DATE: Day 21 Month 4 Year 2026

SUMMARY of
FY2025 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, The University of Osaka

Research Title		Crystal structure of aminoimidazole ribonucleotide synthetase with co-factor and inhibitor complex
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	Present Title	Scientist / Professor
Research Collaborator (Host PI)		Prof. Atsushi Nakagawa

Summary

The enzyme 5-aminoimidazole ribonucleotide synthetase (AIRS; also known as PurM) catalyzes the fifth step of the de novo purine biosynthetic pathway, namely the ATP-dependent conversion of formylglycinamidine ribonucleotide (FGAM) to aminoimidazole ribonucleotide (AIR). This reaction is proposed to require the metal cofactors Mg^{2+} and K^{+} for catalytic activity. AIRS belongs to the PurM superfamily and contains conserved ATPase domains. Due to challenges in preparing protein–ligand complex crystals, no beamtime at SPring-8 BL44XU was utilized in the past year. Nevertheless, by integrating previously collected data from BL44XU with more recent datasets obtained from TPS 05A and 07A, we successfully determined the first high-resolution crystal structures of AIRS from the hyperthermophilic archaea *Pyrococcus abyssi* (*Pa*) and *Pyrococcus horikoshii* (*Ph*). These structures include the apo form as well as complexes with substrate analogs (FGAR), nucleotide ligands (AMPPNP and ADP), and metal cofactors (Mn^{2+}, Mg^{2+}, and K^{+}). Structural analysis reveals that AMPPNP and FGAR bind to the α1–β1 loop and the disordered N-terminal tail, respectively, thereby stabilizing the active-site conformation. These results provide a structural framework for understanding substrate positioning and the catalytic mechanism of AIRS. Notably, a unique non-domain-swapping feature was observed in the first 54 residues of the N-terminal domain in both *Pa*AIRS and *Ph*AIRS, distinguishing them from their orthologs. Further analysis indicates that domain swapping is dependent on the β1–β2 loop and can be classified into two types within the AIRS family: a short-loop, non-swapping archaeal type and a long-loop, swapping bacterial type. Structural, mutational, and biophysical analyses further suggest that the thermostability of these enzymes is supported by enhanced hydrophobic core packing, strengthened dimer interfaces, and shortened surface loops. Collectively, these findings elucidate substrate binding, catalytic mechanism, and domain-swapping behavior in AIRS, while also providing insights into the compact and robust structural features characteristic of thermophilic proteins.

This work has been published in *Intl J. Bio. Macromol.* (2026, 344 (Part 1), 150493). Part of the X-ray diffraction data used in this study was previously collected at BL44XU of SPring-8 under international collaboration with Prof. Atsushi Nakagawa at the Institute for Protein Research at University of Osaka and JASRI.

***Deadline: May 11, 2026**
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