Comparison studies of the structural stability of rabbit prion protein with human and mouse prion proteins

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Abstract

Background: Prion diseases are fatal and infectious neurodegenerative diseases affecting humans and animals. Rabbits are one of the few mammalian species reported to be resistant to infection from prion diseases isolated from other species (I. Vorberg et al., *Journal of Virology* 77 (3), 2003-2009 (2003)). Thus the study of rabbit prion protein structure to obtain insight into the immunity of rabbits to prion diseases is very important.

Findings: The paper is a straight forward molecular dynamics simulation study of wild-type rabbit prion protein (monomer cellular form) which apparently resists the formation of the scrapie form. The comparison analyses with human and mouse prion proteins done so far show that the rabbit prion protein has a stable structure. The main point is that the enhanced stability of the C-terminal ordered region especially helix 2 through the D177-R163 salt-bridge formation renders the rabbit prion protein stable. The salt bridge D201-R155 linking helix 3 and helix 1 also contributes to the structural stability of rabbit prion protein. The hydrogen bond H186-R155 partially contributes to the structural stability of rabbit prion protein.

Conclusions: Rabbit prion protein was found to own the structural stability, the salt bridges D177-R163, D201-R155 greatly contribute and the hydrogen bond H186-R155 partially contributes to this structural stability. The comparison of the structural stability of prion proteins from the three species rabbit, human and mouse showed that the human and mouse prion protein structures were not affected by the removing these two salt bridges. Dima et al. (*Biophysical Journal* 83, 1268-1280 (2002) and *Proceedings of the National Academy of Sciences of the United States of America* 101, 15335-15340 (2004)) also confirmed this point and pointed out that “correlated mutations that reduce the frustration in the second half of helix 2 in mammalian prion proteins could inhibit the formation of PrP^{Sc}”.

Key words: Prion diseases; Immunity; Rabbit prion protein; Molecular dynamics.

1 Introduction

Prion diseases, including Creutzfeldt-Jakob disease (CJD), variant Creutzfeldt-Jakob diseases (vCJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), Fatal Familial Insomnia (FFI), Kuru in humans, scrapie in sheep, bovine spongiform encephalopathy (BSE or ‘mad-cow’ disease) and chronic wasting disease (CWD) in cattle, etc., are invariably fatal and highly infectious neurodegenerative diseases affecting humans and animals. However, for treating all these diseases, there is no effective therapeutic approach ([Aguzzi et al., 2006, Prusiner, 1998, Weissmann, 2004]).

Many marvelous biological functions in proteins and DNA and their profound dynamic mechanisms ([chou, 1984a, 1984b, 1987, 1989, 1994, chou et al., 1988]) can be revealed by studying their internal motions ([Chou et al., 1988]). Likewise, to really understand the stability of rabbit prion molecules and their action mechanism, we should consider not only the static structures concerned but also the dynamical information obtained by simulating their internal motions or dynamic process ([Wang et al., 2009a, 2009b, 2009c, 2008a, 2008b, 2008c, 2009d, 2007a, 2007b, 2009e, 2009f, 2010]). Prion protein molecular dynamics (MD) simulation studies usually have been done on the C-terminal structured region, some C-terminal mutants, copper binding segments (e.g. HGGGW, GGGTH), and amyloid fibril segments (e.g. AGAAAAGA [Zhang, 2010b, Zhang et al., 2010c]). This paper is doing the MD simulation studies on the rabbit prion protein C-terminal structured region.

Rabbits are one of the few mammalian species reported to be resistant to infection from prion diseases isolated from other species ([Vorberg et al., 2003]). Recently, the NMR molecular structures of wild-type, mutant S173N and mutant I214V rabbit prion proteins (124-228) were released into the Protein Data Bank ([Berman et al., 2000]) with PDB ID codes 2FJ3, 2JOH, 2JOM respectively. Zhang (2010a) studied these NMR structures by MD simulations and simulation results at 450 K under low and neutral pH environments confirmed the structural stability of wild-type rabbit prion protein. However, the previous MD simulation results at 500 K under neutral pH environment showed that wild-type rabbit prion protein (124-228) does not have more structural stability than human prion protein (125-228) (PDB ID code 1QLX) and mouse prion protein (124-226) (PDB ID code 1AG2) but the opposite holds ([Zhang, 2009]). In [Zhang, 2010a], the author did not carry on the MD simulations at 450 K under low and neutral pH environments for human prion protein (125-228) and mouse prion protein (124-226); this paper will do this work. Findings of this paper agree with the findings of [Zhang, 2010a], but disagree with the conclusion of [Zhang, 2009]. In order to further confirm the findings, this paper will do longer simulations than [Zhang, 2009, Zhang, 2010a].

The differences between [Zhang, 2009; Zhang, 2010a] are at: (1) Zhang (2009) did not do the simulations under low pH environments, but Zhang (2010a) did; (2) Zhang (2009) did the simulations at 500 K, but Zhang (2010a) did the simulations at 450 K; (3) Zhang (2009) did the simulations by Amber 8, but Zhang (2010a) by Amber 9; (4) Zhang (2009) did not study any mutant of rabbit prion protein, but Zhang (2010a) studied the mutants of rabbit prion protein; (5) Zhang (2009) studied human and mouse prion proteins, but Zhang (2010a) did not study human and mouse prion proteins; (6) In [Zhang, 2009] the time of equilibrations done in constant NPT ensembles under Berendsen thermostat was 20 ns, but in [Zhang, 2010a] the equilibrations were done in constant NPT ensembles under Langevin thermostat for 5 ns; the productions for [Zhang, 2009] were under Berendsen thermostat at 500 K, but for [Zhang, 2010a] the productions were under Langevin thermostat at 450 K.

The infectious prion protein (124-228) (PrP^{Sc}) is an abnormally folded form of the normal cellular prion protein (124-228) (PrP^{C}) and the conversion of PrP^{C} to PrP^{Sc} is believed to involve conformational change from a predominantly α -helical protein (42% α -helix, 3% β -sheet) to a protein rich in β -sheets (30% α -helix, 43% β -sheet) ([Cappai et al., 2004, Daude, 2004, Ogayar et al., 1998, Pan et al., 1993, Reilly, 2000]). This paper studies the NMR structures of wild-type human, mouse, and rabbit prion proteins by computer MD simulations, where the NMR structure of PrP^{C} consists of β -strand 1, α -helix 1, β -strand 2, α -helix 2 and α -helix 3. Simulation results of this paper show that the salt bridges D177-R163, D201-R155 greatly contribute to the structural stability of rabbit prion protein. We also find that the hydrogen bond H186-R155 partially contributes to the structural stability of rabbit prion protein.

2 Materials and methods

Simulation initial structure for the rabbit prion protein was built on RaPrP^C(124-228) (PDB entry 2FJ3). Identical simulations were also done for human prion protein (HuPrP^C(125-228)) and mouse prion protein (MoPrP^C(124-226)). The initial simulation structures of human and mouse prion proteins were built on PDB entries 1QLX ([Berman et al., 2000, Zahn et al., 2000]) and 1AG2 ([Berman et al., 2000, Riek et al., 1996]), respectively. Simulation methods are completely same as the ones of [Zhang, 2010a]. 16 Cl⁻, 14 Cl⁻, 14 Cl⁻, and 5599 waters, 3836 waters, 5909 waters were separately added for the human, mouse, rabbit prion proteins under low pH environment. The step size of 2 fs is typical for the SHAKE algorithm at 300 K. At 450 K, 1 fs was used as the step size. For prion proteins some MD works have been done at 500 K (e.g. [El-Bastawissy et al., 2001, Sekijima et al., 2003, Shamsir et al., 2005, Yang et al., 2005]); this paper does the MD simulations at 450 K and these simulations are not physically relevant.

Simulations were done under low pH and normal pH environments respectively. All the simulations were performed with the AMBER 9 package ([Case et al., 2006]), with analysis carried out using functionalities in AMBER 9 and AMBER 7 CARNAL ([Case et al., 2002]). Graphs were drawn by XMGRACE of Grace 5.1.21, DSSP

([Kabsch et al., 1983]). The AMBER ff03 force field was used. The van der Waals and electrostatic interactions were treated by SHAKE algorithm and PMEMD algorithm with nonbonded cutoffs of 12 angstroms. The systems were surrounded with TIP3PBOX water molecules and neutralized by sodium ions, and optimized to remove bad hydrogen contacts. Then the systems were heated from 100 K to 450 K step by step during 3 ns. The thermostat algorithm used is the Langevin thermostat algorithm in constant NVT ensembles. Equilibrations were done in constant NPT ensembles under Langevin thermostat for 5 ns. After equilibrations, production MD phase was carried out at 450 K for 20 ns using constant pressure and temperature ensemble. All the simulations were performed on the Tango facilities of the Victoria Partnership for Advanced Computing of Australia (<http://www.vpac.org>).

3 Results and discussion

The MD simulations done at room temperature 300 K whether under neutral or low pH environment display very little fluctuation and no variation among rabbit, human and mouse prion proteins. At 450 K there are fluctuation and variation among rabbit, human and mouse prion proteins, but their backbone atom RMSDs (root mean square deviations) respectively calculated from their minimized structures and their radii of gyration do not have great difference even under low pH environment (Fig.s 1-2). Their secondary structures under neutral pH environment at 450 K, under low (Fig. 3) and neutral pH environments at 300 K, do not change very much either. However, the secondary structures under low pH environment at 450 K have great differences between rabbit prion protein and human and mouse prion proteins (Fig.s 4-5): the α -helices of rabbit prion protein were completely unfolded and began to turn into β -sheets but those of human and mouse prion proteins were not changed very much. These results indicate the C-terminal region of RaPrP^C has lower thermostability than that of HuPrP^C and MoPrP^C. Under the low pH environment, the salt bridges D177-R163, D201-R155 were removed (thus the free energies of the salt bridges changed the thermostability) so that the structure nearby the central helices 1-3 was changed for rabbit prion protein.

There always exist salt bridges (where the oxygen-nitrogen distance cut-off calculated for the salt bridges is 3.2 angstroms) between D202-R156, D178-R164 for human and mouse prion proteins, between D201-R155, D177-R163 for rabbit prion protein. The disulfide bond between C178-C213 links the 2nd and 3rd α -helices and keeps several strong salt bridges linked. The salt bridge between D177-R163 keeps the linkage of the middle of α -helix 2 and the coil at β -sheet 2 to α -helix 2. The salt bridge between D201-R155 makes the 3rd and 1st α -helices linked. The salt link distances of D177-R163 and D201-R155 are illustrated in Fig.s 6-8. Except for the short period of break of the salt bridge N202-R156 of human prion protein during 8.5-10.5 ns, the salt bridges are always occupying human, mouse, and rabbit prion proteins. Under low pH environment, all these salt bridges were removed. They did not change the secondary structures of human and mouse prion proteins very much. However, rabbit prion protein is very sensitive to the low pH environment: the remove of these salt bridges led to the collapse of the stable helical structure of wild-type rabbit prion

protein. Structural stability of a protein is determined by factors of hydrogen bond, van der Waals force, hydrophobic interaction, and salt bridge; change from neutral to low pH environments will break the salt bridges only. Thus, we might be able to say that salt bridges D177-R163, D201-R155 greatly contribute to the structural stability of rabbit prion protein. RaPrP^C(124-228) apparently resists the formation of the scrapie form, and the enhanced stability of the C-terminal ordered region especially helix 2 through the D177-R163 salt-bridge formation renders the rabbit prion protein stable. The analyses of human and mouse prion proteins agree with the results of [Dima et al., 2002, Dima et al., 2004], where human and mouse prion proteins were showed lack of stability and correlated mutations that reduce the frustration in the second half of helix 2 in mammalian prion proteins could inhibit the formation of PrP^{Sc}.

The MD simulation experience of the NMR structure of human prion protein variant D178N (PDB ID: 2K1D, released on 03-MAR-2009) ([Mills et al., 2009]) of the author confirmed that the secondary structural performance of the D178N mutant under neutral pH environment at 450 K is same as that of the wild-type rabbit prion protein under low pH environment at 450 K. Though the salt bridge R163-D177 in the structure of prion proteins spans only 14 residues and it should have a rather impact on the local structure, human and mouse prion proteins do not have the stable local structure kept by the salt bridge R164-D178. This agrees with experimental results of [Liemann et al., 1999], which showed a reduced stability if D178 was mutated into N178 for human prion protein. The MD simulation experience of the homology structure of rabbit prion protein of Zhang et al. (2006) also confirmed that rabbit prion protein has a more stable structure than human and mouse prion proteins, and the salt bridge and hydrogen bond between D177 and R163 plays a key important role to this stability.

Recently Zhong (2010) reported that for human prion protein (PDB entry 1QM0) the mutation of H187 into R187 makes the hydrogen bond H187-R156 broken, and the strong electrostatic repulsion between R187 and R156 drives both positively charged side chains away from their original positions leaving their hydrophobic core to be solvent accessible. For rabbit prion protein at 450 K under neutral pH environment, there is the hydrogen bond H186-R155 during more than 26.44% time of the 20 ns production trajectories; however, for mouse (PDB entry 1AG2) and human (PDB entry 1QLX) prion proteins at 450 K under the neutral pH environment, the hydrogen bond between H187 and R156 has been broken during the whole 20 ns production trajectories. The hydrogen bond H186-R155 clearly partially contributes to the structural stability of rabbit prion protein.

Besides pH deviations and the choice of temperatures, point mutations from wild-type can identify more contributing factors for the extraordinary stability of rabbit prion protein. Through the study of wild-type and its S173N variant, recently Wen et al. (2010a) suggested that the ordered loop 165-172 and its interaction with helix 3, together with the unique distribution of surface electrostatic potential, significantly contribute to the unique structural characteristics of RaPrP^C; most recently, Wen et al. (2010b) studied the I214V mutant of rabbit prion protein and suggested that this mutation results in a prominent global shift of surface charge distribution and an in-

crease of backbone flexibility on a ps-ns time scale. Sweeting et al. (2009) pointed out the rabbit prion mutants of S170N, S174N, and S170N/S174N break the hydrogen bond network of residues 170-174.

The choice of temperatures is important and the choosing 450 K for this paper is very significant. At 300 K, the MD results display little difference among rabbit, human and mouse prion protein structures whether under neutral or low pH environments. At 500 K, Zhang (2009) found that under neutral pH environment the structure of rabbit is not more stable than these of human and mouse but the opposite holds. However, at 450 K, this present paper found that the structure of rabbit is much more stable than these of human and mouse; this agrees with the experimental results of rabbit prion protein and shows that the choosing 450 K of this paper is significant.

Recent experimental results on mouse and rabbit prion proteins showed that rabbit specific residues surrounding the GPI anchor attachment site inhibit prion infection ([Nisbet et al., 2010]). We find rabbit prion protein has a C-terminal residue R227 forming a hydrogen bond/salt bridge network with other inner residues but mouse prion protein has no such an arginine residue at the end of C-terminal.

4 Conclusion

The paper is a straight forward MD simulation study of rabbit prion protein (monomer cellular form) which apparently resists the formation of the scrapie form. The analyses of MD simulation results confirmed the structural stability of rabbit prion protein under neutral pH environment. The main point is that the enhanced stability of the C-terminal ordered region especially helix 2 through the D177-R163 salt-bridge formation renders the rabbit prion protein stable. The salt bridge D201-R155 linking helix 3 and helix 1 also contributes to the structural stability of rabbit prion protein. The comparison of the structural stability of prion proteins from the three species rabbit, human and mouse showed that the human and mouse prion protein structures were not affected by the removing these two salt bridges. We also find that the hydrogen bond H186-R155 partially contributes to the structural stability of rabbit prion protein.

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Figure 1: Backbone atom RMSD graphs for human, mouse, rabbit prion proteins.

Figure 2: Radius Of Gyration graphs for human, mouse, rabbit prion proteins.

Figure 3: Snapshots of human, mouse, and rabbit prion proteins at 300 K under low pH environment.

Figure 4: Snapshots of human, mouse, and rabbit prion proteins at 450 K under low pH environment.

Figure 5: Secondary structures of rabbit, human and mouse prion proteins (from up to down) at 450 K under neutral and low (from left to right) pH environments. (red: α -Helix, pink: π - Helix, yellow: 3_{10} -Helix, green: β -Bridge, blue: β -Sheet, purple: Turn, Black: Bend; x-axis: time (0 ns-20 ns), y-axis: residue numbers.)

Figure 6: Salt Bridges of human prion protein at 450 K under neutral pH environment.

Figure 7: Salt Bridges of mouse prion protein at 450 K under neutral pH environment.

Figure 8: Salt Bridges of rabbit prion protein at 450 K under neutral pH environment.