論文の内容の要旨

論文題目　Structural and Functional Analysis of Kinesin Superfamily Protein KIF19A

(キネシンスーパーファミリータンパク質 KIF19A の構造および機能の解析)

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Kinesin superfamily proteins (KIFs) are known as molecular motor proteins, which move along microtubule (MT) filaments. KIFs hydrolyze the ATP to provide the energy to support various cellular functions such as the intracellular transport and the regulation of MT dynamics. In the standardized nomenclature, KIFs are classified into 14 families, from Kinesin-1 to Kinesin-14 and share a highly conserved motor domain sequence. Among all the KIFs, Kinesin-8s are the unique dual functional motors, which can actively walk along the MT to target the MT plus end, where they depolymerize the MT and regulate the MT dynamics. Most of the Kinsin-8 motors play critical roles during the cell division process, such as spindle length regulation, mitotic chromosome alignment control. The structural studies of human Kinesin-8 motor domain KIF18A provided some clues indicating the molecular mechanism of the dual functional Kinesin-8 motor domain. However, the characteristic long loop L2 was missing in the crystal structure of KIF18A because of its flexibility and the rigid KVD finger like motif of Kinesin-13, which is essential for the MT depolymerization, is lacking in the loop L2 of KIF18A. The molecular mechanism of the dual-function of Kinesin-8 is therefore still unclear.

Recently, another member of Kinesin-8 motor, KIF19A was reported as a MT depolymerizing kinesin for ciliary length control, unlike the KIF18A involved in the cell division. *Kif19A*$^{-/-}$ mice displayed hydrocephalus and female infertility phenotypes due to abnormally elongated cilia that cannot generate proper fluid flow. Regarding the different biological functions, to date, the structural mechanisms of the dual functions of Kinesin-8, either common or distinct between KIF18A and KIF19A, remain elusive. To elucidate the molecular mechanism of the dual functions, the MT-based motility and depolymerizing MTs, of Kinesin-8, KIF19A, here I determined the crystal structure of the mouse KIF19A motor domain in the complex with the ADP. I also examined the ATPase activities, the MT-based motilities and MT depolymerization abilities with the wild type motor domain and the mutants, which were based on the structural observation and sequence alignment with other kinesins.

In this study, both the motor domain and the following neck linker were included in the construct, KIF19A353WT (residues from 1 to 353), which was expressed in *E.coli* BL21 (DE3), and purified sequentially by the immobilized metal affinity chromatography (IMAC) and the cation exchange chromatography (CIEX). KIF19A353WT and ADP were co-crystallized in the buffer, which used ethylene glycol and PEG8000 as precipitant. Single crystals were grown at 20°C using hanging drop vapor diffusion method. X-ray diffraction

data at 2.72 Å resolution were collected at BL41XU beamline (SPring-8) and then was processed through HKL2000 program package.

The tertiary structure was tried to be solved by using molecular replacement method. During the structure determination process of KIF19A motor domain, however, I found one atypical helical density that was situated close to the loop L2. It was difficult to assign the certain amino acid sequence to it by the molecular replacement because it was not observed in any other motor domain structures of several KIFs. I combined a sulfur single-wave length anomalous diffraction (S-SAD), which was based on better anomalous signal from protein sulfur atoms obtained at longer X-ray wavelength, to clarify which elements should be assigned to it. As a consequence, the anomalous diffractions of sulfur atom in Met or Cys residues of the protein as well as the phosphorus atom in ADP were successfully detected. Around the corresponding helical density, one strong anomalous signal was detected, indicating that the uncertain helical density was the part of the helix α4 and the anomalous signal came from the diffractions of the sulfur atom at the Cys283 residue. In this way, I could finally determine the complete tertiary structure of KIF19A motor domain with the R-factor of 24.53%.

The overall structure of KIF19A motor domain shared a similar triangle-shape structure with other kinesins, which consists of a central β-sheet of eight strands, sandwiched between six α-helices, three on either side. In comparison with the previously solved motor domains of the various KIFs, remarkable features of KIF19A motor domain seem to be concentrated on its MT-binding side; the disordered helix α4, the short helix α6, the long and wide loop L2 and the flexible Loop L8.

The loop L8 is one of the major MT-binding interfaces and the L8-α3-L9 cluster is retracted from the MT/tubulin in the crystal structure. The tip of L8 remains flexible in the absence of the MT/tubulin. This conformation is conserved between Kinesin-8 subfamily (both KIF18A and KIF19A) and might give a flexibility of the interface of Kinesin-8 to adopt both the straight and curved interface of tubulin-dimers that are found in the MT lattice and the MT plus-end, respectively. The helix α6 takes the unusually shorter form in comparison with the previously solved any structures of KIFs including KIF18A. This conformation might be caused by the KIF19A specific glycine before the highly conserved RAK residues at the C-terminal end of the helix α6. Since the helix α6 serves as the base of the following neck-linker region that is necessary for the MT-based plus-end directed motility as well as the inter-head communication in case of the dimeric motility, this glycine and short form of α6 might produce either positive or negative effect in the motility and the inter-head communication of KIF19A. Further structural and biochemical studies are needed to elucidate the functional role of the L8-α3-L9 and the short helix α6.

Among the structural elements on the MT-binding interface, most prominent conformational change was observed at the switch II cluster (L11-α4-L12-α5) of KIF19A. The helix α4 is atypically distantly positioned from the catalytic core. The protruding α4 was found for the first time among all the solved crystal structures of

KIFs. At the ends of the helix α4, the loops L11 and L12 are destabilized and become flexible so that KIF19A might be able to adjust the position of the helix α4 to fit the two distinct binding partner, the straight tubulin-dimer and the curved tubulin-dimer.

When superimpose the KIF19A structure onto other kinesins, I found that about one turn melted at the N-terminal end of the helix α5. At that point, the highly conserved proline residue among all KIFs except for KIF19A is replaced with the unique asparagine residue (N297). In general, proline residue is often found at the starting or the ending points of the helix to stabilize it. In KIFs except for KIF19A, highly conserved proline is found at the starting point of the helix α5 to stabilize it. Thus, the substitution of asparagine for proline residue might destabilize α5 to be melted into the flexible long loop L12. Therefore, I expected that the flexible positioning of the helix α4 might be caused by the KIF19A specific N297.

This idea was confirmed by the biochemical and biophysical experiments. N297P mutant presented about 30% increase of MT-stimulated ATPase activity and more than 30% decrease of the tubulin-stimulated ATPase activity in comparison with the wild type. As a result, N297P mutation moved microtubules 40% faster than the wild type, whereas N297P mutant decrease the microtubule depolymerizing activity. This indicates that N297P mutant, which fits better to the microtubule than to the curved-tubulin dimer, has the advantage for the MT-based motility. In other words, the KIF19A-specific asparagine residue becomes a key determinant to accommodate the KIF19A interface more to the curved tubulins so that KIF19A has acquired the higher depolymerizing activity at the expense of the motility speed.

Both Kinesin-8 and Kinesin-13, the MT-depolymerizing kinesins, are known to have an extended loop L2 comparing with other motile kinesins, albeit different depolymerizing efficiency. KIF19A and KIF2C have a comparable length of loop L2. Kinesin-13 has a class-specific lysine-valine-asparate (KVD) finger structure that lies in the tip of loop L2 and any residue deletion of the KVD finger will deprive of the effective microtubule depolymerizing activity. Interestingly, the loop L2 in the crystal structure of KIF19A is built up from a hydrophobic tip (I54, L55) sandwiched by the acidic (E49, D50, D52, D53) and basic clusters (R56, H58, R59, R61). The similar order of residue characters (basic-hydrophobic-acidic) in the loop L2 of KIF19A and KIF2C highlight the potential significance of the charged and hydrophobic residues in depolymerizing the MTs. The alanine mutations in each section, in fact, caused the microtubule depolymerizing deficiency. The same subclass Kinesin-8, KIF18A has the 9 residues longer than KIF19A and its charged residues of L2 seem to arrange randomly, resulting in decreasing its depolymerizing activity. What is more, the slender loop L2 and relatively longer antiparallel β-sheet of Kinesin-13 form more rigid protrusion that points down to the MT, just like a sharp dagger, resulting effective depolymerase activity. The long, fan-shaped loop L2 of KIF19A provides the possibility of flexible conformation, reflecting a relatively low microtubule depolymerizing efficiency of the Kinesin-8.

In addition to the role for MT-depolymerization, the basic cluster of L2 of KIF19A also contributes to the processivity of KIF19A when moving along the MTs. The alanine mutations of basic cluster of L2 (PC2A) severely weakened the MT-binding affinity of KIF19A so that MT could not glide on the KIF19A clusters attached on the cover slide. The deletion of E-hook at the C-terminus of tubulins also has the same effect as the PC2A mutation, suggesting that KIF19A might tether the MT using the flexible ionic interaction between the positively charged residues of L2 of KIF19A and the negatively charged residues at the C-terminus of tubulins (E-hook), similar with the interaction between the K-loop of KIF1A and E-hook of tubulins. As described above, KIF19A specific N297 has disadvantage for the MT-based motility. Basic cluster might compensate this disadvantage by increasing the affinity of KIF19A to the MTs.

In summary, this study illustrates the functional anatomy for the dual function of KIF19A. Basic cluster of the loop L2 of KIF19A enables it to tether MTs by the flexible ionic interaction with the E-hook of tubulins so that KIF19A processively walks along the MT-lattice. When KIF19A reaches the plus-end of the MTs, the specific asparagine residue 297 of KIF19A as well as the flexible loop L8 facilitates the binding to the curved protofilament and then, the tubulin dimer is removed from the plus end of MTs by using the energy generated from ATP hydrolysis. The hydrophobic tip of L2 does the critical role during the some step(s) of the depolymerization process. Therefore, KIF19A has acquired the dual function by introducing the multiple strategies, one of which resembles the KIF1A tethering to the MTs or the Kinesin13s depolymerizing strategy, the other of which is KIF19A specific one in which the flexible interface for MT is applied to bind with two distinct binding partners, the straight and curved tubulin-dimers. Further structural studies including the X-ray crystallography of KIF19A in another nucleotide state and the cryo-EM analysis of KIF19A-MT complex as well as the biochemical and cell biological studies are needed to clarify the full biological mechanism of the dual functions of KIF19A.