Truncated forms of the prion protein PrP demonstrate the need for complexity in prion structure

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ABSTRACT. Self-propagation of aberrant protein folds is the defining characteristic of prions. Knowing the structural basis of self-propagation is essential to understanding prions and their related diseases. Prion rods are amyloid fibrils, but not all amyloids are prions. Prions have been remarkably intractable to structural studies, so many investigators have preferred to work with peptide fragments, particularly in the case of the mammalian prion protein PrP. We compared the structures of a number of fragments of PrP by X-ray fiber diffraction, and found that although all of the peptides adopted amyloid conformations, only the larger fragments adopted conformations that modeled the complexity of self-propagating prions, and even these fragments did not always adopt the PrP structure. It appears that the relatively complex structure of the prion form of PrP is not accessible to short model peptides, and that self-propagation may be tied to a level of structural complexity unobtainable in simple model systems. The larger fragments of PrP, however, are useful to illustrate the phenomenon of deformed templating (heterogeneous seeding), which has important biological consequences.

KEYWORDS. prion structure, peptide models, self-propagation, amyloid, fiber diffraction protein aggregation

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The ability of some proteins to fold into alternative, β-rich conformations has been known for over 80 y. Since that time, it has been determined that such β-rich conformations are the defining structural features of amyloid fibrils. The identification of prion rods as amyloid fibrils is more recent, but still well-established. Nevertheless, not all amyloids are prions, indeed, most are not. We must inevitably ask: what are the structural features of prions that distinguish them from other amyloids? What is the structural basis of the protein conformation self-replication that defines prion activity? The answers to these questions have at least 2 important ramifications: conceptually, they will help us to understand the pathological function of prions, and experimentally, they will help us to determine the value of various molecular model systems for prions. The latter is of considerable importance in itself, because of the intractability of most prions to physical and chemical studies.

Because of this intractability, most fiber diffraction studies, all crystallographic studies, and many other structural studies of amyloids have been of small amyloidogenic fragments of biologically important amyloids. These fragments have the cross-β structure common to all amyloids, β-strands running perpendicular to the fibril axis and forming long β-sheets that extend parallel to the fibril axis. However, the β-sheets are arranged in generic stacked-sheet architectures, stacked parallel to one another. These simple arrangements are quite distinct from those of the structurally more complex architectures found in biologically active amyloids. For example, the fungal prion HET-s and putatively the mammalian prion PrP have β-solenoidal structures (Fig. 1A, B), and even the relatively small Alzheimer’s-associated Ab peptide has a U-shaped 2-β-strand architecture. These are cross-β structures, and they are amyloids, but they are qualitatively more complex than simple stacked sheets. Generic stacked-sheet amyloid structures do not appear to share the self-propagating character of structurally more complex prions.

We have recently examined the structures of amyloids formed by a series of fragments of PrP, ranging from the 21-amino-acid fragment PrP21, known to form amyloid fibrils and to be toxic but not self-propagating in cell culture, to the 106-amino-acid fragment PrPSc106, which has been shown to support prion propagation in transgenic mice. We compared these structures to that of PrP27–30 (Fig. 1A). PrP itself consists of approximately 200 amino acids; PrP27–30 has about 65 residues removed from the N-terminus, but retains infectivity and appears to be structurally very similar to the full-length prion protein PrPSc. We found that the structural similarity between the fragments and PrP27–30 depends heavily on the size of the fragment. Despite its experimentally demonstrated toxicity, PrP21 has a generic stacked-sheet structure. A 55-residue fragment, PrP55 (mouse PrP(89–143)P101L), may have stacked-sheet or β-solenoidal conformation, depending on conditions of amyloid formation and fiber diffraction specimen preparation, particularly humidity, but even in the β-solenoidal form, the solenoid appears to consist of only 2 rungs of β-strands, not 4 as in PrP27–30. The diffraction patterns from a larger fragment, PrP89, were under some but not all circumstances consistent with the β-solenoidal structure of PrP27–30, while the patterns from PrPSc106 were strongly reminiscent of those of PrP27–30, including meridional maxima at 9.6, 6.4, and 4.8 A, corresponding to the second, third, and fourth orders of a 19.2-A repeat, the size of a 4-stranded β-sheet.

We concluded that the relatively complex structure of the prion form of PrP27–30 is not accessible to short model peptides, although these models can have some value in understanding the simple generic amyloid stacked-sheet structure. Self-propagation, the definitive property of prions, appears to be tied to a level of structural complexity unobtainable in simple
model systems, so there may not be simple solutions to the structural understanding of prions. Amyloids may be considered in an ascending hierarchy of complexity, beginning with the simplest of stacked-sheet structures formed by peptides as short as 6 amino-acid residues, many of which have been studied by crystallography and fiber diffraction. Under suitable solution conditions, many, perhaps most, polypeptides can form such amyloids. Studies have often been made of small peptide fragments of larger amyloidogenic proteins, but caution should be exercised when considering the amyloid structures of these fragments; it has often been suggested that the amyloid state is accessible to most or all polypeptides under suitable conditions, so there is no reason to assume that the amyloid structure of a fragment is characteristic of the amyloid structure of the complete protein. For example, the β-strands in the structures of short peptide amyloids may be parallel or antiparallel (Fig. 1C, D), although the strands in naturally occurring amyloids have invariably been found to be parallel. The peptide PrP21 formed a 2-strand repeat generic stacked-sheet amyloid, probably with antiparallel strands, under our conditions, although a previous study under nominally identical solution conditions by solid state NMR found a single-strand-repeat, parallel in-register stacked sheet. It is certainly possible that different amyloid structures (parallel and antiparallel) could form even under very similar conditions. Regardless of the structural details, there is no evidence that any generic stacked-sheet amyloid structure of this type is self-propagating, as is required for a prion.

Larger polypeptides or proteins can also form generic stacked-sheet amyloids. Infectious recombinant PrP forms such structures. In most cases recombinant PrP has not been found to be infectious at all, and when it is infectious, it is not clear whether the infectivity is due to a small fraction of molecules, undetected in diffraction experiments but similar in structure to naturally occurring

FIGURE 1. Structural models of amyloids, constructed using UCSF-Chimera. (A) Four-rung β-solenoidal model core (residues 89–174) of PrP (B) Two-rung β-solenoidal structure of HET-s (PDB ID: 2KJ3). (C) Generic antiparallel stacked β-sheet model. (D) Generic parallel stacked-sheet model.
prions, or to a distinctly structured PrP amyloid, which acts as a catalyst for the formation of the fully replication-competent infectious prion structure. This route of infection represents a pathway parallel to the natural etiology and would most likely be caused by a process of deformed templating (heterogeneous seeding).\textsuperscript{17,18,19}

Regardless of the mechanism of infectivity, it is clear that the generic stacked-sheet structure has very little infectivity if any, and is very different, both structurally and functionally, from the $\beta$-solenoidal structure. HET-s exhibits a similar polymorphism:\textsuperscript{20} the wild-type protein at neutral pH and many mutants form $\beta$-solenoids, but other mutants and the wild-type protein at acidic pH form non-infectious\textsuperscript{21} stacked sheets.

There is, however, a more complex stacked-sheet form, which might be seen as intermediate in structure and complexity between the generic stacked sheet form and the $\beta$-solenoidal form. Under low-humidity conditions, PrP\textsubscript{55} adopts a different conformation, in which a $\beta$-solenoid appears to have collapsed into a stacked-sheet structure. The same effect has been seen for HET-s,\textsuperscript{22} and may be the basis of a type of structural polymorphism in the yeast prion Sup35.\textsuperscript{23}

The complexity of the PrP $\beta$-solenoid is such that even another $\beta$-solenoidal structure may not be an adequate model for the full prion structure. PrP\textsubscript{55} does under some conditions appear to acquire a 2-rung $\beta$-solenoidal structure, and it is infectious in transgenic mice expressing PrP(P101L) at a level similar to that of wild-type PrP.\textsuperscript{24,25} Nevertheless, it is too small to form the full 4-rung $\beta$-solenoid, and in this sense, can only be a partial model for PrP\textsuperscript{Sc}. PrP\textsuperscript{Sc106} forms a 4-rung $\beta$-solenoid, and is also infectious.\textsuperscript{14} and of the fragments examined, it may be the best model for PrP\textsuperscript{Sc}. However, both PrP\textsubscript{55} and PrP\textsuperscript{Sc106} exhibit high levels of disorder, and PrP\textsubscript{55} does not consistently form $\beta$-solenoidal structures, so these fragment models offer limited advantages if any over PrP\textsuperscript{Sc} and PrP 27–30 as objects of structural studies.

In a further extension of complexity, there is considerable evidence that prion structure and activity is affected not only by the amyloid structure of the folded protein, but by other parts of the protein, and in particular by the loops at the ends of and often connecting the $\beta$-strands. While there is no structure available in sufficient detail to illustrate this point for PrP, it is evident in the cases of $\text{A}\beta$ and HET-s. Mutations in a region without $\beta$-structure, the connecting loop between the 2 rungs of the $\beta$-solenoidal structure of HET-s (Fig. 1B), can have a large impact on fibrillization kinetics, stability, and structure of the fibril,\textsuperscript{20} as well as infectivity.\textsuperscript{26} In $\text{A}\beta$, residues 21–23 in the boundary between the first $\beta$-strand and the loop connecting it to the second strand are often altered in families exhibiting early-onset Alzheimer’s disease.\textsuperscript{27}

Perhaps the greatest value for prion studies of at least the larger fragments of PrP lies in their use not as structural models for the active prion itself, but as templates that illustrate the phenomenon of heterogeneous seeding or deformed templating.\textsuperscript{17,18,19,28} The infectivity of PrP\textsubscript{55}, for example, may well come from heterogeneous seeding, that is, seeding between distinct amyloid architectures. With two rungs of a $\beta$-solenoid, PrP\textsubscript{55} could easily contain a structured segment that can act as a seed, in distinct contrast to PrP21 and other short, non-infectious fragments. Such seed structures would usually need to contain elements of the PrP\textsuperscript{Sc} structure, and to that extent could represent partial structural models, although they do not contain all of the important molecular interactions found in PrP\textsuperscript{Sc}. Even more significantly, however, the phenomenon of seeding itself has important biological consequences.

The complexity of prion structures can allow a variety of amyloid structures to form, and some of these structures can give rise to prion strains, having different phenotypes. For example, behavioral variation has been observed among PrP strains.\textsuperscript{29} The molecular basis of differences among strains is now widely considered to be structural variation. Although self-propagation of molecular conformation in prions usually faithfully replicates the original prion structure, prions may occasionally act as seeds to form different structures. This is the process of deformed templating, and has been
demonstrated in HET-s, and PrP. Amyloids with distinct architectures can interact with one another, without requiring in vivo cofactors. Deformed templating provides a mechanism for a preferred prion structure to be reached from different initial nucleating agents, although the process may require several passages through animals.

In summary, small fragments of prion proteins have at best limited value as structural models, but infectious fragments, which have at least until now only included fragments of considerable size such as PrP55, may well have value in heterogeneous seeding or deformed templating studies of prion strains, strain adaptation, and interspecies prion transmission.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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