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Limitations of Atom-Centered Dielectric Functions in Implicit Solvent Models

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Many recent advances in Poisson−Boltzmann and generalized Born implicit solvent models have used atom-centered polynomial or Gaussian functions to define the boundary separating low and high dielectric regions. In contrast to the Lee and Richards molecular surface, atom-centered surfaces result in interatomic crevices and buried pockets of high dielectric which are too small for a solvent molecule to occupy. We show that these interstitial high dielectric regions are of significant magnitude in globular proteins, that they artificially increase solvation energies, and that they distort the free energy surface of nonbonded interactions. These results suggest that implicit solvent dielectric functions must exclude interstitial high dielectric regions in order to yield physically meaningful results.

I. Introduction

Continuum solvent models have become an increasingly useful tool in the characterization of biomolecular systems. The most popular such methods employ either the Poisson−Boltzmann (PB) or generalized Born (GB) model, treating the solute as a set of point charges in a low dielectric cavity and the surrounding solvent as a uniform high dielectric medium. The PB model is generally considered to be more accurate and is often used to benchmark GB models. The GB model has found more extensive application in dynamical simulations, however, because it is computationally efficient and more amenable to force calculations.

One of the main challenges in the use of the PB model for dynamics has been the determination of numerically stable and accurate forces. Most PB calculations have used a dielectric boundary based on the molecular surface (MS) as defined by Lee and Richards,[1] which results in forces that are unstable over time, lack analytical definition, converge poorly, and are sensitive to grid discretization.[2] Furthermore, an abrupt dielectric transition results in numerical instability regardless of the location of the boundary. Recent advances in PB methods have avoided these difficulties by using overlapping atom-centered Gaussian or polynomial functions to define the solute surface, resulting in analytically defined, differentiable dielectric functions with smooth transitions between low and high dielectric values.[2,3] These dielectric definitions increase force stability and computational efficiency. However, unless modifications such as those presented by Lu and Luo[4] or Lee et al.[5] are employed, they result in interatomic crevices and buried pockets of high dielectric which are too small for a solvent molecule to occupy.[4−6] It was originally postulated that the consequences of these regions, henceforth called *interstitial high dielectrics*, would be minimal and that either a MS or an atom-centered surface definition should be physically and theoretically equivalent,[2,7] but more recent work has suggested that atom-centered surfaces are physically flawed.[5,6] Nevertheless, implicit solvent models based on unmodified atom-centered dielectric functions are becoming increasingly popular in the biophysical community.[2,3,7−10]

Here, we report results showing that atom-centered surfaces create interstitial high dielectric regions of significant magnitude in globular proteins, increase solvation energies, and distort the free energy surface of nonbonded interactions. Although similar results are expected for most atom-centered smoothed dielectric boundaries, we focus on the spline surfaces (SS's) introduced by Im et al.[3] and implemented in Adaptive Poisson−Boltzmann Solver (APBS),[11] the PBEQ module in CHARMM,[12] and the GBSW model.[8]

II. Methods

The protein surface and energy calculations were performed with APBS 0.3.2 using a grid resolution of 0.2 Å. To facilitate comparison between the different dielectric boundaries, we have chosen to use the Nina et al. optimized radii to define the van der Waals surface (vdWS), MS, and SS.[13,14] For the results with different surface definitions to be comparable, the radii must be rescaled for the MS and particularly for the SS; we have performed the recommended rescaling. To check that results were not specific to Nina et al. radii, they were verified with AMBER optimized radii,[15] parm22 radii,[12] and Bondi radii.[16] The latter two were augmented by the spline window width ($w = 0.3$ Å) for the SS, a generic but very reasonable scaling. APBS versions 0.3.2 and earlier have a flaw in the MS algorithm that, in our testing, overestimates MS volumes by 2−5% and underestimates solvation energies by 1−3%. The results shown

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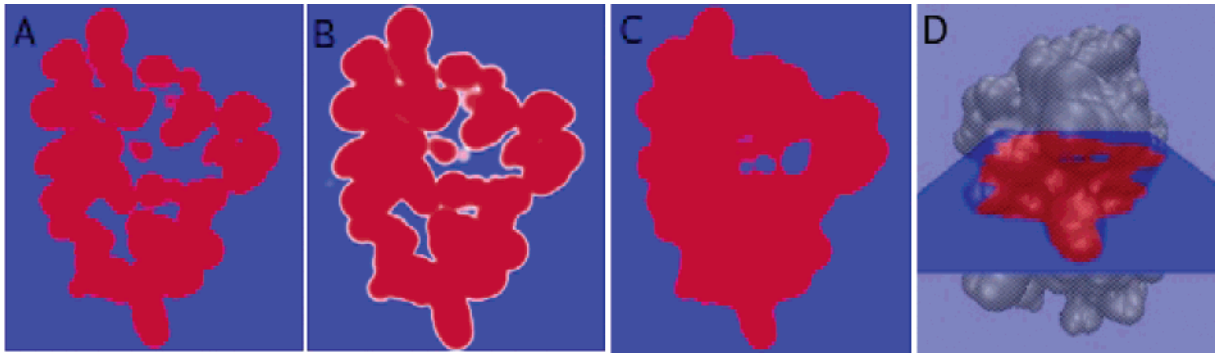

Figure 1. Dielectric maps of IFABP. Dielectric values on a plane intersecting IFABP for the vdWS (A), SS (B), and MS (C). Red regions have $\epsilon = 1$, blue regions have $\epsilon = 80$, and white regions have intermediate dielectric values. The location of the intersecting plane is shown in part D.

TABLE 1: Solute Volumes for Different Surface Definitions[a]

	vdWS	MS	SS[b]	interstitial high dielectric[c]		
protein	$\epsilon = 1$	$\epsilon = 1$	$\epsilon < 80$	$1 < \epsilon < 20$	$20 < \epsilon < 80$	$\epsilon = 80$
1fkg-xtal	14863.3	16087.5	18508.6	1345.8 (8.4%)	502.5 (3.1%)	65.3 (0.4%)
1aki-xtal	17495.8	18954.9	21473.0	1519.4 (8.0%)	608.1 (3.2%)	100.5 (0.5%)
1kzk-xtal	27528.0	29937.1	33885.2	2512.8 (8.4%)	1006.9 (3.4%)	139.7 (0.5%)
1kzk-md	27617.3	30245.4	34081.5	2576.2 (8.5%)	1120.9 (3.7%)	202.3 (0.7%)
1ael-nmr	17589.4	19361.9	21869.6	1532.7 (7.9%)	797.0 (4.1%)	196.8 (1.0%)
1ael-md	19318.5	21585.4	24579.4	1930.0 (8.9%)	1031.1 (4.8%)	262.5 (1.2%)

[a] Solute volumes (Å^3) for FKBP12 (1fkg), lysozyme (1aki), protease (1kzk), and IFABP (1ael). [b] The SS is larger because almost one-fourth of its volume has dielectric (ϵ) values less than 80 but greater than 1. [c] Interstitial high dielectric volumes are measured by the volume within the MS that has high dielectric values in the SS. The total percentage of interstitial high dielectrics relative to the MS volume, shown in parentheses, ranges from 11 to 14%, increasing for the MD and NMR structures.

here were calculated with a modified algorithm that corrects this problem. The energy calculations were performed with zero bulk ionic strength, a temperature of 300 K, a solvent dielectric of 80, a solute dielectric of 1, and charges from the CHARMM22 all hydrogen force field in accordance with the radii.

Implicit solvent potentials of mean force (PMFs) for hydrogen bond formation were calculated by combining solvation, Coulombic, and vdW energies. PB solvation energies were calculated with APBS and the same parameters as those used for protein solvation energies except for a finer grid resolution of 0.1 Å. GBMV and GBSW solvation energies, Coulombic energies, and vdW energies were calculated with CHARMM 31a1. AMBER GB solvation energies were calculated using the igb=1 model in AMBER 8. Because AMBER GB models are not compatible with atoms having zero radius, the hydrogen radii were increased to 0.8 Å. This made the outer surfaces of the hydrogen atoms approximately coincident with the surfaces of the atoms to which they were bound. The explicit solvent PMFs were calculated by WHAM from results of umbrella sampling in TIP3P solvent. Umbrella sampling was carried out using the PMEMD module of AMBER, modified to apply harmonic restraints to only the *y* and *z* coordinates of the peptides. Due to the use of different force fields for implicit and explicit solvent measurements, no quantitative comparison should be made. However, explicit solvent potentials calculated with the CHARMM force field have the same general shape.[17]

III. Results and Discussion

To probe the magnitude of interstitial high dielectric regions in globular proteins, we compared the solute volumes generated by vdWS, MS, and SS. Figure 1 shows the MS and SS dielectric values on a plane intersecting a structure of intestinal fatty acid binding protein (IFABP) taken from a molecular dynamics (MD) simulation. Although MD conformations might be expected to contain more interstitial high dielectrics than NMR or crystal structures, surprisingly similar plots were obtained for all of the systems in Table 1. The solute volumes, reported in Table 1, show a consistent trend across all six structures: substantial interstitial high dielectrics with the SS definition. Within the MS volume, the SS renders 65−262 Å^3 of interstitial high dielectric space with a value of 80 and an additional 502−1121 Å^3 with values over 20. The total interstitial high dielectric space ranges from 12% for the crystal structures to 15% for the NMR and MD structures. The quantitative effects of these regions on electrostatic solvation energies are shown in Table 2; SS energies are overestimated by 11−21%, only slightly less than the overestimation by the vdWS.

TABLE 2: Electrostatic Solvation Energies for Different Surface Definitions[a]

protein	MS	vdWS	SS
1fkg-xtal	−1475.8	−1680.4 (13.9%)	−1638.3 (11.0%)
1aki-xtal	−1724.3	−2034.7 (18.0%)	−1976.7 (14.6%)
1kzk-xtal	−2196.0	−2585.6 (17.7%)	−2499.9 (13.8%)
1kzk-md	−2162.5	−2549.1 (17.9%)	−2475.9 (14.5%)
1ael-nmr	−2247.4	−2838.2 (26.3%)	−2727.0 (21.3%)
1ael-md	−1979.3	−2368.9 (19.7%)	−2301.9 (16.3%)

[a] All energies are reported in kcal/mol. The SS yields energies much larger than the MS and similar to the vdWS. Percentages of vdWS and SS overestimation relative to the MS energies are given in parentheses.

The volume and dielectric value of the interstitial spaces created by the SS can be decreased by using a larger spline smoothing window for SS's or longer Gaussian tails for Gaussian surfaces, but interstitial high dielectrics cannot be eliminated altogether.[2] Unfortunately, this overestimates the size of solvent exposed atoms and creates unphysical bulges around overlapping and adjacent atoms.[5] These expanded dielectric boundaries yield solvation energies and forces that are severely underestimated. For example, when a spline window of 1.0 Å is applied to the systems in Table 1, interstitial high dielectrics with $\epsilon > 20$ are essentially eliminated, but the volume of lowered dielectric outside the MS is increased dramatically and

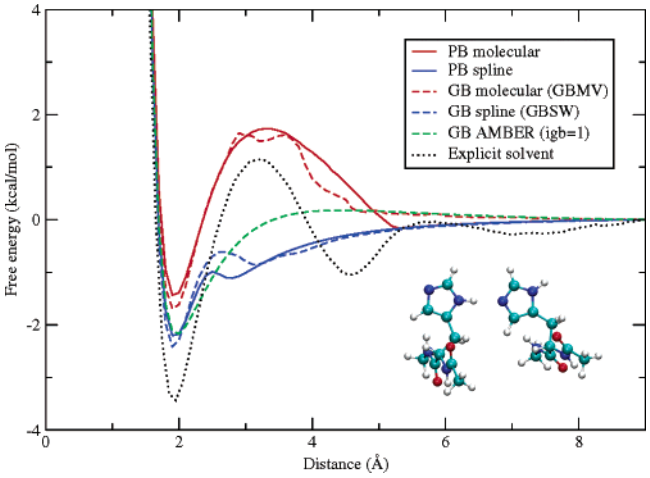

Figure 2. PMF for the illustrated histidine−histidine hydrogen bond. Distances are measured between the hydrogen and nitrogen atoms participating in the bond. Spline-based dielectric boundaries fail to capture the free energy barrier to hydrogen bond separation because they allow interstitial high dielectrics near the hydrogen bond as it is separated.

the solvation energies are underestimated by 35−48%. This over- or underestimation of solvation energies by the SS has also been reported by Lee et al.[6] who used hybrid explicit/implicit solvation energies to test various continuum surface definitions.

While the ability to calculate atomic forces in a PB model is an important advance, such forces can have useful application only if the potential they are derived from accurately represents the physics of the system. In particular, solvation models employed in dynamical simulations must be capable of accurately calculating high energy as well as low energy conformations. Therefore, dynamics may constitute a more demanding test of a solvation model than calculating the solvation energies of static structures, which tend to be dominated by low energy configurations of atoms.

Hydrogen bonds are of particular interest in simulations of biomolecules; since solvation effects make a large contribution to these interactions, the PMF for the separation of a hydrogen bond can be used as a test of the quality of a solvation model. The PMF of hydrogen bonding between the delta hydrogen and the epsilon nitrogen of two delta protonated histidines calculated with a variety of solvation methods is shown in Figure 2. Both PB and GB results based on a MS dielectric boundary faithfully represent the important features of the explicit solvent PMF, a narrow minimum and a significant barrier to separation of the hydrogen bond. The energetic barrier in the MS PMFs comes about because the electrostatic energy rises rapidly as soon as the hydrogen bond participants are separated, but the solvation energy does not substantially increase in magnitude (become more negative) until the bond is sufficiently separated that the solvent probe will fit between the participants. The SS-based implicit solvent models have good performance near the minimum and at long distances but fail to capture the appropriate energetic barrier. At the separation where the MS PMF energy peaks, the SS has large interstitial high dielectrics, which result in a more negative solvation energy. This produces an artifactual minimum—or in less extreme cases, a shoulder—near a location where the PMF should have a maximum. Discrepancies between MS and SS PMFs are most dramatic for interactions between sterically bulky groups, where the magnitude of the interstitial high dielectrics is largest, but are observed to a greater or lesser degree across a variety of hydrogen bond and salt bridge systems (see the Supporting Information). The AMBER (igb=1) GB results, based on the model of Hawkins, Cramer, and Truhlar,[18] illustrate that the lack of a barrier to separation is a general feature of implicit solvent models that allow interstitial high dielectrics. In comparison to the SS results, the AMBER GB PMF is somewhat broader near the minimum but is smoother and avoids the second minimum seen in the SS PMF. For simplicity, apolar solvation contributions have been ignored in the implicit solvent PMFs presented here. A traditional surface area apolar term changes the depth of the minimum but has no appreciable effect on the discrepancies between MS and SS PMFs.

IV. Conclusion

The introduction of atom-centered dielectric functions has been a significant advance for PB force calculations. They can be analytically defined and easily smoothed allowing for numerical stability and increased efficiency. However, this paper demonstrates that atom-centered surfaces produce large volumes of interstitial high dielectrics in globular proteins which artificially overestimate solvation energies and distort the free energy profile of nonbonded interactions such as hydrogen bonds and salt bridges. Dynamical simulations conducted using these dielectric boundaries will sample incorrect conformational ensembles. These findings suggest that, although the optimal surface definition should be smooth and differentiable, it should also exclude interstitial high dielectrics as the MS does. Dielectric boundaries that address this issue, such as those proposed by Luo et al.[4] and Lee et al.,[5] will be critical for further improvement of PB and GB models.

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Supporting Information Available: PMFs of hydrogen bond and salt bridge interactions for additional systems. This material is available free of charge via the Internet at http://pubs.acs.org.

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