BL-1A, BL-5A, AR-NE3A/2017G696

# Structure study on a new pyrophosphate-dependent kinase

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## 1 Introduction

Most kinases use ATP as a phosphate donor, while a few kinases use pyrophosphate (PPi) instead. Three types of PPi-dependent kinase have been reported so far. However, mechanisms of PPi-specific recognition in these enzymes remain unclear.

Here, we identified a new type of PPi-dependent kinase, TM0415, and determined its crystal structures. The structures revealed important residues for the PPi-specific recognition, and these residues enabled us to find additional PPi-specific kinases from the genome database.

## 2 Experiment

Identification of TM0415 was performed using the Dali server [1]. An ATP-dependent *myo*-inositol 3-kinase from *Thermococcus kodakarensis* (MI3K_TK, PDB ID 4XF7) [2] was used as a query structure. Donor specificity of TM0415 was investigated by LC-MS.

Methylenediphosphonic acid (PCP) was used as a PPi analog in crystallization. 2 or 20 mM ammonium sulfate was added for preparing a PCP complex or a sulfate ion complex, respectively. Diffraction data sets were collected at beamlines of BL-1A, 5A, and NE3A. The phases were determined by molecular replacement with the atomic coordinates of an unliganded structure of TM0415 (PDB ID 1VK4).

The additional PPi-specific kinases were discovered using a BLAST search. PPi-dependent activity of candidate PPi-dependent kinases was analyzed by LC-MS and a malachite green assay.

## 3 Results and Discussion

The Dali server showed that the substrate-complex structure of MI3K_TK is the most similar to the unliganded structure of TM0415 from *Thermotoga maritima*. TM0415 has been annotated as a carbohydrate kinase and thought to be involved in *myo*-inositol metabolism. However, this enzyme exhibited no ATP-dependent kinase activity toward various carbohydrates, including *myo*-inositol [3,4]. Structural comparison between TM0415 and MI3K_TK showed that F221, R232, and M266 of TM0415 occupy a potential ATP-binding pocket (Fig. 1A). We investigated the TM0415 activity using ATP, ADP, or PPi as the phosphate donor. This investigation revealed that TM0415 phosphorylates *myo*-inositol using PPi but neither ATP nor ADP.

The PCP-complex structure of TM0415 was determined to elucidate the PPi-binding mode. This structure revealed residues involved in PPi binding (Fig. 1B). R229 is located near PCP, but its side chain was disordered in this structure. In a sulfate-ion-complex structure, which possesses a sulfate ion in the PPi-binding site instead of PCP, R229 interacts with the sulfate ion (Fig. 1C). This suggests that R229 recognizes PPi in the reaction because the sulfate ion is well superposed on one of the phosphoryl groups of PCP. Indeed, a R229A mutation resulted in a drastic decrease in the activity. Three of the PPi-binding residues (K171, R229, and R232) are not conserved in homologous ATP/ADP-dependent kinases, including MI3K_TK. This suggests that these three residues contribute to the PPi-specific recognition. Notably, R232 is also expected to prevent ATP binding as described above. We considered the five residues (K171, F221, R229, R232, and M266) as key residues for the PPi-specific recognition.

We sought additional PPi-dependent kinases from the genome database based on conservation of the five key residues. Fifty candidates of PPi-dependent kinases were found. PPi-dependent activity of four candidates was investigated. Two of them exhibited PPi-specific kinase activity toward *myo*-inositol, suggesting that the five key residues can be collectively used as a signature to discover unidentified PPi-dependent kinases [5].

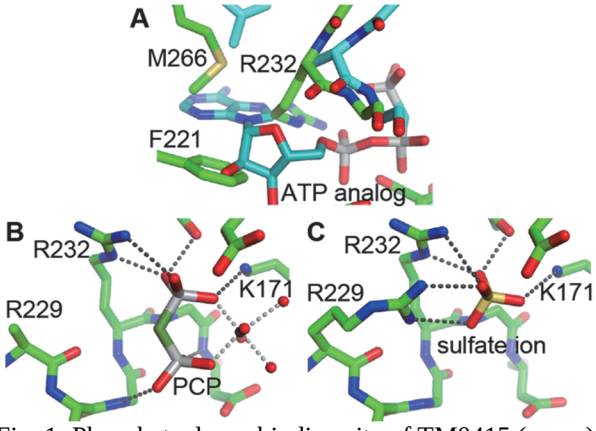


Fig. 1: Phosphate donor binding site of TM0415 (green). (A) Superposition of the substrate complex of MI3K_TK (cyan) on the unliganded TM0415. (B,C) The PCP- or sulfate-ion-complex structure.

## References

[1] L. Holm, *et al. Nucleic Acids Res.* **38**, W545–9 (2010).
[2] R. Nagata, *et al. Biochemistry* **54**, 3494–503 (2015).
[3] I. A. Rodionova, *et al. J. Bacteriol.* **194**, 5552–63 (2012).
[4] I. A. Rodionova, *et al. Environ. Microbiol.* **15**, 2254–66 (2013).
[5] R. Nagata, *et al. Nat. Commun.* **9**, 1765 (2018)

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