NW12A/2008G023

Crystal Structure of the KIF5C Motor Domain Without Any Nucleotide

Shigeyuki Inoue,[1] Ryo Nitta[1,2] and Nobutaka Hirokawa[1,3,*]
[1]Department of Cell Biology and Anatomy, Graduate School of Medicine,
The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan
[2]RIKEN Center for Life Science Technologies, Tsurumi, Yokohama 230-0045, Japan
[3]Center of Excellence in Genomic Medicine Research (CEGMR),
KAU, Jeddah 21589, Saudi Arabia

1 Introduction

The molecular motor kinesin moves along microtubules (MTs) using energy from ATP hydrolysis in an initial step coupled with ADP release. In neurons, kinesin-1/KIF5C preferentially binds to the GTP-state MTs over GDP-state MTs to selectively enter an axon among many processes [1]; however, because the atomic structure of nucleotide-free KIF5C is unavailable, its molecular mechanism remains unresolved. Here, the crystal structure of nucleotide-free KIF5C and the cryo-electron microscopic structure of nucleotide-free KIF5C complexed with the GTP-state MT (GMPCPP-MT) are presented. In silico docking of the crystal structure with the cryo-EM structure revealed the mutual conformational changes of KIF5C and GMPCPP-MT. Nucleotide-free KIF5C complexes with GMPCPP-MT to acquire a new conformation that we termed the "rigor conformation [2]." This conformation of KIF5C not only provides an important missing link in the structural analysis of kinesin, but also elucidates the molecular mechanism of the preferential binding of KIF5C to the GTP-MT.

In this report, we describe the details about the crystal structure of nucleotide-free KIF5C motor domain.

2 Experiment

The KIF5C motor domain purified by immobilized metal affinity chromatography and cation exchange chromatography was dialyzed against crystallization buffer (10 mM PIPES, pH 7.4, 10 mM HEPES, pH 7.4, 100 mM NaCl, 1 mM $MgCl_2$, 1 mM EGTA, 20 % (w/v) sucrose and 100 μM ADP, pH 7.0). Crystallization was done using the hanging-drop vapor diffusion at 20 °C. The reservoir buffer consisted of 2 M ammonium sulfate, 100 mM sodium citric acid, pH 5.5 and 16% (v/v) glycerol. Crystals appear within a week in samples. Subsequently, the C-terminal tail peptide solution of KIF5C (EAVRAKNMARRAHSAQIAKPIRPG) was added to the drop to release bound ADP, such that the final concentration of the tail peptide was 1–2 mM. A few minutes after the addition of the tail peptide, the crystals were frozen in liquid nitrogen. X-ray diffraction experiments were performed at −178 °C on a NW12A beam line at the PF-AR (KEK, Tsukuba, Japan). The data were processed using the program HKL2000. The structures were determined using molecular replacement methods, with the structure of KIF5 in the ADP state (PDB ID: 2KIN) as the initial model. Subsequent rounds of model building and refinement were performed using the program Coot and the program Refmac5. Finally, the model of the nucleotide-free KIF5C motor domain was refined to *R* and *R*-free values of 23.7 and 29.9%, respectively.

3 Results and Discussion

Currently, the atomic structure of any of the KIFs without nucleotide has not been available, except for that of Kin I kinesin [3]. We therefore set out to solve the crystal structure of KIF5C motor domain without any bound nucleotide (Fig.1).

The weakly bound ADP without Mg^{2+} was found in the nucleotide-binding pocket. We thus soaked several additives to the weakly-ADP-bound crystals to further accelerate the release of ADP. In the presence of the C-terminal peptide, only a small round electron density was found in the pocket instead of ADP, although the corresponding density for the peptide was not found in the electron density map possibly because of the peptide's high flexibility (Fig.2).

The crystal structure of the KIF5C motor domain without nucleotide adopts the ATP-like conformation. Especially, the conformations of switch II and the neck-linker are very similar to ATP-like structures. On the other hand, the switch I conformation of this model is slightly different from those in the other ATP-like structures. In this structure, helix α3 rotates so that the C-terminal region of α3 and the following L9 move away from the nucleotide-binding pocket (Fig.3). This rotation observed here breaks the Mg-stabilizer, thus, Mg-ADP is destabilized, which opens the pocket to facilitate the exchange of ADP with Mg-ATP [4] .

References

[1] T. Nakata *et al*., *J Cell Biol* **194**, 245 (2011).
[2] M. Morikawa *et al*., *EMBO J* **34**, 1270 (2015).
[3] K. Shipley *et al*., *EMBO J* **23**, 1422 (2004).
[4] R. Nitta *et al*., *Nat Struct Mol Biol* **15**, 1067 (2008).

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

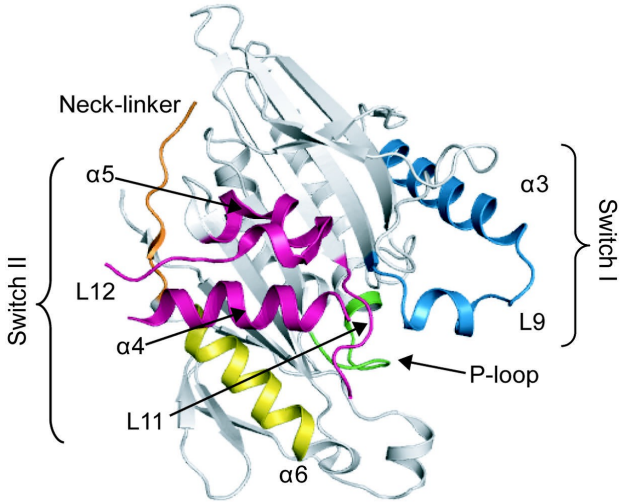

Fig.1 Overall structure of nucleotide-free KIF5C motor domain

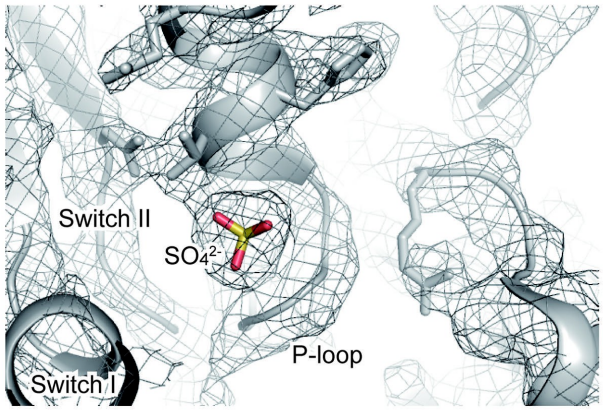

Fig.2 Electron density map of the nucleotide-binding pocket of nucleotide-free KIF5C

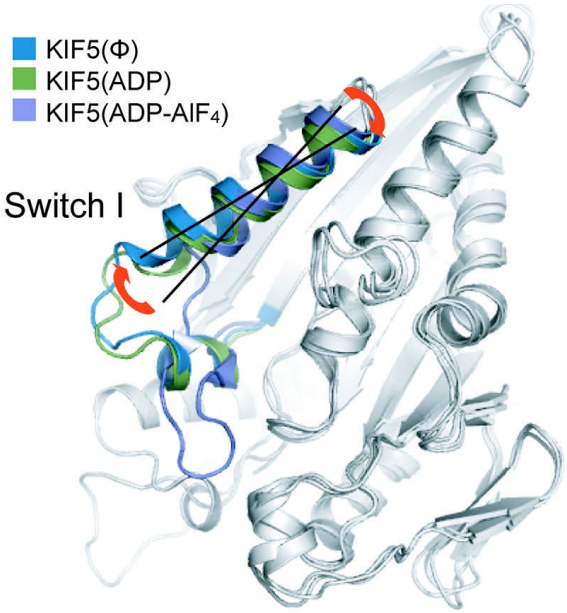

Fig.3 The rotation of helix α3 compared with other state (PDB ID:1BG2 as an ADP state and 4HNA as an ADP-AlF_4 state)