DATE: Day 08 Month 04 Year 2022

SUMMARY of
2021 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		**Structural study of two different types of dUMP hydroxymethylases**
Applicant	**Name**	**Hyun Kyu Song**
	Affiliation	**Korea University**
	Present Title	**Asymmetric structures of the ClpP proteolytic machine in complex with acyldepsipeptides**
Research Collaborator (Host PI)		**Atsushi Nakagawa (Professor)**

Summary

The ClpP serine peptidase is a tetradecameric degradation machine involved in many physiological processes. It becomes a competent ATP-dependent protease with Clp-ATPases. Small chemical compounds, acyldepsipeptides (ADEPs), are known to cause dysregulation and activation of ClpP without ATPases, and have potential as novel antibiotics. Previously, structural studies of ClpP from various species revealed the structural details, conformational changes, and activation mechanism. Although product release by the side exit pores has been proposed, the detailed driving force for product release remains elusive. Here, we report two structures of BsClpP in complex with acyldepsipeptides (specifically ADEP2) at 2.8 and 3.0 Å resolution. Very intriguingly, these two structures have two and five ADEP2 compounds, respectively, bound to each heptameric ring, which is unforeseen because the previous ADEP-bound BsClpP was symmetric 14-ADEP bound. Our two new BsClpP tetradecamer structures have a large difference in their degrees of compression as well as in their numbers of ADEP molecules bound, four and ten. To understand the conformational change for product release, we investigated the relationship between substrate hydrolysis and the pH lowering process. Our data, together with previous findings, provide insight into the molecular mechanism of product release by ClpP self-compartmentalizing protease.