DATE: Day 13 Month May Year 2017

SUMMARY of
2016 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Structural investigation of β-glucosidase mechanism and complexes with substrates.
Applicant	Name	Dr. James R. Ketudat Cairns
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	Present Title	Professor
Research Collaborator (Host PI)		Prof. Dr. Genji Kurisu

Summary

Beta-glucosidases play many roles in animals, plants, fungi and microorganisms and have been applied to biomass conversion, nutritional improvement and medical applications in the case of human genetic disease. In this work, we continued our previous collaboration the structure and function of beta-glucosidases from bacteria and plants.

As we recently published the structure of the glycoside hydrolase family GH116 beta-glucosidase *Tx*GH116, homologous to the human glucosylceramidase GBA2, we continued this work to characterize the complexes of acid/base mutants of this enzyme in complexes with oligosaccharides. Although TxGH116 does not appear to hydrolyze glucosylsphingolipids, the substrates of GBA2 which are thought to have medical significance, its complexes with its preferred substrates, cello- and laminari-oligosaccharides, nonetheless give insights into binding and hydrolysis of substrates by GBA2 and other related GH116 beta-glucosidases. Moreover, they provide further evidence for the role of Asp593 as the catalytic acid/base and its unique geometry of attack, when compared to other retaining beta-glucosidases. This geometry is expected to be conserved in other GH116 beta-glucosidases, as well as in enzymes from the structurally related family GH52. In addition, we collected data on TxGH116 beta-glucosidase that was mutated to imitate known pathogenic mutations in human GBA2, thereby providing insights into how the mutation may affect the enzymes structure and function.

In addition to the studies on TxGH116, we solved structures of the GH1 family enzymes Os3BGlu7 (rice BGlu1) and Os4BGlu18. The rice Os3BGlu7 soaked with manoimidazole was found to have little density in the active site at low mannoimidazole concentration and density for the contaminant glucoimidazole in the active site when soaked at concentration, suggesting mannoimidazole binds very poorly relative to glucoimidazole. For Os4BGlu18, even up to 100 mM coniferin, coniferol or sinapyl alcohol resulted in little ligand electron density in the active site, suggesting that the product alcohols bind very poorly and the substrate coniferin is likely hydrolyzed when it binds the active site. Future work with inactive mutants may be more productive.

In all, we produced 14 useful x-ray crystal diffraction datasets in this study, which allowed us to gain insights into substrate binding, catalytic mechanism and the effects of human pathogenic mutations in GH116 beta-glucosidases, while the datasets the plant GH1 beta-glucosidases generally provided us with insights into the weak binding or the ligands. We have continued to collect data at other sources and expect to be able to generate several papers in this collaboration.

***Deadline: May 19, 2017**
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