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Prion and Non-prion Amyloids of the HET-s Prion forming Domain

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HET-s is a prion protein of the fungus *Podospora anserina*. A plausible structural model for the infectious amyloid fold of the HET-s prion-forming domain, HET-s(218-289), makes it an attractive system to study structure–function relationships in amyloid assembly and prion propagation. Here, we report on the diversity of HET-s(218-289) amyloids formed *in vitro*. We distinguish two types formed at pH 7 from fibrils formed at pH 2, on morphological grounds. Unlike pH 7 fibrils, the pH 2 fibrils show very little if any prion infectivity. They also differ in ThT-binding, resistance to denaturants, assembly kinetics, secondary structure, and intrinsic fluorescence. Both contain 5 nm fibrils, either bundled or disordered (pH 7) or as tightly twisted protofibrils (pH 2). We show that electrostatic interactions are critical for the formation and stability of the infectious prion fold given in the current model. The altered properties of the amyloid assembled at pH 2 may arise from a perturbation in the subunit fold or fibrillar stacking.

Keywords: prion; amyloid; *Podospora anserina*; fungi; thioflavine T

Introduction

Amyloids are fibrillar protein polymers with a cross-β structure.[1] Polymerization of proteins or peptides into amyloid fibrils occurs during a number of protein deposition diseases but also during the physiological assembly of several microbial proteins into cell surface structures. In the particular case of the prion proteins, amyloids or amyloid-like assemblies become self-perpetuating *in vivo* and thus turn into pathological infectious agents or protein-based genetic elements.[2,3] Fungal prions represent ideal model systems to study the process of amyloid formation and its relation to prion propagation.[4] Among these prions are the [*PSI*$^+$], [URE3] and [*PIN*$^+$] yeast prions and the [Het-s] prion of the filamentous fungus *Podospora anserina*. Studies on fungal prion models have demonstrated that the amyloid forms of several prion proteins display prion infectivity.[5–10] This clear connection between amyloid formation and prion propagation raises a central question in prion biology, namely what is the fundamental difference between a prion and a non-infectious amyloid?[11,12] Recent work on the [*PSI*$^+$] yeast prion proposes that prion propagation is governed by two fundamental parameters, the fibril assembly rate and fibril shearing ability.[13] Prion propagation of an

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Abbreviations used: PFD, prion forming domain; ThT, Thioflavine-T; FTIR, ; GuHCl, guanidine hydrochloride; EM, electron microscopy.

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amyloid would occur only if, in combination, these parameters surpass a threshold. This model provides a useful theoretical framework to explain the difference between prions and non-infectious amyloids. Yet the structural basis that differentiates infectious and non-infectious amyloids remains largely unexplored principally because detailed structural information on amyloid assemblies of both types is still very limited and exceedingly difficult to acquire.[14,15]

Both non-infectious and prion amyloids are generally polymorphic. Various examples have been reported of proteins or peptides that are able to adopt variant amyloid conformations depending on the physicochemical conditions. This polymorphism generally translates to differences in fibril morphology, and in some cases polymorphism could be evidenced at the molecular level by solid state NMR.[16,17] Strikingly, polymorphism can become self-propagating *in vitro*.[17,18] Structural polymorphism is the physical basis of the prion strain phenomenon in yeast,[8] and potentially also in mammals.[18] Models proposed for Ure2p and Sup35 yeast prion proteins readily accommodate possibilities of structural polymorphism and are therefore valid models to explain existence of yeast prion strains.[19,20]

The [Het-s] prion of *P. anserina* controls a fungal self–non-self recognition process, known as heterokaryon incompatibility, that occurs when somatic cells of different individuals fuse.[21] The HET-s prion displays a globular α-helical domain appended to a natively unfolded domain termed prion forming domain (PFD) that is responsible for prion propagation and amyloid formation.[22,23] The PFD of HET-s is the C-terminal 218–289 fragment.[22,23] Its amino acid composition is very different from those of the yeast PFDs. Unlike them, the HET-s PFD is not N/Q-rich. Also, while yeast PFDs contain very few charged residues, they are abundant in the HET-s PFD. A combination of hydrogen exchange, solid state NMR and proline-scanning mutagenesis data has led to a model for the infectious amyloid fold of HET-s(218–289).[24] It comprises four β-strands forming a β-roll structure (Figure 1).[24] The β1–β2 and β3–β4 motifs display sequence homology and thus form a pseudo-repeat. They are connected by a large unstructured loop. Recent evidence further supports this β-roll model. Electron diffraction studies have shown that HET-s(218–289) fibrils indeed display a cross-β structure and STEM analyses revealed mass-per-length measurements of one HET-s PFD subunit per 9.4 Å, the value predicted in the model.[25] The existence of this structural model with delimited secondary structure elements enhances the appeal of the HET-s system to study structure–function relationships in amyloid assembly and prion propagation. In contrast to all other known prions, in the case of [Het-s], there is as yet no evidence for the existence of different prion strains. This could have trivial reasons and be related to the lack of adapted reporter systems to detect prion strains of [Het-s] *in vivo*. Alternatively and consistent with the high level of order detected in HET-s(218–289) fibrils by solid state NMR,[26] this lack of strain variants might result from the fact that the fibril structure does not tolerate much variation.

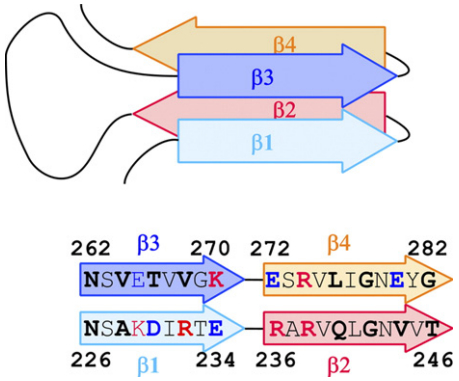

Figure 1. Schematic representation of the β-roll structure model of the HET-s PFD. Four β-strands (β1 to β4) have been demarcated experimentally and are proposed to be stacked perpendicular to the fibril axis in a β-roll fold as two homologous motifs connected by a flexible loop of 15 residues.[24] The sequences of the four β-strands are given. In each β-strand, side-chains are alternately directed toward the solvent (marked in bold) and buried in the interior of the fibril. Positively and negatively charged residues at pH 7 are marked in red and blue, respectively.

Here, we show that the HET-s PFD assembles into several distinct amyloid types *in vitro*. We distinguish three types on the basis of morphology and other properties; namely, bundles of aligned 5 nm fibrils formed at pH 7 in high ionic strength; disordered fibril-containing aggregates formed at pH 7 in low ionic strength; and thicker fibrils (9–15 nm) formed at pH 2, that appear to represent supercoiling of defined numbers (three, six) of 5 nm protofibrils. At pH 7, the amyloids form rapidly, whereas assembly is much slower at pH 2. We show that while fibrils formed at pH 7 are highly infectious, fibrils formed at pH 2 displays little if any prion infectivity in two distinct protein transfection assays.

Results

HET-s PFD fibril diversity

We have reported previously that HET-s(218-289) undergoes a transition from a random coil to a β-sheet rich structure at pH 7 in a buffer of relatively high ionic strength (160 mM acetic acid, 165 mM Tris base).[22] In this buffer, ordered bundles of laterally associated fibrils are detected by EM (Figure 2(a)). The elementary fibrils are ~5 nm in diameter. The bundles are highly variable in width and may exceed 100–200 nm (not shown).

While exploring alternative buffer conditions with lower ionic strength, we found that the same ran-