ACS Chemical Neuroscience

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Soluble Prion Protein Binds Isolated Low Molecular Weight Amyloid-β Oligomers Causing Cytotoxicity Inhibition

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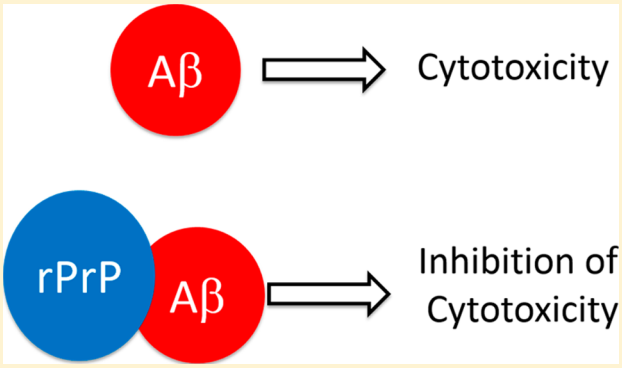
Supporting Information

ABSTRACT: A growing number of observations indicate that soluble amyloid-β (Aβ) oligomers play a major role in Alzheimer's disease. Recent studies strongly suggest that at least some of the neurotoxic effects of these oligomers are mediated by cellular, membrane-anchored prion protein and that Aβ neurotoxicity can be inhibited by soluble recombinant prion protein (rPrP) and its fragments. However, the mechanism by which rPrP interacts with Aβ oligomers and prevents their toxicity is largely unknown, and studies in this regard are hindered by the large structural heterogeneity of Aβ oligomers. To overcome this difficulty, here we used photoinduced cross-linking of unmodified proteins (PICUP) to isolate well-defined oligomers of Aβ42 and characterize these species with regard to their cytotoxicity and interaction with rPrP, as well the mechanism by which rPrP inhibits Aβ42 cytotoxicity. Our data shows that the addition of rPrP to the assembling Aβ42 results in a shift in oligomer size distribution, decreasing the population of toxic tetramers and higher order oligomers and increasing the population of nontoxic (and possibly neuroprotective) monomers. Isolated oligomeric species of Aβ42 are cytotoxic to primary neurons and cause permeation of model lipid bilayers. These toxic effects, which are oligomer size-dependent, can be inhibited by the addition of rPrP, and our data suggest potential mechanisms of this inhibitory action. This insight should help in current efforts to develop PrP-based therapeutics for Alzheimer's disease.



KEYWORDS: *Amyloid-β, Alzheimer's disease, Prion Protein, Oligomers, Neurotoxicity*

Alzheimer's disease (AD) is the most common form of dementia, whose pathological hallmarks include the deposition of extracellular neuritic plaques composed primarily of the amyloid-β (Aβ) peptide, the formation of intraneuronal neurofibrillary tangles composed of tau protein, and neuronal cell death.[1,2] Aβ is produced via the proteolytic cleavage of the amyloid precursor protein (APP) by β- and γ-secretases[3] resulting in the release of a peptide 40−43 amino acids in length. Aβ is an intrinsically disordered peptide that has a high propensity to self-assemble into a heterogeneous mixture of small, soluble, low molecular weight oligomers, protofibrils, and ultimately fibrils.[4] Historically, the amyloid cascade hypothesis posited that Aβ fibrils were the cause of AD and the associated neuronal cell death.[5] However, more recent data indicates that nonfibrillar, soluble oligomers and not mature fibrils correlate best with the severity and progression of the disease.[6,7] These oligomers have been shown to impair synaptic plasticity[8,9] and affect memory in experimental animals;[10] they are cytotoxic[11] and cause membrane leakage[12,13] and lysosomal damage.[14,15]

Despite much progress, the cellular and molecular mechanisms by which Aβ oligomers exert diverse neurotoxic effects are poorly defined, and there is much debate over which species are the primary toxic entities.[1] An important discovery was the finding that the cellular prion protein (PrP^C), a glycosylphosphatidylinositol (GPI) anchored cell surface glycoprotein, can act as a high affinity receptor for Aβ oligomers.[16] Following this finding, a number of reports indicated that the PrP^C receptor plays a major role in the pathogenesis of AD, acting as a critical mediator of the neurotoxic effects of Aβ oligomers, such as impairment of synaptic plasticity (as measured by inhibition of long-term potentiation) and neuronal cell death.[16−20] Even though it was shown that treatment with antibodies targeting PrP^C results in the elimination of Aβ-mediated inhibition of long-term potentiation,[21] the notion that PrP^C plays a critical role in AD has been challenged in some other studies,[22−24] leading to the ongoing dispute. This controversy notwithstanding, there is an agreement among the laboratories that prion protein strongly interacts with Aβ oligomers. Indeed, high affinity binding of oligomeric species of Aβ have been reported both to membrane anchored PrP^C and to the GPI-free recombinant prion protein (rPrP),[16,17,21,22,25,26] and the determinants of this binding have been mapped to the PrP segments encompassing residues ~95−110 and 23−27.[25]

Received: August 24, 2015
Accepted: October 14, 2015
Published: October 14, 2015

DOI: 10.1021/acschemneuro.5b00229
ACS Chem. Neurosci. 2015, 6, 1972−1980

Following these developments, recent studies explored the effect of rPrP on the aggregation pathway and toxicity of Aβ. It was found that both the full-length rPrP and its N-terminal fragment N1 (PrP23−110) are strong inhibitors of Aβ assembly into amyloid fibrils.[26−28] Furthermore, these proteins were reported to effectively block the neurotoxic action of soluble Aβ oligomers.[25,26] The latter finding is of particular interest from the therapeutic perspective, suggesting that PrP-based compounds might have a potential for pharmacological intervention in AD.[25,26]

A critical step toward the development of any therapeutic strategy that utilizes PrP-based compounds is a better understanding of the molecular mechanism by which PrP or its fragments interact with Aβ oligomers and prevent their neurotoxic effects. One of the greatest hurdles in this regard is the heterogeneity of commonly used preparations of Aβ, which typically contain a mixture of species ranging from dimers up to particles consisting of hundreds of monomers. Furthermore, these species are transient in nature, evolving with time into structures of increasing size. An approach that allows isolation, characterization, and quantification with regard to size distribution of individual small oligomeric species of Aβ and other amyloidogenic proteins is photoinduced cross-linking of unmodified proteins (PICUP).[29,30] This approach, which utilizes the Fenton reaction generated by visible light photolysis of a ruthenium(II) complex in the presence of an electron acceptor such as ammonium persulfate, relies on the formation of covalent bonds between closely interacting amino acid side chains without *per facto* modifications of the polypeptides.[31] PICUP cross-linking is achieved in a very short reaction time (~1 s) without the need for long linker molecules, which therefore minimizes the possibility of the potential formation of non-native interpeptide interactions that may result from random collisions of peptides.

Previously, PICUP was applied to test cytotoxicity of isolated Aβ40 monomers, dimers, trimers, and tetramers.[32] It was demonstrated that cytotoxicity was proportional to oligomer order, that larger oligomers (Aβ40 tetramer) had greater toxicity compared with Aβ40 dimer, and that the small oligomers were more toxic than fibrils and monomeric Aβ40.[32] In the present study, we used this methodology to isolate single oligomeric species of Aβ42 and characterize these species with regard to their cytotoxicity and interaction with rPrP, as well as the mechanism by which the latter protein inhibits the cytotoxic action of small Aβ42 oligomers.

RESULTS

Shift in Aβ42 Oligomer Distribution toward Monomeric Species Caused by Increasing Concentrations of rPrP. It is known that even a preparation of freshly solubilized Aβ42 is not entirely monomeric and contains a mixture of small oligomers of different sizes.[29] The first question we asked in the present study is whether rPrP could affect the oligomer size distribution in these preparations, because this could shed light on the mechanism by which rPrP inhibits the toxic action of Aβ. PICUP is the technique of choice to address this issue, since, by covalent cross-linking, it traps individual oligomeric species in their native state, and these species can be separated by gel electrophoresis and quantified by densitometric analysis.

The application of PICUP to freshly dissolved Aβ42 (25 °C) in the absence of rPrP reveals that the most populous species is a monomer (34%) (Figure 1 and Supplementary Figure S1). Dimers, trimers, and tetramers have a similar abundance, each accounting for approximately 12−15% of the total Aβ population. The proportion of higher order oligomers (pentamers, hexamers, and heptamers) is smaller, decreasing gradually with the increase in the oligomer size (Figure 1). It should be noted that the size distribution found in our study is somewhat different than that reported previously by other authors.[29] This discrepancy (which does not affect the major conclusions of this study) could be due to factors such as different sources of the peptide and potential small differences in Aβ42 solubilization or preparation protocols (even though nominally these protocols and chemicals used are very similar). When PICUP was applied to mixtures of freshly dissolved Aβ42 and rPrP, little effect on the oligomer size distribution was found at rPrP to Aβ42 molar ratios up to ~1/40. However, at higher concentrations of rPrP, there was a substantial shift in the oligomer size distributions toward the monomeric species of Aβ42. This was largely at the expense of decreasing proportions of higher molecular weight oligomers including tetramer, pentamer, hexamer, and heptamer, with populations of dimers and trimers remaining unchanged (Figure 1 and Supplementary Figure S1). Thus, the addition of rPrP to the assembling Aβ42 results in a substantial shift in the oligomer size distributions toward the monomer population and away from small soluble oligomers of the order of four and higher.

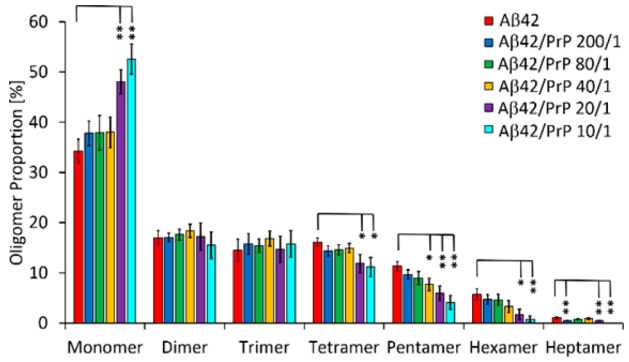


Figure 1. Aβ42 oligomer size distribution in the absence and presence of increasing concentrations of rPrP. Disaggregated Aβ42 (see Methods) was dissolved at a concentration of 20 μM in 10 mM sodium phosphate buffer (pH 7.2) and rPrP at increasing concentrations (0.1, 0.25, 0.5, 1, and 2 μM) was immediately added to the solution prior to cross-linking. Cross-linking reaction was performed within 5 min after peptide solubilization and oligomer size distribution was determined by photoinduced cross-linking of unmodified proteins (PICUP) combined with SDS-PAGE. The numbers indicate molar ratio of Aβ42 to rPrP. No measurable population of the heptamer was detected at the Aβ42/rPrP molar ratio of 10/1. *p < 0.05; **p < 0.01. The absence of any star indicates lack of statistical difference between the population of a given species in the absence and presence of rPrP.

Next we asked the question whether interaction with rPrP could lead to further (noncovalent) self-association of PICUP-isolated oligomeric species. This is an important issue, because such self-association could affect biological properties of these oligomers. Morphological examination of the PICUP-isolated, purified dimers, trimers, and tetramers by AFM reveals the presence of largely spherical particles (Figure 2). Analysis of the heights of randomly selected populations of these particles (three cross sections of the images as illustrated in Supplementary Figure S2) revealed that these heights were <1 nm (Figure 2A), <1.5 nm (Figure 2C), and <2 nm (Figure 2E) for the isolated dimers, trimers, and tetramers, respectively.

DOI: 10.1021/acschemneuro.5b00229
ACS Chem. Neurosci. 2015, 6, 1972−1980

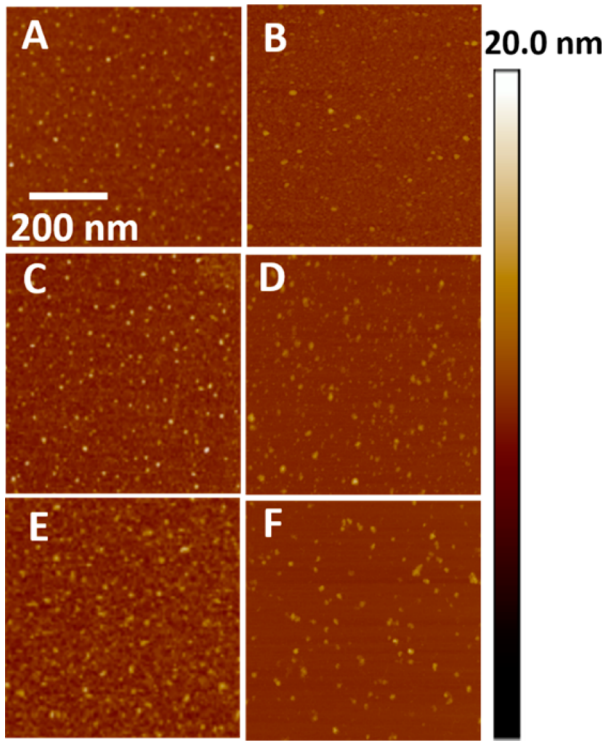


Figure 2. Atomic force microscopy images of isolated Aβ42 dimers, trimers, and tetramers alone and upon incubation with rPrP. Panels A, C, and E represent the isolated Aβ42 dimer, trimer, and tetramer alone, respectively. Panels B, D, and F represent the isolated dimer, trimer, and tetramer, respectively, upon incubation for 168 h with rPrP (Aβ42/rPrP molar ratio of 20/1). The color *z*-scale represents the height scale of 0−20 nm.

Importantly, the incubation of isolated Aβ42 oligomers with rPrP (molar ratio 1/20) for up to 168 h did not significantly alter the morphology or the sizes of the dimers (Figure 2B), trimers (Figure 2D), and tetramers (Figure 2F). The size of PICUP-isolated oligomers was further analyzed by quasi-elastic light scattering (Table 1). As expected, the hydrodynamic radii

Table 1. Hydrodynamic Radii of Isolated Aβ42 Oligomers in Absence and Presence of rPrP as Assessed by Dynamic Light Scattering[a]

	R_{hyd} Aβ42 (nm)	R_{hyd} Aβ42/rPrP (nm)
Aβ42 dimer	3.55 ± 0.05	4.05 ± 0.25
Aβ42 trimer	3.95 ± 0.15	3.8 ± 0.20
Aβ42 tetramer	4.25 ± 0.85	3.6 ± 0.60

[a]PICUP-isolated Aβ42 oligomers in the absence and presence of rPrP (Aβ42/rPrP molar ratio of 20/1) were preincubated for 24 h at room temperature. Data represent mean values ± standard error of the mean. Statistical analysis shows no significant difference between isolated oligomers alone and oligomers preincubated with rPrP (p > 0.1).

(R_{hyd}) of fully hydrated dimers, trimers, and tetramers were considerably larger compared with the heights of respective dried oligomers as measured by AFM. However, as in the case of AFM-based analysis, no significant differences were detected between the hydrodynamic radii of oligomers in the absence and presence of rPrP (molar ratio to Aβ42 of 1/20), confirming that rPrP does not induce further self-association (or clumping) of PICUP-isolated oligomeric species of Aβ42.

Inhibitory Effect of rPrP against Cytotoxicity of Isolated Aβ42 Oligomers in Primary Neurons. Previously, using a neuroblastoma cell model, it was shown that rPrP almost completely blocks cytotoxicity of Aβ42, even at a low rPrP/Aβ molar ratio of 1/20.[27] However, the preparations of Aβ42 used were highly heterogeneous (containing species ranging in size from very small particles such as dimers or trimers to large spherical oligomers), making it difficult to explore molecular mechanisms underlying this protective effect of rPrP. Therefore, here we extended cytotoxicity studies to well-defined oligomers trapped and isolated by PICUP. Cytotoxicity of these species in the absence and presence of rPrP were tested in rat primary hippocampal neurons. The viability of neurons was assessed by measuring the release of lactose dehydrogenase (LDH) into the medium.[27]

Treatment of hippocampal neurons with isolated dimers, trimers, and tetramers of Aβ42 (0.5 μM in each case) resulted in substantial release of LDH, indicating a major cytotoxic effect of all these species (Figure 3). At the quantitative level, the

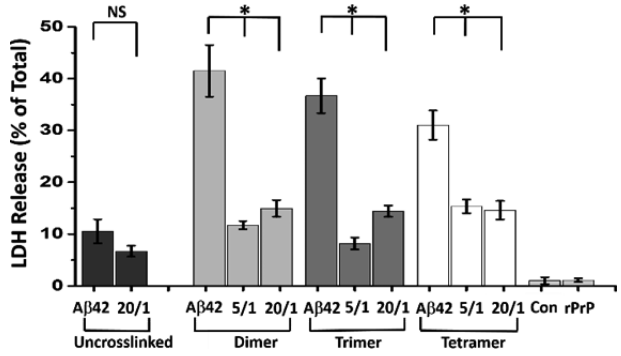


Figure 3. Inhibitory effect of rPrP on the cytotoxicity of different oligomeric species of Aβ42 in rat primary hippocampal neurons. Primary neurons were treated for 48 h with different oligomeric species of Aβ42 (freshly solubilized un-cross-linked preparation and isolated dimer, trimer, and tetramer) in the absence and presence of rPrP. The final concentration of Aβ42 was 0.5 μM in each case. Numbers under the bars indicate molar ratio of Aβ42 to rPrP, and the label "Aβ42" refers to Aβ42 alone (i.e., in the absence of rPrP). Bars labeled "Con" and "rPrP" represent control data for neurons treated with control gel extract of PICUP reaction buffer and neurons treated with rPrP at the highest concentration used in these studies (4 μM). Oligomer concentrations used to calculate the molar ratios represent the concentrations of particular oligomeric species (not the monomer concentrations). Data shown represents the average of at least four independent experiments, and the error bars depict the standard error of the mean. NS, no statistically significant difference (p > 0.05); *p < 0.05.

cytotoxic capacities of the isolated small Aβ42 oligomers show a species size dependency, whereby the smaller oligomers such as dimers have significantly greater capacity to cause cytotoxicity (42% LDH release) compared with the larger trimers (37% LDH release) and tetramers (31% LDH release). The cytotoxic effect of each of these isolated species is much greater than that of freshly solubilized preparation of Aβ42 (11% LDH release). This may not be unexpected considering the high proportion of monomeric Aβ42 in freshly solubilized peptide solution as determined experimentally using PICUP (Figure 1). However, quantitative comparison of cytotoxicity between well-defined isolated oligomers and freshly solubilized solution of un-cross-linked peptide is difficult and may be misleading since the latter preparation contains a multitude of species of different sizes,

DOI: 10.1021/acschemneuro.5b00229
ACS Chem. Neurosci. 2015, 6, 1972−1980

and the concentration of individual species (which evolve with time) is difficult to estimate.

When neurons were treated with Aβ42 dimers preincubated with rPrP, the release of LDH was greatly reduced compared with neurons treated with dimers alone (Figure 3), indicating that rPrP is a strong inhibitor of small oligomer cytotoxicity. This inhibitory effect was observed already at rPrP concentration corresponding to rPrP/Aβ42 molar ratio of 1/20 and was further increased when this ratio was increased to 1/5 (42%, 15%, and 12% LDH release for neurons treated with Aβ42 dimers alone and in the presence of rPrP at the 1/20 and 1/5 molar ratio to Aβ42, respectively). A similar inhibitory effect of rPrP was also observed for the cytotoxicity of trimer and tetramer species, which in the presence of rPrP induced substantially smaller release of LDH than oligomers alone (Figure 3). At the quantitative level, the relative inhibition was significantly stronger for dimers and trimers than larger tetramers (64%, 61%, and 50% inhibition at rPrP/Aβ molar ratio of 1/20 for dimers, trimers, and tetramers, respectively).

The Effect of rPrP on Membrane Permeation Effects of Isolated Aβ42 Oligomers. One of the hypothesized mechanisms with which Aβ has been suggested to cause cytotoxicity and cell death is through permeation of the lipid bilayer of cell membranes.[12,13] Consistent with this view, it has been observed that Aβ42 has a capacity to cause the release of a fluorescent dye, calcein, from biomimetic phospholipid membranes such as large unilamellar vesicles (LUVs).[12] The calcein release assay employs the encapsulation of the calcein fluorescent dye within the lumen of the LUVs at a self-quenching concentration. Upon permeation of the LUVs, the calcein diffuses out of the internal lumen into solution and becomes diluted, whereby the calcein concentration is decreased and a fluorescence increase is observed.

Here, we used the calcein release assay and LUVs composed of DOPC, DOPE, DOPS, and cholesterol (molar ratio of 50/15/5/30) to examine whether PrP has an inhibitory effect against Aβ-induced disruption of the lipid bilayer. The incubation of LUVs (0.5 mg/mL total lipid) with freshly prepared solution of (un-cross-linked) Aβ42 resulted in the immediate permeation of the membrane bilayer and the release of the encapsulated calcein. Over the time course monitored (180 min), ~35% of the total encapsulated calcein was released from the vesicles (Figure 4A), in good agreement with previously published data.[12] When the experiments were performed in the presence of rPrP (Aβ42/rPrP molar ratio of 20/1), the percentage of calcein release decreased to 27%, indicating that rPrP has a modest but statistically significant ($p < 0.05$) inhibitory effect against Aβ42-induced bilayer permeation. The addition of PICUP-isolated Aβ42 dimer to the LUVs resulted in an overall 16% total release of calcein, significantly less compared with vesicles treated with un-cross-linked solution of Aβ42. Remarkably, rPrP almost completely inhibited dimer-induced permeation of the vesicles (4.3% total release of calcein) (Figure 4B). Isolated Aβ42 trimer shows a similar capacity to cause membrane permeation as the dimer. Furthermore, also in this case, rPrP had a strong inhibitory effect, reducing calcein release from 14% to 7% (Figure 4C). Finally, the tetramer produced the lowest total calcein release of the isolated oligomers tested (release of ~8% of the total calcein encapsulated), and in this case no inhibition of the small membrane permeation effect was found in the presence of rPrP (Figure 4D). Collectively, these data demonstrate that smaller oligomers such as dimers have larger ability to cause membrane permeation compared to the larger tetramer oligomers. The inhibitory effect of rPrP against isolated Aβ42-oligomer-induced membrane permeations also appears to be oligomer size dependent, with rPrP being a more potent inhibitor of

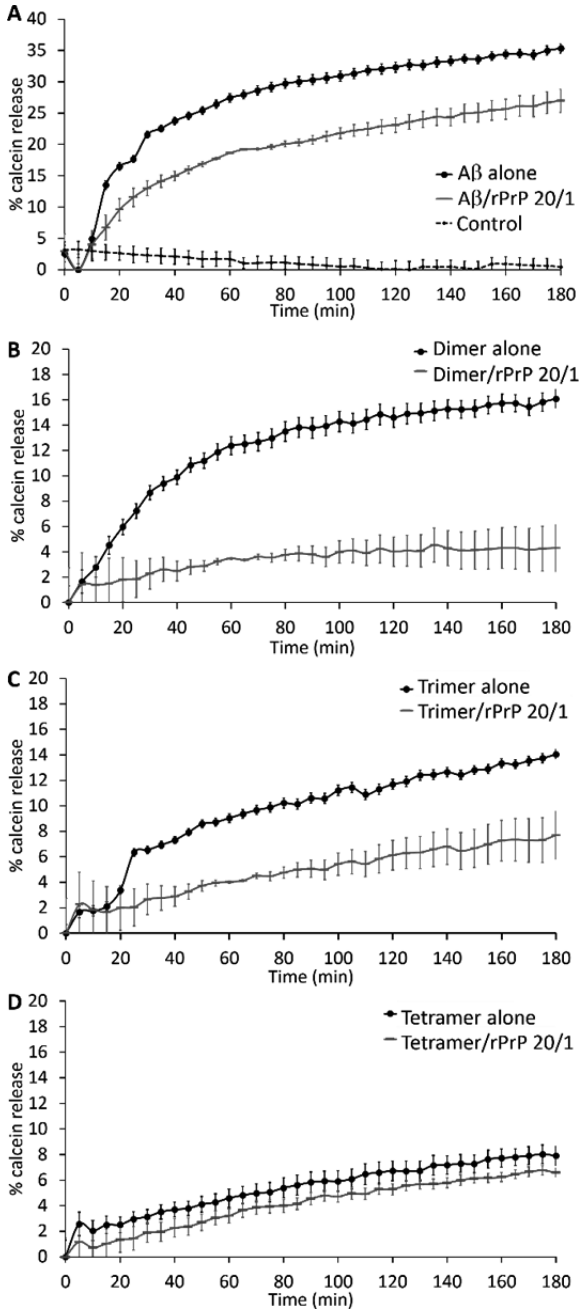


Figure 4. Calcein release from large unilamellar vesicles upon addition of Aβ42 in the absence and presence of rPrP. (A) Un-cross-linked preparation of freshly solubilized Aβ42. (B) Isolated Aβ42 dimer. (C) Isolated Aβ42 trimer. (D) Isolated Aβ42 tetramer. In each case, Aβ42 oligomers (20 μM of each oligomeric species) in the absence or presence of rPrP (1 μM) were added to LUVs (0.5 mg/mL) with encapsulated 200 mM calcein. The concentration of Aβ42 (20 μM) represents the concentrations of particular oligomeric species (not the monomer concentrations). Percentage of calcein release from LUVs is plotted as a function of time and is the average of three independent experiments. Error bars represent the standard error of the mean. "Control" in panel A shows spontaneous calcein release from LUVs in the absence of any additives.

DOI: 10.1021/acschemneuro.5b00229
ACS Chem. Neurosci. 2015, 6, 1972−1980

membrane damage induced by smaller isolated Aβ42 oligomers such as dimers compared with larger trimers and tetramers.

Smaller Aβ42 Oligomers Show Increased Affinity toward rPrP Compared with Larger Oligomers. As shown above, the inhibitory effect elicited by rPrP toward neuronal cytotoxicity and lipid bilayer permeation activity of Aβ42 shows oligomer size dependence. To gain insight into the molecular basis of this phenomenon, we employed surface plasmon resonance (SPR) to determine whether the isolated Aβ42 oligomers show different affinities toward rPrP. Biotinylated S231C rPrP was covalently attached to a streptavidin sensor chip. Solutions of Aβ42 dimer, trimer, and tetramer (2 μM) were then injected into the flow cells, and the association between the oligomer and rPrP was monitored for 120 s. Subsequently, the flow cells were rinsed with phosphate buffer to monitor the dissociation of the Aβ42 from the rPrP.

Representative sensograms for the freshly prepared solution of un-cross-linked Aβ42 and different PICUP-isolated oligomeric species are shown in Figure 5. These sensograms were used to determine the association (k_{assoc}) and dissociation (k_{off}) rate constants, from which the equilibrium dissociation constant (K_D) for binding to rPrP was calculated. As shown in Table 2, the apparent K_D for the un-cross-linked preparation of Aβ42 is 0.36 μM, and a similar value was determined for the isolated dimeric species. The K_D values for the trimer and tetramer are approximately 2- and 4-fold higher compared with that of the dimer. Thus, the affinity of Aβ42 for rPrP shows considerable oligomer size dependency: it is highest for the dimer, followed by the trimer and the tetramer. K_D values obtained in this study for well-defined small oligomeric species are higher than those derived from SPR experiments for heterogeneous oligomeric preparations of Aβ42. It is possible that some larger oligomeric species in the latter preparations have particularly high affinity for PrP.[22,26] However, it should be noted that sensograms obtained in these previous studies were highly unusual in that their dissociation parts were almost horizontal, likely due to factors such as secondary binding events or multivalency of binding (see Discussion in ref 25). This nearly horizontal character may interfere with accurate determination of the dissociation rates that are used for calculation of K_D.

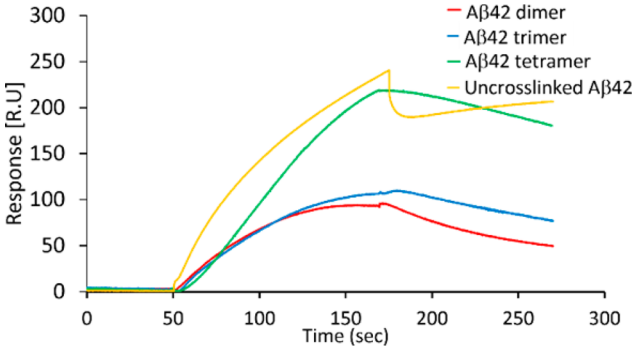


Figure 5. Representative surface plasmon resonance sensograms monitoring the binding between rPrP and Aβ42. Solutions of un-cross-linked Aβ42 and Aβ42 dimer, trimer, and tetramer (2 μM of each oligomeric species) were injected into the flow cell, and the association between the Aβ42 oligomer and rPrP attached to the streptavidin sensor chip was monitored for 120 s. Subsequently, the flow cells were rinsed with phosphate buffer to monitor the dissociation of the Aβ42 from the rPrP. It should be noted that the initial signal decrease in the dissociation part of the sensogram for un-cross-linked Aβ42 is followed by a small gradual increase of the signal. A potential explanation of this atypical behavior is the rebinding to the rPrP chip of a minor population of large oligomeric species (e.g., heptamers or larger) present in the heterogeneous mixture of un-cross-linked Aβ42. A similar rebinding effect was noted for some other proteins.[33]

Table 2. Equilibrium Dissociation Constant between Aβ42 Oligomers and rPrP Derived from Surface Plasmon Resonance Experiments[a]

Aβ42 species	K_D (μM)
un-cross-linked Aβ42	0.36 ± 0.16
Aβ42 dimer	0.38 ± 0.01
Aβ42 trimer	0.70 ± 0.16
Aβ42 tetramer	1.43 ± 0.12

[a]The values reported are based on three independent experiments, and the standard error of the mean is shown.

DISCUSSION

It has been posited that the primary neurototoxic species in Alzheimer's disease are small soluble oligomers,[1,6−11,34] the levels of which have been shown to be around 70-fold higher in AD brains compared with control matched brains.[34] By contrast, the monomeric species appears to be nontoxic, and some reports suggest that it may even have a neuroprotective role.[35] Recent studies indicate that the cellular, membrane-anchored prion protein, PrP^C, may play an important role in AD pathogenesis, acting as a mediator of at least some of the neurotoxic effects of Aβ42 oligomers.[16−19,21,22,36] Furthermore, it has been shown that soluble recombinant PrP and its fragments are strong inhibitors of cytotoxic and synaptotoxic effects of Aβ42, suggesting that rPrP or its fragments may offer new avenues for pharmacological intervention in AD. However, the mechanism by which rPrP prevents Aβ42 toxicity is at present unknown. Studies in this regard are hindered by the large heterogeneity and transient nature of Aβ42 oligomers in commonly used preparations. In an attempt to overcome this fundamental difficulty, we here used well-defined oligomeric species of Aβ42 that were trapped by PICUP. We found that rPrP alters the size distribution of small Aβ42 oligomers and that both the binding affinity between rPrP and Aβ and the protective effects of rPrP against Aβ42 cytotoxicity and membrane damage are oligomer size dependent.

Apart from the issue of the interaction between rPrP and Aβ and the effect of this interaction on Aβ toxicity (see below), our study sheds a new light on the ongoing debate as to which specific small oligomeric species of Aβ42 are most toxic. Various groups maintain that different species possess the greater cytotoxic capabilities, and the Aβ species that have been posited to possess particularly strong toxic capacity include, among others, the dimer,[37,38] trimer,[37,39] and dodecamer.[40] Of particular interest in this regard are previous studies using PICUP-isolated stable oligomers of Aβ40, which found that the isolated Aβ40 tetramer possessed higher cytotoxic potency compared with the trimer and dimer (tetramer > trimer > dimer).[32] In our present study with Aβ42, we observed the opposite oligomer order−toxicity relationship, whereby the Aβ42 dimer showed increased cytotoxicity compared with the trimers and subsequently the tetramers (dimer > trimer > tetramer). This dramatic difference between Aβ40 and Aβ42 in oligomer size dependence of their cytotoxicities could possibly result from structural differences between Aβ40 and Aβ42

DOI: 10.1021/acschemneuro.5b00229
ACS Chem. Neurosci. 2015, 6, 1972−1980

oligomers[41] as well as the different assembly pathways by which the two peptides oligomerize.[29,42] The addition of the extra two amino acids in the C-terminus of Aβ42 has been shown to play a key role in the formation of Aβ42 oligomers,[43] and differences between Aβ40 and Aβ42 are observed in the first contacts that form during monomer folding.[44] The finding of a fundamental difference in oligomer size dependence of cytotoxicity for Aβ40 and Aβ42 is of considerable importance, because it is generally believed that Aβ42 is more relevant to the pathogenesis of AD than Aβ40.[45,46]

As in the case of cytotoxicity in primary neurons, the dimer population of Aβ42 showed a greater ability to cause permeation of model lipid membranes compared with the trimer and tetramer population. However, it should be noted that, in contrast to neuronal cytotoxicity, in the calcein release assay un-cross-linked heterogeneous mixture of Aβ42 possessed greater ability to cause lipid membrane permeation compared with any of the isolated oligomeric species (Figure 4). This indicates that the mechanisms involved in Aβ42-induced cell death are more complex, involving factors other than permeation of the lipid bilayers. In membrane permeation events, Aβ has been shown to form stable defects and pores in lipid bilayers.[13,47] The apparent reduction in abilities to cause membrane permeation observed with the stabilized oligomers could be a result of the cross-links partially decreasing the Aβ pore mobility, since typically the pore is loosely connected by the disordered inner β-sheet, which causes mobility of the peptide subunits forming the pore.[48] Moreover, because the Aβ barrel pores in the lipid bilayers are formed from multimeric chains,[49] having a pure, isolated population of oligomers may in some way hinder the formation of the defined Aβ pores.

Previous studies showed that cytotoxicity of Aβ42 preparations enriched in large spherical oligomers (that are heterogeneous and likely also contain smaller oligomeric species such as dimers, trimers, and tetramers) can be almost completely prevented by rPrP.[27] One of several, mutually nonexclusive possibilities that could explain this protective effect is that rPrP shifts size distribution in the heterogeneous mixture of Aβ42 toward the monomer. This type of mechanism seems to be supported by our present data, which show that rPrP increases the monomer population at the expense of larger oligomeric species such as tetramers, pentamers, hexamers, and heptamers (Figure 1). The shift in size distribution could reduce the overall toxicity of the heterogeneous Aβ42 preparations, because the Aβ monomer is nontoxic and can even have a neuroprotective effect.[35] However, our data also shows that rPrP is an effective inhibitor of the cytotoxic action of stable, PICUP-derived oligomers. Since these oligomers have no possibility for dissociation into the monomers under these conditions, in this case the protective action of rPrP must be due to either direct interaction of rPrP with Aβ42 oligomeric species or due to rPrP binding to the membrane surface that could prevent toxic Aβ42−membrane interaction. The high affinity of PrP for Aβ42 oligomers as observed in previous studies[16,19,21,22,25] and the present report, together with the correlation we found between the inhibitory action of rPrP against the cytotoxic action of stable oligomeric species of different size and the binding affinity of these species to rPrP suggest the primary role of the former scenario.

The mechanism with which direct interaction of rPrP with stable Aβ42 oligomers prevents Aβ42 cytotoxicity is at present unclear. In principle, this could be due to competitive blocking of Aβ42 binding to membrane-associated PrP^C. However, an increasing number of studies indicate that, in contrast to the inhibition of LTP by Aβ42, cytotoxic and membrane-perturbing effects of the peptide are not mediated by PrP^C,[19,22,27] calling for an alternative explanation. From AFM and dynamic light scattering data, it is apparent that rPrP does not result in the nonspecific aggregation of isolated Aβ42 oligomers (Figure 2 and Table 1), suggesting that PrP does not work by sequestering oligomers into larger (potentially less toxic) multimeric complexes. Therefore, it appears that rPrP inhibits cytotoxicity of stable Aβ42 oligomers by causing changes in the structure of individual oligomeric species or by associating with these species in a manner that precludes their toxic interaction with the membranes. Apart from the cytotoxic effects, another activity of naturally formed (un-cross-linked) Aβ42 oligomers that is of direct relevance to Alzheimer's disease is their ability to impair synaptic plasticity.[8,9,16,19,20] Further studies are needed to determine whether individual PICUP-isolated oligomers are also synaptotoxic and whether such activity could be inhibited by rPrP.

While a number of studies have explored which specific amino acid residues of PrP are important for the interaction with Aβ oligomers,[16,25,26] no information is yet available with regard to which part of Aβ42 is involved in this interaction. However, previous studies indicate that the interaction of Aβ with many other ligands, as well as Aβ42 membrane binding and its toxicity are strongly dependent on the N-terminus.[41,50−53] If the Aβ42 N-terminus is also involved in the interaction with rPrP, this interaction could affect the abilities of Aβ42 oligomers to associate with neuronal membranes (either through lipids or specific receptors), resulting in the inhibition of the toxic effects as observed in the present study. Molecular dynamics simulations of Aβ42 shows that the N-terminus significantly changes during the oligomerization of the peptide,[54] and oligomers of different size have differing solvent exposures of the N-terminal residues. These differences in N-terminal exposure could contribute to the observed increased binding of rPrP to the dimer compared with the trimer and tetramer (Figure 5).

In conclusion, the present data suggest that rPrP could cause the inhibition of Aβ42-induced cytotoxicity by two mutually nonexclusive mechanisms: (i) shift in the size distribution of Aβ42 species away from larger oligomers toward the nontoxic, potentially neuroprotecive monomer and (ii) direct interaction with Aβ42 oligomeric species in a manner that renders these species less toxic. This insight should aid in future development of PrP-based therapeutics for Alzheimer's disease.

METHODS

Preparation of Soluble Prion Protein. Expression and purification of recombinant human rPrP and S231C rPrP was performed as described.[55] Protein concentration was determined from the absorbance at 280 nm using an extinction coefficient of 56650 M^{-1} cm^{-1}. For the C-terminal biotinylation of S231C rPrP for SPR, 5 molar excess of TCEP was added to the protein, and the mixture was incubated at 4 °C overnight. The protein was then diluted to 0.2 mg/mL in 50 mM $KHPO_4$ (pH 6), and 20 molar excess maleimide−PEG2−Biotin (ThermoFisher Scientific) was added. Following incubation for 90 min at room temperature, the protein was analyzed by mass spectrometry to verify biotinylation. The mixture was then separated on CM sepharose column and dialyzed against 10 mM sodium acetate, pH 4. The protein was stored at −80 °C until use.

Preparation of Amyloid-β Peptide. Human Aβ42 was purchased from American Peptide Co. (Sunnyvale, CA) and prepared as

DOI: 10.1021/acschemneuro.5b00229
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previously described.[27] Briefly, the lyophilized peptide was solubilized to 2.25 mg mL^{-1} in 1,1,1,3,3,3-hexafluoro-2-propanol and vortexed vigorously until complete solubilization. The solvent was evaporated with N_2 and peptide stored at −80 °C. Preceding use, the peptidic film was resuspended to 400 μM in 10 mM NaOH and subjected to 10 cycles of 10 s sonication at 4 °C. The disaggregated peptide was diluted to 100 μM in 10 mM sodium phosphate, pH 7.4, and the concentration was determined from the absorbance at 280 nm using a Nanodrop spectrophotometer and an extinction coefficient of 1490 M^{-1} cm^{-1}.

Photoinduced Cross-linking of Unmodified Proteins (PICUP). Eighteen microliter aliquots of freshly prepared solution of Aβ42 (20 μM in 10 mM sodium phosphate, pH 7.4) were immediately cross-linked with the addition of 1 μL of 1 mM tris(2,2′-bipyridyl)dichlororuthenium(II)hexahydrate (Ru(Bpy)) and 1 μL of 20 mM ammonium persulfate in a thin-wall PCR tube. The samples were irradiated for 1 s using an HL 150-AY cold source halogen light (AmScope, USA) at a distance of 10 cm using a SLR K1000 Pentax camera (Asahi Opt, Co., Japan), fitted with macro camera bellows (Zykkor, USA). The free radical reaction was quenched upon the immediate addition of 10 μL of tricine sample buffer containing 5% β-mercaptoethanol. For samples containing rPrP, the latter protein was added at the appropriate molar ratio to Aβ42 before the PICUP reaction.

SDS-PAGE and Aβ Oligomer Isolation and Purification. Cross-linked Aβ oligomers were separated by SDS-PAGE and either silver stained or coomassie stained depending upon subsequent experimental use. Samples were run on a NuPAGE 4−12% Bis-Tris gradient gel (Invitrogen, Life Technologies, USA). Gels were silver stained using the SilverQuest staining kit (Invitrogen, Life Technologies, USA) according to the manufactures instructions for densitometry and analyzed using ImageJ.[56] For isolation of pure single Aβ oligomeric species, gels were run as above but stained with EZ Blue gel staining reagent (Sigma-Aldrich Company, USA). Gel bands were excised, underwent three rounds of freeze−thaw, and then were crushed. The crushed gel bands were incubated with 0.1% (v/v) ammonium hydroxide and rotated at 24 rpm for 30 min at room temperature. The gel slurry was then centrifuged at 16000*g* for 5 min, and the supernatant was removed and stored on ice. The gel slurry underwent a second round of incubation with 0.1% (v/v) ammonium hydroxide, and the supernatant was collected. SDS-Out SDS precipitation reagent was then added to the Aβ suspension according to the manufacturer's protocol (Thermo Scientific Pierce, Life Technologies, USA). The Aβ solution was filtered through a Nalgene filter unit and then dialyzed in Milli-Q water for 48 h using a 3500 MWCO dialysis membrane (Spectrum Laboratories Inc., USA) with several changes of the dialysis water. The dialyzed samples were lyophilized and stored at room temperature until use. Isolated oligomers were resuspended in appropriate buffer for each experiment, and their concentration was expressed as the concentration of a particular oligomeric species (i.e., not the monomer concentration).

Atomic Force Microscopy (AFM). Aβ42 oligomers were examined by AFM using a Multimode 8 AFM (Bruker Co., USA) fitted with a NanoScope V controller using software NanoScope 9.1. Images were acquired in ScanAsyst mode using a silicon tip on a nitride lever with a resonant frequency of 70 kHz and a spring constant of 0.4 N/m. Samples were adsorbed to freshly cleaved mica for 60 s and then rinsed with Milli-Q water three times. Adsorbed samples were dried using N_2 and then imaged. Images were analyzed using the NanoScope Analysis 1.5 software (Bruker Co., USA) by taking three cross sections of the micrograph and determining the heights of the oligomers across the sections (Supplementary Figure S2).

Dynamic Light Scattering (DLS). Hydrodynamic radii of PICUP-isolated Aβ oligomers in the absence and presence of rPrP were determined using a DynaPro NanoStar (Wyatt Technology Corp, CA, USA). Proteins and peptides were filtered using a 0.2 μm syringe filter cartridge and then centrifuged at 16000*g* for 30 min to remove any large particulates and contaminants.

Surface Plasmon Resonance (SPR). SPR measurements were carried out on a Biacore T100 system (GE LifeSciences, USA) using a preimmobilized streptavidin SA sensor chip (GE LifeSciences, USA). C-terminally biotinylated S231C rPrP (10 μM solution in 10 mM sodium acetate, pH 4) was immobilized onto the sensor surface at a flow rate of 20 μL/min for a target immobilization of ∼5000 RU. Uncross-linked Aβ42, Aβ42 dimer, Aβ42 trimer, and Aβ42 tetramer (2 μM in each case) in a buffer containing 50 mM $KHPO_4$, 150 mM NaCl, 30 mM EDTA, 0.05% (v/v) Tween, pH 6.4, were injected over the rPrP captured sensor surface at a flow rate of 20 mL/min for an association time of 120 s , followed by rinsing with phosphate buffer. Sensograms were normalized by subtracting the signal from the reference channel without immobilized rPrP. Data analysis was performed using the built-in BIAevaluation software.

Calcein Release Assay. All lipids were obtained from Avanti Polar Lipid, Inc., USA. Large unilamellar vesicles (LUVs) were prepared using previously published protocols with some modifications.[12,57] Briefly, lipid films were prepared by gently evaporating the chloroform from the DOPC/DOPE/DOPS/cholesterol mixture (molar ratio of 50/15/5/30), and the dry lipid films (total lipid concentration of 10 mg mL^{-1}) were suspended by vortexing for 30 min in a buffer containing 10 mM HEPES, 150 mM NaCl, and 200 mM calcein. LUVs were prepared by passing the suspension 19 times through the extruder fitted with two stacked 100 nm polycarbonate membranes (Avestin Inc., Canada). Nonencapsulated calcein was removed using a 1 mL Sephadex G-50 microcolumn. The resulting LUV suspension was diluted to 0.5 mg mL^{-1} in HEPES buffer, pH 7.4, and stored at 4 °C. Fluorescence intensity measurements were carried out in a 96 well, flat bottom plate on an Infinite M1000 PRO (Tecan Systems, USA) using an excitation wavelength of 490 nm and emission of 520 nm. The percentage of calcein release was determined using the equation

$$\%\text{calcein release} = (F - F_0) \times 100/(F_{max} - F_0)$$

where F is the measured calcein intensity, F_0 is the starting calcein intensity, and F_{max} is the maximum calcein intensity measured after complete lysis of the LUVs with 2% (v/v) Triton X-100. Experiments were performed three times and averaged.

Animal Protocol. Isolation of primary neuronal cells from animals was approved by the Institutional Animal Care and Use Committee (IACUC) at Case Western Reserve University for this study.

Primary Neuronal Culture. Primary hippocampal neurons were isolated from embryonic (E18−E21) Sprague−Dawley rats (Charles River Laboratories, USA) using a standard procedure[58] and finally resuspended in B27/neurobasal medium (Life Technologies) supplemented with 0.5 mM glutamine and 5% deactivated fetal bovine serum (GIBCO BRL). Neurons were plated on poly(L-lysine) coated coverslips at a density of ∼3×10^4 cells/coverslip, and after 24 h, the medium replaced with one without fetal bovine serum.

Lactate Dehydrogenase Cytotoxicity Assay. Medium bathing the plated cells was replaced with serum-free neurobasal medium supplemented with 2% (v/v) B27. Hippocampal neurons were subsequently treated with Aβ oligomers in the absence or presence of rPrP. Cell viability was assessed using a lactase dehydrogenase (LDH) assay kit (Takara Bio Inc., Shiga, Japan) after 48 h treatment. Total LDH was determined upon complete cell lysis with 2% Triton X-100. Experimental data (corrected for small spontaneous LDH release in the absence of any additives) was expressed as a percentage of total LDH release under each experimental condition.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00229.

Representative SDS-PAGE gel for separation of oligomeric species in the mixtures of PICUP-cross-linked Aβ42 in the absence and presence of rPrP and analysis of atomic force microscopy images for PICUP-isolated Aβ42 oligomers (PDF)

DOI: 10.1021/acschemneuro.5b00229
ACS Chem. Neurosci. 2015, 6, 1972−1980

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Author Contributions

T.L.W. designed research, performed experiments, analyzed data, prepared recombinant prion protein, and wrote the paper. J.-K.C. prepared primary neuronal cells. K.S. prepared recombinant prion protein and performed cytotoxicity measurements. W.K.S. designed research and wrote the paper.

Funding

This work was supported in part by National Institutes of Health Grants NS074317 and NS083687 and the Spitz Health Innovation Pilot Grant program.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

T.L.W. thanks Dr. Yinghua Chen in the Department of Physiology & Biophysics for the helpful advice and support with SPR.

ABBREVIATIONS

Aβ, amyloid-β; PrP^C, cellular prion protein; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine; GPI, glycosylphosphatidylinositol; LDH, lactase dehydrogenase; LTP, long-term potentiation; LUVs, large unilamellar vesicles; PICUP, photoinduced cross-linking of unmodified proteins; rPrP, recombinant prion protein; RU, resonance units; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SPR, surface plasmon resonance; TCEP, tris(2-carboxyethyl)phosphine

REFERENCES

(1) Benilova, I., Karran, E., and De Strooper, B. (2012) The toxic A beta oligomer and Alzheimer's disease: an emperor in need of clothes. *Nat. Neurosci. 15*, 349−357.

(2) Hardy, J., and Selkoe, D. J. (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science 297*, 353−356.

(3) Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Teplow, D. B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M. A., Biere, A. L., Curran, E., Burgess, T., Louis, J. C., Collins, F., Treanor, J., Rogers, G., and Citron, M. (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science 286*, 735−741.

(4) Uversky, V. N. (2008) Amyloidogenesis of natively unfolded proteins. *Curr. Alzheimer Res. 5*, 260−287.

(5) Lorenzo, A., and Yankner, B. A. (1994) Beta-Amyloid Neurotoxicity Requires Fibril Formation and Is Inhibited by Congo Red. *Proc. Natl. Acad. Sci. U. S. A. 91*, 12243−12247.

(6) Hyman, B. T., Marzloff, K., and Arriagada, P. V. (1993) The lack of accumulation of senile plaques or amyloid burden in Alzheimer's disease suggests a dynamic balance between amyloid deposition and resolution. *J. Neuropathol. Exp. Neurol. 52*, 594−600.

(7) Ingelsson, M., Fukumoto, H., Newell, K. L., Growdon, J. H., Hedley-Whyte, E. T., Frosch, M. P., Albert, M. S., Hyman, B. T., and Irizarry, M. C. (2004) Early Abeta accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. *Neurology 62*, 925−931.

(8) Townsend, M., Shankar, G. M., Mehta, T., Walsh, D. M., and Selkoe, D. J. (2006) Effects of secreted oligomers of amyloid beta-protein on hippocampal synaptic plasticity: a potent role for trimers. *J. Physiol. 572*, 477−492.

(9) Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J., and Selkoe, D. J. (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature 416*, 535−539.

(10) Ford, L., Crossley, M., Williams, T. L., Thorpe, J. R., Serpell, L. C., and Kemenes, G. (2015) Effects of Aβ exposure on long-term associative memory and its neuronal mechanisms in a defined neuronal network. *Sci. Rep. 5*, 10614.

(11) Deshpande, A., Mina, E., Glabe, C., and Busciglio, J. (2006) Different conformations of amyloid beta induce neurotoxicity by distinct mechanisms in human cortical neurons. *J. Neurosci. 26*, 6011−6018.

(12) Williams, T. L., Day, I. J., and Serpell, L. C. (2010) The effect of Alzheimer's Abeta aggregation state on the permeation of biomimetic lipid vesicles. *Langmuir 26*, 17260−17268.

(13) Williams, T. L., and Serpell, L. C. (2011) Membrane and surface interactions of Alzheimer's Abeta peptide–insights into the mechanism of cytotoxicity. *FEBS J. 278*, 3905−3917.

(14) Soura, V., Stewart-Parker, M., Williams, T. L., Ratnayaka, A., Atherton, J., Gorringe, K., Tuffin, J., Darwent, E., Rambaran, R., Klein, W., Lacor, P., Staras, K., Thorpe, J., and Serpell, L. C. (2012) Visualization of co-localization in Abeta42-administered neuroblastoma cells reveals lysosome damage and autophagosome accumulation related to cell death. *Biochem. J. 441*, 579−590.

(15) Al-Hilaly, Y. K., Williams, T. L., Stewart-Parker, M., Ford, L., Skaria, E., Cole, M., Bucher, W. G., Morris, K. L., Sada, A. A., Thorpe, J. R., and Serpell, L. C. (2013) A central role for dityrosine crosslinking of Amyloid-beta in Alzheimer's disease. *Acta. Neuropathol. Commun. 1*, 83.

(16) Lauren, J., Gimbel, D. A., Nygaard, H. B., Gilbert, J. W., and Strittmatter, S. M. (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature 457*, 1128−1132.

(17) Gimbel, D. A., Nygaard, H. B., Coffey, E. E., Gunther, E. C., Lauren, J., Gimbel, Z. A., and Strittmatter, S. M. (2010) Memory impairment in transgenic Alzheimer mice requires cellular prion protein. *J. Neurosci. 30*, 6367−6374.

(18) Kudo, W., Lee, H. P., Zou, W. Q., Wang, X., Perry, G., Zhu, X., Smith, M. A., Petersen, R. B., and Lee, H. G. (2012) Cellular prion protein is essential for oligomeric amyloid-beta-induced neuronal cell death. *Hum. Mol. Genet. 21*, 1138−1144.

(19) Nicoll, A. J., Panico, S., Freir, D. B., Wright, D., Terry, C., Risse, E., Herron, C. E., O'Malley, T., Wadsworth, J. D., Farrow, M. A., Walsh, D. M., Saibil, H. R., and Collinge, J. (2013) Amyloid-beta nanotubes are associated with prion protein-dependent synaptotoxicity. *Nat. Commun. 4*, 2416.

(20) Um, J. W., Nygaard, H. B., Heiss, J. K., Kostylev, M. A., Stagi, M., Vortmeyer, A., Wisniewski, T., Gunther, E. C., and Strittmatter, S. M. (2012) Alzheimer amyloid-beta oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. *Nat. Neurosci. 15*, 1227−1235.

(21) Freir, D. B., Nicoll, A. J., Klyubin, I., Panico, S., Mc Donald, J. M., Risse, E., Asante, E. A., Farrow, M. A., Sessions, R. B., Saibil, H. R., Clarke, A. R., Rowan, M. J., Walsh, D. M., and Collinge, J. (2011) Interaction between prion protein and toxic amyloid beta assemblies can be therapeutically targeted at multiple sites. *Nat. Commun. 2*, 336.

(22) Balducci, C., Beeg, M., Stravalaci, M., Bastone, A., Sclip, A., Biasini, E., Tapella, L., Colombo, L., Manzoni, C., Borsello, T., Chiesa, R., Gobbi, M., Salmona, M., and Forloni, G. (2010) Synthetic amyloid-beta oligomers impair long-term memory independently of cellular prion protein. *Proc. Natl. Acad. Sci. U. S. A. 107*, 2295−2300.

(23) Kessels, H. W., Nguyen, L. N., Nabavi, S., and Malinow, R. (2010) The prion protein as a receptor for amyloid-β. *Nature 466*, E3. Lauren, J., Gimbel, D. A., Nygaard, H. B., Gilbert, J. W., and Strittmatter, S. M. (2010) Reply. *Nature*, E4.

DOI: 10.1021/acschemneuro.5b00229
ACS Chem. Neurosci. 2015, 6, 1972−1980

(24) Calella, A. M., Farinelli, M., Nuvolone, M., Mirante, O., Moos, R., Falsig, J., Mansuy, I. M., and Aguzzi, A. (2010) Prion protein and Aβ-related synaptic toxicity impairment. *EMBO Mol. Med. 2*, 306−314.
(25) Chen, S., Yadav, S. P., and Surewicz, W. K. (2010) Interaction between human prion protein and amyloid-beta (Abeta) oligomers: role OF N-terminal residues. *J. Biol. Chem. 285*, 26377−26383.
(26) Fluharty, B. R., Biasini, E., Stravalaci, M., Sclip, A., Diomede, L., Balducci, C., La Vitola, P., Messa, M., Colombo, L., Forloni, G., Borsello, T., Gobbi, M., and Harris, D. A. (2013) An N-terminal fragment of the prion protein binds to amyloid-beta oligomers and inhibits their neurotoxicity in vivo. *J. Biol. Chem. 288*, 7857−7866.
(27) Nieznanski, K., Choi, J. K., Chen, S., Surewicz, K., and Surewicz, W. K. (2012) Soluble prion protein inhibits amyloid-beta (Abeta) fibrillization and toxicity. *J. Biol. Chem. 287*, 33104−33108.
(28) Younan, N. D., Sarell, C. J., Davies, P., Brown, D. R., and Viles, J. H. (2013) The cellular prion protein traps Alzheimer's Abeta in an oligomeric form and disassembles amyloid fibers. *FASEB J. 27*, 1847−1858.
(29) Bitan, G., Kirkitadze, M. D., Lomakin, A., Vollers, S. S., Benedek, G. B., and Teplow, D. B. (2003) Amyloid beta -protein (Abeta) assembly: Abeta 40 and Abeta 42 oligomerize through distinct pathways. *Proc. Natl. Acad. Sci. U. S. A. 100*, 330−335.
(30) Rahimi, F., Maiti, P., and Bitan, G. (2009) Photo-induced cross-linking of unmodified proteins (PICUP) applied to amyloidogenic peptides. *J. Visualized Exp.*, e1071.
(31) Fancy, D. A., and Kodadek, T. (1999) Chemistry for the analysis of protein-protein interactions: rapid and efficient cross-linking triggered by long wavelength light. *Proc. Natl. Acad. Sci. U. S. A. 96*, 6020−6024.
(32) Ono, K., Condron, M. M., and Teplow, D. B. (2009) Structure-neurotoxicity relationships of amyloid beta-protein oligomers. *Proc. Natl. Acad. Sci. U. S. A. 106*, 14745−14750.
(33) Nieba, L., Nieba-Axmann, S. E., Persson, A., Hamalainen, M., Edebratt, F., Hansson, A., Lidholm, J., Magnusson, K., Karlsson, A. F., and Pluckthun, A. (1997) BIACORE analysis of histidine-tagged proteins using a chelating NTA sensor chip. *Anal. Biochem. 252*, 217−228.
(34) Gong, Y., Chang, L., Viola, K. L., Lacor, P. N., Lambert, M. P., Finch, C. E., Krafft, G. A., and Klein, W. L. (2003) Alzheimer's disease-affected brain: presence of oligomeric A beta ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proc. Natl. Acad. Sci. U. S. A. 100*, 10417−10422.
(35) Giuffrida, M. L., Caraci, F., Pignataro, B., Cataldo, S., De Bona, P., Bruno, V., Molinaro, G., Pappalardo, G., Messina, A., Palmigiano, A., Garozzo, D., Nicoletti, F., Rizzarelli, E., and Copani, A. (2009) Beta-amyloid monomers are neuroprotective. *J. Neurosci. 29*, 10582−10587.
(36) Kudo, W., Petersen, R. B., and Lee, H. G. (2013) Cellular prion protein and Alzheimer disease: link to oligomeric amyloid-beta and neuronal cell death. *Prion 7*, 114−116.
(37) Hung, L. W., Ciccotosto, G. D., Giannakis, E., Tew, D. J., Perez, K., Masters, C. L., Cappai, R., Wade, J. D., and Barnham, K. J. (2008) Amyloid-beta peptide (Abeta) neurotoxicity is modulated by the rate of peptide aggregation: Abeta dimers and trimers correlate with neurotoxicity. *J. Neurosci. 28*, 11950−11958.
(38) Shankar, G. M., Li, S., Mehta, T. H., Garcia-Munoz, A., Shepardson, N. E., Smith, I., Brett, F. M., Farrell, M. A., Rowan, M. J., Lemere, C. A., Regan, C. M., Walsh, D. M., Sabatini, B. L., and Selkoe, D. J. (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat. Med. 14*, 837−842.
(39) Townsend, M., Shankar, G. M., Mehta, T., Walsh, D. M., and Selkoe, D. J. (2006) Effects of secreted oligomers of amyloid β-protein on hippocampal synaptic plasticity: a potent role for trimers. *J. Physiol. 572*, 477−492.
(40) Lesne, S., Koh, M. T., Kotilinek, L., Kayed, R., Glabe, C. G., Yang, A., Gallagher, M., and Ashe, K. H. (2006) A specific amyloid-beta protein assembly in the brain impairs memory. *Nature 440*, 352−357.
(41) Urbanc, B., Betnel, M., Cruz, L., Bitan, G., and Teplow, D. B. (2010) Elucidation of amyloid beta-protein oligomerization mechanisms: discrete molecular dynamics study. *J. Am. Chem. Soc. 132*, 4266−4280.
(42) Barz, B., and Urbanc, B. (2012) Dimer formation enhances structural differences between amyloid beta-protein (1−40) and (1−42): an explicit-solvent molecular dynamics study. *PLoS One 7*, e34345.
(43) Roychaudhuri, R., Yang, M., Deshpande, A., Cole, G. M., Frautschy, S., Lomakin, A., Benedek, G. B., and Teplow, D. B. (2013) C-terminal turn stability determines assembly differences between Abeta40 and Abeta42. *J. Mol. Biol. 425*, 292−308.
(44) Yun, S., Urbanc, B., Cruz, L., Bitan, G., Teplow, D. B., and Stanley, H. E. (2007) Role of electrostatic interactions in amyloid beta-protein (A beta) oligomer formation: a discrete molecular dynamics study. *Biophys. J. 92*, 4064−4077.
(45) Borchelt, D. R., Thinakaran, G., Eckman, C. B., Lee, M. K., Davenport, F., Ratovitsky, T., Prada, C. M., Kim, G., Seekins, S., Yager, D., Slunt, H. H., Wang, R., Seeger, M., Levey, A. I., Gandy, S. E., Copeland, N. G., Jenkins, N. A., Price, D. L., Younkin, S. G., and Sisodia, S. S. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1−42/1−40 ratio in vitro and in vivo. *Neuron 17*, 1005−1013.
(46) Mayeux, R., Honig, L. S., Tang, M. X., Manly, J., Stern, Y., Schupf, N., and Mehta, P. D. (2003) Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: relation to age, mortality, and risk. *Neurology 61*, 1185−1190.
(47) Kagan, B. L., and Thundimadathil, J. (2010) Amyloid peptide pores and the beta sheet conformation. *Adv. Exp. Med. Biol. 677*, 150−167.
(48) Jang, H., Zheng, J., and Nussinov, R. (2007) Models of beta-amyloid ion channels in the membrane suggest that channel formation in the bilayer is a dynamic process. *Biophys. J. 93*, 1938−1949.
(49) Jang, H., Arce, F. T., Ramachandran, S., Capone, R., Lal, R., and Nussinov, R. (2010) beta-Barrel topology of Alzheimer's beta-amyloid ion channels. *J. Mol. Biol. 404*, 917−934.
(50) Jin, M., Shepardson, N., Yang, T., Chen, G., Walsh, D., and Selkoe, D. J. (2011) Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration. *Proc. Natl. Acad. Sci. U. S. A. 108*, 5819−5824.
(51) Luheshi, L. M., Tartaglia, G. G., Brorsson, A. C., Pawar, A. P., Watson, I. E., Chiti, F., Vendruscolo, M., Lomas, D. A., Dobson, C. M., and Crowther, D. C. (2007) Systematic in vivo analysis of the intrinsic determinants of amyloid Beta pathogenicity. *PLoS Biol. 5*, e290.
(52) Ono, K., Condron, M. M., and Teplow, D. B. (2010) Effects of the English (H6R) and Tottori (D7N) familial Alzheimer disease mutations on amyloid beta-protein assembly and toxicity. *J. Biol. Chem. 285*, 23186−23197.
(53) Venkatasubramaniam, A., Drude, A., and Good, T. (2014) Role of N-terminal residues in Abeta interactions with integrin receptor and cell surface. *Biochim. Biophys. Acta, Biomembr. 1838*, 2568−2577.
(54) Meral, D., and Urbanc, B. (2013) Discrete molecular dynamics study of oligomer formation by N-terminally truncated amyloid beta-protein. *J. Mol. Biol. 425*, 2260−2275.
(55) Morillas, M., Swietnicki, W., Gambetti, P., and Surewicz, W. K. (1999) Membrane environment alters the conformational structure of the recombinant human prion protein. *J. Biol. Chem. 274*, 36859−36865.
(56) Abramoff, M. D., Magelhaes, P. J., and Ram, S. J. (2004) Image Processing with ImageJ. *Biophotonics Int. 11*, 36−42.
(57) Hayden, E. Y., Kaur, P., Williams, T. L., Matsui, H., Yeh, S. R., and Rousseau, D. L. (2015) Heme Stabilization of alpha-Synuclein Oligomers during Amyloid Fibril Formation. *Biochemistry 54*, 4599−4610.
(58) Bouyer, P., Bradley, S. R., Zhao, J., Wang, W., Richerson, G. B., and Boron, W. F. (2004) Effect of extracellular acid-base disturbances on the intracellular pH of neurones cultured from rat medullary raphe or hippocampus. *J. Physiol. 559*, 85−101.

DOI: 10.1021/acschemneuro.5b00229
ACS Chem. Neurosci. 2015, 6, 1972−1980