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Morphology and Secondary Structure of Stable β-Oligomers Formed by Amyloid Peptide PrP(106−126)[†]

Patrick Walsh,[‡,§] Jason Yau,[§] Karen Simonetti,[‡] and Simon Sharpe*[,‡,§]

[‡]*Molecular Structure and Function Program, Hospital for Sick Children, Toronto, ON, Canada M5G 1X8, and* [§]*Department of Biochemistry, University of Toronto, Toronto, ON, Canada M5S 1A8*

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ABSTRACT: The formation of nonfibrillar oligomers has been proposed to be a common element of the aggregation pathway of amyloid peptides. Here we describe the first detailed investigation of the morphology and secondary structure of stable oligomers formed by a peptide comprising residues 106−126 of the human prion protein (PrP). These oligomers have an apparent hydrodynamic radius of approximately 30 nm and are more membrane-active than monomeric or fibrillar PrP(106−126). Circular dichroism and solid state NMR data support formation of an extended β-strand by the hydrophobic core of PrP(106−126), while negative thioflavin-T binding implies an absence of cross-β structure in nonfibrillar oligomers.

The formation of fibrillar protein aggregates characterizes many neurodegenerative diseases, including Alzheimer's, Parkinson's, and mammalian prion diseases. While the accumulation of amyloid fibrils, and their deposition in plaques, has long been associated with cell death and disease progression, recent evidence suggests that it is more likely that nonfibrillar protein oligomers are the cytotoxic species (*1*, *2*). Soluble oligomers of the Alzheimer's β-amyloid protein (Aβ) have been observed in vitro and in vivo and induce neuronal cell death more strongly than the fibrillar or monomeric protein (*3*, *4*). Similar oligomers formed by several amyloid proteins have been produced in vitro and share several key features, including a common morphology, the ability to permeabilize model membranes, and cytotoxicity to cultured neurons (*5*−*7*).

On the basis of these observations, a general mechanism for amyloid toxicity has been proposed in which cell death results from the accumulation of nonfibrillar aggregates formed during the early stages of protein misfolding prior to the appearance of amyloid fibrils. However, despite their potential importance in the pathogenesis of amyloid diseases, relatively little is known about the molecular structure of nonfibrillar oligomers. In particular, no structural data have been reported for those formed by peptides other than Aβ.

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*To whom correspondence should be addressed. Phone: (416) 813-7852. Fax: (416) 813-5022. E-mail: ssharpe@sickkids.ca.

The structure of amyloid fibrils formed by several proteins has been described in detail, based primarily on high-resolution solid state nuclear magnetic resonance (NMR) studies (*8*−*10*). X-ray crystallography has also revealed the details of fibril structure for a number of short amyloidogenic peptides (*11*). In all cases, these structures share a common cross-β architecture, in which β-strands run perpendicular to the long axis of the fibril.

The best-characterized nonfibrillar oligomers are those formed by Aβ, for which spherical aggregates ranging from 5 to 35 nm in diameter have been reported, as well as 20−200 μm diameter "β-amy balls" (*5*, *12*). While some of these oligomers have been shown to contain β-sheet structure, their relationship to amyloid fibrils has not clearly been determined. In particular, it remains unclear if the spherical oligomers represent intermediate stages on the pathway to fibril formation or if they are the products of alternate misfolding pathways.

Direct evidence for fibril-like local structure in late stage intermediates of Aβ has been obtained from solid state NMR. Large (>650 kDa), transient oligomers were shown to share a quaternary structure with amyloid fibrils of the same peptide (*13*). More recently, solution NMR spectroscopy has been used to define the local structure of Aβ(1−42) in the context of small (16−64 kDa) SDS-stabilized oligomers, revealing extended β-strands forming a mixture of fibril-like and non-fibril-like intersheet contacts (*14*). The ability of Aβ to form oligomers with very different structural properties suggests that diverse nonfibrillar assemblies are accessible to other amyloidogenic peptides.

Here we describe the morphology and secondary structure of nonfibrillar oligomers formed by PrP(106−126), an amyloidogenic fragment of the mammalian prion protein (PrP). This peptide forms amyloid fibrils and soluble oligomers, induces apoptosis in cultured neurons, and may play a role in catalyzing the conversion of cellular prion (PrP^{C}) to the scrapie form (PrP^{Sc}) (*15*−*17*). We have recently described the structure of amyloid fibrils formed by PrP(106−126) (*18*). These are composed of parallel β-sheets stacked in an antiparallel class 1 steric zipper motif, resulting in 5−7 nm wide untwisted fibrils with the cross-β architecture typical of amyloid. The cytotoxicity of nonfibrillar oligomers of PrP(106−126) has been reported (*2*, *6*), but the details of their structure or morphology have not.

Using a protocol similar to that reported by Kayed et al. (*6*), we have produced oligomers of PrP(106−126) which appear as 5−30 nm spheres by negative stain transmission electron

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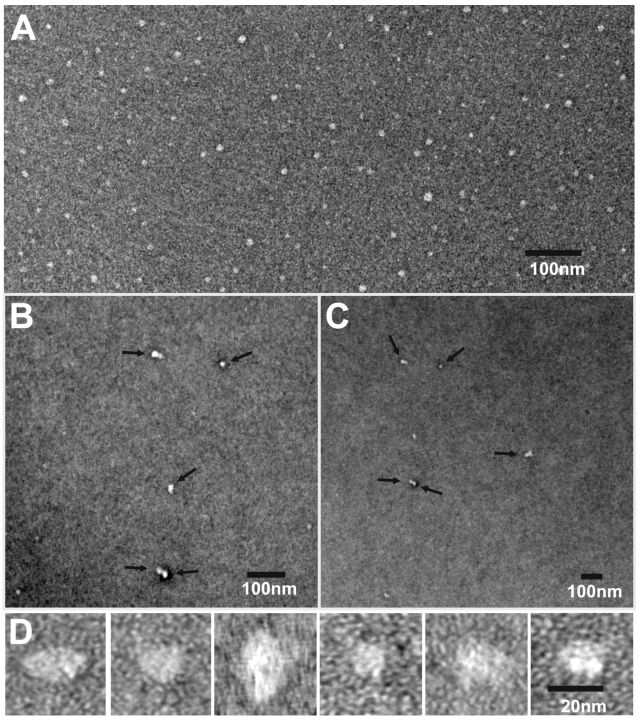

FIGURE 1: Transmission electron microscopy of PrP(106−126) oligomers. (A) Peptide oligomers stained with uranyl acetate and shown at 100000× magnification. (B and C) Lower-magnification images of two samples prepared using a double-carbon technique, with the uranyl acetate stain sandwiched between two carbon films (see the Supporting Information). Because of technical limitations of the specimen preparation for these images, use of more dilute samples was necessary. Examples of peptide oligomers are indicated by the arrows. Apparently elongated objects in panels B and C arise from clustering of two or more small oligomers. (D) Representative TEM images of individual PrP(106−126) oligomers from samples shown in panels B and C.

microscopy (TEM) (Figure 1A), consistent with the reported morphology. However, TEM images of samples embedded in a layer of stain sandwiched between two thin carbon films reveal an asymmetric oligomer morphology, with particles approximately 12−20 nm × 30 nm (Figure 1B−D). This method may reduce some artifacts associated with staining and may therefore provide a more accurate representation of PrP(106−126) oligomers. No fibrils were observed in any TEM images, an observation supported by the lack of thioflavin-T binding in oligomer samples (vide infra). Even after incubation for several weeks, no fibrils or morphological changes are observed in TEM images (Figure S1). Likewise, samples lyophilized for NMR exhibit a similar TEM morphology after reconstitution (Figure S1). To confirm our TEM results, approximately spherical PrP(106−126) oligomers were also observed in unstained samples using atomic force microscopy (AFM) (Figure S2).

The low size distribution of PrP(106−126) oligomers is supported by dynamic light scattering (DLS) measurements (Table S1) in which >97% of the sample mass resides in particles with a hydrodynamic radius of 30−40 nm. This suggests that the oligomers have a mass of >1 MDa, corresponding to more than 500 peptide monomers per oligomer. This is close to the size of large Aβ oligomers recently studied by solid state NMR (*13*), as opposed to the 3−5 nm diameter previously reported for oligomers of PrP(106−126) (*6*). Our results are also consistent with sedimentation velocity experiments performed on the amyloid peptide amylin (*19*), in which no small oligomers were detected, leading the authors to estimate that amylin predominantly forms aggregates of at least 390 kDa.

The cytotoxicity of PrP(106−126) has been debated in the literature; the conflicting reports likely stem from the different aggregated states accessible to this peptide. The oligomers studied here exhibit a potent ablility to disrupt model membranes. Soluble PrP(106−126) oligomers increase the membrane permeability to the self-quenching fluorescent dye calcein (Figure S3). Amyloid fibrils, unstructured monomers (in HFIP), and helical monomers (in TFE) exhibit no activity in this assay, supporting the formation of large soluble oligomers as a potentially important step in the cytotoxicity of PrP(106−126).

We have also defined the secondary structure of PrP(106−126) oligomers using circular dichroism (CD) and solid state NMR. The CD spectra shown in Figure 2A are characteristic of a β-sheet secondary structure, in contrast to the unstructured monomeric peptide. Likewise, the ^{13}C NMR chemical shifts obtained for residues 113−126 of PrP(106−126) are consistent with the presence of an extended β-strand spanning this region of the peptide (Figure 2B and Figure S4). As shown in Figure S5, the ^{13}C line widths observed for residues 113−124 are less than 2 ppm, suggestive of a well-ordered system. A slight increase in NMR line width is observed for ^{13}C resonances from L125 and G126, at the C-terminus, likely indicating an increased level of disorder at the end of the β-strand. Overall, these results suggest a secondary structure similar to that of PrP(106−126) fibrils (*18*).

In contrast to the amyloid fibrils formed by PrP(106−126), addition of nonfibrillar oligomers to thioflavin-T solutions does not result in increased fluorescence emission at 482 nm (Figure 2C and Figure S6). This strongly suggests that the PrP(106−126) oligomers described here lack the characteristic cross-β structure of the fibrillar form. Alternatively, it is possible that the ThT binding sites may be occluded in the nonfibrillar oligomers. In either scenario, the lack of dye binding indicates significant differences in peptide packing relative to amyloid fibrils. This result contrasts with the fibril-like nature of large Aβ oligomers, as monitored by NMR and ThT binding (*13*).

While the relationship between the soluble oligomers of PrP(106−126) and amyloid fibrils is unclear, it is remarkable that no conversion to larger aggregates is observed. The soluble oligomers form rapidly in solution and remain unchanged on a time scale of at least weeks under the solution conditions reported here. No additional loss of peptide mass is observed with extensive dialysis, suggesting that reversion to the monomeric peptide does not occur. The absence of ThT binding at up to 520 μM oligomer indicates a lack of fibril formation (Figure S6) and is supported by TEM analysis of aged samples (Figure S1). Removal of residual monomeric peptide by dialysis results in samples containing only the oligomeric species.

Previous structural studies of amyloid oligomers have relied on trapping a transient state or stabilizing oligomers with detergents (*13*, *14*). Here we demonstrate the formation of a stable, membrane-disrupting oligomeric species by a model amyloid peptide. This system will facilitate investigations of the structure and mechanism of action of nonfibrillar oligomers of PrP(106−126) and may provide insight into the assembly of other amyloids.

On the basis of the importance of nonfibrillar oligomers in the pathogenesis of amyloid diseases, it is essential to develop a detailed understanding of the factors governing the formation, molecular architecture, and activity of nonfibrillar assemblies. In addition to its utility as a model amyloid peptide, PrP(106−126)

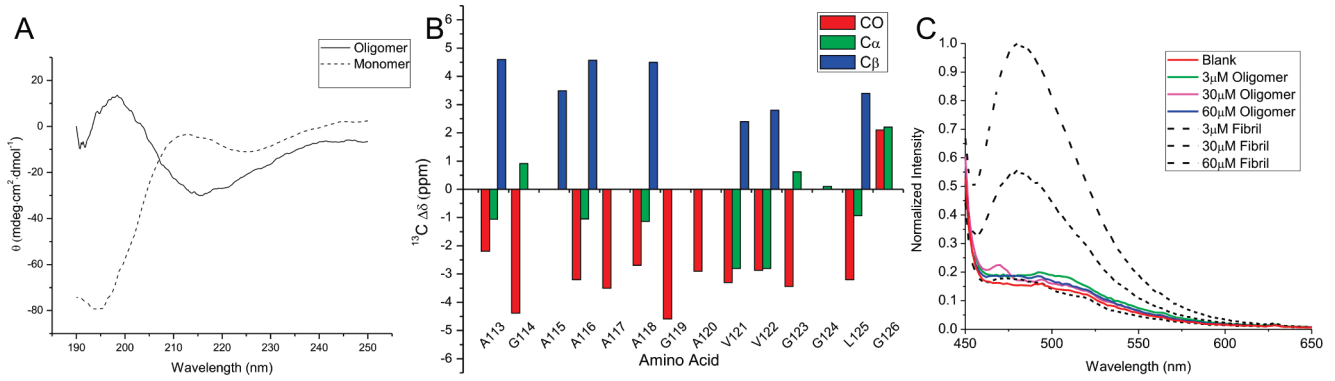

FIGURE 2: Spectroscopic analysis of PrP(106−126) oligomers. (A) Circular dichroism spectrum of nonfibrillar oligomers compared with that of the monomeric peptide in HFIP [monomer spectrum as previosuly reported (*18*)]. (B) ^{13}C secondary NMR chemical shifts for PrP(106−126) oligomers. (C) ThT fluorescence emission spectra of PrP(106−126) fibrils and soluble oligomers as a function of peptide concentration.

has been extensively investigated for its potential role in mediating conversion of PrP^C to PrP^{Sc} in mammalian prion disease. Therefore, a detailed understanding of the aggregated states accessible to this peptide may improve our understanding of the events underlying prion conversion.

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SUPPORTING INFORMATION AVAILABLE

Details of experimental procedures, including ^{13}C solid state NMR resonance assignments, Figures S1−S5, and Tables S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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