Solid State NMR Investigation of 150 kDa oligomers of the 42-residue Alzheimer's β-amyloid peptide

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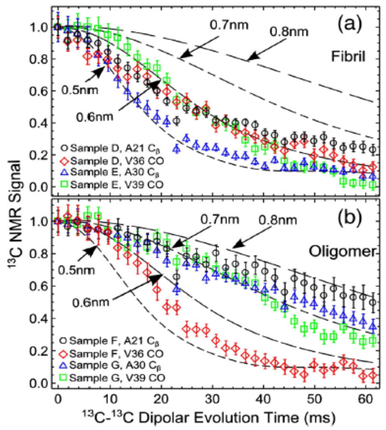
Alzheimer's Disease and the β-amyloid peptide

Amyloid-β (Aβ) is the main component of amyloid plaques (extracellular deposits found in the brains of patients with Alzheimer's disease). Understanding amyloid-β (Aβ) structures and underlying assembly pathways will advance the fundamental understanding of Alzheimer ' s disease (AD) at the molecular level. Recent research suggests that soluble oligomeric forms of Aβ may be causative agents in the development of Alzheimer's disease. It is generally believed that Aβ oligomers are the most toxic. A number of genetic, cell biology, biochemical and animal studies support the concept that Aβ plays a central role in the development of Alzheimer' s disease pathology, however there is no widely accepted structural model for Aβ oligomers. A more detailed understanding of Aβ oligomer assembly is likely to be an important step towards a molecular-level understanding of AD neurotoxicity.

Paravastu lab previous Aβ contributions

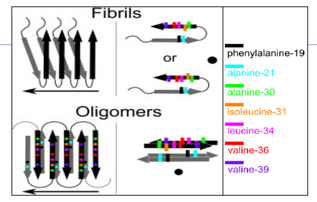
Recently presented a procedure for production of solid state NMR compatible 150 kDa oligomeric samples of Aβ(1-42) (the 42-residue variant), and shown that oligomers and amyloid fibrils differ in intermolecular arrangement of β-strands.



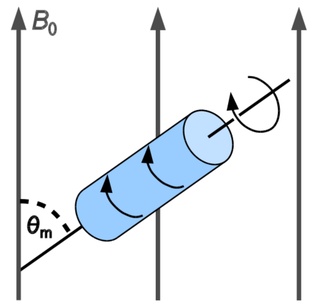
Measurements of intermolecular ^{13}C - ^{13}C magnetic dipole-dipole couplings in C^{β} and CO for Aβ(1-42) fibril (a) and oligomer (b) samples by the PITHIRDS-CT solid state NMR technique. The lines represent predicted decays based on numerical simulations for linear strands of seven ^{13}C nuclei equally spaced by the specified distances. Consistent decays for fibril samples labeled at multiple sites indicate an in-register parallel β-sheet structure. Decreased decays for oligomer samples labeled at the A21 C^{β} , A30 C^{β} , and V39 CO indicate that Aβ (1-42) oligomers are not characterized by in-register parallel β-sheets.

Hypothesis: Aβ42 oligomers are arranged in antiparallel β-strands

The most prominent structural differences between Aβ(1–42) oligomers and fibrils were observed through measurements of intermolecular ^{13}C–^{13}C dipolar couplings observed in PITHIRDS-CT experiments. PITHIRDS-CT data indicate that, unlike fibrils, oligomers are not characterized by in-register parallel β-sheets.



Solid State NMR method: Magic Angle spinning



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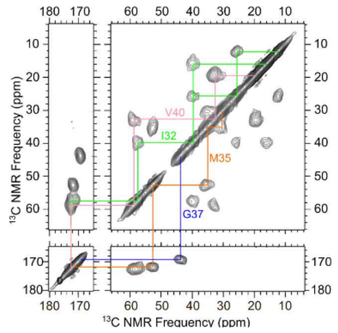
The sample (blue) is rotating with high frequency inside the main magnetic field (B_0). The axis of rotation is tilted by the magic angle θ_m with respect to the direction of B_0. In any condensed phase, a nuclear spin experiences a great number of interactions. The main three interactions often lead to very broad and featureless lines. By spinning the sample at the magic angle θ_m *(ca. 54.74°, where* $\cos^2\theta_m=1/3$*)* with respect to the direction of the magnetic field, the normally broad lines become narrower, increasing the resolution for better identification and analysis of the spectrum.

Results

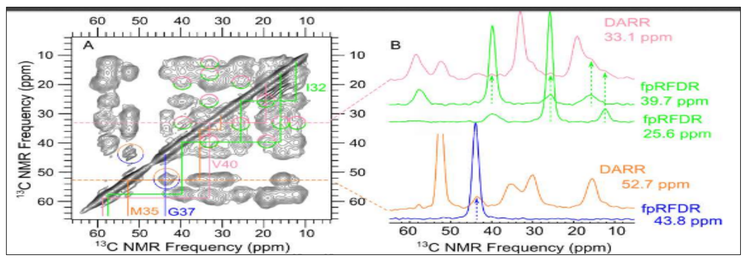
13C NMR chemical shifts are consistent with ordered β-strand structure



Two dimensional ^{13}C-^{13}C fpRFDR solid state NMR spectra of Sample A (IL4: Uniformly labeled ^{13}C at I32, M35, G37, and V40). The off-diagonal crosspeaks represent single-bond correlations which allow residue-specific assignments within the ^{13}C-labeled amino acids, as illustrated by colored lines. Positions of the off-diagonal peaks (chemical shifts) and linewidths indicate that the labeled residues are characterized by well-defined molecular structures, and that Cα and Cβ chemical shifts are consistent with β-strand molecular configurations.

Results

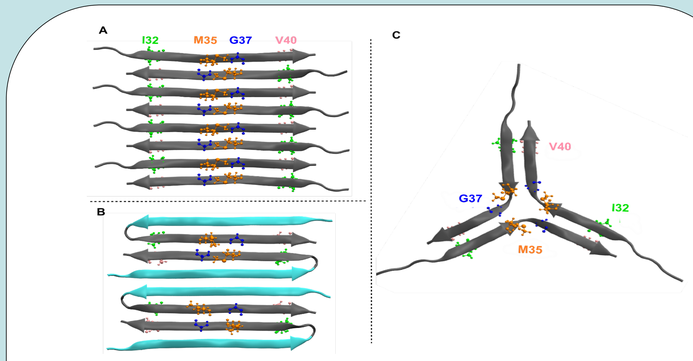
2D DARR data indicate inter-molecular proximities between I32 and V40 as well as M35 and G37



Close spatial proximities between labeled amino acid sidechains were determined using the 2D Dipolar assisted rotational resonance DARR) NMR technique with 500 ms mixing times. These spectra show correlations between atoms separated by distances up to ~0.6 nm. Analysis of the 2D DARR spectrum from Sample A (Figure A and 2B) indicates that the I32 sidechains are near the V40 sidechains and that the M35 sidechains are near the G37 sidechains.

Conclusion

For Aβ(1-42) oligomers, solid-state NMR constraints indicate that the approximate region spanned by residues I32 to V40 adopts an antiparallel β-sheet arrangement. The Aβ(1-42) oligomers are not thought to have a parallel C-terminal β-sheet arrangement due to previous PITHIRDS-CT experiments that show weak decays for three samples ^{13}C –labeled on 1) A21 Cβ, 2) A30 Cβ, and 3) V39 CO. The strong PITHIRDS-CT decay observed for an oligomer sample ^{13}C-labeled on V36 CO is consistent with an antiparallel C-terminal β-sheet "centered" (closest proximity between like residues on neighboring molecules) on residue V36. Specifically, we have shown the antiparallel C-terminus β-sheets are centered around V36. This finding is rationalized by three different molecular models which is an important step toward developing and testing hypothesis about 150kDa Aβ(1-42) oligomer structures.



Possible models of C-terminus arrangements of Aβ(1-42) oligomers based on the solid state NMR characterization. Different colors are representing different amino acid sites as illustrated. A) Antiparallel arrangement of C-terminus β-sheet within the Aβ(1-42) oligomer. B) "Anti" configuration of β-hairpins within in Aβ(1-42) oligomer. C) Triangular configuration of C-terminus β-sheet for Aβ(1-42) oligomer.

References

Danting Huang, Maxwell I. Zimmerman, Patricia K. Martin, Jeremy Nix, Terrone L. Rosenberry and Anant K. Paravastu "Antiparallel β-sheet structure within the C-terminal region of 150 kDa oligomers of the 42-residue Alzheimer's β-amyloid peptide" Paper in preparation.
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