

The Crystal Structure of Microtubule Depolymerizing Kinesin KIF2 in Transitional States

Tadayuki OGAWA¹, Xuguang JIANG¹, and Nobutaka HIROKAWA^{1,*}

¹Department of Cell Biology and Anatomy, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Tokyo, 113-0033, Japan

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 KIF2 were precisely analyzed by X-ray crystallography in combination with the small-angle X-ray scattering (SAXS) at KEK PF beamlines [3][4].

2 Experiment

SAXS analysis indicated that one KIF2 forms a large complex with two sets of tubulin dimers as a transitional conformation [3]; however, it remains unknown how this large conformation of 1:2 complex is produced from the small structural changes of KIF2 molecules during ATP hydrolysis. To investigate the mechanism at atomic resolution, KIF2C core domain proteins in transitional state (KIF2Ccore with ADP-BeFx and ADP-AIFx) were purified from the transitional complex, and crystallized for X-ray crystallography at KEK-PF beamlines.

3 Results and Discussion

Final data sets were collected at the BL-5A and BL-17A beamlines. The crystals belonged to the space group P21212 ($a = 89.45 \text{ \AA}$, $b = 166.59 \text{ \AA}$, $c = 75.50 \text{ \AA}$). The KIF2Ccore:ADP-BeFx structure was determined with the molecular replacement method by using atomic coordinates for the KIF2C:AMP-PNP (PDB: 1V8K). Electron-density maps based on the 2Fo-Fc coefficients were calculated from the phases of the initial model. After subsequent rounds of model building and refinement, the model was refined to R and Rfree values of 20.1% and 26.1%, respectively ($30.0 - 3.10 \text{ \AA}$). The KIF2Ccore:ADP-AIFx structure was similarly determined and refined to R and Rfree values of 19.8% and 23.0%, respectively ($30.0 - 3.43 \text{ \AA}$). As a result, some critical conformational changes were observed. KIF2 has unique structural features, such as a neck, Loop2 (KVD finger), $\alpha 4$, Loop8, and Loop12 [1]. The binding surface with tubulin, Loop8, Loop10, $\alpha 3$ and $\alpha 2a$, were shifted upward in transitional structure (Figure 1A). Loop8 and $\alpha 3$ are trapped in warped structures because of the interaction between the γ -phosphate and switch1 Loop9 (Figure 1B). 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, and these observations serve as direct structural evidence for activation of KIF2 activity at MTs ends (Figure 1C).

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 MTs depolymerization. Therefore, KIF2 functions as a smart molecular machine that efficiently depolymerizes two sets of tubulin dimers with one ATP. Failure to properly regulate microtubule dynamics can cause many diseases, including neurodegenerative diseases and cancers. 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.

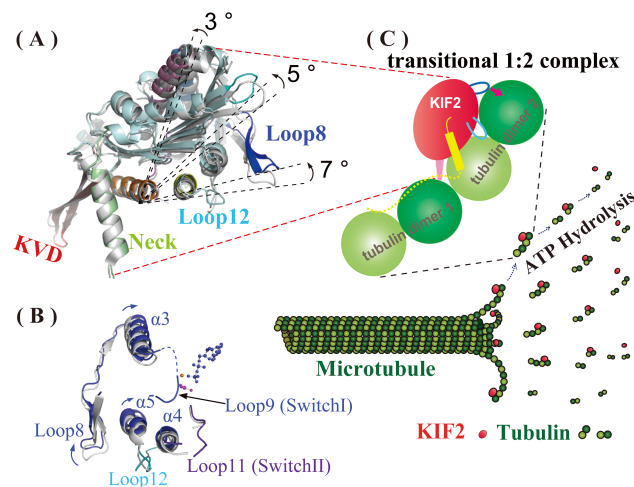


Fig. 1: Transitional Conformation of KIF2

Acknowledgement

PDB accession codes: 5XJB (KIF2Ccore:ADP-BeFx), 5XJA (KIF2Ccore:ADP-AIFx). We thank the all PF staff for help with X-ray crystal data collection.

References

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* hirokawa@m.u-tokyo.ac.jp