The cellular prion protein traps Alzheimer’s Aβ in an oligomeric form and disassembles amyloid fibers

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ABSTRACT There is now strong evidence to show that the presence of the cellular prion protein (PrP<sub>C</sub>) mediates amyloid-β (Aβ) neurotoxicity in Alzheimer’s disease (AD). Here, we probe the molecular details of the interaction between PrP<sub>C</sub> and Aβ and discover that substoichiometric amounts of PrP<sub>C</sub>, as little as 1/20, relative to Aβ will strongly inhibit amyloid fibril formation. This effect is specific to the unstructured N-terminal domain of PrP<sub>C</sub>. Electron microscopy indicates PrP<sub>C</sub> is able to trap Aβ in an oligomeric form. Unlike fibers, this oligomeric Aβ contains antiparallel β sheet and binds to a oligomer specific conformational antibody. Our NMR studies show that a specific region of PrP<sub>C</sub>, notably residues 95–113, binds to Aβ oligomers, but only once Aβ misfolds. The ability of PrP<sub>C</sub> to trap and concentrate Aβ in an oligomeric form and disassemble mature fibers suggests a mechanism by which PrP<sub>C</sub> might confer Aβ toxicity in AD, as oligomers are thought to be the toxic form of Aβ. Identification of a specific recognition site on PrP<sub>C</sub> that traps Aβ in an oligomeric form is potentially a therapeutic target for the treatment of Alzheimer’s disease.—Younan, N. D., Sarell, C. J., Davies, P., Brown, D. R., Viles, J. H. The cellular prion protein traps Alzheimer’s Aβ in an oligomeric form and disassembles amyloid fibers. FASEB J. 27, 1847–1858 (2013). www.fasebj.org

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A key hallmark of Alzheimer’s disease (AD) is extracellular fibrillar amyloid plaques, composed of a 39- to 43-residue peptide, amyloid-β (Aβ). The amyloid cascade hypothesis indicates that Aβ plays a central role in the disease, as genetic alterations underlying familial AD are associated with mutations in, or increased production of, Aβ (1). Studies on the neurotoxic element of Aβ suggest that small diffusible oligomers, rather than mature amyloid fibers, are the toxic form (2, 3).

A number of studies have indicated a direct high-affinity interaction between Aβ and the benign cellular prion protein (PrP<sup>C</sup>). Furthermore, studies with mouse models of AD have indicated that the presence of PrP<sup>C</sup> confers Aβ toxicity. Using an unbiased cDNA expression library screen containing 200,000 proteins, it has been shown that PrP<sup>C</sup> is the strongest candidate to bind to Aβ (4). The same study showed that the interaction between PrP<sub>C</sub> and Aβ<sub>42</sub> oligomers leads to the inhibition of long-term potentiation (LTP) in the hippocampal slices from normal mice expressing PrP<sup>C</sup>. Significantly, studies showed that transgenic PrP-knockout mice were immune to Alzheimer’s disease (AD) pathology (4). Indeed, PrP-knockout mice can develop Aβ plaques, but not neurotoxicity (5). These findings therefore suggested that PrP<sub>C</sub> is the main receptor that mediates Aβ toxicity.

The high-affinity interaction between Aβ and the benign PrP<sup>C</sup> has been reported by a number of groups (4, 6–9). It is believed that PrP<sup>C</sup> selectively binds to Aβ oligomers (4, 6–9), with an affinity in the nanomolar range (4, 7). Furthermore, the Aβ binding seems to involve the N-terminal half of PrP<sup>C</sup> (4, 6, 7). However, the initial report to link Aβ toxicity to PrP<sup>C</sup> has been challenged (8), and certain AD phenotypes have been shown to occur in the absence of PrP<sup>C</sup> (9–11). These conflicting observations may simply reflect the mouse model used and the nature of the Aβ-oligomer prepa-

Abbreviations: Aβ, amyloid-β; Aβ<sub>40</sub>, amyloid-β residues 1–40; Aβ<sub>42</sub>, amyloid-β residues 1–42; AD, Alzheimer’s disease; ATR-FT-IR, attenuated total reflectance Fourier transform infrared; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HSQC, heteronuclear single quantum coherence; mPrP, mouse prion protein; NMDAR, N-methyl-D-aspartate receptor; NMR, nuclear magnetic resonance; PrP, prion protein; PrP<sup>C</sup>, cellular prion protein; PrP<sup>C</sup> (23–231), cellular prion protein residues 23–231; SEC, size-exclusion chromatography; TBST, Tris-buffered saline and Tween 20; TEM, transmission electron microscopy; ThT, thioflavin T; TSE, transmissible spongiform encephalopathy; UV-CD, ultraviolet circular dichroism

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A number of recent studies support the correlation between the presence of PrP<sup>C</sup> and Aβ toxic effects, which impair synaptic plasticity and cause special memory defects and axon degeneration (4, 5, 12–15). Hippocampal primary culture and intrahippocampal injection indicate the cytotoxic effects of Aβ oligomers, which are significantly reduced for PrP-null mice (16, 17). A similar effect is observed in cell culture using cell lines that lack PrP<sup>C</sup> (18). Aβ oligomers also influence PrP<sup>C</sup> trafficking and inhibit PrP<sup>C</sup> endocytosis (19). In addition, aggregates from AD brain extracts have been reported to contain Aβ together with PrP (20, 21). PrP has also been shown to influence the processing of the amyloid precursor protein (APP; ref. 22). A genetic association between AD and the PrP has also been suggested (23, 24), with the polymorphism at residue 129 of PrPC representing a small but significant risk factor in sporadic AD, while others have noted no genetic association (25).

Aβ and PrP<sup>C</sup> are both concentrated at the synaptic terminals (26), and PrP<sup>C</sup> can also misfold into amyloid fibers. This misfolded PrP is associated with transmissible spongiform encephalopathies (TSEs; ref. 27). Mammalian PrP<sup>C</sup>s have a high structural and sequence homology (28, 29) and consist of two structurally distinct domains (30). The C-terminal domain (residues 126–231) is predominantly α-helical, while the N-terminal half of PrP<sup>C</sup> (residues 23–120) is natively disordered (31, 32), contains an octapeptide repeat sequence (residues 58–91), and is notable for its ability to bind Cu<sup>2+</sup> ions (33–36). Interestingly, PrP<sup>C</sup> limits excessive N-methyl-D-aspartate receptor (NMDAR) activity that might otherwise promote neuronal damage (37). Significantly, PrP<sup>C</sup> only affects the NMDA receptor in a copper-loaded state (14). Aβ has a picomolar affinity for Cu<sup>2+</sup> (38), and so may disrupt Cu<sup>2+</sup> binding to PrP<sup>C</sup>, and therefore, in part at least, mediates synaptic injury (14). The mechanism by which PrP mediates Aβ toxicity and NMDA activity is not clear, but may also involve the Fyn receptor (15).

Here, we aim to understand the mechanistic and structural detail of the Aβ:PrP interaction and explain how PrP<sup>C</sup> might influence Aβ oligomerization and toxicity. We show that PrP profoundly inhibits fiber formation by trapping Aβ in an oligomeric form that is rich in antiparallel β sheet. We map the PrP<sup>C</sup> recognition site to specific residues in the natively unstructured N-terminal half of PrP<sup>C</sup>. The ability to trap and concentrate Aβ into toxic oligomers suggests a mechanism by which PrP<sup>C</sup> might confer Aβ neurotoxicity in AD.

MATERIALS AND METHODS

Recombinant mouse PrP (mPrP) expression and purification

Expression and purification is as described previously (39). The coding region of the full-length mPrP(23–231) was cloned into a pET-23 vector to produce a tag-free protein. In addition, recombinant mPrP fragments containing the unstructured half of PrP<sup>C</sup>, mPrP(23–126) and the C-terminus structured domain of the mPrP, mPrP(113–231), were expressed. The C-terminal fragment only contains a His tag, to assist in purification. These recombinant proteins were expressed in minimal medium containing 15N ammonium chloride to produce 15N-labeled proteins suitable for nuclear magnetic resonance (NMR) studies. The recombinant PrP<sup>C</sup> lacks the GPI anchor and glycosylation found in vivo.

Peptide production

All peptides were purchased from Zinsser Analytic (Maidenhead, UK) and Cambridge Research Biochemicals (Cleveland, UK), synthesized using F-moc chemistry, purified as a single peak on HPLC and characterized by mass spectrometry. The purchased peptides included human Aβ peptide, residues 1–40 and 1–42; designated Aβ<sub>40</sub> and Aβ<sub>42</sub>, and shorter PrP peptides, PrP(58–91) and PrP(91–115), human sequence. The N terminus and the C terminus of these two PrP peptides only were acetylated and amidated, respectively, to mimic these residues within the full-length protein.

Aβ solubilization

Aβ<sub>40</sub> or Aβ<sub>42</sub> was solubilized at 0.7 mg/ml in water at pH 10.5 and gently rocked at 4°C for 72 h, with the pH maintained at 10.5 using NaOH. This process generated essentially monomeric, seed-free, Aβ stocks. After solubilization, the absorbance at 280 nm was used to calculate the concentration of Aβ, with an extinction coefficient of 1280 M<sup>−1</sup> cm<sup>−1</sup>. Typically, 10% of the weight was attributed to moisture.

Fiber growth kinetics

The growth of Aβ fibers was monitored using a 96-well microplate BMG Galaxy and Omega FLUOstar fluorescence reader (BMG Labtech, Ortenberg, Germany), with an excitation filter at 440 nm and an emission filter at 490 nm. Each reading consists of 50–100 flashes, prior to each reading, the plate was agitated for 30 s, orbital (3-mm) shaking, every 30 min. Sterile flat-bottomed plates were used and sealed with Starlab polystyrene sealing film (Starlab, Milton Keynes, UK). The wells hold up to 300 μl; these were used to their full capacity to reduce the volume of air in each plate. The pH of a sample was measured before and after each experiment; a variation of ±0.05 pH units or less was observed over the course of the experiment. The amyloid binding fluorophore used was thioflavin T (ThT) of fresh stock (2 mM) in water. The fibril growth experiments were typically carried out using 10 μM of essentially monomeric Aβ<sub>40</sub> or Aβ<sub>42</sub> in the presence of 2 mol eq of ThT. The sample was incubated at 30°C in the presence of 160 mM NaCl and 30 mM 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES) buffer (pH 7.4). Fluorescence detection was made by reading from the top of the well, using an orbital averaging sample reading (4 mm diameter). For the disaggregation experiments, small aliquots of PrP(23–231) were added while the 96-well plate was left on the plate holder within the reader.

Fibril growth-curve fitting

Sigmoidal fibril growth curves were fitted to the following equation, as described previously (40, 41):

\[
y = (y_i + m_x) + \frac{y_u + m_x}{1 + e^{-(x-x_0)/\delta}}
\]

where \(y\) is the ThT fluorescence intensity, \(x\) is the time (h) in hours, and \(x_0\) is the time at which the ThT fluorescence has
reached half maximal intensity ($t_{lag}$). The apparent fiber growth rate ($k_{app}$) is calculated from the following equation:

$$k_{app} = \frac{1}{\tau}$$

The nucleation or lag time ($t_{lag}$) is taken from the following equation:

$$t_{lag} = x_0 - 2\tau$$

### NMR

Main-chain assignments for mouse PrP\textsuperscript{C} amide resonances have previously been reported by ourselves (32). NMR samples contain \textsuperscript{15}N labeled PrP(23–231) (50 \mu M; pH 6.5) in phosphate buffer with essentially monomeric AB\textsubscript{40} (50 \mu M; unlabeled). NMR spectra were acquired at 30°C on a Bruker Avance spectrometer (Bruker Corp., Billerica, MA, USA) operating at 700 MHz for \textsuperscript{1}H nuclei using a 5-mm inverse detection triple-resonance cryoprobe. Phase-sensitive 2D \textsuperscript{1}H-heteronuclear single quantum coherence (HSQC) spectra were acquired using echo-antiecho gradient selection.

### Transmission electron microscopy (TEM)

Carbon-coated 300-mesh grids (SPI Supplies, West Chester, PA, USA) were glow discharged at the start of each experiment. An aliquot (5 \mu l) of the 10 \mu M AB sample was allowed to absorb onto a grid for 1 min before blotting off. This step was then followed by a 5 \mu l aliquot 2% (w/v) of phosphotungastic acid (PTA) at pH 7.4, to be absorbed for 1 min, to produce a negatively stained protein loaded grid. Images of the grids were recorded on a Jelel JEM 1230 electron microscope (Jeol Ltd., Akishima, Japan) operated at 80 kV.

Approximate relationship between protein volume ($V$) and molecular mass ($M$) is as follows: $V = M(kDa) \times 1.27 nm^3/kDa$. Oligomers are assumed to be spherical.

### Size-exclusion chromatography (SEC)

The Tricorn Superdex 200 10/300 analytical gel-filtration chromatography column (GE Healthcare, Little Chalfont, UK) was used on an automated AKTA FPLC system (GE Healthcare), at a flow rate of 0.5 ml/min. The column is capable of resolving proteins below 600 kDa. The column is equilibrated with 1.5 column volumes of buffer (30 mM HEPES, pH 7.4, and 160 mM NaCl), at 4°C. A 0.5-ml injection volume was used to load the protein onto the column (10 \mu M AB). The approximate molecular weight of the AB oligomers was estimated using standard globular proteins.

### Conformational AB oligomer antibody (A11) dot blot assay

AB, typically 30 \mu M, was incubated with and without the presence of PrP\textsuperscript{C} (10 \mu M), then 6 \mu l of these AB preparations was dotted on to a Hybond-ECL nitrocellulose membrane (purchased from Amersham Biosciences, Piscataway, NJ, USA). Once the sample spots were air dried, the membrane was blocked in 10% nonfat dry milk in TBST buffer [containing NaCl, Tris, and Tween 20 as per Invitrogen (Carlsbad, CA, USA) A11 protocol] for 3 h at 4°C. After the incubation, the membrane was washed 3 times with TBST buffer and incubated in 0.5 mg/ml unconjugated rabbit (polyclonal) anti-oligomer primary antibody (A11; cat. no. AHB0052; Invitrogen) in 5% nonfat dry milk in TBST buffer at 25°C for 1 h. After being washed, the membrane was then incubated in the enzyme conjugate (F(ab')\textsubscript{2} fragment of goat anti-rabbit IgG (H+L), alkaline phosphatase conjugate, (F21456; Invitrogen) for 1 h at 25°C. Finally, the membrane was washed once again and then incubated in NBT-BCIP substrate solution (BCBH8024V; Sigma-Aldrich, St. Louis, MO, USA) for 10 min. Stained dots is indicative of the presence of oligomeric species, but not AB monomer or fiber (42).

### Attenuated total reflectance Fourier transform infrared (ATR-FT-IR) spectroscopy

Spectra were obtained using a Bruker IFS 66/s FT-IR-spectrometer. Spectra represent an average of 32 scans, recorded at 25°C in the spectral range from 3000 to 370 cm\textsuperscript{-1}. All samples were prepared in 30 mM HEPES buffer (pH 7.4) and 160 mM NaCl. Small aliquots (5 \mu l) of sample were deposited onto the ATR crystal (ZnSe prism) where it was purged with nitrogen gas to generate a protein film.

### Ultraviolet circular dichroism (UV-CD)

Far-UV CD spectra were recorded between 210 and 260 nm, with a 0.1-cm pathlength and sampling points every 0.5 nm. Three scans were recorded, and a baseline spectrum was subtracted from each spectrum and zeroed at 255 nm. Data were processed using Applied Photophysics Chirascan Viewer (Applied Photophysics Ltd., Leatherhead, UK), Microsoft Excel (Microsoft Corp., Redmond, WA, USA), and the KaleidaGraph spreadsheet and graph package (Synergy Software, Reading, PA, USA). The direct CD measurements (\theta, mdeg) were converted to molar ellipticity, $\Delta \epsilon$ (M\textsuperscript{-1}cm\textsuperscript{-1}), using the equation $\Delta \epsilon = \theta/(33,000-c\ell)$, where $c$ is the concentration and $\ell$ is the pathlength.

### RESULTS

#### Substoichiometric amounts of PrP\textsuperscript{C} will completely inhibit AB fiber formation

In light of the clear interaction between AB and PrP\textsuperscript{C} and its influence on AB toxicity (4, 5,12–15) we wondered whether PrP\textsuperscript{C} might influence the process of AB oligomerization and fiber formation. First we investigated the influence of varying amounts of full-length PrP\textsuperscript{C}, PrP(23–231), on the kinetics of fibril formation of AB\textsubscript{40} and AB\textsubscript{25}, using the well-established ThT amyloid binding fluorescence assay. In the absence of PrP\textsuperscript{C}, AB\textsubscript{40} (10 \mu M) readily forms fibers, exhibiting a characteristic sigmoidal growth curve with a lag-phase (47 h) followed by rapid elongation of fibers (Fig. 1A). In the presence of equimolar concentration of PrP\textsuperscript{C} (10 \mu M) no amyloid fibers are detected even after 450 h (Fig. 1B). The same complete inhibition of fiber formation was observed for 0.1 and 0.05 mol eq of PrP (23–231) (Fig. 1C, D). Remarkably, 1:20 concentration of PrP\textsuperscript{C} (500 nM) relative to AB\textsubscript{40} will strongly inhibit the ability of AB\textsubscript{40} to form fibers. Only after 400 h of incubation with agitation there is a very weak ThT fluorescence signal observed in the presence of 500 nM PrP. At smaller amounts of PrP(23–231), for example, 250 and 100 nM, inhibition of AB\textsubscript{40} fiber formation was not apparent (Fig. 1E, F), and similar lag times, elongation rates, and total ThT fluorescence were observed to those of AB\textsubscript{40} in the absence of PrP\textsuperscript{C} (Supplemental Table S1). The cutoff point at which PrP(23–
PrP<sup>C</sup> inhibits Aβ<sub>40</sub> fiber formation. Kinetics of Aβ<sub>40</sub> fiber formation was monitored by fluorescence upon ThT binding to amyloid. Aβ<sub>40</sub> alone (A), and in the presence of 1 mol eq (B), 0.1 mol eq (C), 0.05 mol eq (D), 0.025 mol eq (E), and 0.01 mol eq (F) of PrP(23–231). Aβ<sub>40</sub> monomer (10 μM) was incubated at pH 7.4 in HEPES buffer (30 mM) and NaCl (160 mM) at 30°C with intermittent agitation. As little as 500 nM of PrP(23–231) completely inhibits Aβ<sub>40</sub> fiber formation over 250 h.

Aβ<sub>40</sub> fiber growth is very pronounced, with a molar ratio of 1:20 (PrP:Aβ<sub>40</sub>) almost completely inhibiting fibril growth, while 1:40 has no detectable influence on Aβ<sub>40</sub> fibril growth. TEM and IR data confirm a profound change in Aβ<sub>40</sub> structure and lack of Aβ<sub>40</sub> fiber content in the presence of PrP<sup>C</sup>, described later.

We also investigated the effect PrP(23–231) has on fiber formation of the more amyloidogenic Aβ<sub>42</sub>. At two concentrations (5 and 10 μM), a very similar effect is observed; both 1:1 and 1:0.1 Aβ<sub>42</sub>:PrP<sup>C</sup> molar ratios inhibit fibril formation (Fig. 2 and Supplemental Fig. S1). When 0.05 mol eq of PrP(23–231) was added, the nucleation period was prolonged, almost doubling the lag time; furthermore, the total maximal fluorescence was also significantly reduced, by more than half. As with Aβ<sub>40</sub>, the presence of smaller amounts, 0.02 mol eq of PrP(23–231), had little effect on the Aβ<sub>42</sub> fibril kinetics.

Aβ fiber inhibition is specific, centered at the N-terminal unstructured half of PrP<sup>C</sup>

Next we investigated how different regions of PrP<sup>C</sup> influence Aβ fiber growth, whether intact full-length PrP<sup>C</sup> is required for fibril inhibition, or whether the effect is isolated to a particular region of the protein. The fibril growth kinetics were measured in the presence of a number of recombinant and synthetic peptide fragments (Fig. 3). PrP(113–231), which contains all the structured elements of PrP<sup>C</sup>, does not inhibit fibril growth even at a 1:1 ratio (Fig. 3B). In particular, there is no increase in the lag time or reduction in total amount of fibers generated (Supplemental Table S2). In contrast, in the presence of only 0.1 mol eq of the unstructured N-terminal domain of PrP<sup>C</sup>, PrP(23–126), complete inhibition of fibril growth is observed (Fig. 3C). This suggests it is the natively unstructured half of PrP<sup>C</sup> that is specific to the PrP-Aβ interaction.

These observations prompted us to look at smaller fragments of the natively unstructured domain of PrP<sup>C</sup>. In particular, the presence of 0.1 mol eq or 1:1 of PrP(58–91), the 4-octarepeat fragment, showed no inhibition of fibril formation (Fig. 3D and Supplemental Table S2). Figure 3E, F also shows fibril growth kinetics measurements for Aβ<sub>40</sub> (10 μM) with different concentrations of PrP(91–115); only at relatively high levels of the PrP fragment do we observe an inhibitory effect at a 1:1 ratio, while no significant inhibition effect is observed at a 1:0.1 ratio.

The effects of various fragments of PrP<sup>C</sup> on Aβ<sub>42</sub> were also investigated (Supplemental Fig. S2). As with Aβ<sub>40</sub>, the N-terminal unstructured domain of PrP(23–126) at just 0.1 mol eq completely inhibits Aβ<sub>42</sub> fiber formation over 250 h of incubation, while the 4-octarepeat fragment has no effect on fiber formation, even at 1:1 molar ratio. It is clear that the Aβ<sub>40</sub> and Aβ<sub>42</sub> fiber inhibition is specific to the natively unstructured N-terminal portion of PrP<sup>C</sup>, PrP(23–126), which has a similar inhibitory effect as full-length PrP<sup>C</sup>.

**PrP<sup>C</sup> causes disassembly of preformed Aβ fibrils**

Next, the effect of PrP(23–231) on preformed mature Aβ fibrils was investigated. Figure 4 shows the fluorescence of ThT-bound fibers at equilibrium, after 240 h of incubation. At this time point, a small aliquot of PrP(23–231) was added to the Aβ fibers to make a 1:1 stoichiometry. The ThT signal rapidly reduces in intensity; almost half (40±3%) of the total ThT Aβ fiber signal is lost within 90 min of PrP<sup>C</sup> addition (see also Supplemental Fig. S3). A parallel control experiment involved adding 10 μM of the PrP fragment, PrP(113–231), to mature Aβ<sub>40</sub> fibrils (Supplemental Fig. S3). As expected, the structured C-terminal half of PrP<sup>C</sup> had no effect on the fiber content, as there was no observable decrease in the ThT fluorescence. This suggests that PrP(23–231) not only stops Aβ<sub>40</sub> fibril formation, but will also disassemble fully formed Aβ mature fibrils. PrP<sup>C</sup> does not dissociate all the Aβ fibers over a 100-h period, as only 40–50% of the total ThT fiber signal is lost. It may be that PrP<sup>C</sup> dissociates Aβ protofibrils rapidly, within an hour, while a subpopulation of more mature fibers is kinetically more resistant to disassembly by PrP<sup>C</sup>(23–231). TEM images of disassembled fibers, described later, suggest that mature...
fibers are broken up into much shorter fragments, which causes a reduction in the amount of ThT binding.

NMR maps the binding site on PrP\textsuperscript{C}

To probe the molecular details of the PrP\textsuperscript{C}:A\textbeta interaction on a per-residue basis, we used 2D \textsuperscript{15}N HSQC NMR spectra of full-length PrP\textsuperscript{C}. Essentially monomeric A\textbeta\textsubscript{40} was incubated with PrP\textsuperscript{C}(23–231) using a 1:1 molar stoichiometry, and \textsuperscript{15}N HSQC spectra were recorded over time, as shown in Fig. 5. Only the amide signals from PrP\textsuperscript{C} are observed in the \textsuperscript{15}N HSQC spectra, as A\textbeta is unlabeled. Initial addition of essentially monomeric A\textbeta\textsubscript{40} has little effect on the PrP\textsuperscript{C} \textsuperscript{15}N HSQC spectra. After 20 h at 30°C, specific PrP\textsuperscript{C} amide resonances start to lose their intensity. In particular, residues from two sections of the natively unstructured N-terminal half of PrP\textsuperscript{C}, between residues T95 and A113, and also residues T33, G35, S43, and T56. While the majority of the signals from PrP\textsuperscript{C} remain unaffected, in particular, none of the C-terminal resonances have their chemical shift or signal intensity affected. Strikingly, the regions of PrP\textsuperscript{C} that are perturbed by A\textbeta binding correlate very closely with the fragments for the N terminus of PrP\textsuperscript{C} that inhibit fiber formation (Fig. 3). \textsuperscript{15}N HSQC spectra of PrP\textsuperscript{C} under similar conditions, in the absence of A\textbeta, remain unchanged for a number of days.

Selected regions of the \textsuperscript{15}N HSQC spectra of PrP\textsuperscript{C} are shown before and after 40 h incubation with A\textbeta in Fig. 5A, B. The peak intensity of some selected amide resonances are plotted vs. time in Fig. 5C. After 20 h incubation, there is also the appearance of new well-resolved PrP amide signals that increase in intensity with time as A\textbeta binds to PrP\textsuperscript{C}. Interestingly, after 40 h, these signals have intensities and line widths comparable to unaffected PrP\textsuperscript{C} amide signals. The appearance of new PrP signals in the presence of A\textbeta indicates slow exchange between free and bound A\textbeta-PrP complex (on the chemical-shift timescale). Furthermore, the comparable intensity of the new PrP signal implies that most or all of the PrP molecules have A\textbeta bound, which suggests that at this stage, a simple 1:1 or 1:2 (A\textbeta dimer) stoichiometric complex has formed.

It is clear from the NMR spectra that PrP\textsuperscript{C} does not interact with disordered monomeric A\textbeta, as NMR spectra recorded for the first 20 h of incubation are unaffected by A\textbeta. A\textbeta must first change its conformation, presumably forming a small oligomeric species (dimeric) or misfolded monomer, before it will bind to PrP\textsuperscript{C}. The PrP\textsuperscript{C} amide line width for the new PrP signals for the PrP:A\textbeta complex are not appreciably increased, suggesting minimal increase in molecular mass. PrP\textsuperscript{C} must therefore be bound to no more than an A\textbeta dimer at this stage of the incubation of A\textbeta with PrP\textsuperscript{C}. We note that the concentrations of PrP\textsuperscript{C} and A\textbeta required for the NMR experiments described are very different from those that were used to monitor fiber formation in Fig. 1. It is therefore not possible to relate the lag times of A\textbeta binding to PrP\textsuperscript{C} (Fig. 5C) with nucleation times for fiber formation.

EM indicates PrP\textsuperscript{C} traps A\textbeta in an oligomeric form and disassembles A\textbeta fibers

Negative-stain TEM was used to investigate the effect of PrP\textsuperscript{C} on the gross structural morphology of A\textbeta fibers.
In the absence of PrPC, incubation of Aβ/H9252 (10 μM, pH 7.4; under the same conditions used for the ThT-binding fiber-growth experiments) generates the characteristic long unbranched fibers, 10 nm in diameter, while thicker mature fibers are 20 nm in diameter (Fig. 6A). However, in the presence of PrPC, TEM images indicate a complete lack of fiber generation throughout the EM grids. Instead, numerous spherical oligomers are observed (Fig. 6B). The small oligomers are typically between 6 and 10 nm in diameter, suggesting spherical oligomers of 100–500 nm³, which roughly approximates to 20–100 Aβ molecules in size (see Materials and Methods). Smaller oligomers may also be present, observed using SEC. Medium-sized oligomers, 25–30 nm in diameter, equate to Aβ oligomers that might contain a few thousand Aβ molecules. The medium-sized oligomers have halos of very small oligomers, 4–5 nm in diameter (approximately hexamers or dodecamers), around them. There are also a few much larger oligomers, 80–100 nm in diameter, containing 5–10 × 10⁴ Aβ molecules (Fig. 6B–D). There are also donut-shaped oligomers observed, 20 nm in diameter (Fig. 6E), which are postulated to form toxic pore-like structures across cell membranes (43).
Remarkably, the addition of PrP C to preformed fibers causes marked disassembly of fibers. The resulting assemblies generated are less spherical than those produced with PrP C incubated with Aβ/H9252 from the start, and have their own distinctive “rice-grain” morphology (Fig. 6F). The oligomers have quite a uniform appearance, typically 60 nm wide and 120 nm long, suggestive of fibers that have been chopped into very short 120-nm rods. Furthermore, under these conditions, some fibers remain after PrP C addition (Supplemental Fig. S4F), as suggested by partial fluorescence on ThT binding (Fig. 4).

**SEC indicates dodecameric Aβ oligomers are formed in presence of PrP C**

We also used SEC to investigate the size of the Aβ oligomers formed. A series of samples obtained from a fibril growth assay after 250 h of incubation was eluted from a Superdex 200 column (Fig. 7). Fibrils produced from 10 μM Aβ eluted in the void volume, as shown in the chromatogram in Fig. 7. In addition, the majority of the fibrils may not even enter the column with a precolumn filter. For Aβ fibers incubated in the absence of PrP C, there is no evidence of small oligomeric Aβ species < 200 kDa in size. Aβ was also incubated with 0.2 or 0.05 mol eq of PrP(23–231); ThT fibril growth assay indicated that under these conditions, no fibers were detected. The resulting chromatograms show two bands, eluting at 60–70 kDa (complex I) and 100–120 kDa (complex II). The N-terminal half of PrP, residues 23–126, also inhibits Aβ fiber formation, and very similar chromatograms were observed (Fig. 7B).

SEC suggests that the incubation of Aβ with either PrP(23–231) or PrP(23–126) results in the formation of two oligomeric species. Assuming one PrP C molecule per complex, this suggests that complex I has an oligomer size of 8–12 Aβ monomers (Aβ is 4 kDa in size), and complex II is 20–24 monomers in size, the typical size for toxic Aβ-derived diffusible ligands (ADDLs). The smaller (complex I) is the more abundant. The larger oligomers, detected by TEM (Fig. 6B) are too large to enter the column or elute within the void volume.

**PrP C-generated Aβ oligomers bind the oligomer-specific antibody**

To further characterize the Aβ oligomers generated in the presence of PrP C, we used the conformational antibody A11, which is specific to toxic oligomers found in vivo, but does not bind to Aβ fibers or low-molecular-mass Aβ (42). Aβ142 that was incubated with PrP(23–231) to generate oligomers clearly showed positive

![Figure 5. 15N HSQC NMR of the PrP(23–231) binding to Aβ40 oligomer. A, B) Selected regions of 2D 15N-1H HSQC of PrP(23–231) alone (black) and PrP(23–231) with 1 mol eq of Aβ40 after 40 h incubation (red). Amide resonances that show a marked loss of signal are labeled in blue. C) Peak intensity plotted against time; 6 new peaks are observed after 20 h (various shades of red), as well as reductions in the intensity of A113 and Q98, while S131 and T187 remain unaffected over 40 h. Spectra obtained at 30°C in 50 mM phosphate buffer (pH 6.5) and 50 μM PrP C and Aβ.](image)

**Figure 6. TEM of Aβ in the presence of PrP C. Negative-stain TEM images of Aβ40 fibers alone (A), Aβ incubated with PrP(23–231) (B–E), and PrP C added to mature Aβ40 fibers (F). Aβ40 (10 μM) samples were incubated at pH 7.4 in 30 mM HEPES and 160 mM NaCl at 30°C with intermittent agitation for 300 h. TEM grids were negatively stained using phosphotungstic acid. Only Aβ oligomers are observed where Aβ is incubated with PrP C (0.1 mol eq).**
respectively. Moreover, Aβ42 on its own did not bind the A11 antibody. Further, Aβ42 oligomers generated by the addition of PrP(23–231) to mature Aβ42 fibers, causing them to disassemble, also bound to the A11 antibody. In addition, the N-terminal unstructured fragment PrP(23–126) had a similar effect when incubated with Aβ40, generating A11 antibody binding to the Aβ oligomers.

Trapped Aβ oligomers contain antiparallel β sheet

Finally, we wanted to understand the structural nature of the Aβ oligomers formed. IR spectroscopy was used to determine the secondary structure conformation within the Aβ:PrP complex. IR spectra were obtained after incubation of Aβ with full-length PrPC (Fig. 9). As expected, the IR spectra of amyloid fibers of Aβ40 and Aβ42 is dominated by a strong amide-I absorption band centered at 1633 cm⁻¹, characteristic of β sheet (Fig. 9A). Aβ incubated with PrP(23–231) (1:0.1 Aβ:PrP) to generate the oligomeric species indicates a significant reduction in the intensity for the amide-I band at 1635 cm⁻¹; instead, the IR spectrum is dominated by a broad band centered at 1695 cm⁻¹, characteristic of β sheet and turns within the polypeptide chain (Fig. 9B). Interestingly, an amide-I band at 1695 cm⁻¹ can be indicative of β sheet in an antiparallel conformation, and the ratio of the intensity of the band at 1695/1633 can indicate the proportion of antiparallel arrangement of the β strands (44). Indeed, this ratio has been used to distinguish Aβ fibrils from the Aβ-oligomeric structure (45). The mixture of Aβ:PrP has contributions from PrP(C) as well as Aβ. The IR spectrum for PrP(C) on its own is also shown (Fig. 9B), although the PrP(C) contribution at a 1:0.1 ratio is minor.

Figure 9C shows the IR spectra for the 60-kDa Aβ-oligomer complex after it has been passed down the size-exclusion column (Fig. 7). In this spectrum, there is a clear amide I band at 1695 cm⁻¹, typical of antiparallel β sheet, with some absorption at 1633 cm⁻¹. A second stronger amide band is very characteristic of α helix and unstructured conformation centered at 1653 cm⁻¹ and may be largely due to a contribution from the α-helical PrPC (although it is not clear what the precise ratio of Aβ:PrP is within the small 60-kDa oligomers).

The final IR spectrum (Fig. 9D) reveals the effect of PrP(23–231) addition to mature fibers. It is clear that there is a profound change in the appearance of the spectra relative to fibers. There is little signal at 1633 cm⁻¹; instead, there is a broad band (1660–1690) typical of turns and β-sheet conformation. The higher ratio of Aβ:PrP used (1:1) means that the contribution to the spectra from PrP is significant, and the PrPC signal has been subtracted out to create a difference spectrum (Fig. 9D).

Aβ42 was incubated with the smaller unstructured N-terminal PrP fragment (residues 23–126), and the IR spectra were obtained (Supplemental Fig. S5). The incubated sample for the Aβ:PrP (23–126) complex has a relatively weak band at 1633 cm⁻¹ and a stronger broad band between 1660–1690 cm⁻¹, again indicative of turns and β-sheet structure.

Similarly, far-UV-CD spectra of Aβ fibers give a strong CD band at 217 nm, characteristic of β sheet. Spectra of Aβ incubated with the natively unstructured PrP fragment, PrP (23–126), show that the extended β-sheet conformation is largely retained within the oligomers (Supplemental Fig. S6). However, CD is not able to distinguish between parallel and antiparallel β strands.

DISCUSSION

Both PrPC and Aβ are concentrated at the synapse (15, 26) and bind to each other with a nanomolar affinity (4, 7). There is now strong evidence to link Aβ neurotoxicity with the presence of PrPC in mouse models of AD and primary cell culture (4,5,12–15). Here we show that PrPC has a profound influence on Aβ fibril growth kinetics. Remarkably, relatively small amounts of PrP

![Image](330x153 to 440x202)

Figure 8. Aβ oligomer antibody binding dot-blot assay. Samples were dotted on a membrane and examined using antibody A11, which is sensitive to oligomers but not to fibers or monomers. A) Aβ40 essentially monomeric. B) Aβ42 (30 μM) incubated with PrP(23–231) (10 μM). C) Aβ42 fibers. D) Disassembled Aβ42 fibers with 1.5 mol eq of PrP(23–231). E) PrP(23–126) (10 μM) only. F) Aβ40 with 0.1 mol eq of PrP(23–126). G) PrP(23–231) (10 μM) only.

![Image](87x613 to 281x740)

Figure 7. SEC of Aβ40 oligomer in the presence of PrP(C). Aβ40 was incubated with PrP(23–231) (A) and PrP(23–126) (B). Traces indicate Aβ40 only (top), Aβ40 with 0.5 μM PrP (middle), and Aβ40 with 2 μM PrP (bottom). Labels I and II indicate oligomers I and II, the complexes formed at ~60 and ~100 kDa, respectively. Aβ40 (10 μM) samples with and without PrP were incubated at pH 7.4 in 30 mM HEPES and 160 mM NaCl at 30°C with agitation for 250 h. SEC was carried out at 4°C and pH 7.4, using a Superdex 200 column.
(23–231), 1/20 mol eq (500 nM), will inhibit Aβ40 and Aβ42 fiber formation, confirming a nanomolar affinity for the interaction.

With the observation that PrP<sup>C</sup> promotes AD pathology, one might have expected PrP<sup>C</sup> to accelerate fiber formation of Aβ rather than inhibit it. However, oligomeric forms of Aβ are thought to be the most toxic to neurons (2,3). Here we show that PrP<sup>C</sup> promotes the formation of oligomeric species over fibers. Indeed, PrP<sup>C</sup> will disassemble mature fibers; this suggests that PrP drives the equilibrium between the fiber and the oligomer to the oligomeric form; trapping Aβ oligomers. Furthermore, we show that PrP<sup>C</sup>-trapped Aβ oligomers bind to the conformational antibody A11, which is specific to toxic oligomers found in vivo (42). Whether the transition from antiparallel oligomer to Aβ fibers is on or off pathway, it is clear that the binding of PrP<sup>C</sup> stabilizes the oligomeric form.

The presence of PrP<sup>C</sup>, even at substoichiometric amounts (1:20 concentration of Aβ), drives Aβ into an oligomeric form, rather than forming amyloid fibers. The ratio of Aβ to PrP to cause complete fiber inhibition implies that a single PrP is sufficient to trap an Aβ oligomer 20 monomers in size, as a 1:1, interaction might leave 95% of Aβ free to form fibers. This is in close agreement with a study by Chen et al. (7), who suggested a binding ratio of 21 Aβ molecules to 1 PrP<sup>C</sup>. Remarkably, preformed fibers are rapidly broken up by the presence of PrP<sup>C</sup>, which may enhance their toxicity, as it has been shown that fibers that have been broken up mechanically have increased toxicity relative to one long fiber (47).

The effect of various fragments of PrP<sup>C</sup> from the N-

**Figure 9.** IR spectra of Aβ oligomers in the presence of PrP<sup>C</sup>. A) Structural characterization using IR amide-I band of Aβ<sub>40</sub> mature fibers (dashed gray) and Aβ<sub>42</sub> mature fibers (solid black). B) Aβ<sub>40</sub> monomer incubated with 0.1 mol eq of PrP(23–231) (solid black) or PrP(23–231) alone (dashed gray). C) Aβ<sub>40</sub> with PrP<sup>C</sup> oligomers eluted by SEC. D) Aβ<sub>40</sub> mature fibers with 1 mol eq of PrP(23–231) (solid black), PrP(23–231) alone (dashed gray), and difference spectra (dotted). Aβ<sub>40</sub> monomer (10 μM) was incubated at pH 7.4 in 30 mM HEPES buffer and 160 mM NaCl at 30°C. Vertical dashed gray lines highlight 1695 and 1653 cm<sup>-1</sup>. Aβ<sub>40</sub> oligomer formed in the presence of PrP<sup>C</sup> shows an increase in 1695 cm<sup>-1</sup> amide-I band.
Figure 10. PrP\textsuperscript{C} stabilizes the oligomeric form of Aβ. Binding of PrP\textsuperscript{C} stops the transition from antiparallel to parallel sheet necessary for a transition from oligomer to fiber. PrP\textsuperscript{C} may stop the rotation of the β-sheet pairing from intramolecular. The energy barrier to go from antiparallel to parallel arrangement of β sheet is likely to be very large. It is therefore more likely that antiparallel oligomeric arrangements largely disassemble to form the in-register parallel sheets formed in fibers. Whether the transition from antiparallel oligomer to fibers is on or off pathway, it is clear that the binding of PrP\textsuperscript{C} stabilizes the oligomeric form.

and C-terminal halves of PrP\textsuperscript{C} indicates that the interaction is specific. The natively unstructured N-terminal half is the key to the Aβ-PrP interaction, while the C-terminal structured domain has no effect on Aβ fibril growth. Furthermore, we have also shown that at a high enough concentration, the short PrP fragment PrP(91–115) is able to strongly inhibit Aβ fibril growth. As the octarepeats alone do not influence Aβ fiber generation, these observations together suggest that the fibril-inhibiting region is centered at residues 91–115 but enhanced by the N-terminal residues 23–58. The Aβ-binding region on PrP\textsuperscript{C} is confirmed by our NMR studies, which show specific amide residues perturbed by the Aβ-PrP interaction, in particular, residues between T95 and A113, but also residues T33, G35, S43, and T56, whereas the C-terminal structured domain is unperturbed by Aβ interactions with PrP\textsuperscript{C}. This agrees very closely with studies mapping the binding region of PrP for Aβ oligomers, which indicate the binding sites of Aβ oligomers to PrP\textsuperscript{C} centered between residues 95–110 (4, 7) and secondary binding enhancement between residues 23–27 (7). In addition to full-length PrP\textsuperscript{C}, which is anchored to the plasma membrane surface, this is also an N-terminal fragment found in vivo, residues 23–113, which is caused by copper catalyzed cleavage (48). This cleaved fragment is able to diffuse within the synaptic space and could also bind to Aβ.

Amyloid fibers and oligomers have common features between proteins, such as exposed hydrophobic residues in an extended β-strand conformation. It has been suggested that the PrP\textsuperscript{C}-Aβ recognition site may be similar to the PrP\textsuperscript{C}-PrP\textsuperscript{Sc} interaction that induces template assisted misfolding in prion replication (6). Interestingly, the natively unstructured residues (residues 90–126) that bind to Aβ have been identified as necessary for prion replication in TSEs (49). Furthermore, like PrP\textsuperscript{C}, the scrapie isoform of PrP may also interact with Aβ (50).

In addition to the PrP\textsuperscript{C}, a number of other protein-binding partners have been indicated for Aβ, including serum amyloid P (SAP), transferrin, apolipoprotein E (APOE), and tau. Furthermore, both the extracellular chaperone, clusterin (51), and serum albumin, the most abundant protein in the cerebrospinal fluid (CSF), inhibit Aβ fiber formation (52), while islet amyloid polypeptide (IAPP) does not (53). PrP\textsuperscript{C} may be unique in its ability to concentrate Aβ into oligomers, while albumin isolates Aβ as a monomer and inhibits larger oligomer formation, as well as fiber generation (52).

The Aβ:PrP interaction represents a major new avenue in understanding Alzheimer’s disease. Potential therapeutics could be designed for the treatment of Alzheimer’s disease that inhibit the interaction of PrP\textsuperscript{C} with Aβ oligomers (12). Residues in the N-terminal portion of PrP\textsuperscript{C} are identified as target residues. Our studies show that small amounts of PrP\textsuperscript{C} have a profound effect on the equilibrium between Aβ fibrils and oligomers. Thus, PrP\textsuperscript{C} traps Aβ in an oligomeric form, which might explain how PrP\textsuperscript{C} can mediate Aβ’s toxic effects.

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Note added in proof: A recent report shows that PrP\textsuperscript{C} will inhibit Ab\textsubscript{42} fiber formation (54). However, no Ab oligomers were observed, and the study concluded that the prion protein is an inhibitor of Ab\textsubscript{42} assembly into toxic oligomers. Clearly, there remains much to be resolved regarding the Ab-prion protein interaction.

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