Electronic Polarization Is Important in Stabilizing the Native Structures of Proteins

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Quantum mechanical computations of proteins based on the molecular fragment approach have been carried out, and polarized protein-specific charges have been derived to provide accurate electrostatic interactions for a benchmark set of proteins. Our study shows that, under the polarized protein-specific force field, the native structure indeed corresponds to the lowest-energy conformation for these proteins. In contrast, when a standard mean-field force field such as AMBER is used, the energies of many decoy structures of proteins could be lower than those of the native structures. Furthermore, MD simulations were carried out and verified that the native structures of these proteins not only are statically more stable but are also dynamically more stable under the polarized protein-specific force field. The present results, together with several recent studies, provide strong evidence that protein polarization is critical to stabilizing the native structures of proteins.

Introduction

Molecular dynamics (MD) simulations can provide detailed information about the structure and dynamics of biomolecules at the atomistic level and uncover their biological roles, for example, in enzyme reactions, protein−ligand binding, and other important biomolecular processes.[1−9] However, to correctly extract structural and dynamical properties of proteins from MD simulations, it is critical that the force field representing the interaction should properly describe important interactions. For example, a protein's native structure is determined by a detailed balance between inter-residue interactions and protein−solvent interactions. Thus, the force field needs to faithfully reflect such a fine balance in order to predict the correct structure. Earlier studies involving mutational and crystallographic experiments suggested that small perturbations of these interactions can cause large conformational changes in a protein's structures. This simply means that a small inaccuracy in the force field can introduce significant errors in predicting a protein's structure and dynamics. At present, a major problem in the structural refinement of proteins is the fact that current force fields often fail to render the native structure as the lowest-energy conformation. In a recent report, Wroblewska and Skolnick (WS) carried out systematic computational studies on an extensive array of protein structures to investigate this issue in detail.[10] In that study, the authors evaluated the energies of many decoy structures for each of the 150 proteins obtained using the AMBER force field and found that many decoy structures actually had energies lower than that of the native structure. This means that the current force field predicts less-stable native structures of proteins. This phenomenon was observed for many of the proteins they studied. Thus, the important question that must be asked is whether the native structure of a protein is inherently less stable or whether some important interaction is simply missing in the current force field. To address this question properly, it is necessary to develop a more accurate force field that is based on more fundamental physics.

In this article, we report a computational study of protein energetics and dynamics based on the polarized protein-specific charge (PPC). The concept of PPC was developed for a more accurate description of electrostatic interactions based on fragment quantum mechanical calculations of proteins in solution.[11−14] PPCs are derived by fitting the ESP (electrostatic potential) from quantum calculations of specific proteins, and therefore, they contain the correct electrostatic polarization effects near native structures. PPCs are protein-specific, and therefore, they are distinct from the amino-acid-specific or mean-field charges typically used in current force fields such as AMBER and CHARMM. These features of the PPC imply that PPCs should give more accurate electrostatic interactions for proteins near native structures. This is extremely important because electrostatic interactions play a significant role in protein structure and function.[15−19] Recent MD studies have shown that PPCs give more accurate pK_a shifts for the ionizable residue in the protein interior for thioredoxin[11] and correctly predict the stable structure in PPARγ−ligand binding.[20] Thus, by explicitly comparing the energetics and dynamics of these proteins under both PPCs and the standard AMBER force field, we hope to provide important answers to the question regarding the relative stability of protein native structures compared to decoy structures.

Theoretical Approach

In the continuum solvation model, the solute (protein) is represented by a charge distribution $\rho(\mathbf{r})$ embedded in a cavity surrounded by a polarizable medium with dielectric constant ε. The solute charge distribution $\rho(\mathbf{r})$ polarizes the dielectric medium and creates a reaction field, which acts back to polarize the solute until equilibrium is reached. By combining the PB (Poisson−Boltzmann) solution for the electrostatic field and the molecular fractionation with conjugate caps (MFCC) calculation for the protein, we obtain the MFCC-PB version for quantum calculations of proteins in solution.[11] The calculated electron densities of the protein fragments are fitted to the ESP (electrostatic potential) using the restrained electrostatic potential

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(RESP) procedure.[22] The quantum chemistry calculation is performed at the level of DFT(B3LYP)/6-31G*. The original AmberFF99 charges were derived from Hartree−Fock (HF)/6-31G* gas-phase calculations. Because dipole moments are systematically overestimated by about 10% from HF calculations, it was believed that this overestimation could approximately account for the solvent polarization effect. For comparison, the MFCC-PB method includes the polarization effect from the protein environment and solvent, and thus, DFT/B3LYP/6-31G* calculations are more appropriate because they accurately reproduce the gas-phase dipoles of small organic molecules.

In constructing the free energy function for structural refinement, the contribution from the configurational entropy of the protein was neglected by assuming that the entropic difference between the native and decoy structures is negligible. This was shown to be basically correct and is consistent with the practice in ref 10 for the proteins of our interest. The protein energy function was represented by the all-atom model of AMBER, the solvent was modeled as a dielectric continuum medium, and the solvation energy was obtained by solving the Poisson−Boltzmann equation. The nonpolar contribution to the solvation energy was obtained as a surface area term. In our study, two sets of charges were used to evaluate the free energy: the AMBER charge with the ff99 parameters and the PPCs calculated from the native structures for the proteins in this study. In these two sets of energy calculations, all other parameters of the AMBER force field remained the same except for the atomic charges. Thus, any difference in the calculated energy between the two sets of force fields is purely electrostatic in nature.

Results and Discussion

A. Relative Energetics between Native and Decoy Structures. WS recently discussed the deficiency of the current force fields for failing to discriminate among native and decoy structures by energy.[10] To quantitatively examine the accuracy of the electrostatic energy for realistic protein systems, the refined "50-set" proteins of WS were used in this study.[10] For each of the 47 proteins, WS picked 50 decoy structures and the native structure for energy refinement by performing multistep MD simulations up to 2 ns. For each decoy or native structure, the authors took 20 snapshots from the MD simulation, giving a total of 1000 configurations for energy evaluation. These configurations were directly taken from the authors' Web site for our energy evaluation in this work.

First, we examined the energetics of the AMBER force field by evaluating the MM-PBSA energy to discriminate the native structure from decoys or misfolded structures for these 47 proteins. To be consistent, we assumed that all decoys adopt the same ionization state as the native structure, as was done in ref 10. We found the lowest energy, $E_{LST}(D)$, from all of the decoy structures and plotted the gap between this energy and the energy of the native state, $E(N)$, i.e., $E(gap) = E_{LST}(D) - E(N)$, for each of the 47 proteins. Figure 1 shows that, in about one-half of the 47 proteins tested, the lowest-energy decoys are lower in energy than the corresponding native states when the AMBER charge is used in evaluating the MM-PBSA energy. The negative energy gaps in Figure 1 range from a few kilocalories per mole to well over 50 kcal/mol, with an average gap of about 30 kcal/mol. This result is consistent with the previous study by WS, who used the generalized Born solvent-accessible surface area (GB/SA) model to investigate the scoring of AMBER force field.[10] They found that the AMBER energy fails to select the native structure from decoy structures in many cases. These decoy structures are compact and well relaxed in energy throughout the MD simulations but are non-native-like. They noticed that the energy in AMBER has a flat and rugged landscape, with many comparable minima away from the native structure. The current PBSA result gives further support to their argument that the AMBER force field is deficient in discriminating the native structure from decoys.

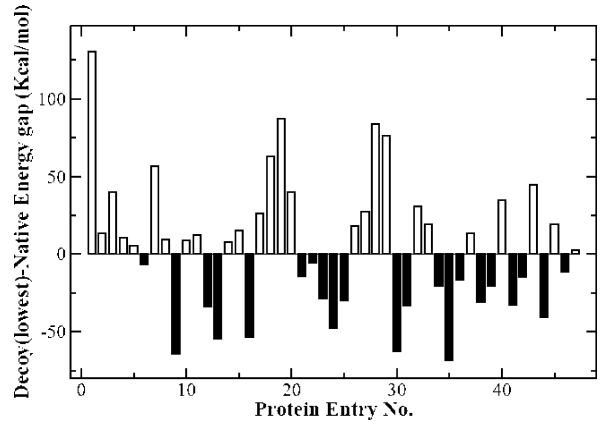

Figure 1. MM-PBSA energy gap, $E(gap) = E_{LST}(D) - E(N)$, between the native and lowest-energy decoys for each of the 47 proteins in the 50-set database obtained using the AMBER force field.

TABLE 1: Average Energy Gap between the Native and the Lowest-Energy Decoy Structures ($E_{gap} = E_{nat} - E_{decoy}$) Obtained Using AMBER and PPC Charges for Proteins

		E_{gap} (kcal/mol)	
PDB	N_{res}	Amber99	PPC
1be9A	115	33.6	−58.7
1befA	177	54.4	−88.3
1bik	110	53.3	−27.4
1bunB	61	28.7	−17.6
1bx7	51	29.6	−7.7
1c1yB	77	33.5	−28.7
1c4zD	144	68.2	−21.7
1cbp	86	30.8	−31.3
1ctf	68	15.0	−26.8
1dtdB	61	40.9	−13.4
1f94A	63	11.4	−31.2
1a19A	89	−13.5	−87.7
1bb9	83	−8.7	−74.2
1bdyA	123	−12.1	−113.3

In contrast, when PPCs are used for the same energy evaluation, the energy of the native state is the lowest in essentially all of the 47 proteins. To generate the PPC, the snapshot of the native structure with the lowest energy is used in the quantum fragment calculation. Table 1 shows a comparison of the gaps between the energy of the native state and the lowest average energy of the decoy structures for 11 proteins obtained using both the AMBER charge and the PPC derived from the native sturcutre. The energy gaps listed in Table 1 are energy differences between the native state and the lowest-energy decoy. Of 11 proteins in Table 1, only three have the native state as the lowest-energy state by about 10 kcal/mol below the lowest-energy decoys when the AMBER charge is used for the MM-PBSA energy evaluation. However, when the PPC is used to replace the AMBER charge for the energy evaluation, all 11 proteins produce the native state as the lowest-energy state, well separated from the lowest-energy decoys, as shown in Table 1. Polarization energy was inclued in the MM-PBSA energy through the polarized charge derived according to the MFCC-PB method.

TABLE 2: Comparison of Energy Gaps[a] with PPCs Derived from Different Protein Structures

PPC from	E_{gap}	PPC from	E_{gap}
1bx7		1ctf	
native	−7.7	native	−26.8
decoy.283	75.5	decoy.292	52.1
decoy.517	64.0	decoy.768	33.0
decoy.612	40.7	decoy.991	28.8
1bunB		1dtdB	
native	−17.6	native	−13.4
decoy.020	57.7	decoy.392	58.8
decoy.300	86.5	decoy.577	78.2
decoy.428	76.4	decoy.778	70.4

[a] E_{gap} defined as in Table 1.

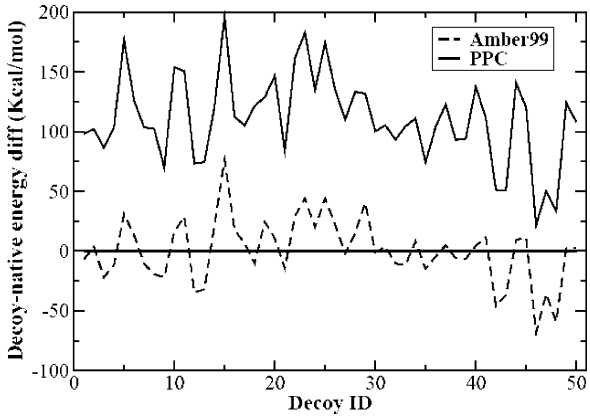

Figure 2. MM-PBSA energy gaps between decoys and the native structure, $E(\text{gap}) = E(\text{D}) - E(\text{N})$, for the protein 1c4zD. The dashed line denotes the results obtained using standard AMBER force field, and the solid line represents the results obtained by replacing the AMBER charge by PPC.

To investigate whether PPCs derived from decoy structures would still give the lowest energy for the native structure, we also calculated some PPCs based on some decoy structures of proteins and compared the energy gaps between the native state and the lowest-energy decoy structures. As shown in Table 2, the energies of these decoy structures were lower than that of the native structures. This confirms that, to find the correct native structure, the charges need to include proper polarization effects of the native structure.

To investigate in more detail the difference in energy between the PPCs and Amber99 charges for individual proteins, we plot in Figure 2 the decoy−native energy gap of protein 1c4zD for all 50 decoys obtained using both sets of charges. As Figure 2 shows, about one-half of the decoys have lower energies than the native state for the AMBER force field. However, when PPCs are used, all decoys have energies higher than that of the native state. In fact, the energy gaps between the native state and the decoys are much larger for the MM(PPC)-PBSA energies than for the MM(Amber)-PBSA energies. In other words, the potential energy landscape in MM(PPC)-PBSA is much steeper toward the native state, whereas that in MM(Amber)-PBSA is much shallower. This phenomenon holds for essentially all 47 proteins that were tested. As was specifically noted by WS, the potential energy landscape in the AMBER force field is quite flat near the native state, and this makes the force field deficient in correctly distinguishing the native state from misfolded structures.[10] The use of PPCs gives a somewhat different energy landscape, changing from flat to steep near the native structure. This correlates well with the expected folding

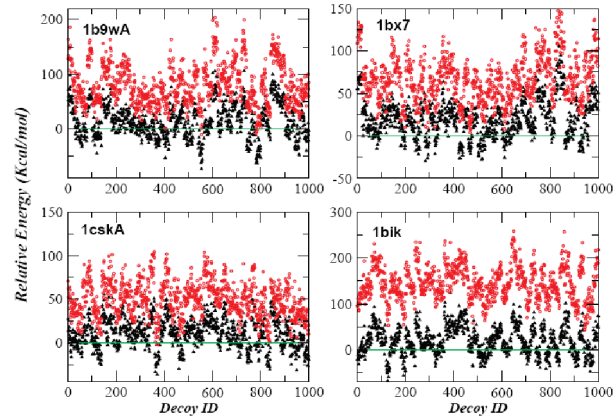

Figure 3. MM-PBSA energies (relative to the native state) of 1000 decoy configurations for proteins 1b9wA 1bx7, 1cskA, and 1bik. The red circles denote energies obtained using PPC, and the solid triangles are those from the standard AMBER force field.

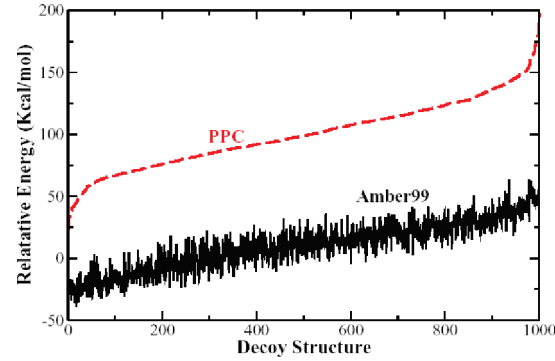

Figure 4. One-dimensional MM-PBSA energy profile of decoys (relative to the native state) for protein 1dtdB. The upper red line is the result obtained using PPC, and the lower curve is the result from the standard AMBER force field. The structures are ordered by energies under the PPC interaction.

process when the system is driven from decoys to the native state quickly along a funnel-like path.[21]

To gain a broad view of the energy profiles of decoys, we plot in Figure 3 the energies of all of the $20 \times 50 = 1000$ snapshots, for each of the four proteins 1b9wA, 1bx7, 1cskA, and 1bik. The results in Figure 3 show clearly that significant fractions of decoy configurations of these proteins have energies lower than that of the native structure under the AMBER potential. In contrast, when the energies are evaluated using PPCs, essentially all decoy configurations have energies higher than that of the native structure. This is purely an electrostatic effect, because the electrostatic interactions are different in the evaluation of configuration energies under AMBER and PPC.

We further plot in Figure 4 the energy profile of 1dtdB under both the AMBER and PPC potentials, with the 1000 decoy configurations arranged according to their energy values evaluated using PPCs. A steep drop of the energy curve occurs near the native state in decoy configuration space as shown by the dashed line in Figure 4. However, when the AMBER charge is used, there is a monotonic decrease of decoy energy passing below the native energy. These results further demonstrate that accurate electrostatic interactions play a critical role in determining the correct energy landscape of proteins and, therefore, in preventing the system from being driven away to some decoy or pseudonative structure because of an inaccurate force field during MD simulations. Thus, if the electrostatic interactions are not sufficiently accurate, distinguishing the native structure from decoys will be extremely difficult.

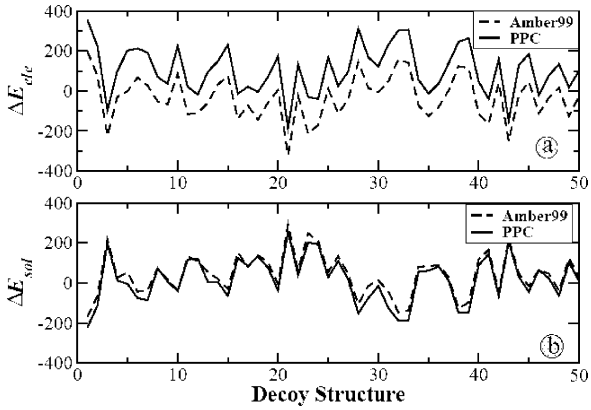

Figure 5. Electrostatic energy component (relative to the native state) of 50 decoys for protein 1c4zD: (a) intraprotein electrostatic energy and (b) electrostatic solvation energy. The dashed lines are results from the standard AMBER force field, and the solid lines are the results obtained using PPC.

This is true because, in the above two different MM-PBSA energy calculations of the proteins, the only difference lies in the charges used. Specifically, AMBER charges are amino-acid-based and mean-field-like, whereas PPCs are protein-specific and carry polarization effects of the protein near the native structure in solution. Thus, the source of energy discrepancy can be analyzed. In the MM-PBSA energy, the difference in energy in the above two sets of energy comes from two components, namely, the electrostatic solvation of the protein and intraprotein electrostatic interaction, because all of the other parameters in the AMBER force field are retained in the PPC energy. We show in Figure 5 the differences in intraprotein electrostatic interaction energy, ΔE_{ele}, and electrostatic solvation energy, ΔE_{sol}, between decoy and native structures for two sets of charges for protein 1c4zD. These are relative energies between the decoy and the native state when different sets of charges are used in the MM-PBSA energy calculation. As can be seen in Figure 5, the difference in solvation energy between AMBER charges and PPCs is relatively small, but the intraprotein electrostatic energy exhibits a large difference. Thus, the gaps in the intraprotein electrostatic energies between the native state and decoys are larger using PPCs than AMBER charges. This can be explained by the fact that PPCs represent polarized electronic states of the proteins and thus help stabilize the native structures.

B. Dynamical Stability of Protein's Native Structure. The above discussion focused on the static picture of proteins in which the energetics of proteins in the native and decoy structures are explicitly compared. An important question from the above analysis emerges. It concerns the dynamical stability of proteins near the native structure in MD simulations, specifically, whether the difference in energetics between PPCs and AMBER charges will directly result in a more stable protein native structure under PPCs in MD simulations. Because NMR relaxation experiments provide direct observations of the dynamic behavior of proteins, validation of the force fields can be accomplished by comparison of protein motions derived from MD simulations and NMR experiments.[23,24] The B3 immunoglobobulin-binding domain of streptococcal protein G (GB3), which has been extensively studied by both NMR experiments[24] and computer simulations,[25] was used in our study. Here, we compare N−H bond order parameters of GB3 derived, respectively, from NMR experiment and MD simulations.

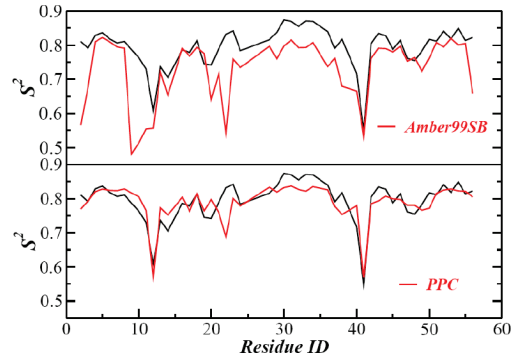

Figure 6. Comparison of NMR spin relaxation order parameters calculated from simulations (red lines) under PPC and AMBER with experimental measurements (black lines).[24]

Further evidence for the stability of the protein's native structure can be seen in our MD simulations of NMR spin relaxation order parameters. For this application, the crystal structure of GB3 (PDB ID: 1IGD) was used in our simulations. Pretreatment of the structure was done according to NMR experiments as described in ref 25. The structure was solvated in a octahedron-like box with 4846 TIP3P water molecules, and the system was neutralized by adding counterions. After heating and equilibration, another 2-ns equilibration run was done at 297 K (*NPT* ensemble). A total of 15 structures were extracted from this 2-ns run, and they were used as starting structures for the subsequent 2.4-ns production runs (*NVE* ensemble). The simulations were done using both PPCs and AMBER charge. A time step of 2 fs was used in the MD simulations with Amber99SB and PPC.

The generalized order parameters were calculated from the MD simulations as ensemble averages according to the expression[2]

$$S^2 = \frac{\varsigma}{2}\langle 3(\mu_i \cdot \mu_j)^2 - 1\rangle$$

where μ_i and μ_j are the N−H bond vectors scaled to unit magnitude at time moments i and j, which are ensemble-averaged. The order parameters were scaled by $\varsigma = 0.89$, and SHAKE was used to constrain chemical bonds involving hydrogen.[26] A comparison of the simulated order parameters and the experimental results is shown in Figure 6. Each of these computed results was averaged from 15 production runs. From Figure 6, one can see that the PPCs do a much better job of reproducing the dynamic behavior of the protein as measured by NMR relaxation experiments. The average deviations of δS^2 are 0.0994 and 0.0357 for AMBER and PPC, respectively. The PPC approach gives order parameters that are in much better agreement with NMR experimental results.

A similar phenomenon was observed by Palmer et al.,[25] that is, many order parameters derived from simulations with the Amber99SB force field were found to give lower values than were obtained in NMR experiments. The lower values of the order parameters indicate overflexibility of the protein and demonstrate that the standard force field might "allow too much motion".[2] The PPC approach properly describes the static polarization effect in proteins through the use of locally polarized protein-specific charges. These results present strong evidence that it is important to include polarization effects in modeling protein structure and dynamics. The PPC technique

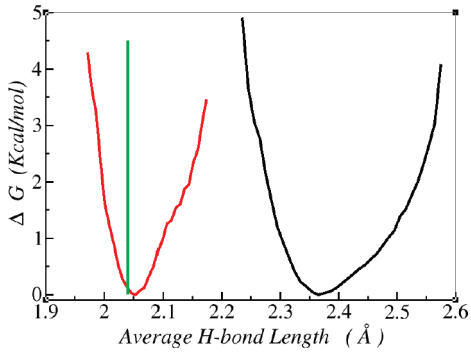

Figure 7. Comparison of the hydrogen-bond free energy landscapes generated under the AMBER potential (black) and the PPC potential (red). Free energies were calculated as a function of average H-bond length using the formula $\Delta G = -kT \ln[H(\text{Length}_{H\text{-bond}})]$, where $H(\text{Length}_{\text{H-bond}})$ is the histogram of average length of main-chain hydrogen bonds sampled during 20-ns MD simulations. The average length of hydrogen bond detected in the X-ray crystal structure is indicated by a green line.

provides a good correction to the current AMBER force field for MD simulations of proteins.

C. Free Energy Landscapes of Proteins. Our analysis indicates that, under AMBER charges, proteins whose decoy configurations have energies that are much higher than that of the native structure might be driven away from the native conformation to some of those decoy conformation as a result of energetic effects. This might cause pseudostability of the system; that is, the system might stabilize at a pseudonative or decoy structure. Another possible outcome is that the system might not converge in MD simulations as a result of the partial denaturing of local structures. Our recent study on PPARγ (peroxisome proliferator-activated receptor, an important protein in adipocyte differentiation and glucose homeostasis) confirmed this phenomenon.[20] In that study, PPARγ was partially denatured during MD simulations under an AMBER potential. However, the same phenomenon did not occur when PPCs were used in the MD simulations.[20]

One of the grand challenges in current computational biology is to predict free energy changes in important biochemical processes (such as enzyme catalysis and protein−ligand binding) at chemical resolution. Thanks to Boltzmann's landmark formula $S = k \ln P$, macroscopic thermodynamic properties and microscopic information sampled from detailed atomistic MD simulations are connected. Free energy landscapes can be constructed directly from the distributions of conformational ensembles sampled from MD simulations. Some advanced sampling techniques (such as umbrella sampling and thermodynamic integration) have been developed for constructing such free energy landscapes.

Here, we construct free energy landscapes of PPARγ in solution as a function of average H-bond length from structures sampled from our previous MD simulations.[20] Figure 7 shows that the conformational ensemble sampled from an MD simulation under a PPC potential is basically focused around crystal structure. In contrast, the equilibrium hydrogen bond length under an AMBER potential is about 0.32 Å longer than that in the crystal structure. Because of the lack of polarization, the final structure of the protein was shifted during the MD simulation under the AMBER potential. Free energy changes calculated from that shifted conformational ensemble would not be accurate. The inaccuracy of the potential energy surface under the AMBER representation would finally be reflected in the free energy landscape. An accurate potential energy surface is the basis for sampling correct conformational ensembles during MD free energy simulations. Our previous work on MD free energy simulations showed that the PPC approach performs very well in predicting free energy changes during the protonation process in proteins whereas AmberFF and CharmmFF strongly overestimate it.[11]

The dynamics result is highly consistent with the energetic analysis under two sets of charges discussed above. Our answer is that, because of the stabilizing effect of electronic polarization, especially those from hydrogen bonds, the native structure is energetically as well as dynamically stabilized. Because PPCs are derived from quantum electronic structure calculations of proteins in solution, they are fundamentally different from AMBER or other standard force fields in which atomic charges are derived for individual amino acids in an inhomogeneous electrostatic environment. It should be mentioned that a number of similar quantum electronic structure methods for protein calculation have also been developed by several groups.[27−29] In particular, the work of Gao and co-workers also aimed to develop atomic charges based on electronic structure calculations of proteins.[27] These new-generation quantum methods for protein structure calculations should provide new insights as well as more accurate descriptions of protein structure and dynamics in computational biology.

Conclusions

Because of the limitation of our theoretical approach, the current study provided strong but still incomplete evidence for the critical role of polarization in stabilizing the native structures of proteins. To provide a complete picture, future theoretical studies will be needed to address the effects of free energy vs enthalpy, explicit water solvation vs implicit solvation, and a completely polarizable force field vs PPCs near the native structures of proteins. Resolving the complete puzzle is not practical in the foreseeable future. We hope, however, that more theoretical investigations along these lines will soon be carried out to address these issues.

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