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Biochemical and Biophysical Research Communications 301 (2003) 31–34
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α-Hairpin stability and folding of transmembrane segments

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Received 19 November 2002

Abstract

Molecular Dynamics (MD) simulations at low dielectric constant have been carried out for peptides matching the double spanning segments of transmembrane proteins. Different folding dynamics have been observed. The peptides folded into the stable helix-turn-helix conformation—α-hairpin—with antiparallel-oriented strands or unstable α-hairpin conformation that unfolded later into the straight helical structure. The peptide having flexible residues in the TM helices often misfolded into a tangled structure that can be avoided by restricting the flexibility of these residues. General conclusions can be drawn from the observed folding dynamics. The stability and folding of some double spanning transmembrane fragments are self-assembling. The following and/or neighboring peptide chains of the protein may support the stability of the hairpin structure of other fragments. The stability of the TM helices containing flexible residues could be maintained due to contacts with neighboring TM segments.
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Keywords: Membranes; Molecular simulations; Protein folding; Self-assembly

Molecular simulations, such as Molecular Dynamics (MD) or Monte Carlo (MC), is a promising approach to studying a protein folding at the atomic level. Recently it was employed for a few simple peptides [1–12] and a short 36-residue protein [13]. Direct simulation of protein folding at the atomic level is still problematic. Studying peptides or small protein segments is the first logical step to a better understanding of the principles of protein folding. Currently two satisfactory approaches are employed to simulate peptide folding in solution: using atomic representation of the peptide–solvent systems [2,8,13] or using atomic representation of the peptide and continuum approaches for the solvent simulation [1,3–5,7,9–12]. Because of the number of variables in a protein–solvent system the former approach is much more time consuming. In implicit models the solvent is represented by an effective energy function in terms of its average effect on a solute. The details of the implementation may vary. In many cases, implicit solvent models are able to substitute for the effects of explicit solvent while increasing the speed of calculations substantially.

This communication focuses on the stability and folding of the hairpin structure of α-helices in non-polar environment. The helical hairpin is a packed pair of α-helices separated by a short turn. Peptide sequences were derived from double spanning fragments of transmembrane proteins. Since many membrane-bound domains in proteins consist of transmembrane (TM) α-helices that traverse the membrane in zigzag fashion, presented studies are important for understanding of general principles of transmembrane protein folding and stability.

Whereas the peptide helix formation in membrane mimicking environment has been examined by MC method [11,12], there have not been conventional MD simulations of the hairpin folding of α-helices at the atomic level in non-polar environment. The only α-hairpin folding of a short model peptide in a water-mimicking environment has been generated by MC trajectories [3]. The folding simulation of a simple β-hairpin structure for short peptides in water using implicit [4,5,7,9,10] and explicit solvation [8] has been described in a few papers.

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doi:10.1016/S0006-291X(02)02994-7

Materials and method

MD simulations were carried out using the CHARMM and Insight package of Accelrys, San Diego, CA. The first stage of the helical membrane protein folding and oligomerization can be usefully conceptualized as the formation and subsequent side-to-side association of independently stable transbilayer helices [14]. This is in agreement with the diffusion–collision model [15] postulating that the first step in folding is the formation of α-helices, which diffuse relative to each other and eventually collide and coalesce with a certain probability. The starting α-helical conformation of the 40–50 residue peptides was generated using the Biopolymer module of Insight with neutral N- and C-termini. The CHARMM22 force field was used for calculation of intramolecular and solvation energies with the different dielectric constants. The Generalize Born (GB) solvation model [16] parameterized within CHARMM force field [17] represented the solvent. The GB model extends the Born model for ionic solvation to multiple charged particles including arbitrary molecules in atomic representations. The GB model is over 35 times faster than the explicit one [17]. The amino acid sequence and length of the peptides were chosen so as to include the residues of the putative interfacial loop and two neighboring transmembrane segments connected by this loop. The MD simulation was run at 300 K with an integration step of 1 fs. The equilibrium configurations of the peptides were recorded every 0.01 ps. The simulations were terminated after the system repeated similar structural motives. The recorded configurations have been analyzed to give a structural description of the folding.

Results and discussion

Multiple simulations were carried out for peptides with the sequence derived from proteins with predicted topology or solved X-ray structure. It turned out that the dielectric constant of 2–2.5 is optimal for α-hairpin structure formation. At a dielectric constant of 1 (vacuum) not a single peptide folded into a α-hairpin conformation. At a dielectric constant that exceeded 3 the peptides tended to form globular structures with some retention of helical segments. Different α-hairpin folding dynamics were monitored during simulations.

Several peptides folded into a stable helix-turn-helix conformation with antiparallel-oriented strands. The typical representative of this group was the 42-residue peptide matching the sequence of the human sodium glucose cotransporter hSGLT1 from residues 147–188. A model of the membrane topology of SGLT1 was determined experimentally and refined by prediction of the approximate transmembrane helix ends by analysis of interfacial hydrophobicity and reverse-turn propensity [18]. According to the analysis, this fragment covers most of putative TM helices 4 and 5 and a connecting extracellular loop located around residues 162–173. The time course of the peptide folding is shown in Fig. 1. Note that the GB model is over 35 times faster than the explicit one [17]. During 60 ps the peptide folded into a stable double strand helical conformation with a short turn formed by Phe-163, Ser-164, and Gly-165 (Fig. 1). After that, all monitored

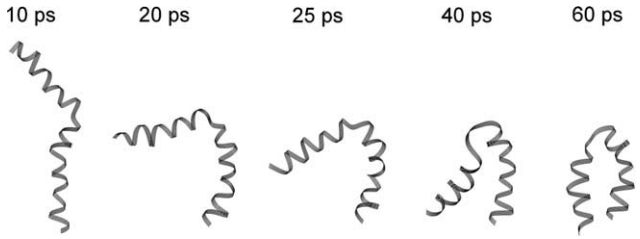

Fig. 1. Time course of folding of the peptide matching the helix 4-loop-helix 5 fragment of hSGLT1. The peptide folded into a stable α-hairpin structure. The N-terminus is on the left.

structures had a similar α-hairpin conformation. The turn site of the fragment developed gradually from a slight kink, which appeared after 10 ps of simulation, starting from an α-helical conformation. The kink divided the structure into two helical regions. The N- and C-parts of the fragment gradually diffused towards each other remaining in helical conformation. At 20 ps the helices reached a perpendicular orientation. After 60 ps the double strand conformation was formed. The peptide remained in this conformation during an observable simulation run. The structure formation of the considered protein segment is an intrinsic property of the double spanning segment and can be characterized as self-assembling. Similar folding dynamics have been observed for the double spanning fragments consisting of helices A and B of bacteriorhodopsin [19], helices 4 and 5 of the rabbit rSGLT1 [18], helices 1 and 2 of the erythrocyte anion transporter Band 3 [20], and others.

An unstable hairpin formation was monitored for the helix 5-loop-helix 6 segment of MsbA. A homolog of the multidrug resistance ATP-binding cassette (ABC) transporters MsbA is organized as a homodimer with each subunit containing six transmembrane α-helices and a nucleotide-binding domain [21]. According to the X-ray structure [21] the TM segments 5 (residues 253–272) and 6 (residues 281–301) consist of α-helices and are connected by an extracellular loop covering the eight residues 273–280. Since only the trace atoms have been solved the structural details of the loop cannot be characterized. It seems that Val-275 is located on the top of the loop. It was first anticipated that this double strand is formed as a result of the interaction exclusively between helices 5 and 6, since helix 6 is not embedded into the protein structure. The conformations during the time course of folding of the peptide at 15, 75, 100, 125, and 150 ps are shown in Fig. 2. After 15 ps a kink appeared at the loop site. It gradually transformed to essential tilt that exceeded 90° at 75 ps. At 100 ps, a hairpin was formed with the turn in the area of Ser-274, Val-275, and Met-276. The hairpin was stable for the following several ps, then unfolded, and at 150 ps the peptide formed the helical structure, which latter started to bend again at the same turn site. Apparently, the

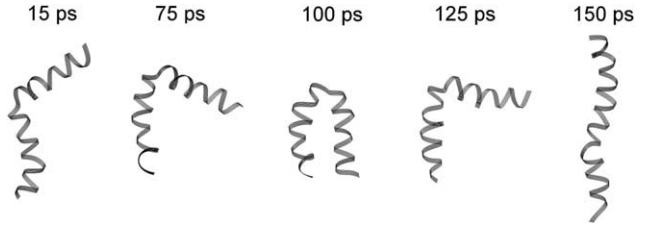

Fig. 2. Time course of folding of the peptide representing the helix 5-loop-helix 6 of MsbA. The peptide folded into an unstable α-hairpin structure.

intramolecular interactions in this segment are not strong enough to provide the antiparallel orientation of the helices 5 and 6. The following peptide chain might "help" to fix the helix 6 in the protein structure. It is not excluded that the flexibility of the unstable fragment could play an important role during folding of the entire protein into the functional structure.

Peptides with flexible residues in TM helix segments often misfolded into a tangled structure that can be corrected by restricting torsion rotations or replacing the flexible residues with more rigid ones. Interesting hairpin structure with the helical loop was generated for the helix 2-loop-helix 3 segment of rSGLT1. The hydropathy analysis of this segment predicted a α-helical hairpin with the extracellular loop covering residues 88–101 [18]. The 46-residue peptide with the sequence corresponding to residues 72–117 of the protein has been simulated. After 170 ps of simulation the peptide formed a tangled conformation containing three helical areas with the loops positioned at two flexible sites containing Gly-80, Ser-81, Gly-82 and Gly-99, Gly-100 (Fig. 3A). Whereas the second site is positioned in the expected loop area, the first one is located in the putative TM helix 2. Obviously, the found conformation is not functional and is probably an artifact of having done the calculations on the isolated peptide. Neighboring TM helices may stabilize the straight helical conformation of the peptide chain at the flexible sites containing glycine. To verify this assumption the flexibility of glycine residues located beyond the putative interfacial loop has been restrained and simulations are repeated starting from an α-helix. After 120 ps the peptide formed a stable conformation in which the N- and C-antiparallel-oriented helices are connected by a short interfacial helix via two bends positioned at Gly-89, Thr-90, Gly-91 and Gly-99, Gly-100 (Fig. 3B). The interfacial helix and bends (residues 89–100) cover most of the residues constituting the predicted extracellular loop [18].

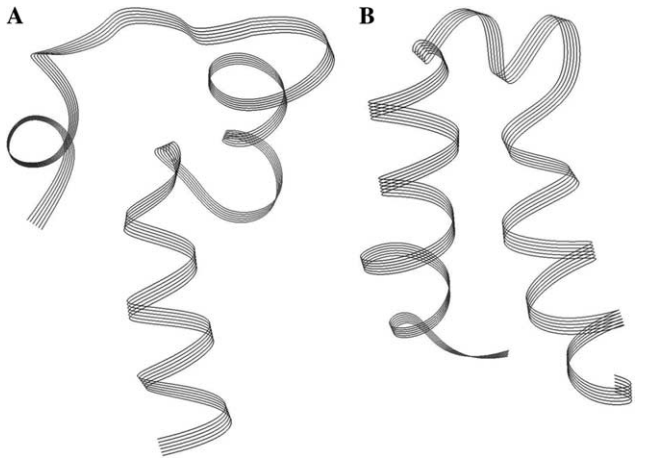

Fig. 3. Folding of the peptide matching the helix 2-loop-helix 3 fragment of SGLT1. (A) Tangled structure. (B) The structure with the restrained flexibility in the putative TM helix 2.

General conclusions may be drawn from the monitored folding dynamics. Folding of some double spanning transmembrane fragments is self-assembling. Following and/or neighboring peptide chains embedded in the protein assist the folding of unstable fragments. Neighboring TM segments may fix the helical structure of the TM segment containing flexible residues.

Acknowledgments

The author thanks Dr. M. Silverman and Dr. R. Reithmeier for support with computational facilities and E. Sevartsidis and D. Krofchick for help in the manuscript preparation.

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