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The diversity and relationship of prion protein self-replicating states

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ABSTRACT

It has become evident that the prion protein (PrP) can form a diverse range of self-replicating structures in addition to bona fide PrP^{Sc} or strain-specific PrP^{Sc} variants. Some self-replicating states can be only produced *in vitro*, whereas others can be formed *in vivo* and *in vitro*. While transmissible, not all states that replicate *in vivo* are truly pathogenic. Some of them can replicate silently without causing symptoms or clinical diseases. In the current article we discuss the data on PK-digestion patterns of different self-replicating PrP states in connection with other structural data available to date and assess possible relationships between different self-replicating states. Even though different self-replicating PrP states appear to have significantly different global folding patterns, it seems that the C-terminal region exhibits a cross-β-sheet structure in all self-replicating states, as this region acquires the proteolytically most stable conformation. We also discuss the possibility of the transformation of self-replicating states and triggering of PrP^{Sc} formation within the frame of the deformed templating model. The spread of silent self-replicating states is of a particular concern because they can lead to transmissible prion disease. Moreover, examples on how different replication requirements favor different states are discussed. This knowledge can help in designing conditions for selective amplification of a particular PrP state *in vitro*.

1. Introduction

According to the protein only hypothesis, the prion protein (PrP) can adopt two alternative structural forms: the normal, cellular form PrP designated as PrP^{C} and the disease-associated, self-replicating form designated as PrP^{Sc} (Prusiner, 1982). To explain the diversity of prion disease phenotypes within the same host, the protein only hypothesis proposes that multiple, stable, conformationally distinct self-replicating PrP^{Sc} states exist and account for different prion strains. Indeed, FTIR spectroscopy and other biochemical assays revealed differences in PrP^{Sc} structure isolated from animals infected with different prion strains (Caughey et al., 1998; Thomzig et al., 2004). It is believed that PrP^{Sc} of different strains share a common folding pattern and that strain-specific differences in PrP^{Sc} conformation are relatively minor.

In the past decade, a number of studies illustrated that the spectrum of structures acquired by the prion protein is much broader than PrP^{C}, PrP^{Sc} or strain-specific PrP^{Sc} variants. Some self-replicating states of the prion protein can only be generated *in vitro*, such as amyloid fibrils produced from recombinant PrP (rPrP) in the absence of cellular cofactors (Baskakov et al., 2002; Bocharova et al., 2005a). Other PrP states, also of amyloid nature, are capable of replicating in the brain and can even be serially transmitted from animal to animal in a laboratory setting. While transmissible, they appear to be neither toxic nor associated with any clinical signs of prion disease. Examples of these include transmissible amyloid plaques in transgenic PrP (P101L) mice induced by material from a Gerstmann–Straussler–Scheinker patient with the P102L mutation (Piccardo et al., 2007); amyloid deposits in transgenic mice expressing GPI-anchorless PrP^{C} (Chesebro et al., 2005); and self-propagating PrP forms characterized by abnormally short, C-terminal PK-resistant fragment (atypical PrPres) observed in animals inoculated with rPrP fibrils (Kovacs et al., 2013; Makarava et al., 2011). In addition, a number of abnormal PK-resistant fragments different from that of *bona fide* PrP^{Sc} or PK-sensitive disease-associated PrP states were observed in human and animals affected by a variety of prion diseases including sporadic CJD (sCJD), iatrogenic CJD, variable protease-sensitive prionopathy and atypical scrapie and BSE (Baron et al., 2008; Biacabe et al., 2007; Satoh et al., 2003; Zou et al., 2003, 2010).

Considering that some abnormal PrP states can replicate silently, *i.e.* without causing clinical symptoms, their spread within or

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between individual organisms can occur undetected. Our work on synthetic prions demonstrated that replication of clinically silent PrP states can give rise to PrP^{Sc} and transmissible prion diseases (Makarava et al., 2011, 2012b). In addition, a rapidly growing number of studies suggests that amyloid states of other amyloidogenic proteins associated with neurodegenerative diseases including Alzheimer's, Parkinson and Huntington's diseases and Amyotrophic Lateral Sclerosis can also spread from cell to cell in a prion-like manner (reviewed in (Jucker and Walker, 2013)). However, it is not known what self-replicating states are truly pathogenic, what states while capable of replicating *in vivo* do not cause pathogenic effects, and what structural features separate pathogenic from non-pathogenic self-replicating states.

While the spectrum of self-replicating PrP states continue to expand, our understanding of structural differences and their relationship to *bona fide* PrP^{Sc} remain primitive. With most of the studies focused on elucidating molecular structure of PrP^{Sc}, several alternative PrP^{Sc} models have been proposed in the past (DeMarco and Daggett, 2004; Govaerts et al., 2004; Langedijk et al., 2006; Stork et al., 2005; Tattum et al., 2006; Wille et al., 2002; Yang et al., 2005). In some aspects the current models contradict each other and are, in part, at odds with growing experimental observations (Requena and Wille, 2014; Shirai et al., 2014). The main challenge of the next decade is to determine the structure of *bona fide* PrP^{Sc} and other self-replicating PrP states. In the current study we discuss the data accumulated up to date on PK-digestion patterns of different self-replicating PrP states and assess possible relationships between these states. We also discuss the possibility of the transformation of self-replicating states within the frame of the deformed templating model and triggering of transmissible prion diseases by silent self-replicating PrP states.

2. Materials and methods

2.1. Expression and purification of full-length rPrP and formation of rPrP fibrils

Human full-length recombinant PrP encompassing residues 23–231 (variant 129 V) was expressed and purified according to previously described procedures (Makarava and Baskakov, 2012). Lyophilized rPrP was dissolved in 5 mM HEPES (pH 7.0) immediately before use. To form fibrils, the rPrP stock solution (0.25 mg/mL) was incubated in 50 mM MES (pH 6.0), 0.5 M GdnHCl at 37 °C under continuous shaking at 600 rpm using a Delfia plate shaker (Wallac). Amyloid formation was confirmed by Thioflavin T fluorescence assay (Makarava and Baskakov, 2012). The work on purification and fibrillation of human rPrP was performed in a Biosafety Level 2 laboratory.

2.2. Preparation of normal brain homogenate

Brains of transgenic mice expressing human PrP^{c} (variant 129 V) were kindly provided by Dr. Wen-Quan Zou (Case Western Reserve University, Cleveland, OH). 10% NBH (w/v) was prepared using brains of healthy transgenic mice expressing human PrP^{c} (variant 129 V) as previously described (Makarava et al., 2011). Briefly, the brains were ground with low speed tissue grinders in ice-cold conversion buffer until homogenous. The conversion buffer consists of Ca^{2+}-free and Mg^{2+}-free PBS, pH 7.5, supplemented with 0.15 M NaCl, 1.0% Triton, and one tablet of Complete protease inhibitors cocktail (Roche, Cat# 1836145) per 50 ml of buffer. The 10% NBH homogenate was pre-cleared by 2 min of centrifugation at 500 × *g* and the supernatant was used for dgPMCAb experiments.

2.3. Protein misfolding cyclic amplification with partially deglycosylated substrate (dgPMCAb)

Protein misfolding cyclic amplification that employs partially deglycosylated PrP^{C} as a substrate is referred to as dgPMCAb. To produce partially de-glycosylated substrate, 10% NBH mice was treated with PNGase F (New England BioLabs, glycerol-free) as follows. After pre-clearance of 10% NBH, 1500 U/ml PNGase F was added to the supernatant, and the reaction was incubated on a rotator at 37 °C for 5 h. The resulting substrate was used in dgPMCAb using the following sonication conditions: the standard sonication program for rounds 0–6 consisted of 30 s sonication pulses delivered at 50% power efficiency applied every 30 min during a 24 h period, while rounds 7–9 consisted of 5 s sonication every 10 min during a 24 h period. The dgPMCA reactions were seeded by adding 10 μL of rPrP fibrils (25 μg/mL) to 90 μL of 10% NBH. For rounds 2–6, 10 μl of the reaction mixture from the previous round was added to 90 μl of 10% NBH; for rounds 7–9, 30 μL of the previous reaction was added to 70 μL of substrate. Each dgPMCAb reaction was carried out in the presence of two 3/32″ Teflon beads (McMaster Carr).

To analyze production of PK-resistant PrP material in dgPMCAb, 10 μL of the sample was supplemented with 5 μL SDS and 5 μl PK, to a final concentration of SDS and PK of 0.25% and 50 μg/mL respectively, followed by incubation at 37 °C for 1 h. The digestion was terminated by addition of SDS-sample buffer and heating the samples for 10 min in a boiling water bath. Samples were loaded onto NuPAGE 12% BisTris gels, transferred to PVDF membrane, and probed with anti-C human PrP antibody (gift from Dr. Wen-Quan Zou, the Case Western Reserve University) that recognizes the epitope 220–231.

3. Results and discussion

3.1. Insight into PrP^{Sc} structure from limited proteolysis

Limited proteolysis with PK produces a truncated form of PrP^{Sc} referred to as PrP27-30 that spans residues ~90–231 for most prion strains (Basler et al., 1986; Oesch et al., 1985). Detailed analysis by mass spectroscopy revealed that each prion strain or isolate is characterized by multiple PK-cleavage sites that are distributed in a strain-specific manner along the region spanning from amino acid residue 74–102 (Fig. 1A) (Parchi et al., 2000; Sajnani et al., 2008). In addition to strain-dependent conformations, the distribution of PK-cleavage sites also depends on PK-digestion conditions including pH, presence of detergents and PK concentration (Notari et al., 2004). Harsher digestion conditions shift the PK sites in the direction of the C-terminus. For instance, upon treatment with high concentrations of PK, additional PK cleavage sites were identified within the central region, including residues 117, 119, 135, 139, 142, and 154 (residue numbers are given for hamster PrP) using limited proteolysis and mass spectroscopy (Fig. 1A) (Sajnani et al., 2008). Subsequent study of PrP^{Sc} devoid of its glycosylphosphatidylinositol anchor and carbohydrates also showed that in addition to the conventional PK-cleavage sites around the residue 90, the following residues within the central region were accessible to PK: 116, 118, 133, 134, 141, 152, 153, 162, 169 and 179 (residue numbers are given for mouse PrP) (Vazquez-Fernandez et al., 2012). In agreement with the data obtained by limited proteolysis and mass spectroscopy, the studies on chemical modifications of tyrosine or lysine residues suggested that in PrP^{Sc} the C-terminal region is considerably less accessible than the central region (residues 90–162) (Gong et al., 2011).

In a manner similar to harsh proteolytic treatment, pretreatment of PrP^{Sc} under partially denaturing conditions exposed new

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