Simulation of the Solvation Free Energies for Methane, Ethane, and Propane and Corresponding Amino Acid Dipeptides: A Critical Test of the "Bond-PMF" Correction, a New Set of Hydrocarbon Parameters, and the Gas Phase-Water Hydrophobicity Scale

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Abstract: We have carried out free energy perturbation calculations to determine the relative free energies of solvation in water for methane, ethane, and propane. Experimentally, the relative solvation free energy of methane and ethane is −0.16 ± 0.01 kcal/mol, and the relative solvation free energy of ethane and propane is 0.17 ± 0.04 kcal/mol. Using the recently described bond-PMF correction and a new set of hydrocarbon parameters, we calculate the relative free energy of solvation of methane and ethane to be 0.03 kcal/mol with Mulliken charges and 0.16 kcal/mol for electrostatic potential derived charges and the relative solvation free energies of ethane and propane to be 0.17 and 0.20 kcal/mol for the two sets of charges. The rather good agreement with experiment for these difficult to calculate quantities is encouraging, in that the methane to ethane free energy is within ~0.3 kcal/mol of experiment and the ethane to propane value is in near-quantitative agreement with experiment. To examine amino acid side chain effects, a simulation was also carried out to mutate *N*-acetylalanine *N*-methylamide to the corresponding valine dipeptide. The free energy difference is 1.1 ± 0.1 kcal/mol, larger than the 0.4 ± 0.1 kcal/mol calculated free energy difference between methane and propane. We show that this difference is due to indirect contributions from backbone atoms. An analysis of these results shows that the use of side chain analogs (e.g. methane → propane to represent alanine to valine) to describe free energy differences is likely to be an excellent approximation only when the presence/absence of the β carbon and the presence/absence of β branching do not change upon mutation.

Introduction

It has been nearly 10 years since the first application of free energy perturbation approaches to the relative solvation free energies of noble gases in water, which accurately reproduced the solvation free energies of Ne, Ar, and Kr.[1] In this time, many interesting applications of free energy methods to complex biomolecular systems, enzyme catalysis, protein-ligand interactions, and nucleic acid stability have been published.[2-6] It is a particular irony that, despite the apparent successes in these complex systems and in noble gas solvation, accurate calculation of solvation free energies of simple hydrocarbons in water has proven difficult.

The reasons for this have been appreciated for some time.[4,7] For free energy calculations which are dominated by electrostatic/H-bond changes (e.g. methanol → ethane), effective two-body potential[8] give free energies that compare very well with experiment. Free energies that are dominated by electrostatic effects and involve change in net charge require a very large nonbonded cutoff or cutoff correction to compare to experiment, but one can achieve reasonably well converged free energies with relative short simulations.[9] This is because dipolar reorientation of the solvent is relatively rapidly achieved and the free energy change is dominated by such reorientation. On the other hand, nonpolar mutations which involve a change in molecular shape are determined mainly by the exchange repulsion and dispersion attraction, which are typically represented by Lennard-Jones 6-12 potentials. To adequately sample the structural changes that accompany such free energy changes requires considerably more computer time, because the solvent must not only reorient but also undergo translational diffusion to fill in gaps or avoid "growing" steric repulsions. The dispersion attraction and exchange repulsion on adding a methyl group to a solute can come close to canceling in determining the free energy charges. This has been noted before by Bash et al.[4] and Singh et al.[10] and is reflected in the fact that the free energies of transfer from the gas phase to water for methane, ethane, and propane are almost identical.[11,12] Water is unique in the large role of the exchange repulsion term, as has been shown in the solvation free energy calculations in nonaqueous solvents by Singh and co-workers.[10] In any case, the accurate calculation of the relative free energies of solvation for methane, ethane, and propane has been difficult. For example, Rao and Singh calculated the relative solvation free energy of ethane and methane to be 0.42 kcal/mol,[13] compared to the experimental value of −0.16 ± 0.01 kcal/mol.[11,12] Fleischman and Brooks calculated a relative solvation free energy of ethane and propane of −0.83 kcal/mol,[7] compared to the experimental vlaue of 0.17 ± 0.04 kcal/mol.[11,12] One could imagine some simple correction to the potential function to reproduce experiment, but the united-atom OPLS (optimized potentials for liquid simulations)[7] model used by Fleischman and Brooks has been calibrated to reproduce enthalpies and densities of hydrocarbon liquids.

An all-atom model for hydrocarbons has recently been derived in our group in a manner analogous to that used to derive OPLS;[14] this model should be about the best one can do within the framework of a simple empirical all-atom potential function. Secondly, another issue manifests itself in the simple example: when one mutates propane to ethane, one carbon becomes a

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hydrogen and three hydrogens disappear (become dummy atoms). How does one correctly evaluate the free energy of shrinking the C–C → C–H bond, and should one shrink the free "disappearing" C–H bonds during the mutation? The calculated free energy should be the same whether one shrinks or does not shrink the C–H bonds which are disappearing, since in either case the attached H becomes a noninteracting dummy atom at the end point and free energy is a state function. Until recently, however, this was not observed in practice. The origin of this difficulty was finally explained in the work of Pearlman and Kollman,[15] who showed that an additional non-negligible "bond-PMF" correction was necessary for thermodynamic perturbations involving bond length changes with only finite sampling time.

Given these two recent developments, it is timely to attempt to calculate the relative free energy of aqueous solvation for methane, ethane, and propane. These calculations are particularly timely for another reason. Sharp et al.[16] have recently rethought the microscopic and macroscopic analyses of the excluded-volume contribution to the free energies of transfer between solvents. Their analysis would seem to suggest that the experimental scale for gas-phase to aqueous free energies of transfer (tabulated by Ben-Naim and Marcus on a molar basis in both the gas phase and water) needed to be corrected for volume fraction effects, i.e., differences in molar volume of solute and solvent. Our theoretical calculation should, in principle, contain all relevant terms in the free energy, and thus, it is of particular interest to compare our calculated free energies with those determined experimentally.

A final issue is how well one can transfer the free energies from model systems (methane to propane) to the analogous peptide and protein systems (alanine to valine). Wolfenden has assumed this transferability,[17] and qualitatively, it has been supported by Bash et al.[4] It is thus also of interest to examine the difference between perturbation of methane to propane by itself and perturbation in the presence of peptide backbone atoms (alanine to valine).

Methods

All the simulations described in this paper were performed using the molecular mechanical simulation package AMBER 4.0.[18] The potential energy function is of the form

$$U_{tot} = \sum_{bonds} K_r(r - r_{eq})^2 + \sum_{angles} K_\theta(\theta - \theta_{eq})^2 + \sum_{dihedrals} \frac{V_n}{2}[1 + \cos(\nu\varphi - \gamma)] + \sum_{i<j}\left\{\left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}}\right] + q_iq_j/\epsilon R_{ij}\right\} + \sum_{H\ bonds}\left\{\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}}\right\} \quad (1)$$

The simulations were carried out at 300 K using an explicit solvent in a cubic cell (18.6 Å) at constant pressure of 1 atm, and a periodic boundary condition was applied. The coupling constants to an external heat and pressure bath were 0.2 ps. The TIP3P water model[19] was used for the solvent. The SHAKE procedure was employed to constrain all the bond lengths.[20] The simulations were carried out using a time step of 1.5 fs and a nonbonded cutoff of 8 Å. Charges were derived through 6-31G* ab initio calculation via Mulliken population analysis for Mulliken charges and electrostatic potential fitting for ESP charges.[21] The AMBER all-atom force field was used for internal parameters, and nonbonded parameters and charges used in simulations are listed in Table I. For the dipeptide perturbation, since we are most interested in the effects of side chains here, we used the averaged backbone charges for the main-chain atoms, and the charges for side chains were chosen to be similar to those of hydrocarbons for the same reason (Figure 1).

Table I. Parameters Used in Simulations for Hydrocarbons

Nonbond Parameters[a]
C: R^* = 1.9082 Å, e = 0.1094 kcal/mol
H: R^* = 1.4872 Å, e = 0.0157 kcal/mol

mol	atom	q(Mulliken)[b]	q(ESP)[b]
CH_4	C	−0.660	−0.464
	H	0.165	0.116
C_2H_6	C	−0.4662	−0.027
	H	0.1554	0.009
C_3H_8	C (CH_3)	−0.476	−0.308
	H (CH_3)	0.157	0.067
	C (CH_2)	−0.302	0.296
	H (CH_2)	0.156	−0.041

[a] The nonbond parameters were taken from ref 14. [b] From 6-31G* ab initio calculations. Mulliken population analysis was used to obtain Mulliken charges, and electrostatic potential fitting was used to obtain ESP charges.[21]

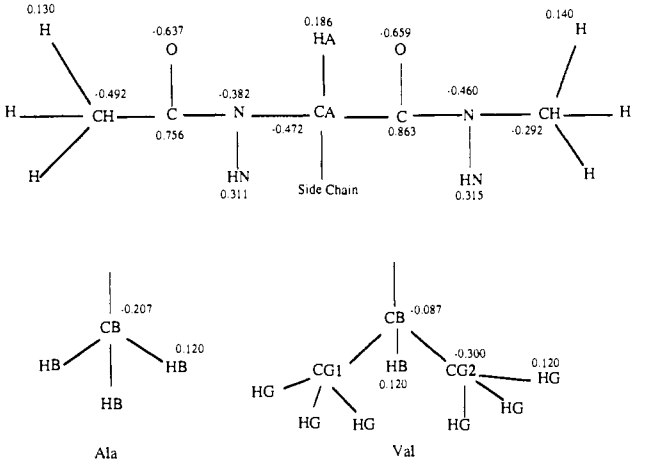

Figure 1. Charges used in simulations for amino acids.

Statistical mechanics free energy perturbation theory[22] allows for the calculation of free energy differences between two states of a system, A and B. The two states A and B are linked together with a coupling constant of λ. That is, the system is represented by a potential function $H(\lambda)$, such that $H(\gamma=0) = H_A$ and $H(\lambda=1) = H_B$, where H_A and H_B are the Hamiltonians of states A and B. The nonbonded-parameter mixing was performed as $\epsilon(\lambda) = \lambda[\epsilon(\text{mixed},\lambda=1)] + (1-\lambda)[\epsilon(\text{mixed},\lambda=0)]$, $r(\lambda) = \lambda[r(\text{mixed},\lambda=1)] + (1-\lambda)[r(\text{mixed},\lambda=0)]$, and $q_1q_2(\lambda) = \lambda[q_1q_2(\lambda=1)] + (1-\lambda)[q_1q_2(\lambda=0)]$. The free energy difference between the states at λ and λ + Δλ is

$$\Delta G_\lambda = -RT \ln \left\langle \exp\left(-\frac{H_{\lambda+\Delta\lambda} - H_\lambda}{RT}\right)\right\rangle_\lambda \quad (2)$$

where R is the gas constant, T is the absolute temperature, and $\langle\ \rangle$ denotes the ensemble average at state λ. The total free energy change between A and B is thus

$$\Delta G = \sum_{\lambda=0}^{\lambda=1} \Delta G_\lambda \quad (3)$$

In the free energy perturbation of propane to ethane, propane ($CH_3CH_2CH_3$) is actually perturbed to $CH_3CH_2HD_3$, where D is a dummy atom with zero van der Waals radii and well depth and zero charge. For perturbations where any bond lengths change

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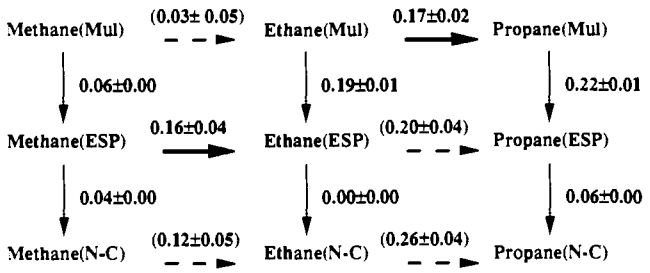

Figure 2. Relative free energies through perturbations. Free energies are in kcal/mol. Mul indicates Mulliken charges, ESP indicates electrostatic potential fit charges, and N-C indicates zero charges. The simulations with bold lines were run in both forward and backward directions; errors are the hysteresis for forward and backward runs. The simulations with plain lines were run only in the forward direction, and errors were estimated from double-wide sampling. The simulations with dashed lines were calculated from the corresponding thermodynamic cycles. The experimental results for methane → ethane and ethane → propane are −0.16 and 0.21 kcal/mol, respectively.[11]

(e.g., here a C–C bond is transferred to a C–H bond), Rao and Singh[23] and Pearlman and Kollman[15] have shown that the standard method of including a change in bond length directly into the calculation suffers from sampling problems and the results are generally unacceptable. Pearlman and Kollman[15] suggested a method to solve this problem—apply SHAKE constraints on the perturbed bonds and then use an explicit potential of mean force (PMF) like calculation to determine the contribution due to bond length changes. Using such a method, they obtained values for the solvation free energies of methane and the relative solvation free energy for neopentane and methane which were independent of whether the disappearing C–H bonds "shrink" during the perturbation processes. We employed this bond-PMF correction for all perturbations involving changes in bond lengths which are described herein. Although AMBER 4.0 has the capability to include both intra- and intermolecular free energy terms in eq 2, we employed the option to include only intermolecular terms. Inclusion of intramolecular terms would have required evaluating eq 2 in the gas phase and would likely have introduced considerably more statistical "noise" in the free energy simulations.

For alanine to valine simulations, the dipeptide was kept in the extended conformation by harmonic constraints of 50 kcal/rad^2 on ϕ, ϕ at −160° and 160°. No effort was made to constrain the side chain dihedral angle. The side chain was initially assigned the trans conformation (IUPAC–IUB rules) and was found to stay in this conformation throughout the simulations. Prior to the perturbations, all systems were equilibrated for at least 25 ps. The "window" method of AMBER/GIBBS was used for all the free energy calculations. For perturbations involving the shrinking or growing of a methyl group, molecular dynamics simulations were run for 180 ps. For perturbations where only the electrostatic distribution changes (changes in charges), the molecular dynamics simulation times were 90 ps. Two 300-ps simulations were conducted to ensure that appropriate convergences had been approached. These simulation lengths seem reasonable in light of the convergence studies of hydrocarbons and dipeptides.[15,24] In both cases, each window consists of 500 steps of equilibration and 500 steps of data collection. The data collections were calculated for both the forward perturbation ($\lambda - \delta\lambda$) and backward perturbation ($\lambda + \delta\lambda$); this is so-called double-wide sampling.

Results

The results of the simulations of hydrocarbons carried out in this study are shown in the thermodynamic cycle displayed in Figure 2. With ESP charges, the results for methane to ethane and ethane to propane are 0.16 and 0.20 kcal/mol, respectively. To determine whether the convergence was reached, a much longer simulation time, 300 ps, was also run for the methane to ethane perturbation with ESP charges. In this case, the result was 0.16 ± 0.01 kcal/mol. This was run only in one direction, and the error is from double-wide sampling. By comparison, for the 180-ps simulation, the results for the forward and backward simulations were 0.18 ± 0.06 and 0.14 ± 0.04 kcal/mol, respectively, with an average of 0.16 kcal/mol. It is clear that, for the systems under study, better statistics could be achieved in 300-ps simulations, although a 180-ps simulation time is probably adequate for the results to converge with relatively small errors. Another observation from Figure 2 is that the hysteresis for the simulations where only charges change is the smallest. As discussed in the Introduction, we see here again that the free energies due to only the electrostatic changes are much less difficult to calculate.

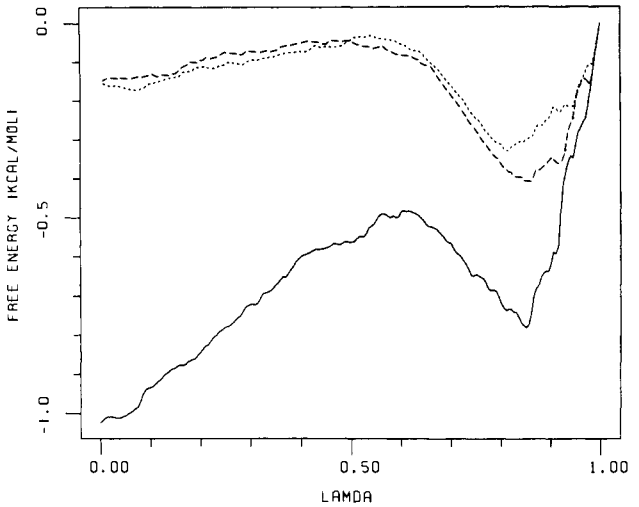

Figure 3. λ dependence of relative free energies: (a) ethane (λ = 1) to methane (λ = 0), short dashed line, ESP charges; (b) propane (λ = 1) to methane (λ = 0), long dashed line, Mulliken charges; (c) *N*-acetylvaline *N*-methylamide (λ = 1) to *N*-acetylalanine *N*-methylamide (λ = 0), solid line.

The representative λ dependence of relative free energies is displayed in Figure 3. As can be seen from the graph, there is a step change at the λ = 1 end, which corresponds to the point where a methyl group is must beginning to shrink. This type of variation pattern has long been observed for alkane solvation in water.[7,13] However, the variation we observed here is much sharper and narrower. This variation is probably due to both the lack of a dominant electrostatic contribution and the competing processes of exchange repulsion and dispersion attraction.

The solvation free energy difference between the the alanine and valine dipeptides was determined to be 1.1 kcal/mol from a 300-ps simulation (1.2 kcal/mol from a 200-ps simulation). The electrostatic contribution to the total free energy was also evaluated by changing only the charges, and the result was +0.1 kcal/mol.

Since the free energy of mutation of methane to propane has been calculated to be 0.4 ± 0.1 kcal/mol, the calculated free energy of alanine to valine mutation (1.15 ± 0.05 kcal/mol, based on the above two simulations) were somewhat of a surprise. However, if we take into account the change of interactions between backbone atoms and water due to the mutation, this difference becomes more understandable. The solvation free energy per unit area derived by fit to experimental solvation data[25] indicates that this parameter for carbonyl oxygen and amide nitrogen is much larger than the parameter for hydrocarbons (−0.100 (kcal/mol)/Å^2 for O/N and 0.004 (kcal/mol)/Å^2 for C from ref 25). Surface area calculation shows that the solvent-accessible area for carbonyl oxygen and amide nitrogen is indeed about 8 Å^2 larger in alanine than in valine, suggesting that backbone atoms contributed to the difference between alanine to valine and methane to propane.

To test this interpretation, another alanine to valine simulation was performed. This time the charges on the backbone atoms were zero except that charges of −0.273 and +0.120 were used

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for the α carbon and the α hydrogen to keep the molecules neutral. The calculated free energy from a 200-ps simulation for the alanine to valine mutation now is 0.5 kcal/mol, nearly identical to the methane to propane result of 0.4 kcal/mol. It is thus likely that what happened in the dipeptide mutation is that the backbone carbonyl and amide atoms are better solvated in alanine than in valine and this provides an extra contribution to the total solvation free energy difference.

How does such a contribution appear in the free energy in eq 2, even though the only terms that contribute to $(H_{\lambda+\delta\lambda} - H_\lambda)$ are the side chain atoms that change upon mutation? ΔG in eqs 2 and 3 is derived exactly from statistical mechanics theory and should represent the free energy difference between two states described by two Hamiltonians H_A and H_B. A reasonable physical picture for what is happening is as follows: The water molecules H-bonding to the amide N—H and carbonyl C=O experience repulsions from the valine C_γ methyls; the repulsions are reduced when valine is mutated to alanine. The small amount of difference in free energy (~0.5 kcal/mol) suggests that the H-bonding pattern is mostly undisrupted by the valine side chain and the loss of the free energy in valine is caused by a decreased optimization in hydrogen bonding and increased repulsions between water and the side chain.

Discussions and Conclusions

We have presented a molecular dynamics/free energy perturbation study of simple hydrocarbons and dipeptides. The all-atom nonbond interaction parameters for hydrocarbons determined by empirically fitting liquid properties were used to calculate the relative solvation free energies. Due to only minor contribution from electrostatic interactions, the solvation free energy of hydrocarbons is a sensitive test of nonbond parameters. Through the use of bond-PMF correction and relatively long simulation times, an accurate and converged value of the required ensemble average has been determined. We consider the absolute error in the calculated ΔG compared to experiment more relevant than the percentage error, because this corresponds directly to a relative error in the experimental data. In this context, the resulting relative free energies of aqueous solvation are encouraging in that the agreement with experiment for ethane → propane is nearly exact (0.17–0.20 kcal/mol calculated, 0.17 ± 0.04 kcal/mol experimental). On the other hand, the free energy of methane → ethane is only qualitative (0.03–0.16 kcal/mol calculated, –0.16 ± 0.01 experimental) in that the deviation between the calculation and experiment is 0.2–0.3 kcal/mol. One has to by aware that there are errors associated with experimental values as well, although they are smaller than those found in the theoretical calculations.

From Figure 2 we find that the charges used become quite important at the level of accuracy under discussion here. When zero charges are used for all three alkanes, the free energy differences of methane to ethane and ethane to propane are 0.12 and 0.26 kcal/mol. The small ESP charges of ethane, compared to methane and propane, lead to methane being even more stabilized than ethane by the solvation of charges. At the other extreme, if Mulliken charges are used for ethane and propane and ESP charges for methane (the carbons of the three molecules then have about the same charges), the free energy differences become –0.03 ± 0.05 kcal/mol for methane to ethane and 0.17 ± 0.02 kcal/mol for ethane to propane. The remaining discrepancy between theory and experiment, besides the effects of charges discussed above and possible differences of intramolecular free energies between solutes in the gas phase and solutes in solution not included in our calculations, could be possibly due to the use of pairwise additive potential functions and classical dynamics. Reproducing experimental results better than ~0.1–0.2 kcal/mol using such a modeling methdology may be too much to ask for.

We have not done a detailed sensitivity analysis of the effect upon changing these parameters by some 10% on either hydrocarbon enthalpies/densities or aqueous solvation free energies. All we can say at this point is that the same set of parameters, when calculated for hydrocarbon properties, performed quite well in aqueous solvation. This provides support for the philosophy inherent in the OPLS force field.[8]

A new hydrophobicity scale has recently been suggested by Sharp et al.[16,26] for gas phase → water transfer free energies, and in the new scale, the methane to ethane and ethane to propane free energies are ~0.7 kcal/mol. In ref 26, there are a number of points made vis-à-vis simulations which deserve comments. We agree that free energy perturbation calculations do not include a "volume entropy" term (ln V). However, on this basis, Sharp et al. suggested that the free energy perturbation results should be compared to $-RT$ ln (molar ratio) plus another "size-correction" term related to the difference in molar volume of solute and solvent. We do not agree with this suggestion. It seems clear to us that, for gas phase to water free energies of transfer, as long as consistent molar units are used to measure the solute concentration in both gas and solution, as described in refs 11 and 27, the calculated free energies should be directly relatable to the experimental free energies determined by $-RT$ ln (molar ratio). The good agreement between our calculated results and those reported in refs 11 and 12 suggested that one does not need an extra "size-correction" term to relate the calculated free energies to experiment.

Finally, we wish to analyze the transferability of data on amino acid side analogs to the relative solubility of amino acids in peptides and proteins. The simulation results have shown that the solvation free energy of the alanine to valine mutation is about 0.7 kcal/mol more positive than that of the mutation of methane to propane. However, since the origin of this difference is the change of interactions between backbone atoms and water, it could be expected that this difference will only be significant when one compares two amino acids with and without a β carbon or with and without β branching. Comparing two amino acids which are similar in their side chain effects on the backbone atoms H-bonding with water should lead to close correspondence between the free energies of the model system and those of peptides. In any case, the side chain analogs should give good estimates of dipeptide free energy changes to within ~0.7 kcal/mol.

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