BL-17A/2022G595, 2020G531

# Structure of kinesin CENP-E motor domain in complex with an ATP analogue

Hideshi YOKOYAMA[1,*], Akira SUZUKI[1], Asuka SHIBUYA[2]

[1] Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, Japan
[2] Faculty of Pharmaceutical Sciences, Josai International University, 1 Gumyo, Togane City, Chiba 283-8555, Japan

## 1 Introduction

Centromere-associated protein E (CENP-E) is a kinesin motor protein essential for mitosis and a new target for anticancer agents with less side effects. To rationally design anticancer drug candidates based on structure, it is important to determine the three-dimensional structure of the CENP-E motor domain bound to its inhibitor.

No CENP-E inhibitors have been approved for use in clinical practice. To efficiently develop CENP-E inhibitors with different chemical structures, it is useful to have structural information on various types of the CENP-E motor domain. However, only two crystal structures of the CENP-E motor domain have been reported, both of which are in ADP-bound form (PDB ID: 1T5C, 6M4I) [1], [2]. There are no crystal structures of the CENP-E motor domain in complex with its inhibitors.

We attempted to obtain the structural information of the CENP-E motor domain bound to ATP-competitive inhibitors by using an ADP hydrolysis enzyme apyrase to replace ADP. Here we determined the first crystal structure of the CENP-E motor domain in complex with adenylyl-imidodiphosphate (AMPPNP), a non-hydrolysable ATP analogue [3].

## 2 Experiment

The CENP-E motor domain was expressed and purified as described previously [2]. The purified protein was mixed with apyrase and AMPPNP at a molar ratio of 1 : 10 : 5, and incubated in 10 mM MES-NaOH (pH 6.0), 4 mM EDTA, and 6 mM $CaCl_2$ at 25 °C for two hours in order to remove ADP from the nucleotide-binding pocket of the CENP-E motor domain. The resulting protein was purified by gel filtration chromatography. Crystallization was performed using the sitting-drop vapor-diffusion method at 4 °C. The 8.3 mg/mL protein solution was mixed with AMPPNP at a molar ratio of 1 : 50. Crystallization drops were prepared by mixing the CENP-E motor domain and AMPPNP solution described above, and the reservoir solution containing 0.1 M MES-NaOH (pH 6.5) and 18% (w/v) PEG3350. Needle-shaped crystals appeared after five days.

A crystal was cryoprotected in a solution containing 25 mM PIPES-NaOH (pH 6.8), 150 mM NaCl, 1 mM $MgCl_2$, 0.5 mM TCEP, 0.5 mM EGTA, 9% (w/v) PEG3350, 500 mM MES (pH 6.5), 2.5% (w/v) sucrose, 5.20 mM AMPPNP and 37.5% (w/v) glycerol, and flash-cooled at 95 K.

X-ray diffraction data were collected and processed and scaled with the program XDS and SCALA. The structure was determined by the molecular replacement method with the program MOLREP in the CCP4 suite. The structure of CENP-E-MgADP (PDB ID: 6M4I) was used as an initial model. Structural refinement was performed with REFMAC5. Manual model fitting was achieved with COOT.

## 3 Results and Discussion

The structure of CENP-E motor domain in complex with AMPPNP was successfully determined at a resolution of 1.8 Å, which is higher resolution than the previously reported structures in ADP-bound form: 1.9 Å resolution [2] and 2.5 Å resolution [1]. The electron density of AMPPNP was observed in the nucleotide-binding site.

Structural differences between the structures of CENP-E-AMPPNP and CENP-E-ADP are observed near the nucleotide-binding site. The helix $\alpha$4 of CENP-E-AMPPNP was longer than CENP-E-MgADP.

The structure of CENP-E-AMPPNP reported in this study was compared with the previously determined structures of some kinesins in complex with AMPPNP. The loop L5 of CENP-E-AMPPNP is shorter than other kinesins, as CENP-E has fewer residues at loop L5. We think that loop L5 is involved with the feature of CENP-E that the release of ADP is a rate-limiting step in the ATPase cycle.

The methods of preparation and crystallization in this study will be useful for crystallization of CENP-E in complex with its inhibitors. Crystallization and crystallographic analysis of CENP-E motor domain in complex with its inhibitors are in progress.

## Acknowledgement

This is the collaborative work with Prof. Akira Asai and his lab members (Graduate School of Pharmaceutical Sciences, University of Shizuoka). We thank the Photon Factory staffs for assistance in X-ray diffraction experiments.

## References

[1] I. Garcia-Saez *et al.*, *J Mol Biol.* **340**, 1107 (2004).

[2] A. Shibuya *et al.*, *Acta Crystallogr. D* **77**, 280 (2021).
[3] A. Shibuya *et al.*, *FEBS Lett.* **597**, 1138 (2023).

* yokoyama@rs.tus.ac.jp