Poisson−Boltzmann Analytical Gradients for Molecular Modeling Calculations

Mark Friedrichs and Ruhong Zhou

Schrodinger, Incorporated, One Exchange Place, Suite 604, Jersey City, New Jersey 07302

Shlomit R. Edinger and Richard A. Friesner*

Department of Chemistry and Center for Biomolecular Simulation, Columbia University, New York, New York 10027

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We present the first analytical gradient Poisson−Boltzmann methodology which is routinely applicable to large biomolecular systems, such as proteins with sizes ranging from 500 to 5000 atoms. Full minimizations of several such systems in the gas phase and in solution are contrasted. Because the solvation free energy term is slowly varying with conformation, it can be evaluated infrequently in the simulation, and hence, reasonable computation times can be obtained even for large solvated systems.

Introduction

The Poisson−Boltzmann (PB) equation has proven to be a surprisingly effective approach to the description of aqueous solvation in biomolecular systems. It has clearly demonstrated that the use of a realistic molecular cavity to describe the dielectric interface, based upon a plausible definition of the solvent-accessible surface, could provide a qualitatively correct description of a wide range of solvation phenomena in proteins and nucleic acids.[1,2] If the parameters of the model—dielectric radii of the atoms, atomic charges, and correct ion terms based on exposed surface—are carefully optimized, the method also appears to be capable of quantitative accuracy for small molecules to roughly the same level as much more expensive explicit solvent calculations, using for example free energy perturbation theory.[1−3] The use of PB calculations has therefore become increasingly widespread and is now a standard tool in, for example, structure-based drug design efforts.

However, a major limitation has been the lack of a practical analytical gradient methodology for PB calculations. A number of preliminary studies have been reported in the literature,[4−6] but these have presented applications only to relatively small molecules, such as the alanine dipeptide. For larger systems, there are significant issues concerning both the accuracy of the gradient methods and the computational effort required to carry out the calculations. To our knowledge, extensive simulations or minimizations of such systems have not yet been reported in the literature.

In this paper, we present a robust and reliable PB gradient methodology which is readily applicable to large biomolecules. The PB energies and gradients are determined via a finite element code, PBF, that we have describe previously[7−9] and which has already been used by us for quantum chemical geometry optimization in solution. Here, we interface this code to the IMPACT molecular modeling program of Levy and coworkers.[10] Substantial modifications of the PBF software were required to run systems of the size examined below; however, most of these modifications involve programming details which are not particularly relevant to scientific issues. The principal substantive change in the model is the use of a Gaussian definition of the solvent-accessible surface of the molecule, which replaces the Connolly surface definition that we (and others) have used previously. The Gaussian surface is far easier to work with and renders several of our numerical algorithms, such as the grid generation code, reliably stable and robust. At the same time, the solvation free energies obtained from the Gaussian surface are very similar to those of the Connolly surface, and there is no particular theoretical reason to prefer one to the other in cases where they are different. In either case, adjustments of the model parameters to fit experimental data are required to correct the quantitatively inaccurate description of the first-shell hydration inherent in any continuum solvation model.

We have chosen below to examine only one utilization of the PB gradient, that of minimization of the solute energy plus solvation free energy of the system. Subsequent publications will discuss molecular dynamics simulations, which are also feasible. Our objective in the calculations shown below is twofold. First, we demonstrate that the gradient technology is tractable and accurate for large systems. Second, we examine the length scales over which PB forces operate, a crucial question for both numerical implementation and physical relevance of the methodology. This leads to some quite interesting conclusions concerning the role of solvation in determining structures.

Theory and Computational Methodology

Finite-Element Poisson−Boltzmann Solver. We start with a review of the methods that are used in our PBF finite-element code to solve the Poisson−Boltzmann equation.[7,8] In the dielectric continuum model, the solute is represented by some fixed charge distribution $\rho(\vec{r})$, contained in a region of low dielectric constant, surrounded by a high dielectric continuum, the solvent. Free ions may be present in the solvent and are assumed to be Boltzmann-distributed at equilibrium. The Poisson−Boltzmann equation is then given by

$$\nabla(\epsilon\nabla\phi) = -\frac{4\pi\rho}{kT} + 4\pi C \sinh(\phi e) \qquad (1)$$

where ϵ is the dielectric constant, ϕ is the electrostatic potential, and ρ is the solute charge density. $C = 2eI/kT$, where I is the

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ionic concentration in the solvent. For weak ionic concentrations, the equation may be linearized and one can write

$$\nabla(\epsilon\nabla\phi) = -\frac{4\pi\rho}{kT} + \epsilon\kappa^2\phi \tag{2}$$

where $\phi(\vec{r})$ is given in units of [kT] and $\kappa^2 = (8\pi\epsilon^2 I)/(\epsilon kT)$ is the inverse Debye length squared. We will focus on the linear form of the Poisson−Boltzmann equation hereafter as the finite-element method is directly applicable to it. Given a complete set of basis functions $\{\psi_i\}$ $i = 1, ..., N$, one can expand the electrostatic potential:

$$\phi(\vec{r}) = \sum_{i=1}^{N} c_i \psi_i(\vec{r}) \tag{3}$$

Substituting back in eq 2 we obtain:

$$\sum_{i=1}^{N} c_i (\nabla(\epsilon\nabla\psi_i) - \epsilon\kappa^2\psi_i) = -\frac{4\pi\rho}{kT} \tag{4}$$

The discretization of the problem is achieved by sampling the three-dimensional space over which the calculation is to be carried out with a set of grid points $\{\vec{r}_i, i = 1, ..., N\}$ such that there is a one to one correspondence between the set of basis functions ψ_i and the set of grid points. Furthermore, the functions ψ_i are only nonzero in some finite region around $\vec{r}_i$ and satisfy the conditions:

$$\psi_i(r_j) = 1 \text{ only if } i = j$$

The coefficients c_i are then the values of the potential at the grid vertices. Hereafter we will refer to the set of these values as the grid potential $\tilde{\phi} = \{c_i\}$, $i = 1, ..., N$. Multiplying both sides of eq 4 by ψ_j and integrating over all space one can write:

$$\sum_{i=1}^{N} c_i \left(\int_V \nabla(\epsilon\nabla\psi_i)\psi_j d^3\vec{r} - \int_V \epsilon\kappa^2\psi_i\psi_j d^3\vec{r}\right) = -\int_V \frac{4\pi\rho}{kT}\psi_j d^3\vec{r} \tag{5}$$

After using Green's theorem and the vanishing condition for the potential at infinity, we obtain a linear equation system, $Ax = b$, which can be solved iteratively

$$\sum_{i}^{N} A_{ij} c_i = b_j \tag{6}$$

where A_{ij} and b_j are defined as

$$A_{ij} = -\int_V \nabla(\epsilon\nabla\psi_i)\nabla\psi_j d^3\vec{r} - \int_V \epsilon\kappa^2\psi_i\psi_j d^3\vec{r}$$
$$b_j = -\int_V \frac{4\pi\rho}{kT}\psi_j d^3\vec{r} \tag{7}$$

The choice of grid points and associated basis functions will determine the structure of the linear system.

The advantage of using a finite-element technology to represent the Laplacian is that one can employ a grid in which the grid points are placed in arbitrary locations. For the present problem, this means that grid points can be concentrated near the surface of the protein where the dielectric is rapidly changing in value. The adaptive mesh used by our code is described in detail in refs7 and 8. Once a grid has been specified, the set of tetrahedral elements must be defined. For this purpose, we employ a Delaunay triangulation algorithm to tile the mesh, as described in ref 8. Initial versions of the code experienced intermittent problems with the Delaunay algorithm for large systems. However, we have eliminated these difficulties (which arose from a subtle mathematical issue leading to unnecessary sensitivity to roundoff error) in the current version, which has been tested for a set of 110 test proteins up to 5000 atoms in size. At this point, the grid generation and triangulation algorithms can be considered stable.

A comparison benchmark for the efficiency of our finite-element approach is the DelPhi program of Honig and co-workers.[6] DelPhi uses a cubical mesh of equally spaced grid points, while PBF uses an adaptive mesh in which the grid points are concentrated at the solvent-accessible surface. This allows a much smaller number of grid points to be used (roughly an order of magnitude for a small protein) while achieving equal accuracy in the solution at the dielectric boundary, which is what determines the accuracy of the solvation free energy.

On the other hand, the finite-element operator has a larger bandwidth (more nonzero elements per row) than the three-point finite different formulas used in DelPhi. This means that the computational cost of applying one row of the operator to a trial vector, the dominant operation in iteratively solving eq 6 from an initial guess, is larger in PBF than in DelPhi; as against this, the size of the matrix is smaller. Overall, we find that the computational efficiency of the current version of PBF is more or less comparable to the most recent versions of DelPhi for equivalent accuracy, although the memory usage is smaller for large systems at high resolution. The present paper, however, does not focus on this issue, as we are principally concerned with the new analytical gradient capabilities.

Polarization Energy Gradient Calculation. The polarization energy can be derived from the fact that within the dielectric continuum model, the polarization contribution is simply the cost of inserting an object of dielectric constant ϵ into an external field. It has been shown in the previous paper[7,8] that the electrostatic contribution to the solvation free energy is given by:

$$G_{\text{pol}} = \frac{1}{2}\int_S \sigma\phi_0 d^2\vec{r} + \frac{1}{2}\int_V \rho_{\text{ion}}\phi_0 d^3\vec{r} \tag{8}$$

As for the gradients of this solvation energy, we have implemented two different approaches, as detailed in the previous paper.[8] The first, mesh operator derivative method (MOD), represents an exact numerical differentiation of the discretized version of eq 6, using Green's function techniques borrowed from ab initio electronic structure theory. The second, Maxwell stress tensor method (MST), is based on a formulation of Gilson, McCammon, and co-workers[5] which is less expensive computationally but also somewhat less accurate (it more or less corresponds to a Hellman−Feynman type approximation to the forces in the context of quantum mechanics). The second method appears to be good enough for our purposes, and hence, it will be used below; however, more study is required to draw a definitive conclusion.

The Maxwell stress tensor method was based on the work of Gilson and co-workers.[5] Starting from the expression for the electrostatic free energy derived by Sharp and Honig,[11] Gilson et al.[5] derived the following expression for the force density associated with the Poisson−Boltzmann equation

$$\vec{f} = \rho^f\vec{E} - \frac{1}{2}E^2\nabla\epsilon - \frac{1}{2}\epsilon\kappa^2\phi^2\nabla\lambda \tag{9}$$

where ρ^f is the fixed charge density associated with solute molecule, proteins in our case, and $\vec{E} = -\nabla\phi$, where ϕ is the electrostatic potential defined in a previous section and solved by the linear matrix equation. It can be shown that the force density $\vec{f}$ can be expressed as the divergence of a tensor, **P**,[12] so that the force acting on a volume V is given by the expression

$$\vec{F} = \int_V \nabla\cdot\mathbf{P}\, d^3\vec{r} \tag{10}$$

where **P** is found to be the sum of the Maxwell stress tensor and a diagonal ionic pressure tensor. This is why we name this method the Maxwell stress tensor method, and it can be written as:

$$\mathbf{P} = \vec{E}\,\vec{E} - \frac{1}{2}\epsilon||\vec{E}||^2 - kT\sum_{i=1}^{N}[(e^{q_i\phi/kT} - 1)c_i]\lambda I \tag{11}$$

In order to obtain the forces acting on the atomic centers as molecular mechanics requires, we must first determine the net force on the dielectric boundary. Applying this to the case where V is the volume within the molecular surface, the difference in the stress tensor above and below the boundary is used to compute the net force on the molecule

$$\vec{F}_b = -\int_S \hat{n}\,(\hat{n}[\mathbf{P}_i - \mathbf{P}_o]\hat{n})\, d^2\vec{r} \tag{12}$$

where $\vec{F}_b$ indicates the force on the boundary. It is easy to prove that the previous equation can be simplified by using electric field $\vec{E}$ rather than a tensor **P** and can be rewritten as

$$\vec{F}_b = -\int_S \frac{1}{2}(\epsilon_0 - \epsilon)\,\vec{E}_0\,\vec{E} d^2\vec{r} \tag{13}$$

Finally, we can relate the integrand $\vec{E}$ to the surface charge density σ and obtain an expression more convenient for numerical evaluation:

$$\vec{F}_b = -\int_S\left[2\pi\sigma(\vec{r})\epsilon_0 + \frac{1}{8\pi}(\epsilon - \epsilon_0)\left\|\vec{E}_0\right\|^2\right]d^2\vec{r} \tag{14}$$

The force is transferred from the dielectric interface to the atoms following a procedure suggested by Gilson and McCammon.[5] Interested users can also find the details of force decomposition in our previous paper.[8]

The remaining contribution to the forces on the atomic centers comes from integrating eq 9 over a volume enclosing all of the fixed charges ρ^f. Since we do not want to include the normal Coulomb interaction between those free charges, we subtract out this vacuum term

$$\vec{F}_s = \int_V \rho^f\,(\vec{E} - \vec{E}_0)d^3\vec{r} = \int_V \rho^f\,\vec{E}^{\,\mathrm{resp}} d^3\vec{r} \tag{15}$$

where $\vec{F}_s$ is the force on atom sources and $\vec{E}^{\,\mathrm{resp}}$ is the response electric field produced by the surface charge density.

Gaussian Definition of the Solvent-Accessible Surface. The definition of the solvent-accessible surface in a discretized PB formulation can be cast in terms of the assignment of each grid point in the mesh to a particular value of the dielectric constant (for example, that of the solute or of the solvent). The Gaussian surface that we use employs the following formula to specify the dielectric of a grid point. First, we define a parameter D from the solute atoms to the mesh point at $\vec{r}$ as

$$D = \sum_i \exp\left(-\alpha\left(\frac{|\vec{r} - \vec{r}_i|}{R_i}\right)^2\right) \tag{16}$$

where the sum is over all atoms i of the solute, r_i is the location of atom i, R_i is the dielectric radius of atom i, and α is a parameter defining the slope of the dielectric interface. The dielectric map is then defined such that if D is less than a cutoff D_0, the dielectric is assigned to be equal to that of the solvent, whereas if it is greater than D_0, the dielectric is assigned to be that of the solute.

The new Gaussian definition of molecular surface will be slightly different from the original Connally molecular surface. However, it is not clear to us that the Connally surface should be superior than the Gaussian surface in describing the continuum solvent boundaries. On the other hand, Gaussian surface is much easier to deal with numerically than the Connally surface, since there are significantly fewer cusps in the molecular surface.

We have experimented with the optimal values of the above parameters and arrived, tentatively, at the following values: $\alpha = 1$ and $D_0 = 0.72$. These values lead to a robust mesh generation protocol while at the same time reproducing solvation free energies of small molecules obtained with the Connally surface definition rather closely. They will be used in all calculations which follow. Further optimization of the parameters with large systems is currently under investigation.

IMPACT Molecular Modeling Code and Interface to PBF. The IMPACT molecular modeling code of Levy and co-workers has been described previously; it can carry out most of the standard protein molecular mechanics functions such as energy and gradient evaluations, molecular simulation and minimization, free energy perturbation, and analysis of trajectories. The new OPLS-AA protein force field of Jorgensen and co-workers[13] has recently been implemented in IMPACT, and we shall use that force field for all calculations that follow.

The interface between PBF and IMPACT that we have designed is straightforward. At a new geometry, PBF receives the molecular coordinates and returns the energy and the analytical gradient. This is then passed to IMPACT where it is added to the usual molecular mechanics energy and gradient. The geometry optimization algorithm in IMPACT, a standard conjugate gradient-type approach, is then performed to minimize the energy with respect to the nuclear coordinates.

A naive assessment of the computational efficiency of the combined methodology would indicate that there is a serious imbalance in the two parts of the calculation: solution of the PB equation is more than 100× slower than evaluation of the molecular mechanics energy. However, several measures can be taken to reduce this computation time substantially. The key is to realize that a typical minimization (or molecular dynamics) step is very small on the length scales over which the PB solvation term varies substantially (as we will discuss further below). The following algorithmic improvements can then be used to accelerate the computations: (1) The PB gradient can be updated relatively infrequently; the criterion we use in the present paper is to recalculate the PB gradient when the maximum displacement of some atom exceeds 0.1 Å as compared to the last location in which the gradient was computed. (2) The solution to the PB equation at the previous geometry can be used as an initial guess at the current geometry. This saves a large number of iterations in the convergence process. (3) In principle, we should be able to reuse the mesh generation from the previous step as well, modifying it where needed. This is more complex technically, and we have not attempted this in the present paper; however, we intend to do this in future versions of the method. (4) There are numerous other strategies, such as the use of much higher mesh densities

TABLE 1: Results of Gas-Phase Minima Followed by a PB Continuum Solvent Minimization for Peptides and Proteins[a]

system	atoms	energy term	start	final	ΔE
2ovo(frag)	169	total	−348.3	−349.4	−1.1
		PB	−185.5	−187.5	−2.0
2mlt(frag)	232	total	−320.4	−321.4	−1.0
		PB	−151.9	−153.4	−1.5
1crn	648	total	−1414.4	−1431.6	−17.2
		PB	−146.1	−148.6	−2.5
2abx	1141	total	−3238.3	−3286.4	−48.1
		PB	−609.6	−616.6	−7.0

[a] All energies are in kcal/mol. The systems are named by PDB file names: frag indicates that only a fragment of the protein was used, with the size specified in the next column. The Energy term total is the total energy of the system including PB solvation energy; PB is the PB solvation energy.

in the active site of the protein than in more distant regions, which can also be used to reduce the computational effort for problems such as binding free energies. Again, we defer the investigation of this to a later publication.

Actual CPU times are presented below in the results section. While there is still a significant penalty as compared to gas-phase simulations, the calculations are at least tractable, even for relatively large proteins, so that initial studies can be carried out.

Results

Specifics of the Calculations. All calculations were carried out using the IMPACT/PBF amalgamation described above. The dielectric constant of the solvent was set equal to 80, while that of the solute was set equal to 2 (the question of what to use for the dielectric constant of the protein is a complex one, which we shall not discuss here; the value of 2 is a plausible choice for illustrative purposes). The OPLS-AA force field of Jorgensen and co-workers was used for the molecular mechanics energy and also to define the dielectric radii of the solute atoms. The latter choice is not optimal for enforcing agreement with experimental solvation free energies; however, it is qualitatively reasonable and thus sufficient for our purposes in this paper.

Solution-Phase Minimization Starting from a Gas-Phase Minimum. One interesting question is how the solvation term would perturb a gas-phase-optimized structure. This is readily examined by carrying out a gas-phase geometry optimization and then turning on the PB solver. Table 1 shows the results of a few protein systems, a fragment of 2ovo, a fragment of 2mlt, 1crn (crambin), and 2abx. Crystal structures from Brookhaven PDB bank are used as the starting point. All systems are minimized in the gas-phase first, then followed by a PB minimization. As we can see from the table, the total energies of the four protein systems have decreased by 1.1, 1.0, 17.2, and 48.1 kcal/mol, respectively; at the mean time, the PB solvation energies have decreased by 2.0, 1.5, 2.5, and 6.9 kcal/mol, respectively. The large decreases in total energy for 1crn and 2abx cases indicate that the PB solvation energy term not only perturbs the structure, but also helps the system to cross energy barriers and minimize to another local minima, even though the solvation energy term might have a longer length scale as we pointed out earlier.

The observation that the solvation term facilitates barrier crossing, leading in both large protein cases to significant changes in the total energy, suggests that for quantitative evaluation of total energies of these systems in solution, gas-phase minimizations followed by single-point solvation free energy calculations are inadequate. While additional test cases certainly need to be run to ascertain how widespread this phenomenon is, the present results provide strong initial evidence as to its importance.

TABLE 2: Results of Peptide and Protein Minimizations Using the Poisson−Boltzmann Gradient Algorithm[a]

system	atoms	minimizer	solvation energy	solute MM energy	total
2ovo(frag)	169	gas−PB	−187.5	−161.9	−349.4
		PB	−187.4	−169.2	−356.6
2mlt(frag)	232	gas−PB	−153.4	−168.0	−321.4
		PB	−150.5	−187.1	−337.6
1crn	648	gas−PB	−148.6	−1283.0	−1431.6
		PB	−160.4	−1313.5	−1473.9

[a] All energies are in kcal/mol. All minimizations were started from the X-ray crystallographic coordinates in the Protein Data Base (PDB). Gas-PB refers to gas-phase conjugate gradient minimization followed by a PB minimization, while PB refers to pure conjugate gradient minimization in PB continuum solvent.

TABLE 3: Computational Performance of the PB Gradient Algorithm[a]

system	atoms	minimizer	total CG steps	PBF calls	CPU (s)
2ovo(frag)	169	gas	1407		43.3
		PB	500	56/3528	1261
2mlt(frag)	232	gas	499		33.2
		PB	352	54/2362	1568
1crn	648	gas	543		149.9
		PB	826	107/6050	11375

[a] Total CG steps refers to number of conjugate gradient steps taken by the minimizer. PBF calls indicates the number of times the PB gradient was reevaluated (upper figure) as compared to the total number of gradient evaluations (lower figure). The CPU timing is obtained from SGI R10K processsors.

Solution-Phase Minimization Starting from an Unminimized State. One might conclude from this discussion that a full minimization with a solvation term on is unnecessary and that one can simply carry out gas-phase minimizations followed by PB minimizations to reorder the minima. However, this is not necessarily always the case. The reason is that gas-phase minimizations may not be able to locate minima which have low energies due to the solvation term.

Many important biomolecular modeling problems, such as ligand binding, loop refinement, and protein folding, require locating the global minimum on the solvated potential surface through some sort of search of phase space. A search that is biased toward the gas-phase minima, for example, simulated annealing in the gas phase, may not locate the lowest energy basin of attraction of the total free energy. What actually happens in practice needs to be ascertained for specific cases by computational experiments. In general, however, one would expect that the inclusion of the solution-phase forces would be important in biasing moves toward the appropriate lowest total free energy basin of attraction.

Therefore, we have carried out solution-phase minimizations of a number of the examples in Table 1, starting from the X-ray structure. For this study, we included only the three smaller systems in Table 1, and results are summarized in Table 2. It can be seen that for all three minimizations, lower energies are reached when the solvation term is turned on at the beginning (as in Table 1, the effects are more modest in the smaller peptides than in the protein). These results again indicate that the use of a solvation term during optimization is critical to properly minimizing solvation free energies in a complicated phase space. Table 3 shows the CPU timing for gas-phase

minimization and the PB solution-phase minimization, along with the number of PBF calls on which the solvation gradients were recalculated.

Conclusion

We have shown that our PB gradient methodology can robustly be applied to optimize peptide and protein structures in solution and that solution-phase minimization can yield different results from gas-phase minimization, as it includes the solvation effect. At present, the additional CPU time required is considerable, on the order of more than 10× larger than that for gas-phase minimization. However, this performance can be improved significantly by optimization of the PB calculation, an effort which is still in progress. Investigation of the impact of PB minimization on the calculation of biologically important properties, such as binding energies, loop geometries, and pK_a's, will be explored in forthcoming publications.

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