Amyloid β Peptides

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An Asymmetric Dimer as the Basic Subunit in Alzheimer's Disease Amyloid β Fibrils **

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Aggregation of monomeric amyloid β peptides (Aβ) into soluble oligomers and insoluble fibrils is one of the major pathological hallmarks of Alzheimer's disease (AD).[1] In the past few years, magic angle spinning (MAS) solid-state NMR spectroscopy has enabled considerable progress in the structural characterization of amyloid aggregates, and a number of structural models have been suggested.[2] Amyloid samples fibrilize in a large variety of morphologies.[3] Differences in fibril morphology have been attributed to differences in peptide conformation.[4] These variations in the molecular structures of Aβ fibrils might be responsible for the variations in the toxicity and deposition patterns of fibrils in AD.[5] Whereas previous NMR studies implied that in all cases a U-shaped peptide fold forms the fundamental structural unit of the fibril,[2a,b,6] cryo electron microscopy studies suggested that different Aβ fibril morphologies can be constructed from a dimeric arrangement of two conformationally distinct peptide molecules in each molecular layer of the protofilament.[7] To date, however, there are no solid-state NMR data that supports the existence of such a structural subunit in Aβ fibrils.

Herein, we employed a seeding protocol to obtain a homogeneous fibril sample in which only one particular polymorph is present. In addition to MAS solid-state NMR spectroscopy, electron microscopy (EM) was employed to characterize and quantify different polymorphs of Aβ fibrils.[7a,8] Quantification was based on the amyloid fibril width and crossover distance, both of which were obtained in a straight forward way from negative-stain EM images.[8a] EM images and the statistical analysis of the fibrils that were employed in the MAS solid-state NMR studies are represented in Figure 1. We found that one polymorph was predominant and was populated with an average crossover distance of 80 nm and a width of approximately 14 nm. This polymorph (polymorph I) made up 76% of the analyzed fibrils. A second polymorph (polymorph II) made up 10% of the analyzed fibrils. Relative to polymorph I, this second polymorph had a similar crossover distance (80 nm), but exhibited a broader width (18 nm). The remaining polymorphs accounted for 14% of the analyzed fibrils. The

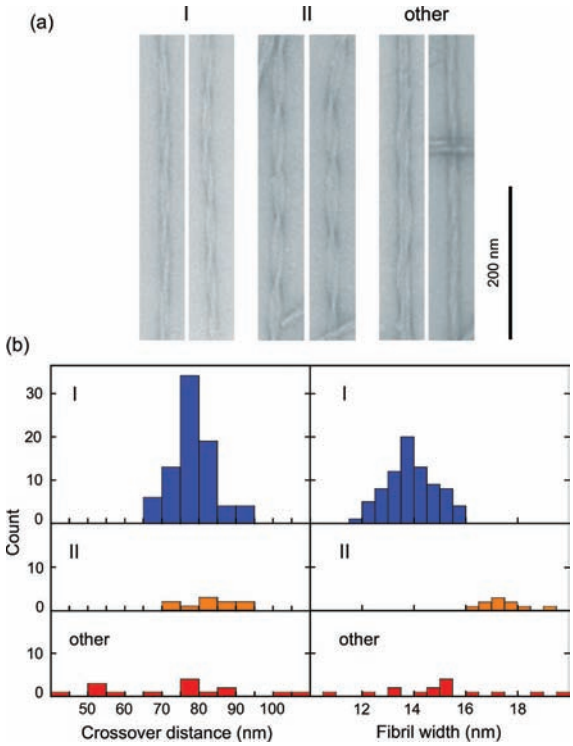

Figure 1. Analysis of fibril morphology. a) Representative negative-stain electron microscopy images of the two dominating fibril polymorphs (I and II) and other polymorphs. b) Statistical analysis of Aβ fibrils employed in MAS solid-state NMR investigations. Polymorph I accounts for 76% of the fibrils present. Polymorph II and all remaining fibril polymorphs account for 10% and 14%, respectively, of the fibrils present.

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dominant polymorph, polymorph I, showed significant similarities to Aβ(1–40) fibril polymorphs 3/4, which were previously analyzed using cryo-EM structural reconstruction by Meinhardt et al.[8a]

To estimate the quality of the solid-state NMR sample, a 2D ^{13}C–^{13}C proton-driven spin diffusion (PDSD) correlation spectrum was recorded (Figure 2, mixing time = 50 ms). Typical line widths of resolved ^{13}C resonances were on the order of 100–130 Hz (see the Supporting Information, Figure S1). Similarly, a 2D ^{13}C–^{15}N correlation spectrum had excellent resolution (see the Supporting Information, Figure S2). The preparation of amyloid fibrils was highly reproducible and different preparations yielded very similar spectra (see the Supporting Information, Figure S3). Broad resonances in the spectra associated with previous solid-state NMR studies on Aβ fibrils were attributed to structural heterogeneity. In fact, subtle differences in the incubation conditions resulted in fibrils with different morphologies.[2b,5a,9] The high spectral resolution observed in this study might in part be due to the employment of the seeding protocol that favors the population of a particular polymorph. Furthermore, we employed a recombinantly expressed Aβ,[10] which was previously shown to have aggregation properties that were slightly different to those of synthetic peptides.[11] Differences in aggregation properties were attributed to amino acid racemization, which may have occurred during peptide synthesis. Similar to our results, Bertini et al. obtained high resolution MAS solid-state NMR spectra when the associated fibrils were prepared from a biosynthetic peptide.[2c] Apparently, the type of morphology and quaternary assembly of fibrils depends on the preparation conditions. When we compared the chemical shifts reported herein with those associated with previous reports[2a,b,5a] (see the Supporting Information, Figure S6), we found that the conformer associated with polymorph I was most similar to that of the two-fold symmetric fibrils produced under agitation.[5a] However, the differences between them were still very large, thus suggesting that the conformation of our fibrils is distinct from that of fibrils reported by Tycko and co-workers.[5a]

^{13}C nuclei were assigned using 3D NCACX and NCOCX experiments. The assigned resonances correspond to residues 12–40 of the Aβ peptide sequence of one conformer (conformer I; Figure 2, shown in black) and residues 21–38 of a second conformer (conformer II; Figure 2, shown in red).

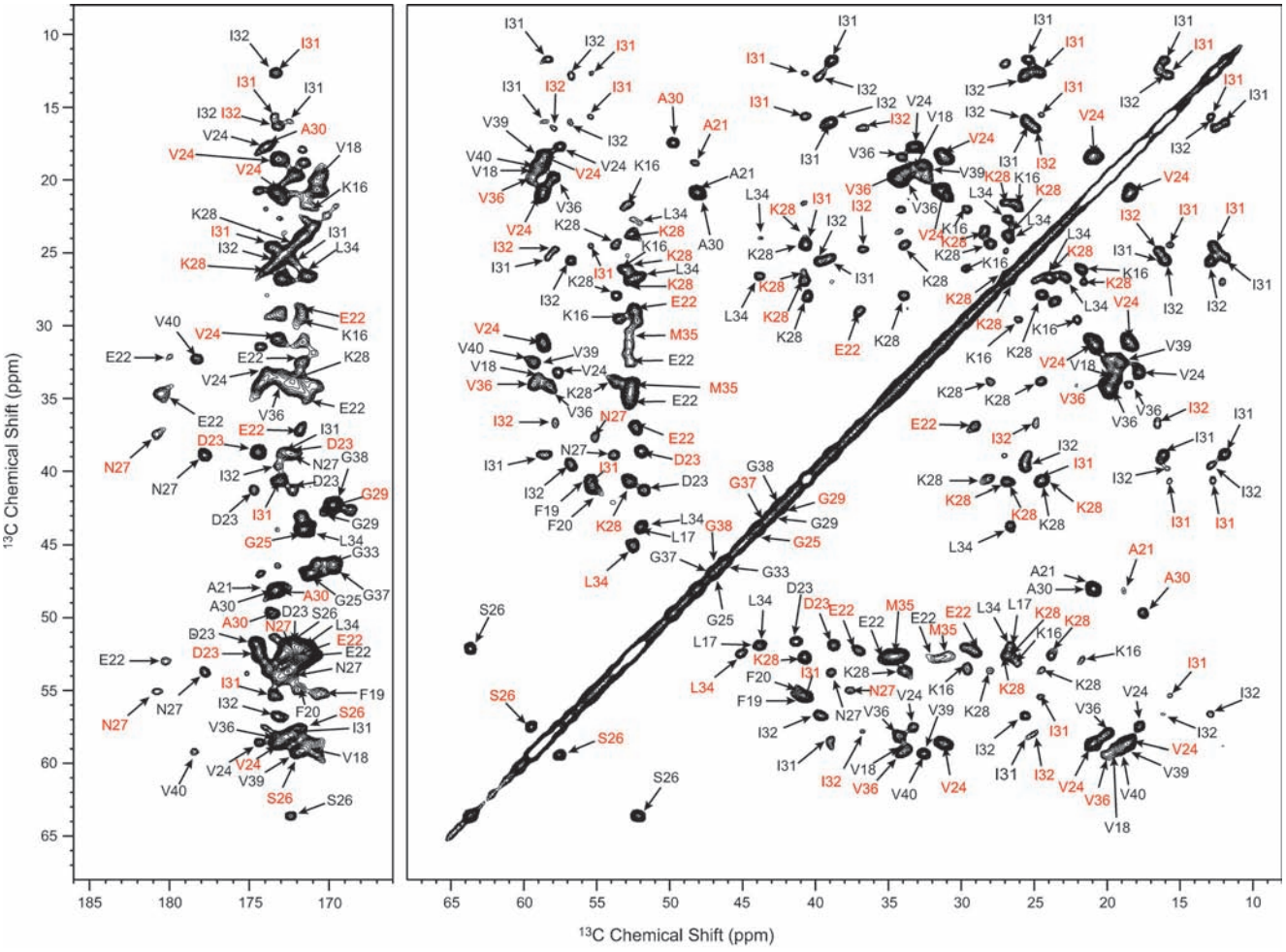

Figure 2. 2D ^{13}C–^{13}C PDSD correlation spectrum of Aβ fibrils. The ^{13}C nuclei assignments were obtained by interpretation of 3D NCACX and NCOCX spectra. Two independent sets of resonances are observed, corresponding to residues 12–40 and 21–38 of the Aβ sequence. Resonances originating from conformer I and conformer II are labeled in black and red, respectively.

The chemical shifts are tabulated in the Supporting Information, Table S1. Residues 1–11 could not be observed by using either CP (Cross Polarization) or INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) NMR experiments. In agreement with previous solid-state NMR experiments, this region of the peptide thus seems to be disordered in the fibrillar state.[6,12] 2D strip plots, which were extracted from 3D NCACX and NCOCX NMR data and focus on the region around residue S26, unambiguously demonstrate that the fibril sample employed in this study contains two conformers (see the Supporting Information, Figure S4).

To obtain site-specific secondary structure information, we analyzed the secondary chemical shifts,[13] that is, the differences in chemical shifts between our assigned resonances and the corresponding resonances found in random coil peptides. The results for fibril conformer I and II are shown in Figure 3. Conformer I shows a pattern indicating a β-sheet/

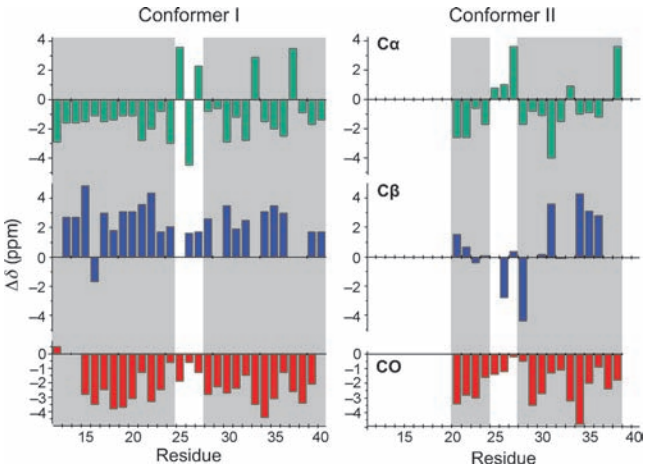

Figure 3. Secondary ^{13}C chemical shift analysis for conformer I and II observed in Aβ fibrils. Δδ indicates the difference between the herein observed and random coil ^{13}C chemical shift values.

turn/β-sheet secondary structure, whereas conformer II shows a shorter structured sequence with a pattern indicating only a β-sheet secondary structure. This result is consistent with previous studies in which a pattern similar to that of conformer I is found for Aβ(1–40) as well as Aβ(1–42) fibrils.[6,12] Analysis of the chemical shifts using the program Delta-2D gave a very similar result (see the Supporting Information, Figure S5).[14] For conformer I, an increased propensity for a PPII (polyproline type II) helix is predicted for the loop region around residue G25, as well as for the region toward the edges of both β sheets. Similarly, in conformer II, the extended β sheet is flanked by residues that have an increased propensity to adopt a PPII helix. PPII motifs are not exclusively found in proline containing peptides and presumably also play an important role in defining the structures of amyloidogenic species.[15]

In principle, there are two possibilities that can account for the two sets of resonances. In the first possibility, Aβ folds into different polymorphs, which are approximately equally populated. This possibility seems very unlikely as fibril seeding would be expected to enrich the sample with a single polymorph. Furthermore, the different polymorphs would then need to have the same nucleation probability, the same fibril stability and the same fibrilization kinetics. Alternatively, in a second possibility, the amyloid fibril unit cell might contain an asymmetric Aβ dimer, yielding two sets of resonances of equal intensity. The EM analysis shows that one polymorph predominates and therefore suggests the presence of a unique conformer in each molecular layer of the protofilament.[4] Minor conformers that are populated to only a small percentage will not be observable by solid-state NMR spectroscopy because the NMR signal intensity is proportional to the number of involved molecules. The high reproducibility in the preparation of the fibrils and the observation of a 1:1 ratio in the cross peak intensities associated with the two conformers (see the Supporting Information, Figure S3) strongly supports the presence of an asymmetric dimer in the fibril unit cell.

Our results herein lend support to the idea that Aβ fibrils can consist of an asymmetric peptide dimer as their fundamental structural unit. Recently, distinct polymorphs of Sup35 and IAPP fibrils have been reported.[16] Other amyloidogenic peptides that crystalize as asymmetric dimers have also been reported.[17] In these reports, the different conformers have been associated with different steric zipper interfaces that enable their assembly into composite β-sheet-rich building blocks that make up the protofilaments.[18] It remains to be determined how the two Aß peptides in our preparation are arranged with respect to one another. A more detailed analysis of the conformation of the two Aß isoforms, or a characterization of their relative arrangement will require samples that are grown on media containing selectively ^{13}C-enriched nutrients,[19] or by using deuterated substrates.[20] Work along this direction is currently in progress.

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