Local orientational disorder in peptide fibrils probed by a combination of residue-specific 13C-18O labelling, polarised infrared spectroscopy and molecular combing

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Local orientational disorder in peptide fibrils probed by a combination of residue-specific ^{13}C-^{18}O labelling, polarised infrared spectroscopy and molecular combing

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A novel combination of site-specific isotope labelling, polarised infrared spectroscopy and molecular combing reveal local orientational ordering in the fibril-forming peptide YTIAALLSPYSGGRADS. Use of 13C-18O labelled alanine residues demonstrates that the N-terminal end of the peptide is incorporated into the cross-beta structure, while the C-terminal end shows orientational disorder

Many different proteins and peptides self-assemble into “amyloid” fibrils. Such fibrils are associated with a range of diseases;[1] in addition, self-assembly of other proteins and designed peptides raises the possibility of their use as potential nanomaterials.[2] Amyloid fibrils share a common core “cross-β” structure, as demonstrated by fiber X-ray diffraction,[3] where strands are arranged perpendicular to the fibril, held together in sheets by hydrogen-bonding between NH and CO groups with bonds running up and down the fibril axis. It is very difficult to obtain more detailed structural information on polypeptide conformation within different fibrils, because in general they are not amenable to analysis by x-ray crystallography.

Infrared linear dichroism (IRLD) uses plane polarized IR light to provide orientational information on the vibrating dipole moments in oriented samples.[4] There are several previous examples of the use of polarized infrared spectroscopy to obtain orientational information relative to the long axis of protein fibres. These include the work of Ambrose and Elliott[5] and Burke and Rougvie[6], who investigated films of fibrous insulin samples, and Hiramatsu and coworkers,[7] who studied fibrils formed from the protein β2-microglobulin and synthetic peptides based on its sequence, using IRLD microscopy on oriented particles within pellets. Recently we have developed the technique of molecular combing to produce reproducible oriented films of amyloid fibrils from hen lysozyme and model peptides.[8] IRLD on oriented amyloid samples gives an Amide I band at around 1630 cm^{-1} polarised parallel to the fibril axis, confirming the cross-β strand conformation.[5-6] In some cases, a higher-frequency peak with orthogonal polarisation is seen, demonstrating the anti-parallel configuration.[7b, 8]

These experiments only give information averaged over the entire protein or peptide, and do not give residue-specific information on backbone conformation. Site-specific data may be obtained with vibrational spectroscopy using isotope labelling, also known as isotope editing, as the change in reduced mass causes a shift in frequency for the labelled site. The combination of isotope labelling with IRLD has been carried out on peptide crystals[9] and membrane proteins in oriented bilayers.[10] In this latter work, the Arkin group has pioneered the use of the ^{13}C-^{18}O label described in this Communication, to determine the orientation of a specific carbonyl group relative to the bilayer normal in membrane-bound proteins. Although labels on unoriented amyloid fibril samples have been used to demonstrate local interactions with standard[11] and 2D[12] IR, no IRLD experiments have thus far been performed on labelled amyloid fibrils. Here, we demonstrate the incorporation of a ^{13}C-^{18}O label into a 17-residue peptide, fibrils of which were oriented using molecular combing methods recently developed in our group.[8] The data obtained are used to probe local orientational ordering.

The peptide YTIAALLSPYSGGRADS has been studied as control for YTIAALLSPYSGGRGDS, which was designed to exhibit the RGD cell-binding motif on the fibril surface.[13] In this application, it is important that the C-terminus is flexible, so that it can interact with cell surface integrin receptors, while the N-terminus assembles into cross-beta fibrils. Residues 1-11 of the peptide sequence were taken from residues 105-115 of transthyretin, a known amyloidogenic protein,[14] and the GG linker chosen to add flexibility to the C-terminus. The RGD and RAD peptide fibrils gave identical small- and wide-angle x-ray scattering patterns,[13] indicating that the single amino-acid difference does not affect the core fibril structure, although interestingly, it does alter the kinetics of assembly and seeding behaviour.[15] The RGDS variant does indeed exhibit cell-binding, and small-angle x-ray scattering measurement of beta-sheet widths suggested that both 17-residue peptide variants were not wholly in the cross-beta confirmation, consistent with expectation.[13] Moreover, the thermodynamic stability of the RGDS variant is the same as that of YTIALLSPYS, the eleven residues of the N-terminus[16], suggesting that the C-terminus does not contribute to the hydrogen bonding that holds together the cross-beta core. However, no more precise details of the chimeric structure were demonstrated, due to the difficulty in obtaining residue-specific information.

We synthesised $^{13}C^{18}O$ alanine from the commercially obtained ^{13}C labelled amino acid; the oxygen labelling was carried out following Arkin's protocol.[14,17] The method yielded approximately 70% labelling (see mass spectra in Supporting Information). Two peptide variants with the sequence YTIAALLSPYSGGRADS were synthesised using standard Fmoc solid-phase synthesis techniques,[18] incorporating the 13C18O-labelled amino-acid into position 5 or 15. We denote these YTIAA*LLSPYSGGRADS and YTIAALLSPYSGGRA*DS respectively. The mass of each peptide was

confirmed by electrospray mass spectrometry which showed a dominant peak at the expected mass of 1743.9 Da (3 Da higher than the expected monoisotopic mass for the unlabelled peptide); each sample was >97% pure following purification by high performance liquid chromatography. Fibrils were formed following published methods13 by dissolving the solid peptides in a solution of 90% water (purified to a resistivity of 18.2 mΩ) and 10% acetonitrile at a concentration of 10 mg/mL. The solutions were vortex mixed for 10 minutes and incubated at 37 °C for 24 hours followed by incubation at room temperature for at least 4 weeks. The two different labelled peptides form fibrils adopting the same morphology as one another, and as the unlabelled peptide fibrils published previously,[13] as shown by the transmission electron micrographs and fiber x-ray diffraction patterns shown in Figure 1. The diffraction patterns both show axial and equatorial reflections at approximately 4.7 and 8.9 Å respectively (exact values given in figure legend), in agreement with the unlabelled fibrils,[13] demonstrating the characteristic cross-beta structure with 4.7 Å between strands and 8.9 Å between beta-sheets. The isotope label itself therefore does not affect the fibrils' internal structure.

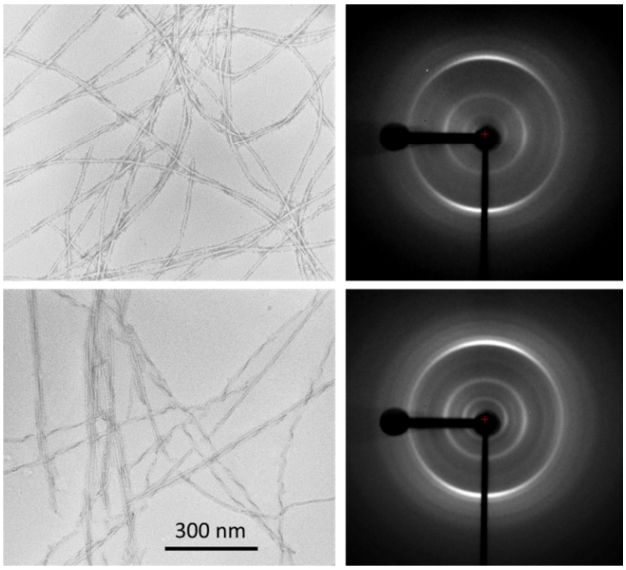

Figure 1: TEM images (left) and fiber x-ray diffraction patterns (right) of fibrils formed from YTIAA*LLSPYSGGRADS (top) and YTIAALLSPYSGGRA*DS. (bottom). Equatorial reflections are at 8.88 ± 0.06 Å and 8.86 ± 0.03 Å respectively. Axial reflections are at 4.72 ± 0.03 Å in both samples. In both x-ray diffraction patterns, the fibril alignment direction is vertical. Both TEM images are shown at the same magnification.

Unpolarised infrared spectra of dried films of the two different labelled peptide fibrils are shown in Figure 2, together with a spectrum from the unlabelled sample. All samples show an intensity maximum in the amide I region at about 1630 cm-1, as expected from the high beta-sheet content of the fibrils.[19] There are, however, significant differences that emerge as a result of the labelled alanine residue. Most importantly, both labelled samples show the additional red-shifted 13C18O stretch at around 1590 cm-1 (illustrated with a star in the figure). Other differences can be seen in the amide I region, especially for the peptide labelled in position Ala-5, YTIAA*LLSPYSGGRADS. This is most probably due to the presence of the label disrupting the coupling between the unlabelled oscillators,[12] suggesting that Ala-5 lies within the extended beta-sheet region.

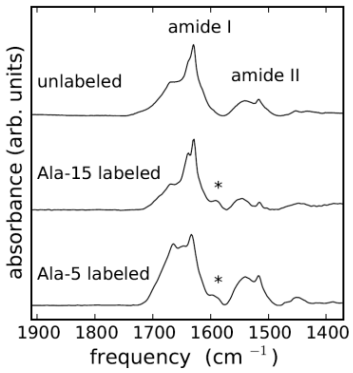

Figure 2: FTIR spectra of fibrils formed from labelled and unlabelled YTIAALLSPYSGGRADS. The CO stretch from the labelled residues, marked with a star in the figure, is visible at approximately 1590 cm^{-1}. Spectral intensities are scaled with respect to the amide I band.

In order to analyse the orientation of the labelled $^{13}C^{18}O$ groups, aligned films of the two different labelled peptide fibrils were prepared using a molecular combing technique that we have described previously.8 Briefly, 10 μL droplets of fibril suspension were blown along individual CaF2 IR windows using a stream of air, which induces fibril alignment in the flow direction. Polarised infrared spectra were then obtained parallel and perpendicular to this axis. The spectra are shown in Figure 3. For both samples, differences between the orthogonally polarised spectra demonstrate some degree of overall sample alignment. In particular, the peaks at around 3280, 1630 and 1540 cm-1 corresponding to the Amide A, unlabelled Amide I and Amide II vibrations respectively show positive, positive and negative peaks in the (parallel-perpendicular) difference spectra. The Amide A NH stretch and Amide I CO stretch are therefore oriented parallel to the fibril axis, while the Amide II, which is predominantly a stretch along the beta-strand, is oriented perpendicular to it. This is to be expected for a cross-beta structure.[8]

In the YTIAA*LLSPYSGGRADS sample, the 1587 cm-1 band from the labelled Ala-5 residue shows significant positive dichroism, indicating that the $^{13}C^{18}O$ group is close to parallel to the fibril axis, and therefore that it too is incorporated into the extended cross-beta structure.

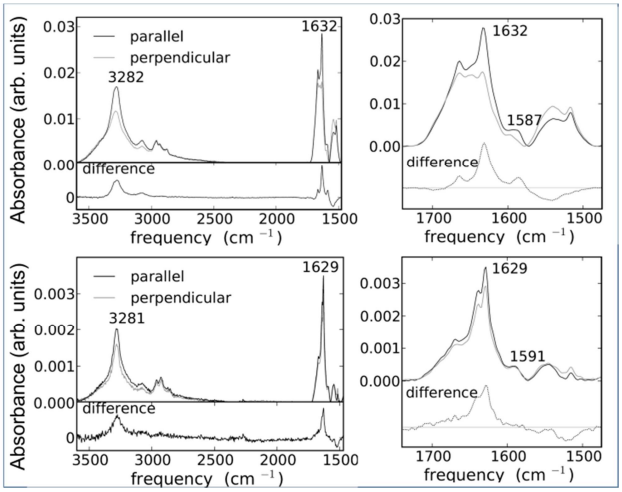

Figure 3: Polarised infrared spectra of aligned fibrils from YTIAA*LLSPYSGGRADS (top) and YTIAALLSPYSGGRA*DS (bottom), showing full spectra (left) and amide I and II region (right). Difference spectra are defined as parallel minus perpendicular, and are scaled with respect to the amide I difference peak.

In contrast, the band from the labelled Ala-15 residue in the YTIAALLSPYSGGRA*DS sample shows no significant difference between the two different polarisations. This indicates that its 13C18O group is either disordered, or else oriented close to the magic angle[20] of arccos(1/√3), approximately 55° from the fibril axis. We believe that a disordered structure, due to the flexibility of the GG residues, is more plausible. This is particularly significant in light of the suggestions that the C-terminus influences the kinetics[15] but not the thermodynamics[16] of the formation of these fibrils.

A proposed structure is illustrated in Figure 4. This is depicted assuming that the peptide strands are parallel rather than anti-parallel, an assumption based on previous suggestions for YTIAALLSPYSGGRADS[13] and the fact that this arrangement has been demonstrated for YTIAALLSPYS[21].

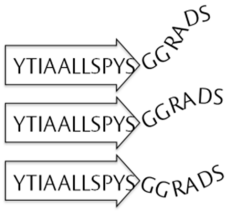

Figure 4: Suggested structure for YTIAALLSPYSGGRADS peptide in a fibril.

Conclusions

Our data confirm that the YTIAALLSPYSGGRADS peptides adopt a chimeric structure in the fibrils, as suggested by previously published x-ray scattering data.[13] The novel combination of molecular combing, polarised spectroscopy and isotope labelling elucidate the nature of this structure, whereby the ordered cross-beta structure is adopted only by the N-terminal part of the peptide.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Mass Spectrum of isotope labelled amino-acid

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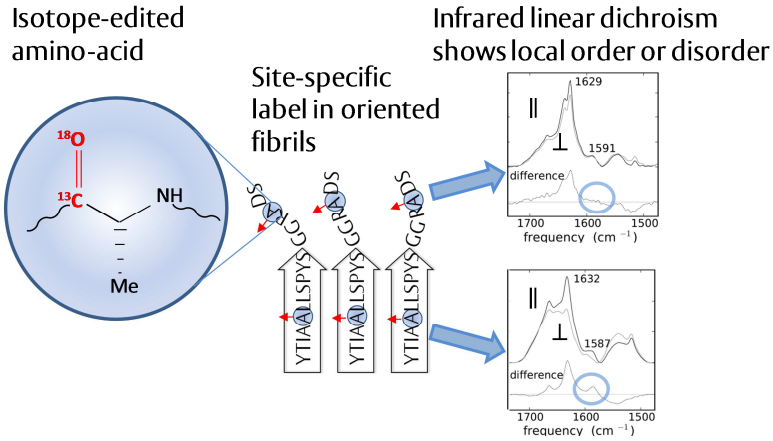
Graphical Abstract



Textual Abstract

Local disorder at the C terminus of a functional fibril-forming peptide is revealed by a novel combination of site-specific 13C-18O labeling, and infrared linear dichroism spectroscopy applied to fibrils macroscopically oriented by molecular combing