Comparative Study of the Folding Free Energy Landscape of a Three-Stranded β-Sheet Protein with Explicit and Implicit Solvent Models

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We present a molecular dynamics (MD) simulation study of the folding thermodynamics for the three-stranded β-sheet protein Betanova. The protein was explicitly described by employing an all-atom model. The solvation was accounted for by two different solvent models: explicit TIP3P water and implicit Generalized Born (GB) with an exterior dielectric of 80. An umbrella sampling technique was utilized to probe thermodynamically relevant states at different stages of folding. The generated data were combined with the weighted histogram method to produce the two-dimensional folding free energy landscape. Sampling of conformational space was carried out in explicit solvent at 275 K and in implicit solvent at 275, 350, and 400 K. The folding free energy surface of Betanova at 275 K was found to be consistent with that in explicit solvent. In particular, the two models agree with regard to the location of the global minimum, the absence of a significant barrier for folding on the folding free energy surface, and the minor role of hydrogen bonding in the folding of Betanova. On the other hand the GB solvent model overestimated the stability of the protein and the folding transition temperature. It also yielded a slightly different shape for the folding free energy surface, compared to the calculations with explicit solvent. Exploration of the temperature dependence of the folding landscape in the GB solvent model yielded a similar overall shape with a shift in the global minimum toward smaller values of the folding reaction coordinate. The inclusion of an explicit surface-area-based treatment of hydrophobic interactions did not qualitatively change the results obtained with the GB model. We conclude that the GB solvent model is sufficient for studying the folding thermodynamics of small polypeptides.

1. Introduction

Solvation plays a critical role in the stability and interactions of biomolecules. Its correct description is necessary for any computational approach to chemical and biophysical problems to be accurate.[1,2] The most commonly used solvent in biophysical studies is water. It has been a subject of considerable interest during the last three decades.[3,4] Among various ways to include water into simulation studies, two major approaches can be distinguished. In the first approach, the solvent nuclear degrees of freedom are treated explicitly and propagated according to the classical equations of motion. The forces acting on the solvent molecules are derived from the intermolecular potentials, which in general include long-range electrostatic and short-range repulsion and dispersion pairwise additive components.[5−8] In more advanced models the nonadditive interactions (polarizabilities) are also included at classical[9−18] or quantum[19−23] levels.

In the second approach, solvent nuclear degrees of freedom are eliminated and the solvation is treated implicitly. The main idea here is to reparametrize the biomolecule's interatomic interactions in such a way that its energetics, dynamics, and structural properties remain the same as in explicit solvent. The major reason for using less rigorous continuum models is to reduce the computational time required to sample the enormous conformational space of large biomolecules. The implicit treatment of the solvent is done in part within the framework of continuum electrostatics theory with such methods as the distance dependent dielectric constant, reaction field methods, numerical solution of Poisson's equation[24,25] and additional terms to account for nonelectrostatic components, e.g., hydrophobic solvation.[26−29] These models can be used to explore the effect of solvent on intermolecular and intramolecular energetics of biopolymers as recently demonstrated by the successful application of such a model to study the folding of a small 16-residue β-hairpin[30] and other small peptides.[31]

One model that provides a continuum solvent description for the electrostatic components of the energy is the generalized Born model introduced by Still et al.[32] This model is based on the Born model for ionic solvation[33] and is extended to treat solutes containing a set of charged sites and having arbitrary molecular shape. The generalized Born model has been extremely successful in reproducing energetics of small molecules and molecular ions,[34−39] although other physical properties such as pK_a shift predictions based on the GB model were not as impressive.[38] Generalized Born models have also been employed in a number of biological applications[36,37,39−41] and found to be the most accurate among various implicit solvation models. They provided reasonably good estimates for the solvation free energy of biomolecules and were capable of reproducing relative free energies of different peptide conformations.

A preeminent problem in biophysics is to understand the folding mechanism of proteins and to predict their three-dimensional conformation under physiological conditions.[42−49] One of the ways to address this problem is all-atom molecular dynamics simulation coupled with the biased sampling technique. Although only relatively small proteins can be studied with this method, it is very powerful in that it allows one to

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investigate the folding free energy landscape along several coordinates (radius of gyration, amount of secondary structure, etc.) and thus provides deeper understanding of folding of small proteins. For example, this method was employed to study the folding free energy landscape for the three-helix bundle fragment B of *Staphylococcal* protein A,[50,51] segment B1 of *Streptococcal* protein G (GB1),[52,53] major cold shock protein A (CspA),[54] and the small designed β-sheet protein which is the focus of this work.[55] The folding coordinates used in these studies, performed using an explicit representation of solvent, included the radius of gyration and the fraction of native contacts. One key result from this work is that the topology of the protein native state influences the folding mechanism. For example, in protein A, which is an all-α protein, the formation of tertiary (native) structure occurs concurrently with the formation of the secondary (collapsed) structure. In contrast, for the mixed α/β protein GB1, the collapse, accompanied by the formation of $\approx$35% of the native structure, occurs first and is followed by evolution toward the native state without significant change in size. This finding appears to be generally true and moreover has experimental corroboration from the observation by Plaxco et al.[56] that the rate of folding varies inverserly with the extent of long-range contacts made in the protein native state.

Recently, we have applied this technique to study the folding free energy landscape of a designed β-protein Betanova.[55] This is a very small polypeptide consisting of 20 residues.[57] It has three β-strands in its native fold and is only marginally stable (0.6$-$0.7 kcal/mol at 278 K). We chose this system due to its small size, the fact that it is a designed sequence, and the nature of its native topology. Our original simulation study was done using an explicit solvent model. The aim of the present work is to explore the GB solvation model, which was developed in ref 39, and compare the results for Betanova, e.g., the shape of the folding landscape, protein stability, protein folding driving force, etc., with those obtained in the explicit solvent. This objective is of particular importance because the GB model of ref 39 was parametrized to yield correct electrostatic solvation free energies but did not include explicit treatment of the hydrophobic interactions, which are believed to be a major factor in the stability of β-proteins.[30,58] We will examine the influence of the empirical energy term for hydrophobic solvation on the folding as well. We will also investigate the temperature dependence of folding thermodynamics of Betanova by employing the GB solvent model.

In what follows we describe the models and methods employed in this study and compare the results on the thermodynamics of folding of the β-protein Betanova, obtained with explicit and the generalized Born solvent models.

2. Methods

The details of the sampling of the folding free energy landscape of Betanova are given in ref 55. Here we just briefly summarize our protocol.

(i) First we characterized the native state of the protein by conducting two native MD simulations at 275 K in explicit TIP3P solvent.[6] These simulations served to define the native side chain contacts and hydrogen bonds. Two residues not adjacent in the sequence were considered to be in contact or hydrogen bonded if the centers of geometry of their side chains were within 6.5 Å or if the distance between their backbone hydrogen and oxygen atoms was within 2.5 Å. In our definition, the native contacts were those present in the native trajectory with relatively high probability (56% or higher for the side chain contacts and 66% or higher for the hydrogen bonds).

(ii) After characterizing the native basin we prepared the initial conditions for biased sampling. This was done by running four high temperature (350, 375, 385, and 400 K) unfolding trajectories in explicit solvent. The generated database of structures was partitioned into 24 bins based on the number of native contacts and radius of gyration. For each bin a hierarchical clustering was performed in order to choose the representative structures that would serve as the initial conditions for the sampling.[50] Overall 74 initial conditions for the sampling were chosen.

(iii) The sampling was performed for each of the initial conditions by employing MD simulations with a biased (umbrella) potential. Each of these simulations was carried out for a duration of 300 ps. A biasing potential with the force constant of 500 kcal/mol and quadratic with respect to the fraction of native contacts was employed.[53]

(iv) Finally, the sampling data were combined by the weighted histogram analysis method (WHAM).[59$-$61] This method provides a robust estimate of the density of states projected on specific reaction coordinates (e.g., radius of gyration, number of native contacts), as has been demonstrated in a number of biophysical applications.[50$-$53,61,62]

In the present study we begin with step (iii) (sampling) using the same initial conditions as in ref 55 and utilizing the GB solvation model of ref 39. We note that we employed a single dielectric constant value of 80 to represent the solvent at all temperatures, thus we are not including the temperature dependence of the electrostatic components of solvent effects. One could, in principle, utilize a temperature dependent dielectric constant, and thereby, include temperature effects beyond those from peptide chain enetropy. However, for the purposes of the present study we choose to examine only this component. It is anticipated that lowering the dielectric constant of the solvent with increasing temperature, as seen experimentally for water, would slightly stabilize the peptide in compact states with increasing temperature. Additionally, to explore the influence of hydrophobic interactions on the free energy surface of Betanova, we augmented the GB interaction potential by a surface-area-based hydrophobic term: γASA. ASA in this expression represents the total solvent accessible surface area of the protein and γ is a constant of 0.005 kcal/mol/Å^2.[26] Sampling for the ASA-augmented energy function was carried out at 350 K.

The restraint potential was calculated by using the native side chain contacts reported in ref 55 (see Table 1a). The molecular dynamics simulations were performed with the CHARMM package using the parameter set TOPH19/PARAM19.[63] The trajectories were integrated with a 2 fs time step using the Verlet leap-frog algorithm.[64] The covalent bonds between hydrogen and heavy atoms were fixed with the SHAKE algorithm.[65] No truncation of the nonbonded interactions was performed. A constant temperature was maintained by reassigning atom velocities from a Gaussian distribution if the average temperature drifted outside of a window $\pm$5 K. The simulations were conducted at 275 K with explicit solvent and at 275, 350, and 400 K with implicit solvent. One set of simulations, in which the GB model was augmented with a hydrophobic term, was carried out at 350 K. All production runs were preceded by an equilibration period of 60-ps duration. Calculations with the GB solvent model were done in parallel on SGI Origin 2000 workstations using the load-sharing facility (LSF) queueing system to control worksharing in this loosely coupled parallel calculation.

TABLE 1: List of Native Contacts for Protein Betanova

no.	first residue	second residue	no.	first residue	second residue
		In Explicit Solvent			
1	Gly2	Ser4	10	Gly8	Tyr10
2	Trp3	Tyr10	11	Lys9	Thr16
3	Trp3	Asn12	12	Lys9	Glu18
4	Trp3	Thr17	13	Tyr10	Thr17
5	Ser4	Gln6	14	Tyr10	Arg20
6	Ser4	Thr11	15	Thr11	Thr16
7	Val5	Gly8	16	Asn12	Gly14
8	Val5	Tyr10	17	Asn12	Thr16
9	Gln6	Thr11	18	Asn12	Thr17
		In Implicit Solvent			
1	Arg1	Asn12	11	Gly8	Tyr10
2	Arg1	Asn13	12	Gly8	Gly19
3	Gly2	Ser4	13	Lys9	Thr11
4	Gly2	Asn12	14	Lys9	Thr16
5	Trp3	Asn12	15	Lys9	Glu18
6	Trp3	Thr17	16	Tyr10	Thr17
7	Ser4	Thr11	17	Tyr10	Gly19
8	Val5	Tyr10	18	Thr11	Thr16
9	Gln6	Lys9	19	Asn12	Gly14
10	Gln6	Thr11	20	Gly14	Thr16

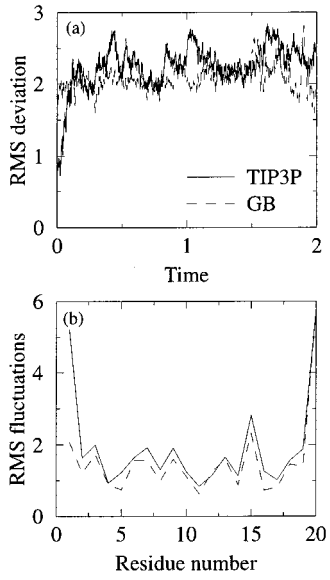

Figure 1. (a) Time evolution (in nanoseconds) of the backbone root-mean-square (RMS) deviation (in angstroms) relative to the reference NMR configuration used to initiate the native simulation of Betanova at 275 K: (solid) with explicit solvent; (dashed) with GB solvent. (b) Residue-averaged root-mean-square (RMS) fluctuations for protein Betanova from 2 ns simulations using two different solvent models: (—) with explicit solvent; (- - -) with GB solvent.

3. Results and Discussion

We start by comparing the properties of the protein Betanova derived from the native trajectories in which explicit TIP3P and implicit GB solvents were employed. In Figure 1a the backbone root-mean-square deviation (rmsd) between the initial and instantaneous protein conformations as a function of evolution time along the trajectory is shown. We see that in both simulations the backbone rmsd reaches a value of 2−2.5 Å very quickly and stays at that level throughout the simulations. This suggests that the native state of Betanova protein is stable though somewhat mobile in our force field. This also means that the simulation utilizing GB solvent is successful in maintaining the protein's native structure. The backbone rmsd between the average structure from the explicit solvent simulation and that from the NMR structure determination is 1.9 Å compared to 2.2 Å for the protein solvated using the GB model. Moreover, as apparent from the RMS fluctuations averaged over individual residues (see Figure 1b), the protein's native state shows similar fluctuations in both solvent models.

We also calculated the native side chain contact maps and compile them in Table 1b. We employed the same probability cutoff, namely, 56%, as in our previous work.[55] Comparing the native contact maps for protein Betanova obtained with explicit and implicit solvation models, we observe a significant amount of overlap between them. This suggests that our choice to use the same initial conditions as in ref 55 to sample the thermodynamics of folding of Betanova in the GB solvent is justified. Furthermore, we note that some of the native contacts listed in Table 1a, such as Ser4-Gln6, Val5-Gly8, and Tyr10-Arg20, are present in the protein with a nonvanishing probability of ≈45% during the native simulation in the GB solvent. Among the native side chain contacts formed by the protein in the explicit solvent, we emphasize the following six: Trp3-Tyr10, Trp3-Asn12, Trp3-Thr17, Val5-Tyr10, Tyr10-Thr17, and Asn12-Thr17. These contacts constitute the hydrophobic "core", as has been observed in the experimental studies on this system.[57] It should be mentioned that in GB solvent two of these contacts are missing: namely, Trp3-Tyr10 and Asn12-Thr17. This does not mean, however, that the native state is less stable in GB solvent, since the aforementioned missing contacts are replaced by neighboring interactions.

Next we consider the folding free energy landscape of Betanova. The potentials of mean force (PMF) as a function of the fraction of native contacts, ρ, and radius of gyration, R_g, obtained with explicit and implicit solvent models at various temperatures, are given in Figure 2. First we compare the PMF surfaces in explicit and implicit solvents at 275 K. The PMF obtained in explicit solvent (Figure 2a) contains three basins corresponding to unfolded, collapsed, and native states. The basin of unfolded states is located in the upper left corner of the PMF plot. It is relatively narrow along the ρ coordinate and extends from 16 to 11 Å along the R_g coordinate. It contains a local minimum, centered at $\rho \approx 0.15$ and $R_g \approx 12$ Å. The basin of collapsed states is wide in terms of both fraction of native contacts and radius of gyration. The former varies between 0.1 and 0.6, and the latter varies between 7 and 11 Å. It is populated by protein conformations having various elements of secondary structure, most noticeably partially or fully formed β-hairpins with nativelike turns. The basin of native states of the protein is located in the lower right corner and corresponds to the global minimum on the folding free energy surface. The basin of native structures is relatively narrow along the R_g coordinate and begins at a value of $\rho = 0.6$. Thus the folding of Betanova in explicit solvent involves two stages of collapse: the first collapse occurs when it enters the basin of collapsed states and the second one when it enters into the native basin.

In contrast to the PMF in Figure 2a, which has a distinctive L-shape, the PMF surface obtained with the GB solvent (Figure 2b) is close to a "diagonal" shape, i.e., suggesting concomitant collapse and native structure formation. Similar to the PMF in Figure 2a, it contains a local minimum, corresponding to a partially folded state ($\rho \approx 0.3$ and $R_g \approx 12$ Å), and a global minimum in the lower right corner of the plot (at $\rho \approx 0.8$), corresponding to the native state. However, unlike the explicit solvent, the GB solvation model does not distinguish unfolded and collapsed states. In Figure 2b they both are merged into a single basin. We also note that in the GB solvent the unfolded and partially folded states are destabilized to a greater extent than in the explicit solvent. To better illustrate this we plot in Figure 3a the potential of mean force along the ρ coordinate

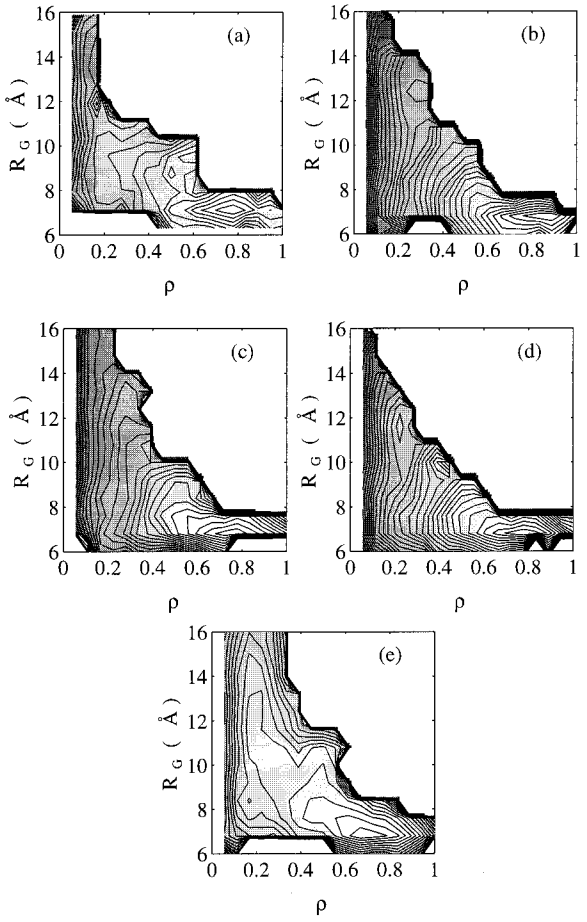

Figure 2. Potential of mean force as a function of radius of gyration, R_g, and fraction of native contacts, ρ. The contours are drawn every 0.5 kcal/mol: (a) with explicit solvent at 275 K; (b) with GB solvent at 275 K; (c) with GB solvent at 350 K; (d) with GB solvent and hydrophobic interaction term at 350 K; (e) with GB solvent at 400 K.

for both of the solvation models. Obviously, the unfolded conformations have a much higher free energy in the GB solvent than in the explicit solvent. We will return to the issue of the native state stability a bit later.

For now we turn our discussion to the effect of temperature on the free energy surface of Betanova. We explore this by conducting two high-temperature simulations employing the GB solvent model. Compared to the PMF at 275 K (Figure 2b), the higher temperature PMF surfaces, shown in Figure 2c,e, become wider and the global minimum is shifted toward smaller values of ρ-coordinate. However, the overall shape of the free energy surface does not change much and remains close to the diagonal form. Also, as expected, the free energy difference between folded and unfolded states becomes smaller as temperature increases. Surprisingly, there is no free energy barrier in a one-dimensional plot of PMF versus ρ (Figure 3a), which would be expected if the folding were to occur via a 2-state process as suggested in the experimental paper.[57] This result indicates that the folding of Betanova is likely to be a diffusion controlled process. However, it would be premature to draw this conclusion, since it could be influenced by deficiencies of the GB solvation model. We will address this problem next.

We already mentioned that the stability of the native state is overestimated in the GB solvent model. Here we discuss this in more quantitative manner. To do so we calculate the cumulative probability, $P(\rho^*)$, for the protein to occupy states with $1 \leq \rho \leq \rho^*$:

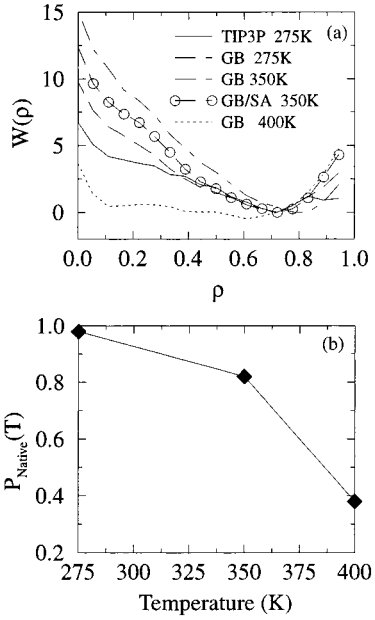

Figure 3. (a) Potential of mean force, W(ρ) (kcal/mol), as a function of fraction of native contacts, ρ: (—) with explicit solvent; (—·—) with GB solvent at 275 K; (- - -) with GB solvent at 350 K; (···) with GB solvent at 400 K; (- - ○ - -) with GB solvent and hydrophobic term at 350 K. (b) Fraction of conformations populating the native basin as a function of temperature from the GB model.

$$P(\rho^*) = \frac{\int_1^{\rho^*} d\rho \exp(-\beta W(\rho))}{\int_1^0 d\rho \exp(-\beta W(\rho))} \quad (1)$$

where $\beta = 1/k_B T$ is the Boltzmann factor and $W(\rho)$ is the potential of mean force. The accumulated population of protein Betanova in the native basin (native population), $\rho > 0.6$, is $\approx$90% in explicit solvent at 275 K. In the implicit GB solvent the native population is 98%, 82%, and 38% at 275, 350, and 400 K, respectively. The corresponding stability of the Betanova native state at 275 K in explicit and implicit solvents is respectively $\approx$1 and 2 kcal/mol. The experimental estimate is 0.6−0.7 kcal/mol at 278 K.[57] Thus the stability of Betanova in the explicit solvent is in good agreement with the experimental estimate while the GB model overestimates this protein's stability. As noted above, this appears to be due to the destabilization of partially folded states in the GB model. From the temperature dependence of the native population, depicted in Figure 3b, we estimate the folding transition temperature, at which the native population is $\approx$59%, to be $\approx$390 K. This is clearly not physical. This high folding temperature is due to the overemphasized stability of the native state in the GB model. In addition, the plot in Figure 3b suggests a certain degree of cooperativity in folding of Betanova.

Another deficiency of the GB model is lack of explicit account for hydrophobic interactions, which are important in folding of β-proteins. To explore the effect of hydrophobic interactions on the PMF surface of Betanova, we augmented the GB interaction potential by the following hydrophobic term: γASA. Here ASA is a total solvent accessible surface area of the protein and γ is a constant of 0.005 kcal/mol/Å^2.[26] The sampling was carried out at 350 K. The computed PMF is given in Figure 2d and one-dimensional projection is given in Figure 3a. First we note that, compared to the simulation without the hydrophobic term, the free energy of unfolded states increases relative to that of the native conformation. This is obviously due to larger exposure of the side chains of nonnative

conformations. Second, the inclusion of the hydrophobic term does not change the PMF surface qualitatively, e.g., the location of the global minimum remains the same and the PMF surface lacks the free energy barrier between nonnative and native conformations. From these results we draw the conclusion that the GB solvation potential itself is sufficient to describe the PMF of Betanova. It would be interesting to test this on bigger proteins, where due to a larger hydrophobic core, an accurate account for hydrophobic interactions becomes of greater importance.

On a final note, in our previous work we found that the interstrand hydrogen bonds do not drive the folding of Betanova but may play a role in stabilizing the native protein topology.[55] We arrived at this conclusion by analyzing the folding free energy as a function of total number of native hydrogen bonds, N_{hb}, and fraction of native contacts, ρ. We observed that the free energy was independent of N_{hb} up to a value of ρ equal 0.6, where a transition between nonnative and native states occurs, and that N_{hb} varies between 3 and 7 in the native basin. We performed a similar analysis for the GB solvent and found that the shape and features of the free energy surface obtained in this model solvent are virtually identical to those in the explicit solvent, suggesting that both solvation models agree; hydrogen bonding plays a minor role in the folding of Betanova.

4. Conclusions

We have examined the PMF surface for the folding of a designed three-stranded β-sheet protein Betanova by all-atom MD simulations, employing two solvation models: explicit TIP3P water and implicit GB with a dielectric constant near that of water. In the explicit solvent the folding of Betanova involves a two-staged collapse and the PMF surface has an L-shape. By contrast, in the GB solvent the shape of the PMF surface is closer to diagonal and the formation of the native fold occurs more concurrently with the protein's compaction (native tertiary and secondary structure formation). The location of the global minimum on the PMF surface, which corresponds to the native state, is roughly the same in both solvation models. The general shape of this free energy landscape does not change with increasing temperature. We found that the stability of Betanova was overestimated in the generalized Born solvent model, while the calculation with explicit solvent was in good agreement with the experimental estimate. According to both solvation models, the folding free energy surface for the protein Betanova does not contain a significant barrier between the basins of native structures and nonnative states, and the interstrand hydrogen bonds do not assist the folding of Betanova, rather to a certain degree they stabilize the native fold. We have also shown that inclusion of a surface-area-based hydrophobic term into the GB potential results in destabilization of nonnative conformations of Betanova. However, this does not bring about qualitative changes in the PMF; e.g., its overall shape and location of the global minimum remain the same.

Our results indicate that despite its drawbacks, such as overemphasized stability of the native state and too high folding transition temperature, the GB solvent model may be usefully applied to study thermodynamics and possibly kinetics of folding of small polypeptides. Its application to larger proteins could be limited by the lack of explicit account for hydrophobic interaction and deserves a separate study.

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