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Spectroscopic Signature for Stable β -Amyloid Fibrils versus β -Sheet-Rich Oligomers

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Abstract

We use two-dimensional IR (2D IR) spectroscopy to explore fibril formation for the two predominant isoforms of the β -amyloid ($A\beta_{1-40}$ and $A\beta_{1-42}$) protein associated with Alzheimer's disease. Two-dimensional IR spectra resolve a transition at 1610 cm^{-1} in $A\beta$ fibrils that does not appear in other $A\beta$ aggregates, even those with predominantly β -sheet-structure-like oligomers. This transition is not resolved in linear IR spectroscopy because it lies under the broad band centered at 1625 cm^{-1} , which is the traditional infrared signature for amyloid fibrils. The feature is prominent in 2D IR spectra because 2D lineshapes are narrower and scale nonlinearly with transition dipole strengths. Transmission electron microscopy measurements demonstrate that the 1610 cm^{-1} band is a positive identification of amyloid fibrils. Sodium dodecyl sulfate micelles that solubilize and disaggregate preaggregated $A\beta$ samples deplete the 1625 cm^{-1} band but do not affect the 1610 cm^{-1} band, demonstrating that the 1610 cm^{-1} band is due to very stable fibrils. We demonstrate that the 1610 cm^{-1} transition arises from amide I modes by mutating out the only side-chain residue that could give rise to this transition, and we explore the potential structural origins of the transition by simulating 2D IR spectra based on $A\beta$ crystal structures. It was not previously possible to distinguish stable $A\beta$ fibrils from the less stable β -sheet-rich oligomers with infrared light. This 2D IR signature will be useful for Alzheimer's research on $A\beta$ aggregation, fibril formation, and toxicity.

Graphical Abstract

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Notes

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Supporting Information

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FT-IR spectra (Figure S1); fit of two summed Gaussian functions to the spectra of each $A\beta$ isoform aggregated for 1 week at pH 2 and 7.5 (Figure S2); individual simulated 2D IR spectra and diagonal traces (Figure S3); eigenvector coefficients (Figure S4); diagonal trace of the simulated spectrum (Figure S5) (PDF)

INTRODUCTION

β -Amyloid (A β) plaques are a hallmark of Alzheimer's disease (AD), and similar β -sheet-rich plaques are associated with over 20 human amyloid diseases.¹ A β is produced as a mixture of 38–43 residue isoforms, with the 40 (A β _{1–40}) and 42 (A β _{1–42}) residue species predominating in humans. Although both spontaneously form amyloid fibrils in vitro, A β _{1–42} is more aggregation-prone and associated with disease states.^{2–4} Therapeutics capable of clearing A β plaques can facilitate recovery from AD symptoms in mice,^{5,6} and reduced endogenous clearance of A β correlates with the onset of AD.^{7–9} There is also evidence that nonfibrillar oligomers may be the most neurotoxic species.^{10,11} The characterization of A β aggregates and fibrils represents major areas of focus in Alzheimer's research, as accurate structural characterization is needed to understand the factors that influence and inhibit aggregation. Toward this end, we demonstrate a method to spectroscopically differentiate stable A β fibrils from other β -sheet-rich oligomers, a distinction that is difficult or impossible to make with other standard methods in the absence of electron microscopy.

A β aggregation is most commonly characterized by circular dichroism (CD), thioflavin-T (ThT) fluorescence, and infrared (IR) spectroscopy. CD provides an estimate of relative β -sheet content, although all β -sheets typically give similar spectra. ThT fluorescence is often used to identify amyloid fibrils, but the dye is also known to bind to other β -sheet-rich aggregates,¹² induce amyloid formation,¹³ be insensitive to major fibril restructuring,¹⁴ and fail to produce signal in the presence of amyloid.¹⁵ IR spectroscopy resolves the β -sheets of amyloids from the β -sheets of soluble proteins according to their frequencies,^{16,17} but typically does not distinguish A β amyloid fibrils from other, possibly off-pathway, oligomers and aggregates that have high β -sheet content.

Two-dimensional IR (2D IR) spectroscopy offers advantages over these other methods. In particular, the 2D IR signals scale nonlinearly with the absorption coefficient,^{18–20} resulting in narrower line widths and improved spectral resolution. In a standard IR spectrum, strong transitions can be obscured by more numerous weak transitions, whereas in a 2D IR spectrum, strong transitions tend to dominate because of the nonlinear scaling. β -Sheets of amyloid fibrils have very strong amide I transitions because many backbone carbonyls vibrate in unison, and thus become enhanced over random coil and other less regular structures. In this report, we resolve a transition at 1610 cm^{–1} that allows clear positive

identification of very stable A β amyloid fibrils in aggregated A β samples, distinguishing fibrils from other β -sheet-rich oligomers.

The idea that the structures of intermediates formed en route to amyloid formation differ from the β -sheet structure of the fibrils is not a new concept. There is evidence for transient α -helical intermediates in the aggregation of A β , α -synuclein, and amylin.^{21–26} More germane to the present study are the observations of restructuring of β -sheet structures present in intermediates preceding amyloid fibril formation. For example, there is evidence for antiparallel structure in intermediates of α -synuclein,²⁷ the SH3-domain,^{28,29} and A β ³⁰ (though we do not observe the standard signature of antiparallel β -sheet structure in the oligomers observed here). Crystal structures for toxic “cylindrin” intermediates in the aggregation of α B-crystallin have also been reported, and a segment of the A β sequence was noted to be compatible with the cylindrin oligomer structure.³¹ Using 2D IR and isotope labeling, our group has demonstrated the presence of a transient β -sheet in amylin that is ultimately part of a disordered loop in the fiber.^{32–34} Thus, it appears that oligomers of amyloidogenic proteins may often adopt a different structure than that of the amyloid fibril. In this study, we find that we can exploit the differences in β -sheet structure, even in the absence of isotope labels, to distinguish β -sheet-rich A β oligomers from A β fibrils.

MATERIALS AND METHODS

Two-Dimensional IR Spectroscopy

The 2D IR spectrometer used has been described previously.^{35–38} A ca. 3.2 W regenerative amplifier (Spectra Physics, Solstice) outputs 800 nm, 100 fs pulses at a rate of 1 kHz, which pump an optical parametric amplifier (OPA, Light Conversion, TOPAS). Signal (1417 nm) and idler (1845 nm) pulses undergo difference-frequency generation to produce 100 fs mid-IR pulses centered at 1600 cm⁻¹, with bandwidth of approximately 150 cm⁻¹ full width at half-maximum. The mid-IR pulses are split into pump (95%) and probe (5%) beams using a CaF₂ wedge. The pump beam passes through a horizontal fully reflective germanium acousto-optic modulator pulse shaper described previously.³⁸ The pulse shaper generates a collinear pair of compressed pump pulses with variable delays that are scanned to generate the pump axis of the 2D IR spectra. Four-frame phase cycling is used to subtract background and suppress pump scatter.³⁷ Pump and probe beams are spatially overlapped and focused at the sample using parabolic mirrors. The temporal delay (t_2) between the pump pulse pair and probe beam is controlled using a motorized delay stage (Newport) and set to zero for the experiments in this article. The probe beam is spatially dispersed onto a 64-element mercury–cadmium–telluride detector array (Infrared Associates), which provides ca. 3 cm⁻¹ resolution along the probe axis.

Sample Preparation

A β _{1–40} and A β _{1–42} were purchased from Anaspec. Proteins were dissolved in deuterated hexafluoroisopropanol to deuterate exchangeable sites and to promote disaggregation of the protein samples, and the hexafluoroisopropanol was removed by lyophilization prior to addition of aqueous buffers. Concentration measurements for each protein were determined in aqueous solution and made via 280 nm extinction coefficients using a NanoDrop 2000

(Thermo Scientific). The A_{280} coefficient used for both proteins was $1490 \text{ M}^{-1} \text{ cm}^{-1}$.³⁹ Proteins aggregated spontaneously upon dissolution in D_2O buffers at pD 7.5 or 2.0 as indicated in the text. Protein samples are placed between a pair of 2 mm thick CaF_2 windows separated by a $56 \mu\text{m}$ Teflon spacer for 2D IR experiments. Transmission electron microscopy (TEM) images were collected on a Phillips CM120 at the UW-Madison Medical School Electron Microscopy Facility.

Simulations of 2D IR Spectra

Two-dimensional IR vibrational spectra were simulated using transition dipole coupling^{40–42} to compute the couplings between amide I modes in structures obtained from the protein data bank (PDB). Couplings between nearest neighbor residues were computed separately from dihedral angles using the map of Jansen et al.⁴³ Diagonal disorder (full width at half-maximum (FWHM) 25 cm^{-1}) was added to the local mode frequencies. The computed stick spectra were convoluted with a two-dimensional Gaussian lineshape (FWHM 18 cm^{-1} along the diagonal) elongated along the diagonal to simulate the 2D IR lineshape at $t_2 = 0$ waiting time, and the local mode frequency was set to 1668 cm^{-1} such that the 1625 cm^{-1} transition observed experimentally matched the frequency of the higher-frequency mode for the calculated structures. We note that the need for a higher local mode frequency (1668 cm^{-1}) than might be expected for an amide I transition is due to the Jansen map local mode site shifts being too far to the red for the dihedral angles associated with β -sheets. All of the calculations were performed in Matlab using a custom Matlab program called coupled oscillator spectrum simulator (COSMOSS).⁴⁴ COSMOSS is an open-source spectral simulation software available on the GitHub: <https://github.com/JJ-Ho/COSMOSS>.

RESULTS AND DISCUSSION

Two-Dimensional IR Spectra and TEM Images of $\text{A}\beta$ Oligomers and Fibrils

Two-dimensional IR spectra of $\text{A}\beta_{1-40}$ and $\text{A}\beta_{1-42}$ are collected over the course of 7 days along with corresponding TEM images to assess the presence of amyloid fibrils. Data are collected at both pD 7.5 and 2.0. At pD 2.0, $\text{A}\beta$ is known to form longer, straighter fibrils that are more similar to those observed in human brain tissue. Low pD also speeds the kinetics, and we used 0.04% NaN_3 in the pD 2.0 solution such that our results could be directly compared to earlier 2D IR studies on $\text{A}\beta_{1-40}$ that used the same solvent.^{45–48} At micromolar concentrations, aggregation of $\text{A}\beta$ can take days or weeks;² our experiments are performed at 1 mM so that the aggregation is complete in 7 days or less. The absorption spectra collected at 1 mM are similar to those previously reported at $100\text{--}500 \mu\text{M}$ ^{45–50} (see Figure S1 for linear spectra from this study), although we note that non-isotope labeled amide I bands are not always sensitive to details of amyloid β -sheets.

Figure 1 shows the representative TEM images and the 2D IR spectra at 30 min, 1 day, and 7 days after initiating aggregation by dissolving lyophilized samples in buffer, along with diagonal cuts through the 2D spectra. In all of the samples, the TEM images at 7 days exhibit fibrils, as expected. In contrast, the TEM images collected 30 min after aggregation is initiated show small amorphous structures consistent with many previous reports of $\text{A}\beta$ oligomers and protofibrils. The TEM images at 1 day are either those of oligomers/

protofibrils, fibrils, or a mixture, depending on the A β isoform and pD. The corresponding 2D IR spectra for all of the samples exhibit a strong set of peaks at 1625 cm⁻¹. These features are from the amide I modes (backbone carbonyl vibrations) of β -sheet transitions that are commonly used to monitor aggregation in the IR studies on A β ,¹⁶ as expected from earlier work on protofibrils/oligomers and fibrils of A β . In samples that contain oligomers/protofibrils, random coil features are also observed in the 1640–1670 cm⁻¹ region. The 1625 cm⁻¹ β -sheet feature is always the dominant feature because the 2D intensities scale with the transition dipole strength to the fourth power,^{18–20} whereas linear spectra scale with the second power, as mentioned above.

Interestingly, in samples that contain fibrils according to TEM, we also observe a second pair of transitions at 1610 cm⁻¹. This 1610 cm⁻¹ transition is observed for both A β _{1–40} and A β _{1–42}. The transition is marked with an asterisk in the diagonal cuts and 2D spectra in the spectra where it is observed. It only appears in samples where fibrils are observed in the TEM images and appears to correlate with the relative amounts of fibril versus amorphous structures, although quantifying morphologies with TEM is very difficult. In the 7 day samples, the intensity is about 25% of the main amide I β -sheet peak at 1625 cm⁻¹, except for A β _{1–40} at pD = 2, where it is about 10%. Figure S2 shows fits to the diagonal traces of the equilibrated samples after 1 week, which are consistent with these relative intensities. In principle, the 2D lineshapes could provide additional information, though we do not fit them here because there is no significant difference in inhomogeneous broadening between the two bands. Though low pD speeds equilibration of the samples to their final state, A β _{1–40} at low pD still shows less intensity at 1610 cm⁻¹ when equilibrated. It thus appears that the 1610 cm⁻¹ transition is indicative of amyloid fibrils and can be used to positively identify the presence of A β fibrils. This band is not resolved in the broader linear infrared spectra of A β fibrils, as it is not visible in published linear spectra of A β fibrils,^{49–51} and we also do not observe it in linear spectra (Figure S1 shows the FT-IR spectra under the same conditions used in Figure 1).

The 1610 cm⁻¹ Absorption is an Amide I Mode

The amide I transitions of most amyloid forming proteins fall between 1610 and 1630 cm⁻¹.¹⁶ Thus, the 1610 cm⁻¹ transition could be created by a backbone vibrational mode (i.e., an amide I mode), but it would be among the lowest frequency amyloid transitions reported. Some side chains also have absorbances that fall in this region. In the A β sequence, only the arginine at residue 5 could exhibit an absorption in this region.⁵² To test if arginine is the origin of this transition, we collected the spectra of the R5G mutant fibrils under the same conditions as above. Shown in Figure 2 are the TEM and 2D IR spectra of the mutant fibrils generated in pD 2 and 7.5 buffers after several days of incubation in buffer. Consistent with the observations for the native sequences, aggregation occurs more quickly at pD 2. In both samples, long fibrils are formed and the amyloid β -sheet transition is also observed at 1625 cm⁻¹, indicating that the R5G mutation has little effect on the structure of the fibrils. In addition, the 1610 cm⁻¹ transition is observed.

We also note that the anharmonic shift of the 1610 cm⁻¹ transition (which is the frequency difference between the blue fundamental transition and the corresponding red sequence band

transition) is about 14 cm^{-1} , similar to that of the 1625 cm^{-1} transition. Anharmonic shifts less than ca. 20 cm^{-1} are typical of delocalized vibrational modes.^{53–55} Thus, we conclude that the 1610 cm^{-1} mode is created by a backbone amide I vibration with an atypically low frequency.

Stability of the 1610 cm^{-1} Species toward Sodium Dodecyl Sulfate (SDS) Independently Confirms It Represents Amyloid Fibrils

We next investigated the correlation of the 1610 cm^{-1} mode with $A\beta$ stability, as measured by the ability of SDS micelles to disrupt the structures present in our aggregated samples. SDS is known to disaggregate/solubilize nonfibrillar β -sheet-rich oligomers but not amyloid fibrils and to stabilize a nonaggregated, partially helical state.^{56–58} The behavior of SDS toward aggregates can cause inaccurate results when studying oligomer sizes,^{59–62} but it is useful for distinguishing amyloid fibrils from other partially aggregated structures.^{57,58} Thus, the addition of SDS to preformed $A\beta$ aggregates is an additional assessment of the presence of amyloid, allowing us to determine if the 1610 cm^{-1} mode corresponds to protofibrils/oligomers versus the presence of amyloid, independent of the TEM measurements in Figures 1 and 2.

SDS micelles are $3.2\text{--}4.2\text{ nm}$ in diameter⁶³ and have an aggregation number of ~ 62 , which means that there are an average of $0.62\text{ }A\beta$ molecules per micelle for a $1\text{ mM }A\beta$ sample in 100 mM SDS .⁶⁴ Figure 3a shows the 2D IR spectra of lyophilized $A\beta_{1-40}$ and $A\beta_{1-42}$ reconstituted in 100 mM SDS without any earlier incubation. Two maxima are observed for each $A\beta$ isoform. The maxima for $A\beta_{1-40}$ are at 1639 and 1647 cm^{-1} , whereas the maxima for $A\beta_{1-42}$ are at 1636 and 1647 cm^{-1} . The spectra also show a higher frequency shoulder representing the disordered peptide structures. The transitions at 1647 cm^{-1} are consistent with α -helical structure as reported from the NMR studies, and the lower frequency transition in each may correspond to a second distinct α -helical structure, or it is possible that a fraction of the protein still adopts a nonamyloid β -sheet conformation under these conditions, as this frequency is also consistent with the range expected for native β -sheets.¹⁶ Comparison to the $A\beta$ spectra in the absence of micelles confirms the absence of extended, amyloid-like β -sheet aggregates.

To study the SDS-induced disruption of aggregates (disaggregation) in samples that have already been allowed to incubate in buffer (thus preforming aggregates), 1 mM solutions of $A\beta_{1-40}$ and $A\beta_{1-42}$ are allowed to incubate in buffer for some time, at which point, an equal volume of 100 mM SDS is added to the solution and 2D IR spectra are collected. These conditions again result in a ratio of $0.62\text{ }A\beta$ molecules per micelle. Figure 3b shows the 2D IR spectra of $A\beta_{1-40}$ allowed to aggregate in $\text{pD }7.5$ buffer for 30 min , at which point SDS micelles are added and the 2D IR spectrum is collected 30 min later. Consistent with our expectations from Figure 1, no 1610 cm^{-1} transition is present after 30 min of aggregation, indicating no fibrils are present in the sample. Within 30 min of adding SDS, the sample is completely disaggregated, judged by the comparison of the 2D spectrum in Figure 3b (right) to those in Figure 3a. Specifically, the peak frequency blue shifts to well above the characteristic β -sheet rich/amyloid region, the line width broadens significantly, and the

anharmonicity increases. The fact that SDS has disrupted the β -sheet structure indicates that only nonamyloid oligomers were present and have been disassembled.

In additional experiments, we allow 1 mM $A\beta_{1-42}$ to aggregate for 24 h in pD 2.0 solution, allowing the fibrils time to mature, and monitor the kinetics of their disaggregation by SDS micelles. In this case, we did observe the 1610 cm^{-1} transition, and TEM images (refer to Figure 1) indicate that fibrils form under these conditions. We use rapid-scan 2D IR to track the course of disaggregation, and kinetic traces are shown in Figure 3c for the 1625 cm^{-1} (both oligomers and fibrils) and 1610 cm^{-1} (fibril only) transitions. Representative diagonal slices through the 2D spectra are shown below the kinetic traces; 30 min into disaggregation, both the 1625 and 1610 cm^{-1} transitions are observed. As disaggregation proceeds, the β -sheet transition partially decays, along with a rise in the higher-frequency region at ca. $1635\text{--}1650\text{ cm}^{-1}$. The 1610 cm^{-1} transition does not decay, indicating that the 1610 cm^{-1} transitions correspond to mature amyloid species that are not disaggregated by SDS micelles. These observations in Figure 3c are representative of our observations in general; SDS is not able to disaggregate the amyloid species corresponding to the 1610 cm^{-1} transition.

We are also interested in whether we can prepare samples so strongly aggregated that SDS micelles cannot disaggregate them to any significant extent. We allow $A\beta_{1-42}$ to aggregate for 6 weeks in pD 2.0 solution. These amyloid fibrils are very stable toward SDS micelle disaggregation, showing very little disaggregation even 3 weeks after SDS micelles have been added (Figure 3d).

The observations in SDS provide further evidence that the 1610 cm^{-1} transition is associated with stable amyloid fibrils. Because the 1625 cm^{-1} mode is always present when there is also a 1610 cm^{-1} mode, they must both be coming from the same fibers, not polymorphs each with a single distinct absorption frequency. It is likely that multiple polymorphs are present, and that one (or more) give(s) rise to transitions at both 1610 and 1625 cm^{-1} , whereas others may only exhibit transitions at 1625 cm^{-1} . We investigate this further with simulations below.

Simulating the 2D IR Spectra of $A\beta$ Fibrils

To test the possible structural origins for the 1610 cm^{-1} mode, we simulate the spectra using structures of $A\beta$ fibrils taken from the protein data bank (PDB). Our goal with these simulations is to establish whether published $A\beta$ structures can produce the 1610 cm^{-1} 2D IR peaks observed experimentally. The 1625 cm^{-1} mode has been simulated many times in the past with model β -sheets.^{53,65–69} Because 2D IR spectra are very sensitive to short-range couplings, we opted to simulate our $A\beta$ 2D IR spectra using the crystal structures formed from fragments of $A\beta$, as X-ray structures generally provide the highest structural resolution (ca. $2\text{ \AA}$). Accordingly, we calculated the 2D IR spectra for several published $A\beta$ crystal structures, and show the results from two structures here (Figure 4b). These two structures are taken from the coordinates of PDB file 2y3k, which is the crystal structure of $A\beta$ fragment 35–42.⁷⁰ This PDB file contains coordinates for four geometries of strands that are only slightly different from one another, but all fall within the crystal structure accuracy. On average, the carbonyl groups for the structure on the left are about 5° more parallel to their

nearest interstrand neighbors, and the strands are spaced ca. 0.3 Å closer together than those on the right. Even though these differences are small, 2D IR spectra are very sensitive to local structure. The simulated spectrum of the left structure has its strongest ($A_{\perp}$, named for the orientation of the transition dipole relative to the β -strands) transition at 1611 cm^{-1} , whereas the A transition of the right structure is at 1625 cm^{-1} (details of the simulations are found in the Materials and Methods section). To reproduce the experimental data, the spectra are added in a 1:4 ratio (vide infra). The 2D spectrum and diagonal slice are shown in Figure 4c. This 14 cm^{-1} difference is caused by the couplings due to differences in dihedral angles, interstrand spacings, and relative amide I mode orientations. The 1611 cm^{-1} absorption is created by interstrand couplings (β_{ISC}) that are about 5 cm^{-1} larger than those for the structure on the right, associated with more parallel β -strands and shorter distances between residues in adjacent strands. The couplings between neighboring residues are very sensitive to the relative orientation and distance between the local mode transition dipoles,^{40–42} such that the narrower interstrand distances and more aligned amide oscillators (mentioned above) of the structure on the left lead to the 5 cm^{-1} average change in interstrand couplings. Because the frequency difference scales as $2*\beta_{\text{ISC}}$, these couplings account for 10 cm^{-1} out of 14 cm^{-1} of the frequency difference between the structures. Couplings between nearest neighbors and other residues account for the remainder of the frequency difference. We note that, across amyloid species, these couplings depend on the structure of the fibril and not necessarily its age or maturity.²⁰ Figure S3 shows the individual simulated spectra, and Figure S4 shows the principle eigenstate responsible for the IR intensity for each in the case with no diagonal disorder. Although a doorway mode analysis^{71,72} could be performed for the case with diagonal disorder turned on, a molecular eigenstate picture is appropriate for describing the spectra of these highly ordered β -sheet structures in which a single mode is responsible for nearly all of the IR intensity. Figure S5 shows an overlay of the simulated diagonal trace with the experimental data. A recent study has also examined the effects of varying coupling constants in simulated amyloid β -sheets on the frequencies and intensities of the amide I transitions.²⁰ Experimentally, solvent exposure may also play a role in shifting the frequencies of the individual β -sheet regions.

Amyloid fibrils often contain multiple β -sheets. Each β -sheet within a fibril will have its own $A_{\perp}$ mode and corresponding intensity and frequency. Coupling between β -sheets is a minor perturbation because apposing β -sheets have small vibrational coupling, and adjacent sheets are separated by loops and turns.⁷⁴ Figure 4a shows a structure for a full-length $A\beta$ fibril to illustrate the arrangements of multiple β -sheets within a fibril (PDB: 2mxu⁷³). With this idea in mind, we added together the 2D IR spectra for the two structures in Figure 4b in a 1:4 ratio to produce the spectrum shown in Figure 4c. Figure 4c is very similar to that observed experimentally for $A\beta$ fibrils (Figures 1 and 2). The addition of the spectra is intended to model distinct β -sheet regions in a full-length $A\beta$ fibril, each having its own $A_{\perp}$ mode. Thus, we conclude that 1610 cm^{-1} is a marker for amyloid fibers and that our experimental results are consistent with an $A\beta$ fibril that has multiple β -sheet regions.

We note that previously reported 2D IR spectra^{45–48} of $A\beta_{1-40}$ contained a transition at 1610 cm^{-1} , although it is not assigned nor mentioned in the article, which focuses on isotopic labeling (see Figure 4 of ref 45). To our knowledge, 2D IR spectra of $A\beta_{1-42}$ have not been previously reported.

SUMMARY AND CONCLUSIONS

In summary, we have used 2D IR spectroscopy to reveal a vibrational transition that allows us to differentiate samples containing A β fibrils from those containing other β -sheet-rich structures, such as oligomers and protofibrils, as verified by TEM and SDS disaggregation. As has been noted previously,^{59–62} the observation that SDS disaggregates β -sheet-rich A β oligomers carries broad relevance for A β and other amyloid aggregation research, where SDS is commonly used to characterize sizes and relative abundances of protein aggregates and fibrils by gel electrophoresis or other methods. Our evidence indicates that the 1610 cm⁻¹ transition stems from a delocalized amide I mode, and spectral modeling of amyloid structures suggests the lower transition frequency of this mode can be explained by differences in β -sheet structure of different regions of the fibril. It is worth noting that similar observations could exist for other amyloid species, as amyloid fibrils are characterized by specific fibril structures that involve multiple β -sheets;^{75–78} the enhanced ability of 2D IR spectroscopy to resolve overlapping lineshapes may continue to prove powerful in this regard. The ability to spectrally distinguish A β fibrils from β -sheet-rich oligomers carries broad implications for AD research into the aggregation, toxicity, and fibril formation of A β . For example, infrared spectroscopy has recently been demonstrated to be a powerful tool for the early prediction and detection of AD by monitoring the amide I spectra of A β extracted from human blood and cerebrospinal fluid using antibodies,^{79,80} and the additional structural sensitivity of 2D IR could allow direct detection of fibrils in antibody-extracted A β samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Two-dimensional IR spectra of 1 mM $A\beta$ aggregated for 30 min, 1 day, and 7 days, diagonal slices through the fundamental transition, and representative TEM images for the following samples: (a) $A\beta_{1-40}$ in pH 7.5 20 mM Tris buffer, (b) $A\beta_{1-42}$ in pH 7.5 20 mM Tris buffer, (c) $A\beta_{1-40}$ in pH 2.0 0.04% NaN_3 solution, and (d) $A\beta_{1-42}$ in pH 2.0 0.04% NaN_3 solution.

Figure 2.

Two-dimensional IR spectra and diagonal slices of 1 mM $A\beta_{1-42,R5G}$ collected after 6 days of aggregation in pD 2.0 0.04% NaN_3 solution and after 14 days of aggregation in pD 7.5 20 mM Tris buffer and corresponding TEM images. Fibril formation in the pD 7.5 buffer took longer than in the pD 2.0 buffer. Similar to the 2D IR spectra of $A\beta_{1-40}$ and $A\beta_{1-42}$ fibrils shown in Figure 1, a transition is observed at ca. 1610 cm⁻¹.

Figure 3.

(a) Two-dimensional IR spectra and diagonal slices of 1 mM Aβ₁₋₄₀ and Aβ₁₋₄₂ reconstituted directly into 100 mM SDS. (b) Two-dimensional IR spectra and diagonal slices of Aβ₁₋₄₀ aggregated for 30 min in pD 7.5 Tris buffer and 30 min after the addition of an equal volume 100 mM SDS. (c) Kinetic traces from rapid scan 2D IR in the β-sheet and α-helical regions for Aβ₁₋₄₂ aggregated for 1 day in pD 2.0 NaN₃ solution, and diagonal slices at various disaggregation times scaled to the maximum intensity. (d) Two-dimensional IR spectra of Aβ₁₋₄₂ aggregated for 6 weeks in pD 2.0 NaN₃ solution and 3 weeks after the addition of an equal volume 100 mM SDS.

Figure 4.

(a) ssNMR structure of an A β fibril obtained from PDB structure 2mxu.⁷³ (b) Atomic structures of A β _{35–42} fragments from PDB file 2y3k from which β -sheets were generated as described in the text. (c) The 2D IR spectrum simulated as a mixture of the two structures from panel (b) added in a 1:4 ratio to simulate the spectrum observed experimentally. Note that the local mode transition frequency was shifted to best match the experimental data, though the difference in frequency is unaffected by this shift. Details of the calculations are given in Materials and Methods section.