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

Project Representative

Yutaka Akiyama   National Institute of Advanced Industrial Science and Technology, Director

Authors

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

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

[*2] Second Department of Physiology, Tokyo Medical University

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. Although the three-dimensional structures of prion proteins have been revealed by NMR spectroscopy, the process underlying the conformational change from PrPC to PrPSc and the dynamics and functions of PrPC remain unknown. 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 ver.7, AMBER ver.8 and MolTreC. (These program differ in the calculation used for long range interactions.) Currently, the vectorization ratios are 98% and the parallelization ratios are 97.9%.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 performed molecular dynamics (MD) simulations on HuPrP90-231 at 300K for 20 ns. Simulations were also undertaken with Pro102Leu known as a disease-associated factor.

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)). 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 associated 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).

More recent conformational conversion models focus on intra- and intermolecular disulfide bonds (6). Some experiments have suggested that intramolecular disulfide bonds in
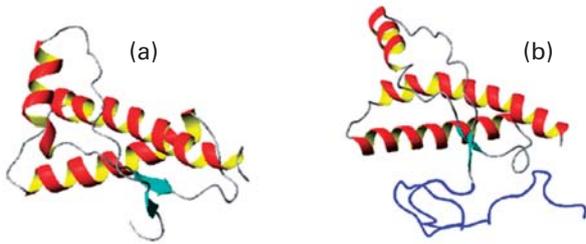
Fig. 1 (a) PrPC determined by NMR (b) Blue colored residues were modeled by this study.

PrPC are required for its conversion to PrPSc (7). To weaken these disulfide bonds a hypothetical molecular chaperone may be necessary (8). 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. (9) 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 (10, 11, 12), 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. (13) 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 (14, 15) 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 300K. Fig. 2 (a) and (b) show temporal history in 3D PCA (Principal Component Analysis) space. Only one amino acid mutation makes many differences. Initial structures of Wild Type and Mutant stay one conformational space. As shown in Fig. 2 (a), structures of Wild Type are stable through the simulation. In 3D PCA space, they fluctuated between -3 and 2 angstrom at a maximum in y component. Fig. 2 (b) shows the process of making
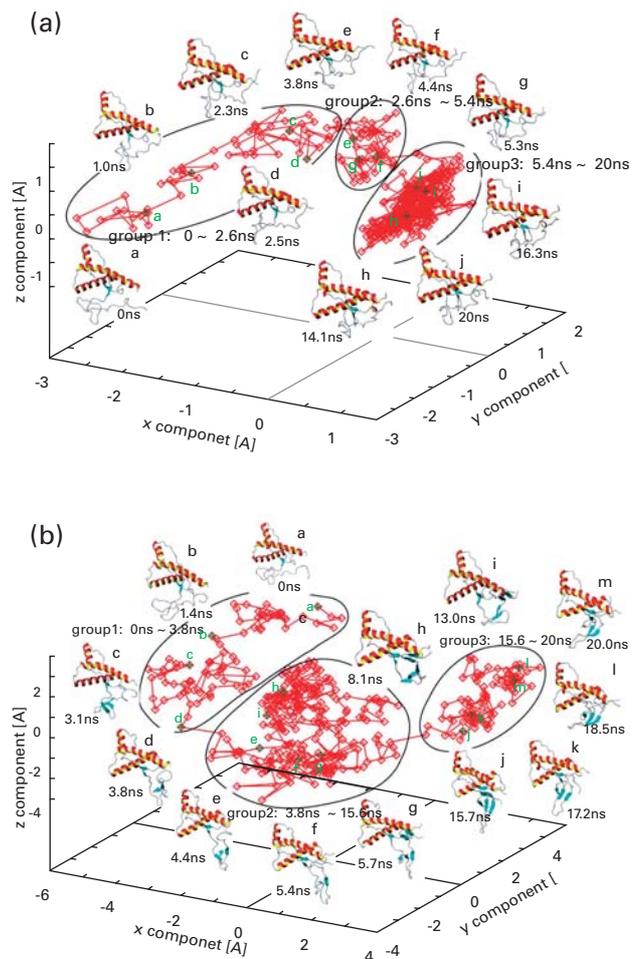

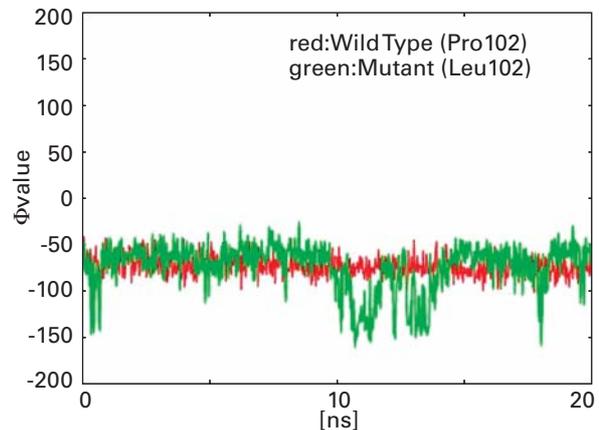Fig. 2 Simulation Results. Temporal history of (a) Wild Type and (b) Mutant (p102L)

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

beta strands. Structures at P102L overcome the energy barrier and fold another structure. Pro102's phi value was very stable, remaining within a small range throughout our simulation. In contrast, Leu102's phi value 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.

Bibliographies

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, r., 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) 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.

7) 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.

8) 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.

9) 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.

10) 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.

11) 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. 20:145-54.

12) 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.

13) 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.

14) 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.

15) 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.

正常プリオンタンパク質から異常プリオンタンパク質への構造転移プロセスの解明に関する研究

プロジェクト責任者

秋山　　泰　独立行政法人産業技術総合研究所生命情報科学研究センター

著者

関嶋　政和*1，本野　千恵*1，野口　　保*1，金子　清俊*2，秋山　　泰*1

＊1　独立行政法人産業技術総合研究所生命情報科学研究センター

＊2　東京医科大学生理学第二講座

　狂牛病、スクレイピーなどの原因物質とされるプリオンタンパク質は、生体内で安定に存在している機能が明らかにされていないタンパク質である。生体内で正常な構造を取っているプリオンタンパク質が、感染型の異常構造を取っているプリオンタンパク質と接触することで、ミスフォールディング(折り畳み誤り)を起こして感染型の異常構造を取りアミロイド化していくことが知られているが、その感染・構造転移の機構は明らかにされていない。本プロジェクトでは、プリオンタンパク質の感染と構造の関係について明らかにすることを目標としている。本プロジェクトでは、正常プリオンタンパク質の異常プリオンタンパク質への構造転移のメカニズムを探るために、シミュレーションターゲットをプリオンタンパク質の一遺伝子置換 (D178N, P102Lなど)を明らかにすることを目指している。この遺伝子置換を行うと、プリオンタンパク質がβストランドの多い異常プリオンタンパク質に構造転移するというものである。分子動力学シミュレーションの結果、野生型の102proがプリオンタンパク質の正常構造をシミュレーションを通して保っているのに対し、変異型の102Leuが異常構造とでもいうべきβストランドが多い構造を示した。そこで、102Proが異常構造への構造転移を抑制しているのか、102Leuの構造転移を加速しているのかを明らかにすることを目指し、P102Lの変異型に続き、P102A, P102VというLeu. Proと同じく非極性残基の変異を導入したシミュレーションを行った。その結果、LeuやVal, Alaでもβストランドを形成した。解析の結果、Proの二面角ϕが著しく取りうる角度の制約を受けているのに対して、Leu, Val, Alaでは比較的自由に様々な角度を取りうることを確認した。

キーワード：プリオンタンパク質，構造転移，分子動力学シミュレーション，狂牛病，蛋白質の安定性