The Smart Mechanism of Microtubule Depolymerizing Nano-Machine

Microtubules (MTs) are dynamic tube-like cytoskeleton composed of tubulin dimers. The proper regulation of the polymerization and depolymerization of MTs is a critical event in cells. KIF2, a type of motor protein, depolymerizes MTs; however, the mechanism by which a small number of KIF2 proteins can depolymerize a large number of MTs remained unknown. The combination of small-angle X-ray scattering and X-ray crystallography revealed that one KIF2 core domain forms a large complex with two sets of tubulin dimers during ATP hydrolysis, suggesting a smart mechanism for depolymerizing a large number of MTs efficiently.

In our cells, proteins efficiently fulfill their physiological functions through their multidimensional features, such as conformational change, interaction, and complex formation. Therefore, precise analyses of their dynamic structures in solution may deliver critical insights into the physiological mechanisms of their functions.

Microtubules (MTs) are dynamic tube-like structures polymerized from large numbers of tubulin dimer proteins. Proper regulation of the polymerization and depolymerization of MTs is critical for fundamental events in cells such as the formation of neurons and cell division. KIF2, one of the kinesin motor proteins (KIFs), is known to regulate MT dynamics by depolymerizing MTs from tip ends [1, 2] [**Fig. 1(A)**]; however, the mechanism by which a small number of KIF2 proteins drive the processive depolymerization of MTs composed of a large number of tubulins remained unclear. To address this issue, we analyzed the transitional states of MT depolymerization via KIF2 by small-angle X-ray scattering (SAXS) and X-ray crystallography, in combination with size-exclusion chromatography (SEC), multi-angle light scattering (MALS), analytical ultracentrifugation (AUC), and cross-linked mass spectrometry (X-link MS) [3, 4] [**Fig. 1(B)-(C)**].

During MT depolymerization via KIF2, the large transitional KIF2-tubulin complex was monitored by high-resolution size-exclusion chromatography (HiRes SEC); therefore, the large complex was further analyzed by SAXS beamline BL-15A2 [5] [**Fig. 1(B)**]. HiRes SEC was connected with the following flow-cell for SAXS measurement, and the separated sample was directly analyzed by SAXS (HiRes SEC-SAXS), thus greatly reducing X-ray damage to the protein complex. SAXS analysis indicated the radius of gyration (R_g) to be 51.5±0.5 Å, with D_{max} of 203 Å. By integration with HiRes SEC-RI-MALS, AUC and X-link MS, the molecular weight of the transitional KIF2-tubulin complex was determined as 257 kDa, which corresponded to the sum of its components: 51 kDa (KIF2) and two sets of 103 kDa (tubulin dimer) (1:2 complex). These measurements directly suggest that one KIF2 forms a large complex with two sets of tubulin dimers. To reveal the structural arrangement and outline of the KIF2-tubulin complex in solution, HiRes SEC-SAXS data was further analyzed, and the SAXS model of the large 1:2 complex displayed a pipe structure with a bulge at one end and had a reasonable volume for its components, one KIF2 and two tubulin dimers [**Fig. 1(D)**].

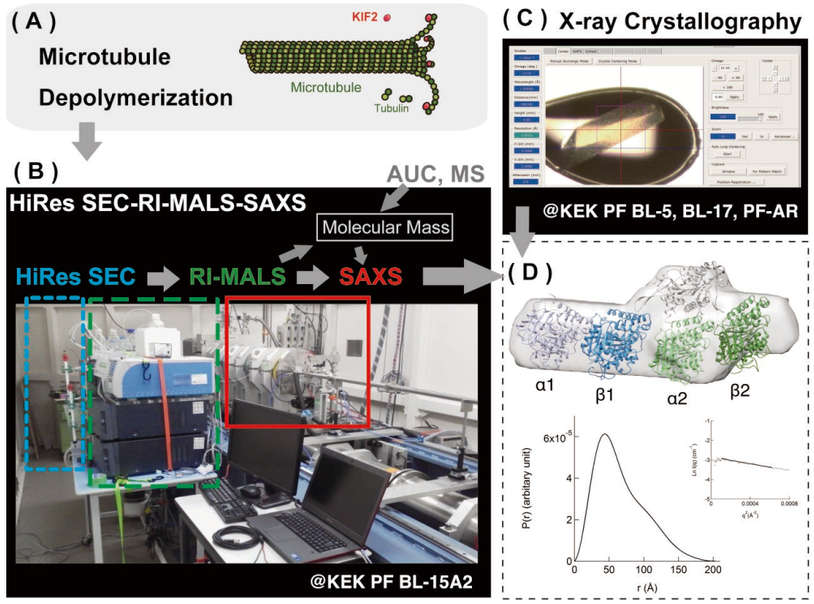

Figure 1: Combination of multiple X-ray based approaches at PF. **(A)** Microtubules (MTs) are dynamic polymer cytoskeletons composed of α-/β-tubulin dimers. KIF2 depolymerizes MTs. **(B)** HiRes SEC-SAXS system at BL-15A2. **(C)** X-ray crystallography beamline. **(D)** SAXS model of KIF2-tubulin complex.

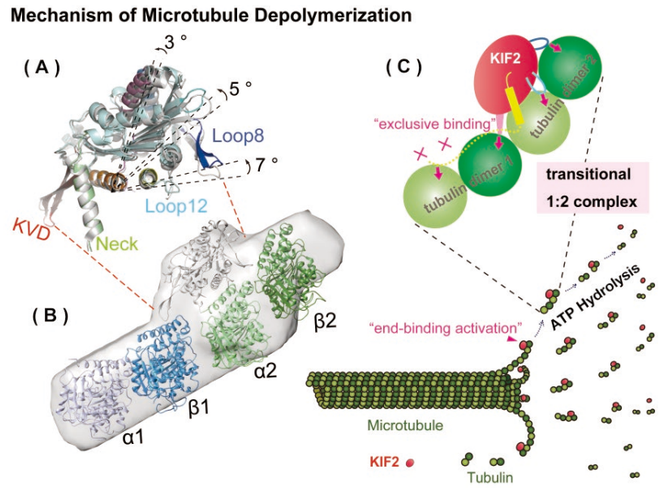

Figure 2: Mechanism of microtubule depolymerization. **(A)** Transitional conformation of KIF2 structure revealed by X-ray crystallography. **(B)** Transitional conformation of KIF2-tubulin 1:2 complex revealed by SAXS analysis. **(C)** Model of the efficient mechanism of microtubule depolymerization via transitional KIF2-tubulin complex.

When one KIF2 core and two tubulin dimers compose the large transitional complex, it was unknown how this large conformation of 1:2 complex is produced from the small changes of KIF2 molecules during ATP hydrolysis. To reveal the mechanism at atomic resolution, KIF2 molecules in the transitional state were purified from the transitional 1:2 complex, and crystallized for X-ray crystallography beamlines [**Fig. 1(C)**]. As a result, some critical conformational changes were observed. KIF2 has unique structural features, such as a neck, Loop2 (KVD finger), α4, Loop8, and Loop12 [1]. The binding surface with tubulin, Loop8, Loop10, α3 and α2a were shifted upward in the transitional KIF2 structure [**Fig. 2(A)**]. These major conformational changes with the greatest curvature were both induced by docking with tubulin dimers and by ATP hydrolysis to stabilize the transitional 1:2 complex conformation [**Fig. 2(B)**]; these observations serve as direct structural evidence for the activation of KIF2 activity at the ends of MTs [**Fig. 2(C)**].

In an in vivo system, large amounts of MTs must be regulated efficiently by using the limited resources of KIF2 proteins and ATP. In this sense, the 1:2 ratio is a fundamental property of the catalytic mechanism of MT depolymerization. Therefore, KIF2 functions as a smart molecular machine that depolymerizes two sets of tubulin dimers with one ATP [**Fig. 2(C)**]. The KIF2 protein works more efficiently to depolymerize a large amount of microtubules when dispersed in a well-balanced fashion, allowing it to bind over the entire tip of the microtubule, instead of gathering in the same spot. Failure to properly regulate microtubule polymerization and depolymerization can cause many diseases, including neurodegenerative diseases and cancers. The mechanism of microtubule depolymerization revealed in this study provides the basis for understanding the pathology and establishing effective methods for treating microtubule-related diseases.

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BEAMLINES

BL-15A2, BL-10C, BL-6A, BL-1A, BL5A, BL-17A and AR-NW12A

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