Microbeam X-ray Diffraction Study on Insulin Spherulites

Insulin is a hormone with a molecular weight of 5.7 kDa, that is composed of two polypeptide chains. In the native state, its secondary structure is primarily α-helical. However, forms a spherical precipitate called spherulite (Fig. 1), which is made of β-amyloid fibrils (Fig. 2), at low pHs and high temperatures (pH 2.0 and 37 - 100 °C). No chemical modification is involved in the assembly of insulin to form spherulite, and insulin retains its three disulfide bridges (two between chains and one within one chain) in spherulite. Amyloids are self-aggregates of insoluble, fibrillar assemblies of protein molecules. Amyloids can be harmful to organisms, causing pathologies such as Alzheimer's disease, Parkinson's disease, Huntington's disease, type II diabetes, and prion diseases. Several nonpathological proteins such as insulin, lysozyme and short peptides self-assemble into amyloid-like fibrils, suggesting that amyloid formation is an inherent property of polypeptide chains. Denatured proteins in amyloid fibers commonly take a cross-β structure even when their native form is α-helical [1].

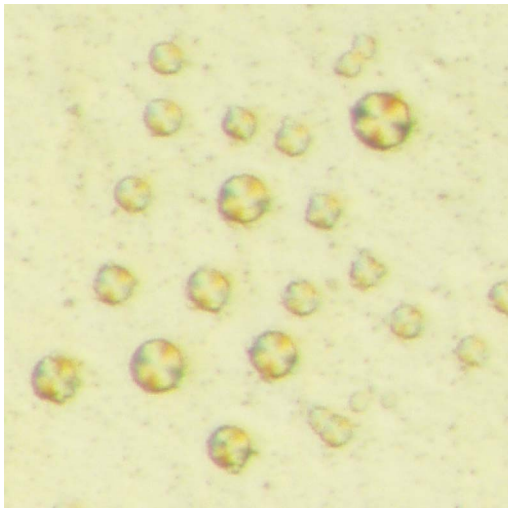

Fig. 1. Bovine insulin spherulites under a polarizing microscope. The diameter of the largest spherulite is about 100 μm.

Although amyloid fibers of various proteins have been studied by X-ray diffraction analysis, the specimens were prepared by orienting fibers by drying and stretching. Thus, the native fully hydrated structure of amyloid fibers has been unknown. We made use of spherulite in which amyloid fibers are naturally oriented. Although each spherulite is only 50-100 μm in diameter, it is possible to investigate regions parts in which fibers are mostly oriented in same direction [2].

The experiment was carried out at beamline **BL40XU** with a 5-μm pinhole. The X-ray energy was 15.0 keV. The X-ray detector was an X-ray image intensifier (V5445P, Hamamatsu Photonics) with a cooled CCD camera (ORCA-II-ER, Hamamatsu Photonics). Small-, medium- and wide-angle diffraction patterns were recorded at different specimen-to-detector distances. Each spherulite was scanned two-dimensionally with 5~20-μm steps.

The small-angle diffraction pattern (Fig. 3) showed a broad oriented peak at a Bragg spacing of 23 nm in the equatorial direction (that is, at right angles to the fibers), which probably corresponds to the distance between the amyloid fibers in spherulite. Since this spacing was constant across the spherulite, the fiber density seems uniform. This supports a model with branching amyloid fibers (Fig. 2, right).

The medium-angle pattern showed broad maxima at Bragg spacings of 3.3 and 1.2 nm. These peaks correspond to the internal structure of an amyloid fiber. A previous electron microscopic study [3] showed that each fiber is composed of about four protofilaments. A helix made of a square arrangement of four cylinders, with a diameter of 2.4 nm and a helical radius of 2.0 nm, can account for the 3.3-nm peak. The 1.2-nm peak probably corresponds to the distance between β-sheets in each protofilament (see below).

The wide-angle pattern showed a meridional (that is, along the fiber axis) arc at a Bragg spacing of 0.48 nm (Fig. 4). This is a peak corresponding to β-sheets that is commonly observed in amyloid fibers, which shows that the hydrogen bonds of the β-sheets are aligned along the axis of the amyloid fibers. No other meridional reflection was observed.

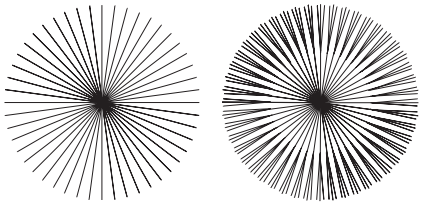

Fig. 2. Arrangement of amyloid fibers in spherulite. The image on the left shows a model without fiber branching, and the image on the right shows a model with fiber branching. Amyloid fibers grow isotropically in three-dimensions from a core whose structure is unknown.

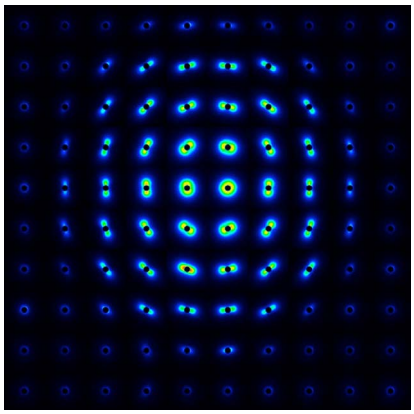

Fig. 3. Galley of small-angle diffraction patterns from an insulin spherulite. A spherulite, located at the center, was scanned with a microbeam with 10-μm steps. Diffraction patterns obtained at each spot were arranged to make this galley. Strong scattering is observed at right angles to the fiber axis. At the center, end-on diffraction from fibers is observed, which is isotropic.

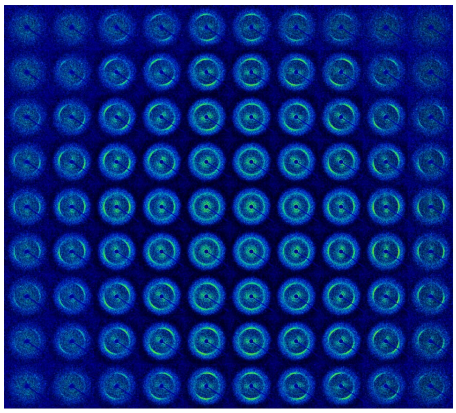

Fig. 4. Galley of wide-angle diffraction patterns from an insulin spherulite. At the peripheries of the spherulite, meridional arcs are observed parallel to the fiber axis.

On the basis of these diffraction patterns and a model of the Aβ peptide of Alzheimer's disease [4], a model of an insulin molecule in the amyloid fiber is proposed (Fig. 5). In this model, the two chains of insulin are aligned side-by-side in a plane perpendicular to the fiber axis. Hydrogen bonds are formed between molecules in neighboring planes making two β-sheets along the fiber axis. The axial separation distance between molecules is 4.8 nm, and the distance between two chains of each molecule within the plane is about 1.2 nm. Since the disulfide bond imposes a strong constraint on the structure, the arrangement of the side chains is mostly deduced.

This is the first study in which an amyloid fiber was investigated in a fully hydrated state. Microbeam X-ray diffraction analysis has advantages over diffraction experiments on dry stretched fiber in that not only can the fibrils be studied in the native state but also the results are not influenced by contaminating materials or other types of aggregate. The structure revealed using this technique will give new insight into amyloid fiber formation and help us understand the mechanism of amyloidosis.

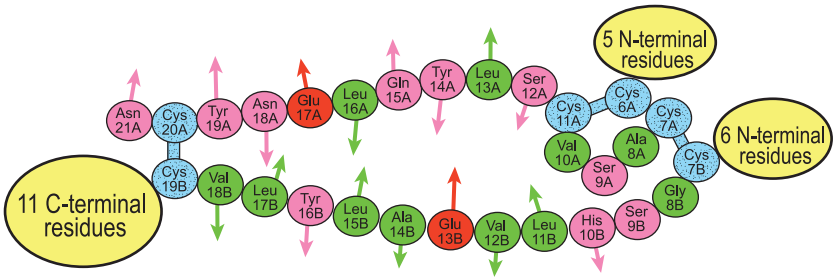

Fig. 5. Structural model of insulin molecule in amyloid fiber. The fiber axis is perpendicular to this drawing. Bovine insulin consists of two chains, namely, A with 21 residues (above) and B with 30 residues (below). There are three disulfide bonds, one within the A chain and two between the chains. The molecule lies in a plane perpendicular to the fiber axis. A portion of the A chain (residues from 12 to 29) and a portion of the B chain (from residues 10 to 18) are connected to the molecules in the planes above and below with hydrogen bonds between the main chains, forming two β-sheets. The arrows indicate the direction of the side chains.

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References

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