Calculation of Chloroform/Water Partition Coefficients for the N-Methylated Nucleic Acid Bases

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The chloroform/water partition coefficients have been determined for five N-methylated nucleic acid bases (9-methyladenine, 9-methylguanine, 1-methylcytosine, 1-methythymine, and 1-methyluracil) by calculating the absolute solvation free energies of the bases in chloroform and using the data for water calculated previously by Miller and Kollman *(J. Phys. Chem.* **1996**, *100*, 8587). Thermodynamic perturbation calculations with explicit solvent were used to determine the absolute solvation free energies. Two separate sets of chloroform simulations were performed differing only in the choice of atomic charges for chloroform and the bases. In the first simulations, labeled as charge set 1 (CS1), RESP was used with the RHF/6-31G(3df,2p) basis set to determine the chloroform charges and the RHF/6-31G* basis set to determine the charges for the nucleic acid bases. For the second simulations, labeled as charge set 2 (CS2), the CS1 chloroform charges were scaled down to reproduce the experimental dipole moment in solution, and the CS1 bases charges were reduced by 10%. The reduction of the atomic charges in the second model was done to account for the less polarizing environment of chloroform (as compared to water) and yielded a significant improvement in the calculated results. The partition coefficients calculated from CS1 yielded an average absolute error of 1.5 log units compared to experiment, where in contrast, CS2 shows good agreement, with an average absolute error of only 0.5 log units.

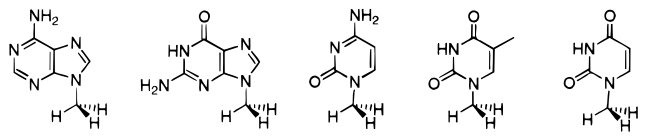
Introduction

The low volatility of the N-methylated nucleic acid bases has precluded an accurate experimental determination of their absolute solvation free energies.[1] In water, the solvation free energies have been estimated only for adenine and thymine,[2] and there is no experimental data for solvation in chloroform. However, it is much easier to determine the partitioning between chloroform and water of the bases, and these partition coefficients have been measured by Wolfenden.[1]

The partition coefficients can also be determined theoretically by calculating the absolute solvation free energies of the bases in both chloroform and water, and these results have appeared recently.[3,4] Computational methods have been used in related work by a number of groups to calculate the solvation free energies in water with both explicit[5−7] and implicit solvent.[3,4,8−12] Much less attention has been paid to solvation in chloroform, with only one implicit[3] and one explicit[4] solvent study to date.

When employing a molecular mechanics representation of such polarizable systems with explicit solvent, careful consideration must be given to the nature of the atomic charges used for both the solute and the solvent. In particular, the dramatic difference in polarity between water and chloroform raises the question as to whether it is appropriate to use the same electrostatic representation (partial charges) of a solute molecule for simulations in chloroform as that used for simulations in water. Classical two body additive force fields such as AMBER[13] with the Cornell et al. force field[14] and OPLS[15] commonly use parameters developed for water simulations for calculations with nonpolar solvents. This may be inappropriate as the partial charges used to represent solute molecules in water simulations are enhanced compared to their gas phase values in order to implicitly account for the polarization by the aqueous solvent.

In this manuscript we report the determination of the chloroform/water partition coefficients for the five N-methylated nucleic acid bases (shown below): 9-methyladenine, 9-methylguanine, 1-methylcytosine, 1-methythymine, and 1-methyluracil.



We have used thermodynamic perturbation simulations to determine the solvation free energies in chloroform and combined these with the previously reported results in water[7] to obtain the partition coefficients. To address the charge set issue, we have run two sets of chloroform simulations. The partial charges used for the bases in the first simulation are comparable to the charges that would be used for simulations in water. The partial charges for the bases and for chloroform were scaled down in the second simulations in an attempt to counteract the overpolarization from using a "water-like" charge set. Additionally, where possible, comparisons are made to experimental[1] and other calculated[3,4] results.

Computational Methods

All of the molecular dynamics and thermodynamic perturbation calculations in this work have been performed using the Cornell et al.[14] force field and the AMBER 4.1[13] suite of programs. The free energy differences were calculated using thermodynamic integration (TI). A detailed explanation of the methods used for these calculations has been presented previously by Miller and Kollman.[7]

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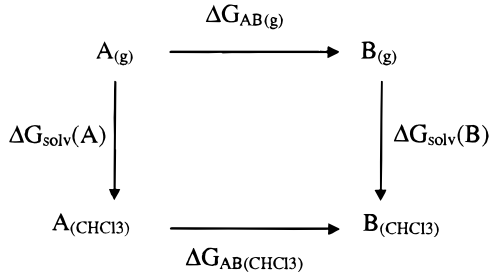

Figure 1. Thermodynamic cycle used to determine the relative solvation free energies of the two molecules A and B.

The chloroform/water partition coefficients were calculated according to the following equation:

$$\log P(\mathrm{CHCl_3/H_2O}) = -\frac{1}{2.303RT}(\Delta G_{\mathrm{CHCl_3}} - \Delta G_{\mathrm{H_2O}}) \quad (1)$$

The absolute solvation free energy (ΔG) of the each N-methylated nucleic acid base was determined by summing the calculated solvation free energy of the base relative to methane and the calculated absolute solvation free energy of methane.

$$\Delta G_{\mathrm{solv}}(\mathrm{Base}) = \Delta\Delta G_{\mathrm{solv}}(\mathrm{Base}\rightarrow\mathrm{Me}) + \Delta G_{\mathrm{solv}}(\mathrm{Me}) \quad (2)$$

The thermodynamic cycle illustrated in Figure 1 was used to calculated the relative solvation free energies, $\Delta\Delta G_{\mathrm{solv}}$(Base-Me), for the bases in chloroform. While the experimental solvation free energies, ΔG_{solv}(A) and ΔG_{solv}(B), are difficult to calculate directly, the relative free energy differences, $\Delta G_{\mathrm{AB(CHCl3)}}$ and $\Delta G_{\mathrm{AB(g)}}$, can be calculated with TI. In this study we have not assumed that the internal contributions to the free energy differences are comparable in the gas phase and in solution, and thus we have calculated both differences explicitly. From Figure 1, the relative free energies of solvation of molecules A and B can be determined as

$$\Delta\Delta G_{\mathrm{solv}} = \Delta G_{\mathrm{AB(CHCl3)}} - \Delta G_{\mathrm{AB(g)}} \quad (3)$$

The absolute solvation free energy of methane (ΔG_{solv}(Me)) was determined by perturbing methane to nothing both in solution and in the gas phase. Previous calculations on the solvation free energies of the bases in water[7] have shown the necessity to use electrostatic decoupling in all simulations.

We performed two separate sets of simulations with the nucleic acid bases in chloroform. The first set of simulations, where the solute and solvent molecules have "full" charges determined from RESP[16] calculations is labeled as charge set 1 (CS1). In the second set of simulations, labeled as charge set 2 (CS2), we scaled down the charges of both chloroform and the bases. For both CS1 and CS2, the bond stretching, angle bending, and van der Waals parameters for the flexible all-atom chloroform model were taken from Fox and Kollman[17] and are given in Table 1. The gas phase experimental geometry[18] was used as the equilibrium structure. The stretching and bending force constants for chlorine were derived by taking the values for the corresponding fluoro parameters in the Cornell et al. force field and scaling them by the ratio of the force constants for Cl and F in the MM2 force field.[17]

For the CS1 simulations, chloroform charges were determined with RESP[16] and the 6-31G(3df,2p) basis set and are shown in Table 1. The calculated dipole moment is 1.36 D. Experimentally, the dipole moment has been measured as 1.1 D in solution[19] and 1.0 D in the gas phase.[20] Pure liquid simulations of chloroform with this model yielded a density of 1.470 g/cm^3, compared to the experimental value[20] of 1.473 g/cm^3. The calculated ΔH_{vap} was 7.04, versus 7.43 kcal/mol measured experimentally.[20]

TABLE 1: Force Field Parameters for Chloroform

atom	R^* (Å)	ϵ (kcal/mol)	CS1[a] charge (au)	CS2[b] charge (au)
C	1.908	0.1094	−0.476	−0.386
C1	2.000	0.2550	0.049	0.040
H	1.187	0.0157	0.329	0.266

parameter	equilibrium values[c]	force constants[d]
C−C1	1.758	232.4
C−H	1.100	340.0
H−C−C1	107.7	38.1
C1−C−C1	111.2	77.7

[a] RESP charges using the RHF/6-31(3df,2p) basis set. [b] RESP charges from the RHF/6-31(3df,2p) basis set, then reduced by 19%. [c] Bonds in Å, angles in deg. [d] In kcal mol^{-1} rad^{-2}.

The atomic charges of the nucleic acid bases were calculated using RESP with the 6-31G* basis set. No additional force field parameters had to be developed for the bases.

The CS2 parameters differ from CS1 only in the atomic charge. In the case of chloroform, we have scaled down the charges from CS1 by 19% to match the experimental solution phase dipole moment of 1.1 D. The revised chloroform charges are shown in Table 1. In pure chloroform liquid simulations the reduced charges have essentially no effect on the calculated density. The calculated ΔH_{vap} is somewhat smaller, 6.87 kcal/mol, compared to 7.04 kcal/mol for CS1, and 7.43 kcal/mol from experiment. The electrostatics contribute only about 6% of the total interaction energy, and it can be seen that moderate changes in the charge model have only a small effect on the pure solvent simulations. There is however a significant change between CS1 and CS2 in the presence of a solute molecule.

For the nucleic acid bases with CS2,we scaled the RHF/6-31G* RESP charges used in CS1 by 0.9. The HF/6-31G* basis set is known to enhance the polarity of molecules, leading to larger calculated dipole moments as compared to experimental gas phase values.[14] This is reasonable for water simulations, but chloroform is not able to polarize the solute to the same extent and thus raises the concern that the magnitude of the charges determined with RESP using this basis set are too large. Caldwell and Kollman,[21] in a study of methanol and *N*-methylacetimide, achieved excellent results by reducing the calculated 6-31G* RESP charges by factors of 0.86−0.88 (this reduced the charges to gas phase values) and adding isotropic atomic polarizabilities. They created a nonadditive model that accurately reproduced the structures and energetics of these pure liquids. In this study, scaling the CS1 base charges by 0.9 yields values that are representative of a slight polarization of the gas phase charges and thus are reasonable to use in the chloroform simulations. We emphasize this scale factor of 0.9 is just an approximate value and no attempt has been made to vary this value to improve agreement with experiment.

The molecular dynamics and TI calculations with CS1 were carried out in the NPT ensemble with periodic boundary conditions, a temperature of 300 K and a pressure of 1 atm. A time step of 1.0 fs was used to ensure stability in the simulations. We used a nonbonded cutoff of 12 Å together with the long-range cutoff correction[22] and updated the nonbonded pair list every 10 steps.[23] Temperature was maintained with the Berendsen coupling algorithm[24] with separate solute/solvent coupling. To reduce temperature fluctuations, it was necessary to randomize the velocities every 500 steps.[17]

Each system consisted of the initial N-methylated base and between 178 and 293 $CHCl_3$ molecules. After 1000 steps of

TABLE 2: Calculated Free Energies for the Base to Methane and Methane to Nothing Perturbations in Water and Chlororform

	ΔG (kcal/mol)	uncertainty (kcal/mol)[a]
	In Chloroform (CS1)	
A to Me	14.44	0.34
G to Me	20.18	0.06
C to Me	15.39	0.20
T to Me	13.15	1.19
U to Me	14.02	0.02
Me to nothing	−0.50	0.14
	In Chloroform (CS2)	
A to Me	11.97	0.21
G to Me	16.95	0.50
C to Me	12.87	0.32
T to Me	11.14	0.13
U to Me	11.39	0.27
Me to nothing	−0.18	0.19
	In Water[b]	
A to Me	13.90	0.14
G to Me	24.34	0.19
C to Me	20.30	0.24
T to Me	14.34	0.14
U to Me	15.92	0.06
Me to nothing[c]	2.47	0.04

[a] Reported as half the difference between forward and backward run (hysteresis). [b] Reference 7. [c] Reference 25.

energy minimization, the systems were heated to 300 K over 20 ps and then equilibrated for an additional 50 ps.

TI simulations were run in the forward and backward direction for 202 ps with 10 ps of equilibration in between. With electrostatic decoupling, a single forward run consisted of two separate simulations, a 202 ps simulation in which the electrostatics were perturbed, and a 202 ps simulation involving the perturbation of the Lennard-Jones parameters. The free energy change for the forward run is taken as the sum of the two individual perturbations. Each simulation was broken into 101 windows, each comprising 1000 steps of equilibration and 1000 steps of data collection. The free energy is reported as an average of the forward and backward runs, and the uncertainty is taken to be half of the difference between them (hysteresis).

The protocol for the simulations with CS2 was slightly modified. It was noted from the simulations with CS1, that the vdW part of the perturbations took longer to converge. For this reason, the vdW perturbations with CS2 were lengthened to 404 ps (101 windows, with 2000 steps of equilibration, and 2000 steps of data collection). The electrostatic perturbations were still run for 202 ps.

Results and Discussion

Solvation Free Energies. The calculated solvation free energies for the bases relative to methane and for the disappearance of methane in both chloroform (CS1 and CS2) and water[7] are shown in Table 2. With both charge sets the free energy change for all of the base to methane perturbations is large and positive. This is expected since the bases are much more polar and hydrophilic than methane. The magnitude of the change is less in chloroform compared to water, with the exception of adenine with CS1. The uncertainty in the simulations varies from 0.02 kcal/mol (for U to Me with CS1) to 1.19 kcal/mol (for T to Me with CS1), and in most cases is less than 0.4 kcal/mol.

With CS1 and CS2, the disappearance of methane in chloroform is favored by 0.50 ± 0.14 and 0.18 ± 0.19 kcal/mol respectively (Table 2). As expected, the free energy change is smaller with CS2 because of the reduced magnitude of the electrostatic interactions. For the solvation free energy of methane in water, we used the calculated value from Sun and Kollman[25] of 2.47 ± 0.04 kcal/mol.

TABLE 3: Calculated Solvation Free Energies (kcal/mol) of the Nucleic Acid Bases in Chloroform

method	A	G	C	T	U
CS1	−13.9	−19.7	−14.9	−12.6	−13.5
CS2	−11.8	−16.8	−12.7	−11.0	−11.2
OPLS/FEP[a]	−14.2	−17.4	−15.3	−14.5	−13.0
SM5.4/A[b]	−13.3	−15.9	−15.5	−9.2	−8.9
AM1-MST[a]	−10.4	−14.2	−11.3	−9.5	−9.1
6-31G*-MST[a]	−10.5	−15.4	−12.4	−10.2	−10.0

[a] Reference 4. [b] Reference 3.

TABLE 4: Calculated Solvation Free Energies (kcal/mol) of the Nucleic Acid Bases in Water

method	A	G	C	T	U
AMBER/TI[a]	−11.4	−21.9	−17.6	−11.9	−13.4
OPLS/FEP[b]	−11.6	−21.7	−20.1	−13.1	−12.8
SM5.4/A[c]	−15.2	−20.1	−19.9	−9.6	−10.5
AM1-MST[d]	−10.8	−21.1	−16.1	−10.1	−10.4
6-31G*-MST[d]	−8.5	−18.1	−15.1	−10.3	−10.9
exptl[e]	−13.6			−(9.1−12.7)	

[a] Reference 7. [b] Reference 6. [c] Reference 3. [d] Reference 4. [e] Reference 1.

The calculated absolute solvation free energies for the nucleic acid bases in chloroform with CS1 and CS2 are shown in Table 3. Solvation in chloroform is favorable for al1 of the bases, with the calculated ΔG ranging from −19.7 kcal/mol for guanine with CS1 to −11.0 kcal/mol for thymine with CS2. Both sets of calculations indicate the same ordering for the solvation free energies: G > C > A > U > T. With CS1 and CS2, guanine has the largest solvation free energy by 4.8 and 4.1 kcal/mol, respectively, over cytosine. Compared to CS1, the calculated solvation free energies from CS2 are less negative by at least 1.6 kcal/mol.

We have also included the calculated solvation free energies from other methods in Table 3. Orozco,[4] using OPLS parameters and Jorgensen's united atom chloroform model,[26] calculated the free energies of the bases relative to thymine, perturbed thymine to pyridine, and then used the experimental absolute solvation free energy of pyridine to determine the absolute solvation free energies of the bases in chloroform. Giesen and co-workers[3] used their SM5.4/A formalism (a continuum solvent method that uses class IV charges, allows for solute/solvent polarization, and accounts for nonelectrostatic solvation effects). Orozco[4] has also performed continuum solvent MST-SCRF calculations at the AM1 and 6-31G* levels of theory, calculating the free energy of solvation as the sum of three contributions: cavitation, van der Waals, and electrostatics, the latter making use of the MST method developed by Tomasi.[27,28]

There is no available experimental data for the solvation free energies of the bases in chloroform, and thus it is not possible to determine which method is most accurate. All of the methods rank guanine and cytosine as the most favorably solvated bases in chloroform. Most of the methods, except for OPLS/FEP, rank adenine as the third most favorably solvated. Our models rank thymine as the least favorably solvated, in contrast to the other methods which give uracil.

The calculated solvation free energies of the nucleic acid bases in water are presented in Table 4. The solvation free energies were determined by summing the results from the base to methane perturbations by Miller and Kollman,[7] with the calculated solvation free energy of methane from Sun and Kollman.[25] The limited available experimental data is also

TABLE 5: Calculated and Experimental Chloroform/Water Partition Coefficients (log *P*) and Average Absolute Error (AAE) As Compared to Experiment

method	A	G	C	T	U	AAE
CS1	1.8	−1.6	−2.0	0.5	0.1	1.5
CS2	0.3	−3.7	−3.6	−0.7	−1.6	0.5
OPLS/FEP[a]	1.9	−3.2	−3.5	1.0	0.1	1.2
SM5.4/A[b]	−1.4	−3.1	−3.2	−0.3	−1.2	0.2
AMl-MST[a]	−0.3	−5.1	−3.5	−0.4	−1.0	0.6
6-31G*-MST[a]	1.5	−2.0	−2.0	−0.1	−0.7	1.1
exptl[c]	−0.8	−3.5	−3.0	−0.4	−1.2	

[a] Reference 4. [b] Reference 3. [c] Reference 1.

presented.[2] The calculated solvation free energy for thymine is well within the range given for the experimental data. We underestimate the solvation free energy of adenine compared to experiment.

Table 4 also contains aqueous solvation free energies calculated by other methods, including OPLS/FEP,[6] and three continuum solvent methods, SM5.4/A,[3] AM1-MST,[4] and 6-31G*-MST.[4] All of the methods agree that guanine has the most negative solvation free energy, and the solvation free energy of thymine and uracil differ by no more than 1 kcal/mol. The largest variance in the results comes with adenine. The calculated solvation free energy from the various methods ranges from −8.5 to −15.2 kcal/mol.

There have been additional calculations of the solvation free energies of the nucleic acid bases in water.[5,8−12] These results cover a wide range of values, in the case of adenine, spanning from −5.1 kcal/mol[12] to −20.9 kcal/mol.[8] We present only the methods in which the solvation free energies have also been calculated in chloroform to allow for comparison in determination of the partition coefficients.

Chloroform/Water Partition Coefficients. The calculated chloroform/water partition coefficients are given in Table 5. The partition coefficients are calculated using eq 1 and the chloroform and water solvation free energies. The AMBER/TI water solvation free energies from Table 4 are used to calculate log *P* for both CS1 and CS2. Direct comparison can be made to Wolfenden's experimental results,[1] also shown in Table 5.

The calculated log *P* values with CS1 (from 0.9 for T to 2.6 for A) are all too positive compared to the experimental results. This is almost certainly because we used an aqueous polarized charge model for the solute and thus artificially enhanced the electrostatic interactions of the bases with $CHCl_3$.

We see a notable improvement with correlation to experiment using CS2. The bases now prefer solvation in water, with the exception of adenine, which still slightly favors chloroform. Guanine and cytosine both strongly favor solvation in water (calculated log *P* is −3.7 and −3.6, respectively), just as the experimental results indicate. The calculated partition coefficients for thymine and uracil are in good agreement with experiment: uracil favors solvation in water more strongly than thymine. This is reasonable, as the additional methyl group should make thymine more hydrophobic. Adenine has the largest deviation; the calculated value of 0.3, differs by 1.1 log units from the experimental value of −0.8.

A comparison can be made to partition coefficients calculated with other methods (Table 5). The OPLS/FEP calculations give results similar to CS1, with too positive values of log P ranging from 0.3 for G to 2.7 for A. The continuum solvent SM5.4/A results provide very good agreement with experiment. The parameters used in the solvation models are optimized by fitting to experimental solvation free energies in both water and chloroform and partition coefficients of a variety of organic molecules (not including the nucleic acid bases).[29] The largest

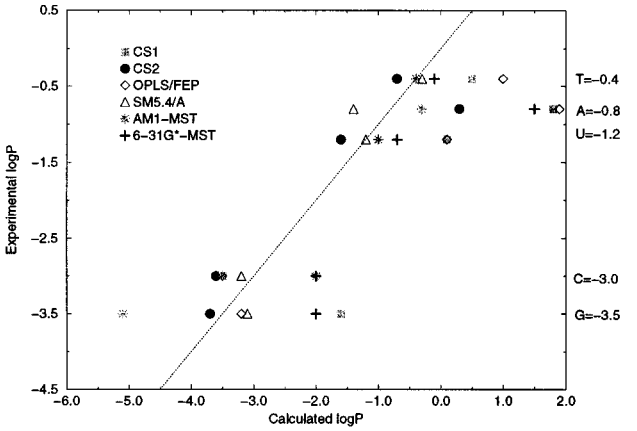

Figure 2. Comparison of experimental versus calculated values of log *P*. At the right side of the graph we indicate the experimentally determined values of the partition coefficients.

deviation with experiment comes with adenine (log *P*(calc) = −1.4, log *P*(exp) = −0.8); here solvation in water is favored too strongly compared to chloroform. The AM1-MST calculations indicate a very strong preference of guanine for solvation in water. Otherwise the results are in good agreement with experiment. The 6-31G*-MST calculations show an almost uniform underestimation of the preference of the bases for solvation in water.

The experimental versus calculated partition coefficients for the various methods discussed above have been plotted and are shown in Figure 2. The calculated partition coefficients are plotted on the *x*-axis, with the corresponding experimental values on the *y*-axis. The experimental partition coefficients are shown on the right-hand side of the plot for each of the nucleic acid bases. A thin dotted line with a slope of one and intercept of zero is also shown in the plot. Perfect agreement between calculated and experimental results would yield data points exactly on that line.

It is noteworthy that the poorest agreement with experiment is found with the two-body additive force fields, CS1 and OPLS (average absolute errors (AAE) of 1.5 and 1.2 log units, respectively), in which the charge model is the same in both solvents. The quantum mechanical continuum models do better, e.g. AM1-MST (AAE = 0.6), 6-31G*-MST (AAE = 1.1), and SM5.4/A (AAE = 0.2), although it is not clear why 6-31G*-MST is so much worse then AM1-MST. The performance of SM5.4/A (AAE = 0.2) is most impressive. The better performance of these models as compared to the force field methods makes sense, since they explicitly include polarization.

Conclusion

We have determined the chloroform/water partition coefficients for the five N-methylated nucleic acid bases using the calculated absolute solvation free energies of the bases in both chloroform and water. Comparisons have been made to the log *P* values determined experimentally[1] and also to other calculated methods.[3,4]

Of particular concern was determining the appropriate solute and solvent partial charges for use in the thermodynamic perturbation calculations. To investigate this, two sets of simulations were performed in chloroform: CS1, in which "water-like" partial charges were used for both the bases and chloroform, and CS2, in which the partial charges of the bases and the solvent were scaled down.

The partition coefficients calculated with the CS1 chloroform absolute solvation free energies show poor agreement with experiment (AAE = 1.5 log units). With CS2, the chloroform

charges were scaled to match the solution dipole of 1.1 D, and the partial charges of the bases were reduced to yield values close to the gas phase. The log *P* values calculated with CS2 show a significant improvement compared to CS1, and the AAE is reduced to 0.5 log units. We have also performed some calculations where the dipole moments of the bases are unmodified, but the charges on chloroform have been scaled to yield the experimental dipole moment of 1.1 D. This model, not presented in detail here, leads to errors roughly half way between CS1 and CS2, thus supporting the importance of an accurate electrostatic model for both the solvent and solute.

All in all, these studies make clear the importance of including explicit polarization effects in molecular mechanical models if one is going to accurately simulate properties of molecules in both polar and nonpolar environments. Our recent, more accurate "gas phase" models for nucleic acids bases,[30] combined with our models for explicit polarization in molecular mechanical force fields[21] makes it a propitious time for us to test such models. Calculations to do so are being initiated in our lab.

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References and Notes

(1) Cullis, P. M.; Wolfenden, R. *Biochemistry* **1981**, *20*, 3024.
(2) Ferguson, D. M.; Pearlman, D. A.; Swope, W. C.; Kollman, P. A. *J. Comput. Chem.* **1992**, *13*, 362.
(3) Giesen, D. J.; Chambers, C. C.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B* **1997**, *101*, 5084.
(4) Orozco, M.; Colominas, C.; Luque, F. J. *J. Chem. Phys.* **1996**, *209*, 19.
(5) Bash, P. A.; Singh, U. C.; Langridge, R.; Kollman, P. *A. Science* **1987**, *236*, 564.
(6) Elcock, A. H.; Richards, W. G. *J. Am. Chem. Soc.* **1993**, *115*, 7930.
(7) Miller, J. L.; Kollman, P. A. *J. Phys. Chem.* **1996**, *100*, 8587.
(8) Cramer, C. J.; Truhlar, D. G. *Chem. Phys. Lett.* **1993**, *202*, 567.
(9) Orozco, M.; Luque, F. J. *Biopolymers* **1993**, *33*, 1851.
(10) Young, P. E.; Hillier, I. J. *Chem. Phys. Lett.* **1993**, *215*, 405.
(11) Mohan, V.; Davis, M. E.; McCammon, J. A.; Pettitt, B. M. *J. Phys. Chem.* **1992**, *96*, 6428.
(12) Gao, J. L. *Biophys. Chem.* **1994**, *51*, 253−261.
(13) Pearlman, D. A.; Case, D. A.; Caldwell, J. W.; Ross, W. S.; Cheatham, T. E., III; Ferguson, D. M.; Seibel, G. L.; Singh, U. C.; Weiner, P. K.; Kollman, P. A. *AMBER 4.1*; University of California, San Francisco, CA, 1995.
(14) Cornell, W. D.; Cieplak, P.; Bayly, C. L; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179.
(15) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657.
(16) Bayly, C. L; Kollman, P. A. *J. Am. Chem. Soc.* **1994**, *116*, 697.
(17) Fox, T.; Kollman, P. A. *J. Phys. Chem.* **1997**, in press.
(18) Harmony, M. D.; Laurie, V. W.; Kuczkowski, R. L.; Schwendeman, R. H.; Ramsay, D. A.; Lovas, F. J.; Lafferty, W. J.; Maki, A. G. *J. Phys. Chem. Ref. Data* **1979**, *8*, 619.
(19) Glasel, J. A. *J. Am. Chem. Soc.* **1970**, *92*, 372.
(20) Weast, R. C., Ed. *CRC Handbook of Chemistry and Physics;* CRC Press: Boca Raton, FL, 1988.
(21) Caldwell, J. W.; Kollman, P. A. *J. Phys. Chem.* **1995**, *99*, 6208.
(22) Allen, M. P.; Tildesley, D. J. *Computer Simulation of Liquids;* Clarendon Press: London, 1987.
(23) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327.
(24) Berendsen, H. J. C.; Potsma, J. P. M.; van Gunsteren, W. F.; Di Nola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684.
(25) Sun, Y. X.; Kollman, P. A. *J. Comput. Chem.* **1995**, *16*, 1164.
(26) Jorgensen, W. L.; Briggs, J. M.; Conteras, M. L. *J. Phys. Chem.* **1990**, *94*, 1683.
(27) Tomasi, J.; Bonaccorsi, R.; Cammi, R.; Olivares del Valle, F. J. *J. Mol. Struct.: THEOCHEM* **1991**, *234*, 401.
(28) Tomasi, J.; Alagona, G.; Bonacccorsi, R.; Ghio, G.; Cammi, R. *Theoretical Models of Chemical Bonding*; Maksic, Z., Ed.; Springer: Berlin, 1991; Vol. 4, p 546.
(29) Chambers, C. C.; Hawkins, G. D.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem.* **1996**, *100*, 16385. Giesen, D. J.; Chambers, C. C.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B* **1997**, *101*, 2061.
(30) Dixon, R.; Kollman, P. A. *J. Comput. Chem.* **1997**, in press.