Role of Hydrophilic and Hydrophobic Contacts in Folding of the Second β-Hairpin Fragment of Protein G: Molecular Dynamics Simulation Studies of an All-Atom Model

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ABSTRACT  Predicting the folding mechanism of the second β-hairpin fragment of the Ig-binding domain B of streptococcal protein G is unexpectedly challenging for simplified reduced models because the models developed so far indicated a different folding mechanism from what was suggested from high-temperature unfolding and equilibrium free-energy surface analysis based on established all-atom empirical force fields in explicit or implicit solvent. This happened despite the use of empirical residue-based interactions, multibody hydrophobic interactions, and inclinations of hydrogen bonding effects in the simplified models. This article employs a recently developed all-atom (except nonpolar hydrogens) model interacting with simple square-well potentials to fold the peptide fragment by molecular dynamics simulation methods. In this study, 193 out of 200 trajectories are folded at two reduced temperatures (3.5 and 3.7) close to the transition temperature $T^\ast \sim 4.0$. Each simulation takes $< 7$ h of CPU time on a Pentium 800-MHz PC. Folding of the new all-atom model is found to be initiated by collapse before the formation of main-chain hydrogen bonds. This verifies the mechanism proposed from previous all-atom unfolding and equilibrium simulations. The new model further predicts that the collapse is initiated by two nucleation contacts (a hydrophilic contact between D46 and T49 and a hydrophobic contact between Y45 and F52), in agreement with recent NMR measurements. The results suggest that atomic packing and native contact interactions play a dominant role in folding mechanism. Proteins 2002;47:154–162.

INTRODUCTION

The β-hairpin motif is one of the basic building blocks of the structures of proteins. Understanding the folding of a β-hairpin is the first step toward the understanding of protein folding. The folding of the second β-hairpin fragment (residues 41–56) of the Ig-binding domain B of streptococcal protein G ($\beta_2$BpG) is of particular interest because it is the only β-hairpin whose folding is proposed to be initiated by hydrophobic collapse,1–5 rather than by the inter-strand hydrogen bonds near the turn region.6,7 The latter has been found to be the folding mechanism of a number of other β-hairpins, such as the 11-residue peptide VVVDPGVVVV,8 the first β-hairpin of tendamistat,9 and a three-stranded antiparallel β-sheet peptide.10 The folding mechanisms of these three peptides were examined by all-atom folding simulations of models interacting with well-established empirical force fields (AMBER,11 CHARMM,12 or GROMOS13) in an explicit9 or implicit8,10 solvent.

Because all-atom folding simulation of $\beta_2$BpG with an empirical force field is not yet feasible, the proposed mechanism was inferred from an equilibrium free-energy analysis,2–5 unfolding simulations1,4 and computational mutation studies.8 In these studies, various empirical force fields with atomic details, in an explicit or implicit solvent, were employed. The proposed mechanism, however, differs from the folding simulation results of lattice14 and off-lattice15 models with a reduced atomic representation, but with sophisticated interaction scheme designed for the same β-hairpin. Both folding simulations suggest that folding is initiated at the turn region. Klimov and Thirumalai used an off-lattice model in which each sidechain is represented by a bead and interacts with each other by an empirical residue-dependent contact energy and van der Waals diameter.15,16 In addition, hydrogen bonds between backbone carbonyl oxygen CO and amide hydrogen NH groups are represented by virtual moieties located between α-carbons,17 and the chirality is maintained by dihedral angle potentials.

There are several possible explanations for the discrepancy between the folding mechanism predicted by all-atom models based on empirical force fields1–5 and by reduced models.14,15 One possibility is that folding may not be the reverse of unfolding at high temperature.18–20 However, the qualitative agreement between the unfolding results1 and equilibrium free-energy surface analysis2,5 of all-atom models indicates that the folding mechanism inferred from unfolding simulations is likely reliable. Another possible reason is the inaccuracy of the hydrophobic
interactions in the reduced models. However, Klimov and Thirumalai\textsuperscript{15} found that variation of the strength of native contacts does not alter the transition state of folding. A similar conclusion was reached by Hoang and Cieplak.\textsuperscript{21} Using C\textsubscript{r}-based models, these investigators found that a β-hairpin is initiated at the turn even when the interaction strength for hydrophobic residues is increased by a factor of 5. The same folding mechanism was found by Kolinski et al.\textsuperscript{14} employing a sophisticated lattice model of β\textsubscript{2}BpG, in which a surface-exposure statistical potential is used to mimic hydrophobic interactions.

The theoretical interest in folding of β\textsubscript{2}BpG is largely spurred by experimental thermodynamic and kinetic measurements.\textsuperscript{6,22} β\textsubscript{2}BpG is found to be relatively stable (42% native backbone hydrogen bonds at 278K)\textsuperscript{22} and its folding time is estimated to be 6 μs.\textsuperscript{6} The folding can be depicted as a two-state process.\textsuperscript{6,7,23} Whereas earlier NMR study suggested the importance of hydrophobic interactions,\textsuperscript{22} more recent results indicated that both hydrophilic (D46 and T49) and hydrophobic (Y45 and F52) residues play crucial roles in folding.\textsuperscript{24}

In this article, we propose that the folding mechanism of β\textsubscript{2}BpG is encoded in the tertiary structure of its native state. The hypothesis is tested using an all-atom (except for nonpolar hydrogen atoms) model interacting with simple discontinuous square-well interactions.\textsuperscript{25} Only native interactions are present in the model (Gō model).\textsuperscript{26} The simplified all-atom model has been applied to study the folding thermodynamics of a three-helix bundle fragment B of staphylococcal protein A.\textsuperscript{25} It was shown that unlike simpler C\textsubscript{r}-based model,\textsuperscript{27,28} specific packing of sidechains yields a strong transition to the native state without an equilibrium molten-globule intermediate state. Using molecular dynamics simulations, we demonstrate that native atomic contacts and packing interactions determine the overall folding pathway of β\textsubscript{2}BpG.

**METHODS**

**Model**

The setup for our theoretical model is as follows.\textsuperscript{25} The initial heavy-atom positions of the native state of the model β-hairpin (Fig. 1) were obtained from the protein coordinates (residues 41–56 of Protein Data Bank 2gb1).\textsuperscript{29} The N-terminus and N-methyl-C-terminus of the polypeptide were capped with acetyl and amine groups, respectively. The total number of atoms is 163. The initial positions of polar hydrogens were generated by the CHARMM program\textsuperscript{12} and were minimized for 100 steps to remove bad contacts using the CHARMM polar hydrogen parameter set 19 with distance-dependent dielectric constant.\textsuperscript{30}

In the present model, all heavy atoms and polar hydrogens (bonded to N or O) are represented by a bead. Two bonded atoms, \(i\) and \(j\), as well as any 1,3 angle-constrained pair and 1,4 aromatic carbon pair, are constrained to a center-to-center distance between 0.9σ\textsubscript{ij} and 1.1σ\textsubscript{ij}, where σ\textsubscript{ij} is the atomic separation obtained by the CHARMM energy minimization. This constraint is accomplished by an infinitely deep square-well potential

\[
u_{\text{bond}}^{\text{ij}}(r) = \begin{cases} 
\infty, & r < 0.9\sigma_{\text{ij}} \\
0, & 0.9\sigma_{\text{ij}} < r < 1.1\sigma_{\text{ij}} \\
\infty, & r > 1.1\sigma_{\text{ij}} 
\end{cases}
\]

We also introduce a “bond” potential for improper dihedral angles to maintain chirality about a tetrahedral extended heavy atom and certain planar atoms. The potential has the form

\[
u_{\text{improp}}^{\omega} = \begin{cases} 
\infty, & \omega > \omega_0 + 20^\circ \\
0, & \omega_0 - 20^\circ < \omega < \omega_0 + 20^\circ \\
\infty, & \omega < \omega_0 - 20^\circ 
\end{cases}
\]

A large 20° fluctuation was used to increase the flexibility and folding rate of the hairpin. The improper angles \(\omega\) are identified using the CHARMM parameter set 19.\textsuperscript{30} In eq 2, \(\omega_0 = 35.26439^\circ\) for chiral-constrained atoms (e.g., an α carbon without explicit hydrogens) and \(\omega_0 = 0^\circ\) for planar-constrained atoms (e.g., a carbonyl carbon). The improper dihedral potential \(u_{\text{improp}}^{\omega}\) preserves the L-form chirality of amino acids and mimics some of the rigidity of peptide units. A nonbonded \(i, j\) pair interacts by a hard-core and square-well potential

\[
u_{ij}(r) = \begin{cases} 
\infty, & r < 0.8\sigma_{ij}^{\text{vdW}} \\
B_{ij}, & 0.8\sigma_{ij}^{\text{vdW}} < r < 1.2\sigma_{ij}^{\text{vdW}} \\
0, & r > 1.2\sigma_{ij}^{\text{vdW}} 
\end{cases}
\]

where \(\sigma_{ij}^{\text{vdW}}\) are the van der Waals parameters from the CHARMM polar hydrogen parameter set 19\textsuperscript{30} and \(B_{ij}\) is the yet-to-be-determined interaction strength. The factor of 0.8 for the hard-core diameters is typical for ratio between the diameter of a hard-sphere reference system and the van der Waals parameter found in the Week–Chandler–Andersen perturbation theory,\textsuperscript{31} while a ratio of 1.5 between the square-well and hard-core diameters is typical for systems of small molecules.\textsuperscript{32} To mimic the original native structure more accurately, initial hard-core overlaps in the CHARMM minimized structure are removed by decreasing \(\sigma_{ij}^{\text{vdW}}\) for those atomic pairs that overlap. There are a total of 108 overlapping pairs, and the averaged reduction in radius is 92%. The native structure has 947 square-well atom–atom overlaps (including both backbone and side-chain contacts). Henceforth, these are referred to as native atomic contacts. The number of nonlocal native atomic contacts for each residue and between a pair of residues facing each other in the hairpin is shown in Figure 1, along with the nonlocal residue–residue contact map.

A Gō interaction\textsuperscript{26} is employed to ensure that the energy of the native structure [Fig. 1(a)] is at the global minimum. In a Gō model, a square-well overlap between two atoms results in an interaction energy of \(-\varepsilon (B_{ij} = -1)\) for a native contact and 0 (\(B_{ij} = 0\)) otherwise. The Gō model is used to determine whether detailed atomic contacts are enough to yield the correct folding pathway identified from earlier unfolding and equilibrium simulation studies based on more sophisticated force fields.\textsuperscript{1–5} Henceforth, the internal energy, \(E^\text{in} = E/\varepsilon\), and temperature, \(T^\text{in} = k_B T/\varepsilon\), are scaled in units of \(\varepsilon\). A reduce time unit \(t^\text{in}\) is also
used ($t^* = t \sqrt{M/\sigma_e^2}$ with $\sigma_e$ is 1 Å and $M$ is the average mass of the atoms).

The all-atom model employed in this case was developed independently from a similar but simpler all-atom model by Shimada et al.\textsuperscript{33} Their model is a heavy-atom model without any dihedral potentials. The model was employed in a Monte Carlo kinetic study of crambin in which unfavorable repulsive (shoulder) potentials were applied to all non-native contacts.

Simulations

The discontinuous molecular dynamics (DMD) simulations of the isolated model β-hairpin are performed in the canonical ensemble. Constant temperature is maintained by collisions with ghost-solvent particles. The algorithm and implementation of DMD are described in earlier publications.\textsuperscript{34,35} Thermodynamic results are obtained by direct simulations over 13 temperatures from $T^* = 1.0$ to

![Residue–residue contact map of the second β-hairpin fragment of protein G (residues 41–56) (Protein Data Bank Accession no. 2gb1). Only nonlocal contacts (i-j) between residues are shown.](image1)

![Number of nonlocal atomic contacts for each residue (labeled inside the circles connected by solid lines) and number of atomic contacts between a pair of residues facing each other in the β-hairpin (labeled inside the circles connected by dashed lines). Locations of main-chain hydrogen bonds are indicated by dotted lines (H1–H6).](image2)

![Native structure. Drawn with MOLSCRIPT.\textsuperscript{41}](image3)
Five independent runs with different initial velocities are performed at each temperature to estimate errors. The thermodynamic properties at temperatures that are not simulated are obtained by using the weighted histogram method, which is a least-square optimization method for extracting the degeneracy factors of the energy levels (and the partition function) from multiple simulations at different temperatures.

The kinetic folding studies were conducted at $T^* = 3.5$ and $T^* = 3.7$, where the native state is stable. The initial coil-like configurations and velocities were obtained from equilibrium simulations at $T^* = 6.0$, and kinetic simulations were performed for $t^* = 50,000$ (~200 million collisions). Each simulation took ~7 h on a Pentium PC (800 MHz) and coordinates were recorded every 10 reduced time units for later analysis. A peptide is considered to be folded if its all-heavy atom root-mean-square deviation (RMSD) from the global minimum structure is <2.5 Å. This value is smaller than the equilibrated average value of 3.2 Å at $T^* = 3.5$, to ensure the completion of folding. Other criteria can also be used but will not change the results presented in this study. A progress variable $Q$, the fraction of native nonlocal ($i - j \geq 3$) contacts between residues, is used to monitor the folding process. Two residues are in contact if there are any native square-well atomic overlaps between them. To facilitate the comparison with earlier unfolding simulations, two additional progress variables (number of hydrogen bonds and core radius of gyrations) were employed. The core radius of gyration, $R_{core}$, is the radius of gyration of three residues W43, Y45, and F52. The number of hydrogen bonds, $N_{HB}$, is defined as the number of backbone–backbone hydrogen bonds excluding the two at the frayed end of the hairpin. In this case, a hydrogen bond exists if there is a square-well overlap between the carbonyl oxygen and the amide hydrogen of the peptide backbone. There are a maximum of five main-chain hydrogen bonds; all are native contacts in the global minimum structure [H1 to H5 in Fig. 1(b)]. H6 is a non-native contact in our model; thus, there is no interaction between the main-chain oxygen of D47 and amide hydrogen of K50, except for hard-core repulsion. Changing H6 from a non-native to a native contact (i.e., has an attractive interaction of $-\epsilon$) does not alter the results presented in this article (see below).

The thermodynamic results and overall folding behavior are found to be independent of the ghost solvent density that is used to control the temperature. However, if the ghost solvent density is too high, it will decrease the rate at
which the system explores its conformational space and thus reduce the folding rate. In contrast, if the ghost solvent density is too low, thermal equilibration will be too slow and a folding simulation initiated at high temperature will become a simulated annealing. We found that a ghost density of 0.01 Å/Å³ leads to only about 4% of total collisions and achieves a thermal equilibration from $T^*$ to $T^*$ within four reduced time units. For comparison, the density of water is 0.03 Å/Å³.

RESULTS AND DISCUSSION

The heat capacity as a function of temperature for the hairpin is shown in Figure 2(a). The transition occurs at $T^* = 4$ based on the weighted histogram method. The distribution of energy at the transition temperature is mostly bimodal, except for a change in curvature at $E^* = -650$ [Fig. 2(b)]. This suggests the possible existence of equilibrium transition state intermediates. A quantitative assessment would require more accurate thermodynamic studies near the transition region—a subject beyond the scope of this study.

A total of 200 folding simulations were performed. All 100 trajectories are folded at $T^* = 3.5$, while 93 out of 100 folded at $T^* = 3.7$ within $t^* = 50,000$. The folding time at which 50% are folded is 12,500 at $T^* = 3.5$ and 17,640 at $T^* = 3.7$, respectively. The fraction of not folded as a function of time is plotted in Figure 3. The data at $T^* = 3.5$ and 3.7 can be accurately fitted to a single exponential function. To estimate the folding time in a real unit, we first estimated the time required for making a nonlocal contact between two residues that are two residues apart (h/Å). The values vary, but on average, they are in the order of 100 reduced time units. A recent experiment found that the contact time between two residues that are 1–9 residues apart is 20–70 ns. Hence, 12,500 reduced time units for folding at $T^* = 3.5$ is about 3 s. This is very close to the 6 s estimated from experimental measurements.

The result for the fastest folding trajectory (run 89) at $T^* = 3.5$ is shown in Figure 4. Its folding time is 3,550 reduced time units. The hydrophobic core collapses quickly to the native value (5 Å) at $t^* = 1,000$ reduced time units. The productive hydrogen bond formation leading to the final formation of the hairpin secondary structure, was initiated at H5 at $t^* = 2,660$ and completed at $t^* = 3,000$. Figure 5 shows the structure at $t^* = 2,500$ in which the contact between hydrophobic
residues Y45 and F52 is established without any native hydrogen bonds.

The probability distribution of conformations sampled in the 193 folded trajectories is plotted as a function of the number of hydrogen bonds and the core radius of gyration in Figure 6. At both temperatures, the folding of βBpG starts with a quick collapse from $R_{\text{core}} \sim 10$ Å (the average value at $T^* = 6$) to $\sim 7$ Å before the formation of any native main-chain hydrogen bonds. The population distribution in $R_{\text{core}}$ and $N_{\text{HB}}$ of the folded trajectories shows at least one partially collapsed species at $R_{\text{core}} \sim 6$ Å and $N_{\text{HB}} \sim 2$. The unfolding simulations by Pande and Rokhsar also yielded an intermediate state (the H state) located at $R_{\text{core}} \sim 5$ Å and $N_{\text{HB}} = 0–2$. At $T^* = 3.7$, another intermediate appears to develop at $R_{\text{core}} \sim 7$ Å and $N_{\text{HB}} = 0$. This is likely to be related to the S state found by Pande and Rokhsar. Pande and Rokhsar defined the S state as a partially solvated state and the H state as a hydrophobic core collapsed state. Lack of explicit solvent perhaps explains the low population of the S state in our all-atom model. Despite the difference, the qualitative agreement between the folding simulation of our all-atom model and the unfolding simulation of all-atom CHARMM model in explicit solvent is striking. Both indicate that the β-hairpin collapses before the formation of native hydrogen bonds.

Figure 7 illustrates the probability distribution in the planes of (a) $Q$ versus $R_{\text{core}}$ and (b) $Q$ versus $N_{\text{HB}}$. The initial collapse is completed at $Q < 0.2$ without any native main-chain hydrogen bonds. The hydrogen bond does not start to form until $Q > 0.3$. The contact maps at different ranges of $Q$ values for the folded trajectories are shown in Figure 8. For the coil state ($Q = 0–0.1$), only occasional contacts between residues D46 and T49 can be seen. The initial collapse at $Q = 0.1–0.2$ and $0.2–0.3$ is driven by the strengthening of the native contacts between D46 and K50 and T51, as well as the contact between D47 and K50—the first hairpin residue–residue contact from the turn region [Fig. 1(b)]. The contact probabilities between hydrophobic residues Y45 and F52 (0.18 at $Q = 0.1–0.2$ and 0.62 at $Q = 0.2–0.3$) initially lag behind that of D47 and K50 (0.57 at $Q = 0.1–0.2$ and 0.78 at $Q = 0.2–0.3$). But, the hydrophobic contact probability is greater after $Q > 0.3$ and serves as the new nucleation site for the growth of the β-hairpin structure. At $Q = 0.5–0.6$, even the contact between T44 and T53, the fourth hairpin residue–residue contact from the turn region, becomes more probable than the contact between D47 and K50. The latter is reduced from 0.88 at $Q = 0.4–0.5$ to 0.85 at $Q = 0.5–0.6$. For $Q > 0.6$, the folding is a downhill zipping both outward and inward (not shown).
Thus, the folding of \( \beta_{9252} \)-BpG involves two nucleation contacts. The first one is between D46 and T49. It leads to a cluster of hydrophilic residues (D46, T49, K50, and T51). The formation of the hydrophilic cluster brings together the hydrophobic contacts between residues Y45 and F52. The latter is the second nucleation site for the final formation of the \( \beta \)-hairpin. These two nucleation sites explain the existence of two partially collapsed kinetic intermediates. The populations of these two kinetic intermediates are low; thus, the overall kinetics is two-state like (Fig. 3).

The probabilities of the main-chain hydrogen bonds [H1–H6; see Fig. 1(b) for definition] at different ranges of \( Q \) values are shown in Figure 9. The hydrogen bond nearest to the turn (H6) is less than 30% at any values of \( Q \). Thus, on average, the hairpin formation is not initiated at the turn region but at H5. To verify this, we have changed the nonnative contact of hydrogen bond H6 to a native one. That is, the square-well potential of hydrogen bond H6 is set to \(-1000\). All 50 folding simulations at \( T^* = 3.5 \) successfully folded to the native state. The maximum probability of H6 increases only slightly from 25% to 31% at \( Q = 0.8–0.9 \). Moreover, there is no significant change in terms of folding rate. Thus, the overall picture is the same regardless whether H6 is an attractive contact or not.

The fact that the folding of \( \beta_{9252} \)-BpG is initiated at the middle of the hairpin, rather than at the turn region, may be explained in terms of number of nonlocal atomic contacts. Residues D46 and F52 have the highest and second highest number of nonlocal atomic contacts, respectively [Fig. 1(b)]. The number of atomic contacts between Y45 and F52 is the highest for a pair of residues facing each other. More atomic contacts between two residues not only lead to stronger contacts between residues but also...
increase the contact probability between them. This explains the importance of residue D46 and the Y45–F52 contact in the folding of β2BpG. Residues with large numbers of nonlocal contacts are also found to serve as nuclei for the folding of acylphosphatase.38,39

To verify the dominant role of packing and native contacts in protein folding, the interaction strength of 28 atomic contacts between the aromatic rings of Y45 and F52 was reduced from −ε to −0.2ε. We performed 50 folding simulations for the new model, with the same initial configurations and velocities as for the original Gō model. We found that 44 out of 50 simulations of the new model were folded at \( T^* = 3.5 \). A slower folding rate is due to the weaker native interaction in the new model. The probability distribution of sampled conformation for the folded trajectories as a function of the number of hydrogen bonds and core radius of gyration (not shown) is found to be essentially the same as that in Figure 6(b). More detail analysis of contact maps as a function of \( Q \) indicates that the folding mechanism for those 44 folded trajectories are similar to that of the original Gō model. The relative stability of the folding mechanism with respect to the “hydrophobic” strength were also obtained by Klimov and Thirumalai15 and Hoang and Cieplak.21

Folding of the new-all atom model of β2BpG is not initiated at turn, in agreement with equilibrium and unfolding simulations of all-atom models with empirical force fields. However, in our new model, the folding involves two nucleation contacts—a hydrophilic contact between D46 and T49 and a hydrophobic one between Y45 and F52. Only hydrophobic interactions were identified in earlier theoretical work.1–5 Moreover, on average, the hairpin formation is initiated at H5. In the equilibrium all-atom simulations based on empirical force fields,2,5 H5 (H6 in their notations) is not very stable. H3 (in ref. 5) or H4 (in ref. 2) is the most stable hydrogen bond. These discrepancies could be attributable to the neglect of the difference between hydrophobic and hydrophilic contacts in our model.

Results of our all-atom model is very consistent with available experimental data. The folding of the β-hairpin is cooperative in agreement with experiments.6−7,23 While the importance of hydrophobic interactions was highlighted in an early experiment,22 a recent mutation study34 indicated that both hydrophilic (D46 and T49) and hydrophobic (Y45 and F52) residues play crucial roles in the stability of the β-hairpin. The hydrophilic residues are found to be essential for stabilizing the rigid loop region.40 These critical hydrophilic and hydrophobic residues are identical to those found in our folding studies. Thus, it appears that the nature of the native contacts (hydrophilic or hydrophobic) is not as important as the contact itself in determining the folding mechanism. More studies are certainly needed in this direction.

CONCLUDING REMARKS

In this article, a recently developed all-atom model (except for nonpolar hydrogen atoms) based on discontinuous interactions25 is applied to fold the second β-hairpin fragment of the Ig-binding domain B of streptococcal protein G. The observed folding behavior (collapse first, rather than turn first) is consistent with the predictions of all-atom unfolding and free-energy surface analysis based on current state-of-art force fields but differs from results obtained from models using a reduced representation. This is true despite the use of sophisticated empirical interactions between residues in those reduced models. Moreover, the new model agrees with a recent NMR experiment in that hydrophobic and hydrophilic residues are both crucial in the folding of β2BpG. Thus, packing and native atomic contact interactions play a dominant role in the folding mechanism of proteins. A similar conclusion was obtained in a separate folding kinetic study of a three-helix bundle fragment B of Staphylococcal protein A (to be published).

NOTE ADDED IN PROOF

After the acceptance of this article, three articles on all-atom equilibrium simulations, folding and unfolding simulations of β2BpG appeared.42−44 Our results are in overall agreement with theirs.

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REFERENCES