



The Molecular Structure of Alzheimer β-Amyloid Fibrils Formed in the Presence of Phospholipid Vesicles**

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Abstract: β-amyloid (Aβ) fibrils are the major species involved in Alzheimer's disease (AD). An atomic-resolution molecular structure of Aβ40 fibrils formed in the presence of lipid vesicles was obtained by using magic angle spinning (MAS) solid-state NMR spectroscopy. The fibril structures formed in the presence of the lipid vesicles are remarkably different from those formed in solution. These results provide insights into the molecular mechanism of Aβ aggregation in the presence of lipid vesicles.

Alzheimer's disease (AD) is characterized by the presence of neuritic plaques and neurofibrillary tangles in the brain. β-amyloid peptide (Aβ) fibrils are the major components of amyloid plaques. A variety of morphologies of Aβ fibrils have been detected by electron microscopy (EM). Previous studies have demonstrated that the toxicity of Aβ fibrils is associated with their morphology and molecular structure.^[1] Studying fibril structures of diverse morphologies and under different formation conditions is thus important to gain structural insights into AD. Structural models of the Aβ fibrils formed in solution with various morphologies have been revealed by solid-state NMR and EM.^[2–5] However, under biological conditions, the interactions between Aβ and membranes play an important role in the aggregation of Aβ^[6–9] because Aβ is generated from the cleaving of the amyloid precursor protein (APP) and is likely bound to the neuronal membrane surface. Moreover, Aβ aggregates probably exert their toxic action through distortion of the neuronal membrane or alteration of the permeability of the neuronal membrane through the formation of ion channels.^[10,11] In spite of the importance, in terms of neuropathology, of Aβ fibrillization in the presence of the membrane, atomic-resolution structural information is lacking.

Solid-state magic angle spinning (MAS) NMR spectroscopy is a powerful tool for providing atomic-resolution structures for insoluble and uncrystallizable amyloid

fibrils.^[12–19] In the past 15 years, MAS NMR has led to encouraging structural insights into the mechanism of Aβ fibrillization in Alzheimer's disease.^[3,5,20–25] However, the broad linewidths typically associated with MAS NMR signals of the fibrils hamper structural characterization by using [¹³C,¹⁵N]-labeled samples and multidimensional MAS NMR experiments. Generally, a number of fibril samples of chemically synthesized Aβ peptides with site-specific labeling are used in MAS NMR experiments. In this study, highly ordered Aβ40 fibril samples were obtained through the fibrillization of recombinantly expressed Aβ40 peptides. Such highly ordered [¹³C,¹⁵N]-labeled fibril samples significantly improve MAS NMR spectral resolution, thus permitting detailed

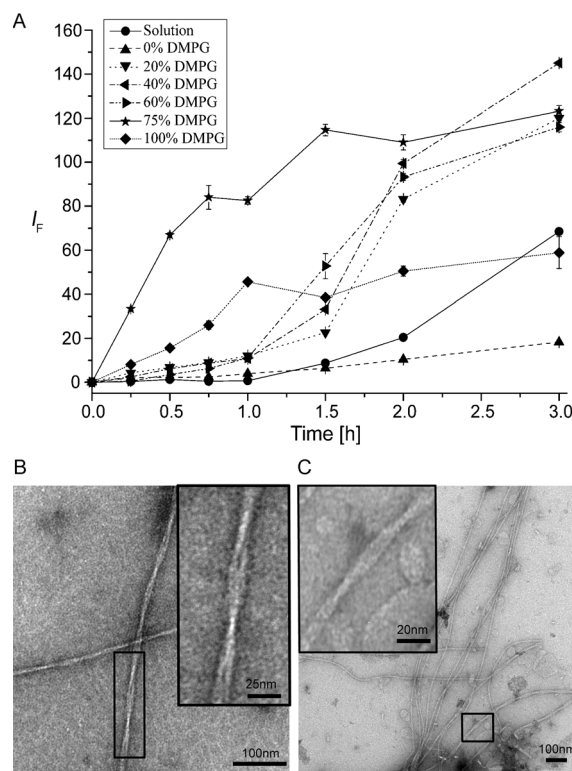


Figure 1. A) Thioflavin T (ThT) fluorescence measured during the aggregation of 50 μM Aβ40 peptide in solution and in the presence of the DMPG/DMPC vesicles with different percentages of the anionic lipid DMPG. TEM images are shown for 50 μM Aβ40 peptide after incubation in solution for 96 h (B) or in the presence of DMPG/DMPC (1:3 molar ratio) vesicles for 2 h (C). Fluorescence intensities were measured at λ_{ex} = 440 nm, λ_{em} = 485 nm. The error bars in (A) are standard deviations of three measurements. Points with no visible error bars represent measurements with tiny variance. DMPG = 1,2-dimyristoyl-sn-glycero-3-phosphocholine, DMPC = 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol.

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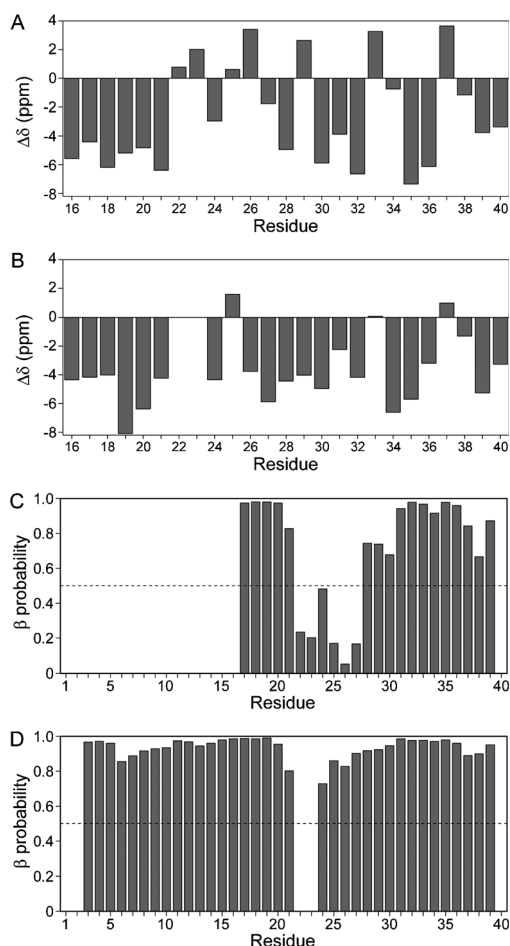


Figure 3. Secondary chemical shifts ($\Delta\delta$) of lipid fibrils (A) and solution fibrils (B). $\Delta\delta$ values were calculated according to Equation 1 in Ref. [27]. The probability of β -strand secondary structure for A β 40 lipid fibrils (C) and solution fibrils (D) predicted by TALOS+^[28] by using MAS NMR chemical-shift assignments. The dashed line indicates a probability of 50%.

restraints for the structure calculations and not all of the torsion angles of glycine residues were included. Therefore, the twists in the β 2 strand in the calculated structure are not derived from TALOS torsion angles of the glycine residues. The twists in the β 2 strand result in nonparallel contact of the β 1 and β 2 strands (Figure 4). This nonparallel contact pattern of the β 1 and β 2 strands has also been reported in the fibrils formed in brain tissues.^[5] However, the details of the contacts are different. By contrast, non-kinked and parallel β 1-strand-turn- β 2-strand structures of solution fibrils were reported by Bertini^[4] and Tycko et al.^[2,3,30] Our MAS NMR data demonstrate that our solution fibrils exhibit similar structure to those reported by Bertini and co-workers.^[4] The similarities of the structures are supported by the surprisingly similar 2D NCA and ^{13}C - ^{13}C correlation spectra (Figure S6) and the similar secondary chemical shifts (Figure S3B) of these two sets of solution fibrils. Furthermore, we have observed a number of distance restraints for defining β 1- β 2 interstrand contacts such as F19-L34 (Figure S7) and F19-V36 (Figure S8). These restraints are also reported for Bertini's solution fibrils.

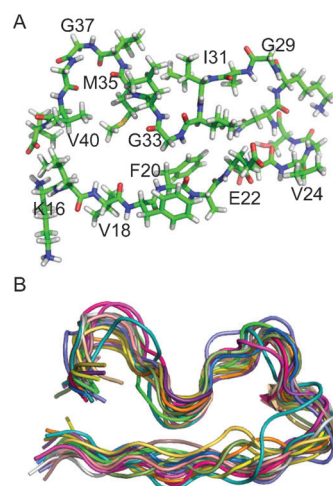


Figure 4. A Structural model of the monomeric unit of A β lipid fibrils. A) The structure with the lowest experimental restraint energy in the Xplor-NIH calculations. B) Superposition of the 20 lowest-energy structures (out of 400 calculated structures) with an average backbone RMSD of 2.15 Å.

To reveal the organization of the monomeric unit of the lipid fibrils, we assigned intermolecular restraints by comparing the 2D ^{13}C - ^{13}C DARR spectra of [^{13}C , ^{15}N]-labeled A β fibrils diluted or undiluted with natural abundant A β molecules. Since the cross peaks in the spectra of the diluted sample belong to intramolecular correlations, cross peaks present in the spectra of the undiluted sample but absent in the spectra of the diluted sample can be assigned to intermolecular correlations. By using this approach, we assigned a number of intermolecular-distance restraints, including V39-G29, V39-A30, V39-I31, G38-I31, and G38-I32 (Figure S9 and Table S5). Since the above restraints are possibly present in three-fold^[3] and antiparallel two-fold symmetric models,^[2,4] more intermolecular restraints are required to define the organization of the monomeric units, and work in this direction is in progress.

The present study demonstrates that the interaction of A β 40 peptides with lipids not only influences the aggregation kinetics but also alters the molecular structure of the resulting fibrils. The key observation is that the structure of A β lipid fibrils is significantly different from that of solution fibrils. The structure difference can be attributed to the template effect of anionic phospholipids in A β 40 fibril nucleation. There are two aspects to this effect. Firstly, the two-dimensional membrane matrix provides a different environment from the solution, in which A β 40 molecules self-associate with relatively few restraints. Secondly, the interactions between A β 40 and the membrane, especially electrostatic interactions, mediate A β 40 intramolecular and intermolecular interactions, which are major driving forces of the self-assembly of A β 40, thus leading to fibrils of a different structure. In recent simulations, electrostatic interactions between anionic lipids and the charged residues of A β were observed to destabilize the β -turn- β motif of A β 40 fibrils and mediate aggregation.^[31] Our experimental results are consistent with this study.

In summary, we have characterized the molecular structure of A β 40 fibrils formed in the presence of lipid vesicles by using [^{13}C , ^{15}N]-labeled samples and multidimensional MAS NMR spectroscopy. The structure of the lipid fibrils is distinct from that of solution fibrils. This study not only provides the first structural model of A β 40 fibrils formed in the presence of the lipid vesicles, but also demonstrates how peptide–lipid interactions influence the fibrillization of the A β 40 peptide, a process that is closely associated with the pathophysiology of AD.

Experimental Section

A β 40 peptides were expressed in the BL21 (DE3) PlyS *E. coli* strain and purified by ion-exchange chromatography. To prepare fibrils in solution, lyophilized A β 40 peptide was dissolved in NaOH (50 mM) and then diluted with NaPi buffer (10 mM pH 7.4) to a final A β 40 concentration of 50 μM . The pH value was adjusted to 7.4 and the solution was incubated at 37°C with gentle shaking for 96 h. To prepare A β 40 fibrils with the DMPC/DMPG vesicles, lipid vesicles (2.5 mM) were added to a 100 μM A β 40 peptide solution to give a final A β concentration of 50 μM and the solution was incubated at 37°C with gentle shaking for 2 h. The pellets were collected into NMR rotors by ultracentrifugation, lyophilized, and rehydrated with 30% water. NMR experiments were carried out on a wide-bore Varian VNMRs 600 MHz (14.1 T) NMR spectrometer with a 4 mm triple-resonance T3-HXY MAS probe and a standard bore Bruker 800 MHz (18.8 T) NMR spectrometer with a 3.2 mm E-free HCN probe.

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