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Review

Prion protein: structural features and related toxicity

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Transmissible spongiform encephalopathies, or prion diseases, is a group of infectious neurodegenerative disorders. The conformational conversion from cellular form (PrP^{C}) to disease-causing isoform (PrP^{Sc}) is considered to be the most important and remarkable event in these diseases, while accumulation of PrP^{Sc} is thought to be the main reason for cell death, inflammation and spongiform degeneration observed in infected individuals. Although these rare but unique neurodegenerative disorders have attracted much attention, there are still many questions that remain to be answered. Knowledge of the scrapie agent structures and the toxic species may have significance for understanding the causes of the diseases, and could be helpful for rational design of novel therapeutic and diagnostic methods. In this review, we summarized the available experimental evidence concerning the relationship among the structural features, aggregation status of misfolded PrP and related neurotoxicity in the course of prion diseases development. In particular, most data supports the idea that the smaller oligomeric PrP^{Sc} aggregates, rather than the mature amyloid fibers, exhibit the highest toxicity to the host.

Keywords prion protein; oligomer; amyloid; toxicity

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Introduction

Transmissible spongiform encephalopathies (TSE), or prion diseases [1,2], is a group of infectious neurodegenerative disorders accompanied by cognitive impairments, extensive brain damage and neuronal dysfunction. The prion protein (PrP) has attracted much attention from researchers with different scientific background. PrP is best known for its important roles in the pathogenesis of prion diseases, including Creutzfeldt–Jakob disease (CJD), fatal familial insomnia, Gerstmann–Straussler–Scheinker syndrome (GSS) and Kuru in humans, bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goat, and chronic wasting disease in elk and deer. Although these diseases are rare, their unique mechanism of transmission and the concerns generated by the recent appearance of a new variant form of CJD, which has been linked to consumption of meat contaminated with BSE, have put prions in the spotlight [3]. Increasing evidence now supports the idea that the central event is the conformational change of PrP through a post-translational process [4–6], from cellular form (PrP^{C}) to disease-causing conformation (PrP^{Sc}). And all of these diseases have a long incubation period before an extremely rapid clinical stage. PrP^{Sc} is found in extracellular deposits in the diseased brain, and sometimes in the lymphoid tissues [1,7], which is best known as the essential constituent of infectious prions [8,9]. However, both the mechanism and the real neurotoxic agent by which the misfolded protein causes brain damage are unknown. Notably, many researches have shown that mice devoid of PrP^{C} are resistant to scrapie and do not propagate infectious prions [10–13]. It suggested that the presence of PrP^{C} is necessary to establish and further evaluate these diseases. Interestingly, experimental evidence in transgenic mice models revealed that the expression of endogenous neuronal PrP^{C} is required to mediate neurotoxic effects of PrP^{Sc}. On the other hand, the toxic activity of PrP^{Sc} and the protective activity of PrP^{C} are interconnected [14].

More and more reports suggested that the soluble oligomers, constituted by comparatively few copies of infectious units, are the most important agent for self-replication of misfolded aggregates [15,16]. Lasagna-Reeves *et al*. [17] found that the most toxic oligomers in Alzheimer disease, existing in stable ring-like shapes, do not go on to become fibrils which act as templates for making new copies if free monomers are available. Growing evidence suggests that soluble protein aggregates, rather than insoluble fibrils or rods, are toxic in other protein misfolding diseases (PMD) [18,19]. However, it is not well elucidated whether the

soluble oligomeric PrP^{Sc} or large aggregate is really infectious in prion diseases. Until recently, toxicity of prions has remained to be a subject under intense scrutiny, and more evidence is required to understand the molecular state of the protein that constitutes the infectious particle as well as the neurotoxicity.

In this review, we summarized the conformational features of prions that are believed to be the main infectious agent in TSE. We also discussed the development of the interesting questions in prion biology: what is the toxic species and how do prions damage humans and animals?

Conformation of Prion Protein and the Prion Strains

Using nuclear magnetic resonance (NMR), Riek *et al.* [20] first established the 3D structure of autonomously folding PrP domain that contains a two-stranded antiparallel β-sheet and three α-helices. While Pan *et al.* [6] demonstrated that PrP^{C} purified from brain homogenates has a high content of α-helices (42%) and almost no β-sheet (3%) by Fourier-transform infrared (FTIR) spectroscopy. Moreover, NMR results showed that the presence of β-sheet in PrP (121–231) is of importance for the initiation of the conformational transition from PrP^{C} to PrP^{Sc}. PrP^{C} is a monomeric, glycosylated, α-helix-enriched protein attaching to the cell surface through glycosylphosphatidylinositol (GPI) anchor [21,22], and is reported to be especially abundant in the central nervous system (CNS) [23]. Although the physiological function of PrP^{C} is still a controversial matter, it is reported to be involved in cell–cell adhesion, intracellular signaling *in vivo*, and may therefore contribute to cell–cell communication in the brain [24]. In contrast, PrP^{Sc}, a scrapie of pathogenic isoform, is a polymeric, β-sheet-rich and insoluble molecule which possesses a higher proportion of β-sheet structure in place of the normal α-helix structure [4]. Although both of the two proteins possess identical primary amino acid sequence and share the same posttranslational modifications [25], they have very different biochemical properties. PrP^{C} is a soluble molecule and susceptible to proteinase K (PK), while PrP^{Sc} is insoluble in detergent and partially resistant to proteolysis, and the protease-resistant core designated as PrP 27–30 is derived [26], which consists of about 142 amino acids and conveys prion infectivity. However, there is also an exception, in which case up to 80%–90% of CJD-related prions are sensitive to PK [27]. Combined with proteases and detergent, PrP 27–30 is readily polymerizes into large amyliodic structure [28] which may contain as many as 1000 PrP molecules [29].

Currently, it is widely accepted that during the aggregation of PrP^{Sc}, alternative folding structures and glycosylation patterns are able to be adopted, which can stably and faithfully replicate and result in different diseases [30,31]. It is very difficult to understand how a protein can possess two stable and completely different folded structures and even one of these conformations is able to convert the other into itself. Moreover, the misfolded form can in turn adopt multiple conformations with distinct properties. Compelling scientific evidence indicates that PrP can have numerous folding patterns, and they can faithfully replicate from generation to generation [30]. In analogy to other infectious agents, these variants have been termed strains. The distribution of infectious prions among different tissues and biological fluids can be dramatically different depending on the species of animal and strains of prions [32,33]. Protein-only hypothesis, in which PrP^{Sc} is considered as the main constituent of prion infectious agent [4,34], has been difficult to explain the existence of multiple prion strains. Animals affected by prion diseases may develop different pathologies. The incubation periods, profile of histological damage, and clinical signs are widely accepted as the main *in vivo* characteristics used to differentiate between prion strains [35]. Since the first evidence about the existence of prion strains was described in scrapie-affected goats by Pattison and Millson in 1961 [36], the existence of the prion strains have attracted more and more attention. Using mouse models, more than 20 strains have been isolated [37], and many of these strains have their origin of different sources including human sources as sCJD and GSS [38–40]. Another more interesting finding is the isolation of different prion strains from the same host after being inoculated with PrP^{Sc} of a single species [30], and the coexistence of two or more prion strains in a same host [41].

Protein Misfolding and Aggregation

Infectious particles-like viruses are dependent on their own genetic material to direct the continuous replication during infection process in the target host cells. In prions, however, PrP^{Sc} is able to replicate itself in an autocatalytic manner by recruiting and converting the normal PrP^{C} proteins into the infectious isoform [4]. The accumulation of PrP^{Sc} in the brain is thought to be the main reason of cell death, inflammation, and spongiform degeneration observed in the infected brains of patients or animals [42]. The classical accumulation of the abnormal protein aggregates is composed of misfolded PrP units (monomers). This amyloid-like structure can exist as soluble monomers, small oligomers, intermediate protofibrils and insoluble fibrils (**Fig. 1**) [43–45].

The formation of fibril structure, which can be specifically stained by dyes such as Thioflavine T or Congo red, starts with a nucleation-dependent polymerization by a lag phase in which nucleation occurs, and is followed by an exponential elongation phase (**Fig. 1**) [46,47]. A critical step in the pathogenesis of prion diseases is the conformational

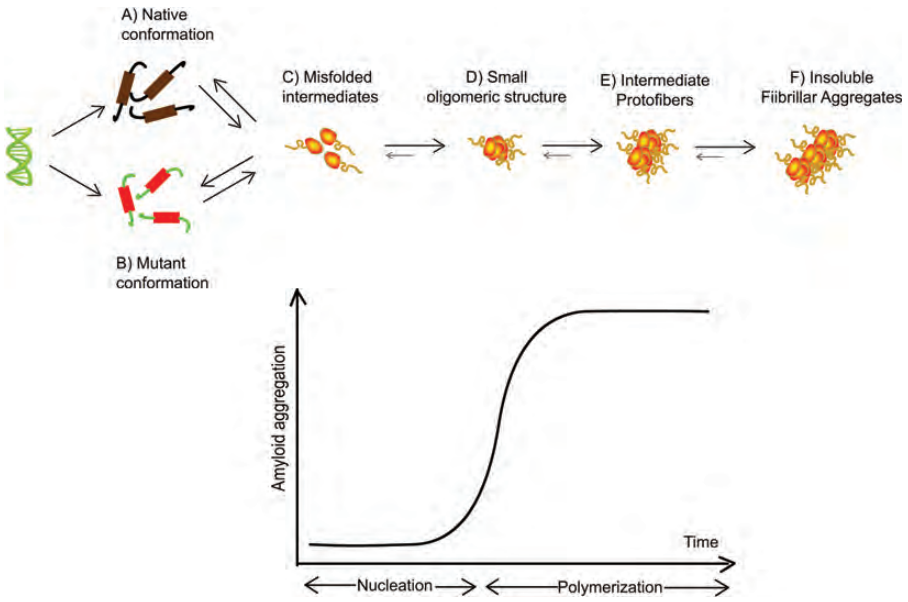

Figure 1 Seeding-nucleation model of prion propagation Wild-type prions can adopt a normal 3D structure by correct folding (A), whereas the spontaneous, perhaps age-related gene mutant may induce the generation of mutant conformation (B). The native PrP^{C} or mutant protein then undergoes a conversion to a misfolded conformation by a post-translational modification (B or C). But this misfolded protein is not stable, and it can polymerize to more stable oligomeric structures (D) by interaction between PrP^{C} and PrP^{Sc} monomers. Given more time, in the presence of PrP^{C}, oligomers then grow in size and aggregate to profilers (E) and fibrillar aggregates (F).

conversion of prion protein [48], through which the initial spontaneous, perhaps age-related formation of misfolded structure is generated. Alternatively, the existence of mutation in the primary structure, which may give rise to significant destabilization of PrP, may act as a mutant precursor, even could generate the mutant prion [49] and facilitate the interaction between PrP^{C} and PrP^{Sc}. Once this oligomeric seeds are formed, PrP^{C} can be recruited, leading to an explosive and exponential growth phase. And in this step, more and more normal PrP^{C} are converted to PrP^{Sc}, producing a huge amount of heterogeneous mixture of polymeric structures [44]. Obviously, in this process, appropriate amount of oligomeric seeds is necessary, which is sensitive to the concentration of available PrP^{C} and the equilibrium distribution between the native and misfolded conformation [8].

Oligomeric PrP^{Sc} are smaller aggregated assemblies that consist of a few monomeric misfolded protein units. And then they can seed a comparatively rapid aggregation into protofibrils [50], which are reported in equilibrium with low molecular weight oligomers and have a secondary structure characteristic of amyloid fibrils [51,52]. A novel annular protofibrils intermediated with unique pore-like properties are reported to be the cause of cell dysfunction and even cell death in amyloid diseases [53,54]. With increasing time, protofibrils are elongated to the most stable species-fibrils or plaques in the presence of PrP^{C} [55]. Since the structure of PrP^{C} is maintained by the accurate activities of chaperone that participates in the biosynthesis of the protein [56], the existence of abnormal structure is also dependent on the impairment in the cell's clearance mechanism.

Toxicity of Prions

The toxicity of prion aggregates is first investigated using purified PrP^{Sc} from infected mouse brain [57], or synthetic PrP peptides (PrP106–126) [58], which is considered as the possible amyloidogenic core of PrP^{Sc}, and extensively used to induce apoptotic cell death. In addition, N-terminally truncated PrP was reported to trigger neuronal death [59], and under certain conditions, PrP has the intrinsic properties to render the protein toxicity. The putative generation of pores in cell membranes [60] and activation of inflammatory responses [61] induced by PrP^{Sc} are commonly associated with the intrinsic toxicity of these structures. However, in some other rare cases, no PrP^{Sc} was detectable in the disease [62] or a large amount of PrP^{Sc} deposition is observed with no disease [63], which suggested that infectivity and pathogenicity are distinct features of abnormal PrP [64]. Considering the particular feature of prions, the most interesting event is to establish a link between toxicity and the physical state of infectious unit with respect to the particle size, PrP content or other potential constituents. However, it is very difficult to establish the original toxic species due to the heterogeneous nature of PrP^{Sc} aggregates, which are comprised of a mixture of mature fibrils and oligomers.

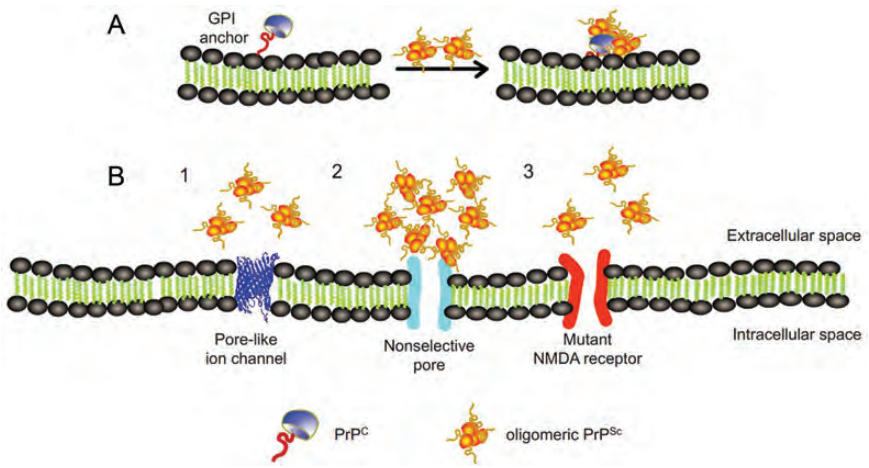

Figure 2 Potential mechanism of PrP-mediated toxicity (A) PrP^{C}-mediated amyloid toxicity. The interaction of PrP^{C} with PrP^{Sc} may facilitate the internalization of prion oligomers. (B) Putative models of prion oligomers induced toxicity. (1) Pore-like structure is generated in the cell membrane, resulting in the imbalance between intracellular and extracellular environment. (2) At high intracellular concentration of misfolded PrP^{Sc}, non-specific membrane pores induce apoptotic signaling. (3) Mutant of NMDA receptor or some other factor that affect its function may also lead to excitoxicity.

Considering the serious risk of prion diseases for public health, in which spongiform PrP^{Sc} deposit is observed in the infected brain, many studies were designed to evaluate the toxicity of PrP^{Sc} aggregates, which has long been considered as a pathological marker in prion diseases [4,65]. Increasing evidence, however, suggests that these amyloid-like aggregates are neither obligatory for prion infectivity nor disease pathogenesis [15,18,66]. Moreover, the mature fibrils are considered to be a protective mechanism to prevent the high toxicity of small oligomers. One possible explanation is that PrP^{Sc} aggregates might be expected to have greater stability, while smaller oligomers have greater converting activity and infectivity [67,68].

A previous study suggested that the intracellular neuronal propagation of pathogenic PrP plays an important role in neurotoxicity [69], and then another study demonstrated that by inhibiting the proteolytic β subunits of the 26S proteasome, the toxic prion oligomers are likely to result in neuronal perturbation and contribute to the widespread neuronal loss [70]. Consequently, the focus of research on pathologic mechanisms has shifted from amyloid fibrils to oligomers, which were found as pre-fibrillar intermediates of the mature amyloid fibrils [71,72]. Kazlauskaite *et al.* [73] described that prion oligomers are toxic to neuronal cells in culture for the first time using pre-fibrillar, recombinant hamster prions. Later, a controversy arose when Baskakov *et al.* [74] found that mature amyloid fibrils prepared from the full-length recombinant prion protein possesses similar toxicity as soluble oligomers to cultured cells as well as primary hippocampal and cerebella neurons. This study indicated that both soluble oligomers and the amyloid fibrils in prion disease are intrinsically toxic to cultured cell, while the endogenously expressed PrP^{C} is required to mediate the toxicity. And surprisingly, a 50% decrease of PrP^{C} expression may reduce the sensitivity of neurons toxicity of PrP^{Sc} [75]. A recent study showed that the disordered N-terminal domain and C-terminal GPI anchor of PrP is required for the PrP^{C}-mediated toxic signaling pathways, without being converted to PrP^{Sc} [**Fig. 2(A)**] [76]. However, by stereotaxically injecting recombinant ovine and murine prion oligamers, Simoneau *et al.* [77] found that the soluble oligomers exhibit considerably higher toxicity in the brain compared with PrP fibrils *in vitro* and *in vivo*, and the toxicity is independent of endogenous PrP expression by the neurons, since they obtained similar results using PrP-knockout mice or normal mice. Interestingly, they can eliminate the toxicity of PrP oligomers by blocking the hydrophobic domain at the surface of the oligomers. A more recent study reported by Collinge's group [78] demonstrated that prions are not neurotoxic but responsible for generating the toxic PrP species from PrP^{C}, which is triggered during the prion propagation.

Although much experimental evidence suggests that neurotoxicity in prion disease is mediated by misfolded protein oligomers, a consensus on the mechanism whereby neurotoxic forms of PrP kill nerve cells remains far from clear. Some models have been proposed to explain the toxicity of prion oligomers or protofibrils based on the interaction between aggregated proteins and cell membranes [**Fig. 2(B)**], and the most often described process is proposed to be the pore formation, resulting in the electric imbalance between the intracelluar and extracellular environment [54,60,79,80]. In this hypothesis, during the process of prion aggregation, soluble oligomers are elongated by recruiting newly misfolded monomers on their ends. Encountered cell membrane, however, instead of forming longer rod, the 'pore-like' assemblies or called 'annular protofibrils', are generated in lipid membranes, which has been confirmed by using molecular dynamic

simulations [81]. Once the discrete ion channels form, the small oligomers are able to translocate the pore directly while large aggregates are prone to bump through the plasma membrane [82], leading to cell dysfunction [53,54]. And the deleterious effect is proposed to be mediated by this membrane poration, followed by non-specific membrane leakage [83] or specific ionic transport through ion channels across bilayer membranes [84,85], and finally leading to the ionic homeostasis destabilization [76]. One study, using prion peptides derived from unprocessed *N*-termini of mouse and bovine prion protein, suggested that in parallel with the cellular trafficking, they can form transient pores leading to toxicity [86]. Moreover, another possible mechanism is the specific PrP-mediated modulation of *N*-Methyl-D-Aspartate (NMDA) receptor, which plays a crucial role in mediating a wide range of important nervous system functions [87,88], while excessive NMDA receptor activity may lead to overloaded cytotoxicity and neuronal damage [89].

Outlook

Prions are best known as the infectious agents associated with prion diseases, the hallmark pathological features of which are the spongiform degeneration in the brain, accompanied by extensive neuronal loss, astrogliosis, and cerebral accumulation of the misfolded and protease-resistant form of prion protein. Recently, an increasing body of experimental data suggests that oligomeric assemblies of misfolded proteins (PrP^{Sc}) are the predominant neurotoxic species in prion diseases. Considering the particular feature of prions, which accumulate in a heterogeneous population of aggregates with different monomeric units, a consensus assumption that smaller oligomeric PrP^{Sc} is responsible for the toxic activities has been accepted. However, it is still a long way to fully understand how exactly these aggregates exert their neurotoxicity, which will be helpful for rational design of novel therapeutic and diagnostic methods for these diseases. It is also expected that novel methodologies will arise in the future to treat many of the most devastating diseases.

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