Modeling Intermediates in Prion Protein Fibril Formation

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Abstract:

The misfolding and aggregation of the prion protein have been implicated in several fatal neurodegenerative diseases. Misfolded prion proteins first aggregate into toxic, infectious protofibrils before forming fibrils. We constructed different protofibril models from molecular dynamics simulations of the human prion protein under misfolding conditions. These protofibril models were compared with experimental data to assess if they are reasonable models for the toxic intermediates.

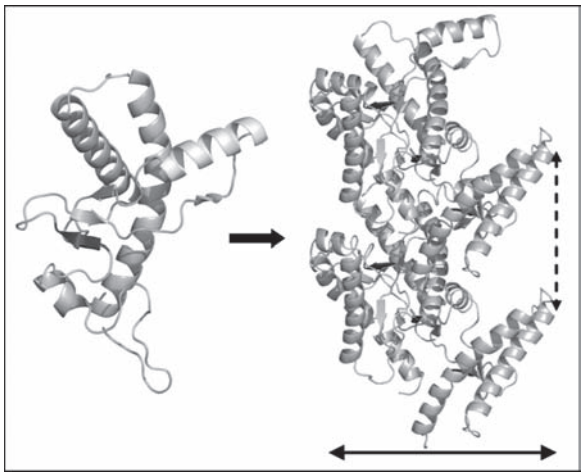

Figure 1: Left: Misfolded human PrP at pH 5. Right: Side view of a protofibril model. The dotted line indicates the rise per turn, while the solid line indicates the diameter of the model.

Introduction:

Aggregates of an abnormal form of the prion protein (PrP) are responsible for a group of fatal neurodegenerative conditions known as prion diseases, which include bovine spongiform encephalopathy (mad cow disease) and Creutzfeldt-Jakob disease. Prion diseases are caused by the misfolding and aggregation of PrP, which may be triggered by low pH or mutations. The structures of misfolded PrP cannot be determined experimentally, so our group used molecular dynamics to simulate the misfolding of PrP at various pH levels and with different mutations.

Misfolded prion proteins first assemble into soluble protofibrils before forming insoluble fibrils (Figure 1). There is evidence that protofibrils are toxic and infectious, and knowing the structure of these protofibrils can help us understand and combat prion diseases. Currently there is no high-resolution experimental data on protofibril structure, but experiments have shown that misfolded PrP aggregates have significantly more β-structure and less α-helical content compared with the natively-folded PrP. Experiments also indicated that residues 98-110 and 136-140 are important for aggregation and are likely on the binding interface between monomers [1].

Building on previous work [2], this project aimed to construct alternative human protofibril models with increased β-sheet content. We have built several models that are consistent with many available experimental data. (See Figure 1)

Methods. Misfolded conformations were selected from molecular dynamics simulations of PrP under misfolding conditions and docked manually to create models with cross-monomer β-sheets [2]. All models were constructed using PyMOL [3].

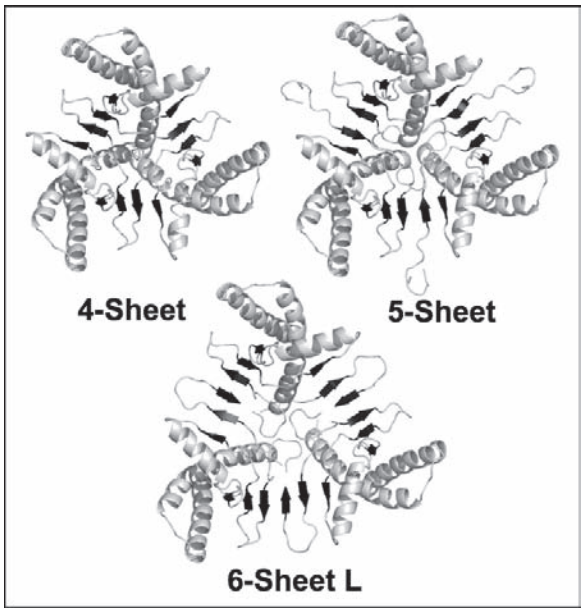

Figure 2: Top views of the left-handed spiral models.

Prion Protofibril Models. The human protofibril models we constructed are named according to the number of strands in the cross-monomer β-sheets. 4-sheet, 5-sheet, and 6-sheet L are left-handed spiral models built from misfolded wild-type PrP at pH 5 (Figure 2). All models have a repeating unit of three monomers. 4-sheet is similar to the previous protofibril models because each β-sheet has four strands. We were interested in building models with more β-structure, so we modeled one and two extra strands in the N-terminus to get 5-sheet and 6-sheet L, respectively.

While 6-sheet L has more β-structure, it also has more gaps in between monomers, which may make the model unstable. To make the model more compact while maintaining β-structure, we adjusted the 6-sheet conformations and fitted them into a right-handed spiral model, 6-sheet R, which has fewer gaps than its left-handed counterpart.

5-sheet D178N, whose cross-monomer β-sheet consists of five strands, was built from misfolded PrP conformations with the disease-causing mutation D178N. The mutated conformations only fit into a right-handed spiral, whereas the wild-type conformations fit readily into left-handed spirals.

To compare the different models, we measured the diameter and the rise per turn (Figure 1). The diameter increased with the increasing number of β-strands, but the rise per turn does not show a clear trend (Figure 3).

	4-Sheet	5-Sheet D178N	5-Sheet	6-Sheet R	6-Sheet L
# β-strands	4	5	5	6	6
Handedness	Left	Right	Left	Right	Left
Diameter (Å)	67.1	72.7	74.3	79.4	81.1
Rise (Å)	37.5	37.5	40.5	34.5	40.5

Figure 3: Comparison of protofibril models.

Comparison with Experimental Data:

To check if known antibody binding sites are accessible in our models, mapped epitopes were projected onto them (Figure 4). All models are accessible to the ICSM18 and H3:2 antibodies and, after some adjustments, also to R1. These three antibodies can bind to both native PrP and misfolded aggregates.

I5B3 and H3:3 bind selectively to misfolded PrP aggregates, and most of our models are accessible to them. Since I5B3 binds to three separate regions at once, the three regions must neighbor one another. This is indeed the case in our models (Figure 4). Furthermore, H3:3 binds to a region that is significantly altered during the misfolding simulations of PrP. The differences in the H3:3 binding region in native and misfolded PrP may explain the selectivity of H3:3 for PrP aggregates.

Finally, all our models can accommodate glycans at the known glycosylation sites, Asn-181 and Asn-197, and all have exposed proteinase K digestion sites at Gly-127.

Conclusions and Future Work:

Structures representing misfolded PrP can be fitted into continuous spiral models with 4, 5, or 6 β-strands. Two spiral conformations, left-handed or right-handed, are possible with 6 β-strands. However, the models with 6 β-strands show significant gaps, which may mean that these structures are unstable. Misfolded PrP with disease-related mutation D178N resulted in a different spiral model than wild-type misfolded PrP. Most of our models agree with available experimental data, indicating that the models are reasonable. More experimental data is needed to further assess if our models are representative of prion protofibrils.

Acknowledgements:

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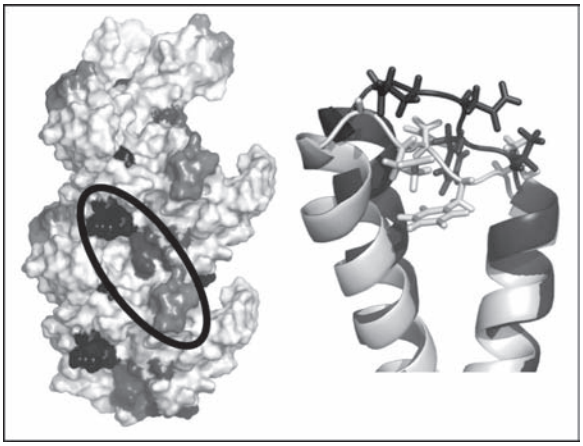

Figure 4: Left: I5B3 binding regions in the 4-sheet model. Right: Changes in the H3:3 binding region in native PrP (light gray) and misfolded PrP (dark gray).