3D Structure of Amyloid Protofilaments of β_2-Microglobulin Fragment Probed by Solid-State NMR

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Amyloid fibrils are highly-ordered filamentous aggregates formed by the self-assembly of peptides or proteins. There are currently approximately 20 known diseases associated with deposition of amyloid fibrils, including Alzheimer's disease, Parkinson's disease and dialysis-related amyloidosis. Additionally, numerous peptides and proteins not directly related to diseases can also form amyloid-like fibrils *in vitro*, suggesting that amyloid fibril formation is a generic property of the polypeptide chain. To obtain further insight into protein folding and misfolding, it is crucial to clarify the mechanism of fibril formation and the structural stability of amyloid fibrils.

We have combined solid-state nuclear magnetic resonance (NMR), X-ray fiber diffraction and atomic force microscopy to reveal the 3D structure of amyloid protofilament-like fibrils formed by a 22-residue K3 peptide (Ser20-Lys41) of β_2-microglobulin, a protein responsible for dialysis-related amyloidosis. Although a uniformly ^{13}C, ^{15}N labeled sample was used for the NMR measurements, we could obtain the 3D structure of the fibrils on the basis of a large number of structural constraints, leading to an atomic-level understanding the protofibril structure and the types of interactions required to stabilize this structure. The conformation of K3 fibrils was found to be a β-strand-loop-β-strand with each K3 molecule stacked in a parallel and staggered manner. The fibrillar conformation was stabilized by intermolecular interactions, rather than intramolecular hydrophobic packing as seen in globular proteins. Together with thermodynamic studies of the full-length protein, formation of the fibrils is likely to require side chains on the intermolecular surface to pack tightly against those of adjacent monomers. By revealing the structure of β_2-microglobulin protofilament-like fibrils, this work represents technical progress in analyzing amyloid fibrils in general through solid-state NMR.

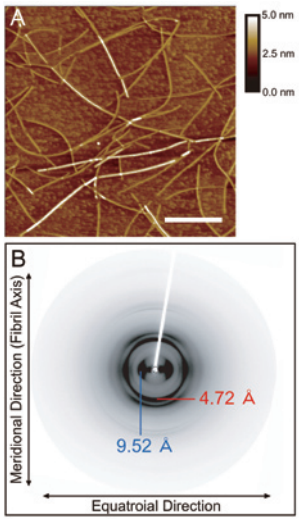

Fig. 1. (A) AFM images of K3 fibrils. The scale bar represents 0.5 μm. (B) X-ray fiber diffraction of the K3 fibrils. The data show a typical cross-β pattern.

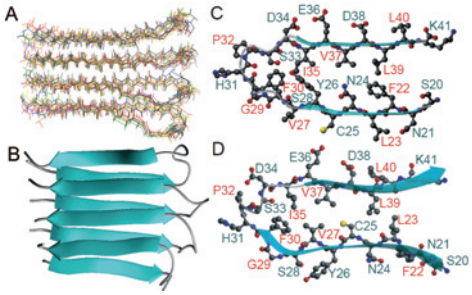

Fig. 2. Calculated ensemble (A) and ribbon model representation (B) of tetrameric structures of K3 fibrils. Comparison of one K3 structure in the fibrillar state (C) and in the native β_2-microglobulin (D).

Plant Aurora Kinase Required for Chromosome Segregation

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Chromosomes change their morphology and position dynamically during mitosis, and the processes of chromosome condensation and segregation play crucial roles in equal separation of genetic information to both daughter cells. Mitosis depends mainly on two post-translational mechanisms: protein phosphorylation and proteolysis.

Although phosphorylation of Ser10 and Ser28 (H3S10ph and H3S28ph) of histone H3 is ubiquitous among eukaryotes, the phosphorylation mechanism during the cell cycle remains unclear. In this study, H3S10ph and H3S28ph in tobacco BY-2 cells were observed in the pericentromeric regions during mitosis (Fig. 1).

Aurora kinases belong to a cell cycle-dependent serine/threonine protein kinase family and are highly conserved from yeast to humans. In *Arabidopsis thaliana*, three genes have been identified as Aurora kinases, AtAUR1 to 3. The Aurora kinase inhibitor Hesperadin inhibited the kinase activity of AtAUR3 in phosphorylating both Ser10 and Ser28 of histone H3 *in vitro*. Consistently, Hesperadin inhibited both H3S10ph and H3S28ph during mitosis in BY-2 cells. These results indicate that plant Aurora kinases phosphorylate not only Ser10, but also Ser28 of histone H3 *in vivo*.

Hesperadin treatment increased the ratio of metaphase cells, while the ratio of anaphase/telophase cells decreased, although the mitotic index was not affected in Hesperadin-treated cells. These results suggest that Hesperadin induces delayed transition from metaphase to anaphase, and early exit from mitosis after chromosome segregation. In addition, micronuclei were observed frequently (Fig. 2). Lagging chromosomes, caused by the delay and failure of sister chromatid separation, were also observed at anaphase and telophase in Hesperadin-treated BY-2 cells (Fig. 3). Our data suggest that plant Aurora kinases and H3S10ph/H3S28ph may have a role in chromosome segregation and metaphase/anaphase transition.

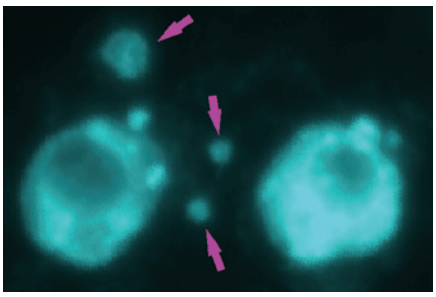

Fig. 2 Hesperadin-induced micronuclei (arrows) at interphase.

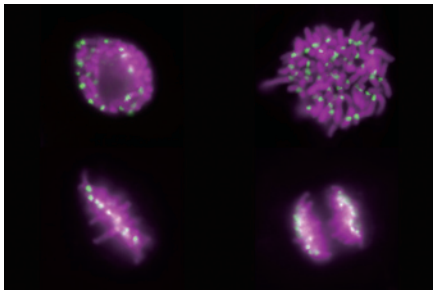

Fig. 1 Phosphorylation of histone H3 at Ser10 (green) on mitotic chromosomes (magenta).

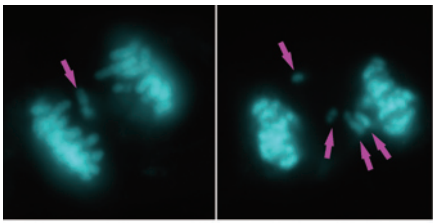

Fig. 3 Hesperadin-induced lagging chromosomes (aroows) at anaphase (left) and telophase (right).