The Molecular Dynamics Simulations of the Conformational Transition of Prion Protein from its Cellular Form to the Anomalous Form using the Earth Simulator

Group Representative

Yutaka Akiyama National Institute of Advanced Industrial Science and Technology

Authors

Masakazu Sekijima [*1] ・ Chie Motono [*1] ・ Tamotsu Noguchi [*1] ・ Kiyotoshi Kaneko [*2]
Yutaka Akiyama [*1]

*1 Computational Biology Research Center (CBRC), National Institute of Advanced Industrial Science and Technology (AIST)

*2 National Institute of Neuroscience (NIN), National Center of Neurology and Psychiatry (NCNP)

Prion protein is known as a disease factor of prion disease such as Bovine Spongiform Encephalopathy (BSE), scrapie, and Creutzfeld-Jacob Disease (CJD). Prion protein is a naturally occurring polypeptide that becomes transformed from a normal conformation to that of an aggregated form, characteristic of pathological states in fatal transmissible spongiform conditions. A central theme in prion protein research is the detection of the process that underlies the conformational transition from the normal cellular form (PrPC) to its pathogenic isoform (PrPSc). In the NMR structures of the prion protein, the N-terminal region is generally disordered. In contrast, the C-terminal region is well ordered and structured. Although the C-terminal region of human prion protein has been revealed through NMR spectroscopy, the process underlying the conformational change from PrPC to PrPSc and the dynamics and functions of PrPC remain unknown. Wille et al. have proposed a model featuring B-helices in the region 90–170 and a two-helix bundle in the C-terminal region 171–231 on the basis of electron crystallography data. To gain insight into the mechanism of this transition, we have characterized the biophysical properties of the recombinant protein corresponding to residues 90–231. In this study, we parallelized and vectorized the molecular dynamics (MD) simulation programs, AMBER and MolTreC. (These program differ in the calculation used for long range interactions.) Currently, the vectorization ratios are 98% and the parallelization ratios are 97.4%. We performed MD simulations on Wild Type and Mutant (Pro102Leu) of HuPrP90–231 at 300K. Dihedral angle phi of Pro102 was very stable, remaining within a small range throughout our simulation. In contrast, dihedral angle phi of Leu102 varied considerably, allowing the possibility of interacting with other residues to form secondary structures, such as a beta sheet. This suggests that Pro102 is critical to prevent the transition from random structure to beta sheet structure in the wild type form. Our planning simulations are wild type and mutant on hexameric prion protein which has residues 90–231. We think Earth Simulator enables us to calculate these large systems.

Keywords: Prion; molecular dynamics simulation; conformational stability; transmissible spongiform encephalopathies

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases attributable to the structural transformation of cellular prion (PrPC) to its anomalous isoform (PrPSc). In humans, these diseases include kuru, Creutzfeldt-Jacob disease (CJD), fatal familial insomnia (FFI), and Gerstmann-Straussler-Scheinker syndrome (GSS), in sheep, scrapie, and in cattle, bovine spongiform encephalopathy (BSE). The most important aspect of prion diseases is the conformational transition of PrPC to PrPSc, both of which are isoforms with identical amino acid sequence. However, comparison of their secondary structures shows that PrPC is ~42% helical with a very low (~3%) β-sheet content, PrPSc, on the other hand, consists of 30% α-helices and 43% β-sheets. While the precise physiological role of PrPC, and the chemical difference between PrPC and PrP remain unknown, it appears that their differences are conformational (1).

The three-dimensional structures of monomeric PrPC from various sources have been determined by NMR spectroscopy (2) and found to be very similar among many species. The N-terminal region (residues 23–124) is flexible, and the C-terminal region (residues 125–228) that contains the globular domains is well structured. All of these structures contain intramolecular disulfide bridges, three α-helices, and a short double-stranded β-sheet (Fig. 1 (a)). Recent X-ray crystallo-

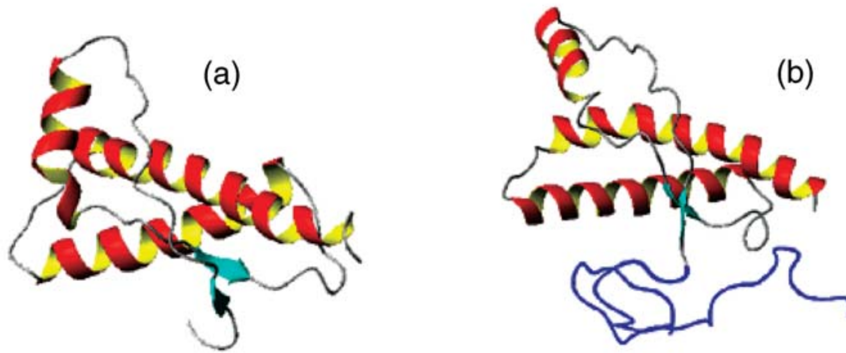

Fig. 1 (a) PrPC determined by NMR (b) Blue colored residues were modeled by this study.

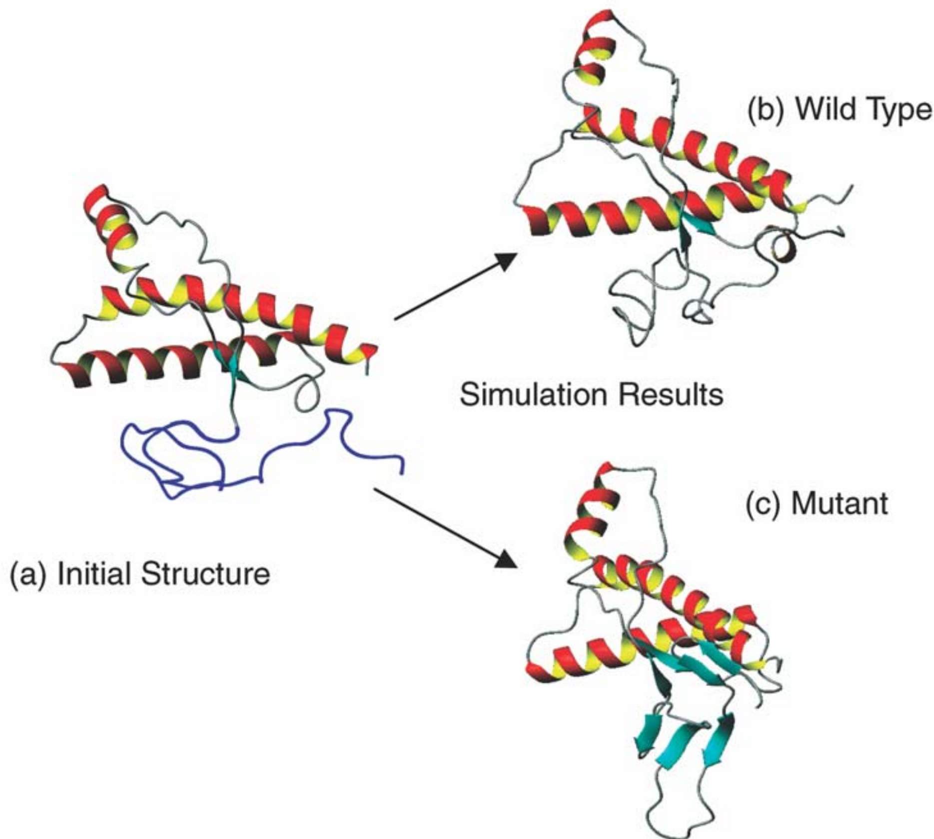

Fig. 2 (a) Initial Structrue (b) and (c) are temporary results of Wild Type and Mutant.

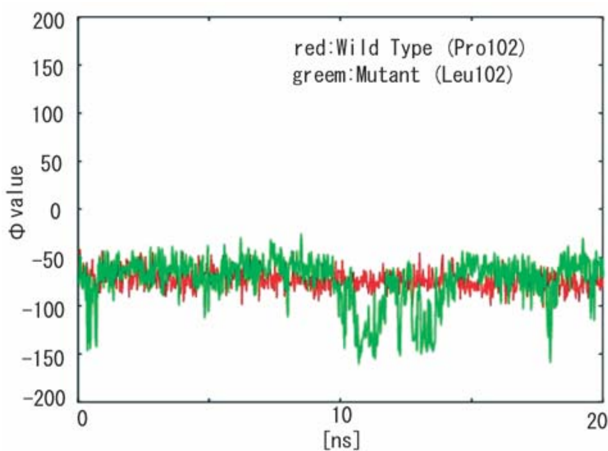

Fig. 3 Phi distribution for the Res. 102 during simulation.

graphic studies determined the dimeric form of human PrPC. The dimer is the result of three-dimensional swapping of the C-terminal helix 3 and rearrangement of the disulfide bonds (Fig. 1 (b)). The transition process from PrPC to PrPSc has been explained by two popular models. According to the hetero-dimer model (3), PrPSc induces the conformational change of PrPC by contact. The nucleation-dependent polymerization model of Lansbury and Caughey (4), on the other hand, suggests that PrPSc acts as a crystal seed at the starting point for crystal-like growth of a PrPSc oligomer and that conformational change occurs via transient interaction between PrPC and PrPSc. Several mutations in the primary structure of PrPC are known to segregate in variety of TSEs. In this study, we selected several mutations known to be asso-

ciated with FFI. In these mutations, the change from a positively charged- to an uncharged residue may affect the hydrogen bonding network and salt bridge (5). Recombinant forms of human and murine PrPC manifest a pH-dependent conformational change in the pH range from 4.4 to 6, a loss of helix, and a gain of strands (6). Lower pH accelerated conversion in a cell-free conversion assay (7). Thus, acidic pH may play a role in facilitating the conformational change that ultimately results in the formation of PrPSc.

More recent conformational conversion models focus on intra- and intermolecular disulfide bonds (8). Some experiments have suggested that intramolecular disulfide bonds in PrPC are required for its conversion to PrPSc (9). To weaken these disulfide bonds a hypothetical molecular chaperone may be necessary (10). However, the function and dynamics of the PrPC remain to be elucidated.

Molecular dynamics (MD) simulations are widely used to simulate the motion of molecules in order to gain a deeper understanding of the chemical reactions, fluid flow, phase transitions, and other physical phenomena due to molecular interactions. Rapidly increasing computational power has made MD simulation a powerful tool for studying the structure and dynamics of biologically important molecules. Taking into account all electrostatic interactions by using the Particle Mesh Ewald (PME) method, relatively long (2–3 ns) simulations with the explicit solvent water box can be carried out. Day et al. (11) have shown that by increasing the temperature, protein unfolding can be accelerated without changing the pathway of unfolding and that this method is suitable for elucidating the details of protein unfolding at minimal computational expense. With these methods, one can obtain proper trajectories that reflect the conformational and dynamic characteristics of molecules at each time point during simulation.

Most reported MD simulations of PrPC have been reported (12, 13, 14), involved short simulation times of less than 2 ns, or were performed using the AMBER ff94 force field, and most of the previously reported simulation targets were the C-terminal region which NMR determined. Higo et al. (15) used the multi-canonical method to show that the ff96 force field reproduces the energy landscape more correctly than does the ff94 force field both in vacuo and in solvent water.

Our past simulation (16, 17) showed the necessity of simulation on N-terminal region to reveal the process that underlies the conformational transition from PrPC to PrPSc. We performed MD simulations on Wild Type and Mutant (Pro102Leu) of HuPrP90–231 at 300 K. Dihedral angle phi of Pro102 was very stable, remaining within a small range throughout our simulation. In contrast, dihedral angle phi of Leu102 varied considerably, allowing the possibility of interacting with other residues to form secondary structures, such as a beta sheet. This suggests that Pro102 is critical to prevent the transition from random structure to beta sheet structure in the wild type form. Our planning simulations are wild type and mutant on hexameric prion protein which has residues 90–231. We think Earth Simulator enables us to calculate these large systems.

References

1) K. M. Pan, M. Baldwin, J. Nguyen, M. Gasset, A. Serban, D. Groth, I. Mehlehorn, Z. Huang, R. J. Fletterick, F. E. Cohen, and S. B. Prusiner. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. Proc. Natl. Acad. Sci. U.S.A. 90 : 10962–10966.
2) R. Riek, S. Hornemann, G. Wider, M. Billeter, R. Glockshuber, K. Wuthrich. 1996. NMR structure of the mouse prion protein domain PrP(121–321). Nature. 382 : 180–182.
3) S. B. Prusiner, Molecular biology of prion diseases. Science. 252 : 1515–1522.
4) P. T. Lansbury, Jr., and B. Caughey. The chemistry of scrapie infection: implications of the 'ice 9' metaphor, Chem. Biol. 2 : 1–5.
5) R. Riek, G. Wider, M. Billeter, S. Hornemann, R. Glockshuber, K. Wuthrich. Prion protein NMR structure and familial human spongiform encephalopathies. Proc. Natl. Acad. Sci. USA. 95 : 11667–11672.
6) W. Swietnicki, R. Petersen, P. Gambetti, W. K. Surewicz. pH-dependent stability and conformation of the recombinant human prion protein PrP(90–231). J. Biol. Chem. 272 : 27517–27520.
7) D. A. Kocisko, S. A. Priola, G. J. Raymond, B. Chesebro, P. T. Lansbury Jr, B. Caughey. Species specificity in the cell-free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier. Proc. Natl. Acad. Sci. USA. 92 : 3923–3927.
8) E. Welker, W.J. Wedemeyer, and H.A. Scheraga. A role for intermolecular disulfide bonds in prion diseases? Proc. Natl. Acad. Sci. USA. 98 : 4334–4336.
9) T. Muramoto, M. Scott, F. E. Cohen, and S. B. Prusiner. Recombinant scrapie-like prion protein of 106 amino acids is soluble. Proc. Natl. Acad. Sci. USA. 93 : 15457–15462.
10) K. Kaneko, L. Zulianello, M. Scott, C. M. Cooper, A. C. Wallace, T. L. James, F. E. Cohen, and S. B. Prusiner. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc. Natl. Acad. Sci. USA. 94 : 10069–10074.
11) R. Day, B. Bennion, S. Ham, and V. Daggett. Increasing temperature accelerates protein unfolding without changing the pathway of unfolding. J. Mol. Biol. 322 : 189–203.
12) J. Zuegg, and J. E. Greedy. 1999. Molecular dynamics simulations of human prion protein: importance of correct treatment of electrostatic interactions. Biochemistry. 38 : 13862–13876.

13) E. El-Bastawissy, M. H. Knaggs, and I. H. Gilbert. Molecular dynamics simulations of wild-type and point mutation human prion protein at normal and elevated temperature. J. Mol. Graph. Model. 20 : 145–154.
14) N. Okimoto, K. Yamanaka, A. Suenaga, M. Hata, and T. Hoshino. Computational studies on prion proteins: effect of ala(117)-->val mutation. Biophys J. 82 : 2746–2757.
15) J. Higo, N. Ito, M. Kuroda, S. Ono, N. Nakajima, and H. Nakamura. Energy landscape of a peptide consisting of alpha-helix, 3(10)-helix, beta-turn, beta-hairpin and other disordered conformations. Protein. Sci. 10 : 1160–1171.
16) M. Sekijima, C. Motono, S. Yamasaki, K. Kaneko, Y. Akiyama. Molecular dynamics simulation of dimeric and monomeric forms of human prion protein: Insight into dynamics and properties. Biophys. J. 85 : 1176–1185.
17) M. Sekijima, C. Motono, S. Yamasaki, K. Kaneko, Y. Akiyama. Molecular Dynamics Simulation of Prion Protein by Large Scale Cluster Computing. *Lecture Notes in Computer Science (LNCS)*. 2858 : 476–485. Springer-Verlag. 2003.