Secondary structure analysis of Lewy bodies in the brain of Parkinson's disease patients

Parkinson's disease (PD) is a progressive neurodegenerative disorder of the central nervous system that mainly affects the motor system. Four motor symptoms are associated with PD: tremor, rigidity, slowness of movement, and postural instability. PD is the most common neurodegenerative disorder after Alzheimer's disease, and there is no basic treatment to control the development of the disease. It had been known for quite some time that Lewy bodies (LBs), abnormal protein aggregates, are formed in the brain of Parkinson's disease patients, and it is thought that LBs play an important role in the onset of the disease. LBs mainly consist of α-synuclein (α-syn), which is a 140-amino acid protein abundant in presynaptic terminals of nerve cells in the brain. α-syn is highly expressed in the central nervous system and erythrocytes, yet its function remains obscure. Although α-syn exists *in vitro* as an intrinsically disordered monomeric protein [1], it transforms into fibrils after agitation at 120 to 1000 rpm for a few days. Because these fibrils have a β-sheet-rich structure and a cross-β conformation, as can be seen by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction, these fibrils are often regarded as 'amyloid fibrils,' which are defined biophysically as fibrillar polypeptide aggregates with a cross-β conformation. Although elucidating the fibrillization process of α-syn is difficult, several studies have indicated that misfolded monomers undergo self-assembly into metastable oligomeric intermediates and finally, into amyloid-like fibrils. Recent epoch-making studies showed that the fibril seeds formed *in vitro* as described above can be propagated in the mouse brain [2]. The hypothesis stating that α-syn fibrils are involved in the pathogenesis of PD is under investigation *in vivo* as well as *in vitro.* On the other hand, the secondary structure of proteins in LBs in the human brain has not been clarified. Several electron microscopy (EM) studies demonstrated that LBs contain a filamentous amyloid-like structure, which is morphologically similar to wild-type α-syn fibrils *in vitro* [3]. In addition, they showed that typical LBs have granular components in the core and radially arranged fibrils in the halo. These results indicate that 'amyloid fibrils' which are pathologically characterized as depositions of protein fibrils with a specific appearance in EM, are part of LBs. However, EM cannot provide information on the secondary structure of a protein, and thus, whether LBs have a β-sheet conformation is unclear. Although Congo red, Thioflavin-T, and Thiazin red can be used for amyloid detection, their staining provides little information on the structure. The secondary structural information on proteins is important for developing treatment drugs. Because LBs cannot be induced *in vivo* or *in vitro*, to confirm that LBs contain amyloid fibrils, it is necessary to verify the abundance of the β-sheet.

Fourier transform infrared microspectroscopy (FTIR) is an established structural analysis method and is sensitive to the secondary structure of proteins. The absorption maxima for α-helix (~1655 cm^{-1}), β-sheet (~1630 cm^{-1}), and random coil (~1645 cm^{-1}) structures are included in the frequency range of the amide I band. FTIR also provides information on the amount of lipids in the beam. Because FTIR produces the spectrum derived from a chemical bond, it provides detailed structural information that cannot be obtained by staining and EM. However, the FTIR measurement of LBs is not easy for several reasons. The main difficulty is that LBs are too small to be irradiated with an infrared beam, and their density is too low to obtain a significant signal. To overcome this, a strong and small infrared beam is required. For this reason, we used the synchrotron radiation SPring-8 **BL43IR**.

Brain tissue samples from three patients (83-year-old female, 76-year-old male, and 74-year-old female) with neuropathologically confirmed PD were studied. The samples were fixed in 4% buffered formaldehyde and embedded in paraffin according to routine tissue processing for pathological examination. For each sample, 10-μm-thick sections were cut and deposited on CaF_2. Tissue sections were immunostained by anti-human phosphorylated α-syn (Ser129) monoclonal antibody. These samples were allowed to dry at room temperature. The brilliance of infrared synchrotron radiation (IR-SR) at BL43IR is more than two orders

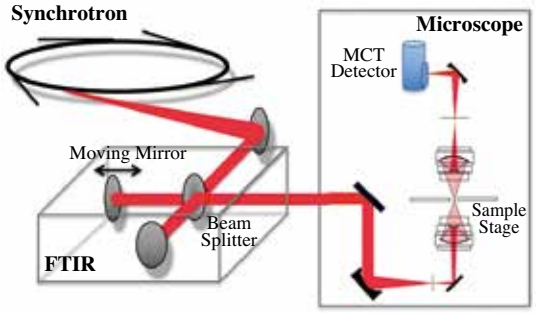

Fig. 1. Optical layout of the microspectroscopic station at BL43IR. The infrared synchrotron light is injected into the FTIR (Bruker VERTEX70) interferometer, and the light then goes to the microscope (Bruker HYPERION2000). Infrared light transmitted through a sample is detected by an MCT (HgCdTe) detector.

of magnitude higher than that of the laboratory source in the fingerprint region of 2000–1000 cm^{-1}. A Fourier transform infrared (FTIR: Bruker VERTEX 70) spectrometer was used with IR-SR as the infrared source (Fig. 1). A tissue sample on an adjustable motorized x–y mapping stage was observed with an optical microscope (Bruker HYPERION 2000). A rectangular region of 100 μm × 100 μm including amyloid deposits or LBs was mapped with an aperture size of 7 μm × 7 μm and 3–5 μm steps in the horizontal and vertical directions. Interferograms were acquired with 400 scans, and signals were averaged and Fourier transformed to generate a spectrum with a nominal resolution of 3 cm^{-1}.

The total protein distribution was evaluated by calculating the sum of the absorbances at 1540 cm^{-1} and 1640 cm^{-1}. The proportion of β-sheet structures was analyzed by curve fitting to the FTIR spectra ranging from 1700 cm^{-1} to 1600 cm^{-1}. Spectrum data were fitted using four Gaussian species centered at 1628 cm^{-1} and 1680 cm^{-1} (β-sheets), and 1648 cm^{-1} and 1661 cm^{-1} (random coils, α-helices, and others) as in previous reports. During the fitting procedure, the peak height was free, whereas the width at half height was maintained at < 25 cm^{-1}. A reasonable fit was obtained as shown in Fig. 2. The lipid distribution was based on the area of the symmetric CH_2 band at 2850 cm^{-1} (2858–2848 cm^{-1}, baseline 3000–2750 cm^{-1}). Our results showed a shift in the infrared spectrum, which indicates the abundance of a β-sheet-rich structure in LBs. Also, 2D infrared mapping of LBs revealed that the content of the β-sheet structure is higher in the halo than in the core, and the core contains a large amount of proteins and lipids (Fig. 3) [4].

Here, we present, to our knowledge, the first data on the secondary structure of LBs obtained using synchrotron FTIR microscopy (FTIRM). Furthermore, β-sheet mapping was performed to elucidate the process of generation of LBs. Our FTIRM approach has the potential for elucidating the pathology of many amyloid-related diseases. However, since the laboratory FTIR instruments do not have sufficient brightness, FTIRM measurement of brain tissues requires synchrotron radiation. This has been limiting the use of this technique, particularly for medical researchers. However, since many synchrotron radiation facilities have recently been constructed worldwide, more researchers now have better access to FTIRM for biological samples.

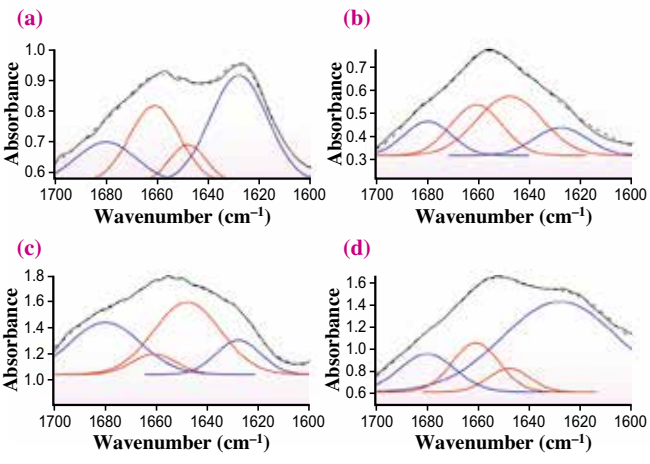

Fig. 2. FTIRM spectra (amide I region) obtained from **(a)** fibrils of α-syn expressed in *E. coli*, **(b)** normal brain tissue from the brain of a patient with PD, **(c)** the core of an LB, and **(d)** the halo of an LB (solid black lines). Blue and red lines represent the contributions of β-sheet structures and non-β-sheet structures (random coils, α-helices, and others), respectively. The dashed lines represent the fitted curves. Data were fitted using a Gaussian species model centered at 1628 cm^{-1}, 1680 cm^{-1} (β-sheets, blue line), 1648 cm^{-1}, and 1661 cm^{-1} (random coils, α-helices, and others, red lines.

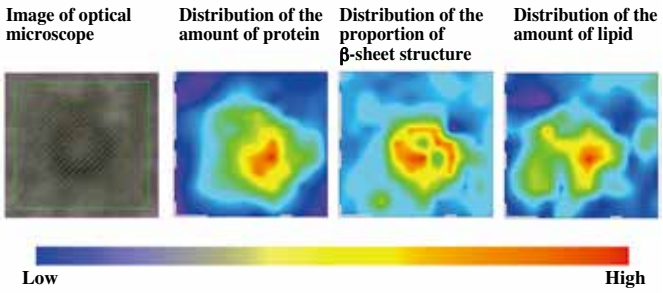

Fig. 3. Visible and FTIR images of typical LBs in the *substantia nigra* of the midbrain derived from the 83-year-old female PD patient. Shown from left to right are a microscope image, the amount of total proteins, the proportion of β-sheet structures, and the amount of lipids. The color bar indicates low (blue) to high (red) contents. The area shaded with green was scanned with 4 μm steps, 12 × 11 pixels = 48 × 44 $μm^2$.

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References

[1] K. Araki *et al.*: Sci. Rep. **6** (2016) 30473.
[2] K. C. Luk *et al.*: Science **338** (2012) 949.
[3] T. Iwatsubo *et al.*: Am. J. Pathol. **148** (1996) 1517.
[4] *K. Araki, N. Yagi, Y. Ikemoto, H. Yagi, C. Choong, H. Hayakawa, G. Beck, H. Sumi, H. Fujimura, T. Moriwaki, Y. Nagai, Y. Goto and H. Mochizuki: Sci. Rep.* ***5*** *(2015) 17625.*