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Introducing critical residues in the human prion protein and its Asp 178 Asn mutant by molecular dynamics simulation

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ABSTRACT

The molecular dynamics (MD) simulation method is used to assess structural details for human prion protein (hereafter PrP_N) and its Asp178 Asn mutant (hereafter PrP_M) which causes fatal familial insomnia disease. The results reveal that the flexibility and instability increase in PrP_M could be related to specific amino acids exposed to the solvent. Solvation free energy of PrP_M is 20 $kjmol^{-1}nm^{-2}$ more than PrP_N that is caused by solvent accessible surface area (SASA) especially hydrophobic area, S_{pho}. The study of time interval properties indicates a number of critical amino acids in prion proteins, which exposed to the solvent. They can be ideal anchor-points for initial intermolecular contacts, or affect metal-ion occupancy. The present achievements may be used in drug design for the prevention or treatment of disease..

Keywords: MD simulations; human prion protein; flexibility, solvation free energy; solvent accessible surface area; covariance matrix

INTRODUCTION

The human prion protein (HPrP) is a soluble cell-surface glycoprotein that is bound to the plasma membrane of neuronal cells via Asn181 and Asn197. Since these amino acid positions are two N-glycosylation sites, they stabilize human cellular prion protein PrP^C and prevent the conversion of PrP^C to pathogenic isomer or abnormal prion protein, PrP^{SC} [1]. Prion diseases arise from undesirable folding and refolding of peptides and are often amyloidogenic, affecting humans and a variety of mammals. They include scrapie of sheep and goats, bovine spongiform encephalopathy (BSE) and several human diseases such as Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker Syndrome (GSS) and fatal familial insomnia [2, 3]. According to the 'protein only hypothesis' the infectious agent is PrP^{SC} and prion diseases are caused only due to conversion of PrP^C to PrP^{SC} that is prone to aggregation. The conversion process is unknown so there is still no cure for the prion diseases [4-8]. Its physiological function is not fully understood at the moment, but evidence points to an involvement in various cellular processes, such as regulation of presynaptic copper levels and protection from oxidative stress [9]. Studies have shown that His155, His187 and Glu196 are a good place for copper ion Cu^{2+} and this connection leads to reduced aggregation and increase in antioxidation activity, while preferential binding of Al^{3+} to Glu and Asp accelerates the conversion of PrP^C to PrP^{SC} , its potency in inducing aggregation being very high [4, 9-11]. This protein consists of a small two-stranded β-sheet, three α-helices, the flexible loop 167–171 and two turns. Helices 1 and 2 are connected together though disulfide bond C179-C214 [1, 12]. Many mutations in the prions have been reported [13-18]. PrP_M is recognized as the pathogenic agent responsible for insomnia diseases, which is a class of neurodegenerative diseases. The articles did not report many changes in the structure of PrP_M but their stability was reduced. Some believe it to be related to a cut of Arg164-Asn178 salt bridge [19, 20], although our studies have shown other reasons.

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Molecular dynamics is a powerful tool used for molecular simulation and records every moment of structural rearrangement. This technique was benefitted from for the investigation of PrP_M and PrP_N in order to obtain information from the early stages of conformational change. The important amino acids are introduced in human prion proteins that can be starters for the conversion process or unfavorable interactions that have not been previously reported.

COMPUTATIONAL DETAILS

All simulations and data analysis were performed using GROMACS 3.3.3 with the united-atom protein force field for MD simulations [21, 22]. Analysis of the secondary structure was done with the DSSP program [23]. Solution NMR structure of the C-terminal domain (125–228) of the human prion protein (Protein Data Bank Accession Code: 1hjm) was chosen as the initial structure for wild-type prion protein (hereafter PrP_N). Simulations including explicit solvent molecules (spce water) were done in an octahedron box. To obtain an electro-neutralized simulation system, appropriate Na^+ ions was replaced by randomly selected water molecules. Temperature has been selected at 310 K for a wider configurational space in the short time simulation, using the Berendsen algorithm [24]. Pressure was maintained at 1 bar, using the Berendsen algorithm. Bond lengths were constrained to their equilibrium values using the shake algorithm for water [25], and the LINCS algorithm for the protein molecule[26]. Dynamic plasticity investigates through covariance matrix. Diagonalization of the covariance matrix yields a set of eigenvectors and eigenvalues. The corresponding eigenvalues indicate amplitude of the correlated motions along these directions [18, 21, 22, 27].

RESULS AND DISCUSSION

Fig 1 shows Root-mean-square fluctuations (RMSFs) for two model structures PrP_M and PrP_N. Based on the obtained results, although PrP_M has larger fluctuations with respect to PrP_N, fluctuations follow a certain pattern. The conformational changes which are due to flexibility and instability can prepare PrP_M for conversion and aggregation. Results show helix 1, the loop 167-172 and the C-terminal end of helix 2 as being more flexible. There is also a notable region in the protein, in which its original structure is retained almost within the simulation time scale. This region consists of a segment located in N-terminal of helix 2 in close contact with a segment in helix 3 by s-s bond.

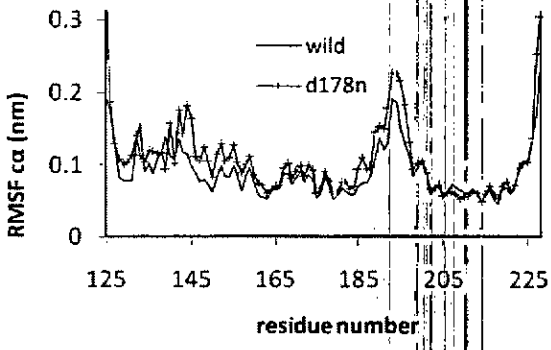

Fig. 1. RMSFs of the Cα-positions as a function of residue number for wild (prp_N) and d178n (prp_M) structures.

To more investigations plasticity, covariance matrix was analyzed. Trace of the covariance matrix after diagonalization is 20.9972 nm^2 for PrP_N and 31.1187 nm^2 for PrP_M. These values can be area displaced by the solvent or measurement of structural changes and high dynamic plasticity. From this result, one can certainly claim that due to the acceptance of multiplicity structures that are in dynamic equilibrium with the native state, the conformational rearrangements and the transition probability are very high in PrP_M.

Solvent accessible surface area(SASA) are shown in Figures 2. Average surface area calculations in the plateau region show that the hydrophobic Solvent accessible surface area, S_{pho} for PrP_M is more than PrP_N.

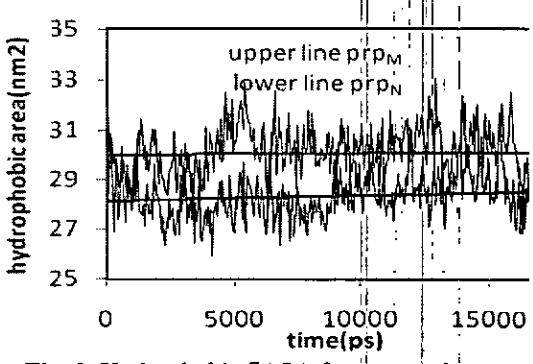

Fig. 2. Hydrophobic SASA for prp_N and prp_M structures.

Thus, due to the increase in area, solvation free energy of PrP_M is 20 $kjmol^{-1}nm^{-2}$ more than PrP_N (Fig 3). By considering the covariance matrix and the SASA results, this propensity is higher for PrP_M as a result of either an unusually high proportion of alternative conformations in dynamic equilibrium, or an unusually high proportion of S_{pho}. If all solvent-protein surface area of side chains are divided into hydrophobic and hydrophilic area, can be stated that:

$$\Delta G_{solvation} = \Delta G_{sol.hydrophilic} + \Delta G_{sol.hydrophobic}$$
$$\Delta G_{solvation} = \Delta H_{solvation} - T\Delta S_{solvation}$$

(Equation 1)

Since the hydrophobic interaction with the solvent (water) reduces the entropy of system, and the enthalpy of solvation is negative, it being especially less negative for hydrophobic interactions، the solvent is predominant [28-31]. As a result, structural changes due to mutation cause some amino acids to be exposed to the solvent so that they can play an important role in misfolding, abnormal intermolecular interactions and aggregation.

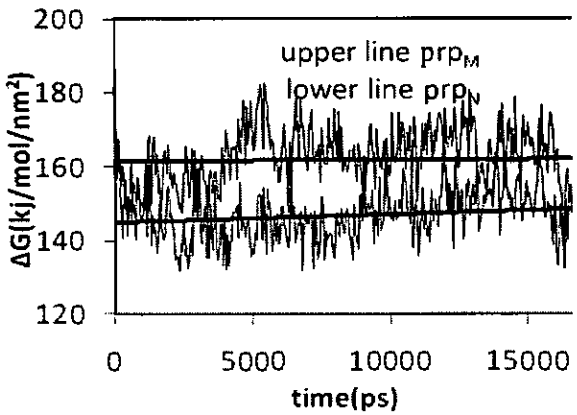

Fig. 3. solvation free energy of side chains as a function of simulation time for prp_N and prp_M structures.

To more investigations of SASA, amino acids in which SASA for the two species PrP_M and PrP_N have tremendous differences are listed in Table 1. Many experimental observations support the hypothesis that an abnormal interaction between prion-prion molecules or prion-lipid membrane may constitute a minimal and sufficient molecular event leading to the process of conversion or aggregation [32].

According to this Table, the amino acids that have a greater share in intermolecular interactions, stacking and the conversion of PrP^C to PrP^{SC} can be identified. Highly surface-exposed ile139 in PrP_N shows it can be both an ideal anchor-point for PrP_N with lipid membrane or initiator for intermolecular contacts. This result is in good agreement with other investigations that showed intermolecular contacts. This result is in good agreement with other investigations that showed that peptide containing residues I138-I-H-F141 are presented at the surface of cellular prion protein and are prone to fast aggregation [33, 34], which is confirmed the simulation accuracy in this work. For PrP_M, Arg136 Glu196 has significant S_{pho} and S_{phi} , in other words this amino acid is exposed to solvent and are therefore critical amino acids in prions. Highly surface-exposed Glu196 can cause changes in copper-ion occupancy, antioxidation activity or even changes the critical role of adjacent residue, Asn197, which is mentioned in the introduction. Also, the most S_{pho} has been seen in Arg 136 which similar to Glu196; can be an ideal anchor-point for initial intermolecular contacts leading to conversion or oligomerization. Another important result is that S_{phi} for residues Glu and Asn have increased in PrP_M, this feature can enhance the link of these residues to Al^{3+} or other ions that efficiently promote misfolding/aggregation of prions. Preferential binding of Al^{3+} to these amino acids and their neutralization with negative charges reduces the negative charge-charge repulsion and thereby allows for a protein structure supporting aggregation. It seems that blocking some of the amino acids listed in Table 1 which are crucial for the onset of the conformational rearrangement, can help us in drug design for the prevention or treatment of disease.

Table1. Solvent accessible surface areas of some critical residues for two model structures, PrP_N and PrP_M

	PrP_M		PrPN	
Residue number	**Hydrophobic area(nm^2)**	**Hydrophilic area(nm^2)**	**Hydrophobic area(nm^2)**	**Hydrophilic area(nm^2)**
arg136	1.53433	0.13778	0.56369	0.09521
ile139	0.11823	0.06444	0.61940	0.19028
tyr150	0.18789	0.09512	0.03524	0.03789
glu152	0.92866	0.13416	0.94833	0.11372
asn153	0.27763	0.11295	0.20868	0.06079
met154	0.60909	0.15322	0.22742	0.11257
arg156	0.59268	0.19995	0.59937	0.14130
asn159	0.68668	0.17615	0.42630	0.13196
asp167	0.42018	0.13599	0.75959	0.11237
tyr169	0.62956	0.14226	0.50334	0.08937
gln172	0.69052	0.14771	0.68979	0.10537
his187	0.57496	0.13903	0.35710	0.11369
thr192	0.45222	0.14886	0.23119	0.06542
glu196	1.02278	0.18779	0.75911	0.12935

CONCLUSION

It can briefly be stated that Root-mean-square fluctuations and covariance matrix calculation show PrP_M being under higher structural instability and high structural deviation. Conformational plasticity having arisen from D178 N mutation may promote the conversion into intermediate states close to the harmful isoform. Time interval properties as a function of residue number indicate a number of critical amino acids in PrP_N and PrP_M, that can be ideal anchor-points for initial intermolecular contacts, or affect metal-ion occupancy and function.

Measurement of the SASA shows that propensity of PrP_M for adopting alternative folds or aggregation is high. Thus it is concluded because structural changes due to mutation can cause some amino acids to be exposed to the solvent, which can play an important role in misfolding, abnormal intermolecular interaction, metal binding and aggregation.

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