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SEC-SAXS Analysis Revealed the Transitional Complex of Microtubule Depolymerizing Kinesin and Tubulin in Solution

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1 Introduction

Microtubules (MTs) are dynamic tube-like structures polymerized from large numbers of tubulin dimer proteins. The proper regulation of the polymerization and depolymerization of MTs is critical for fundamental events in cells. KIF2, a member of kinesin motor proteins (KIFs), is known to regulate MTs dynamics by depolymerizing MTs from tip ends [1][2]; however, the mechanism by which a small number of KIF2 proteins drives the processive depolymerization of MTs composed of a large number of tubulins remained unclear. To address this issue, the transitional states of MTs depolymerization via KIF2 were analyzed by the small-angle X-ray scattering (SAXS), in combination with size-exclusion chromatography (SEC) and multi-angle light scattering (MALS) [3][4].

2 Experiment

During MTs depolymerization via KIF2, the large transitional KIF2-tubulin complex was monitored in high-resolution size-exclusion chromatography (HiRes SEC) [3]; therefore, the large complex was further analyzed by SAXS at beamline BL-15A2 [5]. HiRes SEC was connected with the following flow-cell for the SAXS measurement, and the separated sample was directly analyzed by SAXS (HiRes SEC-SAXS), by which the X-ray damage to the protein complex was greatly reduced. In addition, HiRes SEC-RI-MALS was also conducted to confirm the molar mass value of the complex. In HiRes SEC-SAXS system, the mono-disperse eluate directly flows into a flow-cell holder and serial scattering images were taken with 10 sec exposure. Each ten scattering profiles in the first half of an elution peak were averaged and the scattering profiles of the KIF2-tubulin complex extrapolated to an infinite dilution condition were calculated from these averaged scattering profiles.

3 Results and Discussion

The radius of gyration (Rg) was estimated from the Guinier approximation to be 51.5±0.5 Å, and Dmax was calculated to be 203 Å on the basis of the pair distribution function, P(r). Both HiRes SEC-RI-MALS and HiRes SEC-SAXS estimated the large transitional KIF2-tubulin complex around 250 kDa in molecular weight, which corresponded the sum of its components: 51 kDa (KIF2 core domain) and two sets of 103 kDa (tubulin dimer) (1:2 complex). These measurements directly suggested that one KIF2 forms a large complex with two sets of tubulin dimers as a transitional conformation. To reveal the structural arrangement and outline of the KIF2-tubulin complex in solution, HiRes SEC-SAXS data was further analyzed, and 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, arranged longitudinally (Figure 1).

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 longitudinal 1:2 complex is a fundamental property of the catalytic mechanism of MTs depolymerization. Therefore, KIF2 functions as a smart molecular machine that efficiently depolymerizes two sets of tubulin dimers with one ATP. The microtubule depolymerization mechanism revealed in this study promises to become the basis for understanding the pathology to establish effective treatment methods for microtubule-related diseases.

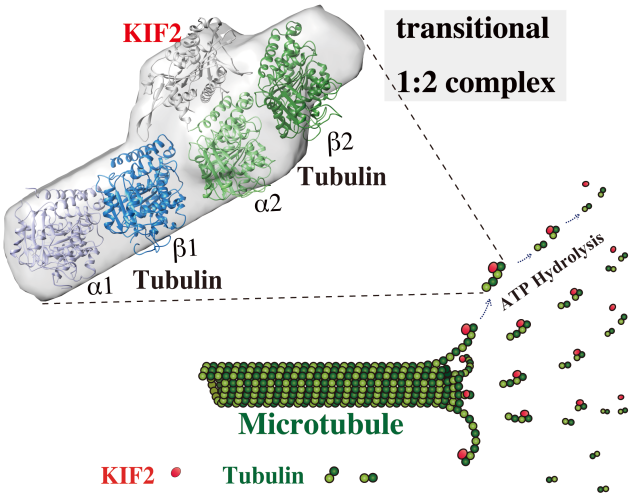

Fig 1: Structure of KIF2-Tubulin Complex in Solution

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