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Structure determinations of kinesin spindle protein Eg5 in complex with its inhibitors

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1 Introduction

Kinesin spindle protein Eg5 is a mitotic spindle motor protein, and plays an essential role in centrosome separation and bipolar mitotic spindle formation during the early stage of mitosis. The inhibition of Eg5 has been shown to cause cell cycle arrest in mitosis with the irregular formation of monopolar spindles and subsequent apoptotic cell death. As the inhibition of mitotic spindles is a pharmaceutically validated strategy for cancer therapeutics, human Eg5 represents an attractive target molecule for novel clinical therapies. A large number of Eg5 inhibitors have been reported. Some of them, such as ispinesib and filanesib (ARRY-520), have been entered into clinical trials as anticancer drugs.

Our group has been previously determined the crystal structure of the Eg5 motor domain in complex with a biphenyl-type inhibitor [1]. The inhibitor binds to the α4/α6 allosteric pocket 15 Å from the ATP-binding pocket, which differs from conventional allosteric inhibitors that bind to the allosteric L5/α2/α3 pocket of Eg5. Binding of the inhibitor to the allosteric pocket induces the deformation of the ATP-binding pocket through the Tyr104 residue. The biphenyl-type inhibitor suppresses the binding of ATP. The allosteric effect of the biphenyl-type inhibitor on the binding of ATP is unique and unprecedented to the best of our knowledge.

Our group has been recently determined the crystal structures of the Eg5 motor domain in complex with *S*-trityl-L-cysteine (STLC)-type inhibitors [2].

2 Experiment

The Eg5 motor domain was expressed in *Escherichia coli* BL21(DE3) CodonPlus RIL as a C-terminal His6 fusion protein using the expression plasmid pCold III. Harvested cells were disrupted by sonication, and the centrifuged supernatant was pufified with a Ni-NTA Agarose resin. And then the eluted proteins were also purified with a HiTrap SP HP cation-exchange column (GE Healthcare). The purified protein was mixed with each inhibitor at a molar ratio of 1 : 5.

Crystallization was performed using the sitting-drop vapor diffusion method at 20°C. Crystallization drops were prepared by mixing 0.5 μL of the protein-inhibitor solution and 0.5 μL of the reservoir solution. In the case of one protein-inhibitor complex, the protein-inhibitor solution contained 17.4 mg/mL Eg5 motor domain, and the reservoir solution contained 30% (w/v) polyethylene glycol (PEG) 3350, 0.1 M 2-morpholinoethanesulfonic acid (MES)-NaOH (pH 6.5), and 0.2 M ammonium sulfate. Rod-shaped crystals grew to an approximate size of 0.1 × 0.05 × 0.05 mm.

A crystal was cryoprotected in a solution containing 30% (w/v) sucrose, and flash-frozen at 100 K. X-ray diffraction data were collected, and data were processed and scaled with XDS and SCALA.

The structures were determined using a molecular replacement method with the program MOLREP in the CCP4 suite. The structure of the Eg5 motor domain in complex with STLC (PDB code, 2WOG) was used as an initial model. Structural refinement was performed with REFMAC5 and PHENIX, and manual model fitting was achieved with Coot.

3 Results and Discussion

The structures were determined at a resolution of 2.6 Å and 2.2 Å. All these structures contain two molecules in an asymmetric unit. The final model contains residues 16–366, one Mg^{2+} ADP, and one inhibitor for each molecules. The structures of the Eg5 motor domain in complex with STLC-type inhibitors were superposed well.

One inhibitor (here called as A) possesses a single *para*-methoxy substituent in one phenyl ring of the trityl group in STLC. By superposing the structures in complex with these inhibitors of STLC and inhibitor A, the structures of the binding site are nearly the same. Due to the presence of the *para*-methoxy substituent of inhibitor A, however, the C atom in the center of the trityl group of inhibitor A was displaced 0.5 Å from that of STLC. For reference, the locations of ADP are nearly identical in the STLC-type inhibitor complexes. Because the *para*-methoxy substituent causes a steric hindrance with the neighboring Right-side residues, the inhibitor molecule is totally shifted by 0.5 **Å** to the Left-side wall when compared with STLC. These differences may contribute to the higher affinity of inhibitor A with Eg5 than that of STLC.

Crystallization and crystallographic analysis of another kinesin spindle protein CENP-E in complex with its inhibitors are in progress.

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References

[1] H. Yokoyama *et al.*, *ACS Chem. Biol.* **10**, 1128 (2015)
[2] H. Yokoyama *et al.*, *ACS Omega* **3**, 12284 (2018).

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