Pyroglutamylated Amyloid-β Peptide Reverses Cross β-Sheets by a Prion-Like Mechanism

Jason O. Matos,‡ Greg Goldblatt,§ Jaekyun Jeon,‖ Bo Chen,‡ and Suren A. Tatulian*‡

†Biotechnology Graduate Program, University of Central Florida, 4000 Central Florida Boulevard, Orlando, Florida 32816, United States
‡Department of Physics, University of Central Florida, 4000 Central Florida Boulevard, Orlando, Florida 32816, United States
§Biomedical Sciences Graduate Program, University of Central Florida, 4000 Central Florida Boulevard, Orlando, Florida 32816, United States
‖Physics Graduate Program, University of Central Florida, 4000 Central Florida Boulevard, Orlando, Florida 32816, United States

ABSTRACT: The amyloid hypothesis causatively relates the fibrillar deposits of amyloid β peptide (Aβ) to Alzheimer’s disease (AD). More recent data, however, identify the soluble oligomers as the major cytotoxic entities. Pyroglutamylated Aβ (pE-Aβ) is present in AD brains and exerts augmented neurotoxicity, which is believed to result from its higher β-sheet propensity and faster fibrillization. While this concept is based on a set of experimental results, others have reported similar β-sheet contents in unmodified and pyroglutamylated Aβ, and slower aggregation of pE-Aβ as compared to unmodified Aβ, leaving the issue unresolved. Here, we assess the structural differences between Aβ and pE-Aβ peptides that may underlie their distinct cytotoxicities. Transmission electron microscopy identifies a larger number of prefibrillar aggregates of pE-Aβ at early stages of aggregation and suggests that pE-Aβ affects the fibrillogenesis even at low molar fractions. Circular dichroism and FTIR data indicate that while the unmodified Aβ readily forms β-sheet fibrils in aqueous media, pE-Aβ displays increased α-helical and decreased β-sheet propensity. Moreover, isotope-edited FTIR spectroscopy shows that pE-Aβ reverses β-sheet formation and hence fibrillogenesis of the unmodified Aβ peptide via a prion-like mechanism. These data provide a novel structural mechanism for pE-Aβ hypertoxicity; pE-Aβ undergoes faster formation of prefibrillar aggregates due to its increased hydrophobicity, thus shifting the initial stages of fibrillogenesis toward smaller, hypertoxic oligomers of partial α-helical structure.

1. INTRODUCTION

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by neuronal and synaptic loss leading to cognitive and memory impairment. Extracellular fibrillar deposits (plaques) of amyloid-β (Aβ) peptide have been found in the AD brain and thought to be causatively related to the disease.1–3 However, currently accumulated evidence identifies the soluble oligomers of Aβ as the main neurotoxic entities.1–8

Aβ is a proteolytic product of the amyloid precursor protein and can contain varying numbers of amino acid residues, with the 40- and 42-residue peptides (Aβ1-40 and Aβ1-42) being the prevalent forms. Circular dichroism (CD) and NMR data indicate that in organic solvents such as hexafluoroisopropanol (HFIP) Aβ1-42 adopts a partially α-helical structure and in the presence of >80% H2O acquires a β-sheet structure.9 In aqueous media, Aβ forms fibrils composed of β-sheets where the strand axis is approximately perpendicular and the H-bonding is parallel to the long fibrillar axis, known as a cross β-sheet structure.10,11 Antiparallel β-sheets were proposed to constitute the core structural motif of fibrils formed by Aβ1-42 or its fragments.12,13 However, solid state NMR studies on Aβ1-42 and shorter peptides identified in-register parallel β-sheet structures,14–16 consistent with models derived from spin-label EPR17 solution NMR,18 and Fourier transform infrared (FTIR) studies.19,20 Apparent inconsistencies might originate from different stages of peptide aggregation in different samples, as Aβ1-42 oligomers and fibrils were shown by FTIR to adopt antiparallel and parallel β-sheet structures, respectively.21

Significant amounts of N-terminally truncated and pyroglutamylated (at Glu3 or Glu11) Aβ peptide (pE-Aβ) have been identified in AD brains and shown to aggregate at increased rates22–26 and to be more cytotoxic than unmodified Aβ.27,28 Even at low fractions, pE-Aβ coaggregates with Aβ by a seeding mechanism and forms structurally distinct and highly toxic oligomers.29 Dot-blot experiments using conformation-sensitive antibodies showed that the highly toxic oligomers containing 5% or less pE-Aβ were structurally different from the mildly toxic unmodified Aβ aggregates of similar size.27 While these studies imply a structural mechanism for augmented toxicity of
pE-Aβ, the underlying structural differences between Aβ and pE-Aβ remain uncharacterized. Solution NMR showed that Aβ_{1−40} in trifluoroethanol/water (2:3) has a reduced α-helical propensity compared to Aβ_{1−40}, consistent with a significantly higher β-sheet content and faster fibrillogenesis of pE-Aβ. Conversely, CD studies identified similar content of β-sheet in both unmodified Aβ and pE-Aβ peptides, and fibrillogenesis of Aβ_{1−40} was reported to be significantly slower compared to Aβ_{1−42}. These conflicting data on fibrillogenesis of unmodified Aβ and pE-Aβ are evidently related to the inherent polymorphism and sensitivity of the Aβ peptides to the experimental conditions and procedures. Since the content of pE-Aβ in AD brains varies in a wide range and a fraction of pE-Aβ is assumed to form hypertoxic aggregates of partial α-helical structure as opposed to mildly toxic β-sheet fibrils.

2. MATERIALS AND METHODS

Materials. The Aβ_{1−42} and uniformly ^13C-labeled Aβ_{1−42} peptides were purchased from rPeptide (Bogart, GA, USA) and were >97% pure. Aβ_{1−42} was from Innovagen (Lund, Sweden) and was 98% pure. The peptides were analyzed by MALDI-TOF mass-spectrometry at the ICBR Proteomics Core Facility of the University of Florida (Gainesville, FL, USA), and the amino acid compositions of all three peptides were confirmed. Salts, buffers, HFIP, and other chemicals were from Fisher Scientific (Hanover Park, IL, USA) or Sigma-Aldrich (St. Louis, MO, USA).

Experimental Procedures. In all experiments, the lyophilized peptides were initially dissolved in HFIP at 200 μM concentration to disperse any preformed aggregates. In TEM experiments, appropriate amounts of the peptides were dried in a glass vial by desiccation for 15 min, followed by incubation in an aqueous buffer of 50 mM NaCl + 50 mM Na,K-phosphate (pH 7.2) at 37 °C with constant stirring for 24 h. TEM samples were prepared following the procedures described by Nilsson, i.e., by deposition of 5 μL of peptide suspension on the grid, incubation for 5 min, and rinsing with 4 μL of distilled/deionized water, followed by staining for 30 s.
with 2 μL of 3% uranyl acetate, washing twice with 5 μL of distilled/deionized water, and air-drying. Grids for the negative control experiments were prepared by identical procedures using 5 μL of blank buffer instead of the peptide suspension. Images were acquired on a JEOL TEM-1011 operated at 80 kV (Ted Pella, Inc., Redding, CA, USA).

In CD experiments, the HFIP solutions of peptides were dried by desiccation in a 4 mm × 4 mm quartz cuvette and spectra were collected between 180 and 330 nm to determine the structure of the dry peptides. Subsequently, an aqueous buffer of 50 mM NaCl + 50 mM NaK-phosphate (pH 7.2) was added to a 50 μM final concentration of the peptides and spectra were acquired consecutively for 24 h to identify secondary structural changes upon fibrillogenesis at 37 °C with constant stirring, using a J-810 spectropolarimeter (Jasco, Tokyo, Japan). To improve the signal-to-noise ratio, the spectra were smoothed using a 13-point Savitzky-Golay linear least-squares algorithm embedded in the Igor Pro 5.03 software.

FTIR experiments were conducted to determine the structure of the peptides in desiccated form, nominally hydrated by atmospheric humidity, and in the presence of excess aqueous buffer. Desired amounts of the peptides were dissolved in HFIP at 200 μM concentration, and 40 μL of the solution was placed on a CaF₂ FTIR window and dried in a desiccator for 15 min. FTIR spectra of the peptide samples were collected while the peptide was allowed to absorb humidity from the atmosphere as monitored by the increase in the H₂O stretching band intensity around 3270 cm⁻¹. Then, 80 μL of aqueous buffer (10 mM NaK-phosphate in D₂O, pH 7.2, corresponding to the pH-meter reading of 6.8) was added to the peptide and the sample was sealed by a second window using a 50 μm-thick Teflon spacer, followed by measurements of spectra of the peptide in aqueous medium. The spectra were measured by coadding 500 scans on a Vector-22 FTIR spectrometer (Bruker Optics, Billerica, MA, USA) equipped with a liquid nitrogen-cooled Hg–Cd–Te detector, at 2 cm⁻¹ nominal resolution at 25 °C, as described earlier. Reference transmission spectra were collected using either a single CaF₂ window or the buffer sealed between two windows and were used to calculate the absorbance spectra. H₂O vapor spectra were measured separately and subtracted from the sample spectra when necessary. The spectra were smoothed as described above, and baseline correction was applied.

### 3. RESULTS

Based on earlier findings that Aβ_{43-42} forms aggregates that are structurally different from the aggregates of Aβ_{1-42} and exert prion-like toxicity on cultured neurons, we hypothesized that Aβ_{pE3-42} modulates the structure of the unmodified peptide reminiscent of prions. Since the content of pyroglutamylated Aβ can vary up to 50% of total Aβ, we studied pE-Aβ/Aβ samples at 1:9 and 1:1 molar ratios in addition to pure Aβ and pE-Aβ peptides. TEM images were acquired at 2, 4, 12, and 24 h of incubation, as described in the Materials and Methods. Most significant differences between Aβ_{1-42} and Aβ_{pE3-42} were detected at the early stages of aggregation. At 2 h, the samples of Aβ_{pE3-42} were dominated by nonfibrillar aggregates of irregular shape and average dimension of 30–100 nm, while the Aβ_{1-42} samples showed well-defined fibrils and a smaller number of small aggregates (Figure 1a and b). The 1:9 and 1:1 molar combinations contained predominantly prefibrillar structures (Figure 1c and d). At 4 h of incubation, the fibrils were seen in all samples, with little morphological differences, with small aggregates still present (Figure 1e–h). While the mature fibrils formed by the Aβ_{pE3-42} peptide seem to be thicker, possibly bundled (cf. part n of Figure 1 with parts m, o, and p), consistent with earlier observations, the TEM data do not allow identification of more distinct, definitive morphological differences between the fibrils of the unmodified and pyroglutamylated peptides and their combinations. Taking into account the clear differences between the early stage assemblies of Aβ_{1-42} and Aβ_{pE3-42}, these data suggest that the fibrillogenesis of the two forms may follow different pathways, leading to fibrils that are similar at the level of morphology. The images obtained in negative control experiments showed clear grids, as expected (not shown).

Earlier TEM studies showed similar morphologies of Aβ_{pE3-42} and Aβ_{1-42} aggregates at the initial stages of aggregation but more "curvilinear and entangled" fibrils of Aβ_{1-42}, at 1–2 days of fibrillogenesis. In the equimolar sample, the fibrils were less entangled, i.e., more like Aβ_{pE3-42} fibrils, suggesting that pE-Aβ might be able to dictate its morphological (and probably structural) features to the aggregates.

It has been recognized that the fibrillar morphology is determined by the molecular structure of the peptides, but CD studies provided conflicting data on the relative secondary structural changes in Aβ and pE-Aβ during fibrillogenesis (see above). To monitor the structural transitions in the peptides during fibrillogenesis, peptide samples dried from HFIP solution were used as a starting point, before the onset of aggregation. CD spectra of Aβ_{1-42}, Aβ_{pE3-42} and their combinations in dry form shown in Figure 2a indicate mostly α-helical structure with two minima around 222 and 208 nm. The spectrum of Aβ_{pE3-42} has a significantly
were dissolved in HFIP and dried on a CaF2 window by 15 min exposure of the peptides to an aqueous buffer. It is remarkable that Aβ1-42 promptly adopts and maintains β-sheet structure, as evidenced by a deep minimum at 215–216 nm of spectra measured at 1 and 24 h of incubation (Figure 2b). The spectra of Aβ1-42, on the other hand, show a wide well between 208 and 222 nm, most likely indicating a combination of α-helical and β-sheet structures (Figure 2c). These data suggest substantially different structures of Aβ1-42 and AβpE3-42 while the former readily adopts β-sheets, the latter shows increased α-helical propensity. The CD spectra of the 1:9 AβpE3-42/Aβ1-42 combination display β-sheet features, i.e., a prominent minimum at 216 nm at 1 h and at 219 nm at 24 h of incubation (Figure 2d). The higher intensity and the red shift of the signal at 24 h may reflect gradual expansion of the peptide into the aqueous medium and decreased solvent accessibility upon aggregation.

It should be noted that the spectra of Figure 2d are dominated by the structural features of Aβ1-42 which are present at a large molar excess (90%). At 1:1 molar ratio, the 1-h spectrum shows a minimum at 209 and a shoulder at 223 nm (Figure 2e), implying α-helix structure, possibly including a β-sheet component, as in the case of pure AβpE3-42 (cf. blue spectra in Figure 2c and e). At 24 h, the spectrum has a β-sheet minimum at 216 nm and a shoulder at 227 nm, likely generated by a turn structure. It is remarkable that AβpE3-42 exerts a dominant structural effect, especially at the early stages of fibrillogenesis. Thus, consistent with the TEM data, CD results indicate that (a) Aβ1-42 and AβpE3-42 evidently follow distinct structural pathways of fibrillogenesis and (b) AβpE3-42 is able to divert the overall path toward less β-sheet and more α-helical intermediates.

While TEM and CD data indicate distinct structural differences between Aβ1-42 and AβpE3-42 and suggest a dominant structural effect of pE-Aβ on Aβ, neither of these methods has the capability of resolving the individual structures of the two peptides in combination and the mutual structural effects. Individual structures of two proteins combined in one sample can be determined by FTIR spectroscopy if their amide II bands are spectrally separated, which is achieved by 13C-labeling of one of the proteins. Despite its resolving power, such “isotope-edited” FTIR spectroscopy has not been used to characterize the concomitant structural transitions of Aβ and pE-Aβ during fibrillogenesis.

To detect the structural changes accompanying formation of amyloid fibrils, FTIR spectra were measured before and after exposure of the peptides to an aqueous buffer. Both peptides were dissolved in HFIP and dried on a CaF2 window by 15 min desiccation. AβpE3-42 adopts an intramolecular antiparallel β-sheet structure (peak at 1634 cm⁻¹ and shoulder around 1695 cm⁻¹), as well as a significant fraction of α-helix and turn structures (broad component(s) between 1685 and 1650 cm⁻¹) (Figure 3a). 13C-Aβ1-42 forms an intermolecular β-sheet (main peak at 1588 cm⁻¹) plus turns and an insignificant α-helix (component at 1617 cm⁻¹) (Figure 3a). These data imply that 13C-Aβ1-42 readily forms a cross-β structure even in the absence of an aqueous medium while AβpE3-42 forms intramolecular β-hairpins and an α-helix. In aqueous (D2O) buffer, both peptides adopt parallel intermolecular β-sheet structure, as evidenced by the major amide I peaks at 1628 cm⁻¹ for AβpE3-42 and 1585 cm⁻¹ for 13C-Aβ1-42 (Figure 3b). However, the prominent component between 1680 and 1650 cm⁻¹ in the spectra of AβpE3-42 indicates that the pyroglutamylated peptide retains significant fractions of α-helical and turn structures. (The small peak in the spectra of 13C-Aβ1-42 at 1673 cm⁻¹ is likely generated by trace amounts of trifluoroacetic acid usually present in synthetic peptide samples).

The amide II spectral region provides additional structural information on proteins and peptides. Flexible secondary structures or open, solvent accessible tertiary structures undergo faster amide hydrogen/deuterium exchange resulting in reduction of the amide II band intensity around 1540 cm⁻¹. A considerable amide II band is retained in the spectra of 13C-Aβ1-42 after a 2 h exposure to D2O while that of AβpE3-42 is lost (Figure 3b), indicating Aβ1-42 forms a rigid secondary structure and/or a tight, solvent-inaccessible tertiary structure, characteristic of a cross-β sheet structure, while AβpE3-42 has a more open tertiary structure and/or more flexible secondary structure.

It has been shown earlier that isotope-edited FTIR can be used to probe the intermolecular interactions of peptides. In the case of closely spaced 13C-labeled peptide units, through H-bonding or through space 13C−12C vibrational coupling between adjacent strands results in a lower frequency (~1590−1594 cm⁻¹) amide I mode whereas 13C−12C coupling between labeled and unlabeled units generates higher frequency (~1600−1604 cm⁻¹) components of enhanced intensity.

FTIR studies were conducted on combined 13C-Aβ1-42 and unlabeled AβpE3-42 to probe the intermolecular interactions and mutual structural effects of the peptides. Since pE-Aβ in AD brain can constitute up to 50% of total Aβ1-42 we studied AβpE3-42/13C-Aβ1-42 samples at 10% and 50% molar fractions of AβpE3-42. Data of Figure 4 indicate that the β-sheet peak of 13C-Aβ1-42 at 1585 cm⁻¹ up-shifts by 3 and 10 cm⁻¹ in the presence of 10% and 50% pE-Aβ, respectively, while the β-sheet peak of AβpE3-42 at 1626–1628 cm⁻¹ up-shifts by 10 and 4 cm⁻¹ in the presence of 90% and 50% 13C-Aβ1-42, respectively, indicating strong interactions and vibrational couplings between the two peptides. Thus, AβpE3-42 and Aβ1-42 form a mixed β-sheet structure with tight intermolecular interactions.

Next, we tested the emerging hypothesis that the pyroglutamylated peptide is able to modulate the structure of the unmodified Aβ during amyloid fibril formation. To assess early structural events in aggregation, combinations of HFIP......
solutions of Aβ₁–42 and ¹³C-Aβ₁–42 were dried on a FTIR CaF₂ window followed by collection of spectra while the sample was allowed to absorb atmospheric humidity. Figure 5a shows the spectra of Aβ₁–42 and ¹³C-Aβ₁–42 combined at 1:9 molar ratio in a wide spectral range. The 1700–1500 cm⁻¹ region corresponds to the amide I and amide II modes and the H₂O stretching band around 3270 cm⁻¹. The broad band (Figure 5a, b) indicates augmented β-sheet formation and hence fibrillation of Aβ. As shown in Figure 5c, in the presence of 50% pE-Aβ, the intermolecular β-sheet peak of ¹³C-Aβ₁–42 shifts from 1588 to 1595 cm⁻¹ (cf. dotted and gray spectra), indicating strong interaction between the two peptides. In the presence of only 10% Aβ₁–42, a smaller shift from 1588 to 1592 cm⁻¹ is observed, Figure 5b. In the presence of an equimolar amount of Aβ₁–42 there is no α-helix to β-sheet conversion of ¹³C-Aβ₁–42 over time, as seen by the similar signal intensity at 1617 cm⁻¹ in gray and black solid spectra in Figure 5c. Most importantly, Aβ₁–42 causes a strong reduction of the intermolecular β-sheet signal of ¹³C-Aβ₁–42 at 1595 cm⁻¹ during longer coincubation (cf. gray and black solid spectra in Figure 5c) paralleled with increased intensity around 1658 cm⁻¹. The spectra of the combination (gray and black solid lines in Figure 5c) indicate an increase in α-helical structure (signals at 1658 and 1617 cm⁻¹) and a decrease in β-sheet structure (signals at 1634 and 1585–1588 cm⁻¹) in both peptides as compared to the weighted sum of individual spectra (dotted line in Figure 5c). Although the component at 1634 cm⁻¹ might partially result from turn structures in addition to the α-helix in Aβ₁–42, these data identify prominent mutual conformational effects of the two peptides; pE-Aβ at 10% delays cross β-sheet formation and hence fibrillation and at 50% reverses the cross β-sheet structure formation of Aβ.

4. DISCUSSION

Our data identify significant differences between Aβ₁–42 and Aβ₁–42 at the levels of morphology as well as secondary and tertiary structures. At the initial stages of fibrillogenesis, the pyroglutamylated Aβ₁–42 peptide forms more prefibrillar aggregates, apparently due to its increased hydrophobicity, and it undergoes fibril elongation slower than the unmodified Aβ₁–42 peptide (Figure 1a,b), in agreement with earlier data. Retardation of fibrillation appears to be imparted to Aβ₁–42 even at low molar contents of Aβ₁–42 (Figure 1c). CD data indicate augmented α-helical and diminished β-sheet propensity of Aβ₁–42 which is transmitted to the mixed assemblies (Figure 2). FTIR indicates that the unmodified peptide readily forms a tightly packed intermolecular β-sheet, while pE-Aβ
forms a less compact β-structure and contains more α-helix and turn structures than Aβ (Figure 3). Furthermore, the pyroglutamylated peptide not only exhibits a significantly lower tendency to form a β-sheet structure but also inhibits cross β-sheet formation in the unmodified peptide through direct interactions (Figures 4 and 5). These structural transitions occur rapidly upon hydration, but they can be captured when the peptides undergo nominal hydration by exposure to atmospheric humidity. These conditions are both technologically beneficial and meaningful because the fibrils formed by Aβ1−40 were shown to contain an extremely low fraction of water, i.e., an average of 1.2 water molecules per β-strand.50 Significant retention of the amide II band in the spectrum of Aβ1−42 in a D2O-based buffer (Figure 3b) is in line with this finding. Furthermore, the rapid loss of the amide II band of Aβ7−42 upon exposure to D2O indicates a more flexible secondary and/or a more open tertiary structure of the pyroglutamylated peptide.

If pE-Aβ eventually forms fibrils that contain β-sheet structure, even though different from the fibrils formed by unmodified Aβ, why do the intermediate structural steps matter? The answer is that the oligomeric, prefibrillar assemblies of Aβ that adopt still poorly characterized "pathological conformation" are the most toxic species.51 For example, the secreted pool of Aβ oligomers exerts its toxic effect partly by binding to a set of receptors, including the insulin receptor that recognizes an α-helical ligand.51 Intracellular oligomers bind to the mitochondrial or endoplasmic reticulum proteins and cause cell damage through oxidative stress or calcium dysregulation before they are secreted.52,53 Since the cytotoxic effect is exerted before formation of the extracellular deposits, characterization of the intermediate "pathological conformations" is crucial.

The structural impact of pE-Aβ on the unmodified peptide even at low molar content (10%) is indicative of a prion-like effect. The pE-Aβ peptide tightens interacts with Aβ, as indicated by efficient 12C—13C vibrational coupling (Figures 4 and 5), and thereby transmits the specific structural features to the unmodified peptide. Data of Figures 2–5 strongly imply that this specific structure is rich in α-helix as opposed to β-sheet. This effect takes place even at 10% pE-Aβ, when pE-Aβ molecules cannot simultaneously interact with a large excess of unmodified Aβ. We therefore propose that once the structural transition occurs in the unmodified Aβ molecule by its interaction with pE-Aβ, it acquires the capability to further transmit the altered structure to other Aβ peptides by direct interaction. This prion-like conformational effect of pE-Aβ may eventually shift the overall path of peptide aggregation toward formation of hypotxic, lower molecular weight aggregates of partial α-helical structure and thus suppress formation of less toxic cross β-sheet fibrils.

■ REFERENCES

(1) Hardy, J.; Allsop, D. Amyloid Deposition as the Central Event in the Aetiology of Alzheimer’s Disease. Trends Pharmacol. Sci. 1991, 12, 383–388.
(2) Pike, C. J.; Burdick, D.; Walencewicz, A. J.; Globe, C. G.; Cotman, C. W. Neurodegeneration Induced by β-Amyloid Peptides in vitro: The Role of Peptide Assembly State. J. Neurosci. 1993, 13, 1676–1687.
(3) Hardy, J.; Selkoe, D. J. The Amyloid Hypothesis of Alzheimer’s Disease: Progress and Problems on the Road to Therapeutics. Science 2002, 297, 353–356.
(4) Kirkridze, M. D.; Bitan, G.; Teplow, D. B. Paradigm Shifts in Alzheimer’s Disease and Other Neurodegenerative Disorders: The Emerging Role of Oligomeric Assemblies. J. Neurosci. Res. 2002, 69, 567–577.
(5) Klein, W. L.; Stine, W. B.; Teplow, D. B. Small Assemblies of Unmodified Amyloid β-Protein Are the Proximate Neurotoxin in Alzheimer’s Disease. Neurobiol. Aging 2004, 25, 569–580.
(6) Bernstein, S. L.; Dupuis, N. F.; Lazo, N. D.; Wyttenbach, T.; Condron, M. M.; Bitan, G.; Teplow, D. B.; Shea, J. E.; Ruotolo, B. T.; Robinson, C. V.; Bowers, M. T. Amyloid-β Protein Oligomerization and the Importance of Tetramers and Dodecamers in the Aetiology of Alzheimer’s Disease. Nat. Chem. 2009, 1, 326–331.
(7) Masters, C. L.; Selkoe, D. J. Biochemistry of Amyloid β-Protein and Amyloid Deposits in Alzheimer Disease. Cold Spring Harb. Perspect. Med. 2012, 2, a006262, 1–24.
(8) Benilova, I.; Karran, E.; De Strooper, B. The Toxic Aβ Oligomer and Alzheimer’s Disease: An Emperor in Need of Clothes. Nat. Neurosci. 2012, 15, 349–357.
(9) Tomasselli, S.; Esposito, V.; Vangone, P.; van Nuland, N. A.; Bonvin, A. M.; Guerrini, R.; Tancredi, T.; Temussi, P. A.; Picone, D. The α-to-β Conformational Transition of Alzheimer’s Aβ(1-42) Peptide in Aqueous Media is Reversible: A Step by Step Conformational Analysis Suggests the Location of β Conformation Seeding. ChemBioChem 2006, 7, 257–267.
(10) Eanes, E. D.; Glenner, G. G. X-Ray Diffraction Studies on Amyloid Filaments. J. Histochem. Cytochem. 1968, 16, 673–677.
(11) Kirschner, D. A.; Inouye, H.; Duffy, L. K.; Sinclair, A.; Lind, M.; Selkoe, D. J. Synthetic Peptide Homologous to Beta Protein from Alzheimer Disease Forms Amyloid-Like Fibrils in vitro. Proc. Natl. Acad. Sci. U.S.A. 1987, 84, 6953–6957.
(12) Petkova, A. T.; Bunkowsky, G.; Dyda, F.; Leapman, R. D.; Yau, W. M.; Tycko, R. Solid State NMR Reveals a pH-Dependent Antiparallel β-Sheet Registry in Fibrils Formed by a β-Amyloid Peptide. J. Mol. Biol. 2004, 335, 247–260.
(13) Chaney, M. O.; Webster, S. D.; Kuo, Y. M.; Roher, A. E. Molecular Modeling of the Aβ1-42 Peptide from Alzheimer’s Disease. Protein Eng. 1998, 11, 761–767.
(14) Petkova, A. T.; Ishii, Y.; Balbach, J. J.; Antzutkin, O. N.; Leapman, R. D.; Delaglio, F.; Tycko, R. A Structural Model for Alzheimer’s β-Amyloid Fibrils Based on Experimental Constraints from Solid State NMR. Proc. Natl. Acad. Sci. U.S.A. 2002, 99, 16742–16747.
(15) Paravastu, A. K.; Leapman, R. D.; Yau, W. M.; Tycko, R. Molecular Structural Basis for Polymorphism in Alzheimer’s β-Amyloid Fibrils. Proc. Natl. Acad. Sci. U.S.A. 2008, 105, 18349–18354.
(16) Goldsbury, C. S.; Wirtz, S.; Müller, S. A.; Sunderji, S.; Wicki, P.; Aebi, U.: Frey, P. Studies on the in vitro Assembly of Aβ 1–40: Implications for the Search for Aβ Fibril Formation Inhibitors. J. Struct. Biol. 2000, 130, 217–231.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: statulial@ucf.edu. Tel.: +1-407-823-6941. Fax: +1-407-823-5112.

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was partially supported by NIH Grant 1R03AI097591 to S.A.T., a SEED grant from the College of Sciences of the University of Central Florida to S.A.T., and the Air Force Office of Scientific Research Young Investigator Award FA9550-13-1-0150 to B.C. The ICBR Proteomics Core Facility of the University of Florida is acknowledged for mass-spectr specification of the peptides.
Peptide in Amyloid Fibrils Studied by Site-Directed Spin Labeling. J. Biol. Chem. 2002, 277, 40810–40815.

(18) Lihrs, T.; Ritter, C.; Adrian, M.; Riek-Loher, D.; Bohrmann, B.; Döbeli, H.; Schubert, D.; Riek, R. 3D Structure of Alzheimer’s Amyloid-β(1-43) Fibrils. Proc. Natl. Acad. Sci. U.S.A. 2005, 102, 17342–17347.

(19) Kim, Y. S.; Liu, L.; Axelsen, P. H.; Hochstrasser, R. M. Two-Dimensional Infrared Spectra of Isotopically Diluted Amyloid Fibrils from Aβ(40). Proc. Natl. Acad. Sci. U.S.A. 2008, 105, 7720–7725.

(20) Paul, C.; Axelsen, P. H. β Sheet Structure in Amyloid β Fibrils and Vibrational Dipolar Coupling. J. Am. Chem. Soc. 2005, 127, 5754–5755.

(21) Cerf, E.; Sarroukh, R.; Tamamzu-Kato, S.; Breydo, L.; Derclaye, S.; Dufrêne, Y. F.; Narayanswami, V.; Goomarghagh, E.; Ruysschaert, J. M.; Raussens, V. Antiparallel β-Sheet: A Signature Structure of the Oligomeric Amyloid β-Peptide. Biochem. J. 2009, 421, 415–423.

(22) Schilling, S.; Lauber, T.; Schaupp, M.; Manhart, S.; Scheel, E.; Böhml, G.; Demuth, H. U. On the Seeding and Oligomerization of pGlu-Amyloid Peptides (in vitro). Biochemistry 2006, 45, 12393–12399.

(23) Schlenz, D.; Manhart, S.; Cinar, Y.; Kleinschmidt, M.; Hause, G.; Willbold, D.; Funke, S. A.; Schilling, S.; Demuth, H. U. Pyroglutamate Formation Influences Solubility and Amyloidogenicity of Amyloid Peptides. Biochemistry 2009, 48, 7072–7078.

(24) Schlenz, D.; Rönike, R.; Cynis, H.; Ludwig, H. H.; Scheel, E.; Reymann, K.; Saito, T.; Hause, G.; Schilling, S.; Demuth, H. U. N-Terminal Oligomerization of Aβ(38) and Aβ(40) Renforces Oligomer Formation and Potency To Disrupt Hippocampal Long-Term Potentiation. J. Neurochem. 2012, 121, 774–784.

(25) He, W.; Barrow, C. J. The Aβ 3-Pyroglutamyl and 11-Pyroglutamyl Peptides Found in Senile Plaque Have Greater β-Sheet Forming and Aggregation Propensities in vitro Than Full-Length Aβ. Biochemistry 1999, 38, 10871–10877.

(26) Witts, O.; Eick, C.; Martens, H.; Harmerie, A.; Geumann, C.; Jawhar, S.; Kumar, S.; Mulhaup, G.; Walter, J.; Ingelsson, M.; Degerman-Gunnarsson, M.; Kalimo, H.; Huiinga, L.; Lannfelt, L.; Bayer, T. A. Identification of Low Molecular Weight Pyroglutamate Aβ Oligomers in Alzheimer Disease: A Novel Tool for Therapy and Diagnosis. J. Biol. Chem. 2010, 285, 4115–4125.

(27) Nussbaum, J. M.; Schilling, S.; Cynis, H.; Silva, A.; Swanson, E.; Wansaunet, T.; Taylor, K.; Wilgen, B.; Hatami, A.; Rönike, R.; Reymann, K.; Hutter-Paier, B.; Alexandru, A.; Jaga, W.; Graubner, S.; Glabe, C. G.; Demuth, H. U.; Bloom, G. S. Prion-Like Behaviour and Tau-Dependent cytotoxicity of Pyroglutamylated Amyloid-β. Nature 2012, 485, 651–655.

(28) Russo, C.; Violani, E.; Salis, S.; Venezia, V.; Dolcini, V.; Damonte, G.; Benatti, U.; D’Arrigo, C.; Patrone, E.; Carlo, P.; Schettini, G. Pyroglutamate-Modified Amyloid β-Peptides – AβN3Eβ – Strongly Affect Cultured Neuron and Astrocyte Survival. J. Neurochem. 2002, 82, 1480–1489.

(29) Sun, N.; Hartmann, R.; Lecher, J.; Stoldt, M.; Funke, S. A.; Gremer, L.; Ludwig, H. H.; Demuth, H. U.; Kleinschmidt, M.; Willbold, D. Structural Analysis of the Pyroglutamylated-Modified Isoform of the Alzheimer’s Disease-Related Amyloid-β Using NMR Spectroscopy. J. Pept. Sci. 2012, 18, 691–695.

(30) Tekirian, T. L.; Yang, A. Y.; Glabe, C.; Geddes, J. W. Toxicity of Pyroglutamylated Amyloid β-Peptides 3pE40 and 42 is Similar to that of Aβ 1-40 and 42. J. Neurochem. 1999, 73, 1584–1589.

(31) Sanders, H. M.; Lust, R.; Teller, J. K. Amyloid-β Peptide Aβp3-42 Affects Early Aggregation of Full-Length Aβ. Peptides 2009, 30, 849–854.

(32) Petkova, A. T.; Leapman, R. D.; Guo, Z.; Yau, W. M.; Mattson, M. P.; Tyccko, R. Self-Propagating. Molecular-Level Polymorphism in Alzheimer’s β-Amyloid Fibrils. Science 2005, 307, 262–265.

(33) Jeong, J. S.; Ansalon, A.; Mezzenga, R.; Lashuel, H. A.; Dietler, G. Novel Mechanistic Insight into the Molecular Basis of Amyloid Polymorphism and Secondary Nucleation during Amyloid Fibrillogenesis. J. Mol. Biol. 2013, 425, 1765–1781.