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The Crowded Environment of a Reverse Micelle Induces the Formation of β-Strand Seed Structures for Nucleating Amyloid Fibril Formation

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Supporting Information

ABSTRACT: A hallmark of Alzheimer's disease is the accumulation of insoluble fibrils in the brain composed of amyloid beta (Aβ) proteins with parallel in-register cross-β-sheet structure. It has been suggested that the aggregation of monomeric Aβ proteins into fibrils is promoted by "seeds" that form within compartments of the brain that have limited solvent due to macromolecular crowding. To characterize these seeds, a crowded macromolecular environment was mimicked by encapsulating Aβ40 monomers into reverse micelles. Fourier-transform infrared spectroscopy revealed that monomeric Aβ proteins form extended β-strands in reverse micelles, while an analogue with a scrambled sequence does not. This is a remarkable finding, because the formation of extended β-strands by monomeric Aβ proteins suggests a plausible mechanism whereby the formation of amyloid fibrils may be nucleated in the human brain.

In Alzheimer's disease (AD), amyloid beta (Aβ) proteins aggregate in the brain to form fibrils with in-register parallel cross-β-sheet structure.[1] It has been suggested that their aggregation is initiated by misfolded "seeds",[2,3] which are induced to form in extracellular spaces or subcellular compartments by macromolecular crowding.[4] However, experimental characterization of these seeds is thwarted by aggregation at the concentrations required for study, while inhibiting their aggregation by cooling to 5 °C reveals only random coils.[5]

To circumvent these problems, we have encapsulated the 40-residue Aβ protein (Aβ40) into reverse micelles and examined them with Fourier-transform infrared spectroscopy (FTIR). Encapsulation limits the available solvent and reduces the entropic cost of folding by reducing the configurational entropy of the unfolded chain. These conditions mimic the crowded conditions that exist in extracellular spaces or subcellular compartments where amyloid fibrils appear to form.[6,7] However, encapsulation also precludes aggregation, thereby making the structures that monomeric proteins form under such conditions available for study.

Aβ40 was custom synthesized by the Keck Biotechnology Resource Laboratory at Yale University. A protein containing the same amino acid residues in a scrambled sequence ($Aβ40_{scr}$) was purchased from rPeptide (Bogart, GA). Both proteins were dissolved in distilled hexafluoroisopropanol (HFIP), divided into aliquots containing 125 μg of protein, and lyophilized overnight. This procedure is effective at removing traces of trifluoroacetic acid remaining from protein purification that can give rise to a nonprotein absorption band at 1674 cm^{-1}.[8,9]

Aβ40 was encapsulated with sodium bis(2-ethylhexyl) sulfosuccinate (AOT) and D_2O in isooctane, by two different methods. In method one, an AOT/D_2O/isooctane mixture was added to the lyophilized protein and sonicated with three 10 s pulses of a probe sonicator. In method two, separate solutions of AOT in isooctane and Aβ40 in D_2O were prepared and sonicated with three 5 s pulses. The two solutions were then mixed and sonicated with a probe for 10 s. For both methods, the water loading ratios (w_0 = [D_2O]/[AOT]) ranged from 11.4 to 30.0, which correspond to water droplet diameters of 4.0−10.5 nm by some estimates[10] and 2.1−5.1 nm by others.[11] Attempts to create reverse micelles with larger w_o were unsuccessful due to turbidity and instability/phase separation. The concentrations of protein, water, and AOT were chosen so that only 1 out of 75 micelles in the final preparation contained a molecule of Aβ40. All preparations and measurements were made at 20 °C.

Samples were injected into a calcium fluoride flow cell with a 15 μm polytetrafluoroethylene (PTFE) spacer. FTIR spectra were collected on a Bio-Rad FTS 6000 spectrometer equipped with a narrow-band MCT detector, operating in rapid-scan mode with 2 cm^{-1} resolution. Each spectrum was derived from 256 coadded interferograms processed with medium Norton−Beer apodization.[12] Baseline spectra were obtained from reverse micelles made without protein. The spectra from reverse micelles shown in Figure 1 were collected from four independently prepared samples, normalized, and averaged. Baseline correction was applied to negate the effects of a broad ester band at 1740 cm^{-1} arising from the AOT, but no smoothing was applied. Circular dichroism studies were not feasible due to light scattering and lower sensitivity.

Monomeric Aβ40 within reverse micelles exhibits a prominent band at 1621 cm^{-1} that is characteristic of extended β-strands (Figure 1) and distinct from a "fully extended" chain in which adjacent peptide C═O bonds are not parallel to each other and thus not strongly coupled. Varying the average diameter of the micelles by a factor of nearly 3 has little effect on the spectra. Dry Aβ40 fibrils also exhibit this band, but

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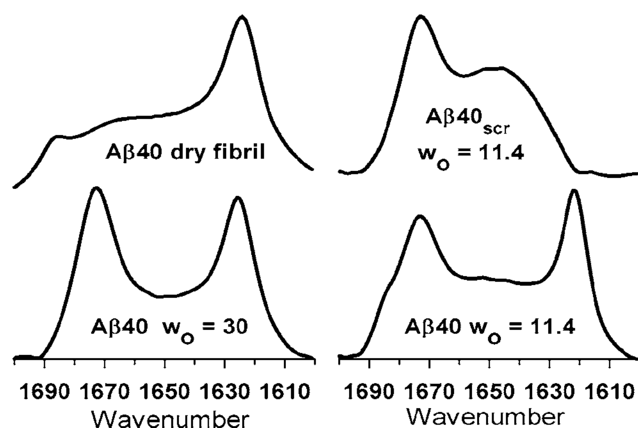

Figure 1. Transmission FTIR spectra of dry Aβ fibrils, monomeric Aβ40 in large and small reverse micelles, and Aβ40 with a scrambled amino acid sequence (A$\beta 40_{scr}$) in small reverse micelles. The low frequency bands at 1621 cm^{-1} arise from extended β-structure in fibrils and reverse micelles. The high frequency bands at 1674 cm^{-1} most likely arise from β turns. The peaks are on the order of 5 milliabsorbance units high.

A$\beta 40_{scr}$ in reverse micelles does not. Both Aβ40 and A$\beta 40_{scr}$ exhibit a prominent band at 1674 cm^{-1}, suggesting that β-turns are present. The proteins within reverse micelles appear to be exclusively monomeric because they were quantitatively recovered in the monomer fraction when a reverse micelle suspension was examined by HPLC.[13,14] Moreover, both the 1621 and 1674 cm^{-1} bands are remarkably narrow, suggesting a homogeneous physical state that would be unlikely if oligomers had formed.

Therefore, we conclude that Aβ40 is monomeric and forms extended β-strands within a reverse micelle. This conclusion is remarkable because it implies that Aβ40 is uniquely capable of forming both extended β-strands within a reverse micelle and parallel β-sheets in amyloid fibrils. A$\beta 40_{scr}$ does not form extended β-strands under the same conditions, nor does it form amyloid fibrils. Therefore, the formation of β-strands by Aβ40 depends both on its sequence and on the chemical environment within a reverse micelle. Both Aβ40 and A$\beta 40_{scr}$ form β-turns in reverse micelles, most likely because the micelles used in these experiments are too small to enclose a fully extended 40-residue chain (~14 nm long).

The factors that induce the formation of extended β-strands within a reverse micelle are not clear. Physical confinement increases the stability of folded proteins by limiting the configurational entropy of their unfolded chains (as in chaperonins). Physical confinement also limits the amount of water available to solvate each protein segment. Depending on the assumed diameter of the water droplet,[10,11] reverse micelles with a water-loading ratio of 11.4 have a volume of no greater than 3.35×10^{-23} L. Therefore, the effective concentration of Aβ40 is very high (at least 20 mM), and the pH drops to 1.7 if even only one free proton is present. Sodium ions, which accompany each of the AOT sulfate groups, make the ionic strength of the micelle interior quite high and may sequester much of the water that is present in their hydration shells.

Each of these factors may be relevant to the misfolding of Aβ proteins in AD because they may be mimicked in the brain. For example, the extracellular environment of the brain is crowded, with protein–water ratios on the order of that in our reverse micelles.[4] Alternatively, brain cells may take up Aβ proteins from the extracellular space and physically confine them in endosomes with limited water.[15] In AOT micelles, the internal surface is lined with anionic sulfate headgroups, which are abundant in brain tissue in the form of lipid sulfatides, and which are inversely correlated to the development of AD.[16,17] Simulation studies have suggested that solvent-mediated interactions between an encapsulated protein and the interior surface of a micelle may affect folding.[18] Indeed, there is abundant evidence that lipid membrane surfaces have a key role in the formation of amyloid fibrils in AD.[19]

Any of these factors may be important, or even essential, for the formation of extended β-strands. Clearly, however, they are not sufficient because A$\beta 40_{scr}$ does not form extended β-strands under the same circumstances. For the same reason, interactions between Aβ40 and AOT or isooctane cannot account for the phenomena we observe in reverse micelles. Therefore, these phenomena critically depend on the sequence of Aβ40 and its interaction with the chemical environment in a micelle.

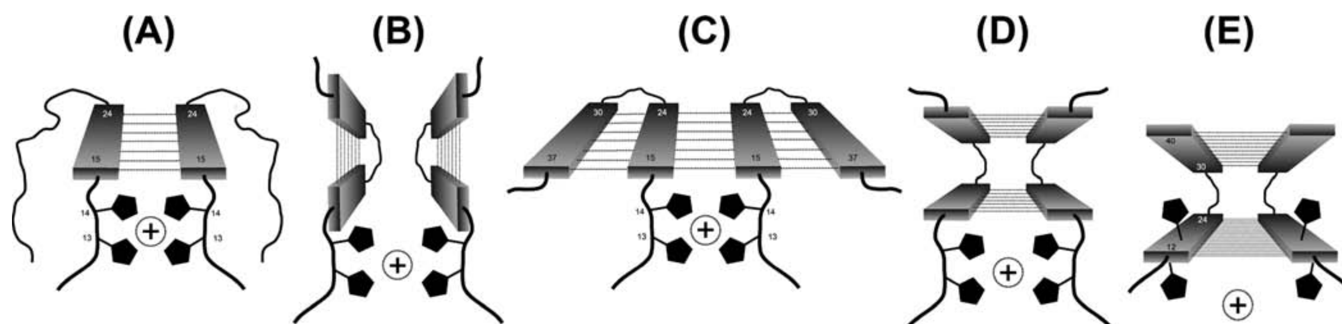

Figure 2. A mechanism for the formation of amyloid fibril seeds. The behavior of monomeric Aβ40 proteins in reverse micelles suggests that a crowded extracellular environment causes them to form extended β-strands, or antiparallel β-sheets consisting of two extended β-strands. If a metal ion is available, it can form a coordination complex with the His residues at positions 13 and 14 on a pair of Aβ40 molecules and induce in-register alignment of the N-terminal residues. Possible configurations for the resulting coordination complex include (A) parallel β-sheet, (B) two antiparallel β-sheets, and (C) mixed parallel/antiparallel sheets. With stability provided by metal-His coordination, these configurations may rearrange through peptide bond rotations and/or the breaking and remaking of hydrogen bonds to yield (D) two parallel β-sheets. (E) Extension of the N-terminal sheet toward the amino terminus eliminates the metal ion because the side chains of adjacent His residues will be on opposite sides of the β-sheet. The resulting structure models our current understanding of amyloid fibril structure and may represent the seed that nucleates the extension of that structure into an amyloid fibril.

Whether or not the extended β-strands we observe in Aβ40 monomers are intermediate forms en route to fibril formation is an open question, but the ability of Aβ40 to form both parallel β-sheets (in a fibril) and extended β-strands (in reverse micelles) is remarkable because it suggests that the formation of in-register parallel β-sheet structure in amyloid fibrils may be nucleated by the edge-to-edge association of two extended β-strands. Such an association may be facilitated by metal ions, especially copper, which are well-known to have a high affinity for Aβ proteins.[20] Metal ions may promote in-register alignment by forming a four-coordinate complex with the His-13 and His-14 side chains on a pair of Aβ40 strands (Figure 2).

The spectra of Aβ40 in reverse micelles may represent two extended β-strands (accounting for the 1621 cm^{-1} bands in Figure 1) joined by a β-turn (accounting for the 1674 cm^{-1} bands in Figure 1) to form an antiparallel β-sheet (Figure 2B). Ordinarily, there is little tendency for isolated polypeptide segments to form antiparallel β-sheets.[21] However, membranes can induce antiparallel β-sheet formation by polypeptides that otherwise would not associate,[22] and their formation would only be further encouraged by the limited amount of water available within the micelle.

If a pair of antiparallel β-sheets formed a metal-ion complex and aligned edge-to-edge, the result would be a mixed parallel–antiparallel β-sheet (Figure 2C). This type of mixed topology has been observed in nature (e.g., plastocyanins) but may not be as stable as a homogeneous topology. Thus, greater stability may drive the exchange of intramolecular hydrogen bonds (in the antiparallel β-sheet of a mixed topology dimer) for intermolecular hydrogen bonds, to yield the parallel β-sheets that are characteristic of an amyloid fibril (Figure 2E).

In conclusion, our observation that Aβ40 monomers form extended β-strands in reverse micelles suggests a plausible mechanism whereby the formation of amyloid fibrils with in-register parallel β-sheets may be nucleated in the extracellular spaces of the human brain. If it operates in human disease, this mechanism could be an important strategic target for mechanism-based therapeutic agents to treat Alzheimer's disease.

■ ASSOCIATED CONTENT

Supporting Information

Polypeptide sequences and sources, reverse micelle compositions, and an illustration of the spectral processing procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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