DATE: Day 07 Month 05 Year 2024

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

Research Title		Crystal structure of glucose-6-phosphate 1-dehydrogenase
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	Present Title	Scientist / Professor
Research Collaborator (Host PI)		Prof. Atsushi Nakagawa

Summary

Glucose 6-phospate-1-dehydrogenase/6-phosphogluconolactonase (G6DP) is an important enzyme that plays a major role in the pentose phosphate pathway (PPP). In the first step of PPP, glucose 6-phosphate is oxidized and NADP is reduced to produce 6-phosphoglucose lactone and NADPH, which is catalyzed by G6DP. It is thus crucial to understand the structural and mechanistic roles of this enzyme. This project aims to determine the crystal structures of G6DP at the highest resolution to elucidate the structural insights of the enzymatic mechanism. We performed crystal screening and collected X-ray diffraction data with a single wavelength at the IPR BL44XU beamline of SPring-8. After several attempts, most of the G6DP crystals diffracted X-ray to about 5 - 6 Å. We are currently optimizing the crystals and trying to improve the resolution for complete structure determination in the future.

During this and previous proposal periods, we also collected several useful X-ray data sets of endoglycosidase Sz and determined the various structures. Monoclonal antibodies (mAbs) have been increasingly important and dominating in the drug markets for various human diseases and cancers. Improvement of the therapeutic activities of mAbs has become a critical issue in the biomedical and biopharmaceutical industry. We have discovered a new enzyme, EndoSz from *Streptococcus equi subsp. Zooepidemicus Sz105*, and applied this novel enzyme to enhance the activities of mAbs with our developed homogenous antibody platform. We demonstrate that the mutant EndoSz-D234M possesses an excellent transglycosylation activity to generate diverse glycoconjugates on mAbs with homogeneous catalysis. We also report the crystal structures of EndoSz-D234M in the *apo*-form and the complex form with the bound G2S2-oxazoline intermediate at high resolutions by utilizing a novel pH-jump method to improve resolution significantly. Characterizations of transglycosylation activities with mutagenesis and crystal structures of EndoSz-D234 in various forms with G2S2-oxazoline intermediate elucidate the transglycosylation mechanism. Based on these findings, we develop a novel homogenous antibody platform with enhanced therapeutic antibody efficacy that can greatly benefit the biomedical and pharmaceutical industries. The paper titled “Structure-based high-efficiency homogeneous antibody platform by endoglycosidase Sz provides insights into its transglycosylation mechanism” was published online at JACS Au, https://doi.org/10.1021/jacsau.4c00004 on April 11, 2024.

***Deadline: May 10, 2024**
***Please submit it to E-mail: tanpakuken-kyoten@office.osaka-u.ac.jp.**
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