Published in final edited form as:

J Mol Biol. 2015 July 3; 427(13): 2319–2328. doi:10.1016/j.jmb.2015.04.004.

Antiparallel β -sheet structure within the C-terminal region of 42-residue Alzheimer's β -amyloid peptides when they form 150 kDa oligomers

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Abstract

Understanding the molecular structures of amyloid- β (A β) oligomers and underlying assembly pathways will advance our understanding of Alzheimer's disease (AD) at the molecular level. This understanding could contribute to disease prevention, diagnosis, and treatment strategies, as oligomers play a central role in AD pathology. We have recently presented a procedure for production of 150 kDa oligomeric samples of A β (1–42) (the 42-residue variant of the A β peptide) that are compatible with solid state NMR analysis, and we have shown that these oligomers and amyloid fibrils differ in intermolecular arrangement of β -strands. Here we report new solid state NMR constraints that indicate antiparallel intermolecular alignment of β -strands within the oligomers. Specifically, 150 kDa A β (1–42) oligomers with uniform ¹³C and ¹⁵N isotopic labels at I32, M35, G37 and V40 exhibit β -strand secondary chemical shifts in 2D fpRFDR NMR spectra, spatial proximities between I32 and V40 as well as between M35 and G37 in 2D DARR spectra, and close proximity between M35 H _{α} and G37 H _{α} in 2D CHHC spectra. Furthermore, 2D DARR analysis of an oligomer sample prepared with 30% labeled peptide indicates that the I32-V40 and M35-G37 contacts are between residues on different molecules. We employ molecular modeling to compare the newly derived experimental constraints with previously proposed geometries for arrangement of A β molecules into oligomers.

Graphical abstract

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Keywords

Alzheimer's β -amyloid; solid state NMR spectroscopy; oligomer

INTRODUCTION

While significant genetic, pathological, and biochemical evidence links Alzheimer's disease (AD) to the self-assembly of the β -amyloid peptide ($A\beta$), complex self-assembly processes produce a diverse array of aggregated structures. Known aggregated $A\beta$ structures include amyloid fibrils and smaller protofibrillar and oligomeric species associated with higher toxicities.^{1–7} Analysis of $A\beta$ amyloid fibrils by solid state nuclear magnetic resonance (NMR),^{8–10} atomic force microscopy,^{9–12} diffraction based measurements,^{13–15} hydrogen/deuterium exchange,¹⁶ mutagenesis,¹⁷ proteolysis¹⁸ and electron paramagnetic resonance (EPR),¹⁹ has revealed that $A\beta$ self-assembly processes can occur via multiple pathways to produce more than one possible fibril structure. Protocols developed to produce different structurally homogenous fibril samples have made it possible to generate multiple experimentally constrained fibril structural models by solid state NMR.^{8–10, 20, 21} In contrast, there is no widely accepted structural model for $A\beta$ oligomers.^{22–25} The existence of oligomers has been documented by size exclusion chromatography (SEC) and sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE), but oligomer preparations tend to be structurally heterogeneous.^{26–30} Oligomers vary in apparent size from 2 to roughly 100 $A\beta$ molecules, tend to be smaller than protofibrils and fibrils, and do not have the elongated aspect ratios or curvilinear appearance of protofibrils.^{28, 31} A more detailed understanding of $A\beta$ oligomer assembly and structure is likely to be an important step towards a molecular-level understanding of AD pathology, but structural characterization requires structurally homogeneous samples. Here we report further on a 150 kDa $A\beta(1-42)$ oligomer (~30–35 $A\beta(1-42)$ molecules) that is isolated as a stable and homogenous preparation.²³ The isolation protocol involves aggregation in dilute SDS and is similar to that used to prepare distinct $A\beta$ oligomers called globulomers.²⁷ Globulomers do not appear to be intermediates leading to fibril formation because globulomers do not readily convert to fibrils and do not accelerate fibril formation when added as potential seeds to initially monomeric $A\beta(1-42)$ solutions.^{27, 28}

The observation of multiple $A\beta$ aggregates raises questions about structural distinctions among oligomers, protofibrils, and fibrils. It is often thought that fibrils are the eventual stable products of $A\beta$ aggregation and that oligomers and protofibrils exist transiently.³² Consequently, different non-fibrillar structures have been argued to be either “on-

pathway”^{33–35} or “off-pathway”²⁸ to fibril formation. Molecules within off-pathway aggregates must dissociate into monomers before eventually being incorporated into fibrils, whereas on-pathway aggregates can convert to fibril structures without dissociation.^{28, 33, 34} There is general acceptance that protofibrils are on-pathway to fibril formation.^{28, 31, 32} If globulomers are off-pathway to fibril formation, there must be a significant structural difference that produces an energetic barrier to conversion between the oligomeric and fibrillar structure. At present, multiple distinct A β fibril,^{9, 10, 21} protofibril,^{34, 36} and oligomer structural models^{22–24} have been proposed. Composed mostly of β -strands, different structures are distinguished in terms of the exact segments of residues involved in β -strands as well as in the organization of β -strands and β -sheets. The majority of accepted fibril models predict β -sheets composed of in-register parallel β -strands.^{9, 37} In contrast, studies of protofibrillar and oligomeric samples using Fourier transform infrared spectroscopy have indicated antiparallel β -sheet structure.^{38, 39} Scheidt *et. al.* and Doi *et. al.* have shown solid state NMR evidence that protofibrils could be composed of molecules that experience intra-molecular hydrogen bonding between distinct β -strands and form antiparallel β -sheets.^{35, 36} While Scheidt *et. al.* suggest that oligomers could have similar structure, others including our own group have proposed incompatible oligomer structural models.^{22–24, 40, 41} More structural knowledge is needed to explain how oligomers differ structurally from protofibrils and fibrils.

We previously reported a solid state NMR structural study on the 150 kDa A β (1–42) oligomers which indicates that oligomers are not composed of in-register parallel β -sheets.²³ In this contribution, we report new solid state NMR constraints on 150 kDa oligomers of A β (1–42). These constraints support the existence of an antiparallel β -sheet formed by residues near the C-terminus. We also present all-atom molecular models which show that the newly derived experimental constraints could be compatible with a subset of previously proposed schemes for arrangement of A β molecules into oligomeric structures.

RESULTS

To obtain new residue-specific structural constraints for the 150 kDa A β (1–42) oligomers, we prepared two oligomer samples with uniform ¹³C- and ¹⁵N-labeling at selected residues and analyzed these samples using two-dimensional (2D) solid-state NMR spectroscopy. The first sample, designated Sample A, included uniform ¹³C and ¹⁵N at I32, M35, G37 and V40 (see Table 1); we refer to this labeling scheme as IL4. These labeled sites were chosen to test the hypothesis that A β (1–42) oligomers are composed of antiparallel β -strands approximately spanning residues 32–40. Evidence for this hypothesis comes from our previous solid-state NMR PITHIRDS-CT experiments on selective ¹³C-labeled samples where a strong decay was only observed for the V36 carbonyl carbon (CO) labeled site.²³ We also conducted an isotopic dilution experiment with Sample B, an oligomer prepared from 30% IL4 monomers and 70% unlabeled A β (1–42) monomers.

¹³C NMR chemical shifts are consistent with ordered β -strand structure

The local molecular configurations of A β (1–42) oligomers were probed using the 2D fpRFDR solid state NMR technique. This technique creates off-diagonal peaks (*cross-peaks*)

that connect directly bonded ^{13}C atoms (distance of $\sim 0.12\text{nm}$), enabling spectral assignments and precise measurements of ^{13}C chemical shifts and line widths.⁴² Fig. 1 is a 2D fpRFDR solid state NMR spectrum of Sample A. Positions of the off-diagonal peaks (chemical shifts) and line widths were determined using nonlinear fitting of cross-peaks to Gaussian functions, and the results are tabulated in Table S1 in the Supplementary Information. The observed ^{13}C NMR line widths indicate a well-defined molecular structure. As we observed previously for the major signals in an identical oligomer preparation with different isotopic labels (denoted IL7),²³ secondary NMR chemical shifts for labeled CO, C_α and C_β sites are consistent with β -strand secondary structure.⁴³ However, in contrast to spectra for IL7 samples, we observed no resolved signals corresponding to minor structures in the present IL4 sample, suggesting a high degree of structural homogeneity.

2D CHHC indicates an antiparallel β -sheet by identifying close proximity ($<0.3\text{ nm}$) between M35 H_α and G37 H_α

A 2D proton-mediated ^{13}C - ^{13}C exchange (CHHC) experiment was used to probe for evidence of antiparallel β -sheets (Fig. 2). In previous studies of amyloid structure with 2D CHHC spectroscopy, a short inter-strand $\text{H}_\alpha/\text{H}_\alpha$ distance ($<0.3\text{ nm}$) was uniquely attributed to the relative orientation of residues brought in close proximity by antiparallel arrangement of adjacent β -strands. This proximity was detected via cross-peaks between non-sequential C_α atoms.^{20, 21} The 2D CHHC spectrum of Sample A in Fig. 2 shows a cross-peak between M35 C_α and G37 C_α , revealing that the corresponding H_α atoms are brought into close proximities by antiparallel β -sheets. For an antiparallel β -sheet, we would also expect a C_α - C_α contact between I32 and V40; however, a cross-peak between these two atoms is not clearly detectable due to their close chemical shifts. Nevertheless, the I32/V40 2D-DARR contacts noted in the following section support the antiparallel β -sheet configuration.

2D DARR data indicate inter-molecular proximities between I32 and V40 as well as M35 and G37

Close spatial proximities between labeled amino acids were determined using the 2D dipolar assisted rotational resonance (DARR) NMR technique with a 500 ms mixing time. Analysis of the 2D DARR spectrum from Sample A (Fig. 3A and 3B) indicates that I32 is near V40 and M35 is near G37. The observed cross-peaks indicate that at least one pair of ^{13}C atoms on different residues are separated by 0.6 nm or less. Strong intra-residue dipolar couplings between directly bonded ^{13}C atoms within each residue prevent us constraining the relative orientation of I32 and V40. The 2D DARR spectrum from Sample B (Fig. 4A and 4B) shows a significant attenuation of the M35-G37 and I32-V40 cross-peak intensities. This signal attenuation corresponds to the dilution of isotopic labels (oligomer formation with a mixture of labeled and unlabeled peptide) and indicates that the observed NMR contacts are the result of proximity between residues on different molecules.

Consistency in peak positions and line shapes for the 2D fpRFDR spectra of Samples A and B confirms the reproducibility of our oligomer sample preparation (see Fig. S1). When we compared peak positions for corresponding signals from Samples A and B, we measured a root mean squared difference in peak position of 0.15 ppm. This amount of deviation is on

the order of the error estimates calculated for the peak fits (± 0.1 ; see Table S1) and less than deviations reported previously between A β aggregate samples which were considered structurally similar.^{44, 45}

DISCUSSION

Emerging molecular structural understanding of A β aggregation suggests that different structures could be categorized in terms of arrangements of β -strands into β -sheets. This level of organization is defined as tertiary structure in the nomenclature of Tycko, which was established to characterize different fibril structures.^{8, 37} It is likely that tertiary structure may be central to the distinctions between fibrillar and nonfibrillar A β species, and classifications based on tertiary structure could provide a basis for distinguishing different aggregation pathways. For the 150 kDa A β (1–42) oligomers that we describe here, solid state NMR data may be summarized in terms of the following constraints: 1) Secondary ¹³C chemical shifts for CO, C α , and C β signals at several residues are consistent with β -strand secondary structure (see Fig. 1, Table S1, and Reference ²³). 2) The 2D-CHHC cross-peak between the M35 C α and G37 C α (Fig. 2) uniquely indicates an antiparallel β -sheet arrangement.²⁰ 3) Antiparallel tertiary structure is further supported by the 2D DARR contacts between residues I32 and V40 and between residues M35 and G37 (see Fig. 3). 4) Reduction of inter-residue 2D DARR cross-peak intensities upon isotopic dilution (see Fig. 4) eliminates any model that predicts that the inter-residue cross-peaks are due to intramolecular proximities.

It should be noted that one additional solid state NMR constraint is known for 150 kDa oligomers: we previously reported a 2D DARR cross-peak between F19 and A30 and I31 residues.²³ Since A30 and I31 are sequential residues and were both isotopically labeled with ¹³C in the previous study, these residues were magnetically coupled and therefore F19 could have been proximate to either residue (or both). Since the previously observed cross-peak was attenuated by isotopic dilution, it must correspond to residues on different molecules.²³ For amyloid fibrils, proximity between these residues is predicted by published models through stacking of parallel β -sheets (quaternary structure).^{9, 10}

A β amyloid fibril structures have been studied most extensively and therefore serve as a basis for interpretation of nonfibrillar structural data. The majority of data on A β fibrils indicate molecular organization into in-register parallel β -sheets,^{8, 20} although one example has been reported of fibrils composed of an A β (1–40) mutant that forms antiparallel β -sheets.²¹ The in-register parallel motif predicts that equivalent β -strands on neighboring molecules align to form β -sheets that maximize overlap between like residues.^{9, 10} Different A β fibril structures have been shown to vary in terms of the precise residues involved in the β -strand regions and the quaternary structure.^{8, 9, 46} The recent model of the fibril formed by the Iowa mutant (D23N) of A β (1–40) proposed by Qiang *et al.* predicts an antiparallel alignment of β -strands within some β -sheets (antiparallel β -sheets). However, fibrils that contain these antiparallel β -sheets appear to be kinetically and thermodynamically less favored than in-register parallel β -sheet fibrils formed by the same peptide.²¹ The belief that fibrils are usually composed of in-register parallel β -sheets has prompted the interpretation that oligomers with in-register parallel β -sheet structures are on-pathway.³³

Previous structural characterizations of globulomer-like oligomer preparations include reports by Yu *et. al.* and Gu *et. al.*^{22, 47} Yu *et. al.* analyzed an A β (1–42) oligomer species (with an extra M residue on the N-terminus) prepared in 0.2% (7 mM) SDS and then concentrated to 1.5% SDS (52 mM) for the final NMR sample.²² They reported solution state NMR data that are consistent with a dimeric structural unit consisting of a pair of in-register parallel β -strands formed by residues near the C-terminus (L34–V40) extending from a β -hairpin (intramolecular hydrogen bonding between nonequivalent β -strands) formed by antiparallel arrangement of two additional β -strand regions (V18–D23 and K28–G33). This proposed molecular configuration cannot be reconciled with our structural constraints on 150 kDa oligomers because our data clearly indicate antiparallel arrangement between adjacent C-terminal β -strands. The report by Gu *et. al.*, on A β (1–42) globulomers presents EPR data indicating 1.15–1.25 nm intermolecular distances between equivalent residues in the vicinity of the C-terminus (G25–V40).⁴⁷ This constraint was shown to be consistent with antiparallel β -sheet structure, though other possibilities were also considered.⁴⁷

Looking beyond data on globulomers, other reports on oligomeric A β propose antiparallel β -sheet structures. Stroud *et. al.* prepared A β (1–42) fibrillar oligomers and analyzed the structure using X-ray diffraction to predict domain swapped antiparallel β -sheets.⁴⁰ Gu *et. al.* reported EPR and X-ray diffraction data on a non-fibrillar aggregate of a GroES-Ubiquitin-A β (1–42) fusion protein. They proposed multiple alternative models based on β -hairpin and antiparallel β -sheet structures for the A β (29–40) segment.²⁵ The proposed β -hairpin model does not agree with our present experimental data because it predicts C-terminal β -strands (residues 28–42) that are oriented parallel to equivalent strands on neighboring molecules.²⁵ Alternative models presented by Gu *et. al.* are consistent with an antiparallel β -sheet configuration for the C-terminal β -strand.²⁵ Finally, while the recent model of Ahmed *et. al.* predicts antiparallel arrangement of β -strands for pentamers prepared at low temperature (4 °C), this model does not predict any hydrogen bonding between β -strands and therefore predicts no β -sheets.

It is important to recognize that our present constraints on 150 kDa oligomers could be consistent with β -hairpin arrangements. Scheidt *et. al.* proposed a β -hairpin structure for A β (1–40) protofibrils stabilized by an antibody-derived fusion protein.³⁵ The structure was reported to include a close proximity of E22 and I31 that is inconsistent with A β (1–40) fibril models but consistent with a β -hairpin.³⁵ Another solid state NMR study by Doi *et. al.* of A β (1–42) protofibrils prepared under close-to-physiological conditions indicated a similar β -hairpin structure.³⁶ Sandberg *et. al.* stabilized β -hairpin structure with an intramolecular disulfide crosslink within an A β (1–42) double mutant (A21C/A30C) and showed that this peptide formed oligomers and protofibrils without proceeding to fibril formation.⁴⁸ Spencer *et. al.* have reported a X-ray crystal structure for a cyclic peptidomimetic analog of A β (17–36), in which twisted β -hairpins are arranged into 3-fold symmetric antiparallel β -strand subunits.⁴¹

Fig. 5 proposes models of C-terminal antiparallel β -sheets arranged into symmetries predicted by published models of A β nonfibrillar structures that could be consistent with our present experimental constraints. These diagrams are representations of all-atom models for

antiparallel β -sheets formed by residues 32–40. We focused our modeling efforts on β -sheets involving residues 32–40 due to lack of sufficient experimental constraints on other residues. To illustrate that all three configurations in Fig. 5 are consistent with our present structural knowledge, atoms subject to experimental constraints are drawn as spheres. These models are based on symmetries of models previously proposed for A β oligomers, although previous models either do not predict residue-specific β -strand alignments or predict alignments which differ from those shown in Fig. 5. Fig. 5A shows an antiparallel β -sheet which is composed exclusively of β -strands spanning residues 32–40, aligned such that V36 CO atoms (red spheres) are in close proximity (0.5 nm). The configuration in Fig. 5A is predicted by our previous report²³, in which we suggested that each A β molecule contributes one β -strand to each of two β -sheets that are stacked to form a single steric zipper, and by Stroud *et. al.*,⁴⁰ who envision an open molecular conformation in which domain swapped β -strands within the same molecule are part of different steric zippers. The Fig. 5A configuration is also similar to that of the antiparallel β -sheet for residues 31–35 within the fibril model proposed by Qiang *et. al.* for the Iowa mutant of A β (1–40), although G33 is at the center of the C-terminal β -sheet for the fibril model.²¹ Fig. 5B depicts an antiparallel β -sheet which is composed of β -hairpins such that the β -sheet includes two distinct β -strands. In this picture, the gray strands are composed of residues 32–40, as in Fig. 5A, and the blue strands represent an N-terminal β -strand. In the absence of experimental constraints, the residues modeled within the turn (28–31) and blue β -strand (14–27) regions of Fig. 5B were chosen arbitrarily. Models with β -hairpins have been proposed by Doi *et. al.* for A β protofibrils³⁶ and Gu *et. al.* for A β oligomers.²⁵ The three-fold symmetric model in Fig. 5C resembles an arrangement of β -strands formed by A β (17–23) within the crystal structure reported by Spencer *et. al.*⁴¹

All of the molecular models in Fig. 5 are consistent with a close spatial proximity between neighboring V36 CO atoms, a constraint that we have previously reported from the observation of a strong PITHIRDS-CT decay of singly ¹³C-labeled A β (1–42) oligomers.²³ Relatively weaker PITHIRDS-CT decays were previously observed for samples selectively ¹³C-labeled at other backbone sites, leading to the suggestion that 150 kDa oligomers are not composed of parallel β -sheets.²³ The 0.5–0.6 nm spatial proximity between V36 CO atoms within presently considered models is illustrated with the red spheres in Fig. 5A–5C. Nuclear spin simulations were performed for our previous PITHIRDS-CT experiment using the coordinates of V36 CO atoms predicted by each model in Fig. 5, and we compared these simulations to the experimental V36 CO PITHIRDS-CT data (Fig. S2). Although each geometry corresponds to a slightly different predicted decay, we consider all of the models to be consistent with the experimentally observed PITHIRDS-CT data. Systematic errors between calculated and measured PITHIRDS-CT decays are often observed, in part because, 1) models tend to be “idealized” configurations that do not consider factors such as twisting of β -sheets that could occur in reality, and 2) limited computational power often limits the number of nuclear spins included in PITHIRDS-CT simulations.^{23, 49} It should be noted that our previous interpretations of PITHIRDS-CT results do not rely primarily on precise agreement with simulations, but rather on comparisons of experimental decays observed for structurally similar samples with different isotopic labels.^{23, 49, 50}

In summary, the present results establish that the C-terminal β -strand is arranged into an antiparallel β -sheet such that the V36 residue is in the closest possible proximity to V36 residues on adjacent molecules. Since A β (1–42) is not known to form fibrils with antiparallel β -sheets, this result could represent an aggregation pathway which differs from the fibril formation pathway. These results therefore provide a molecular level basis for arguing that the 150 kDa oligomer is an off-pathway aggregate, and may explain why this oligomer does not accelerate fibril formation when added to A β (1–42) monomer solutions. For oligomer models with extended β -sheets, it is not clear what would limit the size of the aggregate. However, limits could be imposed if the β -sheets were twisted in a fashion reminiscent of the antiparallel β -sheet oligomers formed by the 11-residue segment of α B crystallin.⁵¹

MATERIALS AND METHODS

Chemicals and A β (1–42) Preparations

All chemicals and solvents were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. A β (1–42) peptide (sequence DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGGVIA) uniformly labeled with ¹³C and ¹⁵N at amino acid residues I32, M35, G37 and V40 (denoted IL4) was purchased from the Keck Biotechnology Resource Laboratory (New Haven, CT). The unlabeled A β (1–42) peptide was synthesized using standard Fmoc solid phase peptide synthesis methods by the Proteomics Core at the Mayo Clinic (Rochester, MN). The HPLC purification step was omitted for the A β (1–42) preparations to conserve peptide,²⁸ and the purity of the peptide was determined by MALDI-mass spectrometry to be > 90%. Lyophilized A β (1–42) peptides, stored desiccated at –80 °C, were purified by SEC as previously described.²³ Briefly, the peptide (5–7 mg) was first dissolved in 400 μ L HFIP, dried as a thin film under a gentle stream of nitrogen, and further dried by centrifugal vacuum concentration. The dried film was completely dissolved in 70 μ L of 0.5 M NaOH, gently vortexed for 30 sec, and diluted with 935 μ L of 20 mM sodium phosphate, pH 7.5 (NaP buffer). Dissolved samples were bath sonicated for 3 min and centrifuged at 18,000 $\times$ g for 10 min before the supernatants were applied to a Superdex 75HR 10/30 column (Amersham Pharmacia, Piscataway, NJ) equilibrated with NaP buffer at a flow rate of 0.5 mL/min. Collected fractions were quantified by UV absorbance at 276 nm (with ϵ = 1450 M^{–1}cm^{–1}).⁵²

A β (1–42) Oligomer Preparations for solid state NMR

A β oligomers were prepared as previously reported.²³ Briefly, aliquots of 100 μ M SEC-purified A β monomer were incubated overnight at room temperature in 50 mM NaCl and 4 mM SDS to produce initial oligomers that we denote 2–4mers.²³ To remove the SDS and reduce the salt concentration, the sample was dialyzed against 20 mM NaP for 48–72 hours and then against 10 mM NaP for an additional 3–4 hours with at least five buffer changes. Evidence of successful removal of SDS is observed in Fig. S3, which shows that ¹³C NMR signals from SDS were not observed in the NMR spectrum of an unlabeled oligomer sample. Sample quality was monitored and confirmed at each step of the preparation by circular dichroism (CD) and thioflavin T fluorescence. Residual or unconverted monomer was

removed by filtering the dialyzed oligomer with an Amicon Ultra 4 centrifugal concentration/filtration device with a MW cutoff of 50 kDa. In order to prepare sufficient amounts for solid state NMR analysis (3–5mg), at least 5 consecutive samples were prepared within 1–2 days. Finally, the preparations were pooled, flash-frozen in a dry ice-acetone bath, immediately lyophilized, and stored at -80°C until use. For isotopic dilution experiments, an oligomer sample was prepared from a mixture of monomers composed of 30% IL4 and 70% unlabeled A β (1–42).

Solid State NMR Spectroscopy

Solid state NMR experiments were performed on a Bruker 11.75 Tesla (500 MHz ^1H NMR frequency) solid state NMR system, equipped with an Avance III console and a 2.5 mm magic angle spinning (MAS) NMR probe. Two-dimensional exchange NMR was performed on IL4 (Sample A) and isotopically diluted (Sample B) oligomer samples. During the 2D exchange mixing periods, ^{13}C - ^{13}C dipolar couplings eliminated by MAS were reintroduced using the fpRFDR, DARR, or CHHC pulse sequence. Decoupling of ^1H using two pulse phase modulation was employed during free evolution and fpRFDR recoupling periods, with a ^1H radio frequency field of 110 kHz. Exchange periods during 2D DARR experiments required continuous irradiation for 500 ms with ^1H fields with powers corresponding to 16.5 kHz nutation frequencies (equal to the MAS spinning rate). The π pulses on ^{13}C required for fpRFDR recoupling were applied using 37.5 kHz radio frequency fields, so that the pulse durations (13.3 μs) were 1/3 of the rotor period at 25 kHz MAS. The 2D fpRFDR spectrum in Fig. 1 and the 2D DARR spectra in Fig. 3 are each the result of 48 hours of signal averaging. Due to isotopic dilution, increased signal averaging (72 hours) was required for the 2D DARR spectrum of Sample B (see Fig. 4). CHHC spectra were acquired at 10 kHz MAS as previously described²⁰ with 150 μs ^{13}C - ^1H and ^1H - ^{13}C cross-polarization periods and a 200 μs ^1H - ^1H spin diffusion period between t_1 and t_2 , and Fig. 2 is the result for 76 hours of signal averaging.

Molecular Modeling

All-atom molecular models were built using the VMD molecular modeling and NAMD molecular dynamics software.⁵³ Initial models were built using linear segments of the A β (1–42) peptide, with $\phi = -180^{\circ}$ and $\psi = 180^{\circ}$. A β (1–42) segments were rotated and translated to desired positions for each candidate structure as a single peptide. Initial candidate structures underwent a brief energy minimization followed by a simulated annealing in implicit solvent with fixed backbone coordinates, where temperatures were increased from 350 K to 1000 K before dropping to 0 K in increments of 5 K with 10 ps simulation time per step. Annealed structures were then energy minimized for 150000 steps at 310 K without fixed backbone coordinates and with artificial hydrogen bonding constraints between β -sheet forming residues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Alzheimer's Association (grant NIRG-10-173755 to AKP), the National High Magnetic Field Laboratory User Collaboration Research Grant Program, and the National Institute on Aging of the National Institutes of Health (award number R01AG045703). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490 and the State of Florida.

ABBREVIATIONS

Aβ	amyloid- β
AD	Alzheimer's disease
CD	circular dichroism
DARR	dipolar-assisted rotational resonance
EPR	electron paramagnetic resonance
fpRFDR	finite pulse radio frequency driven recoupling
HFIP	hexafluoroisopropanol
HPLC	high performance liquid chromatography
MALDI	matrix-assisted laser desorption/ionization
MAS	magic angle spinning
MW	molecular weight
MWCO	molecular weight cut-off
NMR	nuclear magnetic resonance
PAGE	polyacrylamide gel electrophoresis
SDS	sodium dodecylsulfate
SEC	size exclusion chromatography
VMD	Visual Molecular Dynamics
2D	2-dimensional

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Highlights

- A β (1–42) oligomer formation may underpin Alzheimer's disease pathology.
- Solid state NMR data indicate an antiparallel β -sheet within 150 kDa oligomers.
- The β -strands involve residues 32–40 with adjacent residues 36 in close proximity.
- Molecular models were constructed with previously proposed β -strand organizations.

Fig. 1.

A 2D ^{13}C - ^{13}C fpRFDR solid state NMR spectrum of Sample A. The off-diagonal cross-peaks represent single-bond correlations which allow residue-specific assignments within the ^{13}C -labeled residues, as illustrated by colored lines.

Fig. 2.

(A) The aliphatic region of a 2D CHHC NMR spectrum of Sample A, showing a C_α - C_α cross-peak between M35 and G37 at 200 μs ^1H spin diffusion period (colored circles). In the 2D CHHC spectrum, the solid colored lines indicate single-bond exchange pathways or residue-specific assignments for M35 (orange) and G37 (blue) determined from the 2D fpRFDR spectrum in Fig. 1. (B) A horizontal slice of the 2D CHHC spectrum taken at the position of the M35 C_α signal, indicated by the orange dashed line from Panel A (at 52.7 ppm), is compared to a horizontal slice of the 2D fpRFDR spectrum in Fig. 1 taken at the position of the G37 C_α signal (43.8 ppm). The blue vertical dashed line with an arrow head indicates polarization transfer between the G37 and M35 C_α atoms.

Fig. 3.

(A) The aliphatic region of a 2D DARR ^{13}C - ^{13}C spectrum for Sample A (500 ms mixing time). (B) Comparison of selected horizontal slices from the 2D DARR spectrum and the 2D fpRFDR spectrum (Fig. 1). The 2D DARR spectrum in Panel A shows inter-side-chain cross-peaks (correlations between atoms separated by up to ~ 0.6 nm) that indicate proximities between I32 and V40 and between M35 and G37. The solid colored lines in Panel A indicate single-bond exchange pathways or residue-specific assignments for V40 (pink), I32 (green), M35 (orange), and G37 (blue) identified using the 2D fpRFDR spectrum (Fig. 1). The colored circles indicate inter-residue cross-peaks, with specific residues indicated by the colors of the semicircles. The horizontal slices are at positions indicated by dashed lines in Panel A (V40 C_γ slice at 19.4 ppm, pink; M35 C_α slice at 52.7 ppm, orange) and the 2D fpRFDR spectrum (green slices at the C_β and C_γ peak positions of I32 and the blue slice at the C_α peak position of G37). The vertical dashed lines with arrowheads in Panel B indicate polarization transfers between I32 and V40 aliphatic carbons as well as the transfers between M35 and G37 aliphatic carbons.

Fig. 4. (A) Overlaid aliphatic regions of 2D DARR NMR spectra (500 ms mixing time) showing the effect of isotopic dilution. Gray and red contours correspond to Samples A and B, respectively, where Sample B was isotopically diluted (Table 1). Inter-residue I32/V40 and M35/G37 cross-peaks that were clearly attenuated upon isotopic dilution are marked with blue dashed boxes and green dashed circles, respectively. (B) Horizontal slices taken at the frequencies marked with dashed lines (V40 C_γ slice: 19.4 ppm; M35 C_β slice: 52.7 ppm) in Panel A. Loss of inter-residue cross-peak intensities with isotopic dilution (between I32 and V40 and between M35 and G37) is visible for the selected signals marked with vertical dotted lines. Slices in Panel B are scaled to the single bond (intra-residue) cross-peaks present in those slices.

Fig. 5. Models for arrangement of β -strands formed by residues 32–40 into antiparallel β -sheets. Ribbons represent β -strands and spheres represent atoms with NMR-derived structural constraints described in the text. In Panels A–C, spheres are color coded by residue as marked in Panel C. The red spheres, for example, represent carbonyl carbon atoms on V36, for which PITHIRDS-CT data indicate a close interatomic proximity.²³ A) An antiparallel β -sheet composed only of equivalent β -strands (residues 32–40). B) An antiparallel β -sheet composed of β -hairpins with two nonequivalent β -strands (gray ribbons for residues 32–40 and blue ribbons for a presently unconstrained N-terminal β -strand). C) A three-fold symmetric configuration of antiparallel β -strands. D) An enlarged view of the peptide backbone regions defined by the dashed boxes in Panels (A) and (B) with side chains deleted. Experimentally constrained interatomic distances are indicated by arrows. In Panels D and E, atoms are colored by element (C: black; H: white; N: blue; O: red). (E) An enlarged view of the peptide backbones within the dashed box in Panel C, with constrained inter-atomic distances indicated by arrows.

Table 1

Isotopic labeling for 150 kDa oligomer samples.

A β (1–42) Oligomer Sample Designation	Isotopic Labeling Scheme
A	IL4: uniformly labeled with ^{13}C and ^{15}N at I32, M35, G37 and V40
B	30% IL4 and 70% unlabeled A β (1–42) peptide