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Coexisting Order and Disorder Within a Common 40-Residue Amyloid- β Fibril Structure in Alzheimer's Disease Brain Tissue

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Abstract

Fibrils formed by 40- and 42-residue amyloid- β (A β 40 and A β 42) peptides exhibit molecular-level structural polymorphisms. A recent screen of fibrils derived from brain tissue of Alzheimer's disease patients revealed a single predominant A β 40 polymorph. We present solid state nuclear magnetic resonance (ssNMR) data that define its coexisting structurally ordered and disordered segments.

Graphical abstract

Only about half of the 40-residue amyloid- β sequence is structurally ordered in a common fibril structure from Alzheimer's disease brain.

Molecular structures within amyloid fibrils formed by amyloid- β peptides *in vitro* are not determined uniquely by their amino acid sequences. Instead A β 40 and A β 42 fibril structures are sensitive to subtle variations in growth conditions in *de novo* preparations.^{1–3} Multiple distinct structures can also develop simultaneously under a single set of conditions.^{4–7} Moreover, structural differences are self-propagating in seeded fibril growth, *i.e.*, when new fibrils grow from fragments of pre-existing fibrils.^{1, 8} This phenomenon of molecular-level structural polymorphism *in vitro* raises the following questions^{9–12}: Are the A β 40 and A β 42 fibrils that develop in brain tissue of Alzheimer's disease (AD) patients similarly

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Conflicts of interest

There are no conflicts to declare.

polymorphic? Are molecular structural variations in A β 40 and A β 42 fibrils correlated with variations in clinical and pathological characteristics of AD?

To address these questions, our lab developed methods for seeded growth of A β 40 and A β 42 fibrils that can be studied by solid state nuclear magnetic resonance (ssNMR) and transmission electron microscopy (TEM), using amyloid-containing cortical tissue of deceased AD patients as the source of seeds.^{13–15} Experiments by Lu *et al.*¹⁵ found different predominant structures in A β 40 fibrils derived from cortical tissue of two AD patients (patients 1 and 2), indicated by different fibril morphologies in TEM images and different crosspeak signal patterns in two-dimensional (2D) ssNMR spectra. The same predominant polymorph was found throughout the cerebral cortex of each patient. Interestingly, patient 1 had an atypical clinical history, while patient 2 had typical AD symptoms and history. Qiang *et al.* subsequently examined 37 cortical tissue samples from 18 patients, recording TEM images and 2D ssNMR spectra of A β 40 and A β 42 fibrils derived from these tissue samples by seeded growth.¹⁴ For A β 40, a single predominant fibril polymorph was found in most samples from patients from two AD subtypes, namely typical long-duration AD and the posterior cortical atrophy variant of AD. Based on its "fingerprint" in 2D ssNMR, this A β 40 polymorph was the same as the predominant polymorph from patient 2 of Lu *et al.* Thus, we have evidence that most AD patients develop a specific A β 40 fibril polymorph in their cerebral cortex. However, the detailed molecular structure of this polymorph is not yet known.

As a first step towards the development of a full molecular structural model for this predominant A β 40 fibril polymorph in AD, we prepared A β 40 fibrils from seven synthetic peptides with ¹⁵N, ¹³C-labeled residues at the specific positions shown in Fig. 1A. "First-generation" fibrils, prepared by seeding A β 40-sl1 with cortical tissue extracts, were used in ssNMR measurements by Lu *et al.* and Qiang *et al.*^{14, 15} We used one of these tissue-seeded A β 40-sl1 samples (identified as PCA3f in Table 1 of Qiang *et al.* and shown in Fig. 1B) as the source of seeds for "second-generation" fibrils, which were prepared with the remaining six labeling patterns (see Materials and Methods in ESI for experimental conditions). Second-generation A β 40-sl6 fibrils were also used as the source of seeds for "third-generation" fibrils. Second- and third-generation fibrils appear morphologically homogeneous in TEM images with negative staining, with approximately 90% of visible fibrils showing periodic modulations in apparent width with periods approximately 130 $\pm$ 30 nm.

As shown in Fig. 1A, first-generation fibril samples contained a large proportion of extraneous material from the cortical tissue. This proportion was greatly reduced in subsequent generations, becoming nearly absent from TEM images. It is conceivable that the A β 40 fibril structure could be stabilized or otherwise affected by the presence of certain chemical compounds from the tissue. 2D ¹³C-¹³C ssNMR spectra of second-generation A β 40-sl2 fibrils and first-generation A β 40-sl1 fibrils show good agreement of crosspeak signal patterns (see Fig. S1 of ESI), as do 2D ¹³C-¹³C ssNMR spectra of third-generation and second-generation A β 40-sl6 fibrils (see Fig. S2 of ESI). These results indicate faithful propagation of the molecular structure despite the progressive dilution of extraneous material in successive generations.

Figs. 2 and 3 show 2D ^{13}C - ^{13}C and ^{15}N - ^{13}C ssNMR spectra of the second-generation fibrils (see Materials and Methods of ESI for measurement conditions). Crosspeak signals were readily assigned, based on the known labeling patterns and residue-specific chemical shift ranges for labeled sites.¹⁶ When the same residue was labeled in more than one sample (*e.g.*, I31 in A β 40-s12 and A β 40-s13), good agreement of chemical shifts and ssNMR line widths was observed. (Apparent differences in line widths for the same sites in the contour plots in Fig. 2 are due primarily to differences in signal-to-noise for 2D spectra of different samples.)

Importantly, signal intensities and line widths for different residues varied significantly. While certain residues contributed relatively strong, sharp crosspeaks to the 2D spectra (residues 19, 21, 23–25, 27–35), crosspeaks from other residues were weak and broad (residues 2, 7, 9, 12, 17, 20, 22, 26, 38, 39) or undetectable (residues 8 and 10). We attribute these large variations in properties of ssNMR signals to a coexistence of structurally ordered and disordered segments of the A β 40 sequence within a single fibril. The morphological uniformity of the fibrils in our TEM images argues against an alternative interpretation that broad ssNMR signals arise from the presence of multiple distinct polymorphs in our samples, as does the reproducibility of ssNMR signals in multiple independent sample preparations.

In addition to measurements on samples with specific ^{15}N , ^{13}C -labeled residues, we performed three-dimensional (3D) ssNMR measurements on a uniformly ^{15}N , ^{13}C -labeled first-generation A β 40 fibril sample, derived from cortical tissue of patient 2 of Lu *et al.*¹⁵ Strong crosspeaks in 3D NCACX and 3D NCOCX spectra (see Fig. 4 and Fig. S3 of ESI) were observed for residues 18–25 and 28–37, consistent with assignments from specifically labeled samples and providing additional assignments for V18, V36, and G37. The final set of assignments, together with site-specific line widths and predictions of backbone torsion angles from the TALOS-N program¹⁷, are given in Table S1 of ESI.

In previous studies of A β 40 and A β 42 fibril structures by ssNMR and cryo-electron microscopy, certain polymorphs were found to be structurally ordered across the entire peptide sequence^{15, 18–20}, while other polymorphs were found to contain coexisting ordered and disordered segments.^{6, 8, 21–24} Naïvely, one might have expected that the predominant A β 40 fibril polymorph in AD brain tissue would be highly ordered, based on the ideas that the predominant polymorph might be an especially stable one and that a high degree of structural order goes hand-in-hand with high stability. The 2D and 3D ssNMR spectra discussed above clearly contradict this expectation, with only about half of the A β 40 sequence being highly ordered. One might then speculate that partial disorder facilitates the breakage, transport, and propagation of fibrils within brain tissue, so that the predominant fibril structures in AD brain tissue might be partially disordered. However, A β 40 fibrils derived from patient 1 of Lu *et al.* (which we now know to be an uncommon polymorph, based on the results of Qiang *et al.*¹⁴) were found to be highly ordered, exhibiting strong, sharp crosspeaks in 2D and 3D ssNMR spectra from all residues except H14 and E22.¹⁵ Thus, the relationship between structural order and relevance to AD is not yet understood.

Finally, we note that the predominant fibril morphology for our brain-derived A β 40 fibrils, shown in Figs. 1C, 1D, and 1E, is remarkably similar to the morphology of A β 40 fibrils

prepared *in vitro* by Paravastu *et al.*, which have a width modulation period of 120 ± 20 nm.⁸ Moreover, both types of fibrils have mass-per-length values of approximately 27 kDa/nm.^{8, 15} However, ssNMR chemical shifts are significantly different (see Fig. S4A of ESI). We therefore anticipate that the molecular structure of our brain-derived A β 40 fibrils resembles the structure of *in vitro* fibrils prepared by Paravastu *et al.*, but is not the same. In contrast, ssNMR chemical shift differences relative to brain-derived A β 40 fibrils from patient 1 of Lu *et al.*¹⁵ are somewhat larger (see Fig. S4B of ESI). Additional ssNMR data to provide experimental restraints on long-range inter-residue contacts will be required to enable development of a structural model for our brain-derived A β 40 fibrils.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Petkova AT, Leapman RD, Guo ZH, Yau WM, Mattson MP, Tycko R. *Science*. 2005; 307:262–265. [PubMed: 15653506]
2. Kodali R, Williams AD, Chemuru S, Wetzel R. *J. Mol. Biol.* 2010; 401:503–517. [PubMed: 20600131]
3. Qiang W, Yau WM, Tycko R. *J. Am. Chem. Soc.* 2011; 133:4018–4029. [PubMed: 21355554]
4. Goldsbury C, Frey P, Olivieri V, Aeby U, Muller SA. *J. Mol. Biol.* 2005; 352:282–298. [PubMed: 16095615]
5. Meinhardt J, Sachse C, Hortschansky P, Grigorieff N, Fandrich M. *J. Mol. Biol.* 2009; 386:869–877. [PubMed: 19038266]
6. Xiao YL, Ma BY, McElheny D, Parthasarathy S, Long F, Hoshi M, Nussinov R, Ishii Y. *Nat. Struct. Mol. Biol.* 2015; 22:499–505. [PubMed: 25938662]
7. Elkins MR, Wang T, Nick M, Jo H, Lemmin T, Prusiner SB, DeGrado WF, Stohr J, Hong M. *J. Am. Chem. Soc.* 2016; 138:9840–9852. [PubMed: 27414264]
8. Paravastu AK, Leapman RD, Yau WM, Tycko R. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:18349–18354. [PubMed: 19015532]
9. Cohen ML, Kim C, Haldiman T, ElHag M, Mehndiratta P, Pichet T, Lissemore F, Shea M, Cohen Y, Chen W, Blevins J, Appleby BS, Surewicz K, Surewicz WK, Sajatovic M, Tatsuoka C, Zhang SL, Mayo P, Butkiewicz M, Haines JL, Lerner AJ, Safar JG. *Brain*. 2015; 138:1009–1022. [PubMed: 25688081]
10. Condello C, Lemmin T, Stohr J, Nick M, Wu Y, Maxwell AM, Watts JC, Caro CD, Oehler A, Keene CD, Bird TD, van Duinen SG, Lannfelt L, Ingelsson M, Graff C, Giles K, DeGrado WF, Prusiner SB. *Proc. Natl. Acad. Sci. U. S. A.* 2018; 115:E782–E791. [PubMed: 29311311]
11. Rasmussen J, Mahler J, Beschorner N, Kaeser SA, Hasler LM, Baumann F, Nystrom S, Portelius E, Blennow K, Lashley T, Fox NC, Sepulveda-Falla D, Glatzel M, Oblak AL, Ghetti B, Nilsson KPR, Hammarstrom P, Staufenbiel M, Walker LC, Jucker M. *Proc. Natl. Acad. Sci. U. S. A.* 2017; 114:13018–13023. [PubMed: 29158413]
12. Lam B, Masellis M, Freedman M, Stuss DT, Black SE. *Alzheimers Res. Ther.* 2013; 5
13. Paravastu AK, Qahwash I, Leapman RD, Meredith SC, Tycko R. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106:7443–7448. [PubMed: 19376973]
14. Qiang W, Yau WM, Lu JX, Collinge J, Tycko R. *Nature*. 2017; 541:217–221. [PubMed: 28052060]

15. Lu JX, Qiang W, Yau WM, Schwieters CD, Meredith SC, Tycko R. *Cell*. 2013; 154:1257–1268. [PubMed: 24034249]
16. Wishart DS, Bigam CG, Holm A, Hodges RS, Sykes BD. *J. Biomol. NMR*. 1995; 5:67–81. [PubMed: 7881273]
17. Shen Y, Bax A. *J. Biomol. NMR*. 2013; 56:227–241. [PubMed: 23728592]
18. Bertini I, Gonnelli L, Luchinat C, Mao JF, Nesi A. *J. Am. Chem. Soc.* 2011; 133:16013–16022. [PubMed: 21882806]
19. Schutz AK, Vagt T, Huber M, Ovchinnikova OY, Cadalbert R, Wall J, Guntert P, Bockmann A, Glockshuber R, Meier BH. *Angew. Chem.-Int. Edit.* 2014; 53:1–6.
20. Gremer L, Scholzel D, Schenk C, Reinartz E, Labahn J, Ravelli RBG, Tusche M, Lopez-Iglesias C, Hoyer W, Heise H, Willbold D, Schroder GF. *Science*. 2017; 358:116+. [PubMed: 28882996]
21. Qiang W, Yau WM, Luo YQ, Mattson MP, Tycko R. *Proc. Natl. Acad. Sci. U. S. A.* 2012; 109:4443–4448. [PubMed: 22403062]
22. Scheidt HA, Morgado I, Rothmund S, Huster D. *J. Biol. Chem.* 2012; 287:2017–2021. [PubMed: 22130659]
23. Walti MA, Ravotti F, Arai H, Glabe CG, Wall JS, Bockmann A, Guntert P, Meier BH, Riek R. *Proc. Natl. Acad. Sci. U. S. A.* 2016; 113:E4976–E4984. [PubMed: 27469165]
24. Colvin MT, Silvers R, Ni QZ, Can TV, Sergeyev I, Rosay M, Donovan KJ, Michael B, Wall J, Linse S, Griffin RG. *J. Am. Chem. Soc.* 2016; 138:9663–9674. [PubMed: 27355699]

Fig. 1.

(A) Amino acid sequence of Aβ40 and positions of ^{15}N , ^{13}C -labeled residues in the seven selectively labeled samples. (B) Negatively-stained TEM image of first-generation brain-derived Aβ40-sl1 fibrils, used as the source of seeds for second-generation samples. Cyan arrows indicate collagen fibers. (C,D) TEM images of second-generation Aβ40-sl5 and Aβ40-sl4 fibrils. (E) TEM image of third-generation Aβ40-sl6 fibrils, grown from second-generation Aβ40-sl6 fibril seeds. Red arrows indicate the periodicity of apparent width modulation for the most common morphology.

Fig. 2. Two-dimensional ^{13}C - ^{13}C spectra of selectively-labeled, second-generation A β 40 fibrils, with color-coded resonance assignments. Broad crosspeaks are indicated by colored circles and ellipses. Contour levels increase by successive factors of 1.5.

Fig. 3. Two-dimensional ^{15}N - ^{13}C spectra of selectively-labeled, second-generation A β 40 fibrils, with color-coded resonance assignments. Contour levels increase by successive factors of 1.5.

Fig 4. Crosspeak heights in 3D ssNMR spectra of uniformly ^{15}N , ^{13}C -labeled first-generation A β 40 fibrils. Values are shown for N(i+1)/CO(i)/C α (i) crosspeaks in the 3D NCOCX spectrum and N(i)/C α (i)/C β (i) crosspeaks in the 3D NCACX spectrum, where i is the residue number. Values are shown only for residues with detectable, resolved crosspeaks.