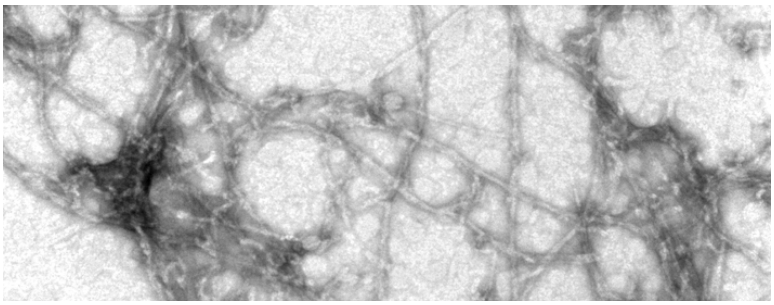
Communication

β Sheet Structure in Amyloid β Fibrils and Vibrational Dipolar Coupling

Cynthia Paul, and Paul H. Axelsen

J. Am. Chem. Soc., **2005**, 127 (16), 5754-5755• DOI: 10.1021/ja042569w • Publication Date (Web): 30 March 2005



J|A|C|S
COMMUNICATIONS

Published on Web 03/30/2005

β Sheet Structure in Amyloid β Fibrils and Vibrational Dipolar Coupling

Cynthia Paul and Paul H. Axelsen*

Departments of Pharmacology, Biochemistry and Biophysics, and Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6084

Received December 10, 2004; E-mail: axe@pharm.med.upenn.edu

Amyloid β (Aβ) proteins are 39−42 residue polypeptides that form fibrils and accumulate in the brain tissue of persons afflicted with Alzheimer's disease. They are the principal component of senile plaques, the pathognomonic lesion in this disease. A detailed structure for the amyloid β fibril is of interest because it is a molecular target for therapeutic intervention.

Early X-ray scattering studies indicated that fibrils possess cross-β structure,[1,2] while infrared spectroscopy detected a widely split amide I band suggesting that this β structure is antiparallel.[3,4] Literature in this field is subject to confusion because different investigations are performed on different forms of these proteins. Hereafter, Aβ40 and Aβ42 designate 40 and 42 residue Aβ proteins, respectively. Aβ_{m-n} designates a polypeptide segment corresponding to residues *m* through *n* of Aβ42, while an amidated C-terminus is designated by *n*′. Accordingly, isotope-edited infrared spectroscopy suggested that fibrils formed by Aβ_{34-42} have an antiparallel β structure.[5] Antiparallel structure was later confirmed by solid-state NMR (SS−NMR) studies in fibrils formed from Aβ_{34-42},[6] A$\beta_{16-22'}$,[7] and Aβ_{11-25}.[8] Therefore, it was surprising when SS−NMR studies of fibrils formed by A$\beta_{10-35'}$[9] and by full-length Aβ40,[10,11] as well as EPR studies,[12] indicated that these longer segments both formed fibrils with parallel in-register β structure.

In view of recent progress in understanding vibrational dipole coupling in isotope-labeled polypeptides, particularly those forming β structure,[13−17] it is of considerable interest to probe the structure of fibrils formed by ^{13}C-labeled amyloid β proteins with infrared spectroscopy. Lansbury previously explored amyloid structure with pioneering studies of this type, but only in a nine residue C-terminal segment, Aβ_{34-42},[5] and pancreatic amyloid.[18]

The introduction of ^{13}C-labeled peptide groups into a polypeptide results in splitting of the amide I band into high frequency ^{12}C components and lower frequency ^{13}C components. The ^{13}C components shift to even lower frequencies when ^{13}C-containing amide I oscillators are aligned and placed in close proximity to each other (Figure 1).[19,20] The intensity of these components, on the other hand, is enhanced when they are aligned with and placed in close proximity to ^{12}C-containing amide I oscillators.[17] Thus, the disproportionately strong signals commonly seen arising from ^{13}C-containing peptide groups in well ordered secondary structure are attenuated when the labels are aligned in adjacent strands.[17]

A$\beta_{10-35'}$ and Aβ40 were synthesized commercially (Synpep) with $^{13}C_1$-labeled amino acids (Cambridge Stable Isotopes) in selected positions. Protein identities and isotopic purity were confirmed by ESI-ion-trap mass spectrometry of tryptic digests. Aggregated proteins were prepared by incubating 10 μM solutions of each protein in D_2O−HEPES pD 7.4 at room temperature for 3 days. Infrared spectra were obtained by evaporating aggregated proteins onto an internal reflection crystal and coadding 1024 scans. A level baseline was set for each spectrum, but no smoothing, deconvolution, or water vapor subtraction was performed.

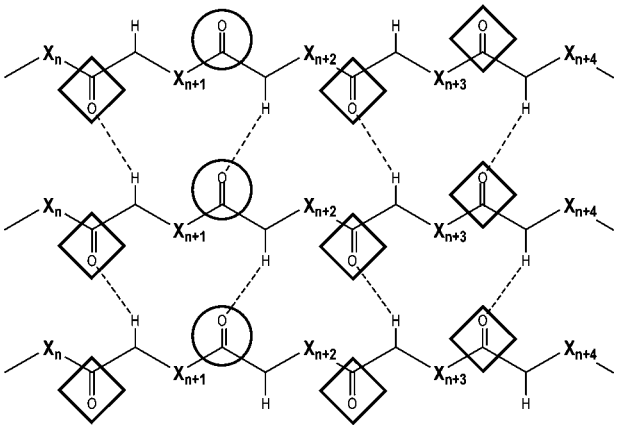


Figure 1. Relationships between ^{13}C-labeled amide I oscillators (C═O groups in circles) and unlabeled amide I oscillators (C═O groups in diamonds) in a parallel β sheet. The β strands are "in-register", meaning that the labels at position X_{n+1} in each strand are aligned. This facilitates interstrand coupling between labeled oscillators and results in a red-shift of the amide I band arising from the labels. Optimal intensification of ^{13}C-containing modes, however, requires optimal interstrand coupling with ^{12}C-containing amide I oscillators, and this is precluded when ^{13}C is present on adjacent strands instead of ^{12}C.

Unlabeled, unaggregated Aβ40 yields a broad, featureless amide I′ maximal at 1658 cm^{-1}, whereas amide I′ in aggregated Aβ40 is maximal at 1624 cm^{-1} and has a high frequency shoulder at 1685 cm^{-1} (Figure 2), as previously described[4] (amide I′: primes indicate spectra collected in D_2O). A $^{13}C_1$ label in the alanine residue at position 21 is difficult to detect in unfibrillized protein, but it has three significant consequences on the spectrum of fibrillized protein: the absorption maximum shifts to 1630 cm^{-1}, a conspicuous shoulder appears at 1612 cm^{-1}, and the high-frequency shoulder at 1685 cm^{-1} nearly vanishes.

The pattern observed in Aβ40 most closely resembles that seen when ^{13}C labels are not immediately adjacent to each other or when they are diluted to some extent with unlabeled protein. This is consistent with the A21/UL spectrum, in which band positions are intermediate between those of the UL and A21 spectra (abbreviations defined in Figure 2 caption).

The A30 spectrum exhibits a 2 cm^{-1} ^{12}C blue shift, a barely discernible ^{13}C shoulder, and an intact high-frequency component. While little can be deduced from such weak coupling, it provides contrast to illustrate that a significant degree of coupling between ^{13}C modes exists in A21. When A21 and A30 are mixed in organic phase and allowed to coaggregate, the result is again virtually a linear combination of A21 and A30 spectra (spectrum not shown), indicating that no significant coupling occurs between these labels in fibrils.

Fibrils formed by A$\beta_{10-35'}$ are unlikely to be significant components in the pathology of Alzheimer's disease but are of interest because they form morphologically similar fibrils despite their abbreviated length (see Supporting Information). Unlabeled A$\beta_{10-35'}$

10.1021/ja042569w CCC: $30.25

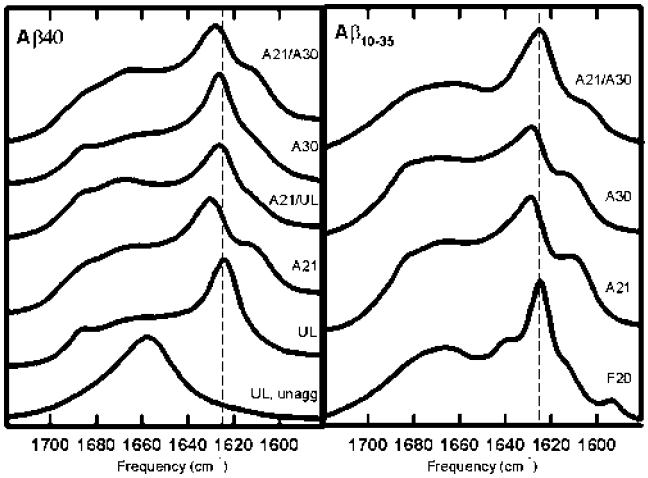


Figure 2. Amide I′ infrared spectra of Aβ40 (left panel) and A$\beta_{10-35'}$ (right panel) proteins. The vertical axes indicate relative absorbance, and dashed lines are drawn at 1625 cm^{-1}. All spectra except the bottom spectrum on the left correspond to aggregated protein. UL: unlabeled. F20, A21, and A30: ^{13}C labels in C_1 of phenylalanine or alanine residues at positions 20, 21, and 30, respectively. A21/UL is one part A21 protein mixed in organic phase with three parts UL protein prior to fibrillization. A21/A30 is a 1:1 ratio of A21 and A30 proteins mixed in organic phase prior to fibrillization.

yields a spectrum that is indistinguishable from unlabeled Aβ40 (data not shown). Labeled A$\beta_{10-35'}$, however, yields three striking spectral findings. First, both the A21 and A30 spectra from A$\beta_{10-35'}$ are similar to the A21 spectrum of Aβ40, suggesting that the environments of residue 30 in A$\beta_{10-35'}$ and Aβ40 are significantly different. Second, when A21 and A30 forms of A$\beta_{10-35'}$ are cofibrillized, the result is not a linear combination of the two individual spectra but one in which the ^{13}C component is further red-shifted to 1605 cm^{-1}. This shift indicates relatively strong dipolar coupling and suggests closer proximity between the A21 and A30 labels when both are present than between A21 or A30 labels when only one is present. Third, the F20 form of A$\beta_{10-35'}$ yields a complex spectrum suggesting the presence of two populations: one with a widely split ^{13}C band at 1594 cm^{-1} and ^{12}C band at 1640 cm^{-1}, and another with a narrowly split ^{13}C shoulder at 1613 cm^{-1} and ^{12}C band at 1625 cm^{-1}. The F20 form of A$\beta_{10-35'}$ is the only protein described herein in which coupling between ^{13}C labels approaches the strength of that observed in other peptides with β structure.[17]

These results support key features of several recently proposed models for amyloid fibril structure,[21,22] but they also add some important caveats. For instance, the presence of dipolar coupling in the A21 and A30 spectra of both A$\beta_{10-35'}$ and Aβ40 is consistent with the conclusion that their β structure is parallel.[9,10] The limited isotope signal enhancement is consistent with simulations of in-register parallel β structure; however, the frequency shifts are not strong as expected.[16,17] This may reflect nonstandard or twisted parallel β sheet structure that yields suboptimal alignment of labeled oscillators.

Another key observation is that residues 21 and 30 differ in the strength of their coupling in Aβ40 but not in A$\beta_{10-35'}$.[23] This finding suggests that Aβ40 and A$\beta_{10-35'}$ have different structures in the vicinity of residue 30. This difference is underscored by the observation that a 1:1 mixture of A21 and A30 is merely additive in Aβ40, but in A$\beta_{10-35'}$ there is evidence of even stronger coupling suggesting proximity between residues 21 and 30 in A$\beta_{10-35'}$ that is not present in Aβ40. This finding supports models in which residues 21 and 30 in a fibril are in close proximity to each other.[21,22,24]

Finally, evidence of two differently coupled subpopulations in F20 spectra of A$\beta_{10-35'}$ is consistent with dual resonances observed in NMR studies of correspondingly labeled Aβ40[11] and the multiple resonances arising from labeling of the adjacent residue (Ala21) in A$\beta_{10-35'}$.[25] At this point, it is not possible to distinguish heterogeneity within an individual fibril from two populations, each with uniform internal structure.

Although the use of vibrational spectroscopy as a technique for macromolecular structure determination is relatively undeveloped, it is likely to receive increasing attention because of its high sensitivity, diversity of suitable sample conditions, and the potential to yield information about molecular orientation. These advantages may prove to be of particular significance to understanding amyloid fibril structure since none of the techniques applied to date help clarify the transverse relationship between cross-β units in a fibril.

Acknowledgment. The authors are grateful to Tomoyuki Oe and Ian Blair for mass spectrometry analysis and Robin Hochstrasser for a discussion of the manuscript. This work was supported by AG20238 from the National Institute on Aging and a grant from the Alzheimer's Association.

Supporting Information Available: Electron micrographs of fibrils and mass spectra of isotope-labeled peptides (PDF, JPG). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Eanes, E. D.; Glenner, G. G. J. *Histochem. Cytochem.* **1968**, *16*, 673−677.
(2) Kirschner, D. A.; Abraham, C.; Selkoe, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 503−507.
(3) Hilbich, C.; Kisterwoike, B.; Reed, J.; Masters, C. L.; Beyreuther, K. *J. Mol. Biol.* **1991**, *218*, 149−163.
(4) Fabian, H.; Choo, L.; Szendrei, G. I.; Jackson, M.; Halliday, W. C.; Otvos, L., Jr.; Mantsch, H. H. *Appl. Spectrosc.* **1993**, *47*, 1513−1518.
(5) Halverson, K. J.; Sucholeiki, I.; Ashburn, T. T.; Lansbury, P. T. *J. Am. Chem. Soc.* **1991**, *113*, 6701−6703.
(6) Lansbury, P. T.; Costa, P. R.; Griffiths, J. M.; Simon, E. J.; Auger, M.; Halverson, K. J.; Kocisko, D. A.; Hendsch, Z. S.; Ashburn, T. T.; Spencer, R. G. S.; Tidor, B.; Griffin, R. G. *Nat. Struct. Biol.* **1995**, *2*, 990−998.
(7) Balbach, J. J.; Ishii, Y.; Antzutkin, O. N.; Leapman, R. D.; Rizzo, N. W.; Dyda, F.; Reed, J.; Tycko, R. *Biochem.* **2000**, *39*, 13748−13759.
(8) Petkova, A. T.; Buntkowsky, G.; Dyda, F.; Leapman, R. D.; Yau, W.-M.; Tycko, R. *J. Mol. Biol.* **2004**, *335*, 247−260.
(9) Benzinger, T. L. S.; Gregory, D. M.; Burkoth, T. S.; Miller-Auer, H.; Lynn, D. G.; Botto, R. E.; Meredith, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 13407−13412.
(10) Antzutkin, O. N.; Balbach, J. J.; Leapman, R. D.; Rizzo, N. W.; Reed, J.; Tycko, R. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 13045−13050.
(11) Balbach, J. J.; Petkova, A. T.; Oyler, N. A.; Antzutkin, O. N.; Gordon, D. J.; Meredith, S. C.; Tycko, R. *Biophys. J.* **2002**, *83*, 1205−1216.
(12) Torok, M.; Milton, S.; Kayed, R.; Wu, P.; McIntire, T.; Glabe, C. G.; Langen, R. *J. Biol. Chem.* **2002**, *277*, 40810−40815.
(13) Brauner, J. W.; Dugan, C.; Mendelsohn, R. *J. Am. Chem. Soc.* **2000**, *122*, 677−683.
(14) Kubelka, J.; Keiderling, T. A. *J. Am. Chem. Soc.* **2001**, *123*, 6142−6150.
(15) Kubelka, J.; Keiderling, T. A. *J. Am. Chem. Soc.* **2001**, *123*, 12048−12058.
(16) Silva, R. A. G. D.; Barber-Armstrong, W.; Decatur, S. M. *J. Am. Chem. Soc.* **2003**, *125*, 13674−13675.
(17) Paul, C.; Wang, J. P.; Wimley, W. C.; Hochstrasser, R. M.; Axelsen, P. H. *J. Am. Chem. Soc.* **2004**, *126*, 5843−5850.
(18) Ashburn, T. T.; Auger, M.; Lansbury, P. T. *J. Am. Chem. Soc.* **1992**, *114*, 790−791.
(19) Krimm, S.; Bandekar, J. *Adv. Prot. Chem.* **1986**, *38*, 181−364.
(20) Parkhe, A. D.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Macromolecules* **1993**, *26*, 6691−6693.
(21) Petkova, A. T.; Ishii, Y.; Balbach, J. J.; Antzutkin, O. N.; Leapman, R. D.; Delaglio, F.; Tycko, R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16742−16747.
(22) Guo, J. T.; Wetzel, R.; Xu, Y. *Proteins: Struct. Funct. Bioinformatics* **2004**, *57*, 357−364.
(23) This difference was of sufficient concern that a second completely new synthesis of A40 with a label at position 30 was obtained and examined. In addition, fibril morphology was verified by electron microscopy, whole protein mass was verified by MALDI-TOF mass spectrometry, and label position was verified in tryptic digests by ESI-MS.
(24) Shivaprasad, S.; Wetzel, R. *Biochemistry* **2004**, *43*, 15310−15317.
(25) Antzutkin, O. N.; Leapman, R. D.; Balbach, J. J.; Tycko, R. *Biochem.* **2002**, *41*, 15436−15450.

JA042569W