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Conformational Study of N-terminal Prion Peptides by Molecular Dynamics Simulations

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The structure of the prion protein(PrP) was analyzed by solution NMR spectroscopy, X-ray crystallography, electron microscopy, and fiber and powder diffraction. Solution NMR studies of the cellular isoform prions protein (PrP^C) show that the N-terminal domain of the protein is flexibly disordered relative to its C-terminal domain, which has a α-helical globular structure.[1] Based on electron microscopy and spectroscopy studies, two different models have been suggested for the assembly of β-sheets in prion fibrils: (i) a parallel β-helix[2,3] and (ii) antiparallel β-chains.[4] The conformational change from the cellular to the scrapie form is the central event in prion replication. A cell-free study shows that the abnormal infectious form (PrP^{Sc}), which predominantly has a β-sheet conformation, must be partially denatured for replication. The known cytotoxicity of the PrP106-126 and PrP111-135 domain is likely structure-dependent.[5,6] The sequence of residues in the PrP106-135 domain among many different animals is mostly homologous. The structural difference between PrP^C and PrP^{Sc} is highlighted by their different sensitivities to digestion by proteinase K: PrP^C is completely digested, whereas PrP^{Sc} generates the undigested 27- to 30-kDa domain.[7] NMR spectroscopy shows that the 90-145 domain within SHa90-231 is flexibly disordered.[8] Thus, the N-terminal domain is likely to be where the structural conformation differs between PrP^C and PrP^{Sc}. We present the results of several 50 ns MD simulations of the N-terminal (111-136) of prion protein in neutral (hereafter PrP_N) and mildly acidic (hereafter PrP_H) conditions. In PrP_N, the arginine is positively charged and the histidine is neutral, whereas in PrP_H, the histidine is positively charged. We decided to focus our attention on the N-terminal histidine residue because human PrP(90-231) exhibits a conformational change that is complete at pH 4.4 but starts at pH 5.5.[9] Our MD simulation may prove useful for evaluating the effect of histidine protonation in determining the conformational behavior of the human prion protein with or without counterions. Both PrP_N and PrP_H MD simulations started from the NMR structure of the recombinant Syrian hamster PrP(90-231) (PDB entry 2PRP).[8] The refined PrP structures PrP(125-228) which were truncated N-terminal conformations (90-124) were now deposited in the Protein Data Bank (PDB entry 1B10), replacing the previously deposited preliminary refined structures (PDB entry 2PRP).

All MD simulations were carried out using the NAMD2 program[10] and the CHARMM27 force field for proteins[11,12] and ions,[13] and using the TIP3P water model.[14] All simulations were performed with periodic boundary conditions at constant temperature and constant pressure with a time step of 2 fs. In all systems, the solvent was relaxed by energy minimization, followed by 3 ns of MD at 298 K, while restraining the protein-heavy atomic positions by using a harmonic potential. Subsequently, all systems were simulated for 50 ns. The long-range electrostatic forces were computed by the particle-mesh Ewald technique.[15] Langevin dynamics with a very weak friction coefficient was used to keep the temperature constant. The Langevin Nosé-Hoover method[16] was used to maintain the pressure at 1 atm. The simulations of the N-terminal PrP peptide with or without counter ions involved ~6,400 atoms and required 53 ns. Secondary structure analyses were carried out by employing the defined secondary structure of proteins (DSSP) method.[17] Structural diagrams were prepared using PyMOL (DeLano, W.L. The PyMOL Molecular Graphics System (2002) on World Wide Web http://www.pymol.org).

To probe how the environment affects the conformational transitions of the N-terminal PrP peptides, four models were designed for the MD simulations. The profile of the secondary structures of N-terminal PrP_H peptides without salts along the trajectory is shown in Figure 1(a). The ribbon indicates the β-sheet at the N-terminal of PrP peptides. Obviously, the β-sheet conformations were observed within a few nanoseconds. The random coil of residues 118-121 was converted into a short β-sheet during the first 5 ns. This simulation revealed that in aqueous conditions and at room temperature (298 K), N-terminal PrP_H peptides without salts consist of ≈ 3% β-sheet, ≈ 52% coil, ≈ 37% bend, and ≈ 3% turn components (Table S1). The β-sheet conformational changes are more pronounced than those in the other models are. When NaCl was added to the full-length PrP(90-231), the experimental CD spectra showed a mixture of α-helical and random coil secondary structures.[18] This finding indicated that the N-terminal PrP peptides favor the random coil structure. However, β-sheet conformations were also observed. Interestingly, the MD simulations indicate that the β-sheet conformations of the N-terminals of PrP peptides oscillated, and many glycine residues can adopt β-sheet

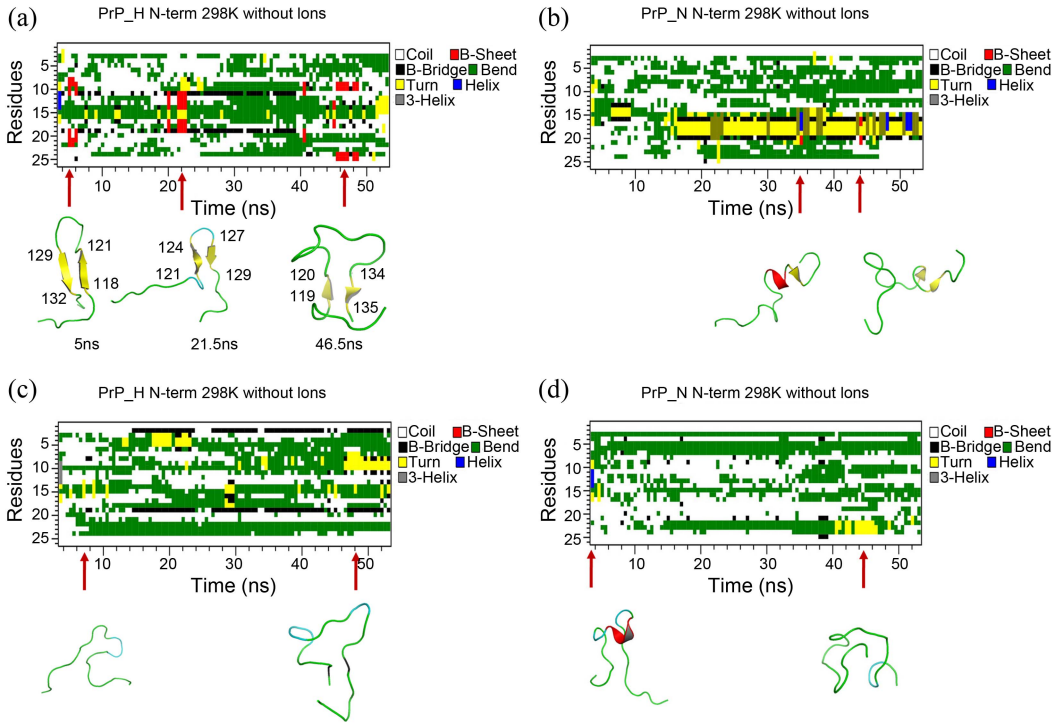

Figure 1. Structural analyses of N-terminal of PrP peptides (a) Secondary structures and snapshot structures extracted from trajectory of PrP_H without salt ions, (b) PrP_N without salt ions, (c) PrP_H with salt ions and (d) PrP_N with salt ions as a function of time, determined with DSSP.

conformations in the trajectories. The glycine residues in the N-terminal of PrP peptides are 119, 123, 124, 126, 127, and 131. The simulations of the fibril model of prion were performed at low pH conditions, and the following residues were involved in the extended β-sheet conformations: 114-122, 125-127 and 129-133, and 135-140.[4] Many of the NMR results show that the N-terminal of PrP peptides is a conformational heterogeneity. The difference in pH may account for this conformational change; however, the protonation of the histidine (111) residue cannot be considered the only effect that is able to stabilize the β-sheet conformation in the N-terminal of PrP peptides.[19] Apparently, many different environmental effects and types of interactions play a role in the conformational behavior of the N-terminal of PrP peptides. In the case of N-terminal of PrP peptides (111-136), β-sheets appear to have short-lived conformations during the MD simulations. It should be considered additional environmental factor that the presence of structured core (125-228) interacting with N-terminal disorder region (90-124) might further stabilize N-terminal β-sheets.

Although it is clear from the results of our MD simulations that N-terminal prion peptides is associated with their environments, this observation suggests that the environment of PrP_H without counter ions should propagate β-sheet conformational changes. N-terminus PrP plays an important role in the $\alpha \rightarrow \beta$ conversion and the molecular pathology of prion diseases.

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