論文の内容の要旨

論文題目　Structural Analysis of Kinesin Superfamily Protein KIF4

（キネシンスーパーファミリータンパク質KIF4の構造生物学的解析）

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Background

In living cells, kinesin superfamily proteins (KIFs) play the central roles for transporting several cargoes along their cellular track, microtubules. Among them, KIF4 belongs to Kinesin-4 family and plays multiple important physiological functions such as the anterograde transport of several cargoes such as the component of ribosomes or the cell adhesion molecule L1, the regulation of apotosis of neuronal cells through the interaction with Polly ADP ribose polymerase, and the regulation of mitotic spindle organization. To achieve these physiological functions, KIF4 not only moves actively along the microtubules but also regulates the microtubule dynamics. However, the molecular mechanism about how KIF4 can play these two distinct roles are still not understood because of the missing of the atomic detail of KIF4 structure. Here we solved first x-ray crystal structure of KIF4 motor domain at 1.8 Å resolution. High resolution atomic structure provided us the many information about the molecular mechanism to achieve these important function.

Materials and Methods

I made the monomer construct KIF4-344 (1-344) which includes the catalytic core and neck-linker. Auto-induction methods enabled me the efficient expression of my target protein. After three purification steps (IMAC, CIEX and SEC), I got high purity of KIF4-344 (99%) for crystallization.

Crystals were grown in the PEG 4,000-based buffers and after the careful optimization of several parameters such as precipitants, pH, additives, and cryo-protectants, high resolution diffraction data could be collected at NW12A (PF) and 41XU (SPring 8). Space group was determined using *Pointless* and *Scala* (space group: $P4_12_12$; unit cell size: 62.78, 62.78,167.66, $\alpha=\beta=\gamma=90.0°$). HKL2000 was used for integration and scaling of the data. Then, I used *Molrep* for molecular replacement and *refmac5* for rigid body refinement and energy minimization. Eg5 structure (3HQD) was used for the reference model. Program *coot* was used for model building and validation. Finally atomic model of KIF4-344 could be solved with R_{work}/R_{free} 17.91%/20.50%.

Results

The overall architecture of KIF4 motor domain is basically similar with other KIF motors solved previously. KIF4-AMPPNP complexed with the ATP analogue AMPPNP has a globular catalytic core with a short strand 'neck-linker' docked on it. The catalytic core has two important elements, the ATPase reaction center and microtubule-binding interface. These two regions are connected by the two mobile elements switch I and switch II. To elucidate the differences between KIF4 and other kinesin structures in the same nucleotide state, I compared the structure of KIF4-AMPPNP with KIF1A-AMPPNP, as both of them were refined to high resolution (1.8 Å for KIF4-AMPPNP and 1.85 Å for KIF1A-AMPPNP). The major differences between them are concentrated on the switch I and switch II, especially the loop L9 in switch I and L12 in switch II. These two loops are ordered and visible in KIF4-AMPPNP whereas they are disordered and invisible in KIF1A-AMPPNP.

Kinesin motor complexed with AMPPNP is considered to represent the ATP state just before the ATP hydrolysis (pre-hydrolysis state). In this state, the nucleotide-binding pocket should be entirely closed. To close the pocket, following two processes are required. 1) Two conserved residues, serine (SSRSH) in switch I and glycine in switch II (DLAGSE) senses the γ-phosphate to trigger ATP hydrolysis. 2) Two conserved residues, arginine (SSRSH) in swtich I and glutamate (DLAGSE) in switch II, form a salt-bridge (backdoor) to close the pocket. In the KIF4-AMPPNP structure, both of these features are observed whereas these two features are only partially found in the KIF1A-AMPPNP

structure (partial bond of salt-bridge) and are not found in the KIF1A-AMPPCP structure. From the KIF1A-AMPPCP structure, to the KIF1A-AMPPNP structure, and finally to the KIF4-AMPPNP structure, the backdoor is apparently closed step by step to bind ATP tightly to prepare the hydrolysis of ATP. Hence, the KIF4-AMPPNP structure represents true pre-hydrolysis ATP state. KIF1A-AMPPCP might represent pre-isomerization state immediately after ATP enters into the pocket and KIF1A-AMPPNP might represent the in-between transition state. With the closure of backdoor, a series of interactions between switch I and switch II are formed to stabilize the loops, L9 in switch I and L11 in switch II.

The important roles of backdoor residues in ATP hydrolysis were further confirmed through the kinetic studies. KIF1A and KIF4 wild type showed microtubule-stimulated ATPase activity, which is consistent with the previous reports. Three mutants of KIF4 backdoor (R212A, R212K and E246D) completely lose their ATPase activity. E246D mutant of backdoor, however, partially rescued the microtubule-stimulated ATPase activity of KIF4. These biochemical experiments further validate the importance of backdoor for ATP hydrolysis of Kinesin motor.

Entire closure of backdoor further induced the docking of neck-linker to the catalytic core. From the KIF1A-AMPPCP structure, to the KIF1A-AMPPNP structure, finally to the KIF4-AMPPNP structure, the neck-linker docks to the catalytic core sequentially from its N-terminus (near neck-initial segment) to its C-terminus. This ATP-binding induced sequential docking of the neck-linker to the catalytic core provided the structural basis for the power-stroke and ATP gating in the dimeric motility moved by the hand-over-hand mechanism.

Previous reports showed that KIF4 not only moves along the microtubule, but also regulates the microtubule dynamics. To elucidate this molecular mechanism, the structural information about the interaction sites between KIF4 and microtubule is helpful. Since the cryo-EM structure of KIF4-microtubule complex is not available, we docked the crystal structure of KIF4-AMPPNP into the cryo-EM structure of KIF1A(AMPPNP)-microtubule complex and KIF5(AMPPNP)-microtubule complex to estimate the binding site of KIF4 for the microtubule. By this in silico docking experiment, KIF4 specific sequences were found on the three major microtubule binding interfaces, the loop L11, the helix α4, and the loop L12. These sequences might contribute to the unique roles of KIF4 such as the regulation of the microtubule dynamics and further structural studies of KIF4-microtubule complex as well as the biochemical experiments using the structure-based several

mutants are needed.

Conclusion

I solved the first KIF4 structure complexed with the ATP analogue AMPPNP at 1.8 Å resolution. This structure adopts the pre-hydrolysis ATP state with the complete closure of the nucleotide-binding pocket which is coupled to the "backdoor" salt-bridge formation. KIFs are thought to be the "backdoor enzyme" so that the backdoor closure not only triggers the hydrolysis of ATP, but also regulates the conformations of switch II and the neck-linker that play the central roles for the microtubule-based motility. In comparison with two KIF1A structures complexed with different ATP analogues, this study provides the structural basis for the molecular mechanism of dimeric motility moved by the hand-over-hand mechanism. I also elucidated the interface of KIF4 for the microtubule, providing the structural information about the KIF4 specific sequences at the microtubule-binding interface that might be crucial to achieve the KIF4 specific function such as the regulation of microtubule dynamics.