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Crystal structures of *Burkholderia thailandensis* nucleoside kinase: insights into catalytic mechanism and nucleoside selectivity

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1 Introduction

Mizoribine (MZR) is a purine nucleoside analogue, and is currently used as an immunosuppressant after renal transplantation, for lupus nephritis, rheumatoid arthritis, and primary nephritic syndrome. MZR is rapidly absorbed and phosphorylated to mizoribine 5′-monophosphate (MZR-P) intracellularly by adenosine (ADO) kinase. MZR-P acts as a competitive inhibitor of inosine 5′-monophosphate dehydrogenase (IMPDH) and guanosine monophosphate synthetase, which are crucial for *de novo* nucleic acid biosynthesis. Because proliferation of T and B cells highly depends on the *de novo* DNA synthesis pathway, MZR-P exhibits immunosuppressive activity. However, MZR dosage should be determined by measuring MZR levels in serum to avoid negative effects.

Nucleoside kinase from *Burkholderia thailandensis* (BthNK; UniProt code, Q2SZE4) belongs to the phosphofructokinase B (Pfk-B) family and catalyzes the phosphorylation of MZR by using ATP as a phosphoryl donor [1]. Among various bacterial ribokinase and ADO kinase homologues tested, BthNK is the only enzyme that effectively converts MZR to MZR-P, and is currently applied to a clinical assay for blood MZR level. To understand the catalytic and MZR binding mechanism, we have undertaken the crystallographic studies on BthNK.

2 Experiment

The expression of BthNK in *Escherichia coli* inhibited cell growth, probably due to the harmful influence of BthNK on the nucleotide metabolism of *E. coli*. Thus, *R. erythropolis* was used as a heterologous expression host [1]. His-tagged BthNK was purified by Ni-affinity chromatography. All crystals were obtained by the hanging-drop vapor-diffusion method at 20°C. The structures were solved by Br-SAD method. The detail experimental procedures were previously described [2].

3 Results and Discussion

The crystal structures of BthNK was determined in ligand-free form, and in complex with inosine, inosine-ADP, MZR-ADP, AMP-Mg-AMP at 2.1-1.55 Å resolution. The structures revealed that typical homodimeric architecture of Pfk-B enzymes in three distinct conformational states: asymmetric dimer with one subunit in an open conformation and the other in a closed conformation (ligand-free form), closed conformation (binary complex with inosine), and fully closed conformation (other ternary and quaternary complexes) (Fig. 1). The previously unreported fully closed structures suggest the possibility that Mg^{2+} might directly interact with the β- and γ-phosphate of ATP to maintain the neutralization of the negative charge throughout the catalytic reaction (Fig. 2). The nucleoside complex structures also indicated that the base moiety of the bound nucleoside is partly exposed to the solvent, enabling the recognition of a wide range of nucleoside bases. Gly170 is responsible for the solvent accessibility of the base moiety and is assumed to be a key residue for the broad nucleoside recognition of BthNK. Site-directed mutagenesis study also supported the importance of Gly170 for broad nucleoside specificity of BthNK.

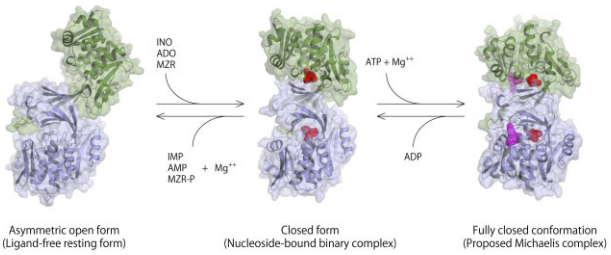

Fig. 1: Three distinct conformational states of BthNK.

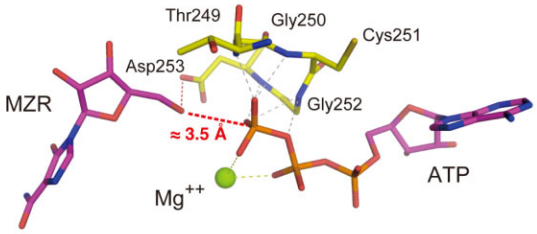

Fig. 2: Putative ES complex model of BthNK.

References

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