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

Jiapu Zhang *

Centre for Informatics and Applied Optimization, Graduate School of Information Technology and Mathematics Sciences, The University of Ballarat, MT Helen Campus, Victoria 3353, Australia

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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) 2003–2009). 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 helixes 3 and 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 (2002) 1268–1280 and Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 15335–15340) 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}".

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 and Heikenwalder, 2006; Prusiner, 1998; Weissmann, 2004).

Many marvelous biological functions in proteins and DNA and their profound dynamic mechanisms (Chou, 1984a, 1984b, 1987, 1989; Chou et al., 1994; Chou and Mao, 1988) can be revealed by studying their internal motions (Chou, 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 and Chou, 2009a; Wang and Wei, 2009b; Wang et al., 2009c, 2009d, 2009e, 2009f, 2008a, 2008b, 2008c, 2007a, 2007b, 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 and Sun, 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

* Tel.: +61 423487360.
E-mail addresses: jiapu_zhang@hotmail.com, j.zhang@ballarat.edu.au

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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, 2010a).

The differences between Zhang (2009) and Zhang (2010a) are: (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; and (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) (Cappaia and Collinsa, 2004; Daude, 2004; Ogayar and Snchez-Prez, 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). $16Cl^{-}$, $14Cl^{-}$, $14Cl^{-}$, 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 and Dalby, 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 and Sander, 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 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 gyrations do not have great difference even under low pH environment (Figs. 1 and 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 (Figs. 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 Figs. 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,