Improvement on a simplified model for protein folding simulation

Ming Zhang, Changjun Chen, Yi He, and Yi Xiao*

Biomolecular Physics and Modeling Group, Department of Physics, Huazhong University of Science and Technology, Wuhan 430074, Hubei, China

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Improvements were made on a simplified protein model—the Ramachandran model—to achieve better computer simulation of protein folding. To check the validity of such improvements, we chose the ultrafast folding protein Engrailed Homeodomain as an example and explored several aspects of its folding. The engrailed homeodomain is a mainly α-helical protein of 61 residues from *Drosophila melanogaster*. We found that the simplified model of Engrailed Homeodomain can fold into a global minimum state with a tertiary structure in good agreement with its native structure.

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I. INTRODUCTION

One of the most challenging problems in biophysics is how proteins fold into biologically active three-dimensional structures from linear amino acid sequences. Significant progress in understanding this process has been achieved, and many simulation methods for investigating it have been developed, from lattice to all-atom ones. By using powerful computers, simulations of protein folding at the all-atom level are now practicable and can give answers to some aspects of the protein folding problem. However, they are still extremely computationally demanding and time consuming. This problem is exacerbated when we need to extract reproducible conclusions from many runs of simulations and from many folding trajectories, possibly under various conditions. Therefore, we still need simpler and computationally faster models to investigate the folding process of proteins, especially larger proteins.

In recent years there has been increasing interest in developing different kinds of simplified protein models. These models are well designed to incorporate the main features of real proteins so as to provide us essential information about the folding process, while remaining simple enough to be computationally feasible.

The early researches on simplified protein models were done along two approaches. One was borrowing the lattice model from spin-glass systems [1]. Such a lattice model represents each amino acid by a single interaction site (bead) corresponding to the C_α atom and can provide valuable insights into the general physical principles of protein folding. Due to its low computational cost compared to other models, the lattice model has been widely used. And some modified versions of the lattice model, such as the face-centered-cubic lattice model [2], triangle lattice model [3], or lattice model with side chains (SCM) [4], were also proposed for different purposes. However, these lattice models have obvious limitations because of their inaccuracy in reproducing protein conformations.

Another approach was proposed by Levitt who emphasized reduction of both the number of effective atoms and the number of degrees of freedom based on real protein conformations [5]. Only those degrees of freedom that have the greatest effects on the conformations are taken into account. Under the same consideration, Irbäck and co-workers proposed a Ramachandran-Sasisekharan model based on the well-known fact that the main degrees of freedom of the protein backbone are the Ramachandran-Sasisekharan torsional angles ϕ_i and ψ_i [6]. This model carries more structural information and is easy to describe essential forces in the folding process. It has been successfully used to fold a de novo protein sequence to its native helix-bundle structure and explore the physical properties during the folding [7].

In their model, each amino acid residue is represented by six (five for Gly) atoms. The simplification here contains two aspects: each side chain is replaced by a single atom C_β and the 20 types of amino acid residues are reduced to a three-letter representation. Their energy function is composed of four terms. The first term is the dihedral potential for angles ϕ_i and ψ_i that uses the traditional three-minimum-energy function with minima at +60°, −60°, and 180°. The second term is for self-avoidance. The last two parts of the energy function are hydrogen bonds and hydrophobic interactions which are the main forces driving proteins to their native structures. Due to the existence of atoms H and O, the energy expression for hydrogen bonds is easy to write. For hydrophobic forces, the model adopted a three-letter representation: "A" denotes residues with hydrophobic C_β, "B" with polar C_β, and "G" (glycine) without C_β. The hydrophobic energy term is counted when two C_β atoms representing hydrophobic side chains are close to each other.

In their original work, this model did well when simulating the folding of a three-helix-bundle model protein. Naturally we hope that we can use this model to predict the structure of a protein directly from its sequence. However, several factors should be noted.

At first, their target protein was de novo designed for facilitating the forming of predetermined structure. The sequence agrees well with the ideal helix sequence pattern and the segment GGG is inserted between helices to form the turn ranges. This prevents us from applying this model to real protein sequences directly.

The other problem is about the setting of the hydrophobic interaction which only exists between two hydrophobic "side

*Corresponding author.

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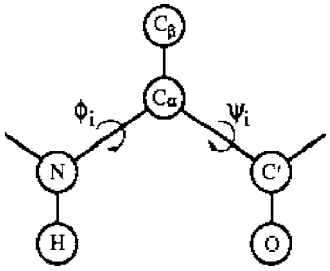

FIG. 1. Schematic representation of one amino acid.

chain atoms." This virtual hydrophobic interaction is meant to simulate the effect of water, which is important in maintaining the tertiary structure. Such a setting can result in the aggregation of hydrophobic side chains and the formation of a hydrophobic core, but does not guarantee that this hydrophobic core is buried in the interior of protein. We can refer to the method used in some lattice models to achieve better simulation of hydrophobic forces.

In this paper we shall make some improvements in this model in order to extend its validity from de novo—designed sequences to real protein sequences.

II. METHOD

A. The model

A detailed description of the Ramachandran-Sasisekharan model can be found in the paper of Irbäck *et al.* [7]. We shall briefly review it in the following. In this model each amino acid is represented by five or six atoms as illustrated in Fig. 1.

As we can see, the two torsional angles are the only parameters of an amino acid. Hence a protein with length of N amino acids has $2N$ degrees of freedom. The side chains of amino acids (C_β) can be either hydrophobic (A), hydrophilic (B), or even do not exist (G).

The energy function consists of four terms [7]

$$E = E_{loc} + E_{sa} + E_{\mathrm{hb}} + E_{\mathrm{AA}}. \tag{1}$$

The first two terms are commonly used local potential (or so-called dihedral potential) and self-avoidance potential:

$$E_{loc} = \frac{1}{2}\sum_i (1 + \cos 3\phi_i) + \frac{1}{2}\sum_i (1 + \cos 3\psi_i), \tag{2}$$

$$E_{sa} = \varepsilon_{sa}\sum_{i<j}\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12}, \tag{3}$$

where φ_i and ψ_i are the Ramachandran-Sasisekharan torsional angles of the ith amino acid. In Eq. (3), ij represents all atom pairs except those being counted into hydrophobic forces. The parameters σ_{ij} in Eq. (3) are given by

$$\sigma_{ij} = \sigma_i + \sigma_j + \Delta\sigma_{ij}.$$

The values of σ_i and σ_j can be found in Table I. $\Delta\sigma_{ij}$ is 0.625 Å for $C_\beta C'$, $C_\beta N$, and $C_\beta O$ pairs and zero for others pairs.

What we want to focus on is the hydrogen-bond term E_{hb} and the hydrophobic term E_{AA}. The potential of hydrogen bonds has orientational preference:

$$E_{\mathrm{hb}} = \varepsilon_{\mathrm{hb}}\sum_{ij} u(r_{ij})v(\alpha_{ij},\beta_{ij}), \tag{4}$$

$$u(r_{ij}) = 5\left(\frac{\sigma_{\mathrm{hb}}}{r_{ij}}\right)^{12} - 6\left(\frac{\sigma_{\mathrm{hb}}}{r_{ij}}\right)^{10}, \tag{5}$$

$$v(\alpha_{ij},\beta_{ij}) = \begin{cases} \cos^2\alpha_{ij}\cos^2\beta_{ij}, & \alpha_{ij},\beta_{ij} > 90, \\ 0, & \text{otherwise}. \end{cases} \tag{6}$$

In Eq. (4), i and j represent H and O atoms, respectively, and r_{ij} denotes the HO distance, α_{ij} the NHO angle, and β_{ij} the HOC′ angle. Any HO pair can form a hydrogen bond.

The hydrophobic component of the energy is given as a Lennard-Jones potential

$$E_{\mathrm{AA}} = \varepsilon_{\mathrm{AA}}\sum_{i<j}\left[\left(\frac{\sigma_{\mathrm{AA}}}{r_{ij}}\right)^{12} - 2\left(\frac{\sigma_{\mathrm{AA}}}{r_{ij}}\right)^{6}\right], \tag{7}$$

where both i and j represent hydrophobic C_β. The other parameters in Eqs. (3)–(7) are listed in Table I [7].

B. Replica exchange Monte Carlo method

In the replica exchange Monte Carlo (REMC) method [8], a number of copies of the model system placed at different temperatures are simulated by a classical Metropolis algorithm. The set of temperatures covers the range from a random coil state to a compact folded globular state. Replicas i and j with neighboring temperatures are swapped with the probability $P_{i,j}$:

$$P_{i,j} = \min[1, w_{ij}],$$

$$w_{ij} = \exp(1/k_B T_i - 1/k_B T_j)(E_j - E_i). \tag{8}$$

As previously demonstrated, this process is very effective in finding the lowest-energy conformation. At low temperatures, the simulations tend to be trapped in local minima on the model energy landscape. The exchange processes move these conformations to higher temperatures where they are

TABLE I. Parameters of the energy function.

	σ_i (Å)							
ϵ_{sa}	N	C_α	C′	H	C_β	O	σ_{hb} (Å)	σ_{AA} (Å)
0.0034	1.65	1.85	1.85	H	2.5	1.65	2.0	5.0

much easier to surmount the intervening energy barriers. In addition, other replicas have the opportunity to sample different regions in conformational space at lower temperatures.

The REMC method is suitable for both finding the global minimum and collecting thermodynamic variables during simulations. We use eight replicas in our simulations with the temperatures ranging from 0.5 to 0.8.

C. Details of the simulations

Previous researches have shown that the ratio of the two parameters ε_{hb} and ε_{AA} is the key factor affecting the simulation results [9]. And the original values were set as 2.8 and 2.2, respectively. So we shall try different sets of values and observe the effects on the folding.

Since our goal is to simulate the folding of the sequences which are derived from real proteins, we need a scheme to map the 20 types of residues into a three-letter representation. The key is to find out which residues are hydrophobic. There are many available hydrophobic scales in the literature which can be classified into two types according to their methods used for constructing the scales. One is based on the proteins with known three-dimensional (3D) structures. The hydrophobicity is derived from the actual interactions between residues and the tendency of residues to be found in the cores of proteins. The other is based on the physicochemical properties of the side chains of amino acids. For simplified models, the first type may be better because we ignore some effects such as interactions between charges, disulphide bonds, etc. Such a reduction is often based on the Miyazawa-Jernigan (MJ) matrix of effective interresidue contact energies [10]. In Ref. [11], the residues CMFILVWY were considered to form the hydrophobic group, while in the work of Irbäck *et al.* [12], the hydrophobic group contains AMFILVWY. The only difference is A and C. Both of the schemes were derived from MJ matrix. In most of our simulations, residues CMFILVWY are taken as hydrophobic group (A). The others except G are counted into polar group (B). And we shall show in the following that this difference in classification does not make significant effect on simulation results.

The original work by Irbäck *et al.* studied the folding of a three-helix bundle model protein [7]. In this paper we choose an ultrafast folding protein Engrailed Homeodomain (PDB ID:1b8i) from *Drosophila melanogaster* [13] as our target. The Engrailed Homeodomain is a eukaryotic DNA-binding domain. It is a mainly α-helical protein of 61 residues which do not require a disulfide bond or ligand in order to fold stably. Its amino acid sequence and three-letter representation are shown below:

Original sequence:

RQTYTRYQTLELEKEFHTNHYLTRRRRI-
EMAHALSLTERQIKIWFQNRRMKLKKEI

Converted sequence:

BBBABBABBABABBBABBBBAABBBBBA-
BABBBABABBBBABAAABBBBABABBBA

It is noted that the converted sequence does not contain any G components. The de novo–designed sequence [7] is listed here for comparison:

BBABBAABBABBAABBGGGBBABBAAB-
BABBAABBGGGBBABBAABBABBAABB

III. RESULTS AND DISCUSSION

When using the original values of the parameters ε_{hb} and ε_{AA} (2.8 and 2.2), the folding simulations of Engrailed Homeodomain with the REMC method cannot find its native conformation. The resulting structure is a long helix conformation. The reason for this misfolding is the missing of GGG segments on turn ranges. The side chains make more limitations on the choice of Ramachandran-Sasisekharan torsional angles ϕ_i and ψ_i [14]. And now, to form turns, it is necessary to overcome higher-energy barriers and we need stronger attractive forces between helices. Intuitively, we can increase ε_{AA} to meet this need. But for a sequence converted from real protein, how do we choose the proper values for the correct folding? In order to investigate the relation between the foldability and the balance of hydrogen bond and hydrophobic force, we carried out a series of simulations for different values of parameter λ, which is defined as the ratio of ε_{hb} to ε_{AA}—i.e., $\lambda=\varepsilon_{hb}/\varepsilon_{AA}$. For simplicity, we just change ε_{hb} and keep ε_{AA} being 2.2. For each value of λ, the conformation with the lowest energy during the entire simulation is kept for comparison. We calculated the contact number and the helix length in these conformations. Two residues

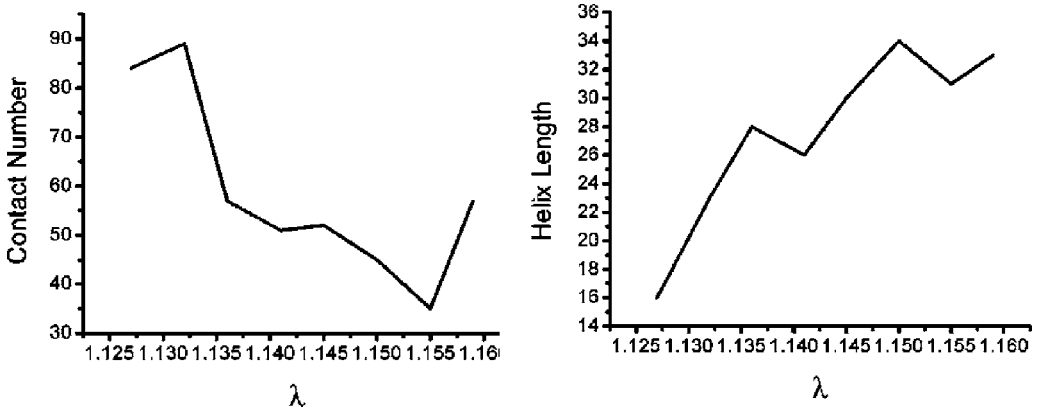

FIG. 2. The contact number and helix length (in unit of amino acid) of the conformations with the lowest energies obtained by folding simulations for different values of λ.

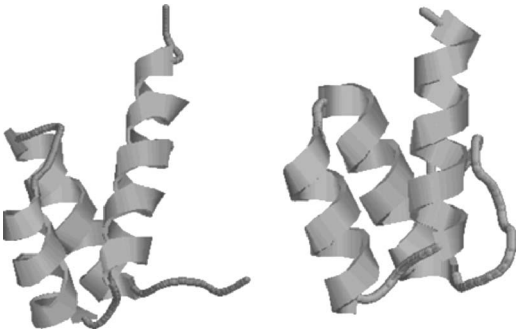

FIG. 3. The native structure (left) and the simulated structure (right) of Engrailed Homeodomain. This result is obtained from the simulation of 1.5×10^5 sweeps with $\lambda=1.15$ ($\varepsilon_{hb}=2.53$ and $\varepsilon_{AA}=2.2$).

are considered to form a contact when the Euclidean distance between the corresponding C_β atoms is lower than 8 Å and at the same time the separation between them along sequence is more than five residues. We also calculated the total "helix length" in the lowest-energy conformation which is the sum of the lengths of each helix segments. If the hydrogen bond energy between residues i and $i+4$ is lower than zero, we think it forms a hydrogen bond. A segment is considered as a helix when all the residues in this segment form hydrogen bonds. The result is shown in Fig. 2.

From the result one can see how hydrogen bonds and hydrophobic forces affect the formation of secondary structures and tertiary structures cooperatively. Stronger hydrophobic forces increase the contact number but reduce the length of helices. So we can try to increase the value of ε_{AA} to overcome the energy expense caused by the turn ranges. ε_{AA} and ε_{hb} are empirical parameters in the original model [7] and depend on the amino acid compositions of real protein sequences—e.g., the ratio and types of hydrophobic and hydrophilic residues and so on. It is difficult to give a quantitative relation between these two parameters and amino acid compositions. Some tries are needed to find suitable values for ε_{AA} and ε_{hb}. However, we find that the simulated results are very sensitive to their values. For a given protein, there is only a very narrow window for these values that can lead the protein fold into a compact conformation. This may correspond to the proper balance between hydrogen bonds and hydrophobic forces for the given protein. This result is in agreement with that of Ref. [15]. Therefore, the simulations in general do not give multiple possible candidates for the native conformation though we have to try different values for the two parameters. For the protein Engrailed Homeodomain, we find $\varepsilon_{hb}=2.53$ and $\varepsilon_{AA}=2.2$ to be a suitable choice.

As mentioned above, the original model only considered the interactions between two hydrophobic residues and omitted those between hydrophobic and hydrophilic residues. This only simulated part of the water effect because it can not guarantee that the hydrophobic core is buried in the interior of protein. This also hindered the formation of correct native conformations. To improve this problem, we can refer to the scheme widely used in lattice models where the interactions between hydrophobic and hydrophilic residues are taken into account to make the hydrophobic core properly buried in the interior of protein. In lattice models the contacts are classified into three types: HH for contacts between hydrophobic and hydrophobic residues, HP for contacts between hydrophobic and hydrophilic residues, and PP for contacts between hydrophilic and hydrophilic residues [16,17]. The weighted factors of the contact energies of HH, HP, and PP are set to be −2.3, −1, and 0, respectively. Such a choice of parameters can be viewed as the sum of a hydrophobic part $\varepsilon_H=-1$ plus a small two-body part $c(H,H)=-0.3$, with hydrophilic part $\varepsilon_P=0$ and $c(H,P)=c(P,P)=0$, where $c(H,H)$, $c(H,P)$, and $c(P,P)$ are two-body parts of the contact energies of the three kinds. Accordingly, we can add another energy term in the Ramachandran-Sasisekharan model:

$$E_{AB}=\varepsilon_{AB}\sum_{i<j}\left[\left(\frac{\sigma_{AB}}{r_{ij}}\right)^{12}-2\left(\frac{\sigma_{AB}}{r_{ij}}\right)^{6}\right], \tag{9}$$

which is counted when a hydrophobic side chain is close enough (<8 Å) to a hydrophilic side chain. The ε_{AB} is set to be $\varepsilon_{AA}/2.3$. We take this choice not only because it has been proven to be reasonable in lattice models but also because we tried it on several small two-helix proteins and all gave satisfiying simulation results. This change can make sure not only of the formation of a hydrophobic core but also make them be properly buried in the interior of protein.

Applying these two changes, we simulated the folding of the converted sequence of Engrailed Homeodomain by using the REMC method. The simulation runs for 1.5×10^5 sweeps with $\lambda=1.15$ ($\varepsilon_{hb}=2.53$ and $\varepsilon_{AA}=2.2$). We found that the protein can fold into a global minimum state with a structure quite similar to the native conformation of this protein. The native structure and the simulated structure of Engrailed Homeodomain are shown in Fig. 3, and their secondary struc-

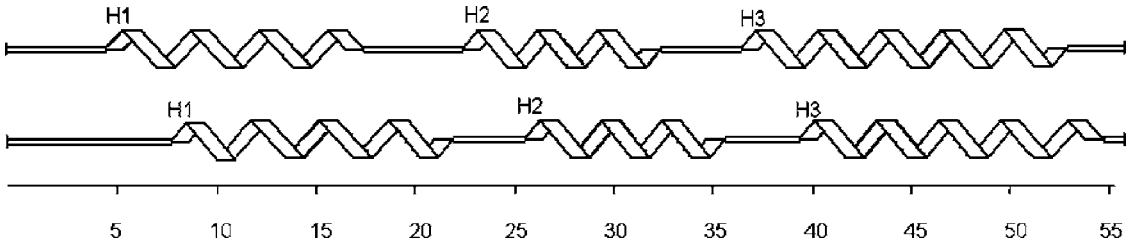

FIG. 4. The secondary structure comparison of the native structure (upper) and the simulated structure (lower) of Engrailed Homeodomain.

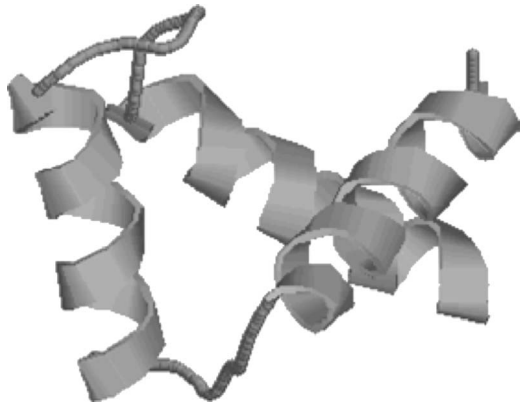

FIG. 5. The structure obtained from simulation of Engrailed Homeodomain with $\lambda=1.15$ ($\varepsilon_{hb}=2.53$ and $\varepsilon_{AA}=2.2$) by taking AMFILVWY as hydrophobic residues.

tures are almost the same except for a little position shift (Fig. 4). The root-mean-square deviation (RMSD) of them is 5.53 Å. One reason for this deviation may be due to the fixed bond length. Though the length difference for each bond is insignificant, together they will affect the long-range distance when accumulated. Another reason may be due to the result of the random coiled end.

To see the effect of different mapping schemes of amino acids, we also ran simulations of Engrailed Homeodomain by taking AMFILVWY as hydrophobic residues. The folding result does not change much, and its topology agrees well with its native structure too (Fig. 5). The RMSD between them is 5.66 Å.

The folding result above is just one aspect for proving the validity of this model. Another important aspect is the thermodynamic property of folding process. Figure 6 gives the temperature dependence of specific heat and gyration radius of the folding process of the 1b8i sequence. The sharp peak of specific heat at temperature 0.53 shows that the system undergoes a significant first-order phase transition from unfolded state to native state. This can also be identified by the significant reduction (to ~12 Å) of the gyration radius around this temperature.

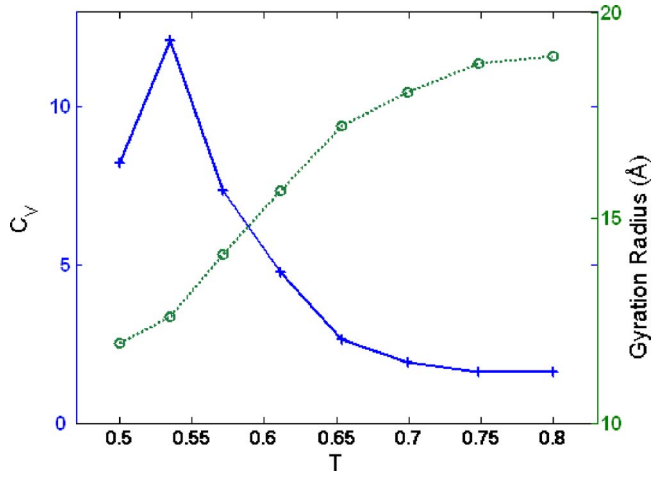

FIG. 6. (Color online) The temperature dependence of the specific heat (+) and gyration radius (o) for Engrailed Homeodomain computed with our improved model. The units for temperature and heat capacity are $1/k_B$ and k_B respectively, where k_B is Boltzmann's constant.

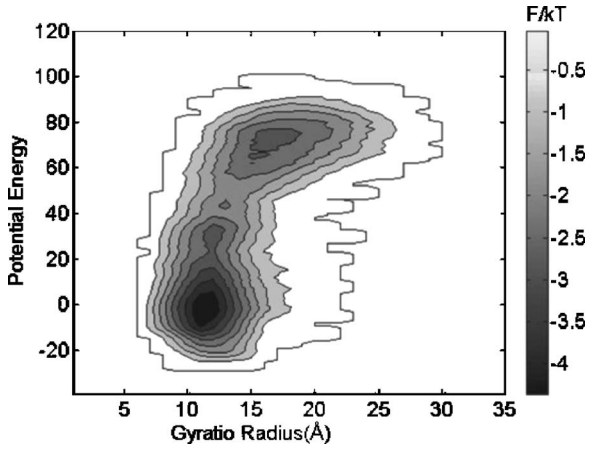

FIG. 7. The free energy (F) as a function of gyration radius and potential energy (in dimensionless unit) for Engrailed Homeodomain computed with our improved model.

We also calculated the free energy as a function of gyration radius and potential energy (Fig. 7). We can see a clear

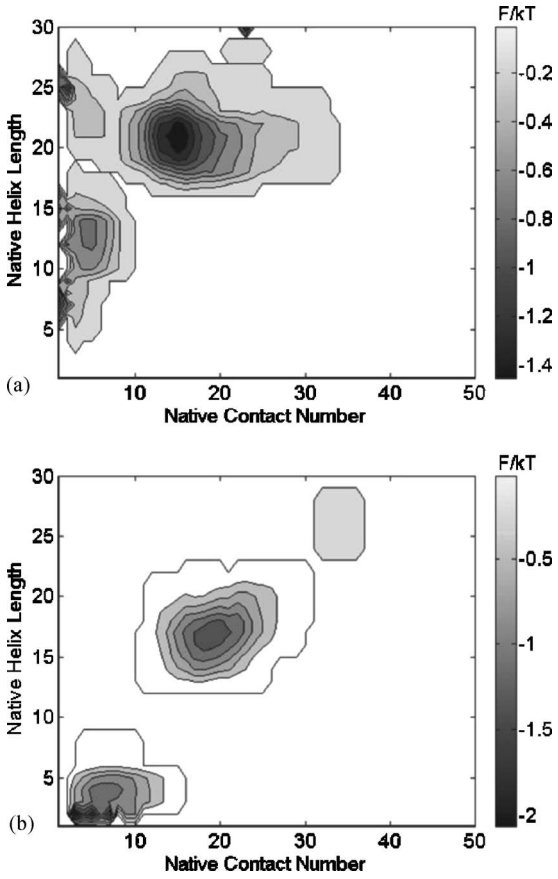

FIG. 8. The folding pathway described by the native contact number and helix length when hydrogen bond is relatively stronger ($\lambda=1.14$) (a) or the hydrophobic force is stronger ($\lambda=0.79$) (b). The figure shows the contours of $-\log(H)$, where H is a histogram from the unfolding simulation. The unit of helix length is amino acid.

energy barrier between the unfolded state and the lowest free-energy (native) state. Furthermore, the gyration radius of the native state is indeed around 12 Å and is in agreement with the specific heat curve. We also find that there exists a local minimum around the potential energy ≈42 and gyration radius ≈12 Å on the free-energy surface. The gyration radius at this local minimum is very close to the native conformation but the free energy is higher. This may correspond to an intermediate state. It is known from the unfolding simulation of the Engrailed Homeodomain protein that intermediate states are indeed present along the folding pathway [13]. This phenomenon was also observed in the folding pathways of another model three-helix-bundle protein [18] and some three-state proteins [19].

From the analysis above, we know how the relative strength of the hydrophobic force affects the resulting structure. Since each protein sequence contains a different percentage of hydrophobic components, we naturally want to see how this difference affects the folding pathway. This can be realized by choosing different values of λ. On the other hand, it is believed that during the reversible denaturation of globular proteins the unfolding pathway is, to a large extent, similar to the folding pathway, with a reversed order of events [20]. So the folding pathway can be explored alternatively from unfolding simulations by this model as well.

Figure 8 is the projection of the unfolding pathway on two parameters that describe the native contact and native helix segment formed in the conformation. The figure shows the contours of $-\log(H)$, where H is a histogram from the unfolding simulations. In Fig. 8(a) the hydrogen bond is relatively stronger ($\lambda=1.14$) and in (b) the hydrophobic force is stronger ($\lambda=0.79$). In both cases we keep $\varepsilon_{AA}=2.2$. It is clear that the folding pathways are different.

Figure 8(a) corresponds to the diffusion and collision model [21]. Protein folding starts with the formation of secondary structures independently of tertiary structure. These secondary structures then assemble into the tightly packed native tertiary structure by diffusion and collision. It is clear that Engrailed Homeodomain belongs to this class. This is in agreement with other calculations [13]. Figure 8(b) agrees well with hydrophobic collapse model of protein folding [22]. The initial event of folding is thought to be a relatively uniform collapse of the protein molecule, mainly driven by the hydrophobic effect. Stable secondary structures start to grow only in the collapsed state.

In summary, we improved the simplified Ramachandran protein model by refining the interactions. Using this improved model, the global minimum conformation of the protein Engrailed Homeodomain is founded to be quite similar to its native one. Furthermore, the thermodynamic property of folding process also agrees well with other works [18,19]. We also investigated how the relative strength of two main driving forces of protein folding affects the folding results. We studied the folding pathway by unfolding simulations and reproduced the two main theories of the folding pathway. Therefore, the Ramachandran-Sasisekharan model, when some improvements are made, is an available simplified protein representation that can be effectively used to explore the physical mechanism of the protein folding.

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