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The Sensitivity of Conformational Free Energies of the Alanine Dipeptide to Atomic Site Charges

Different atomic point charge sets are obtained for the α_R and $C_{7,eq}$ conformations of the alanine dipeptide by fitting the charges of each conformation to the respective ab initio *electrostatic potential surfaces both individually and simultaneously, in both the united atom and the all-atom representations. Using these charge sets, the sensitivity of the relative conformational aqueous free energies to the atomic site charges is investigated. For this particular system, we find that the solute–water contributions to the conformational free energy differences have a rather weak dependence on site charges; the calculated intramolecular contributions, however, show a rather strong dependence on the atomic site charges. It is suggested that the calculated results for the alanine dipeptide using a single, simultaneously fit set of charges for both conformations are in better agreement with experiments than the calculations carried out with charges determined individually for each conformation.* © *1997 John Wiley & Sons, Inc.*

INTRODUCTION

Historically, a topic of considerable interest has been the dependence of aqueous solvation free energies, calculated via computer simulation or other related techniques, on the requisite interaction parameters. A number of studies have suggested that even though short range, i.e., van der Waals, interactions are a factor, the calculated aqueous free energies are primarily sensitive to the electrostatic parameterization used in the calculation (see, for example, Refs. 1 and 2). For instance, Mezei et al. observed that calculated solvation free energy differences between different conformations of alanine dipeptide significantly depends on the charge set employed.[3,4] In another study, Wong and Zhu

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demonstrated, using the sensitivity analysis approach, that calculated free energies can vary significantly with the site charges.[5,6] In his detailed study,[7] Williams investigated the variation of site charges of the alanine dipeptide as a function of different conformations by fitting quantum mechanically generated electrostatic potentials around the isolated molecule. Merz and Kollman, in their study of free energies of enzyme inhibitor binding, arrived at the similar conclusion that small modifications of site charges can change the hydration free energies by as much as several kcal/mole.[8] Similarly, Jorgensen and Gao have shown that when a united-atom representation was utilized, adjustment of site charges for each conformation was necessary to obtain better agreement with the experimental molecular dipole moments,[9] and also that the calculated free energy difference between the *cis* and the *trans* conformations of *N*-methylacetamide was highly sensitive to the site charges. Smith and Karplus observed that gas phase conformational properties of small organic molecules are quite sensitive to the way the electrostatic interactions are handled.[10] Chipot et al.[11] and Dinur and Hagler[12] showed that the site charges should depend on the conformation and developed ways to represent the geometry dependencies. Carlson et al.[13] and Chipot et al.[14] investigated the dependence of the hydration free energies of small molecules and found that the calculated free energies of hydration depend on whether Mulliken charges or charges derived from the ab initio electrostatic potential[15,16] are used. To avoid the poor sampling characteristics of the electrostatic potential arising from charges buried beneath the molecular surface, Kollman and co-workers developed the restrained electrostatic potential fitting scheme and successfully applied it to study the energetics of several sample molecules.[14,17-19]

A possible improvement in the derivation of site charges is found in works by Reynolds et al.[20-22] They derived an algorithm in which the molecular electrostatic potential for each of several different conformations is weighted by the relative Boltzmann factor of each configuration and then a single set of charges is fitted to the set of potentials. Certain physical constraints, such as constraining the charges to reproduce the dipole moments at certain geometries, can be included into the fitting scheme. Intuitively, one would expect a single charge set fit to multiple conformations to be worse than charges separately fit to individual conformations, as the relative number of free fitting parameters appears to be reduced. However, as can be inferred from the variety of parameterizations in use, the usual force field representation, i.e., adding $1/r^6$ and $1/r^{12}$ terms to the electrostatic interactions, is actually redundant in the sense that several different parameterizations reproduce experimental physical properties with similar success. Therefore, because of this redundancy, a single charge parameter set, fit to multiple conformations, can be quite successful. Also, a simultaneously fitted charge set should represent a compromise between electronic distributions peculiar to the individual conformations and should increase the likelihood that the resulting molecular electrostatic potential is accurate over a greater range of conformational space. A difficulty inherent in the approach used by Reynolds and coworkers.[20-22] is that the Boltzmann factors needed initially are often the end results in the calculations, and that if the gas phase Boltzmann factors are utilized these do not generally represent the solution phase. Therefore, unless solution phase experimental data exists, an iterative approach is needed to readjust the calculated charges during the calculations so as to incorporate the correct Boltzmann factors, and these calculations would be impractical. However, where experimental data was available, the approach was shown to be quite successful in theoretical determination of the partition coefficients. Similar success can be expected when the gas and solution phase Boltzmann factors are similar.

There also have been studies to incorporate the effects of solvation on the charge distribution. In their quantum mechanical study, Grant et al.[23] studied the charge distribution of the alanine dipeptide by immersing it into the reaction field of a dielectric continuum solvent. They found that the change in the solute polarization due to the reaction field of the solvent is conformation dependent and that the change can be very substantial. Sharp[24] attempted to include the solvation effects by representing the solvent reaction field as surface polarization charges at the solvent-accessible surface of the solute molecule, and found that the effective charges for different conformations may differ by more than 10% on average. Further information on past investigations of the solvation and of the conformational properties of the alanine dipeptide may be found in a recent review article by Brooks and Case.[25]

Following common practice we will utilize the partial charges obtained from fitting the electrostatic field *outside* the molecule for the calculation of intramolecular electrostatic energy as well. We wish to comment on two consequences of this

practice. First, using such charges for intramolecular interactions, i.e., interactions arising from an effective interaction *inside* the molecule, represents an extrapolation and is thus less reliable by its nature. Clearly, the closer the partial charges are to a representation of the physical reality, the more reliable this approximation will be. Second, it has to be kept in mind that these intramolecular electrostatic contributions are sensitive to the treatment of the so-called 1–4 interactions and our conclusions are limited to methodology utilizing the same formalism to calculate the electrostatic interactions.

Building on these previous studies, we investigate the answer to a basic question concerning the charge set dependence of the conformational free energy differences, using the case of aqueous solvation of alanine dipeptide: Is it possible to use a single, simultaneously fit set of partial atomic charges to represent the molecular electrostatic interactions and still obtain adequate quantitative results for the free energy differences between several conformations of a molecule?

METHODS

The backbone torsion space (Ramachandran) map of alanine dipeptide in aqueous solution has several shallow minima. Experimental solution vibration spectrum of the alanine dipeptide shows many broad peaks, which has been interpreted as corresponding to a multistate conformational equilibrium.[26,27] Furthermore, ir[28] and CD and nmr spectroscopy[29] studies demonstrate that $C_{7,eq}$, α_R, P_{II}, C_5, and $C_{7,ax}$, and possibly other conformations coexist in aqueous solutions. These experimental results are further supported by theoretical predictions.[25]

In this report, the free energy change of going from $C_{7,eq}$ to α_R conformation of the alanine dipeptide is computed. As stated above, these are two of the most frequently occurring conformations of the alanine dipeptide. Note that a more complete investigation should actually consider all of the dominant conformations. However, the associated high cost of the free energy calculations makes such detailed investigations very expensive with the current state of computational technology. Therefore, to keep the computational expense within feasible limits, our study was limited to two of the most prominent conformers. This, however, does not mean that these are strictly the two dominant conformations.

First, conformation $C_{7,eq}$ with backbone torsion angles $(\Psi, \Phi) = (90°, -90°)$ is generally considered to be the minimum of the gas-phase potential surface in the backbone torsion space (from this point on $C_{7,eq}$ will be referred to as C_7). The other utilized conformation, right-handed α-helix α_R $[(\Psi, \Phi) = (-50°, -70°)]$, is of special interest for several reasons. It has now been commonly agreed on that in going from the gas phase to aqueous solution the α_R conformation gets stabilized significantly and the C_7 and C_5 conformations coalesce into a broad β region.[25,30,31] In addition, α_R is set apart from most other conformations by the fact that it occupies an isolated energy minimum of the backbone torsion space.

Atomic site charges of the alanine dipeptide were obtained using the method of fitting partial charges at the atomic positions to reproduce the ab initio electrostatic potential surfaces.[15,16] The molecular geometries used were the same of our previous studies,[32,33] and the electrostatic potential surfaces for both conformations were calculated using the Gaussian-92 molecular orbital program[34] employing the 6–31G^* basis set. Point charges at the atomic positions for each conformation were then determined using a fitting program (P. V. Maye, unpublished program PDCAP), incorporating the Levenberg–Marquardt algorithm.[35] After excluding the grid points falling within the van der Waals surfaces of each conformation, the electrostatic potential evaluated at slightly more than 2000 grid points for each conformation were used in the fitting.

Stouch and Williams[36,37] recently showed that electrostatic potential data is highly correlated such that the least-squares fit is generally singular, i.e., such fits may not allow for determination of all the site charges. The singular value decomposition study of Francl et al.[38] supports the idea that the unrestrained fitting algorithms are rank deficient. Their investigation revealed that for the alanine dipeptide it would be possible to uniquely obtain the charges for up to 17 sites. Therefore, due to the singularity, some of the site charges for all-atom models of the alanine dipeptide having 22 sites are not unambiguously defined. However, since the restrained charge fitting methods have not been well tested in free energy simulations, and since the charges derived using unrestrained electrostatic potential fitting algorithms seem to be rather successful in predicting the free energy differences (see, for example, Refs. 1 and 2), we chose to use an unrestrained fitting algorithm in this study. In this respect, the discrepancies communicated in the later sections can be loosely interpreted as evidence in support of the constrained charge fitting algorithms.

Another concern in the fitting process is the choice of the number of grid points to be used in the least-squares fitting. Although earlier studies showed a dependence on the number of fitting points,[39] a recent report clearly shows that increasing the point density of the fit has little or no influence on the order of the singularity.[38] This justifies our using of approximately 2000 points in the fitting, which corresponds to a grid density of about 1 point/Å^2 on six surface layers between surfaces at one and two times the van der Waals radii, a typical value used in electrostatic potential fitting algorithms.[17]

The atomic charges were determined in two different ways. First, the charges were fit individually for each con-

formation to the corresponding electrostatic potential surface. Second, a single set of charges was determined for both conformations through a simultaneous best fit to the potential surfaces for both conformations. Since the occupational probabilities of the C_7 and α_R conformations in aqueous solution are comparable (see the next section), i.e., the fit weight factors are roughly equal, this second fitting scheme is approximately equivalent to the approach of Reynolds et al.[20-22] for the determination of atomic charges for variable molecular conformations. In addition, the charges were fit both for all-atom (including point charges at hydrogen positions) and for united-atom (point charges excluded at hydrocarbon hydrogen positions) representations. The labels "AAI," "AAS," "UAI," and "UAS" will be used for these four calculations. AA and UA in these labels respectively indicate all-atom and united-atom models. Similarly, *I* and *S* respectively refer to whether the charges are determined by *individual* fits to each conformation or by a *simultaneous* fit to both conformations. A previously used[32,33] united-atom model with the standard AMBER charges[40,41] is also included and will be labeled as "UAA" (in this model, the site charges are conformation independent). These five sets of charges are tabulated in Table I.

In our calculations of the total free energy differences, it was assumed that the intra- and intermolecular degrees of freedom do not couple, giving

$$\Delta A(C_7 \rightarrow \alpha_R) \simeq \Delta A_{intra} + \Delta A_{inter} \qquad (1)$$

This approximation allowed us to use fixed solute conformations in the aqueous simulations. It was also assumed that the intramolecular entropic contribution was the same for all employed charge models, and its value was taken from a previous study[42]: 0.3 kcal/mole in favor of α_R conformation. Even though it would be model dependent, the intramolecular entropic contribution to the conformational free energy differences of the alanine dipeptide is known to be much smaller than the respective intermolecular contribution.[42] Therefore, the error introduced by using a charge model independent intramolecular entropic contribution is negligible for the purpose of this study. While these assumptions are expected to hold to a good degree, the error introduced by them should be quantified by further studies. All calculations reported here used the nonbonded parameters specified by AMBER,[40,41] and the TIP3P model[43] was used to represent water. The solvation free energy differences between the two conformations were calculated by polynomial thermodynamic integration[32,33] using five-point quadratures with an exponent set of {4, 3, 2}. After sufficient equilibration, the integrand at each quadrature point was obtained by running 7×10^6 step Monte Carlo simulations incorporating force biased[44] and preferential[45] sampling. The intramolecular energies were calculated using a factor of 0.5 for the 1–4 interactions (i.e., between atoms separated by exactly three bonds) and a distance-dependent dielectric constant $\epsilon = r$. Further details of the simulation methodology may be found in Refs. 25, 32.

RESULTS

Charge Set Analysis

In his study on the alanine dipeptide,[7] Williams made several observations that are also applicable to our results. The atoms near the periphery of the molecule have better defined charges than the atoms buried within the molecule. Also, the quality of the fit to the electric field slightly worsens if the charges are derived by assuming that they are simultaneously fit to both conformations. The terminal methyl groups have approximately zero total net charge, except that in our case $(CH_3)_3$ is somewhat positively charged. Note, however, that Head-Gordon et al.,[46] found that terminal methyl groups play an insignificant role in the structure and energetics of alanine dipeptide, so this disagreement should not be important. The hydrogens on a given methyl group have nearly the same charges. Carbonyl oxygen charges are almost constant, but the carbonyl carbon charge has a wide charge range. Similarly, imino hydrogen charges are almost constant, but imino nitrogen charge varies considerable.

Calculated ab initio dipole moments are 0.587 and 1.530 $|e| \cdot$Å, respectively, for the C_7 and α_R conformations. While the calculated molecular dipole moments (Table II) are basis-set dependent, the 6-31G^* basis set chosen for this work is generally accepted to be one of the most reliable basis set for obtaining charge distributions.[17-19] All of the four fitted charge sets have molecular dipoles close to the quantum mechanical values with AAI giving the best fit. This may be due to the fact that it involves the largest number of free fitting parameters. However, it is interesting to note that the dipole moment of the C_7 conformation with the standard AMBER charges, UAA, is very different from the rest. Another interesting observation is that if $\Delta\mu \equiv \mu_{\alpha_R} - \mu_{c_7}$, then $\Delta\mu$ (fitted individually) $< \Delta\mu$ (fitted together). In other words, fitting a single set of charges to all conformations resulted in larger dipole moment differences between the two molecular conformations.

Internal Energy Results

The intramolecular conformation energy differences given in Table III were calculated after the

Table I Atomic Site Partial Charges[a]

	UAA	UAS	UAI		AAS	AAI	
Site	$C_{7,eq} = \alpha_R$	$C_{7,eq} = \alpha_R$	$C_{7,eq}$	α_R	$C_{7,eq} = \alpha_R$	$C_{7,eq}$	α_R
$(CH_3)_1$	−0.026	0.076	0.071	0.070	−0.070	−0.059	−0.066
C	—	—	—	—	−0.578	−0.552	−0.530
H	—	—	—	—	0.163	0.172	0.133
H	—	—	—	—	0.168	0.180	0.158
H	—	—	—	—	0.177	0.141	0.173
$C^{(n)}$	0.526	0.585	0.641	0.598	0.770	0.745	0.756
$O^{(n)}$	−0.500	−0.528	−0.572	−0.530	−0.552	−0.576	−0.551
$N^{(n)}$	−0.520	−0.713	−0.731	−0.700	−0.735	−0.696	−0.729
$H^{(n)}$	0.248	0.321	0.339	0.299	0.342	0.353	0.345
CH_1	0.215	0.347	0.296	0.339	0.372	0.364	0.296
C	—	—	—	—	0.332	0.341	0.213
H	—	—	—	—	0.040	0.023	0.083
$(CH_3)_2$	0.031	−0.005	0.013	0.008	−0.073	−0.061	−0.058
C	—	—	—	—	−0.402	−0.354	−0.365
H	—	—	—	—	0.101	0.098	0.106
H	—	—	—	—	0.120	0.089	0.130
H	—	—	—	—	0.109	0.106	0.070
$C^{(c)}$	0.526	0.568	0.572	0.571	0.548	0.501	0.600
$O^{(c)}$	−0.500	−0.534	−0.533	−0.537	−0.523	−0.538	−0.550
$N^{(c)}$	−0.520	−0.688	−0.731	−0.687	−0.501	−0.502	−0.450
$H^{(c)}$	0.248	0.306	0.363	0.304	0.272	0.313	0.299
$(CH_3)_3$	0.272	0.265	0.270	0.265	0.150	0.157	0.108
C	—	—	—	—	−0.162	−0.144	−0.298
H	—	—	—	—	0.099	0.080	0.147
H	—	—	—	—	0.106	0.130	0.123
H	—	—	—	—	0.107	0.091	0.136

[a] The (c) and (n) superscripts that are suffixes for carbonyl and imino sites correspond to the C- and N-terminus, respectively.

structure of an isolated alanine-dipeptide molecule was relaxed to its corresponding α_R or C_7 minimum using the standard AMBER force field[40,41] parameters with our modified charges. Note that the entropic contribution to the intramolecular free energy difference between the two conformations is very small (approximately 0.3 kcal/mole favoring the α_R conformation),[42] so the reported intramolecular energy difference between two conformations is effectively equal to the intramolecular free energy difference between the α_R and C_7 conformations. Also, the contributions from the zero-point vibration energies are assumed to cancel—a reasonable assumption in light of the small entropic contribution to the free energy difference.

The charge set dependence of the intramolecular contribution is rather striking with both individually fit charge sets, UAI and AAI, giving a very large energy difference between the two conformations. As will be shown in the next section, this large intramolecular conformation energy difference is the source of disagreement between the free

Table II Molecular Dipole Moments[a]

	QM	UAA	UAS	UAI	AAS	AAI
$\mu_{C_{7,eq}}$	0.587	0.089	0.552	0.573	0.553	0.586
μ_{α_R}	1.530	1.446	1.552	1.532	1.557	1.529

[a] Dipole moment values are in $|e| \cdot$ Å. To convert to Debye's, multiply by 4.8032. QM results are the *ab initio* dipole moments.

Table III Decomposition of the Conformational Energy Difference $E_{\alpha R} - E_{C_{7,eq}}$ [a]

Energy Term	UAA	UAS	UAI	AAS	AAI
Intramolecular contribution					
ΔE_{intra}	3.88	3.01	7.09	3.35	8.31
ΔE_{bond}	0.29	0.24	−0.05	0.43	0.39
$\Delta E_{nonb,\,vdW}$	−1.38	−0.52	−0.90	−0.84	−1.04
$\Delta E_{nonb,\,Coul}$	4.97	3.29	8.04	3.76	8.96
$\Delta E_{nonb,\,Coul(1-4)}$	1.52	−0.71	−1.95	0.28	9.47
Intermolecular contribution					
ΔE_{inter}	2.66	−7.89	−0.49	−6.13	−1.59
ΔE_{SW}	−9.97	−10.63	−6.37	−9.62	−6.94
$\Delta E_{SW,\,vdW}$	0.27	0.16	−0.42	−0.11	−0.28
$\Delta E_{SW,\,Coul}$	−10.23	−10.79	−5.95	−9.51	−6.67
ΔE_{WW}	12.63	2.75	5.88	3.48	5.36

[a] All energies are in kcal/mole. E_{SW} and E_{WW} respectively correspond to solute–solvent and solvent–solvent contributions. Bonded intramolecular energy includes the bond, the angle, and the tetrahedral energies, as well as the hydrogen-bond and the out-of-plane energies. Intramolecular nonbonded terms utilizes a factor of 1/2 for the 1–4 interactions, and the electrostatic terms are calculated by using a distance-dependent dielectric constant $\epsilon = r$.

energy results for individually fit charge models and the experimental data. Table III shows how the intramolecular conformational energy differences vary with charge sets, and that the observed variations ($\sim$ 5 kcal/mole) are mostly due to the electrostatic term. Since the structures for each charge set relax almost to the same geometries, and since the van der Waals interaction parameters are the same for each set, the bonded and the van der Waals term contributions to the energy difference between two conformations are relatively constant (Table III). As a result, most of the variations arise from the electrostatic term. We would like to point out that strong dependence of the intramolecular energies to the site charges has been observed in other studies as well,[10,14,17-19] and varying the coefficient of the intramolecular 1–4 nonbonded Coulomb term sometimes makes it possible to get better agreement with the experiments. But, in our case changing the coefficient from $\frac{1}{2}$ to $\frac{1}{1.2}$ (following the recipe given by Kollman et al., Refs. 17–19) produced results leading to the same conclusions.

A similar grouping among the charge sets can be observed for the intermolecular interaction energies, except the results for the model with standard AMBER charges, UAA, differ somewhat. We find that the solute–solvent van der Waals energy difference $\Delta E_{SW,vdW}$ is almost constant (within 0.7 kcal/mole) for each set. As for the intramolecular contribution, the calculated electrostatic solute–solvent energies $\Delta E_{SW,Coul}$ of both the individually and simultaneously fitted charge sets, respectively, are similar among themselves. Note that the solute–solvent energy contribution ΔE_{SW} strongly favors the C_7 conformation, and the conformational solute–solvent energy differences vary by more than 2.5 kcal/mole, with simultaneously fit charges giving larger energy differences. In contrast to the solute–solvent energy, the solvent–solvent energy contribution ΔE_{WW} is in favor of the α_R conformation. Comparison of individually fit and simultaneously fit charges shows that the calculated solvent–solvent energy difference between the two conformations is consistently lower with simultaneously fit charges. Since the solute–solvent energy also makes a similar contribution, this results in a considerably larger (>4.5 kcal/mole) total intermolecular conformation energy difference between the different charge models. The calculated ΔE_{WW} for the UAA charge set is unexpectedly large and considerably different than the rest, and this discrepancy is most likely due to the underestimation of the C_7 conformation dipole moment by this charge model.

Free Energy Results

The main objective of this study is to investigate the effects of different ways of deriving the charges on the free energies, i.e., simultaneously vs individually, and united-atom vs all-atom models. Calculated solvation free energy differences (the intermolecular contribution) ΔA_{inter}, its respective energetic (enthalpic) ΔE, and entropic $-T\Delta S$, parts, as well as the intramolecular energy differences ΔE_{intra}, are tabulated in Table IV.

Table IV Free Energy and Energy Differences for $C_{7,eq} \rightarrow \alpha_R$[a]

Set	ΔA_{inter}	ΔE_{inter}	$-T\Delta S_{inter}$	ΔE_{intra}	ΔA_{total}[b]
UAA	-4.35 ± 2.89	2.66 ± 3.58	-7.01	3.88	-0.77
UAS	-4.94 ± 2.66	-7.89 ± 4.25	2.95	3.01	-2.23
UAI	-1.77 ± 2.25	-0.49 ± 4.15	-1.28	7.09	5.02
AAS	-4.23 ± 2.35	-6.13 ± 3.50	1.90	3.35	-1.18
AAI	-3.22 ± 2.20	-1.59 ± 3.11	-1.63	8.31	4.79

[a] All energies are in kcal/mole, and $T = 298$ K. Errors are calculated using block averages and correspond to two standard deviations.
[b] This assumes an intramolecular entropy contribution of 0.3 kcal/mole in favor of α_R conformation, see the discussion in the text. The zero point energy difference contribution is not included.

As Table IV shows, the calculated solvation free energy difference between α_R and C_7 conformations varies by approximately 1 kcal/mole for the all-atom model depending on whether a simultaneously fit single set of charges or individually fit charges are used. Considering the statistical fluctuations in the simulations, this difference is not large enough to indicate significantly different results. For the united-atom models, conformational solvation free energy differences with UAS and UAI charges differ by 3.2 kcal/mole. Although the statistical uncertainties calculated using the method of batch means are of the same magnitude, statistical errors inferred from the closure of a thermodynamic cycle provides further characterization of the error involved. The error for the UAA model was calculated in a previous work[32,33] as $\approx$ 1 kcal/mole. Therefore, we believe the calculated 3.2 kcal/mole difference for the united-atom models is quite reliable. Other trends in Table IV are that the single charge set calculations (UAA, UAS, and AAS) are consistent with each other, and that the individually fit charges (UAI and AAI) predict a lower solvation free energy difference between the two conformations. Comparison of united-atom vs all-atom model calculations reveal smaller changes in the solvation free energy differences: 0.7 kcal/mole for the simultaneously fit charges, and 1.45 kcal/mole for the individually fit charges. Although these values are again too small to be statistically distinct, the trend suggests that an all-atom representation should be preferred when the site charges are derived for each conformation individually. Since it is mainly the methyl groups that are involved in going from all-atom to united-atom representation, the above statement may particularly hold when the solvated molecule is small or when most of the methyl groups lie on the surface, as in the alanine dipeptide.

Another interesting outcome is that the sign of the entropic contribution to the solvation free energy may change depending on the model utilized. The entropic contribution effectively dampens the large enthalpic contribution variation among the models. For this reason, the overall differences observed among the charge sets for the solvation free energies are much smaller than the corresponding differences for the intermolecular internal energies.

Another way to investigate the effects of using different charge sets is to separate the interaction potential into individual terms, and then to study the trends in each contribution. Such a decomposition of the solvation free energy differences is reported in Table V. Since the only difference between the models are the site charges, the contributions of the van der Waals (i.e., 6–12) interactions are almost constant as expected and the electrostatic interaction is the principal cause of the observed differences in solvation energies among the models, as in the case of intramolecular contributions.

DISCUSSION

As discussed in the Methods section, the experimental results,[26,28] particularly the CD spectrum,[29] indicate that α_R is one of the principal conformations of the alanine dipeptide in aqueous solution, and that the C_7 conformation is also present (as coalesced with C_5) at about the same or slightly lower concentration. Therefore, with a loose interpretation, the total free energy difference between the α_R and the C_7 conformations should be zero or should have a small negative value, thus slightly favoring the right handed α-helical structure. It should, however, be emphasized that the interpretation of the experimental results are somewhat ambiguous and, correspondingly, the quantitative results should be treated as having large error bars.

Table V Decomposition of the Solvation Free Energy Differences (Intermolecular Contribution)[a]

	UAA	UAS	UAI	AAS	AAI
ΔA	−4.35	−4.94	−1.77	−4.23	−3.22
ΔA_{vdW}	−1.17	−0.23	−1.00	−1.07	−1.22
ΔA_{Coul}	−3.18	−4.71	−0.77	−3.17	−2.00

[a] All energies are in kcal/mole. Note that this decomposition is for illustrative purposes only; it is *only* the total free energy difference that is independent of the paths followed in the phase space; therefore, the presented decomposition would depend on the employed path.

As Table IV shows, the jointly fit charge sets, namely UAA, UAS, and AAS, all correctly predict the experimental finding that the α_R conformation would be slightly more populated than the C_7 conformation in aqueous solution. In contrast, the individually fit charge sets, UAI and AAI, decidedly favor the C_7 conformation to the point of effectively excluding the α_R conformation. The intermolecular free energy contributions do not vary too much among the charge sets, and the main source of the error in the results obtained with the individually fit charge sets is the intramolecular energy contribution. A closer look at the interaction energy terms (Table III) reveals that the difference effectively arises in the intramolecular electrostatic interactions. A review[25] of ab initio calculations of the difference between the energies of the C_7 and α_R conformations indicates that the intramolecular energy difference is $\approx$ 4 kcal/mol in good agreement with the energies calculated with the simultaneously fit charges.

Thus, there is a clear positive answer to the question posed at the end of the overview section: it is possible to derive a single set of charges to represent more than one conformation of a hydrated molecule. This answer is important, because reducing the number of force field parameters, i.e., using the same parameters at different conformations, allows the preservation of simplicity, which is a desirable attribute for theoretical methodology.

Our study also had an intuitively unexpected result concerning the use of charge sets individually fitted to ab initio electrostatic potential surfaces at certain conformations of an isolated molecule. While one would expect the individually fit charges to be superior to the conformation independent ones, we found, employing the currently standard treatment of intramolecular electrostatics, that the use of site charges derived from the uncontrained fitting to the ab initio electrostatic potential surfaces of isolated molecules at the respective conformations in fact gives worse results (when compared with experiment) and should be avoided. However, it is important to stress that this conclusion is conditional: (a) on the validity of our interpretation of experimental data, and (b) on the treatment of the intramolecular terms and very likely can be considered as an indication that either such treatment may need revision or that it is preferable to treat the intramolecular degrees of freedom quantum mechanically, whenever feasible.

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