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Studies on the structural stability of rabbit prion probed by molecular dynamics simulations

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Abstract: Prion diseases are invariably fatal and highly infectious neurodegenerative diseases that affect humans and animals. Rabbits are the only mammalian species reported to be resistant to infection from prion diseases isolated from other species. At the end of 2007 the NMR structure of rabbit prion (124-228) was deposited into protein data bank (PDB entry 2FJ3). This paper studies the inhibition mechanism of rabbit prion at molecular structural level by molecular dynamics simulations.

1 Introduction

Prion diseases are invariably fatal neurodegenerative diseases that affect humans and animals. Unlike most other neurodegenerative diseases, these can be highly infectious. They include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), Fatal Familial Insomnia (FFI), Kuru in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE or 'mad-cow' disease) in cattle, etc. Transmission across the species barrier to humans, especially in the case of BSE in Europe and CWD (chronic wasting disease) in North America, is a major public health concern. Since 1996, variant Creutzfeldt-Jakob diseases (vCJD) have been found even in young people in UK. However, there is no effective therapeutic approach for treating all these diseases ([6, 8, 1]).

Rabbits are the only mammalian species reported to be resistant to infection from prion diseases isolated from other species ([7]). In 2007, the NMR structure of rabbit prion (124-228) was deposited into Protein Data Bank ([2]) with PDB ID code 2FJ3 ([5]); this gives us a golden opportunity to study the inhibition mechanism of rabbit prion and to find an effective therapeutic approach to prion diseases. This paper studies the inhibition mechanism at molecular structural level.

The N-terminal residues (1-123) of prions are unstructured and not included in this study. The C-terminal residues (124-228) are well structured, with 3 α-helices, 2 short anti-parallel β-sheets, and a disulfide bond between the 2nd and 3rd α-helix (residues number 178 and number 213). The infectious prion (PrP^{Sc}) is an abnormally folded form of the normal cellular prion (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). In this paper we use molecular dynamics (MD) simulations to investigate whether the

resistance of rabbit prion could be due to increased stability of the rabbit prion protein or not.

2 Materials and methods

The initial structure for the rabbit prion simulations was built on RaPrP^{C}(124-228) (PDB entry 2FJ3). The simulations were done starting from more than 3 different initial velocities (we call them seeds) in order to get a conclusion from all observations of simulation results. To further confirm the conclusion, identical simulations were also done for human prion (HuPrP^{C}) and mouse prion (MoPrP^{C}) proteins. The initial simulation structures of human and mouse prion proteins were the NMR structures stored in Protein Data Bank: HuPrP^{C}(125-228) (PDB ID code: 1QLX) and MoPrP^{C}(124-226) (PDB ID code: 1AG2). All the simulations were performed with the AMBER 9 package ([4]), with analysis carried out using functionalities in AMBER 9 and AMBER 7 CARNAL ([3]). Graphs were drawn by XMGRACE of Grace 5.1.21.

All simulations used the ff03 force field of the AMBER 9 package, in neutral pH environment. The systems were surrounded with a 12Å layer of TIP3PBOX water molecules and neutralized by sodium ions using XLEaP module of AMBER 9. The solvated proteins with their counterions were minimized mainly by the steepest descent method and then a small number of conjugate gradient steps were performed on the data, in order to remove bad hydrogen contacts. Then the solvated proteins were heated from 100K to 300K linearly during 300ps and then kept at 300K for 700ps, both in constant NVT ensembles using Langevin thermostat algorithm. The SHAKE algorithm and PMEMD algorithm with nonbonded cutoffs of 12$\mathring{A}$ were used during the heating. The solvated proteins were also heated to 450K in the same way. Equilibrations were done in constant NPT ensembles under Berendsen thermostat for many nanoseconds. After equilibrations, production MD phase was carried out at 300K or 450K for 15ns using constant pressure and temperature ensemble and the PMEMD algorithm with nonbonded cutoffs of 12Å during simulations. Step size for equilibration was 0.5fs and 1fs for the production runs. All simulations were performed on the Tango facilities of the Victorian Partnership for Advanced Computing (VPAC) of Australia. The structures were saved to file every 1000 steps.

3 Results and discussion

The MD simulations done at room temperature 300K displayed very little fluctuation and no variation among three species. In what follows we only show and analyze the results at 450K. Among many seeds of simulations, we found two seeds for which RaPrP differs from HuPrP and MoPrP very much; we denote them as seed1 and seed2 separately. For all backbone atoms of RaPrP^{C}(124-228), Figure 1 shows the root mean square deviations (RMSDs) from the minimized structure. Radii of gyrations of RaPrP^{C}(124-228) are shown in Figure 1 too. We may see that for seed1 the RMSD increases steadily and still tends to go up after 15ns. For seed2, RMSDs increase sdramatically and will still increase after 15ns and the radii of gyrations seem to still steadily go up from 10ns.

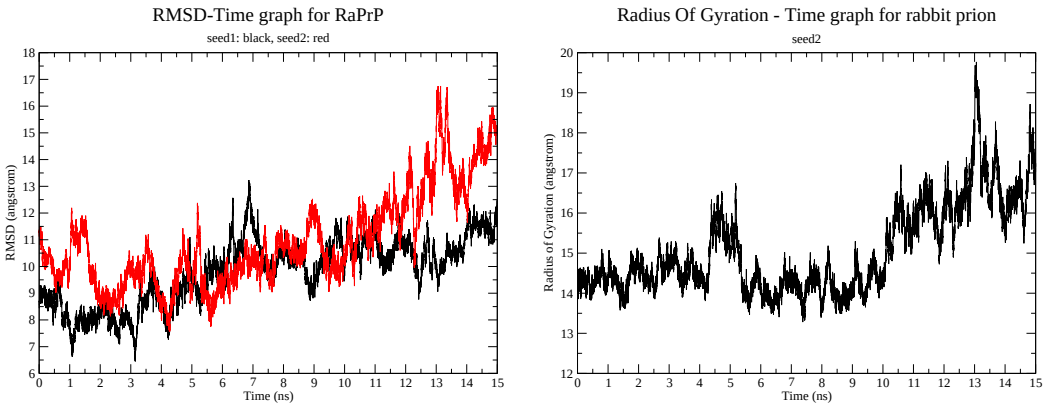

Figure 1: RMSD and Radius Of Gyration graphs for rabbit prion.

In order to make comparisons, the RMSD and radius of gyration graphs of seed1 and seed2 for HuPrP and MoPrP are also shown (see Figures 2 and 3). In Figure 2 we see that HuPrP and MoPrP have leveled-off RMSD values as compared with RaPrP for both seed1 and seed2. With regard to the radii of gyrations of HuPrP and MoPrP, we see that in Figure 3 the values fluctuate around their averaged values (for seed1, RaPrP also has this property).

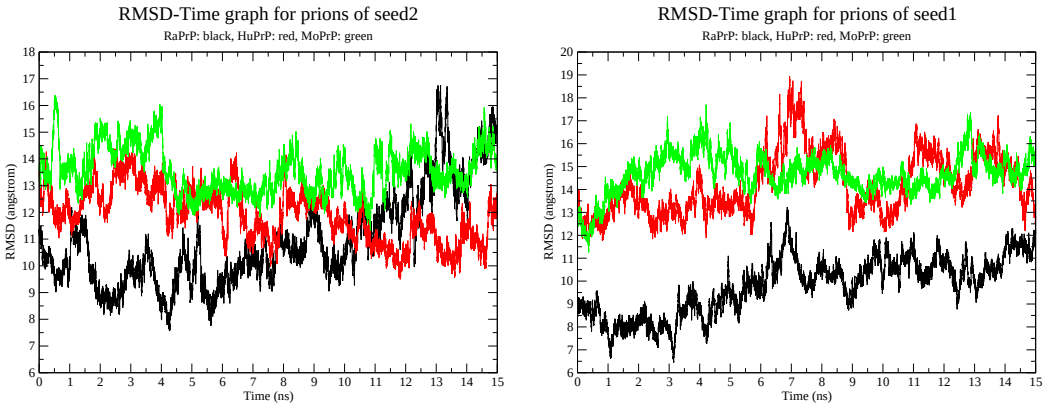

Figure 2: RMSD graphs of rabbit prion, compared with human and mouse prions.

4 Conclusion

MD simulation results show that RaPrP^{C}(124-228) does not have more structural stability than HuPrP^{C}(125-228) and MoPrP^{C}(124-226); instead, the opposite holds. Properties of the structural stability of rabbit prion protein might be found from its N-terminal unstructured region.

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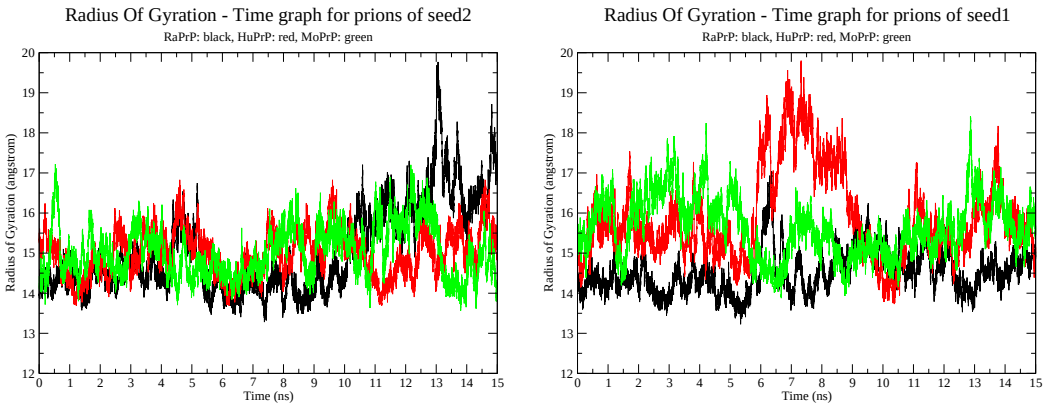

Figure 3: Radius of Gyration graphs of rabbit prion, compared with human and mouse prions.

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