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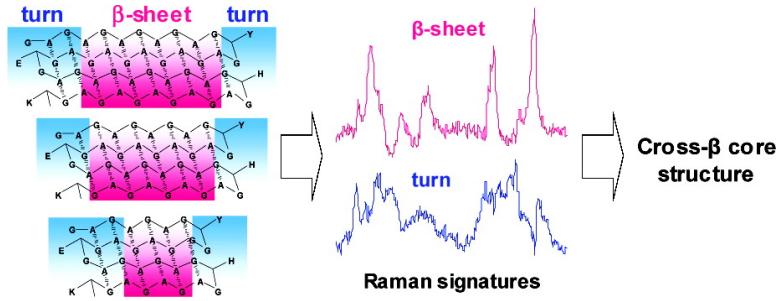

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Genetic Engineering Combined with Deep UV Resonance Raman Spectroscopy for Structural Characterization of Amyloid-like Fibrils

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Structural characterization of amyloid fibrils is crucial for the detailed understanding of fibrillogenesis at a molecular level. The classical tools of structural biology, single-crystal X-ray diffraction and solution NMR, have profound limitations when applied to amorphous or insoluble amyloid fibrils.[1] Transmission electron microscopy (TEM) and scanning probe microscopy (SPM) provide general information on fibril topology and morphology.[1,2] Several specialized techniques have been recently developed for probing fibrillar structure.[3−7] X-ray diffraction can provide atomic-level insight into the β-strand assembly of microcrystals formed from short peptides (5−7 amino acid residues), thereby mimicking the core structure of fibrils formed from amyloidogenic proteins.[8,9] However, similar crystallographic data for full-length proteins are not yet feasible.[6] Deep UV resonance Raman (DUVRR) spectroscopy is a powerful tool for protein structural characterization at all stages of fibrillation.[10,11] We have recently reported on the application of DUVRR spectroscopy combined with hydrogen−deuterium exchange for structural characterization of lysozyme fibril cross-β core.[12] Herein, DUVRR spectroscopy was utilized for the structural characterization of large amyloid-like fibrils prepared from three de novo designed polypeptides consisting of seven repeats of 32, 40, or 48 amino acid repeats, $(GA)_nGY(GA)_nGE(GA)_nGH(GA)_nGK$ (n = 3, 4, or 5, respectively, 32YEHK7, 40YEHK7, or 48YEHK7). The YEHK polypeptides forms well-organized fibrillar structures with topology consistent with the cross-β core structure shown in Figure 1A. The Raman signature of core β-strands was the same for all three types of fibrils, indicating a similar structure. For the first time, the Raman signature of fibril turns was also obtained. No contribution to the fibril Raman spectra from an unordered polypeptide was found. On the basis of Asher's method,[13] the Raman signatures were assigned to an antiparallel β-sheet and, tentatively, β-turns. The Ramachandran angles Ψ were also evaluated. The narrow distribution of the Ψ Ramachandran angles is indicative of the uniformity of the β-strand assemblage. Comparing current results with those reported for lysozyme fibrils allows us to conclude that the structure of the fibril cross-β core depends on the polypeptide sequence.

These studies were enabled by the de novo design of β-sheet-forming repetitive polypeptide blocks with selected amino acids at the turn positions to facilitate control of coacervation and formation targeted amyloid-like fibrils. DNA sequences coding β-sheet-forming polypeptides were prepared utilizing concatenation/block copolymerization in the presence of adaptive DNA sequences containing the recognition sites of type II and IIs restriction endonucleases.[14] The repetitive YEHK-coding sequences were prepared by ligation of the synthetic oligonucleotides coding for the appropriate strand Y ($(GA)_nGY$) and E ($(GA)_nGE$) (n = 3, 4, or 5) in the presence of adaptive DNA sequences. In a similar manner, the H ($(GA)_nGH$) and K ($(GA)_nGK$) (n = 3, 4, or 5) strands were ligated. These two fragments were subsequently joined

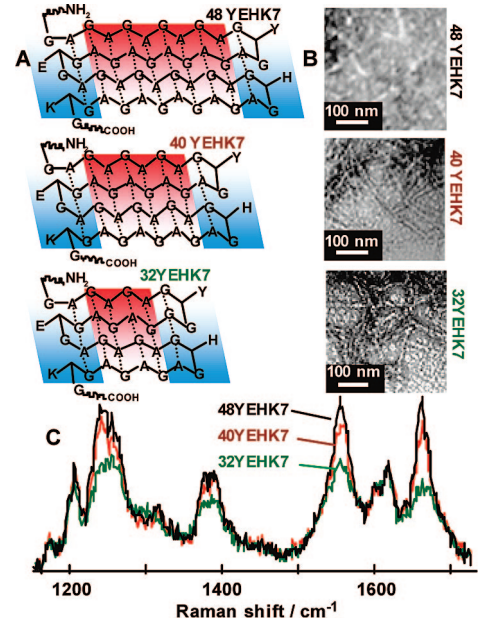

Figure 1. (A) Hypothetical scheme of YEHK fibril cross-β core. (B) TEM micrographs of YEHK fibrils on carbon coated Cu grids. (C) DUVRR spectra of YEHK fibrils.

in the presence of the adaptive DNA to yield the necessary 32, 40, or 48 amino acid building blocks which were oligimerized to the desired heptameric repeat structures. The details of fibril preparation and DUVRR spectroscopic measurements were reported earlier[15,16] and are briefly described in Supporting Information. Fibrils prepared from all three peptides were purified from soluble species by consecutive centrifugation and redispersion.[17] TEM images of the redispersed species have typical fibrillar structures (Figure 1B). DUVRR spectra (Figure 1C) of all three polypeptides exhibit characteristic features of a fibril cross-β core.[12] Consistent with the postulated structural similarity of fibrils derived from the three peptides, the three differential Raman spectra (all possible combinations) were identical to each other within the experimental error (Figure S1). The tyrosine Raman band at 1620 cm^{-1} was used for spectral normalization. The least noisy differential spectrum (48YEHK7−32YEHK7, spectrum "β-sheet" in Figure 2) was adopted as the Raman signature for the β-strand portion of the fibrils. The pure variable method (PVM) was utilized for determining the second component (Figure 2, "turns") of the two-element set, which was assigned as the Raman signature of turns. An excellent fit of all three spectra with the two components (Figure S2) confirmed the structural similarity of the three fibrillar types and hence the validity of our approach.

The obtained Raman signatures of the fibril core were used for evaluating the peptide backbone conformation based on Asher's approach.[13] The dominant 1241 cm^{-1} peak in the Am III region

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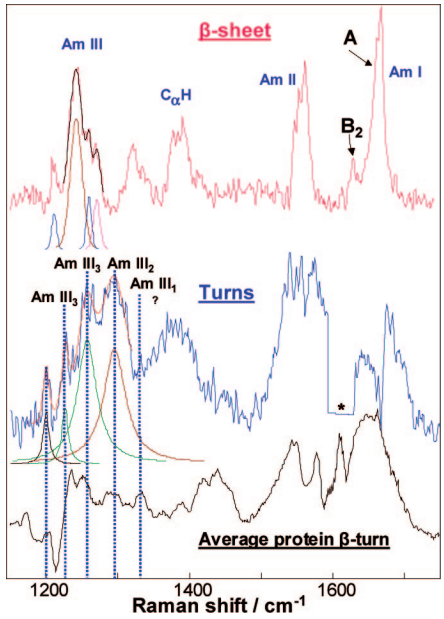

Figure 2. DUVRR spectra of YEHK fibril β-sheet and turns, and the average spectrum of native protein β-turns adopted from ref 18 Amide I (Am I) band is due to carbonyl C═O stretching, with a small contribution from C−N stretching and N−H bending. Am II and Am III bands involve significant C−N stretching, N−H bending, and C−C stretching. The C_α−H band involves C_α−H symmetric bending and C−C_α stretching. (*) Spectral range of strong contribution of aromatic amino acids.

of the β-sheet spectrum (Figure 2) corresponds to a Ramachandran angle of $\Psi = 150°$ as would be associated with an antiparallel β-sheet structure. Additional study would be required to assign the small Raman peaks in the Am III range. It is noteworthy that the β-sheet spectrum does not have a noticeable contribution from the unordered YEHK polypeptide (1299 cm^{-1} band)[17] or turns (Supporting Information). The Am II frequency (1556 cm^{-1}) corresponds with the value assigned to the β-sheet structure of globular proteins[18] and homopolypeptides,[19] although the peak is much narrower in the case of the YEHK fibrils. The Am I region of the β-sheet spectrum comprises two vibrational modes, **A** (1665 cm^{-1}) and $\mathbf{B_2}$ (1629 cm^{-1}), described in detail in Supporting Information.[12] The observed shift of these frequencies in the case of YEHK fibrils, with respect to those found in the study of lysozyme,[12] can be tentatively attributed to stronger hydrogen bonding associated with the YEHK fibrils in comparison to lysozyme fibrils.

The Ramachandran Ψ angular distribution determined from the β-sheet spectrum of YEHK fibrils (Figure S2) is much narrower than that attributed to a "pure" β-sheet prepared from poly-L-lysine-poly-L-glutamic acid.[19] The Am II and Am III peaks from the latter are very narrow as well, 18 and 14 cm^{-1}, respectively, suggesting that the YEHK fibril β-sheet is highly ordered. These findings are consistent with our report on lysozyme fibrils,[12] where no inhomogeneous broadening was found for the cross-β core Raman signature.

The calculated YEHK "turn" spectrum (Figure 2) has many similarities with the "average protein β-turn" Raman spectrum reported by Spiro.[18] The Am III region of the YEHK turn spectrum is well-fitted by three peaks. Two of these features (1228 and 1256 cm^{-1}) correspond well with the Am III_3 peaks of β-turn type I (1223 and 1260 cm^{-1}) or type VIII (1226 and 1260 cm^{-1}) according to the Mikhonin and Asher assignments.[13] The third peak at 1294 cm^{-1} was tentatively assigned to an Am III_2 vibration.[20] There is no known definitive DUVRR spectra assignment of Am III_2 and Am III_1. However, since Ramachandran angles ($\Psi = -30°$, in particular) of $i + 1$ amide bond of a β-turn are close to those of an α-helix, similar Raman bands could be expected for both conformations. Indeed, the DUVRR spectrum[20] of an α-helix has three Am III lines at 1261 cm^{-1} (Am III_3), 1306 cm^{-1} (Am III_2), and 1337 cm^{-1} (weak, Am III_1), two of which are consistent with 1256 and 1294 cm^{-1} bands in the YEHK turn spectrum. A weak Am III_1 band might be obscured by the broad C_αH bending band of the YEHK turns. Three-dimensional modeling of the cross-β core based on the estimated Ψ Ramachandran angles for turns and β-sheet was consistent with the allowed bond distance−angle space of interacting polypeptide chains (Supporting Information). It is not possible at this point to distinguish type I and type VIII β-turns.

DUVRR spectroscopy is a novel method to acquire quantitative information on the peptide backbone conformation in large fibrillar aggregates. The first two examples of cross-β core Raman signatures, those obtained for YEHK polypeptide (this communication) and lysozyme,[12] indicate the very ordered, crystalline-like structure of the antiparallel β-sheet. No inhomogeneous broadening by diverse amino acids is evident, yet Ramachandran Ψ angle differs in the YEHK and lysozyme fibrillar β-sheets. To the best of our knowledge, this is the first direct evidence that the structure of the fibril cross-β core is sequence-dependent. We believe that the controlled tuning of the polypeptide sequence by genetic engineering is a great tool for studies of the relationship between the sequence and the cross-β sheet structure. The data generated via such study might be especially useful in computational modeling of the polypeptide sequence−fibrillar structure relationship.

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Supporting Information Available: Sample preparation and details on Raman spectra statistical analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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