Structural Mechanism of the Microtubule-Activated Nucleotide Exchange of Molecular Motor Kinesin

Molecular motor kinesin moves along microtubules as a result of the energy from ATP hydrolysis. To elucidate the structural mechanism of the nucleotide exchange of kinesin from the ADP to ATP, which is significantly accelerated by microtubules, we solved the series of the crystal structure of monomeric kinesin KIF1A during the release of the ADP. On the basis of new structural data, we propose a model mechanism for microtubule activation of ADP release from KIF1A. Loop L7 of KIF1A acts as a triggering lever for the ADP release. A specific interaction between loop L7 and microtubule triggers the breakage of the link between L7 and the active site, leading to the sequential release of a group of water molecules that sits over the Mg^{2+}, followed by Mg^{2+}, and finally the ADP.

Molecular motor kinesin superfamily proteins (KIFs) utilize the energy of ATP hydrolysis to move along microtubules, and play a critical role in the intracellular transport of various organelles, protein complexes and mRNAs. To elucidate the molecular mechanism of the kinesin motility, we first solved the crystal structures of monomeric kinesin KIF1A and the cryo-EM structures of microtubule-bound KIF1A in two stable intermediate states complexed with ATP and ADP [1]. We then solved three transition structures during the ATP hydrolysis by using several nucleotide analogs [2]. Here we solved four sequential transition structures during the release of ADP by varying the timing to harvest crystals [3]. To collect the X-ray diffraction data on the ADP releasing crystals, we used the AR-NW12A and BL-5A beamlines at Photon Factory. We have now successfully solved all nine intermediate structures of KIF1A during the ATP hydrolysis cycle (Fig. 1) [4].

During the ATPase cycle of KIF1A, the ADP release is the rate-limiting extensively slow step (0.01 s^{-1}), and the microtubules which are the rails of the KIF1A accelerate this step by more than 10,000 times. That is, the microtubules serve as a nucleotide-exchange factor of KIF1A and suppresses the futile consumption of ATP by free kinesin (not on the rail). The atomic structures of KIF1A during ADP release first explained this well known biochemical property.

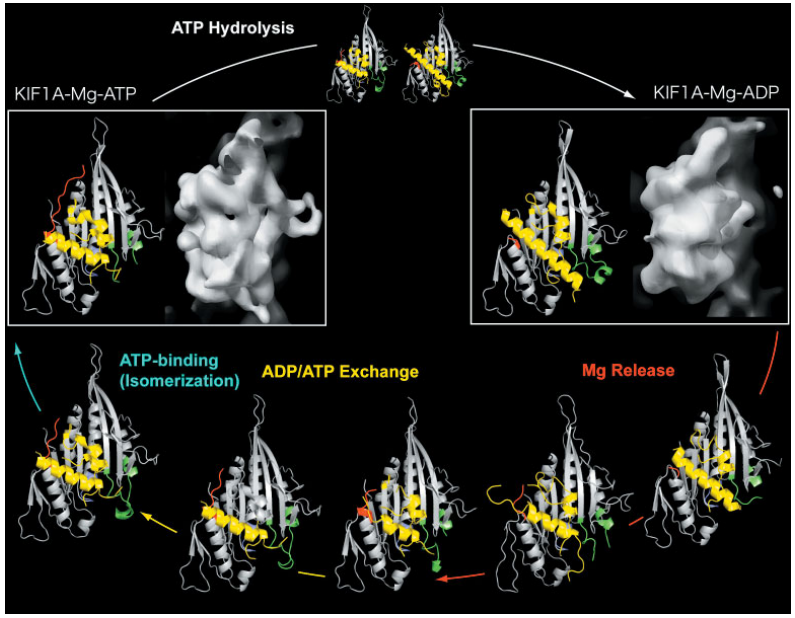

Figure 1
Overall conformational change of monomeric kinesin KIF1A during the ATPase cycle. The motor domain of KIF1A is shown from the microtubule-binding side. Major conformational changes that occurred in the three regions are highlighted in color; switch I (green), switch II (yellow), and the neck-linker (red).

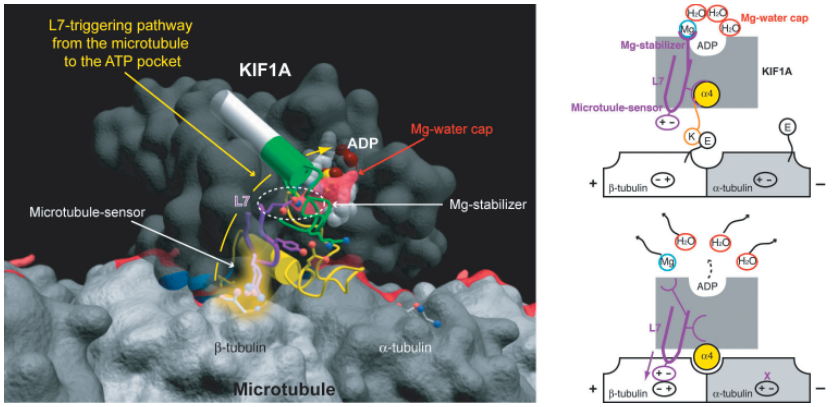

Figure 2
L7-triggering strategy for microtubule-activated ADP release from KIF1A. The KIF1A-microtubule complex is shown from the left side of the microtubules. Before the ADP release (top-right), KIF1A is weakly anchored to the microtubules by the interaction between K-loop (K) of KIF1A and E-hook (E) of the microtubules, and closes the pocket by the Mg-water cap, which is supported by the Mg-stabilizer. The attractive dipole interaction pulls L7 to β-tubulin, but not to α-tubulin (microtubule-sensor) (left panel). The downward movement of L7 breaks the Mg-stabilizer, leading to the sequential release of the water molecules, Mg^{2+} and ADP (bottom-right panel). Mg-water cap, red; L7, purple; switch I, green; switch II, yellow.

Before ADP release, ADP on the surface shallow groove of the ATP pocket is stably covered with an Mg-water cap, a layer of crystal ice formed by the hydrogen bond network around the essential cofactor Mg^{2+} (Fig. 2). This Mg-water cap fills the gap between the β-phosphate of ADP and the proteinous elements of the ATP pocket, so that the ATP pocket is closed in the absence of microtubules. The Mg-water cap is further connected to the root of loop L7 through the linkage namely the Mg-stabilizer, which supports the Mg-water cap to stabilize ADP in the pocket (Fig. 2). This tight ADP trap results in the very slow release of ADP.

For its part, loop L7 takes a long β-hairpin structure. Its root is connected to the ATP-pocket through the Mg-stabilizer as described above, while its tip is exposed on the microtubule-binding surface (Fig. 2). Quite suggestively, the tip of L7 has conserved dipole residues Glu152 and Arg153, which face the oppositely-charged residues Arg158 and Glu159 of helix H4 of β-tubulin of the microtubules. These tubulin residues are well conserved in β-tubulins but not in α-tubulins. This interaction, therefore, would contribute to the discrimination between β- and α- tubulin. Thus, KIF1A can recognize the correct binding site on β-tubulin by using this L7 tip, which we call the "microtubule-sensor." The binding of β-tubulin pulls the sensor loop L7 downward. As the Mg-stabilizer is linked to the root of this sensor loop, the downward movement of L7 triggers the breakage of the Mg-stabilizer, followed by the loss of the Mg-water cap. After the release of the Mg-water cap, the ATP pocket is fully opened and the ADP is readily exchanged into Mg-ATP.

This is the structural mechanism of the microtubule-induced ADP/MgATP exchange of kinesin. By this L7-triggering strategy, KIF1A can effectively use the energy from ATP hydrolysis only when it works on the microtubule rail.

REFERENCES

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BEAMLINES

AR-NW12A and 5A

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