

## $\beta$ -HELIX IS A LIKELY CORE STRUCTURE OF YEAST PRION SUP35 AMYLOID FIBERS

Prions are infectious proteins that propagate by catalyzing the conformational conversion of the native protein to the prion form [1]. However, the conformational conversion mechanism underlying the propagation of the protein-based genetic information is unknown. Various prion proteins, otherwise unrelated, all assemble into structurally similar  $\beta$ -sheet-rich amyloid fibers [2]. Amyloid fibers have also been implicated in several debilitating neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington's diseases [3]. Knowledge of the fibril structure is essential for understanding the molecular mechanism underlying amyloid formation and could lead to the development of agents that inhibit or reverse the process. The yeast protein Sup35 offers a convenient model for studying both amyloid formation and conformational transmission.

We studied the core structure of the amyloid fibers of the yeast prion protein Sup35 [4]. A prion-inducing fragment of yeast Sup35 consists of two domains: a Gln/Asn-rich N-terminal domain (N); and the highly charged medium domain (M). In order to study the core structure of the amyloid fibers and the involvement

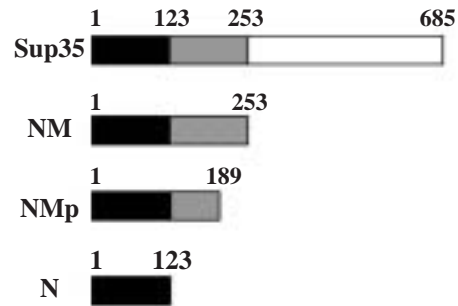


Fig. 1. Domains of Sup35 and Sup35 fragments studied in this work.

of its N and M domains, we prepared three types of fiber from Sup35 fragments: NM (Sup35(1-253)), NMp (Sup35(1-189)), and N (Sup35(1-123)) (Fig. 1). The diameters of the fibers measured by electron microscopy were approximately 120 Å, 80 Å and 60 Å (Fig. 2(a)), respectively.

We collected X-ray fiber diffraction data at beamline BL40B2. X-ray diffraction patterns from dried, oriented fibers (Fig. 2(b)) all show similar set of reflections. The most notable reflection is the sharp

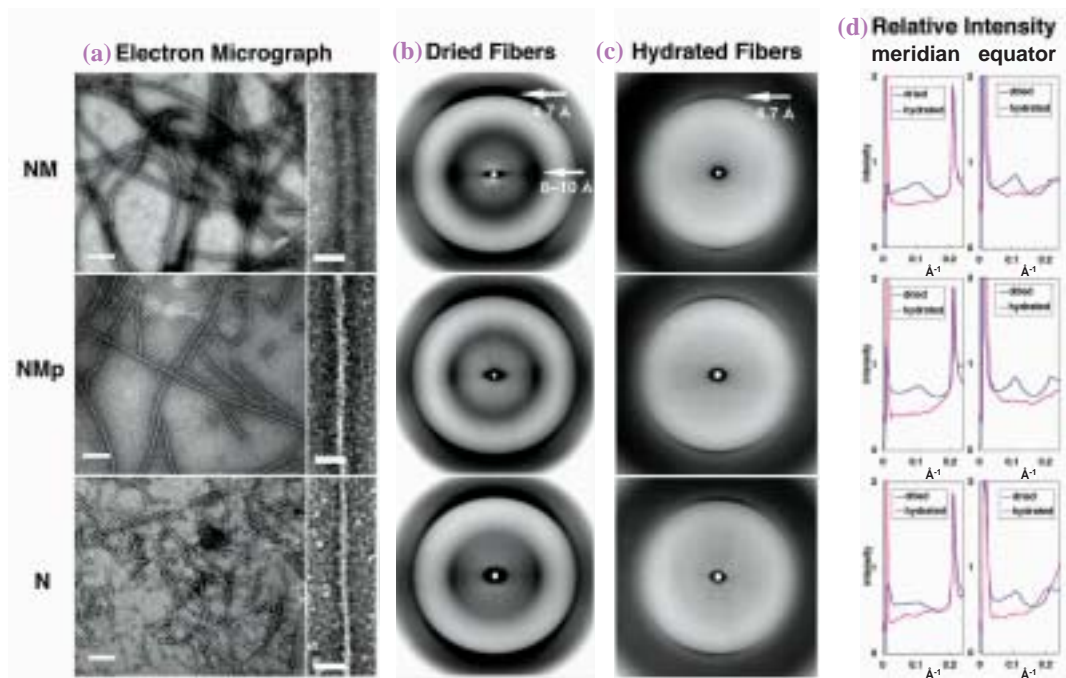


Fig. 2. Structural data obtained from the NM (upper panels), NMp (middle panels) and N fibers (lower panels). (a) Electron micrographs. Magnified images of individual fibers are shown in the right panels. Scale bar, 1000 Å (left) and 250 Å (right). (b) X-ray fiber diffraction patterns obtained from dried oriented fibers. (c) X-ray fiber diffraction patterns from hydrated oriented fibers. (d) Comparison of the meridional (left) and equatorial (right) intensity profiles from dried (blue) and hydrated (red) fibers.

meridional one at 4.7 Å due to  $\beta$ -strands running perpendicular to the fiber axis with an interstrand spacing of 4.7 Å. Because the meridional reflection at 9.4 Å, which is expected to arise from antiparallel  $\beta$ -strands, is not observed, it is considered that the fibers are composed of parallel  $\beta$ -strands. Another notable reflection is relatively diffuse in the equatorial region from 10 Å to 8 Å, arising from  $\beta$ -sheets stacked in the direction perpendicular to the fiber axis. The similarity in diffraction pattern indicates the presence of a common core structure. Because the NMP and N fragments were both generated by removing C-terminal regions of the NM fragment, it would be reasonable to assign the N domain to the region that forms the core structure of the fiber. The M domain is, therefore, likely to be located around the fiber core structure.

Interesting observations came from hydrated, oriented fibers, which are shown in Fig. 2(c). The sharp and strong meridional reflection at 4.7 Å is clearly observed, and the absence of the meridional sharp reflection at 9.4 Å is also clear. However, the equatorial diffuse peak at around 9 Å, which was observed in the dried fibers, is missing in the diffraction data of the hydrated fibers. For a direct comparison between the dried and hydrated fibers, we subtracted circular symmetric backgrounds and scaled the intensity profiles on the basis of the intensity of 4.7 Å reflection. Whereas the dried fibers all show the equatorial diffuse peak at 9 Å (at 0.11 Å<sup>-1</sup> in Fig. 2(d) equator), the hydrated fibers show a flat profile in this region. These results indicate that the stack of  $\beta$ -sheets observed in the dried fiber is an artifact formed during the drying process and the native hydrated fibers do not have the stack of  $\beta$ -sheets in their structure.

Together with the evidence for parallel  $\beta$ -strands, a plausible core structure of Sup35 amyloid fibers is a bundle of  $\beta$ -helix nanotubes [4], as has been proposed by Perutz *et al.* for other amyloid fibers composed of Gln-rich peptides [5]. The central cavity of the  $\beta$ -helix tube would contain water molecules, and when the fibers are dried, these water molecules are removed, resulting in the collapse of the tube and formation of a stack of  $\beta$ -sheets, giving rise to the 9 Å equatorial reflections. The diameter of the  $\beta$ -helix nanotube proposed by Perutz *et al.* [5] is approximately 25 Å, much thinner than the amyloid fibers, and they modeled the fiber to be a twisted bundle of nanotubes. For the M domain of the NM fiber to be on the surface of the nanotube, the M domain has to be located on its one side. The M domain would be helically

arranged on the periphery of the nanotube bundle due to a possible twisting of each nanotube as depicted in Fig. 3 [4].

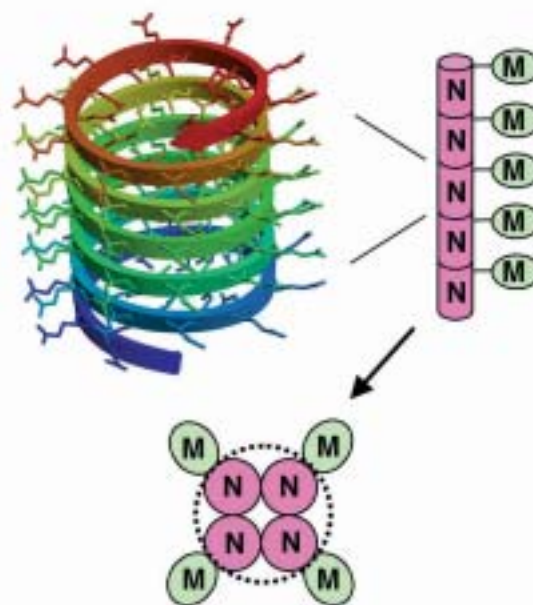


Fig. 3. Model of Sup35 NM fiber.

Aiko Kishimoto<sup>a,b</sup>, Kazuya Hasegawa<sup>c</sup> and Keiichi Namba<sup>b,d,\*</sup>

- (a) Chemical Resources Laboratory, Tokyo Institute of Technology
- (b) Graduate School of Frontier Biosciences, Osaka University
- (c) SPing-8 / JASRI
- (d) Dynamic NanoMachine Project, ICORP, JST

\*E-mail: keiichi@fbs.osaka-u.ac.jp

## References

- [1] S.B. Prusiner: Proc. Natl. Acad. Sci. USA **95** (1998) 13363.
- [2] J.W. Kelly: Curr. Opin. Struct. Biol. **8** (1998) 101.
- [3] C.M. Dobson: Trends. Biochem. Sci. **24** (1999) 329.
- [4] A. Kishimoto, K. Hasegawa, H. Suzuki, H. Taguchi, K. Namba and M. Yoshida: Biochem. Biophys. Res. Comm. **315** (2004) 739.
- [5] M.F. Perutz *et al.*: Proc. Natl. Acad. Sci. USA **99** (2002) 5591.