DATE: Day 19 Month April Year 2024

SUMMARY of
FY2023 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		**Structures of an aminopeptidase P and N-recognins for the Pro/Ndegron pathway**
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	Present Title	**Professor**
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Summary

Gluconeogenesis is a metabolic pathway that generates glucose from non-carbohydrate substrates, and it is a ubiquitous metabolic process in almost all living organisms. In yeast, cells switch from gluconeogenesis to glycolysis to appropriately maintain the level of glucose. Gid ubiquitin ligase complex, composed of 9 subunits (Gid1~9), degrades gluconeogenic enzymes when cells return to the glucose-replete condition. The gluconeogenic enzymes Fbp1, Icl1, Mdh2, and Pck1 bearing N-terminal proline are degraded conditionally by the Gid4-dependent Pro/N-degron pathway. Intriguingly, this Pro/N-degron pathway is expanded by the finding of the coupled aminopeptidase P, such as Fra1 from *Saccharomyces cerevisiae*. Before Pro/N-recognin binds to the Pro/N-degron, Fra1 trims the non-proline residue at the N-terminus to expose Pro at the N-terminus. Therefore, structural information on the Fra1 and N-recognins is indispensable for understanding the functional repertoire of the N-degron pathway. We aim to determine structures of aminopeptidase Fra1 in complexes with prolyl or non-prolyl peptides and designed small chemical compounds for understanding the enzymatic mechanism.

As an initial step, we determined the crystal structure of apo-Fra1 from yeast. One of the characteristic features of Fra1 metalloprotease is its active site, which is formed by two divalent metal ions, especially Mn^{2+}. The electron density map of the active site of Fra1 was quite clear for visualizing the atomic details. In contrast to human and bacterial orthologs, the yeast prolyl-aminopeptidase is a dimer in solution. Based on the current structure, we are working on the mutational study of yeast Fra1 by changing the critical residues at the active site, including metal-coordinating residues. Previously, we established the Fra1 protease assay by monitoring the product with HPLC coupled with mass spectrometry. Furthermore, the derivatives of proline residues were tested to make a complex structure of Fra1 in the presence of inhibitors, which will provide a detailed enzymatic mechanism of this unusual aminopeptidase.

***Deadline: May 10, 2024**
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