Parkinson's disease is a type of amyloidosis characterized by accumulation of amyloid fibrils of α-synuclein

Parkinson's disease (PD) is a progressive neurodegenerative disorder of the central nervous system that mainly affects the motor system with the characteristic symptoms of tremor, rigidity, slowness of movement, and postural instability. It has been known for quite some time that Lewy bodies (LBs), which are abnormal protein aggregates, are formed in the brain of PD 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. Recently, it has been reported that aggregates of α-syn with a cross-β structure (seeds of α-syn amyloid fibrils) are capable of propagating within the brain similarly to prions [1]. However, the presence of cross-β sheet-rich aggregates in LBs of PD patients has not been demonstrated so far (Fig. 1). Nowadays, PD is described as a heterogeneous multisystem neurodegenerative disease because α-syn deposits are not restricted to the central nervous systems; they are also found in the peripheral nerves innervating visceral organs, such as the heart and gut. This observation indicating that α-syn may propagate is interesting.

On the other hand, amyloidosis, a disease in which amyloid fibrils accumulate throughout the body, has been known for a long time. Amyloidosis is incurable because various organs are affected. Because senile plaques of Alzheimer's disease (AD) contain amyloid fibrils, AD is classified as localized amyloidosis. Is PD a type of amyloidosis? By classical histopathological definition, amyloids are Congo Red-stained extracellular proteinaceous deposits with a β-sheet structure. LBs are intracellular deposits and generally not stained by Congo Red. Therefore, PD does not meet the definition of amyloidosis. This seems to contradict the propagation hypothesis of α-syn. Our previous work has demonstrated that LBs are rich in β-sheet structures [4], but it did not provide results that satisfactorily answer the above question. To more accurately answer the above question, the following experiment was performed.

First, we adopted the biophysical definition of amyloid fibrils, that is, amyloid fibrils are fibrillar polypeptide aggregates with a cross-β conformation. We carried out the fine structure analysis of LBs using thin sections of brains of autopsied patients with PD. There are excellent electron microscopy studies that show fibril-like structures in the aggregates. However, they are only morphological observations and do not provide information on the secondary structure of the proteins (Fig. 1). Measurements using real brain tissue are not easy. In addition, LBs are very small with a diameter of 10 μm, so fine structure analysis is very difficult. Therefore, we examined LBs in thin sections of brains of autopsied patients with PD by microbeam X-ray diffraction (XRD) at SPring-8 **BL40XU**.

Brain tissue samples from three patients with neuropathologically confirmed PD were used for our measurements. All samples were collected from the midbrains. The brain samples were fixed in 4% buffered formaldehyde and frozen in accordance with routine tissue processing for histopathological examination. For each sample, 20-μm-thick sections were cut and deposited on Kapton polyimide films. Tissue sections from the PD patient's brain were immunostained with an anti α-syn antibody. Before measurements, these samples were dried at room temperature. Brain sections were scanned with an X-ray microbeam at BL40XU beamline. An X-ray microbeam was obtained using a pinhole with a diameter of 5 μm. The sections were scanned in the X and Y directions with a step size of either 3 or 5 μm. Scans with 10, 20, or 40 steps in each direction were performed. At each point of the scan, a wide-angle X-ray scattering pattern was recorded. The X-ray

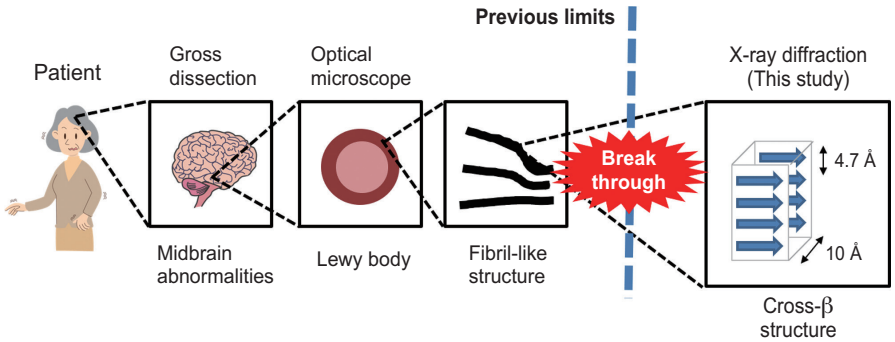

Fig. 1. Abnormal changes in the brain of Parkinson's disease patients.

wavelength was 0.083 nm with a bandwidth of about 2%. The X-ray detector was an X-ray image intensifier (V7739P, Hamamatsu Photonics, Hamamatsu, Japan) coupled with either a CCD camera (C4742-98-24ER, 1344×2018 pixels) or an sCMOS camera (C11440-22CU, 2048×2018 pixels), both from Hamamatsu Photonics. The exposure time was 0.3–0.5 s. The sample-to-detector distance was about 115 mm. No vacuum path was used.

The basic assumption in the data analysis is that LBs, which comprise aggregated amyloid fibrils with high density, should produce stronger X-ray scattering than other regions of the brain. Thus, a map of total wide-angle scattering intensity was compared with a microscopy image of stained sections. For each scan, 100, 400, or 1600 X-ray diffraction patterns were obtained. Total scattering intensity in a diffraction pattern at each point was calculated, and a 2D intensity map was constructed. If the LBs identified by chemical staining contain a high density of amyloid fibrils, a high intensity region is expected to appear in the same area of the 2D intensity map. Brain slices from the patient showed LBs identified by the antibody staining (Fig. 2(e)) and a marked peak in the 2D map of total scattering intensity (Fig. 2(d)).

Then, five points where the highest intensity was observed in the 2D map were searched. These are usually the central part of the identified peak region. The averaged diffraction pattern of the images obtained at these five points was treated as a "top" image (Fig. 2(a)). Similarly, diffraction patterns at half of the points that showed lower intensities than the other half were averaged as a "bottom" image (Fig. 2(b)). The top image was recorded from the region with aggregates of amyloid fibrils, whereas the bottom image represented the background without protein accumulation. A "difference" image obtained by subtracting the bottom image from the top image (Fig. 2(c)) represents diffraction from amyloid fibrils only. The strong rings seen in the top and bottom images are due to the Kapton sheet that was used to mount the brain section. These were completely removed by subtraction. The difference scattering pattern was obtained (Fig. 2(c)), and peaks correspond to amyloid fibrils at q = 6.1 and 13.5 nm^{-1} (d = 1.03 and 0.47 nm) were observed (Figs. 2(c) and 2(f)) in some LBs. These peaks are characteristic of the cross-β structure.

As a result, we found that some of them gave a diffraction pattern typical of a cross-β structure [5]. This finding confirms for the first time that LBs in the brain of PD patients contain amyloid fibrils with a cross-β structure and supports the validity of *in vitro* propagation experiments using artificially formed amyloid fibrils of α-syn. Notably, our finding supports the new concept that PD is a type of amyloidosis, a disease characterized by accumulation of amyloid fibrils of α-syn.

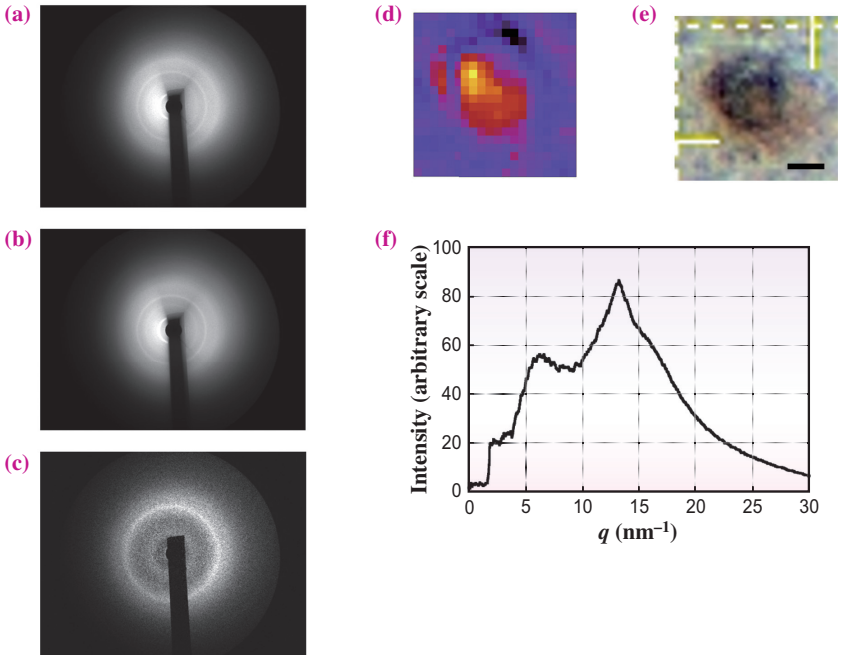

Fig. 2. Analysis of human Lewy bodies showing a sharp diffraction peak corresponding to d = 0.47 nm. **(a)** top image. **(b)** bottom image. **(c)** Difference image obtained by subtracting (b) from (a). **(d)** 2D map of total wide-angle scattering intensity. A 20×20 scan was carried out in 3 μm steps. **(e)** Micrograph of an antibody-stained brain section. Scale bar, 10 μm. **(f)** Circularly averaged X-ray scattering intensity profile of (c).

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