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# Modeling of a propagation mechanism of infectious prion protein; a hexamer as the minimum infectious unit

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**Abstract**

To construct a new model of the propagation mechanism of infectious scrapie-type prion protein ($PrP^{Sc}$), here we conducted a disruption simulation of a $PrP^{Sc}$ nonamer using structure-based molecular dynamics simulation method based on a hypothetical $PrP^{Sc}$ model structure. The simulation results showed that the nonamer disrupted in cooperative manners into monomers via two significant intermediate states: (1) a nonamer with a partially unfolded surface trimer and (2) a hexamer and three monomers. Dimers and trimers were rarely observed. Then, we propose a new $PrP^{Sc}$ propagation mechanism where a hexamer plays an essential role as a minimum infectious unit.


*Keywords:* Go model; Coarse-grained model; Molecular dynamics; Prion disease; Amyloid

Prion diseases include Creutzfeldt-Jakob disease (CJD) in human; bovine spongiform encephalopathy (BSE) in cattle, and so on [1,2]. They are infectious and lethal but there is no efficient treatment at this stage. The major player in prion diseases is a prion protein (PrP). There is much PrP called cellular form PrP ($PrP^{C}$) in the healthy brain. The abnormal isoform termed scrapie form of PrP ($PrP^{Sc}$) has infectious property. Although its infectious property has been well characterized by experimental or epidemiological studies, details of its molecular mechanism remain still unclear.

There proposed various reaction pathway models for prion propagation, but so far there have been no models with the insight into its molecular mechanism. There were two representative hypothetical models for $PrP^{Sc}$ propagation [3]: a seeding model and a refolding model. (A) In the seeding model, a $PrP^{C}$ can convert into monomer $PrP^{Sc}$, which is not stable and not populated. Once a large cluster of $PrP^{Sc}$ is formed, it stably persists and grows to a fibril. The number of seeds increases by breaking of a longer fibril into two or more pieces. In this model, a stable $PrP^{Sc}$ unit is considered to be an oligomer or an amyloid fibril. (B) In contrast, a stable $PrP^{Sc}$ unit is typically a monomer in the refolding model. It is considered that $PrP^{C}$ can convert into $PrP^{Sc}$ only in the presence of $PrP^{Sc}$. Then, a $PrP^{Sc}$/$PrP^{C}$ complex transform into a $PrP^{Sc}$/$PrP^{Sc}$ complex. Then the complex will separate into two $PrP^{Sc}$ monomers. This model is sometimes called heterodimer model [4] or template-assistance model [5].

Although the structure of $PrP^{Sc}$ has not been precisely determined in atomic resolution, Govaerts et al. recently proposed a model structure of $PrP^{Sc}$, a trimer with left-handed β-helices [6]. Although their proposed model is hypothetical, no other model is more realistic, and it apparently includes the structural information. Thus, to model the $PrP^{Sc}$ propagation mechanism with physical flavor including chain topological information, we conducted simulations using a coarse-grained model with a structure-based potential referencing the hypothetical $PrP^{Sc}$ structure.

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## Model and method

We conducted molecular dynamics simulations using a coarse-grained chain model. The chain contains only $C_\alpha$ atoms virtually linked sequentially. The potential for the simulations were the off-lattice version of the Go model [7,8], which uses conformational information but does not discriminate the amino acid types. Here we regard the $PrP^{Sc}$ as the native-like conformation. In the model, we considered pairs of amino acid residues in the native conformation which are in close distance and called them native contact pairs. In this paper, we present only the results from one native contact condition where native contact is judged to exist if the distance between any non-hydrogen atoms belonging to residues, $i$, $j$ ($j > i + 3$) at the native conformation, falls within 6.5 Å. Temperature was controlled by Berendsen thermostat [9]. Above simulation method is similar to the one reported previously [10,11], except the treatment for the disulfide bond between 179th and 214th residues [12,13]. The bond was treated as same as other virtual covalent bonds.

We used the model $PrP^{Sc}$ structure proposed by Govaerts et al. [6] for setting attractive residue pairs. The chain length of the monomeric $PrP^{Sc}$ utilized was 139 (from 90th to 228th, this numbering is for mouse PrP). We made a nonamer structure by simple translocation of the trimer coordinates, as proposed by Govaerts et al. [6], here so as to make the distance between inter-molecular strands similar to that between intra-molecular strands at the β-helix.

To detect the status of protein(s), we employed two indices: the $Q$-value and $n$. $Q$-value is given by

$$(Q\text{-value}) = (\text{number of native contacts formed at a given conformation})/(\text{total number of native contacts at the native conformation}).$$

We interpreted that native contacts are formed when the distance between $C_\alpha$ of amino acid residues, $i$, $j$ is within 1.2 times the distance of $C_\alpha$ atoms of native conformation. $n$ represents the number of monomers in the maximum complex. If at least one native contact is formed between molecules, we judged that they are associating, otherwise, they are dissociating.

## Results and discussion

### *Thermodynamics of a $PrP^{Sc}$ monomer*

We initially simulated the folding–unfolding dynamics of a monomeric $PrP^{Sc}$ and characterized its thermodynamical properties. Figs. 1A–C show the conformations of the $PrP^{Sc}$ monomer at different stages: the given hypothetical $PrP^{Sc}$ conformation (Fig. 1A), a partially unfolded conformation (Fig. 1B), and an unfolded conformation (Fig. 1C). Note that the contacts between C-terminal helical regions were retained during simulations even at the unfolded state (Fig. 1C) because of conservation of the disulfide bond during simulations. We calculated the temperature dependence of heat capacity (Fig. 1D) with the weighted histogram analysis method (WHAM) [14], using many trajectories obtained by simulations at different temperatures. The heat capacity curve had a unique peak. Here, $T_f$ is defined as the peak temperature of heat capacity. $T_f$ of monomer $PrP^{Sc}$ is denoted by $T_f^{mono}$. Temperature is described in units of $T_f^{mono}$, even for nonamer simulations. Fig. 1E shows the time evolution of the $Q$-value, the ratio of native contacts formed, during monomer simulation at $T_f^{mono}$. At this temperature, the folded state had a wide spectrum of conformations from the given $PrP^{Sc}$ conformation to partially unfolded ones (Fig. 1B). Fig. 1F shows free energy profiles calculated with WHAM, indicating that this monomer $PrP^{Sc}$ is not stable at all at temperatures higher than 1.05 $T_f^{mono}$.

### *Disruption simulation of a $PrP^{Sc}$ nonamer*

Next, we conducted nonamer disruption simulations in order to gain an insight into the $PrP^{Sc}$ propagation process. Fig. 2A shows the time evolution of a nonamer disruption process obtained at 1.30 $T_f^{mono}$, which is the lowest temperature for nonamer disruption simulations used in this report. At this temperature, it took roughly two months for nonamers to break into nine monomers, when calculated by a PC with Intel Pentium D CPU 2.8 GHz using one core for one job. By extrapolating the temperature-dependence of average disruption time as shown later, that average disruption time at 1.05 $T_f^{mono}$ can be estimated to be more than 10-fold that at 1.3 $T_f^{mono}$, indicating that computation time required for complete disruption at 1.05 $T_f^{mono}$ may be more than one year, and a nonamer seems not to disrupt during initial few months of calculation. This suggests that a nonamer is stable enough at the temperature where a monomer is not stable anymore within the currently available computational technologies.

At the initial stage of simulation, a nonamer with essentially the same structure as the initial one persisted for a while (Fig. 2B). Then three molecules on the surface of the nonamer unfolded simultaneously at the N-terminal end (Fig. 2C). After short time, these three unfolded molecules dissociated into monomers, while a hexamer of $PrP^{Sc}$ persisted for a relatively long time (Fig. 2D). Next the hexamer disrupted into six monomers almost at the same time (Fig. 2E). The red line in Fig. 2A represents the evolution of the number of monomers in the maximum complex, $n$. This clearly demonstrates the cooperative disruption of a hexamer to monomers without the accumulation of any intermediate oligomeric state between them. We can also recognize the partially unfolded nonameric state (Fig. 2C) by comparing the evolution of the $Q$-value as a function of $n$. Note that results of histogram (Fig. 2F) and time course of $n$ (Fig. 2A) were not sensitive against definition of association (data not shown), although here we judge that molecules are associated when at least one native contact is formed between them, because the disruption processes proceeded in cooperative manner or monomers were separated from an oligomer quickly after losing its stability.

To examine the reproducibility of simulation, we conducted 52 simulations under the same conditions except for initial velocities. All 52 trajectories showed a similar cooperative transition pattern in the time evolution profile of the $Q$-value (data not shown). Fig. 2F shows the histogram of $n$. During the disruption process from a hexamer to monomers, no other complex between $n = 2$ and 5 was observed practically. This result indicates that hexamers are more stable than smaller oligomers between $n = 2$ and 5. Interestingly, Silveira et al. reported that complexes