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Structure-function analysis of enzymes related to *myo*-inositol phosphorylation

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1 Introduction

Archaea has many metabolic pathways that differ from bacterial and eukaryotic ones. For example, *T. kodakarensis* is known to synthesize 3-phosphoglycerate from AMP via ribose-1,5-phosphate.

TK2285 is an enzyme from *T. kodakarensis* that phosphorylates *myo*-inositol [1]. Only two *myo*-inositol kinases have been identified so far, and little is known about these enzymes. Especially, which of the six hydroxy groups of *myo*-inositol is phosphorylated has not been identified, because the mirror symmetry of *myo*-inositol makes the identification of phosphorylated hydroxy group by HPLC or NMR difficult. Thus, we attempted to determine the crystal structures of TK2285 protein in complexed with substrates and with products.

2 Experiment

Crystals of the substrates-complex were obtained using AMP-PCP and *myo*-inositol as ligands and using polyethylene glycol 3350 and ammonium iodide as precipitants. Crystals of the products-complex were prepared using ATP and *myo*-inositol as ligands. The mixture of TK2285 and ligands were incubated at 85 ºC to allow the enzyme to produce ADP and the phosphorylated *myo*-inositol product, and crystallized using polyethylene glycol 3350 and ammonium iodide as precipitants. The crystals were flash-frozen and the diffraction datasets were collected at the beamline NE3A. Both types of crystals were belonged to the space group $P2_12_12_1$ and the cell constants were a = ~77 Å, b = ~81 Å, and c = 81~82 Å. The resolutions were 1.93 Å and 2.08 Å for substrates-complex and products-complex, respectively. The phases were determined by Fourier synthesis using 3W4S structure (unliganded TK2285 [1]) as the model. Both of the structures were refined to R and R_{free} factors of 20%~21% and ~25%, respectively [2].

3 Results and Discussion

Overall structures were shown in Figure 1. The lid-domain of both complexes were closed comparing to the unliganded structure. The substrates-complex showed that the 3-OH group is nearest to the γ-phosphate of AMP-PMP (Figure 2A). The structure suggests that the 3-OH group is phosphorylated by TK2285. The 3-OH phosphorylation was confirmed by the products-complex (Figure 2B). 70% of the 3-OH of the *myo*-inositol was phosphorylated in the crystal structure. The other 30% is non-phosphorylated *myo*-inositol. HPLC with chiral column and NMR analyses of the TK2285 product also identified that the enzyme produces *myo*-inositol-3-phosphate. Thus, we named TK2285 *myo*-inositol 3-kinase (MI3K) [2].

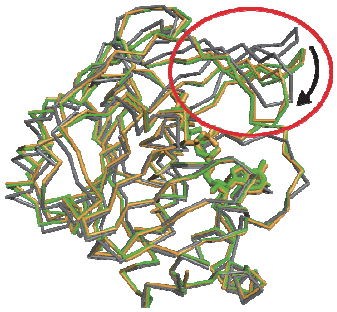

Fig. 1: Superposition of overall structures of TK2285. The structures of the substrates-complex, the products-complex and the unliganded structure are shown in orange, green and gray, respectively. The red ellipse and the arrow show the lid domain and its motion, respectively.

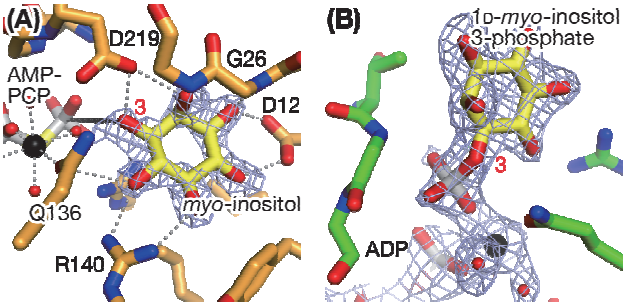

Fig. 2: F_o-F_c omit map contoured at 3σ superposed on the ligand binding sites of the substrates-complex (A) and the products-complex (B). Water molecules and Mg^{2+} ion are shown as red and black spheres, respectively. Gray broken lines show interactions regarding Mg^{2+} ion and *myo*-inositol. A black broken line is drawn between the 3-OH group of *myo*-inositol and the γ-phosphate of AMP-PCP. Red characters represent the atom numbering.

References

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[2] R. Nagata, M. Fujihashi, T. Sato, H. Atomi, and K. Miki, *Biochemistry* **54**, 3494 (2015).

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