Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014; 39 (2) ; 188–195
doi: 10.5505/tjb.2014.21033

Research Article [Araştırma Makalesi]

Yayın tarihi 30 Haziran, 2014 © TurkJBiochem.com

[Published online 30 June, 2014]

Dynamics study on the stability of animal prion proteins

[Hayvan prion proteinleri üzerine dinamik çalışma]*

Xin Chen,
Danhui Duan,
Shuyan Zhu,
Yafang Liu

[1]nstitute of Environmental and Analytical Sciences, College of Chemistry and Chemical Engineering, Henan University, Kaifeng 475001, Henan, China

Yazışma Adresi
[Correspondence Address]

Xin Chen

Institute of Environmental and Analytical Sciences, College of Chemistry and Chemical Engineering, Henan University, Kaifeng 475001, Henan, China
Tel.
Fax.
E-mail. xin_chen@henu.edu.cn

***Translated by [Çeviri]** Dr. Ebru Saatçi

Registered: 3 December 2013; Accepted: 15 January 2014
[Kayıt Tarihi: 3 Aralık 2013; Kabul Tarihi: 15 Ocak 2014]

ABSTRACT

Aim: Structural conversion of normal cellular prion protein (PrPc) into the scrapie isoform (PrPsc) is the central event in the development of prion diseases.

Materials and Methods: To get more insight into the molecular basis of the stability of animal prion protein, 10 ns molecular dynamics (MD) and flow molecular dynamics (FMD) simulations of turtle prion protein (tPrPc) and bank vole prion protein (bvPrPc) have been performed in this paper.

Results: The dynamics and mechanical properties of the two model proteins have been studied.

Conclusion: Various motions of β-sheet appeared in the two proteins, such as twisting, elongation and unfolding. For α-helix, it is more readily to unfold in bvPrPc system. Furthermore, the protective wall staggered with helix is found to be strong enough to stabilize PrPc under the shear flow.

Key Words: Stability, dynamics, prion, shear flow, molecular dynamics (MD) simulation

Conflict of Interest: Authors have no conflict of interest.

ÖZET

Amaç: Normal hücre prion proteininin (PrPc), Scrapie izoformuna (PrPsc) yapısal dönüşümü prion hastalıklarının gelişmesinde merkezi bir olaydır.

Gereç ve Yöntemler: Hayvansal prion proteinin moleküler temelde yapısal kararlılığını anlamak için, kaplumbağa prion proteini (tPrPc) ve bank vole prion proteini (bvPrPc) üzerinde 10 ns moleküler dinamik (MD) ve akış moleküler dinamikleri (FMD) simülasyonların gerçekleştirilmiştir.

Bulgular: Bu iki model proteinin dinamikleri ve mekanik özellikleri incelenmiştir.

Sonuç: Her iki protein içinde, bükülüm, uzama ve açılım gibi β – pilili tabakası hareketleri ortaya çıkmıştır. α-helix için, bvPrPc sistemi daha karasızdır. Bundan başka, heliksle çakışmayan koruyucu duvarın, kesik akış altında PrPc dengelemek için yeteri kadar güçlü olduğu bulunmuştur.

Anahtar Kelimeler: Stabilite, dinamikler, prion, kesik akış, moleküler dinamik (MD) simulasyon

Çıkar Çatışması: Yazarlar çıkar çatışması bulunmadığını beyan ederler.

Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of fatal neurodegenerative diseases [1]. This group includes Creutzfeldt-Jakob disease (CJD), Kuru in humans, as well as sheep scrapie and bovine spongiform encephalopathy [2-4]. The pathogenic mechanisms of prion diseases are diverse, while the conformational conversion of the normal cellular prion protein (PrPc) into the scrapie isoform (PrPsc) is a central etiological event [3, 5]. The two isoforms of prion protein can be distinguished by the composition of their secondary structure: PrPc was about 42% helical with a very low (~3%) β-sheets content, whereas PrPsc contains of 30% α-helices and 43% β-sheets [6-8]. The conversion of PrPc into PrPsc includes an obvious conformational transition of secondary structure. Then the kinetics of the folding of PrPc is essential to the understanding of the molecular pathogenesis of prion diseases. So far, the structure of protein have been elucidated by many experimental methods, such as X-ray diffraction (XRD), nuclear magnetic resonance (NMR) analysis, electron microscopy (EM) and some prediction techniques [9-11]. While both X-ray and NMR techniques have produced useful information on the protein's structure, each method has its limitations. For example, X-ray structures are static glimpses of structures often restrained by crystallographic environment. Alternatively, NMR spectroscopy is useful because the structures are obtained in solution without packing constraints, but because most proteins have a tendency towards aggregation, either specific pH condition or low concentrations must be used. Yet charges in pH can also affect the structure. Then it enables a broad sampling of the conformational states of a protein under controlled conditions and therefore delivers additional understanding of the protein dynamics [12]. Simulation of molecular dynamics (MD) could provide continuous dynamics information on proteins at an atomic level [13, 14]. However, the folding and unfolding time of protein varies from 10μs to 1ms [15, 16], it is difficult to observe this process by pure MD simulation. Flow molecular dynamics (FMD) simulation is an effective method to probe the mechanical properties of biomolecules, by which the dynamic characteristics of protein can be obtained quickly. The FMD method has become a powerful tool for complementing in vitro single-molecule experiments [17, 18].

In this work, MD and FMD simulations were combined to study the thermostability and the dynamics of the bank vole prion protein (bvPrPc) and the turtle prion protein (tPrPc). TSE has not been observed in turtle, and turtle prion protein is concluded as one of the prion proteins of TSE resistant species [19, 20]. The bank vole (*Clethrionomys glareolus*) has recently attracted interest due to unexpected features revealed by its use as a new laboratory animal for the study of prion diseases. When compared to the mouse and the Syrian hamster, the bank vole is found to be highly susceptible to infection by sheep and goat scrapie, and various human strains of TSE also show a low transmission barrier [4, 21, 22]. The structure stability of the bvPrPc and tPrPc were revealed by comparative studies of the internal interaction and mechanical properties of protein. It is expected to reveal the pathogenic mechanism of prion diseases from the structural features of protein, as well as to provide guidance to the drug design of prion diseases.

Materials and Methods

In this study, all MD and FMD simulations were performed with NAMD version 2.7 [23] using Charmm27 force filed [24]. The initial simulation structures of tPrPc and bvPrPc were obtained from the protein date bank (PDB) and the PDB entries were 1U5L [25] and 2K56 [26], respectively. The purified proteins were immersed in a periodic water box with TIP3P [27] model water molecules. Periodic boundary conditions were employed with a solvent shell of 10 Å. The water box was selected to be large enough to accommodate the structure changes during both MD and FMD procedures. And the volume of the water boxes of tPrPc and bvPrPc were $72.58 \times 61.66 \times 56.76$ Å^3 and $72.50 \times 57.80 \times 48.09$ Å^3, respectively. All simulations were carried out with a time step of 2 fs and the van der Waals (VDW) and columbic interactions were truncated at 10 Å. The long-range electrostatic interactions were calculated using the Particle Mesh Ewald (PME) summation scheme. During the MD simulations, the Langevin method was turned on to control the constant temperature at 310 K and the constant pressure at 101.3 kPa. Energy minimization was performed to optimize the geometry of the two protein molecules and then MD simulation was applied to equilibrate the system for 10 ns.

Following the MD simulations, a series of FMD simulations were carried out to investigate the flexibility of the protein dynamic structures. The sub-stable structure of PrPc obtained from MD simulation was employed as the initial structure of FMD simulations. In principle, FMD simulation is carried out by attaching a harmonic restraint to one or more water atoms in the system, and then varying either the stiffness of the restraint or the position of the restraint to pull the atoms along [28]. Constant velocity pulling (PCV) method is one of the basic operations of FMD, and it was used in this work to disturb the PrPc system with external forces. In the PCV simulation, the FMD atoms are attached to a dummy atom via a virtual spring and this dummy atom moves at a constant velocity in the desired direction. This dummy atom moved at constant velocity and then the force between them is measured using:

$$\vec{F} = -\nabla U \tag{1}$$

$$U = \frac{1}{2} k[vt - (\vec{r} - \vec{r}_0) \cdot \vec{n}]^2 \tag{2}$$

Where ∇U stands for the potential energy, k is the spring constant, v stands for pulling velocity, t means the time, $\vec{n}$ is the direction of pulling, $\vec{r}$ and $\vec{r}_0$ are the instantaneous position and the initial position of the FMD atoms, respectively. Since the protein could be roughly described in a rectangular box, the six faces of box were selected as the six different starting orientations of FMD directions. And the initial orientation of PrPc was denoted as +x. Then the other five orientations were named as -x, -y, +y, +z, -z, respectively. In FMD simulations, a slab of water molecules with a thickness of 3 Å was tagged as the FMD atoms and the external force was applied to them along the selected directions. The other water molecules and PrPc were free.

The snapshots of tPrPc and bvPrPc in direction +x were shown in Figure 1a and Figure 1b as an example, respectively. As shown in Figure 1, the tertiary structures of the two PrPc were similar. It could be found from Figure 1a that three α-helices and an antiparallel β-sheet were the main secondary structures of tPrPc. Three helices could also be found in Figure 1b of bvPrPc.

In FMD simulations, all the parameters were carefully adjusted according to the rigidity of the protein itself. And the spring constant k was set to be 30 kcal·mol^{-1}·Å^{-2} and the pulling velocity v was fixed at 5×10^{-4} Å·fs^{-1} to obtain good FMD observation window. The pulling distance was set to be from 45 Å to 69 Å according to the system size and the periodic boundary conditions. One thousand frames of trajectories were deposited during the FMD simulation.

Results and discussion

MD simulation

MD simulation is essential to achieve equilibrium state and generally there are several criterions to judge whether it has achieved equilibrium or not, i.e. the potential energy of protein, the root mean square deviation (RMSD) and the radius of gyration (Rg) of protein during the simulation procedure [29, 30]. The PrPc was immersed in a water box and the energy expression was shown to be complicated. In order to describe the final state of PrPc more exactly, the potential energy of PrPc was extracted individually from the simulation system.

The tPrPc system

The potential energy of tPrPc during the 10 ns MD simulation was shown in Figure 2a. It was discerned from the graph that the fluctuation amplitude of potential energy was small and it vibrated around -1336.7 kcal·mol^{-1}, which indicated that the system of tPrPc had achieved an equilibrium state. It could also be found from Figure 2b that the RMSD of tPrPc converged in the last 2 ns, which showed one of sub-stable states of tPrPc has obtained. Then the equilibrium structure of tPrPc could be used as the initial structure of FMD simulation.

As shown in Figure 3a, it could be found that the average value of RMSD of α-helix was larger than that of β-strand. Among the three helices, the first α-helix (Pro144-Asn153, named as α1) was the shortest one and it changed most dramatically. However, the RMSD of α1 was found to be changed with small amplitude and kept under 3 Å. Previous experimental and theoretical studies have also demonstrated that isolated α1 of mammalian PrPc is very stable [31-33]. With the analysis of MD trajectory it was found that α1 was away from the other two helices, which resulted in the more flexibility of α1. The RMSD value of α3 was the largest one and it was resulted by the unfolding of the terminal residues Ser225, Ala224 and Leu22 (Figure 3b). The H-bond formed between Tyr221 and Ser225 was broken from 1.7ns to 5.4ns. Two β-strands (yellow belt) were gradually lost in the MD process. It was prone to be conversed from β-sheet to coil. The elongation of β-sheet was found in the unfolding process, which is common to PrPc, and it is shown to be more evident at acidic PH values [34, 35].

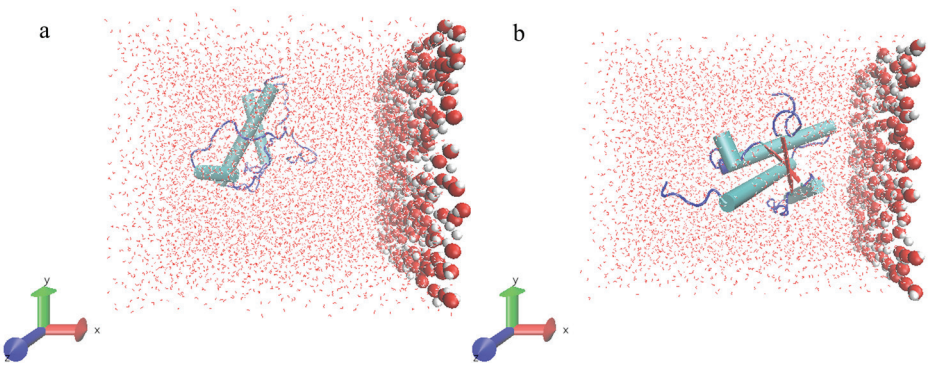

Figure 1. Snapshot of the initial state of (a) tPrPc and (b) bvPrPc in water box with the tagged FMD molecules in the direction of +x. FMD water molecules were coloured in red with VDW model. The free water molecules were shown with the line model. Proteins were displayed with the cartoon model. Helix, sheet and loop were coloured in cyan, red and blue, respectively.

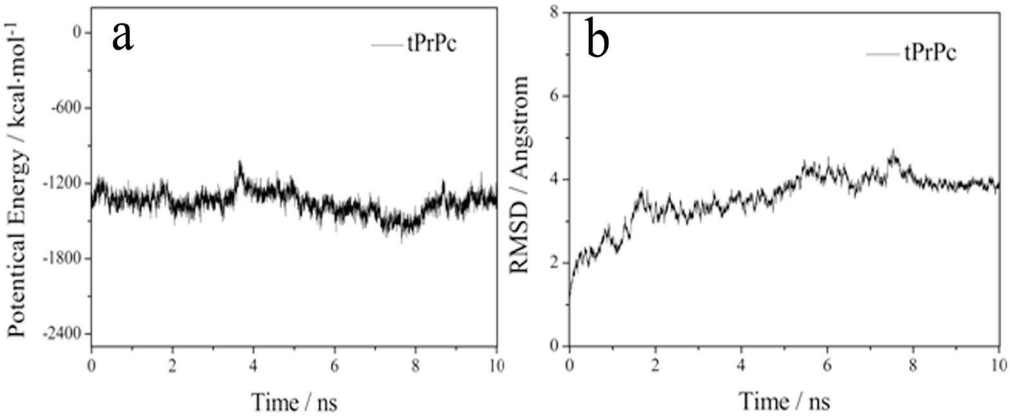

Figure 2. (a) Potentical energy of tPrPc and (b) RMSD of the backbone of tPrPc with respect to the MD simulation time.

Hydrogen bond (H-bond) was one of the most important interactions to maintain the stability of tPrPc. To get more insight into the thermostability of tPrPc, the backbone RMSD (Figure 3a), secondary structure evolution (Figure 3b) and key H-bonds (Figure 4) of helix and sheet were analyzed.

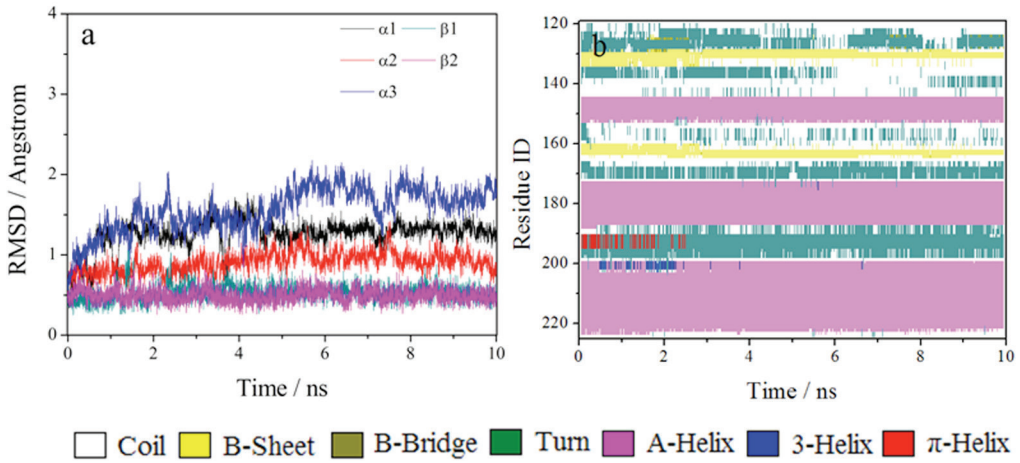

Figure 3. (a) Time evolution of RMSD of secondary structure of tPrPc (b) Secondary structure evolutions of tPrPc as function of time.

It was also found from the H-bond graphs (Figure 4) that there were three kinds of H-bond dynamics of the antiparallel β-sheet. It was discerned in Figure 4a that the H-bond distance changed gently and fluctuated at the value of 2.0 Å. And the change of the H-bond distance in Figure 4b was found to be jumping. The distance was keeping steadily at 2.1 Å at most simulation time, while there were three obvious jumping peaks during the MD process. The two wide peaks were appeared from 1.3 ns to 1.48 ns and from 2.4 ns to 3.14 ns, respectively. And both the distance values changed from 2.1 Å to 7.78 Å. With the help of trajectory analysis, it could be found that part of β1 was reversed and the H-bond formed between two β-strands was broken. The decrease of the H-bond distance was happened with the recovery of β1. The third peak was emerged at 5.0 ns just like an impulse with the increasing amplitude of 8 Å. And this change was also happened with the reversal of β-strand at short time. However, the H-bond composed of Ser132 and Val161 maintained the distance around 4.32 Å (Figure 4c) and it almost kept the value more than 3.5 Å during the whole simulation. According to the common definition of H-bond, 3.5 Å is the boundary of H-bond distance. Then the three kinds of H-bond were named as stable H-bond, meta-stable H-bond and unstable H-bond, respectively. It was observed from the trajectory movie in VMD that the antiparallel β-sheet was twisting with the reversal motion during the whole MD simulation. And the twisting dynamics was composed of the three kinds of H-bond thermodynamics motion.

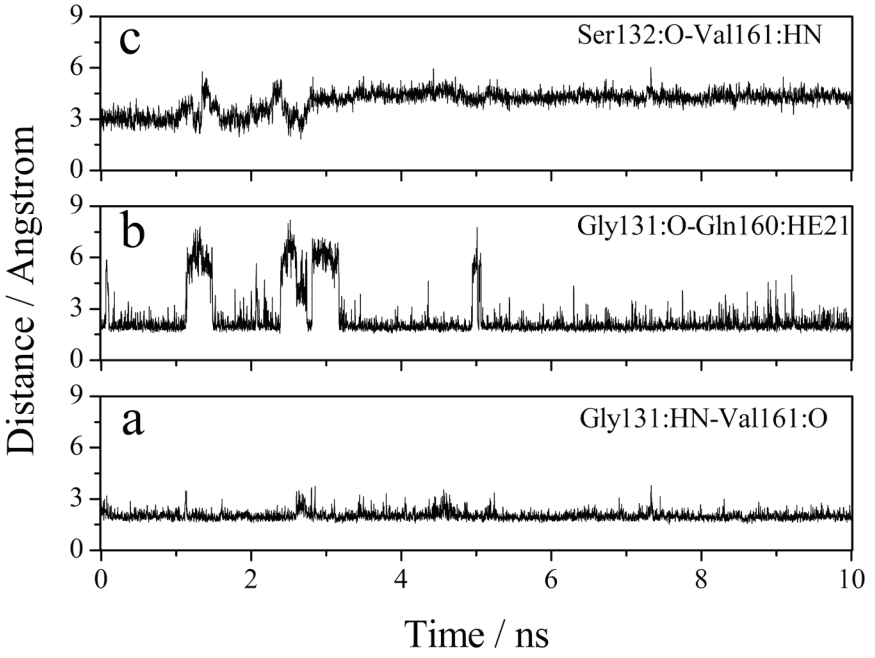

Figure 4. H-bond distance of β-sheet in tPrPc system with respect to MD simulation time. (a) stable H-bond (b) meta-stable H-bond (c) unstable H-bond.

The bvPrPc system

In bvPrPc system, the RMSD and Rg of the protein backbone had been dispalyed in Figure 5a and 5b. Both the two criterions proved that the bvPrPc system had reached an equilibrium state.

As shown in Figure 6, the two yellow belts of β-sheet in bvPrPc were rather stable during the simulation. Which were different from that in tPrPc. Helix 1 and 3 were more readily to unfold from one terminus. Residues 154-156 in α1 was frequently conversed to the turn structure before 7.8 ns. The C-terminal of protein was prone to unfold from α-helix (magenta) to turn (cyan). The distances of H-bonds Glu152-Arg156 and Glu221-Tyr225 involved in the unfolding of α1 and α3 were displayed in Figure 7.

The distance of Glu152-Arg156 was fluctuated around 4.0 Å before it gradually dropped to about 2.0 Å from 7.8 ns, and it was kept on that value to the end of simulation. It was consistent with the active motion of the Arg156-terminus of α1 shown in Figure 6. Similarly, the Glu221-Tyr225 curve could explain the dynamics of α3 shown in the structural evolution. It was fluctuated between 2.0 Å and 5.8 Å before 4.2 ns, and then converged to about 2.1 Å.

Comparation of the two systems

Average RMSD values of tPrPc and bvPrPc during the last 2.0 ns were 3.88Å and 3.30Å, respectively. And it indicated that the overall flexibility of tPrPc is greater than that of bvPrPc. Both the primary and tertiary structures of the two proteins were similarly, however, the dynamics of them were quite different during the 10 ns MD process. The β-sheet of tPrPc was frequently twisting during the simulation, however, it was rather stable in bvPrPc system. For α-helix, the α1 and α3 in bvPrPc were readily to unfold and more active than that in tPrPc.

FMD Simulation

In the two PrPc systems, after 10 ns MD simulation without any disturbances applied to the system, an equilibrium state was relegated to the FMD simulations as the starting state. During the FMD simulation, the FMD water atoms lie in the outermost layer of the water box and the external forces used as probe tips were applied on them. The FMD atoms were moving at a constant velocity and the other water molecules moved freely under periodic boundary conditions.

The force analyses of tPrPc for six directions with respect to simulation time were shown in Figure 8 as an example. The force peaks was related with the rigidity of the part of protein that FMD water molecules encountered in the simulation. The six curves was twisted and converged in the end of simulation. The first peak of each curve was the largest one, and it was resulted from the zero-beginning applying force. Except for the first peak, the other peaks were various in different directions. From 20 to 70 ps, the peaks of –z direction was notable, which was resulted by the rigidity of the helix crossed part of tPrPc. From 60 to 120 ps, the black curve of direction +x possessed the largest fluctuation among all the curves, which was also resulted by the encounter of helix-crossed part of tPrPc. In –y direction, the FMD waters could pass through the interval of helices and the value of the dark green line of this direction was

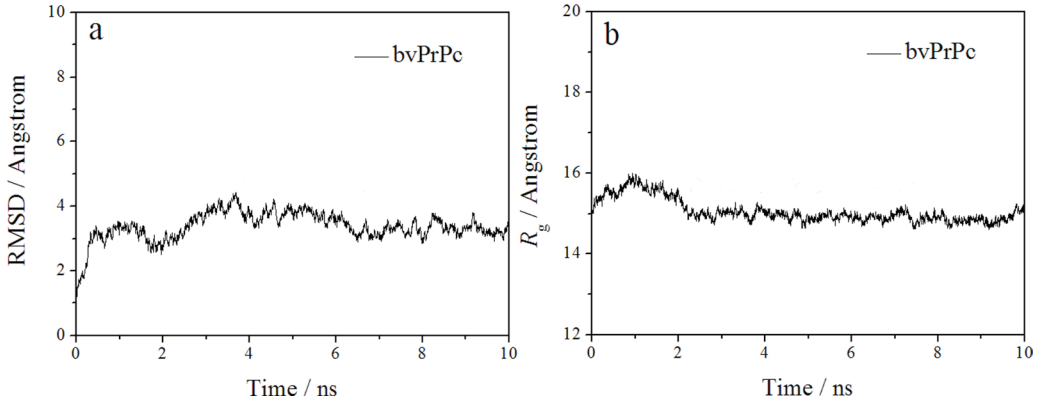

Figure 5. Time evolution of (a) RMSD and (b) radius of gyration of bvPrPc.

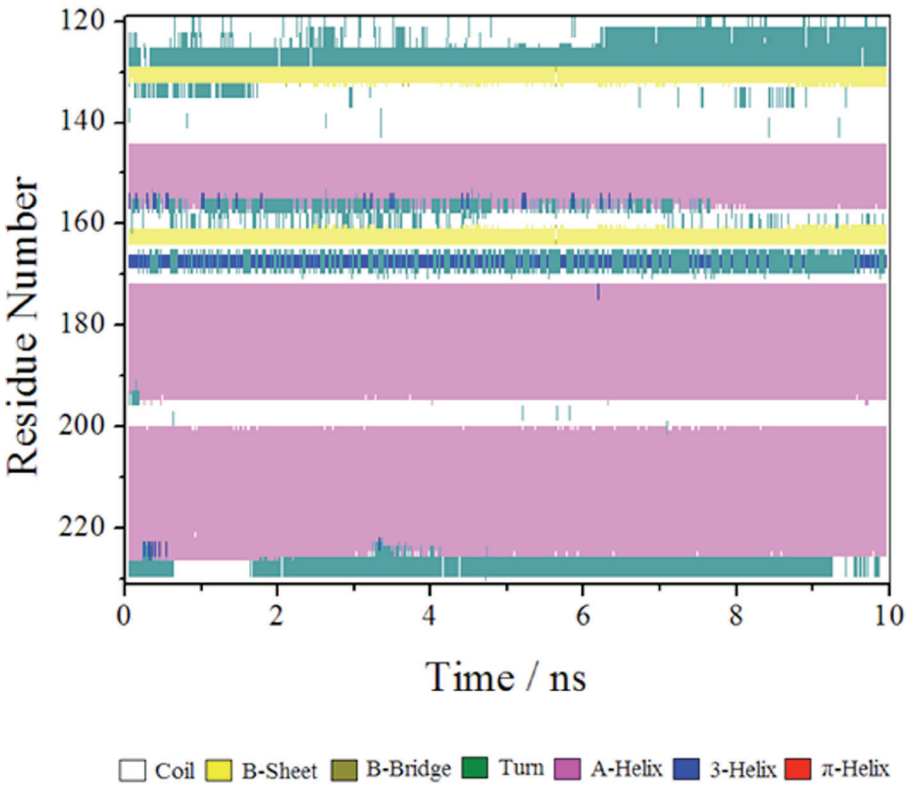

Figure 6. Secondary structure evolutions of bvPrPc as function of time.

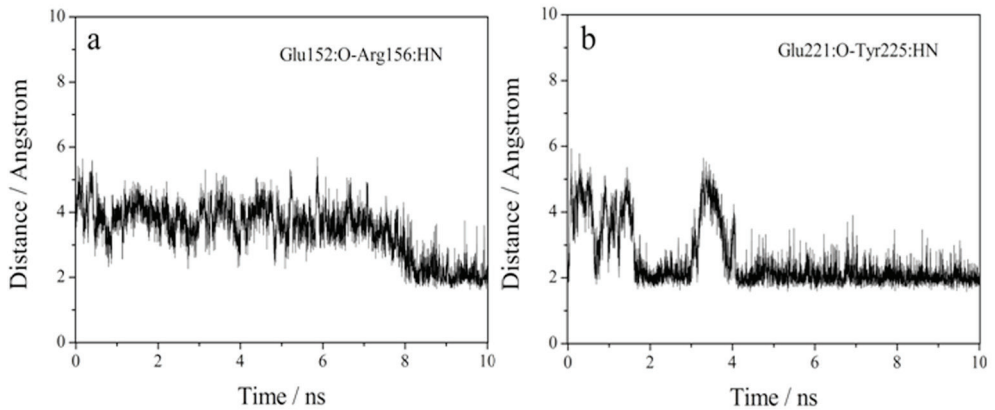

Figure 7. Time evolution of H-bond distances in bvPrPc system.

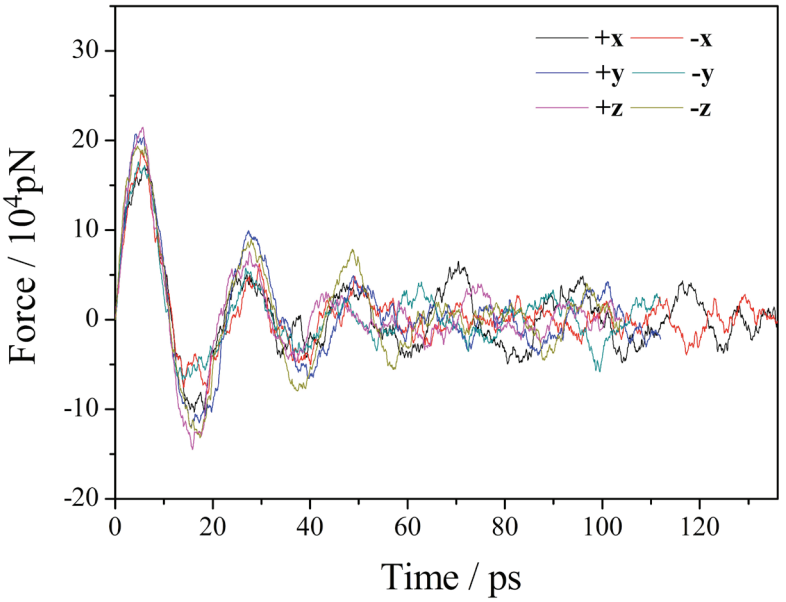

Figure 8. Time evolution of force of tPrPc in all the six directions during the FMD simulation.

small and covered in the force lines. With the analysis of FMD trajectory, it could be found that the three helices were staggered to form a protective wall in –z and +x directions to resist the external disturbance. Then the external force must be enlarged to keep the FMD waters moving in constant velocity. Yet the structure along –y direction was loose and the water molecules could easily pass through. In all the FMD simulations, there was no obvious structure deformation of tPrPc to be found from trajectory movie, which showed that the compact structure of tPrPc was rigid enough to resist the shear flow applied in this work. The compact helix wall was also found in rabbit prion proteins [36]. Similarly, the stability anisotropy was also found in bvPrPc system.

Conclusion

In this paper, MD and FMD simulations were combined to study the stability of tPrPc and bvPrPc at atomic level. Energy minimization and MD simulation were used to get the equilibrium state of protein. And FMD simulations were carried out to probe the rigidity of protein in different directions. It was found that the β-sheet was rather unstable in tPrPc and readily to be twisted during the MD process. Yet the helix in bvPrPc was more active than that in tPrPc and prone to be unfolded to the turn structure. It was also found that the global C-domain of the two model prion proteins was stable enough to resist the water turbulence, and the protective wall staggered with α-helix played key roles on stabilizing PrPc. However, the 10 ns MD simulation was short in this work. Optimized initial structure of FMD simulation obtained from long MD simulation may inspire the unfolding of helix. Further studies on this topic would be of significance for the understanding of the pathogenesis of prion diseases.

Acknowledgement

This work was financially supported by the National Natural Science Foundation of China (Grant No. 21003037 and No. 30900236) and the National Science Foundation of the Education Department of Henan Province (13A150085).

Conflict of Interest: The authors declare that there is no conflict of interest.

References

[1] Ji HF, Zhang HY. β-sheet constitution of prion proteins. Trends Biochem Sci 2010; 35(3):129-134.

[2] Caughey B, Chesebro B. Transmissible spongiform encephalopathies and prion protein interconversions. Adv Virus Res 2011; 56:277-311.

[3] Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982; 216(4542):136-144.

[4] Prusiner SB. Prions. Proc Natl Acad Sci USA 1998; 95(23):13363-13383.

[5] Aguzzi A, Polymenidou M. Mammalian prion biology: one century of evolving concepts. Cell 2004; 116(2):313-327.

[6] Stahl N, Baldwin MA, Teplow DB, Hood L, Gibson BW, Burlingname AL, Prusiner SB. Structural analysis of the scrapie prion protein using mass spectrometry and amino acid sequencing. Biochemistry 1993; 32(8):1991-2002.

[7] Caughey BW, Dong A, Bhat KS, Ernst D, Hayes SF, Caughey WS. Secondary structure analysis of the scrapie-associated protein PrP 27-30 in water by infrared spectroscopy. Biochemistry 1991; 30(31):7672-7680.

[8] Pan KM, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlorn I, Huang Z, Fletterick RJ, Cohen FE. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion protein. Proc Natl Acad Sci USA 1993; 90(23):10962-10966.

[9] Baker D, Sali A. Protein structure prediction and structural genomics. Science 2001; 294(5540):93-96.

[10] Marassi FM, Opella SJ. A solid-state NMR index of helical membrane protein structure and topology. J Magn Reson 2000; 144(1):150-155.

[11] Petsko GA, Ringe D. Fluctuations in protein structure from X-ray diffraction. Annu Rev Biophys 1984; 13:331-371.

[12] Legge FS, Budi A, Treutlein H, Yarovsky I. Protein flexibility: Multiple molecular dynamics simulation of insulin chain B. Biophys chem 2006; 119(2):146-157.

[13] Mayor U, Guydosh NR, Johnson CM, Grossmann JG, Sato S, Jas GS, Freund SM, Alonso DO, Daggett V, Fersht AR. The complete folding pathway of a protein from nanoseconds to microseconds. Nature 2003; 421:863-867.

[14] Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K. Scalable molecular dynamics with NAMD. J Comput Chem 2005; 26(16):1781-1802.

[15] Daggett V. Molecular dynamics simulations of the protein unfolding/folding reaction. Acc Chem Res 2002; 35(6):422-429.

[16] Rathore N, Yan Q, de Pablo JJ. Molecular simulation of the reversible mechanical unfolding of proteins. J Chem Phys 2004; 120(12):5781-5788.

[17] Jaspe J, Hagen SJ. Do protein molecules unfold in a simple shear flow? Biophys J 2006; 91(9):3415-3424.

[18] Szymczak P, Cieplak M. Hydrodynamic effects in proteins. J Phys Condens Matter 2011; 23(3):033102-033115.

[19] Santo KP, Berjanskii M, Wishart DS, Stepanova M. Comparative analysis of essential collective dynamics and NMR-derived flexibility profiles in evolutionarily diverse prion proteins. Prion 2011; 5(3):188-200.

[20] Chen J, Thirumalai D. Helices 2 and 3 Are the Initiation Sites in the $PrP^{C} \rightarrow PrP^{SC}$ Transition. Biochemistry 2013; 52(2):310-319.

[21] Perez DR, Damberger FF, Wuthrich K. Horse Prion Protein NMR Structure and Comparisons with Related Variants of the Mouse Prion Protein. J Mol Biol 2010; 400(2):121-128.

[22] Collinge, J. Prion diseases of humans and animals: their causes and molecular basis. Annu Rev Neurosci 2001; 24:519-550.

[23] Kale L, Skeel R, Bhandarkar M, Brunner R, Gursoy A, Krawetz N, Phillips J, Shinozaki A, Varadarajan K, Schulten K. NAMD2: Greater scalability for parallel molecular dynamics. J Comp Phys 1999; 151(1):283-312.

[24] MacKerell AD, Bashford D, Bellott, Dunbrack RL, Evanseck JD, Field MJ, Fischer S, Gao J, Guo H, Ha S, Joseph-McCarthy D, Kuchnir L, Kuczera K, Lau FTK, Mattos C, Michnick S, Ngo T, Nguyen DT, Prodhom B, Reiher WE, Roux B, Schlenkrich M, Smith JC, Stote R, Straub J, Watanabe M, Wiorkiewicz-Kuczera J, Yin D, Karplus MJ. All-atom empirical potential for molecular modeling and dynamics studies of proteins. Phys Chem B 1998; 102(18):3586-3616.

[25] Calzolai L, Lysek DA, Perez DR, Guentert P, Wuethrich K. Prion protein NMR structure of chickens, turtle and frog. Proc Natl Acad Sci USA 2005; 102(3):651-655.

[26] Christen B, Perez DR, Hornemann S, Wuthrich K. NMR structure of the bank vole prion protein at 20 degrees C contains a structured loop of residues 165-171. J Mol Biol 2008; 383(2):306-312.

[27] Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of simple potential functions for simulating liquid water. J Chem Phys 1983; 79(2):926-935.

[28] Izrailev S, Stepaniants S, Isralewitz B, Kosztin D, Lu H, Molnar F, Wriggers W, Schulten K. Steered Molecular Dynamics. *In* (Deuflhard P, Hermans J, Leimkuhler B, Mark AE, Reich S, Skeel RD) Lecture Notes in Computational Science and Engineering 1998; pp. 39-65, Springer-Verlag, Berlin.

[29] Raffaini G, Ganazzoli F. Molecular dynamics simulation of the adsorption of a fibronectin module on a graphite surface. Langmuir 2004; 20(8):3371-3378.

[30] Raffaini G, Ganazzoli F. Simulation study of the interaction of some albumin subdomains with a flat graphite surface. Langmuir 2003; 19(8):3403-3412.

[31] Ji HF, Zhang HY. A comparative molecular dynamics study on thermostability of human and chicken prion proteins. Biochem Bioph Res Co 2007; 359(3):790-794.

[32] Ji HF, Zhang HY, Shen L. The role of electrostatic interaction in triggering the unraveling of stable helix 1 in normal prion protein. A molecular dynamics simulation investigation. J Biomol Struct Dyn 2005; 22(5): 563-570.

[33] Santini S, Derreumaux P. Helix H1 of the prion protein is rather stable against environmental perturbations: molecular dynamics of mutation and deletion variants of PrP (90–231). Cell Mol Life Sci 2004; 61(7-8): 951-960.

[34] Gu W, Wang T, Zhu J, Shi Y, Liu H. Molecular dynamics simulation of the unfolding of the human prion protein domain under low pH and high temperature conditions. Biophys Chem 2003; 104(1):79-94.

[35] DeMarco ML, Daggett V. Mechanism for low pH triggered misfolding of the human prion protein. Biochemistry 2007; 46(11):3045-3054.

[36] Chen X, Jin XF, Liu YF, Zhang JL. Study on stability of rabbit prion protein by molecular dynamics simulation. Chemical Research 2012; 23(6):58-63.