J|A|C|S
COMMUNICATIONS

Published on Web 02/25/2004

Core Structure of Amyloid Fibril Proposed from IR-Microscope Linear Dichroism

Hirotsugu Hiramatsu,[†] Yuji Goto,[‡] Hironobu Naiki,[§] and Teizo Kitagawa*,[†]

Center for Integrative Bioscience, Okazaki National Research Institutes, Myodaiji, Okazaki 444-8585, Japan, Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan, and Department of Pathology, Fukui Medical University, Matsuoka, Fukui 910-1193, Japan

Received September 2, 2003; E-mail: teizo@ims.ac.jp

In recent years, the amyloid fibril has been recognized as another stable phase of peptide chains and also an origin of some kinds of diseases. Generally, it shows needlelike morphology (typically ~μm in length and ~10 nm in thickness[1−3]). Cryoelectron microscopy proved the twisted nature of the needle for the SH3 domain of the phosphatidylinositol-3′-kinase[4a] and insulin[4b] fibrils. X-ray diffraction measurements[5a,b] on several fibrils showed the presence of the integrative β-sheet structure at the core of the fibril as a common feature, where the peptide chain runs perpendicular to the long axis of the fibril. The presence of a similar β-sheet core in β_2-microglobulin amyloid fibril was deduced from the H/D exchange efficiency.[6] Solid-state NMR experiments provided the information on dihedral angles of the peptide chain,[7] which elucidated the conformation of Aβ peptide within the fibril. We report here a new approach based on IR linear dichroism analysis of amyloid fibrils using a microscope.

An amyloid fibril of β_2-microglobulin (β_2-m) with four additional residues at the N-terminus (Glu^{-4}-Ala^{-3}-Tyr^{-2}-Val^{-1}-Ile^{1}) has been prepared by the extension reaction with intact β_2-m fibrils at pH 2.5.[8] The β_2-m amyloid fibrils prepared by the extension reaction[8] could be one of the most homogeneous fibrils available, without contamination by amorphous aggregates. The extended fibril solution was centrifuged (15 000 rpm at 4 °C for 45 min) repeatedly, and the precipitation was imaged by thioflavin T staining (458 nm excitation, 480−520 nm probe). The specimen includes a lot of tiny particles as shown by panels a and b of Figure 1, where fluorescence intensity was measured at each particle using excitation light with two different polarizations (the direction of polarization is designated by an arrow). Some particles exhibited appreciable differences for the polarization dependent measurements (indicated by a broken circle in panels a and b of Figure 1). Panels c and d of Figure 1 show the expanded image of the highly oriented part. By using an IR microscope (Thermo Nicolet, Continuum), linear dichroism was observed for these oriented particles (see below), while no dichroism appeared on the others. Thus, it became clear that the alignment of the peptides in the amyloid fibril accompanies orientation of dye molecules. The stacking nature of the dye on the fibril can be obtained from the analysis of the IR linear dichroism and will be discussed separately.

The broken line in Figure 2 shows the IR absorption spectrum of the oriented part of the original fibril in the amide I and II regions (Figure 2a), which is a linear combination of the two perpendicularly polarized spectra, and the difference (Figure 2b) between the two polarized spectra. Note that the fibril sample in H_2O has been measured without staining because the dichroism of the dye disturbs the IR analysis. The dichroism in amide I (~1630 cm^{-1}) and amide II (~1550 cm^{-1}) are opposite, which is consistent with the theoretical expectation for these modes. This fact provides us evidences for alignment of the fibril.

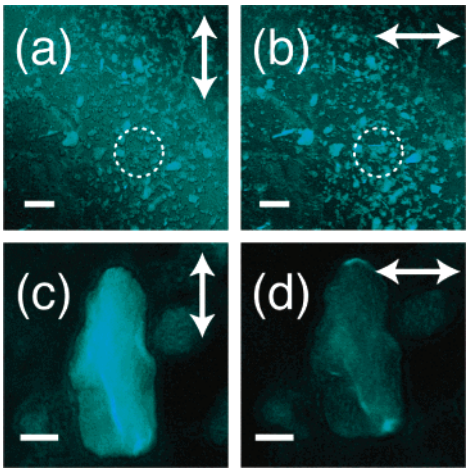

Figure 1. Fluorescence image of a pellet prepared with thioflavin T for β_2-microglobulin amyloid fibril. Scale bar denotes 200 μm (a,b) and 16 μm (c,d). Arrows indicate the polarization direction of excitation light.

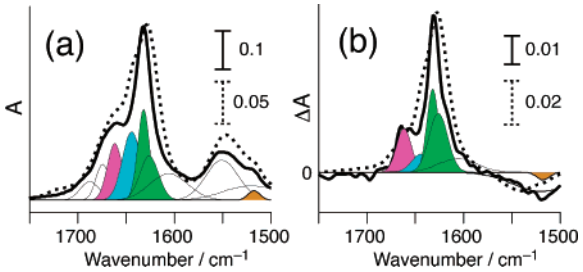

Figure 2. (a) IR absorption spectrum (solid thick) and decomposed contributing bands (solid thin), and (b) parallel-minus-perpendicular difference spectrum (solid thick), and decomposed bands (solid thin) of the #21−31 peptide fibril. Pink, blue, green, and orange correspond to the bands at 1662, 1644, 1632 + 1626, and 1517 cm^{-1}, respectively. Broken lines denote the spectra of β_2-microglobulin oriented fibril before staining.

To get further insight into the structure of the oriented fibril, the amyloidgenic core fragment of β_2m, #21−31 peptide (NFLNCYVSGFH),[9] was examined with the same method. The #21−31 part is a core of this fibril, and occupies the β-strand (N-terminal side) and loop (C-terminal side) in the native structure.[10] Thus, it serves as a good model of the β_2-m that consists of the β-strands and random parts. This peptide was synthesized[11] and purified, and after the fibrilization reaction at pH 7.5,[9] the aligned fibril pellet was prepared. IR linear dichroism was measured on this pellet (Figure 2, solid line); (Figure 2a) the IR absorption and (Figure 2b) the difference between the two polarized spectra. The spectral features

[†] Center for Integrative Bioscience, Okazaki National Research Institutes.
[‡] Institute for Protein Research, Osaka University.
[§] Department of Pathology, Fukui Medical University.

10.1021/ja0383017 CCC: $27.50

Table 1. Parameters Obtained from Infrared Linear Dichroism Measurement

	amide I				Tyr
peak position/cm^{-1}	1662	1644	1632	1626	1517
assignment	—[a]	random	β-sheet	β-sheet	Y19[a]
number of residue	2.3	3.5	2.9	2.3	
dichroic ratio	1.12	1.04	1.14	1.22	0.88
α (deg)	32	47[b]	27	0[c]	90

[a] β-turn or β-bulge. See text. [b] Random coil is expected to give 54.7°. However, this result is different from the ideal case because the number of residues is so small that the sum of the transition dipole moment vectors is not equal to zero. [c] Set to be zero, $f = 0.067$.

Figure 3. Plausible structures of the amyloid core in the fibril of β_2-microglobulin #21−31 peptide.

of the whole protein and model peptide are indeed alike. Accordingly, the structure of the peptide fibril is discussed in detail below.

The amide I band has been decomposed into several Gaussian bands. By following a reported procedure,[12−16] the type of secondary structure and the number of residues therein have been estimated.[16] Parameters obtained are summarized in Table 1. The IR spectrum suggests that the peptide includes two kinds of β-sheet,[17] random coil and an unknown structure. The CD spectrum indicated dominance of the β-sheet structure in the fibril and that this is a common feature of the fibril. Hence, the α-helical content is ignored in this analysis. Concerning the unknown structure, the presence of a β-turn is suggested from the bands at 1662 cm^{-1} [15,16] and 1280 cm^{-1} (data not shown),[15] although, it is not conclusive because of the absence of some expected bands at the higher wavenumber region of the amide I band. β-bulge is the other possible assignment because both of these two bands are assigned to the vibrations of the C═O bond out-of-plane with respect to the adjacent β-strand plane.[12]

The IR linear dichroism has been analyzed by applying Fraser's formulation[18] to each band of the difference spectrum, and angles (α) of each C═O bond against the fibril axis were determined as shown in Table 1. In this analysis, the parameter *f*, which indicates the degree of the alignment, was determined to be 0.067 by assuming the angle to be 0° for the band at 1626 cm^{-1}, for which the observed dichroism was largest. Directions of individual C═O bonds have been derived using this assumption; two bonds in the β-sheet make 0° with the fibril axis, three C═O bonds in the β-sheet (1632 cm^{-1}) are inclined by 27° with respect to the fibril axis, four residues (1644 cm^{-1}) in the random coil by 47°, and two residues in the possible β-bulge structure (1662 cm^{-1}) by 32°. The side chain of the Y26 gives a phenyl stretching band[19] at 1517 cm^{-1}. The observed dichroism means that the side chain is directed perpendicular to the fibril axis. Therefore, this residue is not included in the random but in the stiff parts.

Two plausible models compatible with the present observation are illustrated in Figure 3. In one (Figure 3a), the two β-sheets are connected by the bulge, e.g., the N21∼N24 is contained in the random part, the C25∼Y26 in the β-sheet (0°), the V27∼S28 in the bulge, and the G29∼H31 in the β-sheet (27°). In the other (Figure 3b), the two β-sheets are connected directly, e.g., the N21 and G29∼H31 are located at terminal random part, the F22∼N24 is in the β-sheet (27°), the C25∼Y26 in the β-sheet (0°), and the V27∼S28 in the bulge. A reported preference for the β-bulge formation[20] is assumed here. Since truncation of the two residues at the N-terminus reduces the amyloidgenic property while that at the C-terminus does not affect it,[9] model (Figure 3b) could be more likely. Both parallel and antiparallel stackings of the chains are possible. Model (Figure 3b) is very close to the conformation in the native structure. This result implies that the secondary structure in the native form may be preserved to some extent in the fibril structure, which gives us a key suggestion to analyze the result of the full-length protein fibril. Isotope substitution experiments for key residues would determine the alternative, and are underway in this lab. Thus, this study demonstrates that IR-microscope technique provides detailed structural information on individual peptide groups in amyloid fibrils.

Acknowledgment. This study was supported by JSPS Research Fellowships for Young Scientists to H.H. and by Grant-in-Aid for Specifically Promoted Research (14001004) from the Ministry of Education, Culture, Sports, Science and Technology, Japan to T.K.

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(16) A brief explanation of this estimation is as follows: Assume that the extinction coefficient is the same for the C═O stretching band arising from each secondary structure. Then the absorption intensity (area of band) is proportional to the number of the C═O groups involved, i.e., the number of the residues. Therefore, the intensity ratio among the bands is proportional to that of the number of residues contained in the particular secondary structure. Hence, the band intensity obtained from the fitting analysis is converted to the number of residues with the knowledge that there are 11 residues in total. See: Dong, A.; Huang, P.; Caughey, W. S. *Biochemistry* **1990**, *29*, 3303−3308.
(17) β-sheet structure also gives the band at ∼1690 cm^{-1}, and actually we observed a band at 1688 cm^{-1} (not shown in Table 1). We did not count its contribution since the derived angle α was not equal to the theoretically expected value (90°). It is ascribed to an incompleteness of band decomposition. However, the conclusion is little changed even if this band is taken into consideration.
(18) Infrared linear dichroic ratio *R* is related to the angle α and the order parameter *f* by

$$R(\alpha,f) = \frac{f(\cos^2\alpha) + {}^1/_3(1-f)}{{}^1/_2 f(\sin^2\alpha) + {}^1/_3(1-f)}$$

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JA0383017