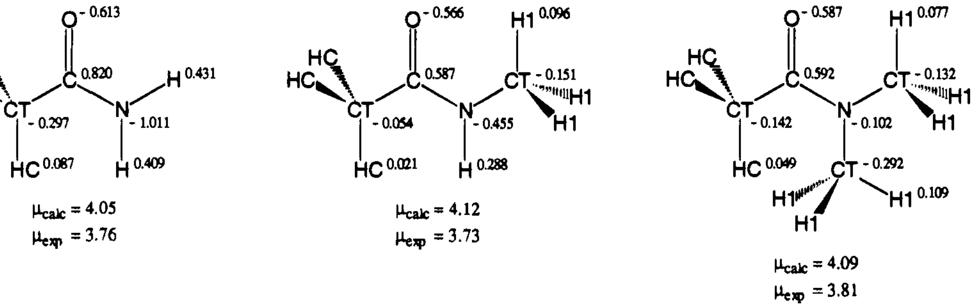
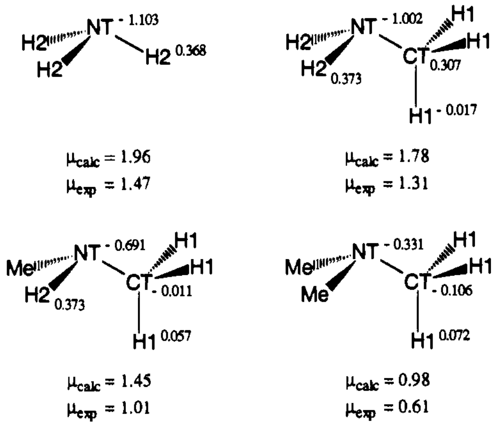


Chart 2



non-solvent water molecule, the OX and HX atom types were used. They are identical to the OW and HW types used for the TIP3P water model[21] but do not include the H−H "bond" used in the solvent H_2O to maintain a rigid internal geometry. The reason for creating this flexible "TIP3P-like" model is to enable calculations of a $H_2O \rightarrow NH_3$ mutation to be done without artefacts caused by mutating a rigid geometry of H_2O into a flexible one for NH_3. This flexible TIP3P model gave essentially the same free energy of solvation as rigid TIP3P.[4d] Most of the bond, angle, and torsion parameters were taken from the Weiner *et al.*[13] all-atom force field. Additional bonded parameters were taken from the Cornell *et al.*[22] force field or adapted from existing parameters and are presented in Table 1. The bonded parameters for the dummy atoms were chosen identical to the corresponding "real" atom parameters. For the ammonia to water perturbation, two non-standard bond angle stretching parameters have been used for H2−NT−H2 and HX−OX−DH; they are listed in Table 1.

Table 1. Additional Force Field Parameters

bond	K_r (kcal mol^{-1} Å^{-2})	r_{eq} (Å)	ref
CT−NT	367.0	1.471	22

angle	K_θ (kcal mol^{-1} rad^{-2})	θ_{eq} (deg)	ref
CT−NT−CT	60.0	109.5	22
NT−CT−H1	35.0	109.5	
CT−NT−H2	35.0	109.5	
H2−NT−H2	100.0	109.5	
HX−OX−DH	100.0	109.5	
HX−OX−HX	100.0	104.5	

dihedral angle	*idivf*	$V_n/2$ (kcal mol^{-1})	γ (deg)	*n*	ref
X−NT−CT−X	6	1.0	0.0	3	22

van der Waals	R^* (Å)	ϵ (kcal mol^{-1})
NT	1.875	0.1700
H2	0.689	0.0157

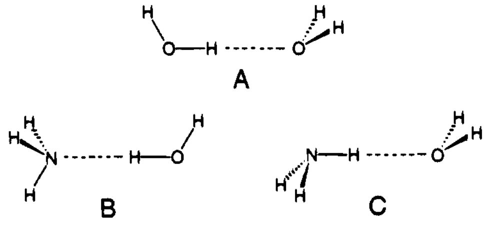


Figure 3. Structure of the three complexes used to develop the new van der Waals parameters for the amine's N−H group.

Van der Waals (VDW) parameters used in this study are those developed for the new force field[22] and have been previously published,[5] or come from the OPLS force field.[23] Preliminary amine calculations have been performed using the OPLS van der Waals parameters for nitrogen and the amide-H VDW parameter for the amino hydrogen. However, given the amine data and in order to be sure that we did the best we could for these calculations, new van der Waals parameters for the NT type (sp^3 nitrogen) and for the H2 type (amino hydrogen) have been developed. These parameters have been obtained, following the procedure of Veenstra *et al.*,[24] by adjusting the R^* values to reproduce hydrogen bond distances for NH_3−water complexes. The relation between A_{ij} and B_{ij} (eq 1) on one hand and R^* (van der Walls radii) and ϵ (well depth) on the other is described in ref 16. Quantum mechanical calculations have been carried out at the 6-31G* MP2 level using the Gaussian 92 program and molecular mechanic calculations have been performed with the ANAL module of the AMBER 4.0 package. Three complexes were studied (Figure 3). The water dimer (Figure 3A) was used to calibrate the MM versus QM results. The $H_3N\cdots H{-}OH$ (Figure 3B) and the $H_2N{-}H\cdots OH_2$ (Figure 3C) complexes were used to determine the best R^*(NT) and R^*(H2) values, respectively. For the complexes A and C, the water molecules have been moved along the axis containing one hydrogen−heteroatom bond and the water hydrogens were oriented to avoid any strong repulsions. In the case of complex B, the axis was defined by the nitrogen lone pair direction and the water molecule was approached with a hydrogen pointed toward this lone pair. The geometry of each molecule has been frozen. For the water molecule, TIP3P geometry and charges have been chosen. The NH_3 geometry has been derived from the standard bond lengths and bond angles found in the AMBER standard parameters list. The NH_3 charges were those shown in Chart 2. For the three complexes, the minimum energy distances have been found by sampling the energies at 0.1−Å intervals. The minimum values have been refined using a parabolic fit. The MM and QM water dimer calculations have shown that TIP3P underestimated the O−O distance by 0.16 Å. This value has been subtracted from the N−O distances obtained by the QM calculation of complexes B and C to give the "calibrated" values of 2.83 and 3.08 Å, respectively. Then, the R^*(NT) parameter has been adjusted in order to reproduce the "calibrated" N−O distance for complex B. The same procedure has been applied for the R^*(H2) parameter and complex C, using the

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Table 2. H-Bond Energies and Distances, Obtained with the NT and H2 Adjusted R^*, for NH_3–Water Complexes

complex[a]	ΔE (kcal mol^{-1})	distances (Å)
$H_3N\cdot\cdot H{-}OH$	-6.4^b	$r_{N\cdot\cdot O} = 2.83$
	$(-6.7)^c$	$r_{N\cdot\cdot H} = 1.87$
$H_2N{-}H\cdot\cdot OH_2$	-3.3^b	$r_{N\cdot\cdot O} = 3.08$
	$(-2.4)^c$	$r_{H\cdot\cdot O} = 2.07$

[a] See Figure 3. [b] MM minimum energies. [c] QM calibrated energies (see text).

adjusted R^*(NT) value. This two-step process has been repeated one more time to refine our R^* values. For the complexation energies, the TIP3P dimer has been found 1.4 kcal/mol less stable than the QM water dimer. The calibrated energy values for complexes B and C have been obtained by adding 1.4 to the QM energies and are shown in parentheses in Table 2. No attempt has been made to find better MM energies by adjusting the ϵ values. The new NT and H2 van der Waals parameters are given in Table 1, and the H-bond energies and distances obtained for the two NH_3–water complexes are presented in Table 2.

Molecular Dynamics/Free Energy Perturbation Calculations. Throughout all MD and FEP calculations, a constant dielectric ϵ of 1.0 and a 8 Å cutoff were used. Scale factors[5] of 0.83 (SCCE = 1.2) an 0.5 (SCNB = 2.0) were employed for the 1–4 electrostatic and 1–4 VDW interactions, respectively. All simulations were carried out at a constant temperature of 298 K and at a constant pressure of 1 atm using periodic boundary conditions and individual molecule center of mass pressure scaling (NPSCAL = 1). Unless otherwise mentioned, all the other control parameter values were those defined by default in the MINMD and GIBBS modules of AMBER 4.0.

Each solute molecule was placed in the center of a cubic box containing between 209 (for NH_3) and 355 (for *N,N*-dimethylacetamide) TIP3P water molecules. This system was first minimized using 100 cycles of steepest descent followed by 1000 cycles of conjugated gradients, then 20 ps of molecular dynamics were carried out to equilibrate the system. A time step of 2.0 fs was used and the SHAKE[25] procedure was applied to constrain all bonds involving hydrogen.

Most of the FEP calculations were carried out using the standard window growth method with 101 windows ($\Delta\lambda = 0.01$) and a time step of 2.0 fs. For each window, 1000 equilibration steps and 1000 collection steps were performed for a total of 404 ps. The SHAKE procedure was applied to constrain all bond lengths to their equilibrium values and bond-PMF correction[9] (NCORC = 1) was employed for all perturbations involving bond length changes. All regular runs (i.e., without electrostatic decoupling) were performed forward (λ: 1 → 0) and backward (λ: 0 → 1) and errors correspond to the observed hysteresis. In the case of the amides, many test runs were carried out. The first test was used to check the convergence and the number of windows has been doubled, all other parameters being unchanged. The slow growth method was used for the second test and a 400-ps perturbation has been performed using a time step of 2.0 fs. The other tests consist of charge perturbations and only the electrostatic contribution to the free energy has been calculated (IELPER = 1). In these cases, the window growth method has been employed and 21 windows of 500 equilibration/500 collection steps have been used with a time step of 2.0 fs. These perturbations have been performed only in the forward direction and errors were estimated from the double-wide sampling.

Absolute solvation free energies of H_2O, NH_3, and $MeNH_2$ have been determined using the so-called electrostatic decoupling scheme[8] in which two runs have to be performed, one for the electrostatic and the other for van der Waals contributions. The conditions described above for the regular runs were applied but, in this case, the perturbations have been carried out only in the forward direction.

Results

Table 3. Relative Free Energies of Solvation for the Amide Perturbations

perturbation	no. of windows	total time (ps) equil	total time (ps) coll	$\Delta\Delta G_{solv}$ (kcal mol^{-1})	expt[a]
ACT → NMA	101	202	202	2.09 ± 0.11	−0.40
ACT → NMA[b]	201	402	402	2.19 ± 0.01	
ACT → NMA	slow growth		400	2.41 ± 0.02	
NMA → NNDMA	101	202	202	1.05 ± 0.02	1.53

[a] Experimental numbers (in kcal/mol) from Wolfenden, ref 7. [b] Perturbation performed only in the forward direction.

$$\begin{tikzcd}
\mathrm{ACT}_{(\mathrm{RESP})} \arrow[r, "2.09 \pm 0.11"] \arrow[d, "-0.77 \pm 0.02"'] & \mathrm{NMA}_{(\mathrm{RESP})} \arrow[r, "1.05 \pm 0.02"] \arrow[d, "-0.72 \pm 0.00"'] & \mathrm{NNDMA}_{(\mathrm{RESP})} \arrow[d, "-0.85 \pm 0.00"'] \\
\mathrm{ACT}_{(\mathrm{ESP})} \arrow[r, "{[2.14 \pm 0.13]}"] & \mathrm{NMA}_{(\mathrm{ESP})} \arrow[r, "{[0.92 \pm 0.02]}"] & \mathrm{NNDMA}_{(\mathrm{ESP})}
\end{tikzcd}$$

Figure 4. Perturbation of the RESP (wk fr/st eq) charges into ESP (un ap) charges for the amide series. The $\Delta\Delta G_{solv}$ values of the upper horizontal processes come from Table 3. The three vertical processes correspond to the charge mutations. The numbers in brackets are calculated from the corresponding thermodynamic cycles. Free energies are in kcal/mol.

A. Amides. We have carried out relative solvation free energy calculations on acetamide (ACT) and on its two *N*-methylated derivatives, *trans*-*N*-methylacetamide (NMA) and *N,N*-dimethylacetamide (NNDMA). The results are presented in Table 3. Surprisingly, the substitution of the first N-bonded hydrogen of the acetamide molecule by a methyl group leads to a large increase of the hydrophobicity, in complete disagreement with experiment,[7] which finds NMA slightly more hydrophilic than ACT. To determine whether the convergence was reached, a longer simulation (804 ps) was run with twice the windows but the same number of equilibration and collection steps per window. This perturbation, which was performed only in one direction, gives roughly the same result as the shorter one, confirming that the 404-ps and 101-windows conditions were adequate to obtain proper sampling. In order to check if the difference between calculated and experimental relative solvation free energies was not due to an artefact of the window growth method, we have also carried out a 400-ps perturbation using the slow growth protocol. In this case, the result is slightly more positive than in the window growth case, but in the same order of magnitude, clearly indicating that these values are consistent with our charges and van der Waals parameters.

To study the influence of the amide charges on their relative solvation free energies, we have performed a series of perturbations where only the charges were modified. First, we have mutated, for each amide, the RESP (wk fr/st eq) charges into the ESP (un ap) changes. The results are shown in the thermodynamic cycle presented in Figure 4. However, mutating RESP to ESP charges has only a very small effect on the relative solvation free energy since the net stabilization of ACT versus NMA is only 0.05 kcal/mol.

As the earlier calculations of Bash *et al.*[8] had led to a very similar solvation free energy for ACT and NMA, it was interesting to see how the new van der Waals parameters and the bond PMF correction, as they are use in actual free energy calculations, would modify this result. To this aim, we have done the mutation of the RESP charges into the Bash charges. The results are presented in Figure 5. The use of the Bash charges, which had been scaled to reproduce the experimental electric dipole moments, has a much more destabilizing effect on acetamide than on the *N*-methylacetamide molecule, but the value obtained for the ACT → NMA perturbation, although

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$$\begin{tikzcd}
\mathrm{ACT}_{(\mathrm{Bash})} \arrow[r, "{[1.30 \pm 0.14]}"] & \mathrm{NMA}_{(\mathrm{Bash})} \\
\mathrm{ACT}_{(\mathrm{RESP})} \arrow[u, "1.68 \pm 0.02"] \arrow[r, "2.09 \pm 0.11"] \arrow[d, "-1.31 \pm 0.00"] & \mathrm{NMA}_{(\mathrm{RESP})} \arrow[u, "0.89 \pm 0.01"] \arrow[d, "-1.18 \pm 0.01"] \\
\mathrm{ACT}_{(\mathrm{AMBER})} \arrow[r, "{[2.22 \pm 0.12]}"] & \mathrm{NMA}_{(\mathrm{AMBER})}
\end{tikzcd}$$

Figure 5. Influence of the charges on the relative solvation free energy of *N*-methylacetamide versus acetamide. The upper part corresponds to the mutation of the RESP (wk fr/st eq) charges into the Bash[8] charges. The lower part corresponds to the perturbation of the RESP charges calculated from the 6-31G* geometries into the RESP (wk fr/st eq) charges derived from the AMBER optimized geometries. The numbers in brackets are calculated from the corresponding thermodynamic cycles. Free energies are in kcal/mol.

smaller than the previous ones, is still 1.7 kcal/mol more positive than experiment.

Charge density being closely related to geometry, we have also calculated the RESP charges for the AMBER geometries and perturbed the original 6-31G*/RESP charges to the AMBER/RESP charges. The results are shown in Figure 5. In this case, we have found a relative solvation free energy of 2.22 ± 0.12 kcal/mol, which is very close to the value of 2.09 ± 0.11 kcal/mol obtained for the 6-31G*/RESP charges. This result shows that our relative solvation free energies are only slightly dependent on the geometry used to derive the charges.

The NMA to NNDMA perturbation, which corresponds to the mutation of the NMA amide hydrogen to a methyl group, gives a relative solvation free energy 0.5 kcal/mol less positive than experiment[7] (Table 3). The use of the ESP charges stabilizes *N,N*-dimethylacetamide more than *N*-methylacetamide and leads to a greater difference between experimental and calculated data (Figure 4). From these results and those obtained for the ACT → NMA perturbation, it can be seen that our calculations predict an increasingly hydrophobic product as we substitute amide hydrogens by methyl groups.

B. Amines. As for the amides, experimental relative solvation free energies of amines[26,27] show that the substitution of the first N-bonded hydrogen by a methyl group leads to a better solvated product (i.e., a more hydrophilic molecule), while subsequent substitutions decrease the hydrophilic character. Therefore, it was interesting to see if we were able to reproduce this trend or if, as in the amide case, a monotonic increase of the hydrophobicity would be observed. The results of these relative free energy calculations are reported in Table 4. As one can see, the calculated $\Delta\Delta G_{\mathrm{solv}}$ are again in disagreement with the experimental results. The ammonia-to-methylamine perturbation gives a positive free energy and further methyl substitutions lead to an increase of the hydrophobicity far more than found in the experimental data. It must also be noted that, in contrast to the amide series where the first addition of a methyl group is responsible for the largest increase of the hydrophobic character and the second produces a more modest change, in the amines, the more methyl groups we add, the larger the hydrophobic effect.

Table 4. Relative Free Energies of Solvation for the Amine Perturbations

perturbation	no. of windows	total time (ps) equil	total time (ps) coll	$\Delta\Delta G_{\mathrm{solv}}$ (kcal mol^{-1})	expt[a]
$NH_3 \rightarrow MeNH_2$	101	202	202	0.62 ± 0.05	−0.26
$MeNH_2 \rightarrow Me_2NH$	101	202	202	1.62 ± 0.01	0.27
$Me_2NH \rightarrow Me_3N$	101	202	202	2.34 ± 0.02	1.06
$NH_3 \rightarrow Me_3N$	201	402	402	4.36 ± 0.05	1.07
$NH_3 \rightarrow H_2O$	101	202	202	−2.17 ± 0.00	−2.01

[a] Experimental numbers (in kcal/mol) from Ben-Naim and Marcus, ref 26, and from Jones and Arnett (for Me_2NH and Me_3N), ref 27.

We have also performed an ammonia to trimethylamine perturbation, where the three ammonia hydrogens were simultaneously mutated to three methyl groups. Due to the greater structural change, we have used twice the windows and a total simulation time of 804 ps. In this case, we have obtained a $\Delta\Delta G_{\mathrm{solv}}$ close to the sum of the three single methyl additions, which is equal to 4.58 ± 0.08 kcal/mol as shown in Figure 6A, where the cycle closure error (indicated in a box) is close to zero. This result suggests that the calculations have converged and that our amine parameters are consistent inside the series.

At this point, it was interesting to relate this amine series to another molecule for which the calculated solvation free energy was known to be in good agreement with experiment. To this end, we have first carried out the ammonia-to-water perturbation (Table 4) and then the water-to-Nothing perturbation (Table 5). The calculation of the water absolute solvation free energy has been reported for many different water models[4] and, by using the TIP3P model and our perturbation conditions, we have found a value in excellent agreement with the experimental value published by Ben-Naim *et al.*[26] From this result and the value obtained from the $NH_3 \rightarrow H_2O$ perturbation, an absolute free energy of solvation of −4.17 ± 0.14 kcal/mol can be deduced for the NH_3 molecule. This value is surprisingly close to the ammonia experimental solvation free energy, which is equal to −4.31 kcal/mol, and tends to prove that, at least for ammonia, our calculations are able to reproduce the experimental ΔG_{solv}.

Two other absolute free energies of solvation have been calculated by mutating ammonia and methylamine to Nothing, respectively, and the results are presented in Table 5. In the ammonia case, the ΔG_{solv} (−4.16 ± 0.11 kcal/mol) is in excellent agreement with the value of −4.17 ± 0.14 kcal/mol deduced from the $NH_3 \rightarrow H_2O \rightarrow$ Nothing set of perturbations (Figure 6B). For the methylamine molecule, the absolute free energy of solvation is found about 1.0 kcal/mol less than the experimental values. However, this result is of particular interest. First, it shows qualitatively the same trend as those observed for our ammonia-to-methylamine perturbation calculation, i.e., that the $MeNH_2$ molecule is found less hydrophilic than NH_3. Secondly, the relative solvation free energy deduced from the ammonia and methylamine ΔG_{solv}, which is equal to 0.59 ± 0.24 kcal/mol, is in remarkable quantitative agreement with the value of 0.62 ± 0.05 obtained for the $NH_3 \rightarrow MeNH_2$ simulation (Figure 6C).

Discussion and Conclusions

We have presented a molecular dynamics/free energy perturbation study of simple amines and amides and their methylated derivatives. Our calculations show that successive methyl additions to ammonia and to acetamide led to a monotonic increase of the hydrophobicity, in complete disagreement with experimental results. However, by performing specific perturbations, we have demonstrated that our calculations were internally consistent. In particular, we have obtained an excellent agreement between the NH_3 absolute solvation free energy, calculated by mutating ammonia to Nothing (−4.16 ±

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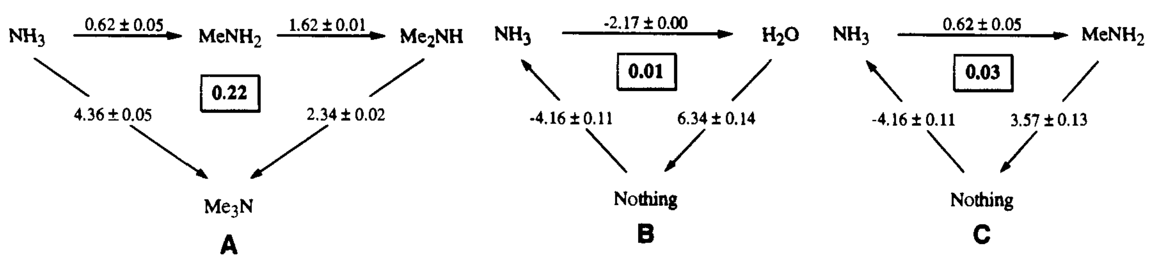

Figure 6. Thermodynamic cycles for the amines and amine/water perturbations. The cycle closure errors are indicated in the boxes. Free energies are in kcal/mol.

Table 5. Absolute Free Energies of Solvation

	free energies (kcal mol^{-1})			
perturbation[a]	electrostatic	van der Waals	ΔG_{solv}	expt[b]
Nothing → NH_3	−6.14 ± 0.02	1.98 ± 0.09	−4.16 ± 0.11	−4.31
Nothing → $MeNH_2$[c]	−5.62 ± 0.01	2.05 ± 0.12	−3.57 ± 0.13	−4.57
Nothing → H_2O	−8.65 ± 0.00	2.31 ± 0.14	−6.34 ± 0.14	−6.32

[a] The perturbations have been performed in the "solute-to-Nothing" direction. [b] Experimental numbers (in kcal/mol) from Ben-Naim and Marcus, ref 26. [c] The calculation of the van der Waals contribution has been divided in three parts. For the first part ($\lambda = 1.0 \pm 0.03$), 98 windows and a $\Delta\lambda$ of 0.01 have been used. The second part ($\lambda = 0.03 \pm 0.01$) has been calculated using 41 windows and a smaller $\Delta\lambda$ (0.0005). For the last part ($\lambda = 0.01 \pm 0.0$), 51 windows have been used ($\Delta\lambda = 0.0002$).

0.11 kcal/mol), and the value derived from the $NH_3 \rightarrow H_2O \rightarrow$ Nothing set of perturbations (−4.17 ± 0.14 kcal/mol). A very good agreement has also been obtained for the $NH_3 \rightarrow MeNH_2$ perturbation since the value given for the direct perturbation (0.62 ± 0.05 kcal/mol) is very close to the absolute free energy difference (0.59 ± 0.24 kcal/mol) of the two compounds. Even in the $NH_3 \rightarrow Me_3N$ case, in which three methyl groups were created simultaneously, the relative solvation free energy obtained for the direct perturbation (4.36 ± 0.05 kcal/mol) is close to the value of 4.58 ± 0.08 derived from the three sequential methyl additions. In each case, the free energy along the closed thermodynamic cycle is very close to zero (Figure 6). The disagreement between our calculated relative solvation free energies and the experimental values is even more surprising since calculations on methane, ethane, propane,[3] methanol,[5] and dimethyl ether[6] are known to give $\Delta\Delta G_{solv}$ values in excellent to very good agreement with experiment.

For the methane, ethane, and propane perturbations, Sun *et al.*[3] have shown that relative solvation free energy could be calculated within ∼0.2−0.3 kcal/mol of experiment even though the simulation of small nonpolar mutations is very difficult due to a delicate balance between positive exchange repulsion and negative dispersion effects. In the case of dipolar molecules, the electrostatic term being dominant, simulations are usually more accurate and require shorter time to converge.

Indeed, the experimental behavior of the amines is very strange and it is interesting to compare the H_2O, MeOH, Me_2O series to our amines. The experimental relative solvation free energy for the $H_2O \rightarrow MeOH$ transformation is equal to 1.2 kcal/mol[26] whereas the corresponding methyl addition to NH_3 leads to a negative experimental $\Delta\Delta G_{solv}$ value of −0.26 kcal/mol, but to a calculated relative solvation free energy of 0.62 kcal/mol. The same observation can be done for the second methyl group addition where the experimental free energy values obtained for the $MeOH \rightarrow Me_2O$ and for the $MeNH_2 \rightarrow Me_2NH$ transformations are ∼3.2[28] and 0.27 kcal/mol, respectively, while our calculated $\Delta\Delta G_{solv}$ for the amines is equal to 1.6 kcal/mol. We also note that excellent accord with experiment has been observed for methanol to methane,[5] methanol to ethane,[5] and methanol to dimethyl ether[6] perturbations. In each case, addition of a methyl group leads to a loss of hydrogen-bonding capability and, as one can see, for the amines NH_3 and $MeNH_2$ we obtain calculated free energies for methyl substitution corresponding to about half of the experimental (or calculated) values reported for the substitution of methyl groups in water and methanol. These results seem reasonable, since the O−H group ability to make hydrogen bonds is greater than that for N−H, but they are in contradiction to experiment for the amines.

Why are the amines different? One possible cause could be the pK_a differences among the amine series. However, according to Ben-Naim,[26] the experimental solvation free energies have been corrected and are given for amines in their un-ionized state. Could unusual polarization effects be responsible for such a difference in the amine behavior? Our charges are derived from the 6-31G* basis set, which overestimates gas phase dipole moments by an amount of 10−20%. This overestimation, which is even slightly larger for the two-stage RESP (wk fr/st eq) charges,[5] is consistent with the TIP3P "effective two-body" water model as it contains approximately the amount of polarization included in this model. As we can expect, the dipole moments of the amines (Chart 2) are comparably enhanced over the experimental gas phase values. However, there is no necessary reason for the solute to be polarized as much as the solvent in aqueous solution[29] and some subtle error in our representation of relative polarization effects cannot be excluded. Another possible source of error in these free energy calculations is our neglect of intrasolute free energies (i.e., the assumption that the free energies due to such terms are identical in the gas phase and solutions). We have demonstrated that this is an excellent approximation in various mutations involving MeOH,[30] NMA,[30] and dimethyl ether.[6] That this is a source of error is made further unlikely by the fact that "rigid" Monte Carlo calculations, which neglect intramolecular free energies,[1,2] do an excellent job in reproducing experimental free energies of solvation for many molecules.

The absolute solvation free energy obtained for ammonia (−4.16 ± 0.11 kcal/mol) is very close to the experimental value of −4.31 kcal/mol, suggesting that our representation (i.e., charge and VDW parameters) of NH_3 is correct. The methylamine absolute free energy of solvation is calculated 1.0 kcal/mol less negative than the experimental value, and along the amine series, we can see that we greatly overestimate the destabilization produced by the successive methyl additions. This result could suggest an unusual methyl effect, although to our knowledge, there is no precedence for such an effect. Moreover, Orozco *et al.*[31] calculations of absolute solvation free energies, using the Miertus−Scrocco−Tomasi SCFR method as well as the MD/FEP protocol, lead also to a methylamine much more hydrophobic than ammonia.

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Acetamide has both large proton-donor (PD) and proton-acceptor (PA) regions, favorable to fit into the water structure. Duffy *et al.*[32] have found an average of 1.6 H-bonds for the PD site (NH2 group) and 1.74 H-bonds for the PA site (carbonyl oxygen). Intuitively, addition of a methyl group, by decreasing the acetamide H-bonding capability, should make the *N*-methylacetamide more hydrophobic, contrary to the experimental observation. Our calculations show a destabilizing effect twice as large for the first methyl addition as for the second addition. These results suggest that the first methyl not only removes an H-bond site but also disrupts the highly organized structure of the water molecules around the solute, while the second methyl addition occurs in a less organized environment and mainly acts by occupying the last PD site of the amide. It should be noted that Jorgensen *et al.*[33] have found a total of 3.0 H-bonds (C=O: 2.1; N−H: 0.9) for the *N*-methylacetamide and 1.55 H-bonds for the *N,N*-dimethylacetamide. It is surprising that for the NMA case, the net loss of H-bonding capability (−0.34 H-bond) is relatively small compared to the large positive $\Delta\Delta G_{\mathrm{solv}}$ we obtain for the ACT → NMA perturbation, whereas for NNDMA, the loss of 1.8 H-bonds is responsible for a smaller effect on the $\Delta\Delta G_{\mathrm{solv}}$ in the NMA → NNDMA transformation. We should note in this regard that our model[5] has found a relative free energy of *N*-methylacetamide and CH_4 of 11.6 kcal/mol, compared to the experimental value (using Wolfenden's solvation free energy[7] of NMA of −10.1 kcal/mol and the methane value of 2.0 kcal/mol[26]) of 12.1 kcal/mol. Our calculated solvation free energy of methane in TIP3P water is 2.7 kcal/mol with no long-range-effect correction and 2.5 kcal/mol with long-range cutoff correction.[34] Using the uncorrected value leads to a calculated solvation free energy of −8.9 kcal/mol for NMA and, using the results from Table 3, a calculated solvation free energy of −11.0 kcal/mol for ACT. Both deviate from their experimental value in opposite directions by about 1.2 kcal/mol. Carlson *et al.*[35] has found ESP free energies for ACT of ∼−13.4 kcal/mol. The difference between the two ACT values likely stems from the use of TIP4P rather than TIP3P water, the different van der Waals parameters (probably most important is the use of $R^* = 0.0$ Å for amide hydrogen in OPLS and $R^* = 0.6$ Å here) and (Figure 4) the less negative solvation free energy of RESP than ESP by 0.8 kcal/mol. Within the OPLS model,[36] the addition of methyl groups to ACT reduces the solvation free energy by 2.6 kcal/mol (ACT → NMA) and 1.3 kcal/mol (NMA → NNDMA) very similar to the methyl effects found here.

Since this work was submitted for publication, we have learned of three other complementary studies which have addressed the solvation free energies of some of the molecules considered here. Tannor *et al.*[37] have used a combination of *ab initio* calculations and a continuum solvation model (GVBcon) to study the relative solvation free energies of ACT, NMA, $MeNH_2$, Me_2NH, and Me_3N; Cramer and Truhlar[38] have applied the combined semiempirical quantum mechanical/continuum model SM2 to ACT, NMA, NNDMA, $MeNH_2$, Me_2NH, and Me_3N and Ding *et al.* (DFEP) have applied both additive and non-additive molecular mechanics/free energy calculations to NH_3, $MeNH_2$, Me_2NH, and Me_3N and additive models to ACT, NMA, and NNDMA with a different force field than used here (MFEP). All the models agree that the relative solvation free energies of ACT and NMA are in the range of 2−3 kcal/mol: MFEP (2.1−2.4); GVBcon (2.0); SM2 (2.1) and DFEP (3.0). The agreement is also very good for NMA → NNDMA: MFEP (1.1); SM2 (0.8); DFEP (0.9). For $NH_3 \rightarrow MeNH_2$, MFEP finds 0.6 kcal/mol and DFEP finds 1.1 kcal/mol (additive) and 0.3 kcal/mol (non-additive). For $MeNH_2 \rightarrow Me_2NH$, MFEP finds 1.6 kcal/mol, GVBcon 1.6 kcal/mol, SM2 1.9 kcal/mol, and DFEP 3.2 kcal/mol (additive) and 2.5 kcal/mol (non-additive). For $Me_2NH \rightarrow Me_3N$, MFEP finds 2.2 kcal/mol, GVBcon 1.5 kcal/mol, SM2 1.7 kcal/mol, and DFEP 1.9 kcal/mol (additive) and 0.6 kcal/mol (non-additive). For the relative solvation free energy of NH_3 and Me_3N, MFEP finds 4.3 kcal/mol and DFEP finds 6.2 kcal/mol (additive) and 3.4 kcal/mol (non-additive) compared to the experimental value of 1.1 kcal/mol. Thus, all the models are generally consistent for the amides and in disagreement with experiment for ACT → NMA. Ding *et al.*[39] and Meng and Kollman[40] find that non-additive effects bring the solvation free energies of the amines in closer agreement with experiment, but a significant discrepancy remains for $NH_3 \rightarrow Me_3N$ (calculated 3−4 kcal/mol and experiment 1.1 kcal/mol).

At this point, we cannot argue why/if experiments are wrong or if our calculations suffer some unusual artefacts on amines and amides not seen with hydrocarbons, alcohols, and ethers. Nevertheless, in this paper we have presented results on solvation free energy calculations of amines and amides which have been proven to be internally consistent, but in qualitative (and quantitative) disagreement with the existing experimental data. These results highlight the need of further examinations from both fundamental theories and experiments. It should be mentioned that it is tricky to measure the ΔG of vaporization of the highly soluble amides and, perhaps, the way the neutral amine solvation free energies are corrected for the presence of the protonated form needs to be reexamined.

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