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RESEARCH ARTICLE

Molecular Dynamic Perspective of Misfolded Construction and Effect of Changes on Secondary Structural Elements of Sheep Prion Protein

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Abstract

Prion diseases (transmissible spongiform encephalopathies) are rare degenerative diseases of the brain caused by a protein that converts to an abnormal form called prion. Scrapie is a fatal, degenerative disease that affects the nervous system of sheep and goats. This disease is one of several transmissible spongiform encephalopathies (TSEs), which are often related to bovine spongiform encephalopathy (BSE or "mad cow disease"). The objective of the present study was to investigate the differences in the molecular properties of sheep prion protein due to their misfolded construction and as well as the effects on changes in their secondary structural elements. In this study, the percentage and the position of secondary structural elements of the protein peptide was predicted by molecular dynamics showed solvent-dependent internal stabilizing forces of the structure and evidenced a higher mobility of the residues following the end of helix H1. This structural duality of the peptide was reminiscent of the overall conformational transition of PrP (Prion protein) from helix to β-sheet. From the result of SOPMA, it was known that 19-21st (coil region) residues were predicted as strand region. During the pathogenic conversion, helix H1 and its two flanking loops of the normal prion protein were thought to undergo a conformational transition into a β-like structure. So the result confirmed that if increase the molecular dynamics studies to this protein would reveal the sheet conformation in the particular region of the protein. The experimental results obtained in the present work showed that this peptide can also fold into the helix H1 conformation when dissolved in a TFE/PB mixture.

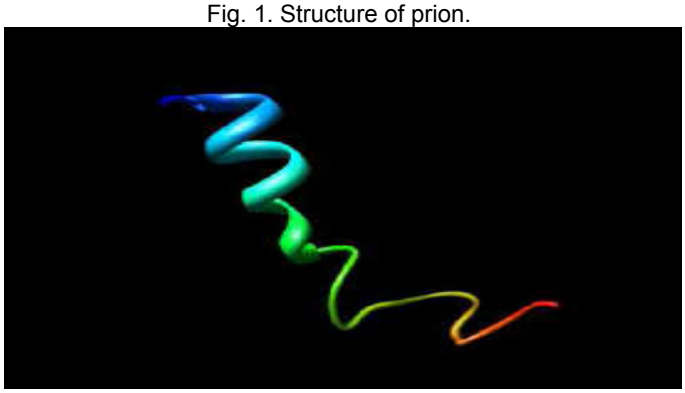
Keywords: Prion diseases, spongiform encephalopathies, molecular dynamics, SOPMA, GROMACS.

Introduction

Prion is a proteinaceous as well as an infection causing particle that lacks nucleic acid. Prions are much smaller than viruses and differ from all living cells because they do not contain any genetic material. A normal protein, cellular prion protein (PrPC) changes shape and becomes an abnormal protein molecule called scrapie prion protein (PrPsc) (Prusiner, 1997). PrPC has 42% of its residues folded in alpha-helices and 3% as beta-sheets, whereas PrPsc is composed of 30% alpha helices and 43% sheets. Evidences showed that this abnormal conversion was due to the conformational transition into a β-like structure from helix H1 and its two flanking loops of the normal prion protein. The newly formed prion then converted nearby PrPC into PrPsc prions and the process was spontaneous. Also these prions never convert back into PrPC (Manuelidis *et al.,* 2007). This altered structure was extremely stable and accumulate in infected tissue, causing tissue damage and cell death (Prusiner, 1991; Christopher *et al.,* 2004). This had been noticed in Bovine Spongiform Encephalopathy (BSE, also known as "Mad Cow Disease") in cattle, Creutzfeldt-Jakob disease (CJD) in humans and Scrapie Disease in sheep.

Prions are resistant to denaturation by chemical and physical agents, making disposal and containment of these particles difficult (Wells *et al.,* 1987). Proteins showed prion-type behavior are also found in some fungi and this has been quite important in helping to understand mammalian prions. However, fungal prions are not appear to cause disease in their hosts and may even confer an evolutionary advantage through a form of protein-based inheritance (Douglas *et al*., 2002). Prions are hypothesized to infect and propagate by refolding abnormally into a structure which is able to convert normal molecule of the protein into the abnormally structured form (Andrew *et al*., 2006). These dynamic studies of prion protein provide insights to detail the mechanism of scrapie, Creutzfeldt-Jakob disease (CJD), Bovine Spongiform Encephalopathy (BSE) and Gerstmann-Straussler-Scheinker syndrome (GSS) (Christl and Donnelly, 2004). The present study was aimed to investigate the difference in the molecular properties of the prion protein models due to their misfolded construction as well as the effects of changing their secondary structural elements in its domain.

Fig. 1. Structure of prion.



Materials and methods

Experimental design: The prion protein of sheep was taken from the Protein Data Bank (PDB: 1M25) (Berman *et al.,* 2007) (www.rcsb.org/pdb) which is a repository of x-ray and NMR determined 3D data (Fig. 1). The sequence length of this protein is 26, starts from glycine (G) and ends with cysteine (C). The non-pathogenic cellular isoform of the prion (PrPc) is a strongly conserved cell surface glycoprotein expressed in all mammalian species. The PDB file was converted to GROMACS readable file format and the topology file was written by using specific command pdb2gmx. The protein was then solvated using genbox command, this genbox program added the correct number of water molecules needed to solvate the protein. Energy minimization was then performed using Conjugate Gradient and Newton-Raphson methods (Stitch *et al.,* 1989; Fujiwara *et al*., 2005). Before molecular dynamics simulation, it is necessary to remove the bad contacts like overlapping atoms and distortion bond angles. The reason is that if start with the molecular dynamics with the bad contacts, the energy in that region will be high and that can either crash the simulation or cause the trajectory to proceed in an unrealistic direction. Even if there are no obvious bad contacts, it is still recommended to run a short energy minimization to relax the structure. Thus, the protein was minimized using conjugate gradient algorithm for about 10 ps.

To initialize dynamics, the solvated and minimized protein must be brought up to the temperature of interest. This was done by assigning velocities at low temperature and then running dynamics according to the equations of motion. After few number of iterations of dynamics, the temperature was scaled upwards. The most common means of temperature scaling is velocity scaling. This was done systematically during the equilibration stage. A typical time step of 1 fs (10-15), equilibration was run for at least 5 ps and often for 10 or 20 ps. To run a molecular dynamic simulation following are required:

- A file containing the coordinates for all atoms.
- Information on the interactions (bond angles, charges, Van der Waals).
- Parameters to control the simulation.

The .pdb or .gro file contained the coordinates for all atoms and this was the input structure file for MD simulation. The interactions were listed in the topology (.top) file and the input parameters were put into .mdp file.

Softwares
Operating system: LINUX CENTOS 5.0
Secondary structure prediction tools: SOPMA (Geourjon and Deleage, 1995).
Molecular dynamics: GROMACS 3.3.3 (Erik Lindahl *et al.,* 2001).
Visualization tools: Sirius (Singh and Thornton, 1990) XMGR 4.2.3 UCSF Chimera (Petersen *et al.,* 2004), PyMOL (DeLano, 2002).

Results and discussion

Energy minimization: Molecular Dynamics simulation was performed using GROMACS with NTP (Moles, Temperature and Pressure) ensemble and these parameters are conserved in dynamics. The initial energy minimization and equilibration for 20 pico sec was done and the results were presented. The bad contacts were removed by performing energy minimization. The energy minimization was performed in two stages. In the first stage, only the water molecules were minimized and the protein was restrained. In second stage, the whole system was relaxed. The favorable least energy was attained as a result of 500 cycles of Steepest Descent, followed by 500 cycles of Conjugate Gradient Minimization techniques. The energy and RMSD (Root Mean Square Deviation) values are presented in Table 1.

Table 1. Energy and RMSD value of prion protein.

Energy Profile	Energy value		RMSD value	
	Initial	Final	Initial	Final
Prion Protein (PrP sc) Stage I	-2.1719E +04	-3.4371E +04	1.2166E +02	1.5004E +00
Prion Protein (PrP sc) Stage II	-3.4647E +04	-3.6739E +04	4.4806E +00	2.5011E -01

Equilibration: Berenson coupling was used for both equilibration and production phase of the system. Figure 2 showed that the temperature was raised from the Zero Kelvin and reached the equilibrium (300 K) at about 7.2 ps and remained same thereafter.

Production Run (MD): Figure 3 depicted the reliability and stability of the simulated systems. The kinetic energy varied from 1165.7914 KJ/mol to 5709.1628 KJ/mol, the potential energy varied from -333149.2726 KJ/mol to -27909.9723 KJ/mol and the total energy varied from -31983.4813 KJ/mol to -22423.2928 KJ/mol.

Fig. 2. Temperature (in K) Vs Time (in ps) plot.

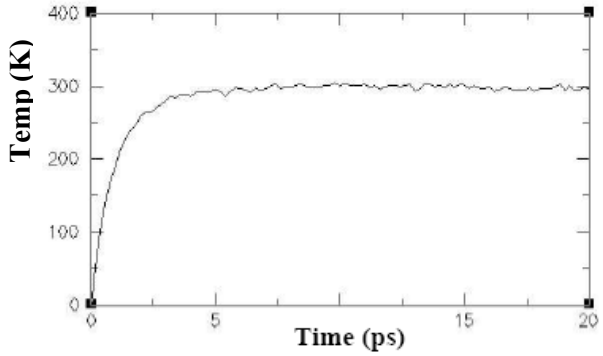
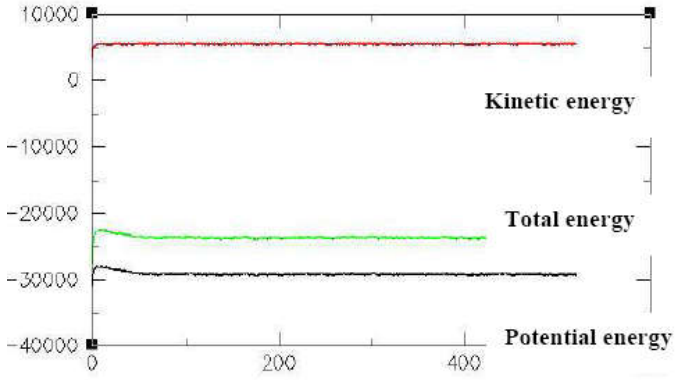


Fig. 3. Time (in ps) Vs Total, Potential & Kinetic energy for prion protein (in KJ/mol).



Backbone RMSD comparison: Figure 4 showed that the conformational fluctuation in prion protein. These fluctuations represent the formation of sheet structure. Each frame was trapped at every 0.5 ps. Further simulation was carried out for 11-16th residues (Fig. 5). From Fig. 6, it was visibly seen that the residues from 11-16th (green and red color) at 1 nano second and they were broken down which lead to the formation of sheet structure.

Fig. 4. Backbone RMSD of prion protein.

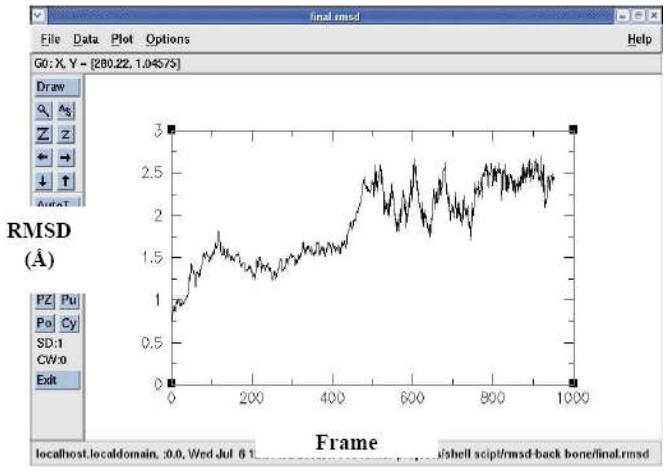


Fig. 5. Region of 11-16th residues in helix structure at the initial stage (before reconstruction).

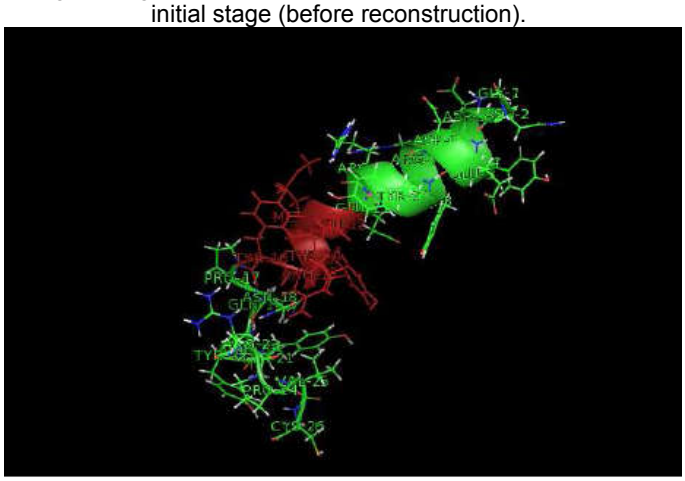
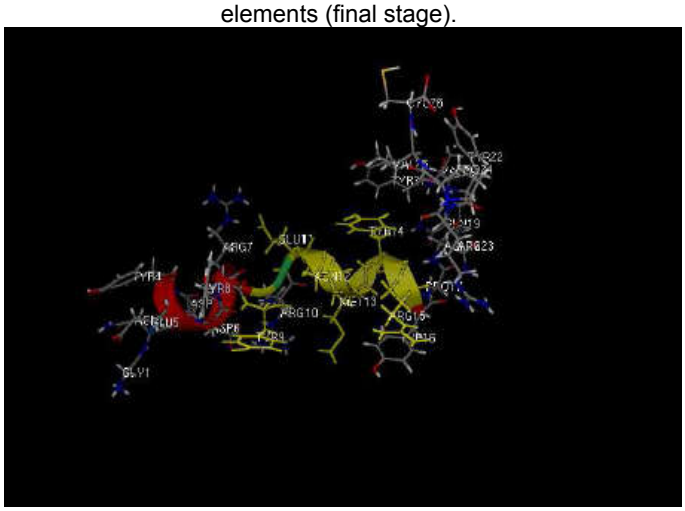


Fig. 6. The reconstructed secondary structural elements (final stage).



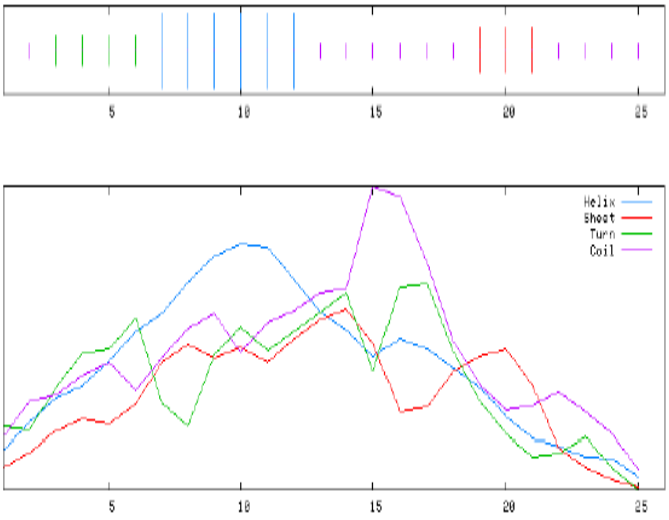
Secondary structure prediction result for the above protein using SOPMA: From the following result of SOPMA, it was concluded that the total sequence length of prion protein was found to be 26 and the secondary structure (i.e. Helix) was found to be in the region of 10-14th residues. The extended strand was found to be in the region of 20-23 residues (Fig. 7).

```
    10        20
     |        |
GNDYEDRYYRENMYRYPNQVYYRPVC
Ccttttthhhhhhcccccceeecccc
```

Screen results Of SOPMA:

Alpha helix (Hh)	:	6 is 23.08%
310 helix (Gg)	:	0 is 0.00%
Pi helix (Ii)	:	0 is 0.00%
Beta Bridge (Bb)	:	0 is 0.00%
Extended strand (Ee)	:	3 is 11.54%
Beta turn (Tt)	:	4 is 15.38%
Bend region (Ss)	:	0 is 0.00%
Random coil (Cc)	:	13 is 50.00%

Fig. 7. SOPMA result for the coil region (19-21 residues) predicted as strand region.



Conclusion

The result showed that if further go in for the depth of molecular dynamics, would reveal the sheet conformation in the particular region of the protein. This dynamics studies had given an outline of the possibilities to use computational tools for the analysis of sheep prion protein. Molecular modeling studies on prion protein gave a simplified view of a complex system (a molecular structure), using visualization software to uncover its remarkable features. The same way simulation software gave a general impression of the behavior and the possible conformations of the prion protein. The dynamic behavior in terms of RMSD of the prion protein had higher deviation showed the instability of its nature, probable reason for its pathogenic form conversion. Further increase of dynamic simulations would reveal the complete beta sheet conformation.

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