Solvation Free Energies of Peptides: Comparison of Approximate Continuum Solvation Models with Accurate Solution of the Poisson−Boltzmann Equation

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We have compared solvation free energies obtained from a number of approximate solvation models with an accurate solution of the Poisson−Boltzmann equation for a large data set of peptide structures, ranging from a single amino acid to a peptide sequence of length nine. The models are assessed for their ability to predict relative energetics of different peptide conformations (of the same sequence) as determined from the Poisson−Boltzmann results. We find that the widely used distance dependent dielectric model yields qualitatively erroneous results; in contrast, the generalized Born model of Still and co-workers, an approximation to the Poisson−Boltzmann equation, provides reasonably good solvation free energies and performs rather well in rank ordering of conformations. A surface area based model produces results of intermediate quality. Our results suggest that the generalized Born model is presently the clearly preferred alternative if one wishes to carry out molecular dynamics simulations with a fast, approximate solvation model.

1. Introduction

The calculation of solvation free energies via dielectric continuum methods is a crucial technology in the molecular modeling of biological systems. Simulations involving explicit water, while feasible, are very expensive computationally, which makes applications to practical problems such as drug design difficult; furthermore, there is no compelling evidence that the current implementations of such methods are more accurate than continuum models, at least with the present generation of force fields.[1] In addition to increasing the cost of evaluation of the energy and gradients due to the presence of a large number of solvent molecules, explicit solvent models require averaging over a huge number of local minima for the solvent in order to obtain free energies. This not only increases computation time drastically; it also introduces a substantial amount of random noise into various simulation methodologies, such as free energy perturbation. Continuum methods avoid these difficulties and hence are the method of choice—provided that sufficient accuracy can be obtained.

Driven by these computational exigencies, a number of approximate continuum approaches have been developed. However, only one of these methods is based upon rigorous physics: this is the Poisson−Boltzmann (PB) equation, which has been cast in a form suitable for computing molecular solvation free energies by the development of parametrized models for the solvent cavity.[2−10] It is possible to derive a Poisson−Boltzmann equation of this form directly from statistical mechanical theories of the liquid state such as the hypernetted chain equation, using a set of model approximations. With suitable empirical parametrization, accurate results have been obtained for small molecule test cases and a number of impressive applications to biological systems have been presented.[11−15] While the precise accuracy available for larger systems of any particular parametrization is at present unclear—there are significant issues of transferability, nonadditivity, and short-range hydrogen bonding energetics,[1] and the problem of modeling the solute charge distribution and polarizability is highly nontrivial—one can be confident that the functional form of the equations is reasonable.

However, obtaining accurate numerical solutions to the PB equations for a large system such as a protein has a significant computational cost. In particular, if one wishes to use a continuum solvent model in a simulation (e.g. molecular dynamics or free energy perturbation), the cost of solving the PB equations and computing an analytical gradient thereof is currently at least 1 order of magnitude more expensive than evaluation of the molecular mechanics energy and gradient. Therefore, efforts have been made to develop inexpensive continuum solvation models to be used for this purpose.

Despite the widespread use of many of these models, there have as yet been few attempts to assess their reliability for large systems, for example, by comparison with PB solvation free energy results. Accuracy for a limited set of small molecule test cases is no guarantee of good results for large, flexible molecules; the former is typically explicitly achieved via the use of quite a few adjustable parameters. To succeed in plausibly representing larger systems, the functional form of the approximation must be suitable to the task at hand. This is particularly questionable in cases where the model has been postulated in an ad hoc fashion, as opposed to being derived from a more rigorous foundation.

The goal of the present paper is to carry out extensive tests of three approximate solvation free energy models, all of which are readily applicable to simulations of large systems: distance dependent dielectric, a model based upon exposed surface area, and the generalized Born (GB) model, an approximation to the PB equation. Our tests are performed for a wide range of

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TABLE 1: Statistical Comparison of the Different Solvation Models in kilojoules per mole[a]

set		PBF/GB-AN			PBF/GB-NU			PBF/DDD			PBF/SA			
		mean	sd	*r*	mean	sd	*r*	mean	sd	*r*	mean	sd	*r*	*n*
1	neutral	−6.05	4.62	0.999	−1.28	3.21	0.999	−139.31	117.72	0.591				20
2	zwion	−21.29	5.80	0.999	−8.39	5.50	0.999	−592.97	123.29	0.327				20
3	lys	−19.31	2.07	0.981	−3.65	2.33	0.977	−769.62	33.12	0.498				233
4	dimer	−34.76	3.42	0.969	−19.26	3.75	0.968	−988.72	48.48	0.803				328
5	trimer	−34.99	4.74	0.913	−18.61	3.93	0.940	−1045.3	50.57	0.608				247
6	pent	−49.73	5.98	0.808	−32.70	5.11	0.855	−1497.7	53.28	0.316				113
7	hept	−38.60	7.24	0.832	−30.95	6.73	0.860	−1667.3	48.27	0.831				20
8	npep	−43.24	9.38	0.837	−32.60	7.87	0.889	−1942.5	43.48	0.402				50
9	$pent_N$	−24.15	5.61	0.875	−17.28	3.77	0.945	−525.7	12.29	0.666	−32.41	14.38	0.7114	359
10	$npep_N$	−47.03	9.48	0.730	−36.47	6.45	0.888	−870.6	17.69	0.489	−83.73	16.45	0.645	177
11	arg_{df}	4.84	5.12	0.997	8.89	4.02	0.997							
12	arg_{sm}	3.59	2.87	0.988	8.12	2.76	0.988							
13	arg_{tw}	-0.23	4.13	0.998	7.42	3.78	0.998							

[a] Neutral, 20 neutral amino acids; zwion, 20 zwitterionic amino acids; lys, zwitterionic, net charge = +1; dimer, tyr-glu (zwitterionic), net charge = −1; trimer, tyr-glu-ala (zwitterionic), net charge = −1; pent, tyr-glu-ala-asp-gly (zwitterionic), net charge = −2; hept, tyr-glu-ala-asp-gly-arg-leu (zwitterionic), net charge = −1; npep, tyr-glu-ala-asp-gly-arg-leu-val-thr (zwitterionic), net charge = −1; $pent_N$, ala-asn-phe-val-trp (neutral ends), net charge = 0; $npep_N$, ala-asn-tyr-val-trp-ile-gly-val-leu (neutral ends), net charge = 0; arg_{df}, arginine with only one charge, different values; arg_{sm}, arg with only one charge, constant value, net charge: 0.5; arg_{tw}: arg with two charges, equal and opposite in value net charge: 0; PBF/GB-AN, PBF vs GBSA; PBF/GB-NU, PBF vs GBSA with numerical Born radii; PBF/DDD, PBF vs distance dependent dielectric; PBF/SA, PBF vs surface area model; sd, standard deviation.

peptide structures and conformations, up to systems as large as nonapeptides. The methodology is straightforward; using the same set of atomic point charges and dielectric radii (the latter is relevant only for the GB model), we calculate the total free energy of each of the three approximate models and compare these values with that obtained from a method that employs an accurate numerical solution of the PB equation for the solvation free energy. Drastic differences in performance are obtained for the three models, with the GB model yielding qualitatively superior results, as might be expected given its sounder underlying physical basis.

The paper is organized as follows. In section 2, we briefly describe the theoretical methods utilized in the calculations. Section 3 presents results for peptides ranging in size from one to nine amino acids. Section 4, the conclusion, summarizes the results and suggests future directions for research.

2. Methods

Solvation Models. Three different models were used to calculate the hydration free energies of peptides solvated in water. The first model, based on approximate solvent accessible surface areas (SA), was proposed in the early days of molecular modeling of biomolecules and is still widely used.[16] The total free energy of solvation of the peptide in this representation is

$$G_{\mathrm{sol}} = \sum_k c_k A_k$$

where A_k is the total solvent accessible surface of all atoms of type k and the constant c_k represents the contribution of atom type k to the solvation energy. The parameters A_k are typically determined by fitting to small molecule solvation free energy data.

The second model employs a distance dependent dielectric constant (DDD) of the form $\epsilon = R_{ij}$ in the computation of the electrostatic energy.[17,18] The physical rationalization for this approximation is the idea that as groups become more distant, dielectric screening increases in effectiveness. However, the actual functional form represents a completely ad hoc assumption and hence can only be justified empirically.

The third model uses a mixed generalized Born/surface area (GB/SA) continuum solvation model.[19] The free energy of solvation in this scheme is written as a sum of three terms:

$$G_{\mathrm{sol}} = G_{\mathrm{cav}} + G_{\mathrm{vdW}} + G_{\mathrm{pol}}$$

a solvent−solvent cavity term G_{cav}, a solvent−solute van der Waals term G_{vdW}, and a solvent−solute polarization term G_{pol}. The first two terms account for the penalty incurred by placing a nonpolar solute of specified geometry into water. This is represented by the sum:

$$G_{\mathrm{cav}} + G_{\mathrm{vdW}} = \sum_k \sigma_k \mathrm{SA}_k$$

where SA_k is the total solvent accessible surface area of all atoms of type k and σ_k is an empirical solvation parameter.

The final term, G_{pol}, which represents the electrostatic solvation free energy, has the form of the Born equation and approximates the solution to the PB equation as has been described previously.[19]

$$G_{\mathrm{pol}} = -166\left(1 - \frac{1}{\epsilon}\right)\sum_{i=1}^{n}\sum_{j=1}^{n}\frac{q_i q_j}{[r_{ij}^{\,2} + \alpha_{ij}^{\,2}\, \mathrm{e}^{-D}]^{1/2}}$$

where

$$\alpha_{ij} = [\alpha_i \alpha_j]^{1/2}$$

$$D = r_{ij}^{\,2}/(2\alpha_{ij})^2$$

r_{ij} is the distance between atom i and atom j, and α is the effective Born radius for atom i with charge q in a dielectric medium with constant ϵ. The effective Born radius, α_i, of an atom i is defined as the radius of a spherical dielectric cavity which would yield the same electrostatic polarization energy of an atom i in the actual molecular cavity.

Determination of the α_i may be carried out by numerical volume integration of a $1/r^4$ polarization term as described in ref 16. We refer to this as the GB-NU model. Alternatively, MacroModel allows specification of a fast analytical approximation to the integral.[20] We refer to this as the GB-AN

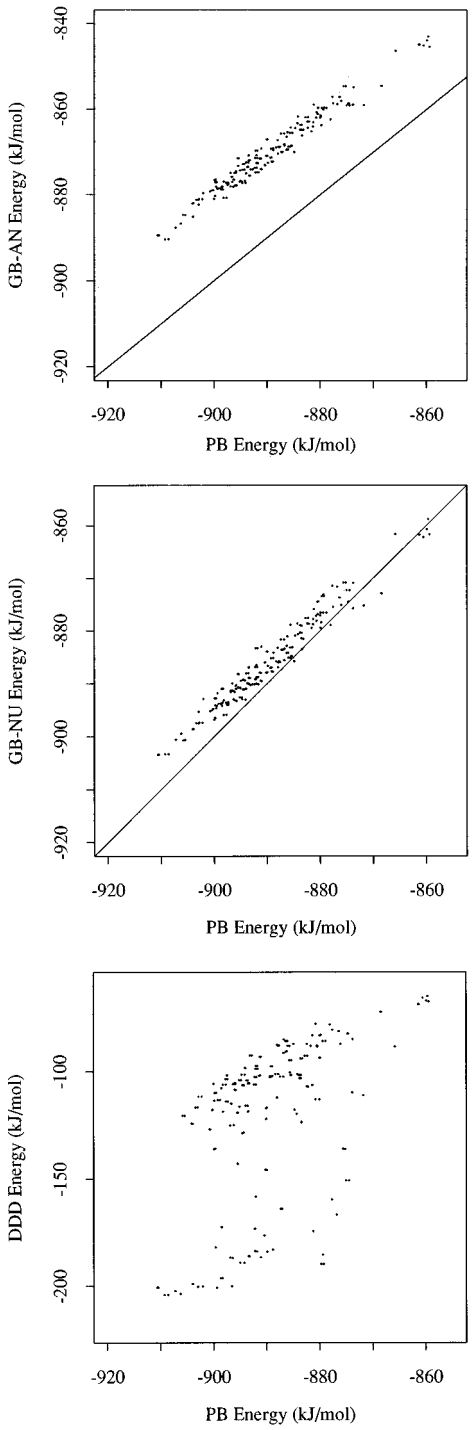

Figure 1. Sets 3−233 of conformations of lysine: (a) PB/GB-AN energy comparison; (b) PB/GB-NU energy comparison; (c) PB/DDD energy comparison.

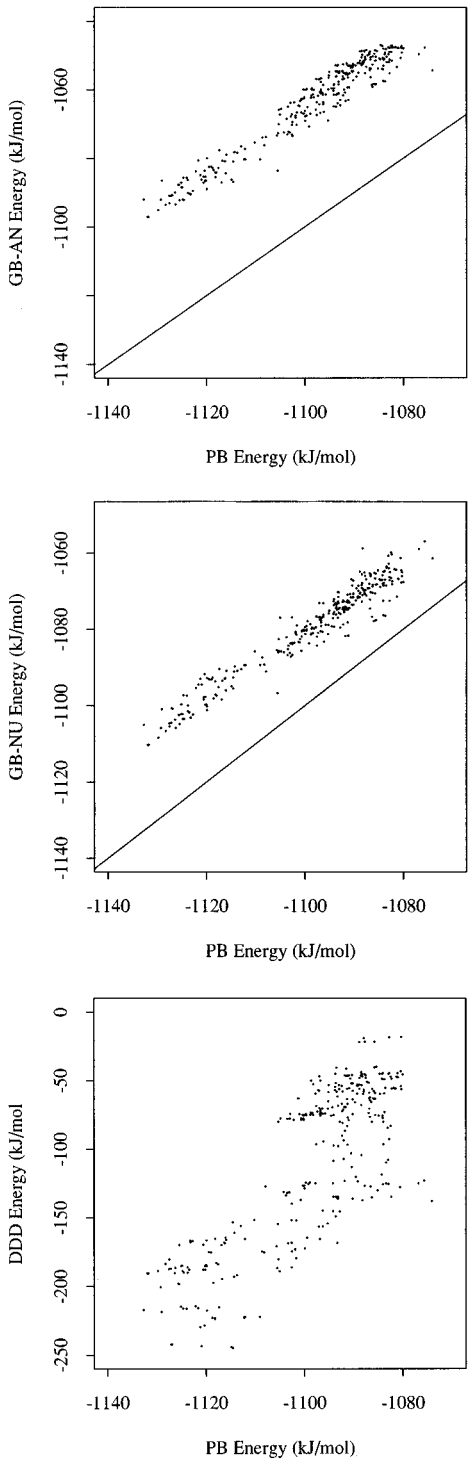

Figure 2. Sets 4−328 of conformations of a dipeptide; (a) PB/GB-AN energy comparison; (b) PB/GB-NU energy comparison; (c) PB/DDD energy comparison.

model. The numerical method is expected to produce a result in closer conformance to the accurate numerical solution to the PB equation for atom i in the actual molecular cavity.

The total free energies from these three models were compared to results based on a PB treatment of the peptide/water system, using a PB finite element solver, PBF, which was introduced into BatchMin. The finite element algorithms used in PBF are described in detail in ref 17. PBF produced the solvation free energy, the sum of the reaction field energy, and a cavity term, which was then added to the molecular mechanics gas phase energy provided by BatchMin.

The polarization energies calculated by PBF were initially compared to those calculated by Delphi, a PB finite difference solver, to ensure that the PBF and BatchMin codes had been merged accurately and were producing correct energies. Extensive tests indicate that the PB results presented here constitute an accurate solution to the Poisson−Boltzmann equation within 0.5 kcal/mol, which is more than adequate for the purposes of this paper.

All three of the above models were implemented using Batchmin, the batch mode molecular modeling facility of MACROMODEL molecular modeling package, with the AMBER* force field used to represent the solute−solute

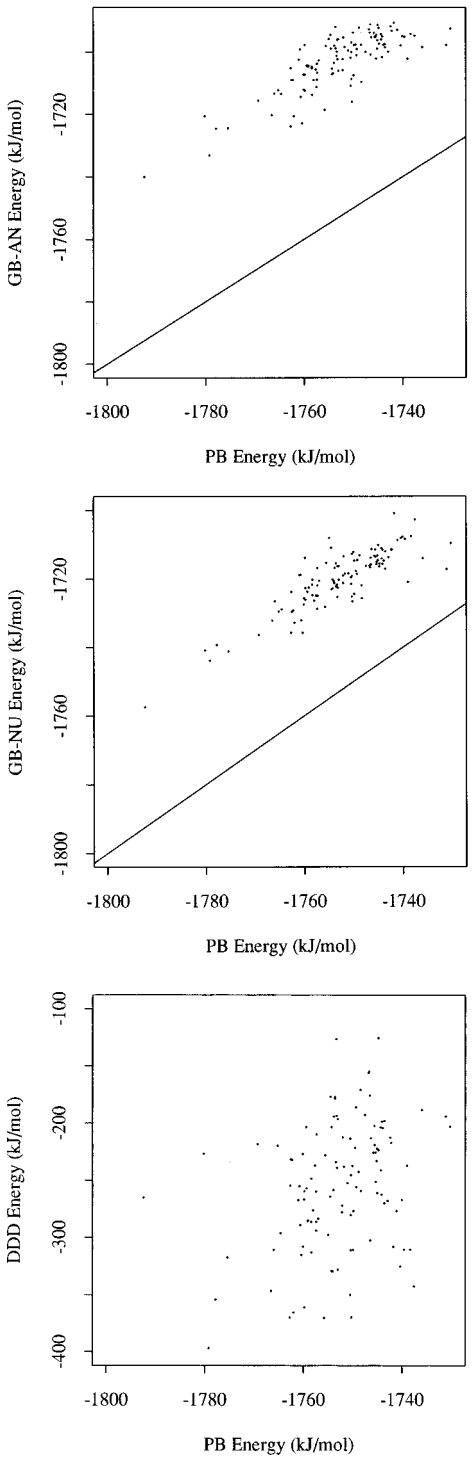

Figure 3. Sets 6−113 of conformations of a pentapeptide: (a) PB/GB-AN energy comparison; (b) PB/GB-NU energy comparison; (c) PB/DDD energy comparison.

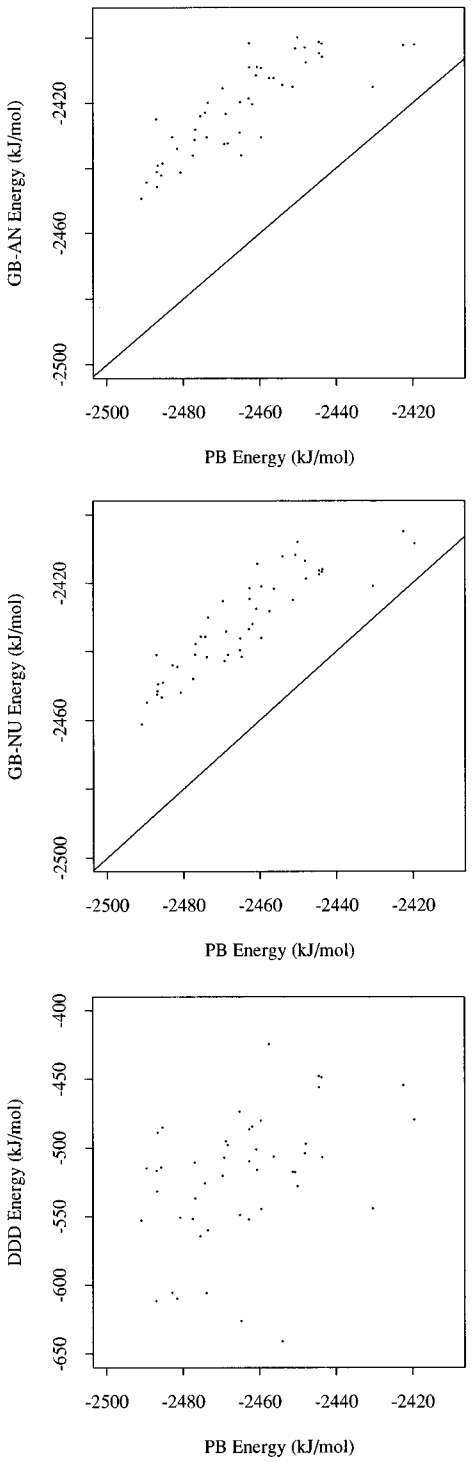

Figure 4. Sets 8−50 of conformations of a nonapeptide: (a) PB/GB-AN energy comparison; (b) PB/GB-NU energy comparison; (c) PB/DDD energy comparison.

interactions, infinite cutoff distances for the nonbonded interactions, and a value of 1.0 for the interior dielectric constant.

All energies reported below are a sum of the solvation free energy computed by a given model for the conformation in question and the internal molecular mechanics energy of that conformation. As the gas phase molecular mechanics energy is identical for each of the four methods, the comparisons represent a suitable test of the ability of the approximate solvation free energy methods to reproduce the solution phase conformation stability predictions of the more reliable PB approach.

Test Sets. A total of 13 test sets of molecular structures have been used in our comparisons.

Sets 1 and 2 are composed of the twenty biological amino acids and differ only at the termini with set 1 neutral and set 2 zwitterionic. Sets 3−10 each contain one group of peptide conformers, ranging in size from one to nine residues; the peptide is either zwitterionic or neutral in character. The amino acid sequence of each peptide is presented in Table 1. All of the conformers were generated using the Monte Carlo conformational search feature of BatchMin; the number of structures generated for each peptide ranged from 20 to approximately 360.

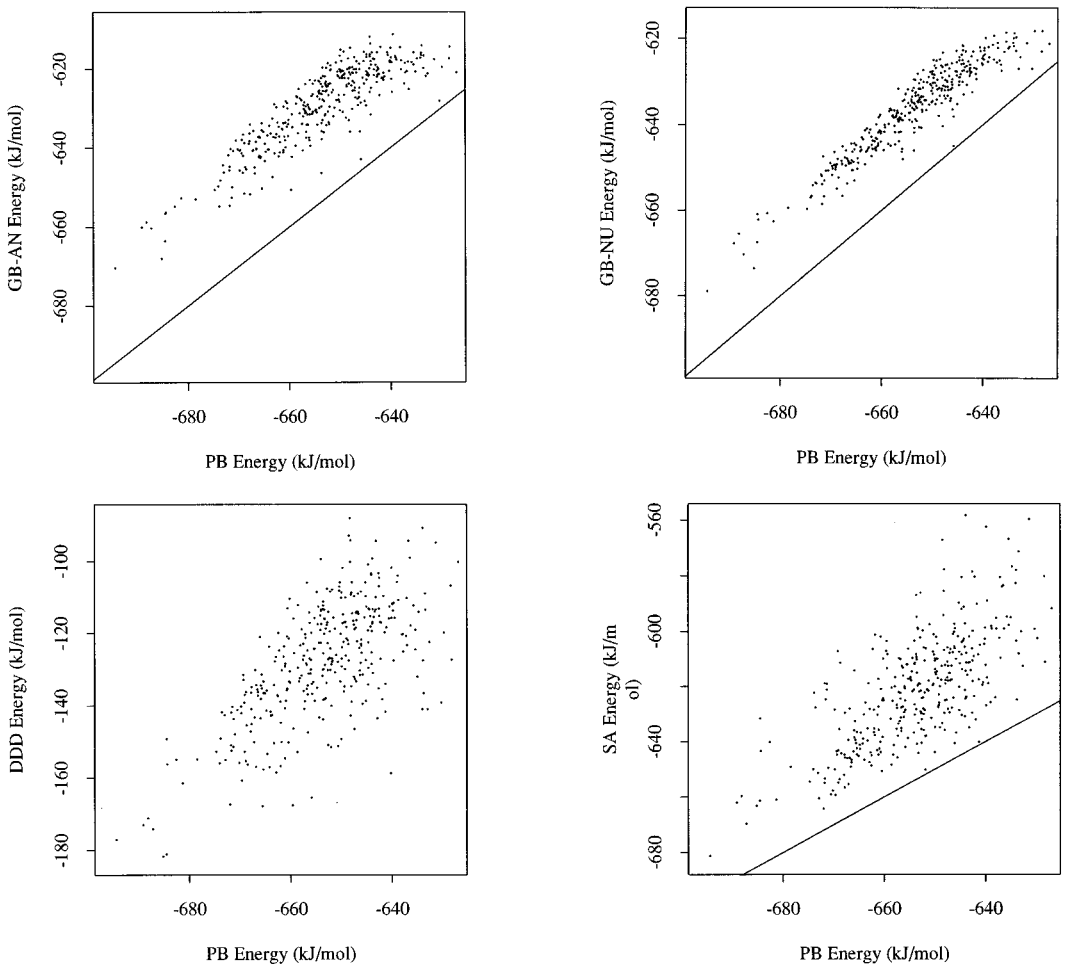

Figure 5. Sets 9−359 of conformations of a neutral pentapeptide: (a) PB/GB-AN energy comparison; (b) PB/GB-NU energy comparison; (c) PB/DDD energy comparison; (d) PB/SA energy comparison.

The last three test sets, 11−13, were designed to examine the effect the presence of net charge on a molecule has on the accuracy of the calculated solvation free energy. These comparisons were all carried out using the amino acid arginine. In set 11, all of the partial charges of the atoms in arginine were set to zero, except one which was left unchanged. Since only charges with a magnitude larger than 0.25 eu were considered, there were 17 different structures. Set 12 used a constant charge of 0.5000 eu, which was successively displaced throughout the molecule, occupying one of the twenty possible atomic positions in turn. Last, in set 13, all possible permutations of two equal and opposite charges of 0.50 eu in the molecule were tested.

3. Results

The total solution phase conformational energies (i.e., molecular mechanics conformational energy plus solvation free energy) of the molecules calculated with BatchMin using the DDD, SA, and two GB models are plotted in Figures 1−9 against PB energies, which are used as the reference. The line $x = y$, reflecting perfect agreement between the two models, is depicted in these plots by a solid line. The difference in energy between the PB model and the other models for each conformation in a set was averaged over the entire set, and the results are presented in Table 1.

From these plots and from the calculation of the mean differences, certain general trends emerge. First, the distance dependent dielectric model performed significantly worse than the GB models, even in the case of a single amino acid (Figure 1). While the GB graphs 1a,b display a linear relationship with the PB results, the DDD graph, 1c, does not show a high degree of correlation with the PB energies. For the DDD model, the mean energy difference with the PB results increases with the size of the system, from −769.92 kJ/mol for lysine to −1942.5 kJ/mol for the nonapeptide. The standard deviation remains constant except for sets 1−2 which contain energies for all 20 biological amino acids, where it is larger, possibly reflecting the greater variation in sequence, size, and structure among the molecules in those sets. The distinction between the DDD and GB models becomes sharper as the systems increase in size; there is very little correlation between the DDD model and the PB energies for the largest peptide tested, a nonapeptide (Figure 4c).

We next consider the SA model, which employs Scheraga's classification of atom types and constants c_k[16] and which we have tested only on the neutral peptide sets 9 and 10. While the overall energetic trends are reasonable in comparison to the PB results, the detailed ordering of the structures displays substantial errors. This is reflected in all regions of the distribution, although the errors are somewhat smaller at low energy.

The GB model, in contrast, displays quite reasonable predictive power with regard to rank ordering of structures for all cases examined. From the graphs of the dimer, pentamer, nonamer, and the neutral pentamer (Figures 2−5), it is obvious that both the PB and GB models selected the same structure as the minimum, and the top 10 lowest energy structures are consistently the same for both models. In the cases of lysine,

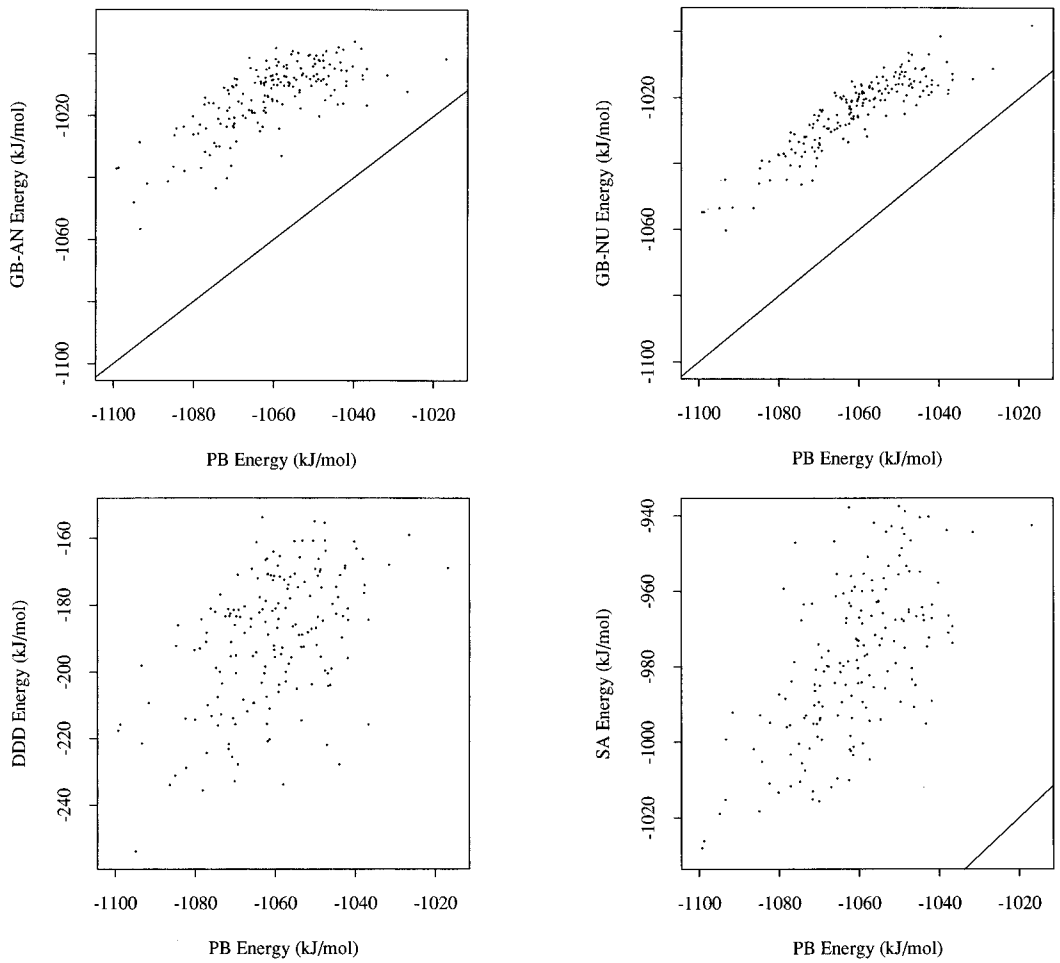

Figure 6. Sets 10−177 of conformations of a neutral nonapeptide: (a) PB/GB-AN energy comparison; (b) PB/GB-NU energy comparison; (c) PB/DDD energy comparison, (d) PB/SA energy comparison.

the trimer, and the neutral nonamer the PB model selected a different structure than the GB model as the lowest energy one; however, these fell as numbers four, five, and three, respectively, in the GB rankings. Furthermore, when GB was used with numerically calculated Born radii, the rankings with respect to PB improved in all cases except for the neutral nonamer.

There is, however, an apparent linear increase in the mean energy difference between the GB and PB models as the size of the peptide increases and a concurrent decrease in the correlation between the two models, which is immediately obvious from Table 1. This linear trend is broken by the pentamer, where there is a noticeable increase in the mean error, −49.73 kJ/mol, which is greater than the error for both the trimer (−34.99 kJ/mol) and heptamer (−38.60 kJ/mol) that precede and follow it in size. This may be due to the net charge of −2 on the pentapeptide as opposed to a net charge of −1 for all of the other truncated peptides.

An additional effect that appears to be due to the net charge on the molecule is an offset manifested in the scatter plots of the PB vs GB energies. This offset is most obviously apparent in the lysine, dimer, and nonamer plots (Figures 1, 2, and 4), all of which have a net charge of −1.

To investigate this effect further, the free energy of an arginine residue with only a single charge was calculated (Figure 7). Since it is difficult to conclude from these graphs if there is an offset, and if so whether it should be attributed to the presence of the charge or to the placement of the charge, a constant charge of 0.5000 eu was tested next (Figure 8). Here, the scatter plots of the GB vs PB energies show a marked negative displacement. Finally, the placement of two equal and opposite charges throughout the molecule was tested (Figure 9). With the disappearance of the net charge, the offset seen in Figure 8 is also eliminated. This suggests that the present GB model has a systematic error for systems with net charge; further investigation of the phenomenon is currently in progress.

The difference between the performance of the two GB models can also be seen from Table 1. The model that uses the numerically calculated Born radii produces energies closer to the PB calculated energy than the model that uses the analytical radii. As the size of the molecules increases, the difference between the two schemes decreases somewhat; for the dimer the numerical radii have errors more than twice as small, but for the nonapeptide the mean energy difference between GB-NU and the PB energy was −32.60 kJ/mol, whereas for GB-AN the energy difference was −43.24 kJ/mol. However, in the case of arginine with one or two charges, the GB model with numerical radii actually performed worse than the model using analytical radii. This again suggests a systematic error in the formalism underlying the current GB model.

In summary, the GB/SA model displays qualitatively superior performance with regard to agreement with the PB results as compared to the DDD and SA models. This performance is reflected in a variety of measures summarized in Table 1: the *r*-factor correlation coefficients, mean errors, and standard deviations. The performance of the DDD model appears to be uniformly poor for all molecular sizes and structures. The SA model has a somewhat better performance but is still lacking in quantitative precision.

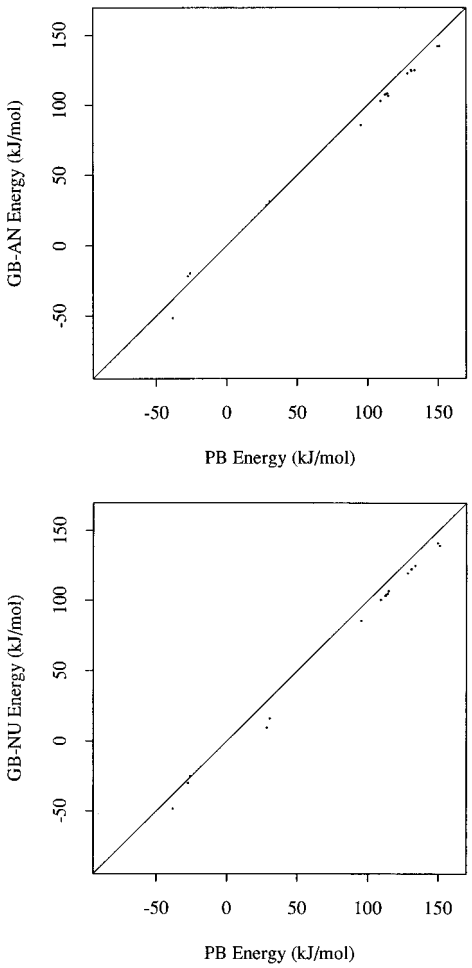

Figure 7. Set 11 for arginine with a varied movable charge: (a) PB/GB-AN energy comparison; (b) PB/GB-NU energy comparison.

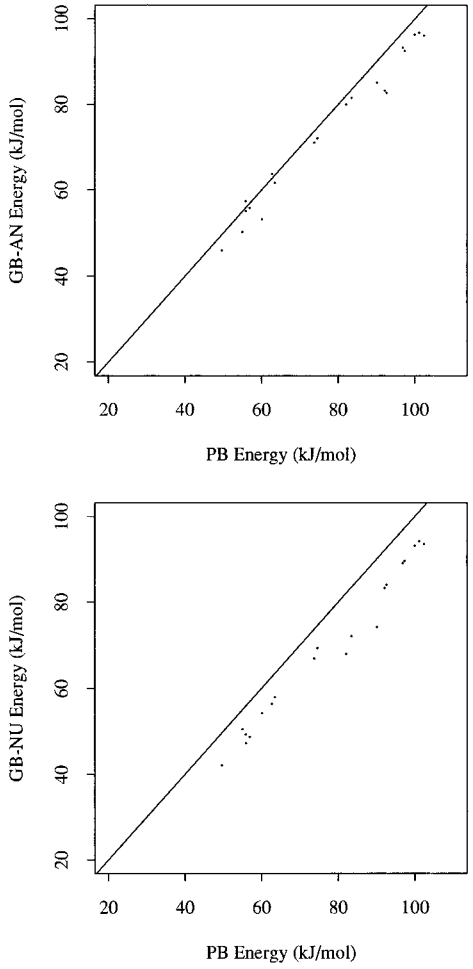

Figure 8. Set 12 for arginine with a constant movable charge: (a) PB/GB-AN energy comparison; (b) PB/GB-NU energy comparison.

4. Conclusion

The above results demonstrate unambiguously that, at least in their specific implementation here, distance dependent dielectric based models are incapable of properly modeling solvation free energies for large, flexible molecular systems. Not only are there significant quantitative errors as compared to the PB results, but predictions of rank ordering of conformers are sufficiently poor to not be useful even for preliminary screening of structures. The surface area model is perhaps of qualitative utility for crude structural predictions; more detailed investigations will be required to ascertain this.

In contrast, the GB model provides reasonable quantitative prediction of solvation free energies for all of the structures investigated, and excellent energetic ranking of conformers for a given peptide. There are obviously improvements to make, in both the analytical model (whose performance could be brought up to that of the numerical model) and in the overall methodology, for example, in correcting the energy offset as compared to PB for species with a net charge. Despite these defects, the GB model, as it stands, is clearly a useful technology for determination of peptide structure in solution.

An appealing approach suggested by the present results is to use the GB model for conformational searching and follow with a PB calculation of the relative energies of the low-lying minima found by the search, carrying out analytical gradient minimization of the PB model if necessary. This approach requires a robust PB gradient method, which we and others[21,22] have recently developed; calculations along these lines are in progress in our laboratory.

The studies presented here establish the validity of the GB approximation only for systems up to the size of a small peptide. For larger structures, such as proteins, there may be new effects which come into play that have not yet been tested. We are currently investigating this issue by calculations analogous to those presented here.

Throughout this paper, we have been assuming that the PB results are themselves accurate predictors of conformational relative solvation free energies. The great majority of work in validating PB calculations has been for small, rigid molecules, where extensive comparisons with experiment and with molecular simulations (both Monte Carlo and molecular dynamics) have been made.[1,23] It is clear that, with suitable parametrization, the PB results can be made to yield very good agreement with experiment: our latest self-consistent reaction field PB model, which includes first shell hydrogen bonding corrections, gives results to within 1.5 kcal/mol of experiment for most cases,[1] as good or better than current results with molecular modeling force fields such as AMBER and OPLS using explicit solvation simulations.

However, there has been little work to verify that PB results agree with simulations for relative conformational energies for larger systems, the focus of the present paper. There are a few studies of the alanine dipeptide, e.g., results reported in ref 20, which display reasonable agreement. This is clearly inadequate to establish the level of quantitative precision, particularly for cases with net charge which, as we have seen in this paper, are much more sensitive to the quality of the model. Studies to elucidate this issue are currently ongoing in our research group.

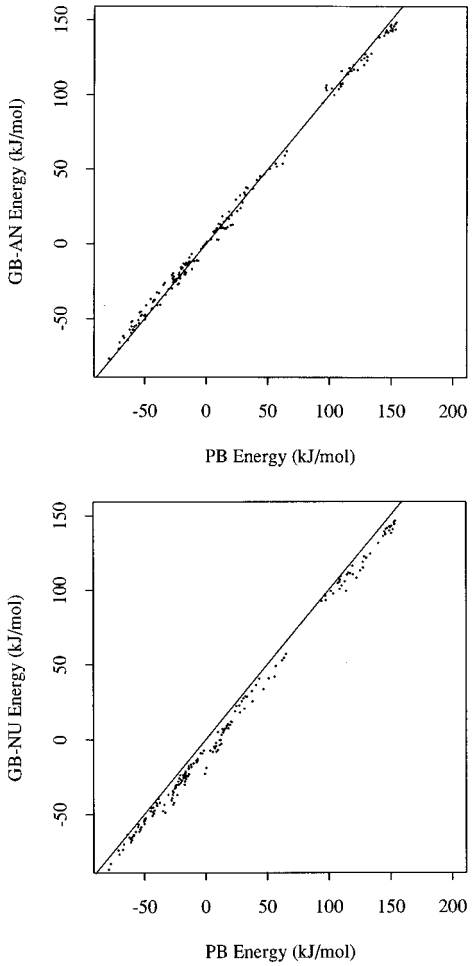

Figure 9. Set 13 for arginine with two equal and opposite charges: (a) PB/GB-AN energy comparison; (b) PB/GB-NU energy comparison.

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