DATE: Day 03 Month 06 Year 2020

SUMMARY of
2019 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		**Structure of p62 autophagy receptor**
Applicant	**Name**	**Hyun Kyu Song**
	Affiliation	**Korea University**
	Present Title	**Crystal structures of N-demethylase A and B**
Research Collaborator (Host PI)		**Atsushi Nakagawa**

Summary

Many bacteria have enzymes that degrade environment-derived exogenous compounds, such as single aromatic rings or multiple benzene rings fused to polycyclic aromatic hydrocarbons. They are widely dispersed in the environment and have detrimental biological effects, including toxicity and mutagenicity. Rieske nonheme iron oxygenases (ROs) catalyze the initial oxygenation reaction of aromatic compounds by enantio- and regiospecific reactions. The soil bacterium *Pseudomonas putida* CBB5 can use caffeine (1,3,7-trimethylxanthine) as a sole carbon and nitrogen source by degrading it to xanthine via sequential N-demethylation. Recent studies have reported a novel type of RO in *P. putida* CBB5, consisting of NdmA, NdmB, and NdmC, which specifically detach methyl groups from the N-1, N-3, and N-7 positions of methylxanthine derivatives, respectively. The environmentally friendly enzymatic reaction products, methylxanthines, are high-value biochemicals that are used in the pharmaceutical and cosmetic industries. However, the structures and biochemical properties of bacterial N-demethylases remain largely unknown.

Here the crystal structures of N-demethylase A and B from *P. putida* CBB5 are reported. By comparing the apo- and substrate-bound structures, we investigate the N-demethylation mechanism and regio-specificities for substrates. In addition, we reveal that N-demethylase A and B prefer to produce a heterohexamer with the α3α′3 configuration (NdmAB), which is a novel type of RO oligomerization state. The improved efficiency of NdmAB for serial caffeine N-demethylation is confirmed by biochemical studies. Moreover, from the complex structure of the NdmD ferredoxin domain with NdmA, we provide information about the interactions and the electron transport pathway between RO components during N-demethylation. These findings provide the substrate specificity and reaction mechanism of bacterial N-demethylation, and they are also potentially useful for many caffeine- and other methylxanthine-related industrial applications.

***Deadline: May 15, 2020**
***Please submit it to E-mail: tanpakuken-kyoten@office.osaka-u.ac.jp.**
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