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Structural Variations in the Cross-β Core of Amyloid β Fibrils Revealed by Deep UV Resonance Raman Spectroscopy

Ludmila A. Popova,[†] Ravindra Kodali,[‡] Ronald Wetzel,[‡] and Igor K. Lednev*[,†]

Department of Chemistry University at Albany, SUNY, 1400 Washington Avenue, Albany, New York 12222, and Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Avenue, Pittsburgh, Pennsylvania 15260

Received October 24, 2009; E-mail: lednev@albany.edu

Abstract: Understanding fibrillogenesis at a molecular level requires detailed structural characterization of amyloid fibrils. The combination of deep UV resonance Raman (DUVRR) spectroscopy and post mortem hydrogen−deuterium exchange (HX) was utilized for probing parallel vs antiparallel β-sheets in fibrils prepared from full-length Aβ_{1-40} and Aβ_{34-42} peptides, respectively. Using previously published structural data based on solid-state NMR analysis, we verified the applicability of Asher's approach for the quantitative characterization of peptide conformation in the Aβ_{1-40} fibril core. We found that the conformation of the parallel β-sheet in the Aβ_{1-40} fibril core is atypical for globular proteins, while in contrast, the antiparallel β-sheet in Aβ_{32-42} fibrils is a common structure in globular proteins. In contrast to the case for globular proteins, the conformations of parallel and antiparallel β-sheets in Aβ fibril cores are substantially different, and their differences can be distinguished by DUVRR spectroscopy.

Introduction

The structures and properties of amyloid fibrils are of considerable interest, due to their associations with a number of neurodegenerative diseases.[1,2] Amyloid fibrils are noncrystalline and insoluble aggregates and thus are not amenable to analysis by conventional X-ray crystallography and solution NMR, the classical tools of structural biology.[3] One of the key structural features of the fibril core, the parallel vs antiparallel arrangement of the polypeptide backbone, has been probed only by solid-state NMR (SSNMR), EPR, and fluorescence spectroscopy.[4−7] These techniques have the limitation, however, of requiring that protein samples be modified prior to analysis by incorporating isotopes (for SSNMR) or special probes (for EPR or fluorescence). Deep UV resonance Raman (DUVRR) spectroscopy is a powerful tool for protein structural characterization at all stages of fibrillation, and it does not require labeling.[8] In particular, the structure of the fibril cross-β core can be probed by combining DUVRR spectroscopy with post mortem hydrogen−deuterium exchange (HX).[9]

In this work, we describe the use of DUVRR spectroscopy to probe parallel vs antiparallel β-sheets in fibrils prepared from *unlabeled* full-length Aβ_{1-40} peptide (previously shown to be parallel[4,7]) and Aβ_{34-42} fragment (previously shown to be antiparallel[10]). The availability of the detailed conformation[11] of the Aβ_{1-40} backbone in fibrils obtained by solid-state NMR[7] allowed us to verify the applicability of Asher's approach[12] for the quantitative characterization of peptide conformation in the fibril core using DUVRR spectroscopy. In addition, we demonstrate here that the conformation of the parallel β-sheet in the Aβ_{1-40} fibril core is different from that of the β-sheet in globular proteins, while in contrast, the antiparallel β-sheet in Aβ_{32-42} fibrils is quite typical. In contrast to the case for globular proteins, the conformations of parallel and antiparallel β-sheets in Aβ fibril cores were substantially different from each other, and this difference can be distinguished by DUVRR spectroscopy.

Materials and Methods

Sample Preparation. Wild-type human Aβ_{1-40} with the sequence DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV was obtained in purified form through a large-scale custom synthesis from the Keck Biotechnology Center at Yale University. Fibrils were prepared as described previously,[4,13,14] but using a slightly modified protocol. Briefly, Aβ_{1-40} peptide powder was

[†] University at Albany, SUNY.
[‡] University of Pittsburgh School of Medicine.

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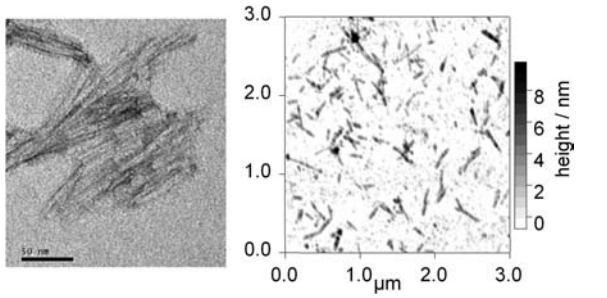

Figure 1. TEM image of Aβ_{1-40} fibrils (left panel) and AFM topology of Aβ_{34-42} fibrils (right panel). The scale bar on the left panel is 50 nm.

dissolved in 2 mM NaOH and the solution was sonicated for 5 min to dissolve the aggregates. This solution was mixed with an equal volume of 20 mM sodium phosphate buffer, and the final pH was adjusted to 7.4. A 1 mL portion of peptide solution at 210 μM was agitated continuously at 23 °C in a glass vial using a small stir bar on a magnetic stir plate from Thermolyne set at a stir rate of 4. Fibril formation is confirmed by using the ThT binding assay.[15] When the ThT reading reached a plateau, typically within 2−3 days, fibrils were harvested by centrifugation at 20 000*g* for 60 min in a tabletop centrifuge.

Aβ_{34-42} peptide with the sequence LMVGGVVIA was obtained in purified form through custom synthesis from the GenScript Corp., Piscataway, NJ. Fibrils were prepared as described previously.[13] Briefly, 1 mg of Aβ_{34-42} peptide powder was dissolved in 50 μL of 88% formic acid and the resulting solution was diluted with water to obtain a 10% formic acid solution. This final mixture was slowly evaporated for 24 h at 24 °C in an Eppendorf tube. Mature fibrils were separated from nonaggregated peptide by centrifugation at 20 000*g*. The presence of fibrils was confirmed by the ThT fluorescence assay[15] and atomic force microscopy (AFM). The resulting suspension was lyophilized. The separated fibrils were redispersed in either H_2O or D_2O for Raman spectroscopic characterization. Aβ_{34-42} and Aβ_{1-40} fibrils were characterized by AFM and transmission electron microscopy (TEM), respectively, and selected images are presented at Figure 1.

Electron Microscopy. An aliquot of the aggregation reaction mixture was taken at the end of the reaction and diluted 10 times in deionized water. A 3 μL sample was placed on a freshly glow discharged carbon-coated grid, adsorbed for 2 min, and washed with deionized water before staining with 2 μL of 1% uranyl acetate for 30 s. Excess stain was blotted using filter paper. The grid was imaged on a Tecnai T12 microscope (FEI Co., Hillsboro, OR) operating at 120 kV and 30 000× magnification and equipped with an UltraScan 1000 CCD camera (Gatan, Pleasanton, CA) with a postcolumn magnification of 1.4×.

Atomic Force Microscopy. A lyophilized powder of fibrils was suspended in deionized H_2O. A 5 μL sample was placed on freshly cleaved mica and adsorbed for 10 min. Excess solution stain was blotted using filter paper, and the sample was dried under a nitrogen flow. The sample was imaged on an Asylum Research MFP atomic force microscope (Santa Barbara, CA) in AC (noncontact) mode using an Olympus AC160TS silicon cantilever with a tip diameter of <20 nm.

DUVRR Spectroscopy. The details of DUVRR spectroscopic measurements have been reported elsewhere.[8,9] Briefly, a 197 nm laser beam was focused into a spinning Suprasil NMR tube containing 100 μL of sample solution or suspension and a Teflon stirring bead. Scattered radiation was collected in backscattering geometry, dispersed using a home-built double monochromator, and detected with a liquid nitrogen cooled CCD camera (Roper Scientific). The spectrum was recorded for 10 min in consequent

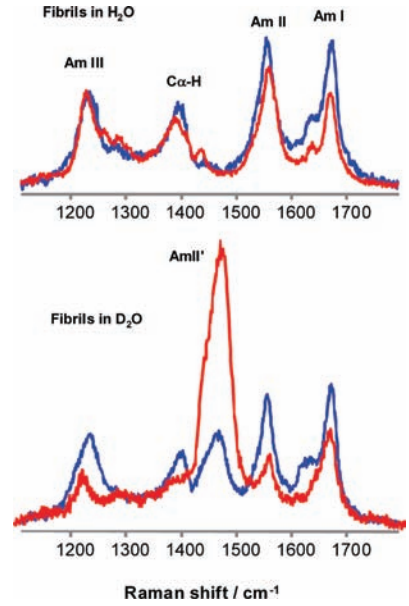

Figure 2. Raman spectra of Aβ_{1-40} (blue) and Aβ_{34-42} (red) fibrils in water and D_2O. The contribution of tyrosine was quantitatively subtracted where appropriate. The Amide I (Am I) band is due to carbonyl C═O stretching, with a small contribution from C−N stretching and N−H bending. The Am II and Am III bands owe to significant C−N stretching, N−H bending, and C−C stretching. The C_α−H band is derived from C_α−H symmetric bending and C−C_α stretching.

30 s strips. GRAMS/AI (7.01) software (Thermo Fisher Scientific) was used for Raman spectroscopic data processing. Typically, 20 individual 30 s accumulations were compared to ensure sample integrity and then averaged. The contribution of Suprasil, solvent, and buffering salts was quantitatively subtracted. In hydrogen−deuterium exchange experiments, peptide fibrils were concentrated by centrifugation at 20 000*g* for 30 min and then resuspended in either D_2O or H_2O. This procedure was performed twice. D_2O-based samples were incubated for 3 h at 25 °C prior to recording to ensure significant HX.

Results and Discussion

Effect of Hydrogen−Deuterium Exchange on the Fibril Raman Spectrum. Figure 1 shows TEM and AFM images of Aβ_{1-40} and Aβ_{34-42} fibrils, respectively. The morphologies observed are consistent with previously reported data.[7] To characterize the fibril core structure, we utilized DUVRR spectroscopy combined with post mortem hydrogen−deuterium exchange (HX).[9] Efficient HX of the −NH hydrogen results in two major changes in the DUVRR spectrum of the peptide backbone. First, the Amide II band is shifted to lower frequencies (Amide II′).[9,16] Second, the Amide III band practically disappears in the DUVRR spectrum of a deuterated peptide[9,16] due to the loss of coupling[17] between C−H bending and N−H bending vibrations. Therefore, any Amide III peak present in the DUVRR spectrum of fibrils in D_2O should be assigned to the contribution from the protonated, HX protected part of aggregated peptides. Figure 2 illustrates changes in the DUVRR spectra of fibrils upon deuteration. Aβ_{1-40} fibrils showed relatively little HX, consistent with previous reports indicating that the majority of amino acid residues in this fibril polymorph are involved in the cross-β core and hence are strongly

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protected.[18] In contrast, the Aβ_{34-42} fibrils exhibit a substantial degree of HD exchange. Nevertheless, even in the case of the Aβ_{34-42} fibrils, it is clear from the significant residual AM II peak in the D_2O Raman spectrum (Figure 2) that these fibrils possess an HX-resistant fibril core. The presence of an exchange-protected core in both types of fibrils confirms[18,19] that the fibrillation is an essentially irreversible process on the time scale of the experiment (about 3 h), since a significant monomer−fibril dynamic exchange would otherwise result in complete HX. It is known that in strongly hydrogen-bonded structures the rate of HX is greatly diminished.[20,21] Consequently, combining hydrogen−deuterium exchange with DUVRR spectroscopy allows us to focus on the spectral contributions of the strongly hydrogen bound fibril core, while the contributions of the D_2O-exchangeable, unordered parts of protein fibrils are filtered out.[9]

The Amide III (Am III) band is essentially the same in the spectra of Aβ_{1-40} and Aβ_{34-42} fibrils in H_2O. In contrast, the Am III peak shows a noticeable shift in the D_2O spectra (Figure 2), indicating a difference in the Raman signatures of the fibril cores of these fibrillar samples. Relating these Raman spectra to the known Aβ_{1-40} fibril core structure is the objective of the following considerations.

Rationale for Calculating the Fibril Core Raman Spectrum from the Known Ψ Dihedral Angles. The Amide III vibrational mode is one of the most structurally informative parts of the protein Raman spectrum. It involves significant C−N stretching, N−H bending, and C−C stretching vibrations.[12] The Amide III vibrational mode shows a distinct lack of coupling between adjacent amide chromophores.[16,22] This results in a nearly independent contribution from all amino acid residues to the Raman spectrum in the Amide III region. We exploited this additive property of resonance Raman signals in the Amide III region to calculate a spectrum of the Aβ_{1-40} fibril core as a sum of spectral contributions from individual amide chromophores. The DUVRR spectrum of an amide chromophore can be calculated using the Asher approach[12] if the Ψ dihedral angle is known for the corresponding peptide bond.

Asher and coauthors[12] have examined the dependence of the Am III_3 band frequency on Ψ dihedral angle and found that this dependence resulted from coupling between the peptide N−H and C_α−H in-plane bending vibrations. The coupling changes sinusoidally with Ψ angle due to the change in distance between C_α-H and N−H protons:[12]

$$\nu^{\beta}_{\mathrm{AmIII}}(\Psi) = 1239(\mathrm{cm}^{-1}) - 54(\mathrm{cm}^{-1})\sin(\Psi + 26^{\circ}) \quad (1)$$

In contrast, the frequency of the Am III band is only marginally dependent on Φ dihedral angle.[23,24] We utilized eq 1 to model the Am III Raman band for the Aβ_{1-40} fibril core using Ψ angles from published SSNMR studies[7] on the same polymorphic form of Aβ_{1-40} fibrils. To calculate the Raman spectroscopic contribution of an individual amide chromophore (averaged over the ensemble of fibrils in the sample), we used the set of residue-specific Ψ angles reported for the Aβ_{1-40} fibril core.[7] We also assumed that the integral Raman cross-section of the Amide III band is independent of frequency (or Ψ angle)[25] to normalize the calculated spectral contributions of individual amino acid residues by the peak area. While the additivity of individual amino acid Amide III resonances would appear to offer a tremendous opportunity for spectral prediction, this approach has some caveats due to potential complications from various possible broadening mechanisms. In the following sections we discuss these caveats in some detail.

Effect of Peptide Bond Hydration. Since it is known that the presence of a water−peptide hydrogen bond (HB), in addition to the peptide−peptide HB, at the same polypeptide backbone site results in an Amide III_3 shift[12] of about 5 cm^{-1}, the presence of a significant number of water molecules in the fibril core could affect the application of eq 1 to Raman spectrum modeling. This does not appear to be a major concern, however, in the present case. The β-strands of Aβ_{1-40} fibrils consist of mainly hydrophobic residues, with the exception of D23 and K28.[7] Numerous structural data on various peptide microcrystals[26,27] and fibrils[28] clearly show that an anhydrous interface between peptide β-sheets is a common feature of such structures. This appears to be the case for Aβ fibrils as well. Additionally, using two-dimensional infrared spectroscopy, Hochstrasser and co-workers[29] recently probed the presence and the location of water molecules in the Aβ_{1-40} fibril core.[29] They found the amount of embedded water in the Aβ_{1-40} fibril core to be only 1.2 molecules per β-strand,[29] a result that places a strict limit on the possible hydration of peptide bonds in the Aβ_{1-40} fibril core. Thus, the expected low level of H-bonded H_2O in the Aβ_{1-40} fibril core justifies the use of eq 1, which has been developed for anhydrous, internal β-strands of the polypeptide backbone.

Effect of Raman Spectral Broadening. The Amide III Raman band of an individual amide chromophore should have a certain shape, which is potentially the result of averaging over an ensemble of fibrillar species in the sample. In general, the shape and width of a Raman band is determined by the contribution from homogeneous and inhomogeneous broadening. The relaxation time T_1 of the vibrational excited state, the "pure" dephasing time T_2*, and the orientational relaxation Γ_{or} are the major components of the homogeneous bandwidth Γ.[30] It is known[30] that in the case of homogeneous broadening the Raman peak has a Lorentzian shape, and its bandwidth is typically smaller than that for inhomogeneous broadening. The overall homogeneous Lorentzian bandwidth of the Amide III resonance Raman peak has been measured[25] to be 7.5 cm^{-1} for crystals of a Gly-Ala-Leu tripeptide which exists in a single hydrogen-

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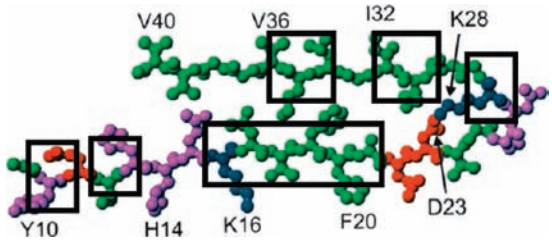

Figure 3. Model of Aβ_{1-40} fibril adapted from Petkova et al.[7] The residues protected from hydrogen−deuterium exchange,[14] for which the conformation is known from SSNMR,[7] are indicated with boxes.

bonded conformation. On the other hand, the experimental DUVRR Amide III bands in both fibrillar samples core exhibit Gaussian contours with a half-width at half-maximum (HWHM) of about 13 cm^{-1}. The latter value is not limited by the characteristic spectral resolution of our instrument, which is 6 cm^{-1} at 100 μm slit width.[8] HWHM of about 13 cm^{-1} is significantly larger than the homogeneous bandwidth (7.5 cm^{-1})[25] of a single peptide conformation, indicating that the inhomogeneous broadening determines the Raman band shape. This conclusion based on Raman spectroscopic characteristics of the Aβ_{1-40} fibril core is in good agreement with the SSNMR data,[7] as discussed below.

According to Tycko[31] the experimental bandwidth in magic angle spinning NMR spectra of 1 ppm or higher is due to the dominant inhomogeneous broadening that results from structural disorder within the sample. ^{13}C SSNMR data[7] reported for the Aβ_{1-40} fibril core have a bandwidth of 1.5 ppm or higher that is indicative of the structural inhomogeneity of Aβ_{1-40} fibrils. This implies that different conformations of the peptide chain in different fibrillar species contribute to a distribution of Ψ angle for each particular amino acid residue.

Thus, one can assume that the shape of the Amide III DUVRR band of the individual peptide residue of the fibril core should be represented by a Gaussian curve with standard deviation corresponding to the uncertainty of Ψ angle of individual peptide residues in the sample. In our calculations, we used an average Ψ dihedral angle uncertainty $\delta\Psi$ of 13°.[7] This value is in good agreement with the uncertainties determined for dihedral angles in a similar fibrillar system.[32] According to eq 1, the average Ψ dihedral angle uncertainty above corresponds to the distribution of vibrational frequency of 13 cm^{-1}.

Calculated Raman Spectrum. Only peptide residues, which constitute β-strands[7] and are resistant to HX,[14] are used for calculating the Raman spectrum of the protonated part of Aβ_{1-40} fibrils in D_2O. These residues include Y10, V12, K16-A21, K28, A30-I32, L34, and M35, as indicated with boxes in Figure 3. The residues G9, D23, and V36, which have been assigned[7] to β-strands, are exchangeable and are therefore excluded from the calculation. According to SSNMR structural data,[7] residues V24, G25, and G29 from the loop connecting two β-strands exhibit ^{13}C chemical shifts which are inconsistent with β-sheets; thus, these are also excluded from the calculation. These loop residues have significant variation in chemical shift values, which can be attributed to a high degree of conformational flexibility. Consequently, these residues are expected to result in a broad Raman band of low intensity that should not interfere substantially with the relatively narrow and intense Am III_3 peak.

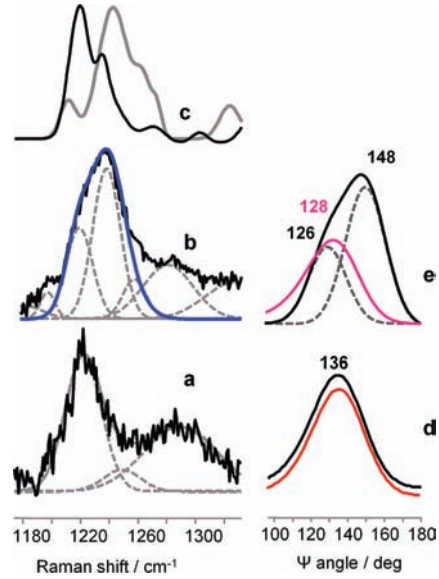

Figure 4. Fibril core DUVRR spectra (left column) and corresponding distributions of Ψ dihedral angle of the peptide backbone (right column): (a) Raman spectrum of Aβ_{34-42} fibril core (solid line) and the best fit with Gaussian peaks (dotted lines); (b) Raman spectrum of Aβ_{1-40} fibril core (solid line) and best fit with Gaussian peaks (dotted contours)and the calculated spectrum based on SSNMR data[7] (blue line); (c) Raman spectra of the fibril core of hamster prion protein[33] (black) and artificial YEHK polypeptide[34] (gray); (d) Ψ angle distribution in Aβ_{34-42} fibril core (black) and in average antiparallel β-sheets in globular proteins[36] (red); (e) calculated Ψ angle distribution in Aβ_{1-40} fibril core (black), its best fit with Gaussian contours (dotted lines), and Ψ angle distribution in average parallel β-sheet in globular proteins[36] (pink).

Comparison of Calculated and Experimental Raman Spectra of the Fibril Core in the Amide III Region. Figure 4a,b shows the Amide III parts of the Aβ_{34-42} and Aβ_{1-40} fibril core spectra. The calculated spectrum (Figure 4b, blue line) is in excellent agreement with the experimental spectrum (black line). This similarity is especially significant because of the substantial difference between the Raman spectrum (Figure 4b) for Aβ_{1-40} fibrils and the spectra of other protein fibrils including Aβ_{34-42} (Figure 4a), hamster prion protein,[33] and artificial YEHK peptide[34,35] (Figure 4c). This result represents the first experimental verification of Asher's approach[12] for using DUVRR spectra for quantitative characterization of peptide conformations *in the amyloid fibril core*.

A closer comparison of experimental and calculated spectra indicates that the major difference between these two spectra is in an additional band at about 1280 cm^{-1}. A similar band is evident in the spectrum of Aβ_{34-42} fibrils (curve a). A minor band around 1250 cm^{-1} is also present in both spectra. However, all these bands are located in the area outside the β-sheet region. Most probably, these relatively small peaks do not belong to the Am III_3 mode and thus do not interfere with the analysis of peptide backbone conformation in the fibril core.

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The black lines in Figure 4d,e represent the distribution of Ψ angles in $A\beta_{34-42}$ and $A\beta_{1-40}$ fibril cores calculated from Raman data using eq 1. We compared these distributions with the statistical distribution of Ψ angles found for antiparallel and parallel β-sheets of globular proteins, respectively.[36] Hovmöller et al.[36] have concluded by analyzing over 1000 globular protein subunits from the PDB that the parallel and antiparallel β-strands have quite similar conformations. The average torsion Ψ angle is 128° for parallel and 136° for antiparallel β-sheets with a standard deviation of 15° for strands with two partners.[36] These parameters were used to calculate Gaussian distributions of Ψ angle for antiparallel (Figure 4d) and parallel (Figure 4e) β-sheets in globular proteins. Figure 4d illustrates a striking resemblance between the Ψ angle distributions found for antiparallel β-sheets in the $A\beta_{34-42}$ fibril core and for globular proteins. Both curves consist of a single Gaussian peak centered at 136° that corresponds to a 1222 cm^{-1} Raman shift for antiparallel β-sheets. In contrast to $A\beta_{34-42}$ fibrils, the $A\beta_{1-40}$ fibril core shows a DUVRR Amide III peak consisting of two major bands centered at 1214 and 1233 cm^{-1} (Figure 4b). It is likely that both of these bands originate from a single vibrational mode (most probably, anhydrous β-sheet Amide III_3), since the spectrum calculated from SSNMR data closely resembles the Amide III peak. Despite the fact that Raman Amide III bands overlap for fibrils with parallel and antiparallel β-sheets, the bands are significantly shifted relative to each other and can be easily distinguished. The Ψ angle distribution in the $A\beta_{1-40}$ fibril core consists of two bands centered at 148 and 126°. A comparison between Ψ angle distributions for parallel β-sheets in $A\beta_{1-40}$ fibril core and globular proteins (Figure 4e, gray dotted and pink lines) shows very little resemblance that is in agreement with previous studies of native and fibrillar transthyretin.[37] In fact, if the smaller band centered at 126° shows a substantial overlap with the globular protein peak, the larger band centered at 148° is shifted substantially relative to the globular protein peak. This means that the majority of amino acid residues in the fibril core, which are not subject to hydrogen−deuterium exchange, have uncommon conformations in comparison with parallel β-sheets in globular proteins.

Conclusions

Deep UV Raman spectroscopy combined with hydrogen−deuterium exchange and Asher's approach relating Ψ dihedral angle with Amide III band frequency is a powerful tool for structural characterization of the fibril core. The application of this method to Aβ fibrils reveals that parallel and antiparallel β-sheets in the fibril core have sufficiently different distributions of Ψ dihedral angles and, as a result, can be distinguished by DUVRR spectroscopy. Furthermore, while the conformation of the antiparallel β-sheets in $A\beta_{32-42}$ fibrils is quite similar to that of antiparallel β-sheets in globular proteins, the same cannot be said about the parallel β-sheets of the $A\beta_{1-40}$ fibril core, which represent a conformation atypical for parallel β-sheets in globular proteins. The applicability of the above conclusions to other types of fibrils remains to be established.

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