Aβ(1–42) fibril structure illuminates self-recognition and replication of amyloid in Alzheimer’s disease

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Increasing evidence has suggested that formation and propagation of misfolded aggregates of 42-residue human amyloid β (Aβ(1–42)), rather than of the more abundant Aβ(1–40), provokes the Alzheimer’s disease cascade. However, structural details of misfolded Aβ(1–42) have remained elusive. Here we present the atomic model of an Aβ(1–42) amyloid fibril, from solid-state NMR (ssNMR) data. It displays triple parallel-β-sheet segments that differ from reported structures of Aβ(1–40) fibrils. Remarkably, Aβ(1–40) is incompatible with the triple-β-motif, because seeding with Aβ(1–42) fibrils does not promote conversion of monomeric Aβ(1–42) into fibrils via cross-replication. ssNMR experiments suggest that C-terminal Ala42, absent in Aβ(1–40), forms a salt bridge with Lys28 to create a self-recognition molecular switch that excludes Aβ(1–40). The results provide insight into the Aβ(1–42)-selective self-replicating amyloid-propagation machinery in early-stage Alzheimer’s disease.

Fatal neurodegenerative diseases such as Alzheimer’s disease (AD) and prion diseases are linked to misfolding of disease-specific amyloidogenic proteins1. These proteins misfold into toxic amyloid fibrils, which self-replicate in vitro and in vivo1–4, acting as pathogenic ‘seeds’ for amyloid-plaque formation. Plaques formed by misfolded amyloid-β (Aβ) are a hallmark of AD. Because cytoxicity is triggered by misfolding of Aβ, intensive efforts have focused on elucidating the structures of amyloid fibrils5–12 and other aggregates1,13–17. Among the Aβ species present in AD, the 42-residue Aβ(1–42) is generally considered to be the most pathogenic species18,19. Aβ(1–42) exhibits notably higher toxicity and aggregation propensity than the more abundant 40-residue Aβ(1–40)20–22, even though their sequences differ only slightly. The Aβ(1–42) fibril is the initial and predominant constituent of amyloid plaques23–25 despite Aβ(1–40) being more abundant in plasma. Increased production of Aβ(1–42) relative to Aβ(1–40) has been reported for numerous pathogenic mutants of γ-secretase, which are linked with early onset of AD26. For the less aggregation-prone Aβ(1–40), a handful of high-resolution structural models have been proposed by ssNMR methods1,7–9. Most of these structures are characterized by a U-shaped strand-loop-strand (β-loop-β) or ‘β-arch’ motif27, in which two parallel β-sheets are connected by a short curved loop region (between residues Asp23 and Gly29) and are often stabilized by a salt bridge between Asp23 and Lys28 side chains5,7–9,28. In contrast, for the more pathogenic Aβ(1–42) fibril, the structural details have been poorly defined despite intensive efforts3–6,10,11,14,18,29. Owing to its high misfolding propensity, Aβ(1–42) fibrils typically show structural and morphological heterogeneity10,11, which limits subsequent analyses. Thus, there are only a few low-resolution or computational models for Aβ(1–42) amyloid fibrils, and experimental conformational details and tertiary structures have remained elusive5,10,11,28,29. Another key question in AD regards the interaction between Aβ(1–42) and Aβ(1–40) amyloid states. A lower ratio of Aβ(1–42) to Aβ(1–40) in the plasma is a known indicator of AD30,31; this presumably suggests depletion of soluble Aβ(1–42) by selective aggregation of Aβ(1–42) species. However, it has been unclear why misfolded Aβ(1–42) does not trigger misfolding of Aβ(1–40) via cross-seeding at an early stage of AD. Beyond in vitro kinetics studies32 and recent studies in mouse models33, there has been no mechanistic or structural understanding of these prion-like cross-propagation properties between Aβ isoforms.

Here, we have elucidated the first atomic model, to our knowledge, for a structurally homogeneous Aβ(1–42) fibril by ssNMR, a powerful tool for amyloid and other noncrystalline proteins34–37. The molecular dynamics (MD)-based structural modeling unveils distinctive structural features of the Aβ(1–42) fibril, which were not identified in previous studies of Aβ(1–40) fibrils. The results provide direct evidence that Aβ(1–42) can misfold into an amyloid fibril via a different path from that of Aβ(1–40), indicating notable structural differences between misfolded Aβ(1–42) and Aβ(1–40) in AD. The structural features of the Aβ(1–42) fibril also provide insight into how tertiary folds of amyloid proteins can define prion-like cross-propagation properties in AD and other amyloid diseases through discrimination of similar amyloid proteins adopting alternative states.
RESULTS
Seeded Aβ(1–42) fibril displays structural homogeneity
We first established a protocol to prepare structurally homogenous amyloid-fibril samples for Aβ(1–42) and observed the morphology of the Aβ(1–42)-fibril sample by using transmission electron microscopy (TEM) (Fig. 1a). We prepared the sample by incubating an Aβ(1–42) solution for 24 h with a 5% (w/w) solution of seeded amyloid fibrils. For reproducible preparation of Aβ(1–42)-fibril samples, we carefully optimized the purification protocol, sample concentration and incubation times. We obtained seeded fibrils in the fourth generation (G4) by repeating this protocol for three successive generations after an initial incubation without a seed (generation 1 or G1) (Online Methods). The seeded fibrils showed elongated filament-like shapes with a diameter within 10 nm, with homogeneous morphology throughout the samples. Many of them appeared bundled together. We confirmed that samples collected after 24–72 h of incubation with the seeding protocol produced fibrils with nearly identical morphologies after up to 13 generations.

In order to examine atomic-level structures and heterogeneities, we performed ssNMR for 13C-15N isotope-labeled Aβ(1–42) in fibrils prepared according to the seeding protocol. Through observation of chemical shifts, which sensitively reflect conformations, specific structural heterogeneity can be monitored from the NMR spectra of the fibrils. We collected 2D 13C-15N chemical-shift-correlation ssNMR spectra (Fig. 1b,d) and 2D 13C-13C ssNMR spectra (Fig. 1c,e,g) for three Aβ-fibril samples with uniformly 13C- and 15N-labeled amino acids introduced at different residues. The data indicated the presence of a single conformer in the seeded fibril. For example, the spectra for sample 1 (Fig. 1b,c) show a single set of cross-peaks for all the directly bonded 13C-15N or 13C-13C pairs for Phe20, Ala21, Val24, Gly25 and Leu34, except for a few very weak minor peaks. Because chemical shifts are sensitive indicators of protein conformations, a single set of chemical shifts for each residue implies that Aβ(1–42) in the fibril had mostly a single conformation (Supplementary Table 1). We observed similar trends for sample 2 and sample 3 (Fig. 1d–g). In contrast, Aβ(1–42) samples prepared without the seeding protocol exhibited two or more sets of cross-peaks (Supplementary Fig. 1a) suggesting the presence of polymorphs. Neglecting the polymorphs in a structural analysis by hydrogen/deuterium-exchange solution NMR or other methods may result in a misleading structure. The homogeneous Aβ(1–42) fibril that we used for the structural analysis is equivalent to a pure Aβ(1–42) 'amyloid strain'. Thus, the system can also be used as a model to study self-propagation and cross-propagation of Aβ(1–42), as discussed below.

Figure 1 Structural homogeneity and morphologies analysis of Aβ(1–42) amyloid fibrils. (a) TEM images of seeded Aβ(1–42) fibrils. The sample was obtained 24 h after fourth-generation (G4) incubation of an Aβ(1–42) solution with seed Aβ(1–42) fibrils (5% in weight). (b,d) 2D 15N-13C correlation ssNMR spectra and (c,e,g) 2D ssNMR 13C-13C correlation spectra of seeded fibril samples uniformly labeled with 13C-, 15N at Phe20, Ala21, Val24, Gly25 and Leu34 (b,c), Ala2, Gly9, Phe20, Val39 and Ile41 (d,e) and Phe4, Val12, Leu17, Ala21 and Gly29 (f,g). In b,d,f, 2D DARR spectra with a mixing time of 50 ms present single intraresidue cross-peaks for each 13C-15N pair, indicating a single conformer. The base contour levels were set to 4–6 times the r.m.s. noise level. The contour levels in the 2D 13C-13C correlation spectra were set to 5% (b), 7% (d) and 10% (f) of the diagonal signals of 13Cα of Ala21 (b,f) or Ile41 (d).
Figure 2  ssNMR-based structural constraints for the Aβ(1–42) fibril.  
(a–c) Superimposed aromatic-aliphatic cross-peaks in 2D $^{13}$C–$^{13}$C ssNMR spectra of the same fibril samples obtained with 200-ms (red) and 50-ms (black) mixing times. Observed inter-residue long-range contacts: Phe20-Ala21 and Phe20-Val24 (a); Phe19-Ala30 and Phe19-Ile32 (b); and Phe19-Ala30 and Phe19-Ile31 (c). The samples were uniformly labeled with $^{13}$C, $^{15}$N at Phe20, Ala21, Val24, Gly25 and Leu34 (a), Phe19, Ala30, Ile32, Gly38 and Val40 (b) and Phe19, Ala30, Ile31, Gly33 and Val36 (c). The base-contour levels were at 4–5 (red) and 6–8 times (black) the r.m.s. noise levels.  
(d) Dephasing curves by frequency-selective REDOR$^{45}$ for measurement of the distance between Ala42 $^{13}$CO and Lys28 $^{15}$N for a 100%-labeled sample (black filled squares), in comparison with simulated dephasing results for a 50%-labeled sample (red filled squares), at 4.0 Å (green dashed line), 4.0 Å (black line) and 4.1 Å (blue dashed line). The best-fit data were obtained for the simulated result for 4.0 Å. The carrier frequency for the selective $^{15}$N pulse$^{45}$ was set to 35 p.p.m. near the $^{15}$N resonance. Open black circles represent control experiments in which $^{15}$N was irradiated at off-resonance at 200 p.p.m. The control results show no effects due to $^{13}$CO and neighboring amide $^{15}$N groups. The error bars were estimated from the noise level of the spectra.

showed single sets of strong cross-peaks (Fig. 1d,e) indicating high structural order and lack of mobility at the C terminus. It is also noteworthy that many of the cross-peaks are weak or missing for the residues located at the N-terminal region at Ala2, Phe4, Gly9 and Val12 (Fig. 1c–g) and at His13 and His14 (data not shown). Thus, we inspected only a handful of residues in the N-terminal region in the analysis. These results establish an overall structural homogeneity of the obtained fibril sample with well-defined conformations at the hydrophobic core and C-terminal residues, and dynamic N-terminal residues.

On the basis of the assigned $^{13}$C and $^{15}$N chemical shifts of the Aβ(1–42) fibril from Figure 1 and other data (Supplementary Table 1), the secondary-structure analysis by TALOS-N$^{41}$ indicated the presence of three extended $\beta$-strand regions at Val12–Phe20, Asn27–Ile32 and Val36–Ile41 connected by two loop regions at Ala21–Ser26 and Gly33–Met35 (Supplementary Fig. 1b,c). Additionally, measurement of interstrand $^{13}$CO–$^{13}$CO distances for Aβ-fibril samples selectively labeled at $^{13}$CO of Ala30 and Leu34 indicated CO-CO distances of 5.0 Å ± 0.1 Å at both residues (Supplementary Fig. 2a), with errors estimated from the noise levels. This finding reveals a fibril made of three stretches of in-register parallel-$\beta$-sheet regions. Although early ssNMR studies of Aβ(1–42) fibrils also reported in-register parallel-$\beta$-sheet formation$^{14,42}$, major structural differences between Aβ(1–42) and Aβ(1–40) fibrils were not identified. In previous studies for in vitro-prepared Aβ(1–40) fibrils, the fibril structures were commonly characterized by a $\beta$-loop-$\beta$ motif, in which two stretches of parallel $\beta$-strands are connected with a single curved non-$\beta$-strand region near Asp23–Gly29 (refs. 2,8,9). Aβ(1–40) fibrils seeded with brain amyloid from atypical AD inherit a U-shaped $\beta$-arch motif$^4$, which is different from the motif of the Aβ(1–42) fibril. Thus, importantly, the triple-$\beta$-motif indicated for the fibril structure of Aβ(1–42) is markedly different from the structure of Aβ(1–40).

S-shaped triple-$\beta$-motif is stabilized by a salt bridge

In order to elucidate the packing of the multiple $\beta$-strands in amyloid fibrils, we examined long-range inter-residue contacts by 2D $^{13}$C dipolar-assisted rotational resonance (DARR)$^{43}$ ssNMR experiments, using an extended $^{13}$C–$^{13}$C mixing period of 200 ms (red spectra in Fig. 2a–c) with an additional $^{13}$C–$^{15}$N distance measurement (Fig. 2d).

We observed multiple long-range inter-residue $^{13}$C–$^{13}$C contacts within a distance of ~6 Å. We observed correlation only within residues or adjacent residues with a shorter mixing time of 50 ms (black spectra in Fig. 2a–c) under the same mixing condition as in Figure 1. Superimposed ssNMR spectra with 200-ms mixing and 50-ms mixing highlight long-range cross-peaks between Phe19 or Phe20 side chains and other amino acids. The observed inter-residue contacts are Phe20-Ala21, Phe20-Val24 (Fig. 2a), Phe19-Ala30 (Fig. 2b,c), Phe19-Ile32 (Fig. 2b) and Phe19-Ile31 (Fig. 2c). We confirmed that these are intramolecular contacts with experiments using isotope-labeled Aβ mixed with unlabeled Aβ (example in Supplementary Fig. 2b,c).

From the chemical shifts, dihedral angles predicted from the $^{13}$C and $^{15}$N shifts (Supplementary Table 1) and long-range distance restraints, we elucidated a multi-$\beta$-segment atomic model with the aid of MD simulations (Fig. 3a–c). The structural model (Fig. 3a) is characterized by S-shaped triple-$\beta$-strand regions that are connected by major coil-and-turn regions at residues 19–23 and 34–35; the results are largely consistent with the above mentioned secondary-structure prediction. Moreover, we identified a new contact between Lys28 and Ala42. The identified side chain contacts (Fig. 3b) not only show good agreement with experimentally observed long-range distance restraints but also explain unobserved long-range contacts for distances beyond 5 Å, which we also used as constraints (Supplementary Table 2). The undetected contacts include those for Phe19-Leu34, Phe19-Val36, Phe19-Gly38, Phe19-Val40 and Asp23-Lys28, many
Figure 3  Structural details of the Aβ(1–42) fibril revealed by the ssNMR analysis. Disordered residues 1–10 are omitted for clarity. Green arrows throughout indicate the fibril axis. (a) View from the fibril axis, showing three β-strand regions (cyan, residues 12–18; yellow, 24–33; green, 36–40) connected by short coil or turn (silver) regions. The unique salt bridge between Lys28 (blue) and Ala42 (red) is shown. (b) Side chain contacts for a single Aβ chain with a van der Waals surface and polarity diagram for the rest of the Aβ chains. Residues are colored as green, hydrophobic; cyan, polar; red, acidic; and blue, basic. Observed long-range side chain intramolecular contacts (purple arrows) and intermolecular contacts (blue arrow) are shown. (c) The side view in ribbon diagram. The in-register parallel-β-sheet arrangement was confirmed by measurements of intermolecular $^{13}$CO-$^{13}$CO distances of ~4.8 Å at Ala30 and Leu34 (purple arrows). (d,e) STEM images of seeded fibril filaments. (e) The diameters of the fibril filaments ranged between 4.5 and 6.0 nm for thinner filaments (right and left) and between 6.0 and 14.0 nm for wider filaments (middle).

of which were previously reported for Aβ(1–40) fibrils with similar β-loop-β motifs2,8 or for Aβ(1–42) fibrils13,14. The structure met nearly all the structural restraints, including those for unobserved contacts, with a few minor violations (Table 1) and reproduced the $^{13}$C and $^{15}$N chemical shifts well, according to ShiftX2 (ref. 44) (Supplementary Table 3) at a level comparable to those in a previous study of Het-s prion fibrils35 (Online Methods). More interestingly, our initial efforts of MD-optimized modeling suggested that with the ssNMR distance constraints, Lys28 cannot maintain a salt bridge with Asp23, although this salt bridge was observed for many of the models for Aβ(1–40) fibrils. Instead, the data suggested a contact between Ala42 and Lys28 (Fig. 3a,b). Thus, we performed an additional long-range distance measurement between the $^{13}$CO$^-$ terminus of Ala42 and the $^{15}$NH$_3^+$ side chain of Lys28 by monitoring $^{13}$C signal dephasing in frequency-selective rotational-echo double resonance (REDOR) experiments15 (Fig. 2d and Supplementary Data Set 1). The value $S/S_0$ represents the signal intensity in the REDOR experiment (S) normalized by that in the control REDOR experiment without $^{15}$N pulse ($S_0$). The measured intramolecular $^{13}$C-$^{15}$N distance was 4.0 ± 0.1 Å, a result suggesting the formation of a unique salt bridge between Lys28 and Ala42. The distance was unaffected (4.1 ± 0.1 Å) in the same experiment for a sample in which labeled and unlabeled Aβ(1–42) samples were mixed in a 1:1 ratio. This confirmed that the salt bridge was formed primarily via an intramolecular contact. From a separate long-range DARR experiment, we also observed contacts between Gly29 and Ile41, which we assigned to intra- and intermolecular contacts (Supplementary Fig. 2b). On the basis of the presence of the intramolecular contact between Lys28 and Ala42, we attributed the intermolecular contacts to the contacts of Gly29 and Ile41 in the next neighboring Aβ chains, but we did not include them in the structural calculations. We reoptimized the preliminary model with the new restraints, including them between Lys28 and Ala42 (Fig. 3a–c, Supplementary Fig. 3 and Online Methods). The stabilization by this salt bridge between Lys28 and Ala42 explains why the unique S-shaped triple- or multi-β-sheet motif is observed for only Aβ(1–42) fibrils. Because Ala42 does not exist in Aβ(1–40), such a structure is not likely to be stable for Aβ(1–40). The structure also exhibits Gly29-Ile41 contacts. This evidence suggests the possibility that Aβ(1–42) constitutes a distinct amyloid strain that has different propagation and structural properties from that of Aβ(1–40).
The high-resolution negatively stained scanning TEM (STEM) image (Fig. 3d,e) for fibrils gently washed with deionized water shows twisted single strands that exhibit a periodic modulation in diameter between 6.5 ± 1 nm and 13 ± 1 nm (Fig. 3e). We also observed thinner filaments that show a modulation approximately between 4.5 and 6.0 nm (Fig. 3e). The range agrees with the dimensions of the ssNMR-based structural model, which exhibits similar dimensions of 4.5 nm by 3.5 nm perpendicular to the fibril axis (Fig. 3b). An alternative model made of dimeric protofilament elements also explains the morphological properties (data not shown), whereas the use of negative staining makes it difficult to elucidate the exact mass per length from the STEM data. The thicker filaments may be attributed to a hydrophobic assembly of multiple basic protofilament units shown in Figure 3b. Although further analysis by ssNMR and other complementary methods is needed to define the detailed protofilament arrangements of Aβ(1–42), the obtained atomic model reproduces the morphological features of the amyloid fibril.

Aβ(1–42) fibril does not template Aβ(1–40) fibril formation

Previous in vitro kinetics studies and recent studies in mouse models have suggested distinct propagation properties for Aβ(1–42) and Aβ(1–40) fibrils. However, these studies used amyloid fibrils for which structural profiles and homogeneity were not well defined. More importantly, there has been no molecular-level mechanism that explains the differences in amyloid propagation of Aβ(1–40) and Aβ(1–42) fibrils, which mimic different amyloid strains. By taking advantage of the structurally homogeneous fibril of Aβ(1–42), which is equivalent to a pure Aβ(1–42) amyloid strain, we analyzed the propagation of amyloid formation from a seed Aβ(1–42) fibril to an Aβ(1–40) fibril by using thioflavin T (ThT) fluorescence, which is an indicator of amyloid-fibril formation. Incubation-time dependence of ThT fluorescence for Aβ(1–40) monomer based on ssNMR (Fig. 4a) showed that fibril formation for a control sample containing only Aβ(1–40) monomer required a lag time of 13.0 ± 0.1 h until the ThT fluorescence started to increase. This is explained by a multistep misfolding mechanism in which monomeric Aβ requires time for conversion to fibril form via oligomeric intermediate states. We observed substantially faster fibril growth for another control experiment in which the Aβ(1–40) monomer sample was incubated with seed Aβ(1–40) fibril as a seed. The lag time became nearly zero when we added Aβ(1–40) fibril as a seed. This is typically interpreted as evidence that monomers are directly converted to the fibril at the terminus of the seed fibril by using the seed fibril as a template. Of particular interest is that when we added the Aβ(1–42) fibril (G3 incubated for 3 d) as seed to an Aβ(1–40) monomer solution (Fig. 4b), we found that the lag time (12.8 ± 0.2 h) showed nearly no deviation from that for the control without any seeds. Our preliminary analysis showed that 2D 13C ssNMR spectra of Aβ(1–40) fibril samples prepared with or without Aβ(1–42) seed fibrils displayed few differences (data not shown). These results suggested that the fibril structure of Aβ(1–40) is not replicated from the cross-seeded Aβ(1–42) fibrils. Therefore, despite the high sequence similarity, monomeric Aβ(1–40) is incompatible with the distinct tertiary fold of the Aβ(1–42) fibril.

DISCUSSION

In this work, we have demonstrated what is to our knowledge the first atomic structural model of Aβ(1–42) fibril based on ssNMR data for structurally homogeneous samples, which have been hitherto unavailable. Despite the moderate resolution, the structure displays some remarkable features, which are summarized below along with their biological significance. First, the Aβ(1–42) fibril structural model elucidated by this work shows a unique triple-β motif, which is made of three β-sheets encompassing residues 12–18 (β1), 24–33 (β2) and 36–40 (β3). The suggested structure is distinct from a β-loop-β motif, which commonly characterizes the reported high-resolution structural models of in vitro Aβ(1–40) fibrils. This structure is also notably different from the recently reported structure of a brain seed Aβ(1–40) fibril, which largely retains a U-shaped topology of the β-arch motif with an Asp23-Lys28 salt bridge but involves larger non-β regions at residues 25–33 and 37–40 (ref. 4). Our results clearly show that, despite the minimal sequence difference, Aβ(1–42) misfolds into fibrils with a markedly different tertiary fold from that observed for Aβ(1–40) fibrils in past studies (Supplementary Fig. 4). The structure of the Aβ(1–42)-specific amyloid fibril with a unique tertiary fold provides a new perspective in AD research because fibrils of Aβ(1–40) and Aβ(1–42) have often been considered to be very similar. Second, we identified a salt bridge between the Lys28 side chain and the Ala42 N terminus in the Aβ(1–42)–fibril structure. Major differences in the stabilizing interactions between Aβ(1–42) and Aβ(1–40) fibrils explain why Aβ(1–42) can misfold into fibrils in a distinct pathway from that of Aβ(1–40) and offer a mechanistic clue to early-stage misfolding of Aβ isoforms. Third, the obtained structural features explain Aβ(1–42)-selective misfolding at an early AD stage and the lack of cross-propagation of Aβ(1–40) fibrils from Aβ(1–42) fibrils. Although recent developments have made it possible to delineate the structures of Aβ(1–40) fibrils seeded from the brains of patients with AD, no structural details have been provided even for synthetic Aβ(1–42) fibrils. This work suggests that cross-propagation barriers are probably caused by major tertiary-structural differences between the Aβ(1–40) and Aβ(1–42) fibrils and the
structural incompatibility of monomeric Aβ(1–40) and the Aβ(1–42) fibril, the latter of which uses Ala42 as a stabilizing salt-bridge contact. Such cross-propagation behavior between slightly different amyloid proteins is considered to be critical in propagation of prions across different mammalian species. Indeed, recent studies have shown that inoculation of synthetic Aβ(1–42) fibrils in mouse models prompted formation of plaque-like aggregates that were primarily composed of Aβ(1–42) without involving Aβ(1–40) as a major species. The present study has provided a stimulating initial example that explains how a tertiary fold of an amyloid fibril can be used as a self-recognition machinery and pose a structural barrier between amyloid or prion proteins even among those having high sequence similarity. Finally, Aβ is known to form various polymorphs, as indicated in the present and previous studies. Indeed, some of the side chain contacts, such as Phe19-Leu34, which were indicated in the previous ssNMR studies of Aβ(1–42) fibrils, are missing in the present Aβ(1–42)-fibril structures. Thus, this study represents only the first step toward revealing previously unknown structural details and structural variations of Aβ(1–42) fibrils, which are likely to be more relevant to the pathology of AD than well-studied Aβ(1–40) fibrils.

In conclusion, the previously undescribed structural and kinetic features of Aβ(1–42) fibrils in the present work offer a new perspective of how tertiary folds of amyloid fibrils critically influence amyloid propagation in AD and possibly in other neurodegenerative diseases. They also suggest that drugs designed to optimally obstruct the Aβ(1–40) β-arch motif may not work as well against AD, which can be caused by the more toxic Aβ(1–42) fibrils with the triple-β-motif discovered here.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. Coordinates have been deposited in the Protein Data Bank under accession code 2MXU.

Y.I. contributed to structural modeling and design. Y.X., D.M., B.M., R.N. and Y.I. wrote the paper. All authors were involved in the editing of the manuscript.

COMPETING FINANCIAL INTERESTS

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ONLINE METHODS

Sample preparation. Aβ(1–42) peptide (sequence DAEFR-HDSGY-EVHHQ-KLVFF-AEDVG-NKGAIGLM-VGGVY-1A) was chemically synthesized by an Applied Biosystems (ABI) model 433A automated peptide synthesizer (Life Technologies) with Fmoc-protected 13C- and 15N-labeled amino acids (Sigma Aldrich and Cambridge Isotope Laboratories) at selected sites13 and was purified by reversed-phase HPLC (Shimadzu Scientific Instruments), with an Agilent ZORBAX 300 Extend-C18 column. Fmoc protection of the labeled amino acids (Sigma-Aldrich and Cambridge Isotope Laboratories) was performed at the UIC Research Resource Center (RRC). Purity of the Aβ samples was determined to be approximately 85% and 95% before and after the HPLC purification, respectively, on the basis of mass analyses with an ABI 4700 MALDI TOF/TOF mass spectrometer at the UIC RRC. The lyophilized peptide after HPLC purification was weighed and then completely dissolved at 2 mg/mL in an aqueous solution containing 30% acetonitrile (Fisher Scientific) and 0.1% trifluoroacetic acid (TFA; American Bioanalytical) at 4 °C; the solution was subsequently lyophilized again. The lyophilized peptides were stored with drying reagents in a freezer at −20 °C. Before each incubation, the peptide was warmed to room temperature and dissolved in hexafluoroisopropanol (HFIP) (Sigma-Aldrich) at 4 °C; the solution was subsequently lyophilized.

The HFIP-treated peptide was first dissolved in a 10 mM NaOH solution (Fisher Scientific) to 0.6 mM, and then the Aβ solution was diluted to 60 μM at pH 7.4 with a 10 mM phosphate buffer. The fresh Aβ(1–42) peptide solution was filtered by centrifugation with a 50-kDa molecular-mass-cutoff filter (EMD Millipore Amicon Ultra-15 filter with regenerated cellulose membrane) at 4.8 × 10^5 g for 3 min in order to remove any undissolved peptide or preformed aggregates. The final Aβ monomer concentration was typically ~50 μM. It was confirmed by TEM analysis and ThT assay that no aggregated Aβ remains in the solution at the beginning of the incubation. The peptide solution was agitated by a continuous slow rotation at room temperature for 3–4 d. The generation 1 (G1) fibril sample was sonicated in an ice-water bath for 2 min and then was seeded (5% [w/v]) to a newly prepared Aβ(1–42) solution that was dissolved and filtered as described above. The seeded solution (G2) was incubated for 3–4 d. Subsequently, Aβ(1–42) solution in the generation n + 1 (Gn+1), sample was seeded with 5% seed fibrils from generation n (Gn) and incubated for 3–4 d. The fibril morphology was monitored by TEM and STEM. As a result of optimization to achieve both improved structural homogeneity and experimental efficiency, 13C- and 15N-labeled Aβ fibril samples were typically harvested after incubation at G4 or at a later generation for 1 d to 1 week. The fibril samples were pelleted by centrifugation at 9,000g for 45 min at 24 °C and were subsequently lyophilized after removal of the supernatant. The lyophilized fibril samples (5–10 mg) were packed into 2.5-mm ssNMR MAS rotors (10 R of 20 kHz. During the initial CP from 1H to 13C, the 13C radio frequency (RF) amplitude was swept from 49 to 66 kHz at the average of 57.5 kHz, following a tangential shape, while the 1H RF amplitude was kept constant at 57.5 + 0.7 kHz, where ν1 is the spinning speed. 13C signals were observed under 1H TIPPP decoupling at 90 kHz with phase alternation of ±12.5° unless otherwise mentioned. The same 1H TIPPP decoupling scheme was also used during the 15N-13C and 13C-13C dephasing and mixing periods. Recycle delays were 2–3 s unless otherwise specified. All assignments are listed in Supplementary Table 1. All the 1D and 2D data were processed by Bruker Topspin and NMRPipe52, respectively.

For the 2D 13C-15N correlation data in Figure 1c.e.g. a pulse sequence with a 50-ms DARR mixing43 was used. During the 13C-13C dephasing period, a 1H RF field was applied with a constant strength matched to ν1 at 20 kHz. A total of 130 complex t1 points were recorded with a t1 increment of 50 μs. For each t1 point, 64–144 scans were accumulated with an acquisition period of 10.29 ms. The obtained NMR data were processed by NMRPipe52. The data were apodized with a Lorentz-to-Gauss window function with an inverse exponential narrowing (IEN) of 10 Hz and a Gaussian broadening (GB) of 130 Hz in the t1 and t2 domains, and with a Lorentz-to-Gauss window function with IEN of 50 Hz and GB of 100 Hz in the t1 domain. The overall experimental time was 12–24 h.

The for the long-range 2D 13C-15N correlation data in Figure 2a–c and Supplementary Figure 2, the same pulse sequence was used at a varied spinning speed of 12–14.5 kHz with a 200-ms DARR mixing, where a 1H RF field was matched to ν1. A total of 120 complex t1 points were recorded with a t1 increment of 50 μs. For each t1 point, 64–144 scans were accumulated with an acquisition period of 10.29 ms. The data were apodized with a Lorentz-to-Gauss window function with IEN of 80 Hz and GB of 150 Hz in the t1 and t2 domains. The overall experimental time was 24–48 h. The contour levels in Figure 2 for 200-ms mixing were set to 11% (a), 10% (b), and 12% (c) of the diagonal signals of 13Cα of Ala21 (a) or Ala30 (b) for 50-ms mixing, they were set to 5% (g, f, 7% (a), 10% (b), and 10% of the diagonal signals of 13Cα of Ala21 (a) or Ala30 (b).

To collect the 2D 13C-15N correlation data in Figure 1b,d.f. we monitored 13N chemical-shift evolution during the t1 period and detected 13C signals after CP from 13N to 13C spins at ν1 of 20 kHz. During the initial CP from 13N to 13C spins in a period of 1.5 ms, the 13N RF field strength was swept from 30 to 40 kHz while the 1H RF strength was kept constant at 55 kHz. During the 13N-13C CP period of 2.5 ms, an 13N RF-field strength was fixed at 15 kHz.
while a $^{13}$C RF strength was swept from 30 kHz to 40 kHz with adiabatic CP. A total of 80 complex $t_1$ points were recorded with a $t_1$ increment of 100 µs. For each $t_1$ point, 64–144 scans were accumulated with an acquisition period of 5.17 ms. The data were apodized with a Lorentz-Gauss window function with IEN of 20 Hz and GB of 100 Hz in the $t_1$ and $t_2$ time domains. The overall experimental time was 24–48 h each.

The frequency-selective $^{13}$C-$^{15}$N REDOR experiments in Figure 2d were carried out at $v_0$ of 8,000 Hz ± 3 Hz with the pulse sequence in ref. 45, with minor modifications. A $^{15}$N π-pulse train with a XY-16 phase cycle53 was rotor-synchronously applied for a REDOR mixing with two $^{15}$N π-pulses in each rotor cycle; the $^{15}$N π-pulse width was 16.66 µs. For selective $^{13}$C-$^{15}$N dipolar dephasing, selective inversion Gaussian pulses for $^{13}$CO$_2$ and $^{15}$NH$_3$ groups centered in the 1,500-µs period were sandwiched by the two identical REDOR mixing sequences. The total time of the REDOR mixing was up to 18 ms. The pulse widths of the Gaussian π-pulses were 1,250 µs and 500 µs for $^{13}$C and $^{15}$N, respectively. $^{1}$H TIPPM decoupling with an RF field strength of 90–100 kHz was applied during the acquisition, REDOR mixing, and selective pulse periods. The details of $^{13}$CO-$^{15}$CO interstrand distance measurements by ssNMR are included in the Supplementary Note. Fitting of the NMR data for the $^{13}$C-$^{15}$N or $^{13}$CO-$^{15}$CO distance measurements to the best-fit simulated curve was confirmed by a $\chi^2$ analysis. The ranges of the uncertainty in the site-specific distance measurements were found to be within ±0.1 Å at the 90% confidence level.

Structure calculation and analysis. In our preliminary MD-assisted structural-modeling efforts, the peptide dihedral angles were systematically changed to minimize the deviation of experimental chemical shifts and those calculated from SHIFTX2 (ref. 44). The stable structural models that meet NMR constraints have been calculated with the CYANA program by adopting a similar approach used for the Het-s prion fibril51. The initial model of a 12-mer for residues 11–42 of Aβ-$\beta$-protein (Aβ) was built with the CYANA 2.1 program by adopting a similar approach used for the Het-s prion fibril. The initial model of a 12-mer for residues 11–42 of Aβ-$\beta$-protein (Aβ) was built with the CYANA 2.1 program by adopting a similar approach used for the Het-s prion fibril. The initial model of a 12-mer for residues 11–42 of Aβ-$\beta$-protein (Aβ) was built with the CYANA 2.1 program by adopting a similar approach used for the Het-s prion fibril. The initial model of a 12-mer for residues 11–42 of Aβ-$\beta$-protein (Aβ) was built with the CYANA 2.1 program by adopting a similar approach used for the Het-s prion fibril. The initial model of a 12-mer for residues 11–42 of Aβ-$\beta$-protein (Aβ) was built with the CYANA 2.1 program by adopting a similar approach used for the Het-s prion fibril. The initial model of a 12-mer for residues 11–42 of Aβ-$\beta$-protein (Aβ) was built with the CYANA 2.1 program by adopting a similar approach used for the Het-s prion fibril. The initial model of a 12-mer for residues 11–42 of Aβ-$\beta$-protein (Aβ) was built with the CYANA 2.1 program by adopting a similar approach used for the Het-s prion fibril.