Structural bioinformatics

Observation of multiple folding pathways of β-hairpin trpzip2 from independent continuous folding trajectories

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Received on October 26, 2007; revised on December 21, 2007; accepted on January 18, 2008

Advance Access publication January 23, 2008

ABSTRACT

Motivation: After 10-year investigations, the folding mechanisms of β-hairpins are still under debate. Experiments strongly support zip-out pathway, while most simulations prefer the hydrophobic collapse model (including middle-out and zip-in pathways). In this article, we show that all pathways can occur during the folding of β-hairpins but with different probabilities. The zip-out pathway is the most probable one. This is in agreement with the experimental results. We came to our conclusions by 38 100-ns room-temperature all-atom molecular dynamics simulations of the β-hairpin trpzip2. Our results may help to clarify the inconsistencies in the current pictures of β-hairpin folding mechanisms.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 INTRODUCTION

β-Hairpin is one of the most important structural elements in proteins. Since its formation involves long-range interactions, understanding its folding mechanism will help us to understand those of proteins (Galzitskaya et al., 2002; Pande and Rokhsar, 1999). Up to now, different folding mechanisms of β-hairpins have been proposed. Munoz et al. (Munoz et al., 1997, 2006) suggested a ‘zip-out’ model from their experiments of the C-terminal β-hairpin of the B1 domain of protein G. This model assumes that the folding initiates at the turn and propagates toward the tails by forming cross hydrogen bonds sequentially and so that the hydrophobic cluster forms relatively later. This folding pathway was supported by later experiment and simulation evidences (Du et al., 2004; Kolinski et al., 1999; Mousseau et al., 2004; Zhang et al., 2006). However, Munoz et al. (Munoz et al., 1997) indicated that this is just the most probable way to form hairpin structure and other mechanisms could play a role too. They mentioned a ‘zip-in’ like pathway with two ends first approaching each other to form a loop. Dinner et al. (Dinner et al., 1999) proposed a ‘middle-out’ model. This model suggests that the folding proceeds by forming a partial hydrophobic cluster and then the hairpin hydrogen bonds propagate outwards in both directions from the partial cluster. This kind of folding pathway was also supported by other simulation studies (Bolhuis, 2003; García and Sanbonmatsu, 2001; Zagrovic et al., 2001; Zhou and Linhananta, 2002). Zhou and co-workers (Zhou and Berne, 2002) found that the hydrophobic core and the inter-strand hydrogen bonds could also form at the same time. Some simulations based on reduced models even suggested a ‘reptation’ pathway (Wei et al., 2004; Imamural and Chen, 2006).

From above we can see that there are inconsistencies in the folding mechanism of β-hairpins. Experiments strongly support the zip-out model, while most simulations prefer the hydrophobic or zip-in model (Munoz et al., 2006). One of the reasons may be that all-atom level simulations usually did not observe enough folding events. Another reason is that the experiments mainly observed the most probable pathway. Due to the requirement of cooperation of side chains of residues, it is difficult to simulate the complete folding of β-hairpin at all-atom level with standard molecular dynamics (MD) simulation method (Snow et al., 2004; Zagrovic et al., 2001). Therefore, most simulation works above were carried out by supplementary methods (Alexandre et al., 2000; Andrec et al., 2005; Bryant et al., 2000; Ding et al., 2003; Galzitskaya et al., 2000; Lee and Shin, 2001, 2003; Liwo et al., 2005; Yang et al., 2004), such as replica-exchange method, transition-path sampling and reduced model. These methods provide a convenient way to view the folding behavior of short peptides. For example, replica-exchange method is a multi-replica and multi-temperature method and leads to high sampling efficiency. With long enough equilibrium time and proper statistical strategy, it could produce some excellent thermodynamic variables, which is in agreement well with experiment, such as heat capacity.

Although peptide folding has its own behavior in the nature, its actual presentation on the computer simulation is strongly dependent of the strategies we apply, especially the force field or energy model we use. In the past, many famous all-atom force field models have been proposed, like CHARMM (MacKerell et al., 1998), AMBER (Cornell et al., 1995), GROMOS (Saares et al., 2005) and OPLS (Jorgensen et al., 1996). A lot of successful applications of these force fields are
published in various journals. But actually these fields have their own preferred models. Okamoto and co-workers test these four typical force fields on the folding of short α-helix and β-hairpin with generalized ensemble method (Yoda et al., 2004). After comparing the secondary structure content in different replicas, they conclude that AMBER94 have excellent performance in α-helix simulation and GROMOS96 behaves well in β-hairpin simulation. AMBER96, CHARMM22 and OPLS-AA/L present proper tendencies to both of them. So, it is clear that for different types of peptides, choose a special force field is quite critical. Sometime it determines the final success of the simulation. Recently, AMBER has made a great improvement in the potential energy form and related parameters to fit the experimental data and high-level QM computations, like AMBER99 (Wang et al., 2000) and AMBER03 (Duan et al., 2003). To compare the performance of these AMBER force fields and their revised versions: AMBER99m1, AMBER99m2, AMBER99off, Lwin and Luo (2006) carry out many simulations for C-terminal β-hairpin from protein G (Blanco et al., 1994; Fesinmeyer et al., 2004). They discuss the structure distribution, folding thermodynamics and folding pathway in different conditions. The final results show that AMBER99ci and AMBER03 produce a good agreement with experiment data, like nuclear Overhauser effect (NOE) and native contacts. Furthermore, the free energy landscapes for these two force fields are a little different. AMBER99ci has a partial folded state in the landscape and AMBER03 does not. But this difference does not change their general agreement in various thermodynamics properties.

In this letter, we report successful simulations of the folding of hairpin trpzip2 (Cochran et al., 2001) by using all-atom molecular dynamics simulation method (Chen and Xiao, 2006) at room temperature. The sequence of the trpzip2 is SWTWENGKWTKW (Fig. 1A). Its native β-hairpin structure has an obvious hydrophobic core composed of two aromatic side-chain pairs (Fig. 1B) and so is similar to the experimentally investigated C-terminal β-hairpin of the B1 domain of protein G. The strong hydrophobic interactions of two aromatic side-chain pairs make the trpzip2 more stable and easier to simulate its folding dynamics by simulation. Therefore, trpzip2 is a very ideal model for investigating the general folding mechanisms of β-hairpins.

Recently, some MD simulations in explicit water for trpzip2 have been proposed. Simmerling group solvated trpzip2 into a period box with TIP3P water molecules, and modeled the global system in the NPT ensemble with 1 atm and 300 K (Okur et al., 2003). With both AMBER94 and AMBER99 force fields, they find that the majority of the hairpin structure is stable in the native state except the terminal residues, which transform from extended state to helical state. Pitera and co-works also simulated trpzip2 in explicit water, but with pretty high temperature, 425 K (Pitera et al., 2006). AMBER96 was selected as the force field. Among numerous folding and unfolding events, they put forward a viewpoint that hydrophobic collapse and turn formation is the dominant mechanism during the hairpin folding. Although explicit solvent produces a more realistic environment for peptide folding, it cost too much computation effort. This greatly affects extensive sampling in the conformational space, especially at the room temperature. So, we apply the implicit solvent in our work to enhance the sampling efficiency. Previous study shows that proper implementation of implicit solvent model could perfectly rebuild experimental data for β-hairpins, such as stability of native state and various thermodynamic properties (Lwin and Luo, 2006; Lwin et al., 2006). And moreover, for the same peptide trpzip2, we could compare the result in implicit solvent with explicit solvent discussed above, make some benefit conclusions.

2 METHODS

In order to obtain enough folding events under the normal conditions, we simulate the β-hairpin with AMBER PARM96 force field (Cornell et al., 1995) and GB/SA implicit solvent model (Still et al., 1990; Qiu et al., 1997). We have done 38, 100-ns simulations for the trpzip2 by using Tinker (See: http://dasher.wustl.edu/tinker/). All the simulations are carried out at 298 K, which is controlled by Nose–Hoover thermostat. The time step is 1 fs. The initial structure is an extended β-strand (Fig. 1B).

For illustration of the folding processes, we select three order parameters: the root-mean-square difference (RMSD), radius of gyration of aromatic pairs (Rg) and inter-strand hydrogen bonds (Nh). The RMSD indicates the similarity between any structure and the native state and it only involves the Cα atoms in the backbone. The radius of gyration of aromatic pairs (Rg) is the value corresponding to size of the hydrophobic core.

\[
R_g = R_g(2, 11) + R_g(4, 9),
\]

where \(R_g(2, 11)\) is the radius of gyration of pair Trp2-Trp11, and \(R_g(4, 9)\) is that of pair Trp4-Trp9 (only side-chain atoms are included).

The hydrogen bond is assumed to be formed when the distance between carbonyl oxygen O and amide hydrogen H in the backbone is shorter than 3.0 Å and the angle of Donor-Hydrogen-Acceptor is larger than 120°. For simplicity we describe all the hydrogen bonds as \(iO–jH\). It represents a hydrogen bond between the carbonyl oxygen in the residue i and amide hydrogen in the residue j. For example, the hydrogen bond network of the native conformation of trpzip2 is (5-8, 8-5, 3-10, 10-3, 1-12).

3 RESULTS

3.1 Free energy landscape

To check the distribution of conformations for the trpzip2 in the folding process under normal conditions, we analyze
Multiple folding pathways of β-hairpin trpzip2

Fig. 2. Free energy landscape for peptide trpzip2. The two-order parameters are selected as RMSD and radius of gyration of aromatic pairs (Rg). The points N and M1 are corresponding to native state. The points M2–M6 indicate the local stable states.

Fig. 3. Free energy profile for peptide trpzip2 along RMSD reaction coordinate.

The total 38 100-ns trajectories and construct the free energy landscape for it (Bryngelson et al., 1995). Totally the accumulated time is 3.8 µs, this is above the characteristic time for trpzip2 (1.8 µs) in Fluorescence experiments (Mousseau et al., 2004). The relative free energy between two states is calculated by

$$F(x) - F(y) = -RT \ln \left( \frac{P_1(x)}{P_2(y)} \right)$$

where $P(x)$ is the corresponding probability distribution function, $x$ is any set of order parameters (Zhou et al., 2001). We set the order parameters as the backbone RMSD from the native structure and radius of gyration of aromatic pairs ($R_g$).

In actual calculation, the reference state in the free energy surface is the highest free energy state, and the free energies of other states in the surface have taken this value out. Figure 2 shows the free energy landscape is very rough. It is not easy for the short peptide to fold from unfolded states. Totally there are seven minima in the landscape. N is the native state and M1–M6 are the local stable states.

The state M1 has similar conformation as the native state and the only difference is the side-chain packing. So it could also be viewed as native state. The states M2, M3 and M4 are hairpin-like structures but with non-native aromatic side-chains conformations. The state M5 is an asymmetric hairpin in which three hydrophobic residues (Trp4, Trp9 and Trp11) form a non-native hydrophobic core. Finally, the last state M6 is a α-helix structure.

To investigate the free energy profile in the hairpin folding process in more detail, we also plot the free energy versus RMSD in Figure 3. From the figure, we could easily find that globally the trpzip2 has a two-state folding mechanism. One is the folded state around RMSD = 0.4 Å and the other is the unfolded state around RMSD = 2.2 Å. The basins corresponding to these two states are at almost the same height. This is similar to the simulation of other groups (Ulmschneider et al., 2006; Yang et al., 2004). Furthermore, the free energy curve is not smooth. There are many other local minima in the landscape, which increase its roughness. This is also claimed in Gruebele’s work (Yang et al., 2004). Finally, the free energy barrier between the folded and unfolded state is about 0.9 kcal/mol. It is lower than that noted in Gruebele’s paper. We think this discrepancy comes from different MD implementations. In our work, we use traditional MD and independent trajectories. And they use replica exchange MD and related trajectories.

3.2 Folding pathways

To investigate the folding pathways of trpzip2, we focus on the 10 successful folding events from the total 38 ones. A folding event is defined as the folding from RMSD larger than 3.0 Å to lower than 1.0 Å. From these 10 folding events we are surprise to find that they belong to four different pathways according to the formation order of backbone hydrogen bonds: zip-out, zip-in, middle-out and non-zipper. In the following, we select four typical folding events to describe these pathways respectively.

3.2.1 Zip-in pathway Figure 4 shows the RMSD, number of inter-strand hydrogen bonds, radius of gyration of aromatic pairs and distances between the inter-strand hydrogen bonding atoms via time and Figure 5 shows some key conformations in the folding pathway. The folding proceeds as follows: After quick collapse (Fig. 5A and B), the peptide falls into a hairpin-like structure. In this structure, two aromatic pairs Trp2-Trp11 and Trp4-Trp9 are formed but located between two β-strands. This prohibits the formation of inter-strand hydrogen bonds. In the following (0.3–2.1 ns), the trpzip2 tries to adjust the aromatic pairs toward outsides. During this process, only inner aromatic pair is broken and its gyration increases to 7 Å (Fig. 4A). The most outside hydrogen bond (1-12) between the two end residues formed in this step (Fig. 4B). At 2 ns, the hydrophobic core is just in the native conformation. The RMSD decreases to 1.0 Å. Then, the native inter-strand hydrogen bonds (3-10, 10-3) and (5-8, 8-5) are formed at 2.1 and 6.9 ns, respectively (see Figs 4B and 5D). Finally, the ‘turn’ region adjusts to the correct configuration (Fig. 5E). These show the native hydrogen bonds form in the order: (1-12)→(3-10, 10-3)→(5-8, 8-5). It is a ‘zip-in’ pathway. The forming of the hydrogen bonds is triggered by forming an outer partial hydrophobic core (Dinner et al., 1999; Imamura and Chen, 2006; Wei et al., 2004).
3.2.2 Zip-out pathway

Figures 6 and 7 also illustrate a folding event. In this case, after initial collapse (Fig. 7A and B), trpzip2 also forms a hairpin-like structure that is approximately a mirror of the native state like the local state M3 (Fig. 7C). All the aromatic side-chains are placed on the other side of the plane of the backbone. In this structure, the native hydrogen bond (5-8) is already formed (see Fig. 6B). At about 27 ns, the peptide extends by breaking the outer aromatic pairs but keeping the inner pairs. Figure 6A shows that the radius of gyration of outer aromatic pairs (Trp2-Trp11) increases suddenly over 7 Å at 27 ns while the gyration of inner aromatic pair (Trp4-Trp9) keeps lower than 5 Å. Finally at about 50 ns, trpzip2 re-collapses and the aromatic pairs aggregate at the right direction. Then, the native inter-strand hydrogen bonds form from the turn to the tail in the order: (5-8) → (8-5, 3-10, 10-3, 1-12). In this process ‘turn’ region, i.e. the inner aromatic pair (Trp4-Trp9) and inner hydrogen bond (5-8), is very crucial to the hairpin folding. It is clear that this folding pathway is a ‘zip-out’ one (Munoz et al., 1997, 2006).

3.2.3 Middle-out pathway

Figures 8 and 9 illustrate another folding event. After initial fast collapsing period, trpzip2 forms a hairpin-like structure with the two hydrophobic pairs opposite to the native one as above (Fig. 9A and B), but in this case any native hydrogen bonds are not formed (see Fig. 8B). In the following process, the trpzip2 costs about 30 ns to adjust the hydrophobic pairs by extending (Fig. 9C) and breaking both aromatic pairs (Fig. 8A). At about 41 ns, the peptide re-collapses and the two aromatic pairs form almost simultaneously. At the same time, the middle native hydrogen bonds (3-10, 8-5, 10-3) are also formed sequentially (Fig. 8B). Then the two most outer native hydrogen bonds (5-8, 1-12) form approximately at about 47 ns. In this process, the two aromatic pairs form a hydrophobic core at the middle and then initiate the formations of inter-strand hydrogen bonds.
propagating outwards in both directions. This just likes a 'middle-out' pathway (Imamura1 and Chen, 2006).

3.2.4 Non-zipper pathway: Figures 10 and 11 illustrate a very fast folding pathway. In this case, the peptide quickly collapses into a hairpin-like structure (Fig. 11A and B) similar to the native one. Then, the peptide adjusts its conformation and folds into the native state in less than 0.2 ns (Fig. 10A). The two hydrophobic pairs and the five native hydrogen bonds are formed almost simultaneously. It is clear that this folding pathway is not a zipper one. Zhou and co-workers also suggested such a pathway based on their simulations of the C-terminal /C12-hairpin of protein G with a highly parallel replica exchange method (Zhou and Berne, 2002).

Among the ten folding events, there are five 'zip-out', one 'zip-in', one 'middle-out' and three 'non-zipper' pathways. This indicates that 'zip-out' is the most probable folding pathway, i.e. it is the mostly-observed pathway. This may explain the results of recent experiment and some Monte Carlo simulations that the turn formation is the rate-limiting step for /-hairpin folding. However, this does not exclude other mechanisms, although with lower probability of occurrence. It is noted that our results show that the fast non-zipper pathway also occurs with large probability. However, this non-zipper pathway may be very difficult to observe experimentally because the folding is very fast. We did not observe the 'reptation' pathway. The reasons may be that we do not have enough folding events or that reptation moving is difficult to realize for real peptides since it has only been observed in reduced peptide models without side-chains.

It is noted that the frequency of observations of the zip-out versus zip-in (and middle-out) is likely proportional to the relative strength of hydrogen bonds and hydrophobic interactions. The hairpin trpzip2 is not natural but designed, and have stronger hydrophobic interactions due to the two aromatic pairs. Besides, hydrophobic interactions are typically overestimated with implicit solvent models. The stronger hydrophobic interactions produced a rough landscape with many local stable states, as shown by Figure 2. Most of these local states are stabilized by hydrophobic cores. This makes trpzip2 fold more probably along zip-in or middle-out pathways. If we reduce the strength of hydrophobic interactions, e.g. mutating the inner aromatic pair and keep the outer one (similar to 2gb1), we would obtain a smoother landscape which makes the zip-out mechanism more probable. These indicate that the
zip-out mechanism may be more pervading in nature than observed in our simulations.

In Fluorescence experiments, the characteristic time for trpzip2 is 1.8 μs (Mousseau et al., 2004). But all the successful folding events in our simulations finished within 70 ns. There are two reasons due to this difference. One is that we use the implicit solvent model. Because less of frictions, this model could greatly enhance the mobility of peptide, and thus reduce the folding time. The second reason is that the limitation of simulation time. Due to the complexity of all-atom force field, we could only view the folding behavior of trpzip2 in first 100-ns, so only 10 of 38 trajectories are found to fold into native state in this time period. Because of these reasons, we think the all-atom simulations are more suitable for the study of folding pathway or folding mechanism.

In summary, we observed multiple folding pathways for the trpzip2, depending on how the two hydrophobic pairs approach to their native conformations. All the previously proposed folding pathways may occur but with different probabilities. The zip-out pathway is the most probable one. This is in agreement with the experimental results. Furthermore, the zip-out pathways are also initiated by the formation of a partial hydrophobic core. This is partially confirmed to the simulation in the explicit solvent (Pitera et al., 2006), which presents that hydrophobic collapse and turn formation is a dominant mechanism during hairpin folding process. Our results may help to clarify the inconsistencies in the current pictures of β-hairpin folding mechanisms.

ACKNOWLEDGEMENT

This work is supported by the NSFC under Grant No. 30352037 and 30470412.

Conflict of Interest: none declared.

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