What Controls Partitioning of the Nucleic Acid Bases between Chloroform and Water?

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The free energies of solvation for six nucleic acid bases in water and chloroform are predicted using the SM5.4/A quantum mechanical self-consistent-field solvation model. We obtain a mean unsigned deviation from experiment of 0.2 $\log_{10}$ units in the partition coefficients, lending extra credibility to the predicted solvation free energies. Predictions are then made for an additional six unnatural nucleic acid bases for which no experimental data are available. Functional group contributions to the solvent−solvent partitioning phenomenon are examined, and the validity of fragment-based partitioning models is assessed.

1. Introduction

Association and binding of the nucleic acid bases to diverse substrates plays a critical role in the structure, transcription, and replication of genetic material and in the regulation and sequence specificity of processes involving DNA and RNA.[1,2] The energetics of association and binding include the free energy cost (or gain) for the desolvation of individual nucleic acid bases by solvent water. In principle, this desolvation energy could be measured directly from the vapor pressure of the nucleic acid bases over an aqueous solution of known concentration (i.e., from the measurement of Henry's constant). However, the results of such an experiment have never been reported, presumably because the extremely low volatility of these compounds makes the measurement too difficult.

In the absence of experimental data, a number of theoretical groups have predicted the aqueous free energies of solvation of the nucleic acid bases[3−15] using both explicit[15−18] and implicit[18−22] representations of the surrounding aqueous solvent. However, the range in the predicted free energies of aqueous solvation for individual bases has been disturbingly large. A recent compilation of results from different calculations[13] includes predicted free energies of aqueous solvation spanning 15 kcal mol^{-1} for 9-methyladenine, 11 kcal mol^{-1} for 9-methylguanine, 7 kcal mol^{-1} for 1-methylcytosine, 6 kcal mol^{-1} for 1-methylthymine, and 5 kcal mol^{-1} for 1-methyluracil.

An alternative to measuring the standard-state free energy of solvation in aqueous solution is to measure the free energy of partitioning between water and an organic solvent. Indeed, to the extent that nucleic acid self-association may be viewed as a partitioning between the aqueous phase and an organic biopolymer phase, this measurement may be the more relevant to the association process. Cullis and Wolfenden[23] reported such measurements in 1981 for the chloroform/water partitioning of methylated adenine, guanine, hypoxanthine, cytosine, thymine, uracil, and a number of simpler heterocycles. In the present article, we calculate partition coefficients for individual solutes as

$$\log K_{\mathrm{CHCl_3/H_2O}} = \frac{\Delta G^\circ_{\mathrm{H_2O}} - \Delta G^\circ_{\mathrm{CHCl_3}}}{2.303RT} \qquad (1)$$

where ΔG°_S is the standard-state free energy solvation of the solute in solvent S, R is the universal gas constant, and T is the temperature (298 K), and we compare to the results of Cullis and Wolfenden for six methylated purine and pyrimidine bases present in nucleic acids. On the basis of the good agreement of our predictions with experiment, we further predict partition coefficients for the mutagen 5-bromouracil and five unnatural methylated nucleic acid bases (Figure 1) that find use in the design of artificial oligonucleotides;[24] experimental data are not yet available for these molecules. Finally, we decompose the molecular partition coefficients into functional group contributions in order to evaluate the degree to which popular fragment models for partitioning and/or solvation free energies[25−27] may be expected to be succesful in molecules as complex as the methylated nucleic acid bases. In particular, we focus on the degree to which adjacent functional groups may interact in a nonlinear fashion to influence the solvation and partitioning free energies.

2. Theory

We begin with a brief description of the SM5.4/A solvation model[28−31] that we employ here. This model represents the solvent as a dielectric continuum (water with dielectric constant $\epsilon = 78.3$ and chloroform with $\epsilon = 4.71$) that surrounds a solute cavity of realistic shape (in particular a cavity formed from the union of atom-centered spheres). The electrostatic component of the solvation free energy (ΔG_{ENP}) is calculated for a set of atom-centered charges by using the generalized Born approximation[32−36] to the Poisson equation.[22] The charges employed are class IV charges[37] and hence the ".4" in the model name. Such charges are derived in this case from Austin Model 1[38,39] (AM1, hence the "/A" in the model name) semiempirical quantum mechanical wave functions in a fashion that has been shown to provide outstanding agreement with experimental charge distributions and high-level theoretical calculations.[37,40] The solution of the generalized Born approximation is accomplished in a manner that allows the solute and solvent to mutually polarize in a self-consistent fashion; that is, the optimal gas-phase charge distribution of the solute is allowed to distort in the reaction field of the surrounding dielectric.[21,22,33,41,42] This relaxation can be very significant; it has been estimated in particular to account for 20−40% of the total free energy of solvation for the nucleic acid bases.[6,11,14] The molecular geometries are also fully relaxed in the presence of the solvent reaction field.

In order to account for non-electrostatic solvation effects (cavitation, dispersion, solvent structural perturbations, the hydrophobic effect, etc.) as well as for the extent to which electrostatic effects in the first solvent shell deviate from bulk

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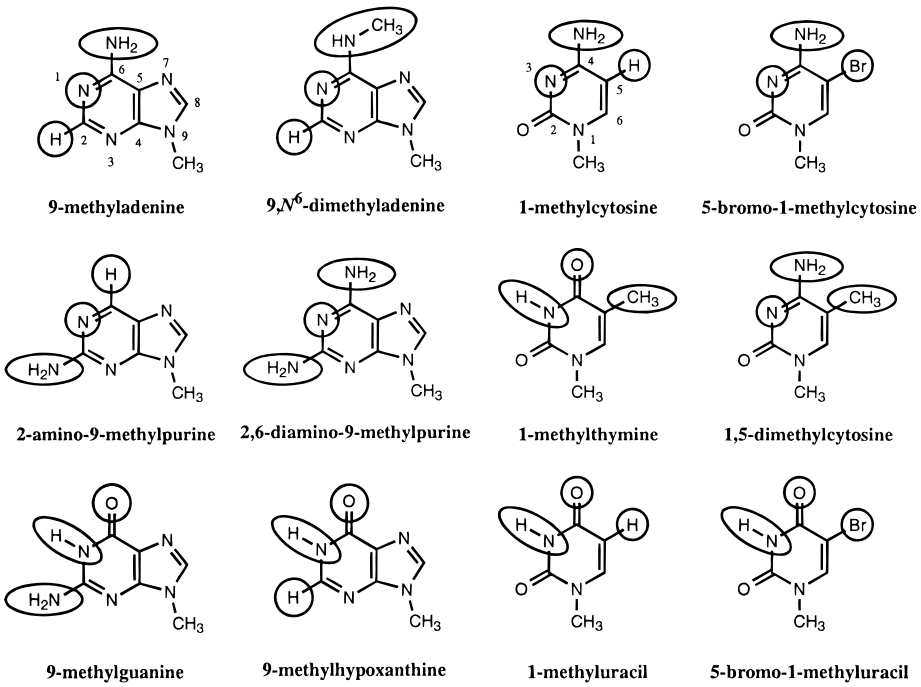

Figure 1. The twelve methylated nucleic acid bases for which partition coefficients were calculated. The numbering schemes for purines and pyrimidines are shown for 9-methyladenine and 1-methylcytosine, respectively. The functional groups that differ from one molecule to the next within the purine and pyrimidine series are circled and correspond to fragments 1, 2, and 3 of Table 2 in a clockwise fashion beginning from the leftmost group; in every case the uncircled remainder is what is meant by "remainder" in Table 2.

solvent, we include additional free energy corrections (whose sum is denoted G_{CDS}) that depend on the degree to which each solute atom is exposed to the first solvent shell. The "SM5" in the model name denotes the functional dependence of these terms on solute geometry. In the SM5 formalism,[28] the contribution associated with each atom of the solute depends on its atomic number, its surface area, and the nearby atoms of the solute according to atomic surface tensions that are expressed in terms of solvent-dependent surface tension coefficients. These coefficients are optimized by fitting to experimentally measured standard-state free energies of solvation and/or free energies of partitioning. The parameters of the SM5.4/A model were obtained in three stages. Fitting was carried out first for water for a data set consisting of 215 free energies of solvation;[28] this stage of the fitting established the parameters for the electrostatic part of the calculation, the dependence of the atomic surface tensions on solute geometry (i.e., the SM5 functional forms), and the numerical values of the surface tension coefficients for aqueous solution. Second, keeping the solute parameters of the electrostatic part of the calculation fixed at the values established for water and using the same SM5 functional forms for the geometry dependence of the surface tensions, we obtained a set of general surface tension coefficients for organic solvents, where any organic solvent is assumed to be completely described by five solvent descriptors (index of refraction, hydrogen bond acidity and basicity, macroscopic surface tension, and dielectric constant); this step was based on fitting 1784 experimental free energies of solvation for 205 solutes in 90 solvents.[29,30] These parameters are taken as final for all solvents except benzene, toluene, and chloroform, for which modified surface tension coefficients were obtained in stage 3. We note that this stage is not independent of the second stage but rather depends on the breadth and diversity of the stage-2 parametrization for much of its robustness. The stage-3 modifications were accomplished using one strategy for benzene and toluene[30] and another strategy for chloroform.[31] Fitting for chloroform was carried out for a data set including 88 heavily weighted free energies of solvation in chloroform, 26 free energies of chloroform/water partitioning, and 385 free energies of solvation in other solvents, which are relevant to the chloroform fit because all solvents are described by the same five solvent descriptors.

The standard-state (1 M) free energy of solvation in solvent S is then calculated as

$$G_S^\circ = \Delta G_{ENP} + G_{CDS} \qquad (2)$$

The mean unsigned errors of the SM5.4/A models for the water[28] and chloroform[31] free energy of solvation data sets were 0.50 and 0.43 kcal mol^{-1}, respectively. An important point with respect to the right-hand side of eq 2 is that, although they are intrinsically nonobservable quantities, considerable effort has gone into making the separated ENP and CDS components of the solvation free energy as physical as possible for the SM5.4 models, and we will have reason to discuss below the extent to which changes in solute functional groups may influence one more than the other. It is important to keep in mind, however, that ΔG_{ENP} and G_{CDS} are model-dependent quantities, not thermodynamic state functions.

3. Results and Discussion

Table 1 lists calculated free energies of solvation and chloroform/water partition coefficients for the 12 methylated bases shown in Figure 1. As noted above, standard-state free energies of solvation have not been directly measured for any of these bases, so it is difficult to assess carefully the calculated values. However, by using available thermochemical data and estimating certain unknown quantities, Ferguson et al.[7] have estimated "experimental" free energies of aqueous solvation of 9-methyladenine and 1-methylthymine to be -13.6 ± 1.1 and -10.4 ± 1.3 kcal mol^{-1}, respectively; the SM5.4/A calculated values are -15.2 and -9.6 kcal mol^{-1}, respectively. Furthermore, the aqueous solvation free energies all fall within the range of previous calculations[13] for 9-methyladenine, 9-methylguanine, 1-methylcytosine, 1-methylthymine, and 1-methyluracil (the only bases for which several other predictions have been made). Finally, our calculated standard-state free energy of solvation for 9-methyladenine in chloroform (-13.3 kcal mol^{-1}) agrees

TABLE 1: Standard-State Free Energies of Solvation and Chloroform/Water Partition Coefficients

	SM5.4/A ΔG_S° (kcal mol^{-1})		log K_{CHCl_3/H_2O}			
solute	H_2O	$CHCl_3$	SM5.4/A	Young et al.[a]	Orozco et al.[b]	experiment[c]
9-methyladenine	−15.2	−13.3	−1.4	−0.6	−0.3	−0.8
9,N^6-dimethyladenine	−12.7	−12.4	−0.2			
2-amino-9-methylpurine	−15.6	−13.6	−1.4			
2,6-diamino-9-methylpurine	−18.4	−15.5	−2.1			
9-methylguanine	−20.1	−15.9	−3.1	−1.3	−4.8	−3.5
9-methylhypoxanthine	−17.3	−13.9	−2.5		−1.4	−2.5
1-methylcytosine	−19.9	−15.5	−3.2	−1.1	−3.4	−3.0
5-bromo-1-methylcytosine	−16.7	−14.4	−1.6			
1-methylthymine	−9.6	−9.2	−0.3	−0.8	−0.4	−0.5
1,5-dimethylcytosine	−18.3	−15.4	−2.2			
1-methyluracil	−10.5	−8.9	−1.2		−1.0	−1.2
5-bromo-1-methyluracil	−10.0	−9.6	−0.3			
mean unsigned error			0.2	1.2	0.6	

[a] References 9 and 44. [b] Reference 14. [c] Reference 23.

well with a value derived from free energy perturbation calculations carried out by Blake and Jorgensen (−12.7 kcal mol^{-1}).[43]

The experimental chloroform/water partition coefficients of Cullis and Wolfenden[23] for the six bases that they measured are also included in Table 1. The mean unsigned error in the predicted SM5.4/A partition coefficients is only 0.2 log units (following the usual convention, all logarithms in this article are to the base 10; this error thus corresponds to 0.3 kcal mol^{-1} for the difference in free energy of transfer between the two solvents). The largest deviation, 0.6 log units, is observed for 9-methyladenine. The high quality of the predicted partition coefficients in these cases where experimental results are available validates the accuracy of the models and supports interpretation of the results for the remaining methylated nucleic acid bases (vide infra).

If we neglect the solute geometry relaxation in each solvent, the mean unsigned error in log K increases from 0.2 to 0.4 log units, and if we further neglect the solute electronic relaxation in each solvent, this mean unsigned error increases to 1.3 log units. These observations show the crucial importance of using a polarizable solute model for quantitative work and justify the effort that we and the Barcelona group[14] have put into parametrizing quantum mechanical models with a polarizable solute, as well as the considerable efforts currently being expended in other groups to add polarizability to other kinds of models, such as force fields.

Young et al., using a self-consistent reaction field model implemented at the HF/6-31G** level of electronic structure theory (single-center multipole expansion through $l = 7$ in an ellipsoidal cavity), have provided electrostatic free energies of solvation in both chloroform[44] and water[9] for 9-methyladenine, 9-methylguanine, 1-methylcytosine, and 1-methylthymine. The log K values calculated from these electrostatic free energies of solvation are provided in Table 1. This approach leads to a substantially larger mean unsigned error (1.2 log units) in comparison to experiment than is found with SM5.4/A. A portion of this error must be ascribed to the failure of these calculations to account for the non-electrostatic components of solvation. However, the corresponding SM5.4/A electrostatics-only log K values for 9-methyladenine, 9-methylguanine, 1-methylcytosine, and 1-methylthymine are −2.0, −3.1, −3.7, and −1.8 log units, respectively; these values are all 1−2 log units more negative than those calculated by Young et al.[9,44] We speculate that these differences reflect the inadequacy of an ellipsoidal cavity for modeling the solvation energies of complex molecules, the difficulty in converging the multipole expansion for molecules containing multiple polar functional groups,[21] the intrinsic uncertainty in the atomic radii assumed for the electrostatic calculation, and/or inaccuracies in their method of calculating partial charges. With regard to the second of these points, Dillet et al.[45] have noted for formamide, for example, the considerably faster convergence of a distributed multipole representation of the density compared to a single-center expansion. The generalized Born model involves a distributed monopole representation of the density, and this special case of a distributed multipole expansion is probably more adequate than a truncated single-center multipole expansion for large molecules. With regard to the third point, we note that, in general, electrostatic calculations are very sensitive to the radii assumed for the atoms because this determines the boundary at which the dielectric medium is assumed to begin (this radius is intrinsically uncertain because the dielectric boundary is actually a fluctuating boundary of finite width), and in most methods currently employed by other workers, including the method used in refs 9 and 4, this means that the calculated free energies of solvation are also sensitive to this choice. Electrostatic calculations are also sensitive to errors in the partial atomic charges. However in SM*x* solvation models, the calculated free energies of solvation are much less sensitive to the assumed radii and method of calculating charges than are the individual ENP and CDS components, because—by design of our parametrized methods—since the uncertainty in the radius affects only the first solvation shell, the semiempirical determination of the surface tension coefficients *for a given set of electrostatic radii and method of calculating partial atomic charges* makes up to a large extent for the intrinsic uncertainty in the location of the dielectric boundary and for any systematic errors in the charges.

We note that Orozco et al. have also recently calculated chloroform/water partition coefficients for the same six methylated bases as here, using a different surface-tension-corrected polarizable-solute continuum dielectric model built upon the AM1 Hamiltonian.[14] They obtained a mean unsigned error of 0.6 log units (or 0.8 kcal mol^{-1}). However, the model of Orozco et al., in addition to having a somewhat larger error, has not been analyzed in terms of fragment contributions. The SM5.4/A model, on the other hand, by being based on atomic charges and atomic surface tensions, allows a straightforward decomposition of the total solvation free energy into atomic and group contributions; we next focus upon this decomposition using the previously described method I.[6]

4. Analysis of Group Contributions

Within the respective classes of the 9-methylpurines and 1-methylpyrimidines, the bases differ one from another at only three positions. These positions are circled in Figure 1: the uncircled atoms in each group preserve their identity *and* their hybridization in all structures of a given class. Table 2 lists

TABLE 2: Fragment Contributions to Molecular Partition Coefficients

	fragment[a]			fragment log K_{CHCl_3/H_2O}			
solute	1	2	3	1	2	3	remainder
9-methyladenine	H	N	NH_2	0.6	−1.5	−0.8	0.3
9,N^6-dimethyladenine	H	N	$NHCH_3$	0.5	−1.2	0.1	0.3
2-amino-9-methylpurine	NH_2	N	H	−0.8	−1.2	0.5	0.1
2,6-diamino-9-methylpurine	NH_2	N	NH_2	−0.8	−1.5	−0.8	1.0
9-methylguanine	NH_2	NH	O	−1.0	−1.8	−1.7	1.4
9-methylhypoxanthine	H	NH	O	−0.2	−1.1	−1.7	0.5
1-methylcytosine	N	NH_2	H	−2.3	−0.9	−0.2	0.2
5-bromo-1-methylcytosine	N	NH_2	Br	−2.1	−0.7	0.8	0.4
1-methylthymine	NH	O	CH_3	−0.6	−1.2	0.9	0.6
1,5-dimethylcytosine	N	NH_2	CH_3	−2.3	−0.9	0.6	0.2
1-methyluracil	NH	O	H	−0.6	−1.4	0.2	0.6
5-bromo-1-methyluracil	NH	O	Br	−0.5	−1.2	0.9	0.5

[a] See Figure 1.

the fragment contributions that all of these groups make to the calculated chloroform/water partition coefficient for each solute, as well as the sums of the contributions from the remaining fragments that all members of the class have in common.

An instructive first case to consider with respect to the utility of fragment decomposition is a comparison of 9-methyladenine to 9,N^6-dimethyladenine. These molecules differ only by methylation of the 6-amino group. As might be expected from consideration of the hydrophobic effect, the change in the partitioning of that fragment upon methylation is +0.9 log units; that is, its solvation in chloroform is improved relative to water. The remaining fragments are much less sensitive to methylation, but do contribute another +0.3 log units to the total change in partition coefficient. A very similar analysis applies to the replacement of an H atom with a methyl group at the pyrimidine 5-position in 1-methylcytosine and 1-methyluracil (to make 1,5-dimethylcytosine and 1-methylthymine, respectively). In each case, the hydrophobic effect changes the partition coefficient of this fragment by about +0.8 log units, while the remaining fragments are not significantly affected. Finally, it is of some chemical interest to note that when the same H atom is replaced with Br instead of a methyl group, roughly the same effect is observed; that is, we predict that bromine is net hydrophobic in this position to about the same extent as a methyl group.

A biologically more significant example is the partitioning of NH_2 fragments, since deamination of cytosine, adenine, and guanine is the most important base transformation leading to *in vivo* DNA damage and has significant potential to cause heritable change.[2] An exocyclic amino group appears as a fragment in Table 2 eight times: three times at the purine 2-position, three times at the pyrimidine 4-position, and twice at the purine 6-position. Interestingly, in spite of the significantly different chemical structures to which these amino groups are attached, the contribution of this group to log *K* ranges over only 0.3 log units, from −0.7 to −1.0. This is a particularly important observation insofar as it appears to validate a fundamental assumption of certain empirical models of solvent/solvent partitioning, namely, that individual groups make fragment contributions to the overall partition coefficient that will be transferable from one molecule to the next.[25−27,46] One interesting feature of this observation for the amino group is that while it appears to be valid for the chloroform/water partition coefficient, it does not hold well for the standard-state free energies of solvation in either solvent. In water, the contributions of this group to the solvation free energy range from −4.3 to −5.6 kcal mol^{-1} and in chloroform from −3.4 to −4.2 kcal mol^{-1}. However, the variations in the individual solvents track each other sufficiently closely that differences in the fragment partition coefficients are damped.

This same trend is observed for the fragment contributions of N at the purine 2-position (four cases, span of 0.3 log units), N at the pyrimidine 3-position (three cases, span of 0.2 log units), carbonyl O at the purine 6-position (two cases, both −1.7 log units), and carbonyl O at the pyrimidine 4-position (three cases, span of 0.2 log units). However when one compares these two fragments (N and O), purine to pyrimidine, it is evident that transferability begins to break down. The O fragments prefer water over chloroform to a lesser degree in the pyrimidines compared to the purines by about 0.5 log units; the N fragments prefer water over chloroform to a lesser degree in the purines compared to the pyrimidines by a substantial margin, nearly 1 log unit. This reflects the nonlinear interactions of these fragments with other functional groups in the different solute molecules.

An example that offers further insight into transferability is provided by analysis of the partition coefficients associated with the common remainder fragments. In the pyrimidines, this partition coefficient spans only 0.4 log units, while in the purines it spans 1.1 log units. The difference appears to arise from the differing extent to which strongly interacting groups of the remainder fragment are exposed to the solvent. In the pyrimidines, the carbonyl group at the 2-position, *N*-methyl group at the 1-position, and H at the 6-position, which dominate the solute−solvent interactions of the remainder fragment, are all exocyclic substituents reasonably well exposed to the solvent. As a consequence, the partition coefficient associated with this fragment is less sensitive to other functionalities in the molecule. For the purines, on the other hand, there are very strong interactions of the heterocycle N atoms at the 3- and 7-positions with surrounding solvent; since these atoms are sometimes poorly exposed to solvent as a result of substitution at either the 2- or 6-positions, their influence on the remainder fragment partitioning can change markedly. So, in the four purines that are substituted at only one of the 2- or 6-positions, the remainder fragment partition coefficient ranges from 0.1 to 0.5, while in the two purines characterized by 2,6-disubstitution, the remainder fragment partition coefficients are 1.0 and 1.4. Thus, the greater the extent to which the hydrophilic heterocycle N atoms are descreened by local solute functionality, the more positive the remainder fragment partition coefficient.

Perhaps not surprisingly, then, the extent to which a fragment partition coefficient is transferable from one molecule to the next appears to depend strongly on the extent to which the functional group is isolated from other interactions. And even for this fairly restricted situation, transferability only applies for solvent/solvent partitioning; predominantly because of the long-range nature of electrostatic interactions, fragment contributions to standard-state free energies of solvation appear to be still less transferable, as noted above for the case of the amino group.

Finally, we focus on an interesting trend in the partition coefficients associated with NH fragments when the N is either at the 1-position in the purines (9-methylguanine and -hypoxanthine) or the 3-position in the pyrimidines (1-methylthymine,

1-methyluracil, and 5-bromo-1-methyluracil). In all of the pyrimidines, this fragment's partition coefficient is about −0.6, in 9-methylhypoxanthine it is −1.1, and in 9-methylguanine it is −1.8; that is, the fragment increasingly prefers water over chloroform as one progresses through this series. This effect derives almost entirely from the ENP component of the partitioning free energy and can be rationalized on the basis of the nature of the interactions of the NH fragment with surrounding groups. In the pyrimidines, the NH fragment is srrounded by two carbonyl oxygens. This arrangement of the functional groups provides optimal intramolecular electrostatic interactions between the partially positive NH proton and the partially negative carbonyl oxygens. Solvation screens this favorable interfragment interaction, so the net preference of the NH group for water over chloroform is small. Another way of viewing this is atomistically. Solvent molecules cannot simultaneously orient to optimally solvate adjacent negative and positive partial charges, so the overall solvent structure is frustrated. The reason that there is a preference for water at all arises from the more favorable direct interactions of the polar NH group with the solvent having the higher dielectric constant, an interaction that might be called "*intra*fragment" to contrast it with the interfragment screening just described. In the case of 9-methylhypoxanthine, one adjacent carbonyl group is replaced by an H atom that only weakly polarizes the solvent, so the favorable solvent interaction of the NH group is increased in a high as compared to a low dielectric solvent. Finally, in the case of 9-methylguanine, the weakly polarizing H atom is replaced by an amino group, thus positioning *additional* partial positive charge in the same region of space. This removes any frustration of the solvation shell around this portion of the solute, and the net preference of the NH fragment for a high dielectric environment reaches its maximum. This analysis of cooperatively between groups is analogous to that presented by Jorgensen et al.[47,48] in rationalizing the strength of multiply hydrogen-bonded systems, where close proximity of one or more donor groups (or acceptor groups) leads to stronger interactions with a hydrogen-bonding partner. In the hydrogen-bonding case, preorganization is achieved by synthetic means, and the energetic cost of that preorganization is paid as part of the synthetic process; in the case of solvation, an energetic cost is required to organize the solvent shell at the point of dissolution, but once organized, favorable interactions with multiple groups may be possible.

5. Concluding Remarks

In conclusion, we have illustrated the high predictive accuracy that can be otained for chloroform/water partition coefficients calculated from the SM5.4/A polarizable-solute continuum solvation models. Importantly, the models take into account nonlinear interactions between different solute functional groups that are typically not included in partitioning models based on summing fragment contributions. The excellent accuracy of the present approach means that it may be possible to use it for rational design of novel nucleic acid bases so as to influence such oligonucleotide properties as helix stability, base-pairing fidelity, binding constants, tendency toward triple-helix formation, and other biological properties and functions sensitive to solvation and desolvation effects.

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References and Notes

(1) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

(2) Watson, J. D.; Hopkins, N. H.; Roberts, J. W.; Steitz, J. A.; Weiner, A. M. *Molecular Biology of the Gene*, 4th ed.; Benjamin/Cummings: Menlo Park, CA, 1987; p 343.

(3) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230.

(4) Bash, P.; Singh, U. C.; Langridge, R.; Kollman, P. A. *Science* **1987**, *236*, 564.

(5) Katritzky, A. R.; Karelson, A. *J. Am. Chem. Soc.* **1991**, *113*, 1561.

(6) Cramer, C. J.; Truhlar, D. G. *Chem. Phys. Lett.* **1992**, *198*, 74; **1993**, *202*, 567(E).

(7) Ferguson, D. M.; Pearlman, D. A.; Swope, W. C.; Kollman, P. A. *J. Comput. Chem.* **1992**, *13*, 362.

(8) Orozco, M.; Luque, F. J. *Biopolymers* **1993**, *33*, 1851.

(9) Young, P. E.; Hillier, I. H. *Chem. Phys. Lett.* **1993**, *215*, 405.

(10) Elcock, A. H.; Richards, W. G. *J. Am. Chem. Soc.* **1993**, *115*, 7930.

(11) Gao, J. L. *Biophys. Chem.* **1994**, *51*, 253.

(12) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; 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.

(13) Miller, J. L.; Kollman, P. A. *J. Phys. Chem.* **1996**, *100*, 8587.

(14) (a) Orozco, M.; Colominas, C.; Luque, F. J. *Chem. Phys.* **1996**, *209*, 19. (b) Orozco, M. Personal communication.

(15) Gao, J. In *Reviews in Computational Chemistry*; Lipkowitz, K. B., Boyd, D. B., Eds.; VCH: New York, 1996; Vol. 7, p 119.

(16) Kollman, P. *Chem. Rev.* **1993**, *93*, 2395.

(17) van Gunsteren, W. F.; Luque, F. J.; Timms, D.; Torda, A. E. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 847.

(18) Orozco, M.; Alhambra, C.; Barril, X.; López, J. M.; Busquest, M. A.; Luque, F. J. *J. Mol. Model.* **1996**, *2*, 1.

(19) Cramer, C. J.; Truhlar, D. G. In *Quantitative Treatments of Solute/Solvent Interactions*; Politzer, P., Murray, J. S., Eds.; Elsevier: Amsterdam, 1994; Vol. 1, p 9.

(20) Tomasi, J.; Persico, M. *Chem. Rev.* **1994**, *94*, 2027.

(21) Cramer, C. J.; Truhlar, D. G. In *Reviews in Computational Chemistry*; Lipkowitz, K. B., Boyd, D, B., Eds.; VCH: New York, 1995; Vol. 6, p 1.

(22) Cramer, C. J.; Truhlar, D. G. In *Solvent Effects and Chemical Reactivity*; Tapia, O., Bertrán, J., Eds.; Kluwer: Dordrecht, 1996; p 1.

(23) Cullis, P. M.; Wolfenden, R. *Biochemistry* **1981**, *20*, 3024.

(24) Brennan, C. A.; Van Cleve, M. D.; Gumport, R. I. *J. Biol. Chem.* **1986**, *261*, 7279.

(25) Hine, J.; Mookerjee, P. K. *J. Org. Chem.* **1975**, *40*, 287.

(26) Cabani, S.; Gianni, P.; Mollica, V.; Lepori, L. *J. Solution Chem.* **1981**, *10*, 563.

(27) Leo, A. J. *Chem. Rev.* **1993**, *93*, 1281.

(28) Chambers, C. C.; Hawkins, G. D.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem.* **1996**, *100*, 16385.

(29) Giesen, D. J.; Gu, M. Z.; Cramer, C. J.; Truhlar, D. G. *J. Org. Chem.* **1996**, *61*, 8720.

(30) Giesen, D. J.; Cramer, C. J.; Truhlar, D. G. To be published.

(31) Giesen, D. J.; Chambers, C. C.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem.* **1997**, *101*, 2061.

(32) Hoijtink, G. J.; de Boer, E.; Van der Meij, P. H.; Weijland, W. P. *Recl. Trav. Chim. Pays-Bas* **1956**, *75*, 487.

(33) Jano, I. *C. R. Acad. Sci. Paris* **1965**, *261*, 103.

(34) Tucker, S. C.; Truhlar, D. G. *Chem. Phys. Lett.* **1989**, *157*, 164.

(35) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.

(36) Cramer, C. J.; Truhlar, D. G. *J. Am. Chem. Soc.* **1991**, *113*, 8305, and 9901(E).

(37) Storer, J. W.; Giesen, D. J.; Cramer, C. J.; Truhlar, D. G. *J. Comput.-Aided Mol. Des.* **1995**, *9*, 87.

(38) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

(39) Dewar, M. J. S.; Zoebisch, E. G. *J. Mol. Struct. (THEOCHEM)* **1988**, *180*, 1.

(40) Storer, J. W.; Giesen, D. J.; Hawkins, G. D.; Lynch, G. C.; Cramer, C. J.; Truhlar, D. G.; Liotard, D. A. In *Structure and Reactivity in Aqueous Solution*; Cramer, C. J., Truhlar, D. G., Eds.; American Chemical Society: Washington, DC, 1994; p 24.

(41) Rinaldi, D.; Rivail, J.-L. *Theor. Chim. Acta* **1973**, *32*, 57.

(42) Tapia, O. In *Quantum Theory of Chemical Reactions*; Daudel, R., Pullman, A., Salem, L., Viellard, A., Eds.; Reidel: Dordrecht, 1980; Vol. 2, p 25.

(43) Blake, J. F.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1990**, *112*, 7269.

(44) Young, P. E.; Hillier, I. H.; Gould, I. R. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1717.

(45) Dillet, V.; Rinaldi, D.; Angyán, J. G.; Rivail, J.-L. *Chem. Phys. Lett.* **1993**, *202*, 18.

(46) Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175.

(47) Jorgensen, W. L.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008.

(48) Pranata, J.; Wierschke, S. G.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 2810.