BL-1A, NW12A/2012G059, 2014G026

Crystal Structure of KIF19A Motor Domain with ADP

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1 Introduction

The kinesin-8 motor, KIF19A, accumulates at cilia tips and controls cilium length. *Kif19a*$^{-/-}$ mice displayed hydrocephalus and female infertility phenotypes due to abnormally elongated cilia that cannot generate proper fluid flow[1]. Uniquely among kinesins, KIF19A possesses the dual functions of motility along ciliary microtubules and depolymerization of microtubules. However, the molecular mechanisms of the dual-functions of kinesin-8 proteins remain to be determined.

To elucidate the molecular mechanisms of these functions we solved the crystal structure of its motor domain and determined its cryo-electron microscopy structure complexed with a microtubule. The features of KIF19A that enable its dual function are clustered on its microtubule-binding side [2].

In this report, we describe the details about the crystal structure of KIF19A motor domain with ADP.

2 Experiment

The coding sequence of the mouse KIF19A motor domain (1-353, referred as KIF19AMD) was cloned into pET21b (+) with a 7XHis-tag at the C-terminal of the motor domain. The KIF19A motor domain was purified immobilized-metal affinity chromatography and ion exchange chromatography and dialyzed against crystallization buffer (10mM MOPS, pH 7.0, 100mM NaCl, 1mM $MgCl_2$, 0.1mM ADP, 15% Sucrose). The hanging drop vapor diffusion method was used. One microliter of KIF19A motor protein sample at 10 mg/ml containing 0.1 mM ADP was mixed with 1 µl reservoir buffer and incubated at 20°C. Single crystals are grown in 10% ethylene glycol, 2% PEG8000, 50 mM Tris-Bicine (pH 8.5), 9 mM $MgCl_2$ and 9 mM $CaCl_2$. The crystals were frozen in liquid nitrogen within two weeks after the crystal grew up. X-ray diffraction data were collected using X-ray diffraction data were collected using a BL41XU beam-line (SPring-8, Japan), at a wavelength λ=1.0 Å. The anomalous diffraction data were collected using a BL-1A beamline (Photon Factory, Japan), at the wavelength λ=2.7 Å. The HKL2000 program package was used to index, integrate and scale the data. The structure of KIF19A motor domain was solved by molecular replacement using the Crystallography & NMR System (CNS) and the atomic coordinates of KIF18AMD (PDB: 3LRE)[3] as a search model. Several rounds of iterative model building and refinement were performed using COOT and Phenix. The final crystallographic model of KIF19A motor domain was refined to R_{work}/R_{free} of 0.222/0.302.

3 Results and Discussion

At the initial stage of the KIF19AMD structure determination process, the residues of the switch II helix α4 could not be determined because there was no corresponding density at the site where α4 is usually located at the center of the MT-binding interface. Instead, we found an unmodeled helical-like density, which was more distant from the KIF19A catalytic core (Figure 1). Long wavelength X-ray diffraction experiment was thus performed to investigate the property of this density, because in the switch II helix α4, one cysteine residue exists. It successfully depicted the anomalous diffractions of sulfur or phosphorus atoms. Among them, one strong anomalous signal was detected close to the center of the corresponding helical density. When the Cys283 residue was assigned to this anomalous signal (Figure 2), all the residues in α4 and the following loop L12 were reasonably determined.

The overall structure of the KIF19AMD motor domain shared a similar triangle-shape with other kinesins (Figure 3). In the KIF19AMD atomic structure, Mg^{2+}-ADP was found embedded in the nucleotide-binding pocket. In comparison with previously solved motor domains of various KIFs, the remarkable features of the KIF19A motor domain are concentrated on its MT-binding side and include the long and wide L2 loop, the flexible L8 loop, the disordered α4 helix, and the short α6 helix (Figure 3).

The destabilized switch II helices contribute to the adaptation to the two distinct interfaces of straight and curved MTs. The rotatable L8-α3-L9 cluster also supports adaptation to the two interfaces. The basic cluster of L2, as well as the basic residues of L12, enables KIF19A to tether to both straight and curved MTs via flexible ionic interactions with the acidic residues of H12 or the E-hook of tubulins. This assures its motility along the MT, although motility speed may be decreased. The hydrophobic tip of L2, as well as the surrounding basic and acidic clusters, plays critical roles in MT depolymerization. These residues stabilize the inter-tubulin-dimer interface of the curved MT protofilament. Thus, the curved conformation of MT ends is stabilized by L2, resulting in the depolymerization of the MTs. In this way, KIF19A has acquired dual functions by

introducing multiple strategies. The resulting KIF19A is a slow plus-end directed motor combined with mild MT-depolymerizing activity.

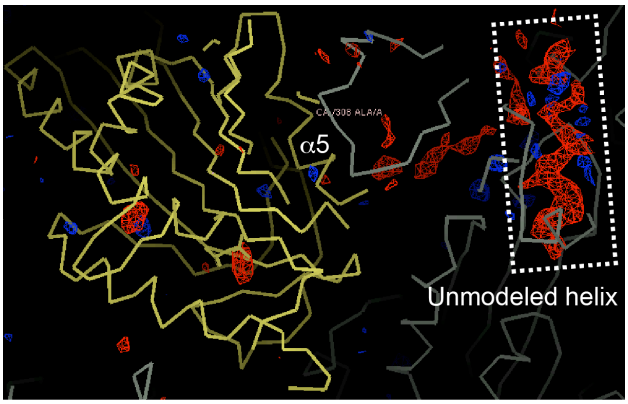

Figure 1 The unmodeled helix

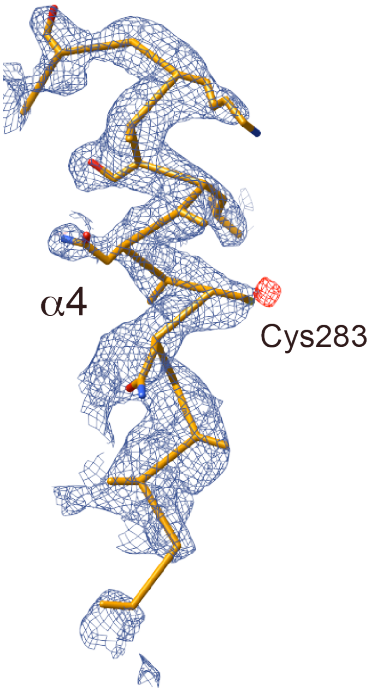

Figure 2 The anomalous signal found around the unmodeled helical density.

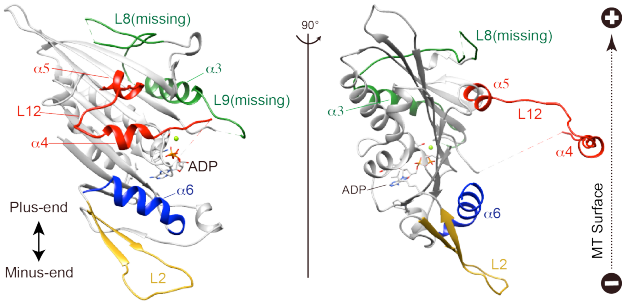

Figure 3 The overall structure of KIF19AMD

Acknowledgement

We are grateful to N. Matsugaki from PF for the technical support. We thank members of the Hirokawa lab for assistance and discussions.

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