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Review Article

Treatment of Electrostatic Effects in Macromolecular Modeling

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INTRODUCTION

The relative stabilities of different conformations of a protein are determined by the balance between a variety of forces, and these same forces influence the association constants for the binding of proteins to small ligands and to other macromolecules. A detailed understanding of how these forces influence protein structure and interactions is thus an essential component of rational strategies for protein engineering.[1] Electrostatic effects are among the most important factors in determining the conformation of proteins in solution and in determining the energetics and kinetics of binding of other molecules to proteins.[1-4]

There are a variety of computer algorithms for manipulating and refining models of proteins and for examining their interactions with other molecules.[5] These include energy minimization, molecular dynamics, and Brownian dynamics, all of which are examples of molecular mechanics. If these methods are to fulfill their potential as powerful tools for protein design, they must be able to faithfully simulate the physical properties of macromolecular systems. To do this requires an accurate description of how the potential energy of the system depends on the relative positions of the constituent atoms. This description, contained in a potential energy function, constitutes the heart of quantitative modeling algorithms.

Given the importance of electrostatic effects in macromolecular systems, it is unfortunate that the largest errors in potential energy functions of the molecular mechanics type are made in those terms for electrostatic interactions. There are two reasons why these errors are so large. First, the other interaction energies can be described relatively accurately by simple mathematical functions involving only a few atoms, whereas electrostatic interactions are very complicated for macromolecules in solution. Second, electrostatic forces are very long range in comparison to the forces associated with other interactions.

These problems, which are serious enough when one is modeling proteins, are severely compounded when treating nucleic acids. To begin with, nucleic acids are polyanions, whereas proteins are usually modeled at or near the isoelectric point. Further, nucleic acids have much larger surface-to-volume ratios than globular proteins. Almost every atom is near the boundary between the high dielectric solvent and the macromolecule, which is essentially an organic material of relatively low dielectric constant. It is near this boundary where errors are largest. These difficulties were vividly illustrated by Mike Levitt, who responded, when asked why he set all atomic charges to zero in the first molecular dynamics simulation on DNA,[6] "Because, when I included electrostatic forces, the molecule flew apart."

This review is intended to identify the basic issues in modeling electrostatic effects in macromolecular systems, to describe the principal methods that have been developed to treat those effects, and to discuss the strengths and weaknesses of various approaches. Although I will include the relevant equations, my treatment will be descriptive rather than mathematical.

The paper is divided into six principal sections. The first is a discussion of the basic issues in electrostatics problems, with an introduction to the difference between treatments at the microscopic and macroscopic levels and a presentation of the equations that apply in different classes of problems; this section is written primarily for the newcomer to the field. The next two sections give brief descriptions of the various methods that are used to model electrostatic effects in macromolecular systems; the methods are divided into microscopic and continuum (macroscopic) treatments. The fourth section describes the results of tests that have been run on the various algorithms. Next follows a section on the controversy over the value of the dielectric constant inside a protein. Finally, the discussion section is intended to provide a personal view of the relative merits of the different approaches, a summary of problems with each of them, and likely directions for future development.

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BASIC ISSUES IN ELECTROSTATICS

Microscopic and Macroscopic Properties

In a homogeneous, continuous medium, the electrostatic potential at a distance r from a point charge q is given by Coulomb's law:

$$U = Cq/(Dr) \tag{1}$$

where C is a constant that depends on units[7] and D is the dielectric constant, a quantity that indicates the extent to which the electrostatic effect of the charge is shielded by the medium. Note that the dielectric constant is a macroscopic property.

In many electrostatic problems, real materials are treated as if they were simple continua, with the effects of the underlying microscopic structure of the material being incorporated into the dielectric constant. On the microscopic level, the shielding of the charges arises from the polarizability of the material.

A variety of atomic and molecular properties contribute to the polarizability. Principal among these are the reorientation of permanent dipoles in an imposed field and the electronic polarizability, which gives rise to induced dipoles in an external electric field. Permanent dipoles may be entire molecules (water molecules, for example) or pieces of them, such as the polar side chain of an amino acid.

It should be noted that the dielectric "constant" is actually frequency dependent. Let us briefly consider the case of liquid water. The water molecule has a large dipole moment, the same as that of charges of $+0.4e$ and $-0.4e$ at a separation of 1 Å, where e is the proton charge. The dipole moment arises from the sp^3 hybridization of the oxygen atom and its electronegativity. This combination results in a substantial positive partial charge on one side of the molecule (the hydrogens) and a corresponding negative charge on the other side (the lone pair electrons).

In a static or slowly oscillating electric field, water dipoles tend to be aligned with the field. Water is therefore a very polarizable material, which is reflected macroscopically by a large dielectric constant, about 80 at 20C. Note that there is a competition between the electrostatic force, which favors alignment of the dipoles with the field, and the thermal motion, which favors random orientations. The net orientation is actually very small; Debye[8] showed that it is equivalent to a 180° rotation of only one molecule in a million, corresponding to an average rotation of 10^{-4} deg per molecule.

At high frequencies, the reorientation of the permanent dipoles does not contribute to the polarization of water. This is because the reorientation of the water dipoles is due to thermal motion, and the electric field is only a small biasing factor in the process of rotational diffusion. As a consequence, if the oscillating electric field is of very high frequency, the water molecules cannot reorient rapidly enough to follow the field. The high frequency dielectric constant of water is due to the electronic polarizability of the molecules, and it has a value of about 4 at 20C.[9,10]

The frequency dependence of the dielectric constant of water must be kept in mind when considering the rigorous treatment of electrostatic effects in modeling studies.[11] The dielectric constant has the low frequency value up to about 2 GHz, then drops off to the high frequency value above about 200 GHz. The broad dispersion is centered at 16 GHz at 20C, corresponding to a rotational relaxation time for the water molecule of about 10 psec. This is a potential hazard for methods like molecular dynamics, where motions on the time scale of 1–100 psec are simulated.

The Equations of Classical Electrostatics

The fundamental problem of classical electrostatics is to find the electrostatic potential (or, equivalently, the electric field) at every point in space for a given distribution of charges. In its most general form, the problem covers cases where the dielectric constant is different in different regions of space. For macromolecular modeling studies, it is also extended to treat the effects of nonzero ionic strength.

In regions of uniform dielectric constant that have no free charges, the electrostatic potential U must satisfy LaPlace's equation at every point r,

$$\nabla^2 U(r) = 0 \tag{2}$$

where ∇^2 is the LaPlacian operator representing the second derivative with respect to spatial coordinates. If there are charges present, Eq. (2) is replaced by Poisson's equation,

$$\nabla^2 U(r) + 4\pi\rho(r)/D = 0 \tag{3}$$

where $\rho(r)$ is the charge density at the point r. The solution to this equation around a point charge q leads to Coulomb's law, Eq. (1).

If there also are mobile ions present (in the case of nonzero ionic strength, for example), their positions can only be described in terms of probability distribution functions, and their electrostatic effects must be described statistically. The appropriate modifica-

tion of Eq. (3) leads to the standard Poisson–Boltzmann equation,

$$\nabla^2 U(r) - \kappa^2 \sinh[U(r)] + 4\pi\rho(r)/D = 0 \quad (4)$$

where κ is the Debye–Hückel parameter,

$$\kappa^2 = 8\pi N e^2 I/(1000 DkT)$$

N, e, k, and T are Avogadro's number, the charge on the proton, Boltzmann's constant, and the absolute temperature, respectively. I is the ionic strength, and the factor of 1000 enters because the ionic strength is expressed in moles/liter, while all other quantities are in cgs units. κ has the dimensions cm^{-1}, and $1/\kappa$ is the Debye screening distance, a measure of the distance over which electrostatic effects are damped out by the mobile ions.[12] Note that this distance is inversely proportional to the square root of the ionic strength. At physiological ionic strength, about 150 mM, the Debye distance is about 8 Å.

The textbook form of Eq. (4) often assumes low ionic strength, in which case the linear approximation $\sinh[U(r)] \approx U(r)$ is assumed.

Finally, we consider the most general case, in which the dielectric constant and the charge density vary from one region of space to another. In this case, Eq. (4) must be modified to give the complete non-linear Poisson–Boltzmann equation,

$$\nabla \cdot [D(r)\nabla U(r)] - \bar{\kappa}^2(r) \sinh[U(r)] + 4\pi\rho(r) = 0 \quad (5)$$

In this last equation, $\bar{\kappa}$ is the modified Debye–Hückel parameter, $\bar{\kappa} = D^{1/2}\kappa$. Equation (4) is often simplified to the linear form of the Poisson–Boltzmann equation, which is a reasonable approximation except in regions of very high charge density,

$$\nabla \cdot [D(r)\nabla U(r)] - \bar{\kappa}^2(r) U(r) + 4\pi\rho(r) = 0 \quad (6)$$

Equations (2–6) fall into the class of differential equations that are typical of boundary value problems. Analytical solutions to these equations exist for some simple geometries, but more complicated cases can be treated only by approximate numerical methods. It is essential that algorithms implemented to numerically solve these equations be tested for accuracy by applying them to test cases where analytical solutions are known.

MICROSCOPIC MODELS

Microscopic models are those that treat the system at an atomic level of detail. The only continuum approximation that enters these models is the use of a dielectric constant or effective dielectric constant in the Coulomb's law calculation of electrostatic energies and forces.

There are a variety of computer programs that are used to model macromolecular structure and dynamics,[5] many of which are related to molecular mechanics.[13] These programs are built around a function that calculates the potential energy of the system as a function of the atomic positions. A typical potential function for such algorithms is of the form

$$E = E_b + E_\theta + E_\phi + E_{vdw} + E_{es} \quad (7)$$

The first three terms in this expression describe the energetic costs for deforming the covalent structure; they are, respectively, bond stretching (b), bond angle bending (θ), and rotations about bonds (ϕ). The last two terms represent noncovalent interactions between pairs of atoms that are separated by more than three covalent bonds. These are traditionally broken into a van der Waals term (vdw) and an electrostatic term (es).

This section discusses algorithms that use a molecular mechanics type of potential function with a normal or slightly modified Coulomb's law representation for the electrostatic term in Eq. (7). The most accurate treatment, in principle, ought to be molecular dynamics (MD), because of the explicit description of atomic motions, so most of the discussion focuses on a brute force approach of very long MD simulations covering the macromolecule and associated solvent.

Molecular Dynamics— Complete Representation

Equation (7) describes the energy of a classical mechanical model for molecules, with each atom taken as a point mass. If one adds Newton's laws to this equation, the resulting set of equations contains all the information necessary to determine the trajectory of the system, that is, the position of each atom as a function of time. All that is required is a set of initial positions (atomic coordinates, usually taken from the crystal structure or an appropriate model) and initial velocities (atomic velocities, generated randomly according to the Maxwell–Boltzmann distribution). MD is an algorithm for numerically solving the equations of motion.

In principle, MD simulations with explicit solvent and using a potential function like that of Eq. (7) might be expected to provide an adequate description to allow for accurate modeling of all relevant factors, even those arising from such complicated and long-range forces as electrostatic and hydrophobic effects. If the functional form of each term of the potential function and the values of the parameters (force constants, partial atomic charges, and so on) are chosen to match known experimental properties,

then a sufficiently long molecular dynamics simulation should sample all relevant conformations with appropriate probabilities, and proper averages of all properties of the model should converge to those of the real system.

With regard to the electrostatic effects, a simple Coulomb's law relationship with a dielectric constant corresponding to that of the average electronic polarizability should be a good first approximation, because the simulated motions would, over the long term, produce the correct screening effects due to the average reorientation of the dipoles of water molecules and of the permanent dipoles of the protein backbone and side chains. The principal error in the electrostatic part of the potential function would then probably arise from the failure to treat induced polarization effects. Modifications to treat these[3,14–16] would be possible refinements to increase the accuracy of the treatment.

However, while such a brute force method is potentially quite accurate, in practice this approach would be computationally overwhelming. Many macromolecular properties fluctuate on time scales of nanoseconds, microseconds, or longer, and these time scales are far beyond those of traditional MD simulations. Convergence could not be obtained in any reasonable time. This is a completely intractable problem if one is interested in solvent effects, since the translational diffusion of water and ions is so slow. One would have to decide where to initially place ions, wait for diffusion to bring the system to equilibrium, and then run a simulation taking averages over a period much longer than diffusional relaxation times.

The long-range nature of electrostatic effects creates further complications, because they are calculated as part of the pairwise nonbonded interactions. Whereas the number of covalent interactions in a system of N atoms grows linearly with N, the number of nonbonded interactions grows as N^2. Molecular dynamics programs usually restrict the calculation of nonbonded interactions to those that occur within a specified cutoff distance, typically 7–12 Å. Calculating these interactions out to distances of two or three times the Debye screening distance would be computationally very demanding, but contributions from these long-range interactions are large.

An additional problem with the above approaches is the observation[17] that existing models of liquid water, which have been parameterized against such properties as the radial distribution function and the temperature dependence of the density, are not particularly accurate in reproducing the dielectric properties of water. This is a critical consideration for studies aimed at understanding electrostatic effects.

Finally, the detailed treatment of induced polarization effects[3,14–16] would probably be necessary to obtain really accurate results in many applications. These would, of course, further increase the computational load.

For all of the above reasons, a brute force approach using an all atom representation for both the macromolecule and the solvent leaves much to be desired. One option is to reduce the level of detail, making suitable approximations to lower the computational burden. This possibility will now be discussed.

Molecular Dynamics— Reduced Representations

The first MD simulations on macromolecular systems were done in the gas phase, because of the limited power of computers in those days. Full representation of the solvent typically requires the use of an array processor or supercomputer, so gas phase simulations are still quite common.

The absence of explicit solvent molecules produces two kinds of errors. First are packing errors associated with motions into the voids left by the missing molecules and dynamic errors due to the absence of viscous damping by the solvent. Second are problems arising from the failure to correctly treat the dielectric discontinuity between the protein and the solvent. These problems can be ameliorated by including explicit solvent molecules in regions of interest. This hydration shell is often surrounded by a region of water molecules that may be fixed or that move according to the diffusive dynamics of the Langevin equation.[18–20]

As argued above, MD simulations using a Coulomb's law approximation with a dielectric constant corresponding to that of the electronic polarizability of the protein should be sufficient to treat electrostatic effects inside the protein, if one includes solvent molecules in the model. But if those molecules are not explicitly included, how to effectively simulate the effects of the high dielectric solvent and counterion screening? One common approach has been the use of a distance-dependent dielectric constant, often of the form $D(r) = r$, where r is the distance between the atoms of interest, measured in Ångstroms.[21] The rationale for this approach is that two atoms inside the protein and close to one another have an interaction that is largely unaffected by the solvent, while interactions between atoms separated by larger distances are more affected. The functional form for $D(r)$ is based on an ad hoc choice that is simple and differentiable, so both energies and forces can be easily calculated.

Another approach is the scaling of partial atomic charges. It is common to represent counterion screening in nucleic acid simulations by reducing the charges on ionized atoms. Typically, phosphate charges are scaled from -1 (in units of proton charge) to a value around -0.2[22–24] For globular proteins, reducing charges near the surface of the

protein has been used to mimic solvent screening effects.[25] Again, this is an ad hoc procedure based on plausibility rather than any experimentally measurable effect.

Distance-dependent dielectric constants and scaled partial charges can mimic solvent screening effects on electrostatic *energies*, at least qualitatively. They are also sufficient to stabilize nucleic acids in MD simulations so the molecules do not fly apart,[24,26] as they do in simulations with $D = 1$ and full partial charges.[6] But they cannot correctly treat electrostatic *forces*. The dielectric discontinuity between the protein and the solvent produces polarization effects that result in a noncentrosymmetric force between two charges inside the protein. Any electrostatic treatment based on pairwise interactions and a Coulomb's law relationship will necessarily produce centrosymmetric forces, and this must give incorrect results if solvent is not explicitly included.

Energy Minimization

All of the foregoing problems are compounded for modeling studies that use energy minimization instead of molecular dynamics. In the absence of thermal motions, entropic effects are not simulated. When energy minimization is used to "optimize" a molecular structure, the system is simply quenched to a local minimum energy structure, and the energy function that is minimized is a potential energy rather than a free energy.

Regarding the treatment of electrostatic effects, the absence of simulated motions makes it impossible to produce proper thermal averaging for dielectric screening effects, although these errors might be somewhat reduced if averages are taken over several structures that are taken from a suitably long equilibrated MD simulation and then minimized.

Energy minimization therefore has extremely limited utility for examining electrostatic phenomena in molecular modeling studies.

Explicit Treatment of Atomic Polarizabilities

The ordinary potential functions of molecular mechanics (Eq. 7) treat partial atomic charges as fixed and independent of conformation. The dielectric constant is assumed to be uniform throughout the molecule, or, if a distance-dependent dielectric constant is used, the same functional form is used throughout the molecule. These assumptions ignore the variations in electronic polarizability of different atoms and groups of atoms.

The basic method for treating the differences in electronic polarizability of individual atoms within the macromolecule is due to Warshel and Levitt.[14] In addition to the ordinary Coulombic term in the potential function, point dipoles are associated with each atom. The magnitude and direction of each dipole are determined in an iterative scheme. To begin with, the electric field acting on each atom and due to the fixed charges in the system is calculated, and a zeroth order value for the induced dipole is calculated from the standard electrostatic relationship: the dipole moment is the product of the atomic polarizability and the electric field. The field acting at a given atom is recalculated as the sum of the field due to fixed charges and that arising from all of the induced dipoles on other atoms. This allows the recalculation of the induced dipole on the atom of interest. After iterating for a few cycles, the values for the induced polarization field converges.

A similar approach was used by Lybrand and Kollman for treating induced polarization effects in a model for liquid water.[15] This approach was recently extended to the interactions of small organic molecules with ions.[16]

Langevin Dipole Model for Water

The computational burden imposed by full treatment of all-atom models of liquid water has long been recognized. Warshel and Levitt[14] introduced a model for the bulk solvent that is intended to reduce this burden while mimicking the effects of the reorientation of water dipoles in the presence of fields arising from charges and dipoles inside the macromolecule and within an explicitly treated shell of hydration.

The region surrounding the volume within which all-atom simulations are carried out is represented by a grid of point dipoles. The density of grid points can be taken to be equivalent to the density of water molecules in the bulk liquid, in which case the dipoles represent those of the water molecules. Alternatively, around critical regions, a higher density of grid points can be used, with an appropriate change in the polarizability associated with each dipole. All-atom simulations led to the conclusion that the mean dipole strength at each grid point can be approximated by a Langevin function, with the orientation of the dipole along the direction of the polarizing electric field. Rather than using an iterative procedure, the magnitude of the field at each grid point is reduced by an effective screening function that has been derived from more detailed simulations.

This Langevin dipole (LD) method for treating water has been combined with the protein dipole (PD) approach for modeling atomic polarizability, described in the previous section. A more complete discussion of this combination, the PDLD method, has been given by Warshel and Russell.[3]

CONTINUUM MODELS

The various forms of the Poisson–Boltzmann equation, Eqs. (4–6), provide a rigorous approach to the calculation of the electrostatic potential if the macromolecular charge distribution can be treated as a collection of point charges (and point dipoles, if

desired) and if effects of polarization and ionic strength can be treated with continuum approximations. Unfortunately, the Poisson–Boltzmann equation does not possess analytic solutions except for extremely simple cases. Two approaches have been used for modeling macromolecular systems.

The first approach, usually identified with Tanford and Kirkwood,[27] assumes a spherical macromolecule. The system must be divided into concentric spherical regions of uniform dielectric constant, a region of low dielectric constant for the macromolecule surrounded by a region of high dielectric constant for the solvent. Similarly, the system must be broken into a region of zero ionic strength (the interior of the macromolecule and, if desired, a boundary layer of solvent inaccessible to ions) and a second region with an ionic strength equal to that of the bulk solvent. The solution to the Poisson–Boltzmann equation can then be written as an infinite series of spherical harmonics whose coefficients are determined by the boundary conditions at the surfaces of successive regions. This approach is discussed in the next section.

For more complex shapes the electrostatic problem can only be solved numerically. One approach is the use of numerical methods to solve the relevant differential equation. To do this, a regular grid is imposed onto the system, and the equation is solved by an iterative numerical method. An alternative numerical approach involves the use of Gauss' law to determine the density distribution of the polarization charge at points on a grid covering the molecular surface. These numerical methods are discussed in the section after the next.

Tanford–Kirkwood Theory and Its Modifications

The earliest model for electrostatic effects in proteins was aimed at understanding protein titration curves. It assumed that the protein was an inpenetrable sphere of low dielectric constant with the charged groups distributed uniformly over the surface of the molecule, immersed in a continuum of high dielectric constant.[28] A more detailed model, with the charges located at specified positions, was developed by Tanford and Kirkwood,[27] following a formalism that had been developed to explain the differences in dissociation constants of various related organic acids where the ionization of one group is affected by a charge or dipole elsewhere in the molecule.[29,30]

The Tanford–Kirkwood model assumes that all ionizable groups are independent except for electrostatic interactions, and that all members of each class of ionizable group (the ϵ-amino group of all lysines, for example) are characterized by a single intrinsic pK_a. A spherical geometry is assumed, with an inner sphere of low dielectric constant, then a spherical shell of high dielectric constant but from which mobile ions are excluded, and finally the bulk solvent region, with a high dielectric constant and a Debye–Hückel parameter corresponding to the bulk ionic strength. By confining themselves to a spherical geometry and considering only the case where all charged groups are at the same distance from the center of the protein, Tanford and Kirkwood[27] were able to write the solution to the Poisson–Boltzmann equation as the sum of spherical harmonics whose coefficients can be determined from the positions of the charged groups. Since this was before the first protein crystal structures were available, they examined a number of models with simple geometries for the charge locations.

One important result of this investigation was the demonstration that the total energy is extremely sensitive to the depth of the charges beneath the interface between the protein and the solvent.[31] A depth of about 1.0 Å for charges and 1.5 Å for dipoles was found to be suitable for reproducing the experimental dissociation constants of various organic acids.

With the availability of protein crystal structures, Tanford–Kirkwood theory was modified by Gurd and co-workers to include information on both the positions and solvent accessibilities of charged groups in proteins.[2,32,33] This approach retains the assumption of spherical geometry, with the same three regions as in Tanford–Kirkwood theory. The position of each charge is projected onto the surface of the molecule. Effects due to the depth of each charge are taken into account by scaling the interaction energy of a pair of charges q_i and q_j,

$$W'_{ij} = W_{ij}(1 - SA_{ij})$$

where W_{ij} is the unscaled interaction energy calculated from Tanford–Kirkwood theory and SA_{ij} is the average fractional solvent accessibility of sites i and j. Solvent accessible surface areas are calculated for the crystal structure using the algorithm of Lee and Richards[34] and converted to fractional values that range from 0 for a completely inaccessible group to 1 for a group that has the same accessibility as in a model tripeptide, where it is assumed to reach its maximum hydration.

A different modification to the Tanford–Kirkwood approach, treating the charge positions more exactly, was recently proposed by States and Karplus.[35] A spherical geometry is still assumed, but all charges are placed at their true positions. The boundary between the region of low dielectric constant and that of the high dielectric constant is taken to be the radius at which the density of protein atoms has fallen to about half that of the core region. This means, of course, that some of the macromolecular charges are placed in the region of low dielectric constant while others are located in the region of high dielectric constant. Ions are excluded

from the region of low dielectric constant, but there is no shell of high dielectric constant from which they are excluded. The electrostatic potential is again written as the sum of spherical harmonics, and the coefficients of the different terms are calculated in closed form from the boundary conditions.

Numerical Approaches

For systems with complicated geometries, the electrostatic differential equations can be solved only by numerical approximation methods. In all of these, some sort of grid is set up. For solving the Poisson equation (zero ionic strength) or the Poisson–Boltzmann equation (nonzero ionic strength), iterative procedures are necessary. An alternative approach in the case of zero ionic strength is to calculate the polarization charge density at the boundary between the low and high dielectric regions. Each of these will now be described.

One of the earliest applications of a numerical approach to molecular electrostatics problems was that of Orttung,[36] who described an application of the finite element method to solve Poisson's equation. Orttung was particularly interested in being able to treat cases where the polarizability varies from atom to atom, and most applications of his method have been to small molecules. I have carried out a careful literature search, and I cannot find any applications to macromolecular systems. A second and more widely used algorithm for numerically solving Poisson's equation is that of Warwicker and Watson.[37] They place the system of interest into a cubic box divided into a regular Cartesian grid and use the method of finite differences to find an iterative solution. Each of the small cubes that define the grid is assigned a value of the dielectric constant that corresponds to that of the macromolecule or to that of the solvent. For the finite difference method, a value of the dielectric constant must also be assigned to each of the lines connecting grid points (the edges of the small cubes), and this is taken to be the average value of the dielectric constants of the four small cubes that meet at that edge. A Gauss–Seidel procedure, with an option for successive overrelaxation, is used to solve the finite difference equations. This approach was first used to show how α-helix dipoles can stabilize a charged substrate bound to the active site of an enzyme.[37]

A similar algorithm has been developed by Honig and co-workers to solve the linearized Poisson–Boltzmann equation,[38,39] thereby allowing the treatment of the effects of ionic strength. In addition to assigning a dielectric constant to each of the lines connecting the grid points, each point of the grid must be given an appropriate value for the Debye–Hückel parameter. A straightforward procedure for assigning these values has been developed. Since there is necessarily a tradeoff between accuracy (which requires a fine grid) and computational speed (which favors a coarse one), a focusing method has been developed that allows the use of finer grids in regions of critical interest. Another refinement is the use of a rotational averaging scheme to reduce the errors inherent in associating the macromolecular charges with grid points. In this procedure, the quantities of interest are calculated for several different orientations of the macromolecule within the grid system, and suitable averages are taken. A package of programs, called DelPhi, has been developed with these options and is available for distribution.[39]

Zauhar and Morgan[40,41] have described a method for the case of zero ionic strength, a method that does not require solving the Poisson equation. The molecular surface is covered with a collection of small curvilinear elements, and the distribution of polarization charge density on those elements is found by applying Gauss' law and the boundary condition requiring the continuity of the normal component of the electric displacement vector across the dielectric boundary. Because the grid is used only on the surface of the molecule, the macromolecular charges are assigned their true positions, rather than being represented by charges placed on a regular three-dimensional lattice. Furthermore, the grid can be easily designed to have a fine mesh in regions of particular interest and a coarser mesh where lower resolution is appropriate.

TESTS OF VARIOUS ALGORITHMS

Methods for modeling electrostatic effects should be tested on two kinds of problems whenever possible: experimental cases, and problems with simple geometries that can be solved in closed form. In evaluating the relative merits of the various methods reviewed here, it is unfortunate that not all methods have been tested on simple theoretical cases where a rigorous error analysis is possible. It is also regrettable that there have been so few comparative studies with different modeling approaches being applied to a single system where experimental results are available.

Two of the continuum modeling methods have been carefully examined in tests on simple problems where analytic solutions are available. Error analyses of these tests reveal the importance of grid size in obtaining accurate results. Zauhar and Morgan[41] have shown that their boundary element method gives errors on the order of 1% or less for the polarization charge density and for the electrostatic potential outside a sphere with a point charge 1.5 Å below the surface. When Honig's DelPhi program for numerically solving the Poisson–Boltzmann equation was tested on a similar problem, errors of less than 5% were found everywhere except within a few Ångstroms of the point charge,[39] where errors in the calculated potential may be as large as 25%. These errors are partly a consequence of the grid represen-

tation of the smooth spherical boundary; their magnitude can be reduced by the focusing method that uses a finer grid in critical regions.[39] Another source of error is the fact that the algorithm is based on a finite difference method, which is not rigorously accurate when the second and higher order spatial derivatives of the potential are large. It should be emphasized, however, that even the largest errors represent values between correct values just inside and just outside the boundary, so they are inaccurate interpolations between accurate potentials.[39] A recent modification of the algorithm allows the treatment of the full, nonlinear Poisson–Boltzmann equation, Eq. (5), which should reduce some of these errors.[42]

Perhaps the most rigorous comparative test of the ability of various theoretical methods to reproduce experimental effects is the recent study by Gilson and Honig.[45] They compared three classes of continuum methods: a Coulomb's law treatment with both constant and distance-dependent dielectric constants, the Tanford–Kirkwood treatment in both its original form and with the modification that corrects for solvent-accessible surface, and the numerical method for solving the linearized Poisson–Boltzmann equation. The test problem was the interaction of ionized residues with a histidine in the active site of subtilisin, where experimental data are available on the effects of point mutations on the histidine pK_a over a range of ionic strengths.[46] For this case, involving a compact globular protein, both the Poisson–Boltzmann approach and the original Tanford–Kirkwood theory were found to be in reasonable agreement with experiment, while the other methods were less successful. Using just the Poisson equation gives reasonable results at low ionic strength,[47] but only the full Poisson–Boltzmann equation can predict the dependence on ionic strength.[45,48]

Another case where the Poisson–Boltzmann method has been compared with other continuum approaches is the reaction between the superoxide anion and superoxide dismutase.[38,49,50] The ability of the Poisson–Boltzmann approach to reproduce the experimental dependence of reaction rate on ionic strength is impressive. It has also been shown that simpler models do not work unless they are empirically parameterized to do so.[50,51]

Warshel's PDLD model has been tested on a number of experimentally characterized systems in the years since its original development.[3,14] Among the data that have been reproduced are the catalytic free energy in chymotrypsin[52,53] and the pK_a values of ionizable sidechains in pancreatic trypsin inhibitor.[54]

Van Belle et al.[55] have examined the contributions of induced dipole moments to electrostatic energies in proteins using the Warshel algorithm.[3,14] In a gas phase simulation (no solvent), they find that such contributions are substantial and cannot be mimicked by conventional molecular mechanics potential functions using either a single valued or a distance-dependent dielectric constant. Unfortunately, the absence of solvent in the simulation left them unable to compare their results with experimental data, such as pK_as of ionizable sidechains, and unable to determine the actual contributions of induced dipoles. Since the dielectric discontinuity between macromolecule and solvent has such large effects, they could not determine whether "including the contribution from induced protein dipoles is really worth the trouble for any practical purposes."[55]

The same study[55] also reports that the choice of partial charges is more critical than the choice of values for the atomic polarizabilities. This is not surprising, since the electric field from a monopole falls off as $1/r$, whereas that of a dipole falls off as $\cos\theta/r^2$, where θ is the angular position with respect to the direction of the dipole. The results of other studies show that the contributions from induced dipoles are generally, but not always, much smaller than those from permanent dipoles in the protein, and that the largest effects are due to solvent dipoles (see, for example, Fig. 6 of ref. 54). Similarly, it has been noted that if one is treating solvent screening effects, the screening of dipoles, either permanent or induced, is a second-order effect that can frequently be ignored.[56]

WHAT IS THE DIELECTRIC CONSTANT INSIDE A PROTEIN?

One area of frequent controversy has to do with the dielectric constant inside a protein. Values ranging from two to over a hundred have been reported in a variety of studies.[2–4,32,33,47,57–60]

The controversy arises because of different points of view about two kinds of phenomena, the screening of charge–charge interactions in proteins and the "solvation" of charges when they are buried inside a protein. Each of these phenomena will be discussed below. It must be remembered, however, as far as the intrinsic polarizability in the presence of an external electric field is concerned, experiment and theory both point to a low dielectric constant for proteins, as for most other organic materials. Let us briefly consider this evidence.

The dielectric properties of proteins have been measured over the full range of water contents, including dry powders, hydrated powders, and in solution, and over the full range of frequency from 0 Hz to 20 GHz.[61–63] All of these studies are consistent with a dielectric constant in the range of 2–4; there is only a small frequency dependence, with a weak dispersion in the megahertz range, probably due to rotation of solvated side-chain dipoles on the surface of the molecule.[62]

The electronic polarizabilities that are used as the basis of most molecular mechanics potential func-

tions, and that are used in the model for explicitly treating atomic polarization,[3,14] have values in the range of 1–2 Å^{-3}. For materials with the densities of proteins, a simple calculation using the Claussius–Mosotti equation[64] shows that this corresponds to a dielectric constant in the range of 2–4. Furthermore, Gilson and Honig[59] have shown that Kirkwood–Fröhlich theory gives an estimated dielectric constant between 2.5 and 4 for a collection of randomly oriented α-helices having a numerical density of dipolar groups equal to that found in real proteins.

Experiment and theory are thus in agreement about the low dielectric constant of proteins. However, even though the low value of the dielectric constant of proteins would appear to be well established, there are two phenomena that have led to arguments for much higher values. These are the screening of charge–charge interactions, and the "solvation" of buried charges.

Electrostatic Screening Inside Macromolecules

Many authors choose to use an "effective dielectric constant," D_{eff}, calculated from an observed energy of interaction between two charges and the use of a Coulomb's law approximation for that energy:

$$E = Cq_1q_2/(rD_{eff})$$

The use of effective dielectric constants as a convenient shorthand description of electrostatic screening has a long history. It is in the spirit of this tradition that distance-dependent dielectric constants are frequently used in molecular mechanics calculations.[5,21]

Large effective dielectric constants in proteins frequently arise from charge screening due to the solvent. This well-known phenomenon is easily described in the framework of classical electrostatic theory; a particularly lucid discussion of its consequences for macromolecules has been given by Gilson et al.[56] One important observation is that for charges on opposite sides of a sphere of low dielectric constant (D_1) immersed in a medium of high dielectric constant (D_2), the electric field lines travel around the outside of the sphere, and the very long path length produces a low interaction energy for the charges. The resulting effective dielectric constant will be larger than either D_1 or D_2. An example of this is shown in Figure 1.

One use of effective dielectric constants is due to Hill,[65] who observed that, for an ion pair in solution, energies calculated from Debye–Hückel theory can be expressed in a Coulomb's law expression with a distance-dependent effective dielectric constant of the form[12]

$$D_{eff} = De^{\kappa r}$$

The case described by Hill is particularly well suited to this kind of concept. The system is a homogeneous electrolyte solution in which we consider two point charges to be set at a fixed distance r from one another, and the effects of charge screening are statistical averages over all possible configurations of the rest of the ions in the system. A macroscopic viewpoint is therefore appropriate.

For problems of charge screening in macromolecules, however, the description in terms of an effective dielectric constant is less attractive. For two point charges separated by a specific distance in a macromolecule in aqueous solution, the electrostatic interaction energy depends on the shape of the macromolecule and the exact positions of the charges; this well-known result has been pointed out many times.[2–4] Figure 1 illustrates the variation of effective dielectric constant with position in one particular case. Furthermore, electrostatic interactions have different effects in different kinds of processes, so the relevant interaction energy, and hence the effective dielectric constant, are also dependent on the specific process being examined.[2–4]

For these reasons, the effective dielectric constant for one application will generally not be transferable to any other. For example, measuring the change in pK_a of an ionizable side chain in one part of a protein due to a mutation at another site does not allow the prediction of the ΔpK_a due to a mutation at a third site. Nor does it provide guidance on, say, the choice of an appropriate dielectric function to be used in a molecular mechanics simulation on the same molecule. To describe the changed pK_a in terms of an effective dielectric constant is therefore not particularly informative. Another example arises in the description of the reversal of charges in a mutation in aspartate aminotransferase,[60] where the interaction for a pair of charges in the $(-+)$ arrangement is described by a dielectric constant of 13, while that for the reversed $(+-)$ arrangement corresponds to a value around 80.

Thus, it seems clear that one should avoid the use of what has been properly called[3] the "ill-defined concept" of an effective dielectric constant wherever possible. It is only truly useful in those cases where results from one study can be used as a reasonable simplification in others.[3,56,66] In these cases it is probably better to refer to this function as an effective screening function rather than an effective dielectric constant to emphasize that it represents contributions from a variety of sources.[66] Further, if such a function is to be used, one should clearly state in which circumstance(s) it is applicable.

Protein Dielectric Constants and Charge "Solvation"

There is another reason why it is often argued that the effective dielectric constant of a protein must be quite high. This is related to the so-called solvation of charges by the macromolecule.

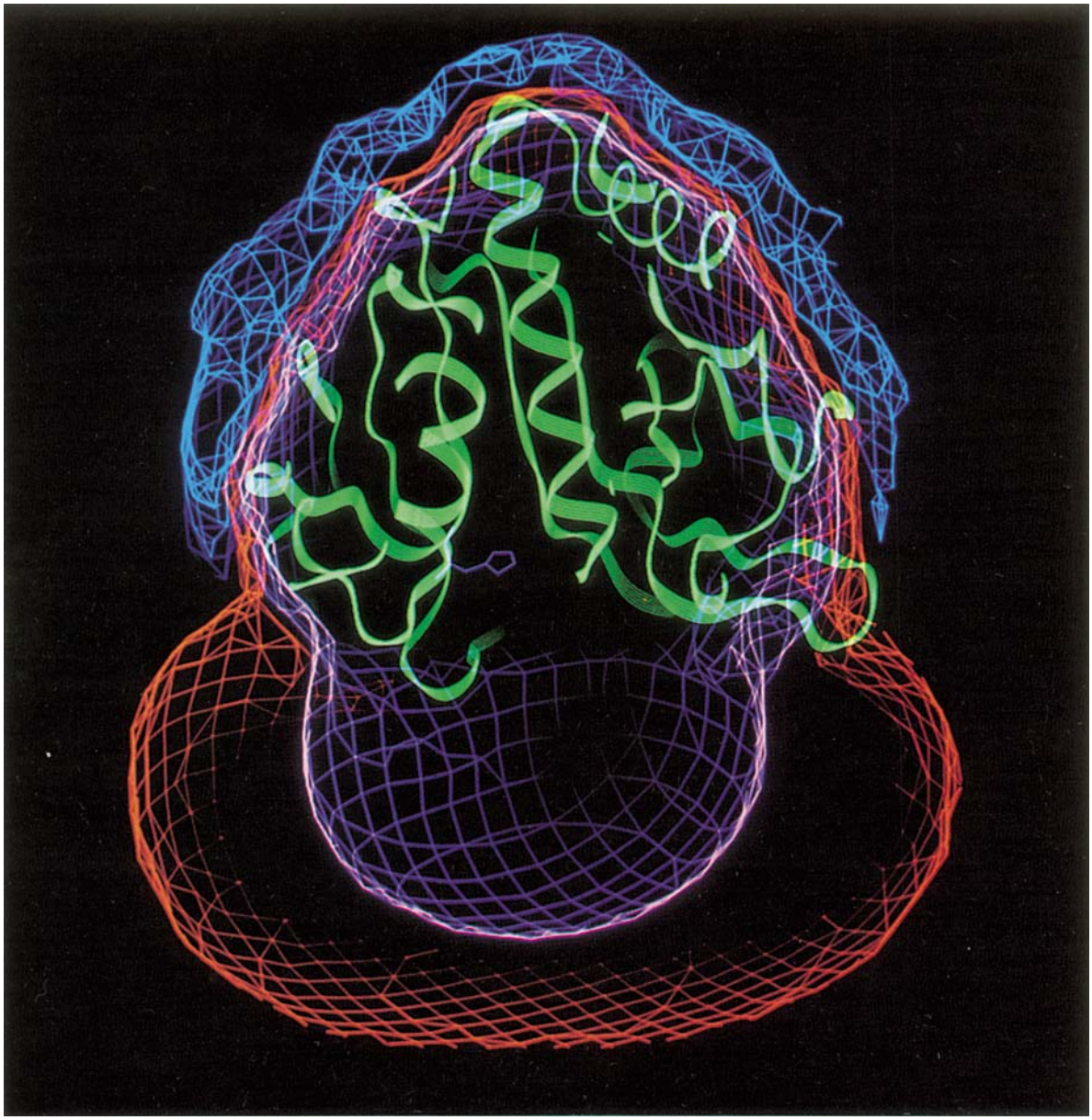

Fig. 1. Electrostatic interactions are influenced by the shape of the boundary between a low dielectric medium (protein) and a high dielectric medium (water). The electrostatic potential due to a charged residue (histidine 66 in subtilisin, shown in magenta) was calculated as a function of position using the finite difference method for solving the Poisson-Boltzmann equation[38,39,45] with a protein dielectric constant of 2 and a solvent dielectric of 78.6. Here the results are compared with the energies calculated by a conventional Coulomb's law treatment, assuming a uniform dielectric of 78.6 throughout. Regions within the red or magenta contour have a greater electrostatic potential (at least 10% greater for the red, and at least 30% greater for the magenta) than for histidine in water, while regions within the blue contour have a smaller potential (at least 10% less) than for histidine in water. The former could be described as having a low effective dielectric constant (less than 70 for the red, and less than 55 for the magenta), and the latter could be described in terms of a high effective dielectric constant (greater than 87), illustrating how the effective dielectric constant depends on position. Note that the region of increased potential is not only in the protein interior, where the dielectric constant is low, but it also extends out a considerable distance from the active site histidine into the high dielectric solvent. This is an example of electrostatic focussing. Also note that regions where there is more effective screening than with solvent alone lie on the opposite side of the protein. These result from the exclusion of electric field lines from the protein into the solvent. If a test charge were placed in the blue region, the electrostatic interaction path length would be longer than for the case with solvent alone. Therefore the effective dielectric constant is greater than the solvent dielectric constant even though the region between the charged histidine and the test charge is filled with material that has a low dielectric constant. Figure provided by Drs. Kim Sharp and Barry Honig.)

The free energy change in moving a body of radius r and charge q from a region of dielectric constant D_1 into a region of dielectric constant D_2 is given by the Born approximation[67]

$$E = Cq^2(1/D_2 - 1/D_1)/(2r)$$

If we use a dielectric constant of 80 for water, and if we assume a radius of about 1.5 Å, we find that the energy cost is roughly 25–50 kcal/mol for moving an atom carrying a charge of $\pm e$ from water into a protein of dielectric constant in the range of 2–4 (where e is the charge on a proton).[7] If the charge on the atom is reduced to $\pm 0.1e$, a value typical of the partial atomic charges in proteins, the energy is still on the order of kT, the thermal energy.

This simple treatment could lead to the argument that proteins must be intrinsically very unstable from an electrostatic point of view. Every atom in the molecule would prefer to be buried in the strong dielectric, water, rather in the weak one, the protein interior. Since, in fact, proteins prefer the folded or globular state to a completely unfolded state, the effective dielectric constant is apparently very high.

The resolution of this problem lies in three observations. First, almost all fully ionizable groups in a protein are in fact on the surface[68]; less than 5% of ionizable groups in 36 globular proteins are not accessible to the solvent when the algorithm of Lee and Richards[34] is used to examine accessibility. Second, as pointed out in the previous section, charge–charge interactions inside proteins are, in fact, substantially screened by the solvent even when charges are not actually on the surface. The energy difference is not nearly as large as that predicted by the simple model in the previous two paragraphs. Third, the ionizable groups that are buried are almost all involved in salt bridges, hydrogen bonds, or interactions with permanent dipoles.[68] These charge–charge interactions are sometimes described as representing an effective "solvation" of ionizable groups by the protein.[3,58]

One particularly pathological case is that of the heme group in cytochrome *c*: the electrostatic self energy of the heme group could be described in terms of a low effective dielectric constant, but interactions of the heme with nearby charges represent a high effective dielectric constant.[69]

While it is clear that proteins have structures that optimize electrostatic interactions to provide stability, and although one could describe these interactions in terms of a high effective dielectric constant, I think that again it is advisable to avoid this "ill-defined concept."[3] In introducing an effective dielectric constant, one mixes microscopic and macroscopic viewpoints in a way that is confusing and that does not add information. Since the effective dielectric constant differs for every case, it is cleaner and simpler to avoid describing these interactions in this way.

DISCUSSION AND CONCLUSIONS

Applicability of Various Methods

The importance of electrostatic effects in questions of structural stability and intramolecular interactions makes their proper treatment in molecular modeling studies extremely important. Different modeling methods are suitable for different kinds of problems, and one is often faced with the need for balancing speed against accuracy. The level of detail chosen for the model should be appropriate to the specific problem being attacked.

The amount of computational effort that is appropriate for treating electrostatic effects will be influenced by the relative importance of other kinds of effects; there are a number of problems where limited accuracy in the treatment of other factors may make it appropriate to use relatively simple approximations for treating electrostatic effects. If solvent effects are not treated at all, any reasonable and computationally convenient form for intramolecular electrostatic effects is probably as good as any other, since such large errors are being made by the omission of solvent. In all atom models for the macromolecule, if explicit solvent is included or if a reduced representation is used for the solvent (a Langevin dipole model or a Poisson–Boltzmann approximation, for example), there will be many applications where the remaining uncertainties in the calculations make the rigorous treatment of atomic polarizabilities irrelevant, and a uniform, low dielectric constant will be an adequate treatment for those polarizabilities. In studies of large-scale conformational changes, current methods for treating the hydrophobic effect are so crude that it may be superfluous to pursue an extremely accurate treatment of electrostatic contributions. Finally, the assumption of static macromolecular structures can introduce large errors: recent Brownian dynamics simulations on the interactions of a polyvalent cation with cytochrome *c* have shown that fluctuations in the protein structure can give differences in collision rate that are as large as those produced by using different methods for calculating the electrostatic potential (J.B. Matthew and S.H. Northrup, personal communication).

As examples, Tanford–Kirkwood theory and its modifications are reasonable approaches for determining the titration curves of globular proteins, even though they do not rigorously treat the overall molecular shape or the correct positions of the charges. On the other hand, all atom models including explicit water molecules are necessary for determining relative association constants using free energy perturbation calculations based on molecular dynamics.[5,15,16,53,66,70–72] In some cases, the conventional potential functions need to be modified to include atomic polarization effects before reasonable answers are obtained.[15,16,53,66,72]

Between these extremes, models of intermediate detail—either microscopic or macroscopic—have been shown to be useful. Thus, a model that explicitly treats atomic polarizabilities inside the macromolecule and that uses the Langevin dipole approximation for simulating solvent effects (the PDLD model[3,14]) can calculate pK_as of ionizable side chains[54] and determine the catalytic free energy of some enzyme reactions.[52,53] And programs that numerically solve the Poisson–Boltzmann equation can reproduce the effects of site-specific modifications on the ionization of nearby side chains,[47,48] and on rates of reactions of proteins with substrates[49,50] and with other proteins.[73] Furthermore, there have been recent modifications of Honig's algorithm to allow the calculation of self energies and solvation energies,[74] leading to the hope that this method will prove suitable for the rigorous calculation of electrostatic energies and forces in molecular mechanics and molecular dynamics.

Shortcomings of Various Methods

The conventional treatment of electrostatic effects in molecular mechanics potential functions, using the Coulomb's law approximation given in Eq. (1), is seriously flawed. This is because of the many-body nature of electrostatic interactions, where Eq. (1) describes only pairwise interactions. It is a common assumption that this approximation can be somewhat improved by replacing the dielectric constant D with an appropriate distance-dependent screening function $D(r)$. An alternative approach would be to more rigorously calculate electrostatic energies and forces using one of the methods described above. Here I summarize the problems in using distance-dependent screening functions, then I briefly point out the flaws in the various alternative methods.

If a simplified model is being used with a distance-dependent screening function or some other effective dielectric constant, it is essential that the uncertainties associated with this choice be considered. In particular, the effects of solvent screening depend on macromolecular shape, the location of the charged group of interest, and the process being examined. As a consequence, the issue of the transferability of screening functions from one study to another must be addressed. For example, Gilson and Honig have shown that the conventional "distance-dependent dielectric constant" often used in molecular mechanics potential functions, $D(r)=r$, underestimates solvent screening of charges near the surface of macromolecules,[45] and it is an underestimation in a number of other problems as well.[58]

One further caution about using a Coulomb's law approximation for electrostatic effects is appropriate. If the solvent is not modeled correctly, the use of a screening function, whether of fixed value or distance dependent, may give reasonable estimations of relative electrostatic interaction *energies* under some circumstances, but the electrostatic *forces* calculated from such an approach will generally have serious errors. This is a consequence of the centrosymmetric nature of Coulombic forces. Dielectric boundaries will necessarily produce interfacial polarization charge distributions that cannot be treated in the framework of Coulomb's law, so serious errors in the calculated forces are unavoidable without rigorous treatment of solvent effects. This problem is compounded at nonzero ionic strength, because then one needs to also calculate the contributions arising from the free ions. If unattainably long molecular dynamics simulations are to be avoided, it is necessary that the electrostatic effects of both solvent and free ions be correct statistical averages.

Because of these problems, it would seem logical to work toward the incorporation of more rigorous treatments of electrostatic effects in macromolecular simulations. Unfortunately, all of the methods described here have some shortcomings. Those are briefly listed here.

The Langevin dipole model for water is a logical approach to presenting suitably averaged contributions of the solvent, particularly if an explicit boundary solvation layer is included.[3,14] Unfortunately, the procedure that is used iterates on the magnitude of the Langevin dipole, but not on its direction. The direction of the electric field at any individual point dipole is not updated during the calculations, which means that contributions to the field from other point dipoles are not rigorously treated. This approximation was introduced because simulations using a grid of rotatable solvent dipoles[75] converge much more slowly.[3] Furthermore, ionic strength effects are not explicitly included in this method.

While Tanford–Kirkwood theory and its modifications[27,31–33,35] treat ionic strength effects rigorously, they suffer from the assumption that the protein/solvent interface is a spherical boundary. With the exception of the algorithm of States and Karplus,[35] they also have the problem that they do not include the true charge positions. The attempt to approximate charge burial effects by correcting interaction energies by a factor related to the solvent-accessible surface[32,33] is flawed because of the failure to treat the self energy term,[3,58] and by the assumption that the intrinsic pK_a of each group is constant.[76] This modification to Tanford–Kirkwood theory would predict that there is no energy cost to move a charge from a polar medium like water to a nonpolar medium like benzene. The success of Tanford–Kirkwood theory in predicting protein titration curves even though the self energy term is ignored is probably due to the fact that, for charged groups on the surface of the molecule, the self energy term is not extremely important. The lack of correlation between changes in pK_a and depth of burial supports this argument.[2] Thus, while this method can be used

for protein titration curves, it does not offer the prospect for calculating electrostatic energies and forces for molecular mechanics.

Numerical methods for solving the electrostatic equations[36–41] must all deal with the difficulties of imposing a grid onto the molecular system. This raises the problem of how to distribute the atomic charges onto the grid for some methods.[36–39] For all of them there is also the issue of errors arising from grid coarseness. Further, neither the algorithm of Warwicker and Watson[37] nor that of Zauhar and Morgan[40,41] can treat the effects of ionic strength. The nonlinear formulation of the Poisson–Boltzmann equation[38] gives substantial errors in regions near charges located at the macromolecular surface, although recent advances[39,42,74] should lead to substantial reduction of these errors.

Future Directions

I believe that both microscopic and macroscopic methods offer promise for improving the treatment of electrostatic effects in macromolecular modeling studies in the future, subject to continuing growth in the power of computers.

Regarding microscopic approaches, it is likely that there will be steady improvements in the treatment of induced polarization effects, both in brute force algorithms that include explicit solvation[5,15,16,70,71] and in algorithms that use reduced representations to circumvent the convergence problem.[3,14,53,66,72,75] The principal challenge facing the developers of these algorithms, it seems to me, will be the realistic treatment of ionic strength effects in a way that provides appropriate statistical averaging.

There are two continuum methods, either one of which could eventually solve the electrostatic problem for many molecular modeling studies. The algorithm of Zauhar and Morgan[40,41] has the advantage of using the true positions of charges within the macromolecule; here again the challenge will be to include the treatment of ionic strength effects. The numerical solution of the Poisson–Boltzmann equation[38,39] is the most direct way of treating ionic strength effects if the problems associated with the use of a grid can be solved. Recent advances, including rotational averaging,[39] focusing,[39] the ability to treat the full nonlinear Poisson–Boltzmann equation,[42] the ability to calculate self energies and solvation energies,[74] and methods that improve the rate of convergence of the calculation[77] are reasons for optimism.

In addition to questions of rigor and computational efficiency, there is the question of availability of programs and their acceptance by the scientific community at large. Several laboratories have implemented programs to numerically solve the Poisson equation, while others have written algorithms for the Poisson–Boltzmann equation. One of these packages, DelPhi,[39] is available for distribution. The PDLD (Protein Dipoles–Langevin Dipoles) algorithm[3] is available in POLARIS, a part of MOLARIS, a multipurpose macromolecular modeling package.[78]

Thus, at the present time there are two truly competitive methods for calculating electrostatic energies in molecular modeling studies, Warshel's POLARIS program and Honig's DelPhi package. R.L. Baldwin (personal communication) has repeatedly raised the question of how well any method could solve the classic problem of the prediction of differences in pK_a for a variety of simple organic acids.[30] One of Warshel's algorithms has been shown to give reasonable agreement with the experimental data on this problem,[75] and a recent test of the Honig algorithm also shows a favorable comparison with experiment (B. Jayaram and B. Honig, personal communication). It would be instructive if both packages were to be tested on a series of identical test cases, both experimental cases and theoretical cases for which closed-form solutions are available. Such tests would enable the molecular modeling community to determine which algorithm is more suitable for various classes of problems.

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