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X-ray Crystallography and SAXS Analysis of Calaxin, a Ca^{2+}-Dependent Regulator of Flagellar Motility

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1 Introduction

In many species, the female gamete or its associated structures release chemoattractants to attract spermatozoa. The chemoattractant increases the intracellular Ca^{2+} concentration of sperm, which is required for the directional changes of the sperm flagellum toward the egg. An axonemal Ca^{2+}-binding protein from ascidian *Ciona intestinalis*, named calaxin, regulates the propagation of Ca^{2+}-induced asymmetric flagellar bending through binding to a heavy chain (HC) of outer-arm dynein and tubulin in a Ca^{2+}-dependent manner [1]. To elucidate the relationship between the Ca^{2+}-dependent regulatory mechanism and structural features of calaxin, we solved crystal structures of calaxin in the Ca^{2+}-bound (active) and Mg^{2+}-bound (inactive) forms. In addition, small-angle X-ray scattering (SAXS) was performed to obtain the evidence of structural transition in solution.

2 Experiments

Calaxin was crystallized in the presence of Ca^{2+} or Mg^{2+}. For crystallographic phasing, the crystal was soaked in a solution containing $SmCl_3$, and the single anomalous diffraction (SAD) data were collected at the Sm peak wavelength of 1.600 Å on the BL-17A beamline. The X-ray diffraction data of the Mg^{2+}-bound calaxin were collected at a wavelength of 1.000 Å on the AR-NW12A beamline. All data were processed with the XDS program to determine the crystal structures of calaxin. The SAXS experiments were performed on the BL-10C beamline. All data were collected at a wavelength of 1.488 Å and processed with the FIT2D program.

3 Results and Discussion

We determined the crystal structures of calaxin in the Ca^{2+}- and Mg^{2+}-bound forms at 1.85 and 2.64 Å resolutions, respectively [2]. Calaxin was composed of 11 helices and contained three EF-hand motifs that bound Ca^{2+}. The Ca^{2+}-bound structure adopts the different conformations between two calaxin molecules in an asymmetric unit: One molecule is in the open state and the other is in the closed state (Fig. 1). Compared with the open state, the closed state exhibited the inward movement of the helices α7, α8 and α10 and reduced the exposure of the hydrophobic surface. The crystal structures are similar between the Ca^{2+}- and Mg^{2+}-bound forms (RMSD 0.516 Å), and the open and closed states were also observed in the Mg^{2+}-bound form. In the open state, calaxin bound Mg^{2+} at the three EF-hand motifs. However, no electron density of Mg^{2+} was observed in third EF hand motif (EF3) of the closed state, and the side chains of the loop between E-helix and F-helix seem to be flexible because of poor electron density. These structural findings indicate that the affinity of EF3 for Mg^{2+} is different between the open and closed states: Mg^{2+} prefers to bind to the open state over the closed state.

To estimate the population of open and closed conformers under equilibrium in solution, we performed SAX experiments and fit the intrinsic scattering curves, which were theoretically calculated with crystal structures of the two states of calaxin, to experimental scattering curves [2]. Ca^{2+}-bound calaxin adopts the closed and open sates with abundance ratio of 18% and 82%, respectively (Fig. 1). In contrast, the existing rate of Mg^{2+}-bound calaxin was limited to only open state (100%). These results suggest that the Ca^{2+} binding functions to shift open-closed structural transition toward the closed state. In addition, the open state may be stabilized by a selective binding of Mg^{2+} to EF3 in the absence of Ca^{2+}.

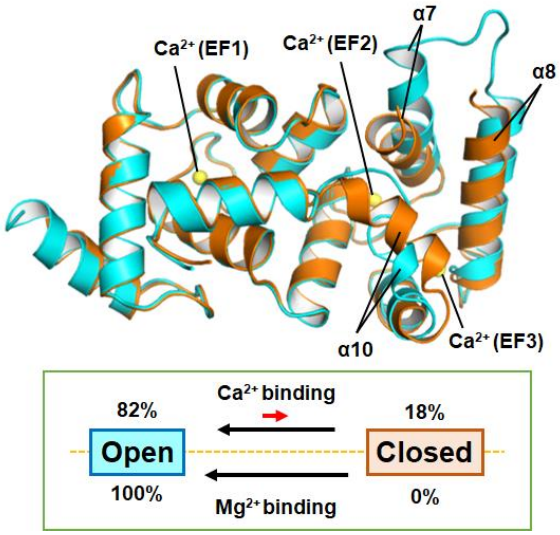

Fig. 1: Open-closed structural transition of calaxin

References

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