Catalytic Mechanism of Cysteine Glycosidase Revealed by X-Ray Crystallography

Even though glycoside hydrolases have massive diversity with their substrate specificities, they have very limited and similar architectures as catalytic machineries. We discovered the first glycoside hydrolase that has an unusual cysteine residue as the catalytic center. In collaboration with research groups in Europe, we uncovered the detailed catalytic mechanism of metal-assisted chemical reaction.

Certain fruits, such as pineapple and papaya, can be used to tenderize meat because they have plenty of protease activity. Proteases in fruits are called 'cysteine protease' because they use a cysteine as a key nucleophilic residue in the catalysis. In contrast, glycoside hydrolases, which degrade carbohydrates, use only two kinds of similar amino acids with a side chain carboxylic acid (glutamate or aspartate) for the hydrolytic reaction in most cases. This has been an enigmatic fact because glycoside hydrolases display enormous diversities with various substrate specificities and more than 160 enzyme families.

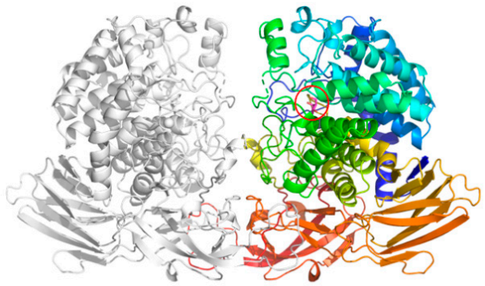

Figure 1: The structure of HypBA1. Its active site is indicated with a red circle.

Bifidobacteria are prominent gut microbes in healthy infants and adults that express various glycoside hydrolases to utilize carbohydrates not consumed by their hosts (humans). A novel glycoside hydrolase, HypBA1, was identified from *Bifidobacterium longum* by Dr. Kiyotaka Fujita at Kagoshima University in 2011. HypBA1 is a β-L-arabinofuranosidase that degrades β-arabinooligosaccharides in plant cell wall glycoproteins. In a collaborative effort with Drs. Kiyotaka Fujita and Akihiro Ishiwata at RIKEN, we determined the crystal structure of HypBA1 in 2014, using the macromolecular crystallography beamlines of the Structural Biology Research Center (SBRC) (**Fig. 1**) [1]. The crystal structure was determined in complex with its reaction product, β-L-arabinofuranose, revealing that Cys417 is in an appropriate position for nucleophilic attack on the substrate's scissile glycosidic bond (**Fig. 2, left**). Cys417 is involved in the unique coordination of a Zn^{2+} ion, which was identified via anomalous scattering analysis using the synchrotron beamline of SBRC (**Fig. 2, right**). Based on these results, we proposed a reaction mechanism in which Cys417 acts as the catalytic nucleophile, with the aid of acid/base catalysis by a glutamate residue (Glu322, **Fig. 3**). This unprecedented mechanism remained controversial because (i) the structure of the covalent intermediate had not been elucidated, and (ii) the strong nucleophilicity of the cysteine thiolate group may impair efficient catalysis at the deglycosylation step.

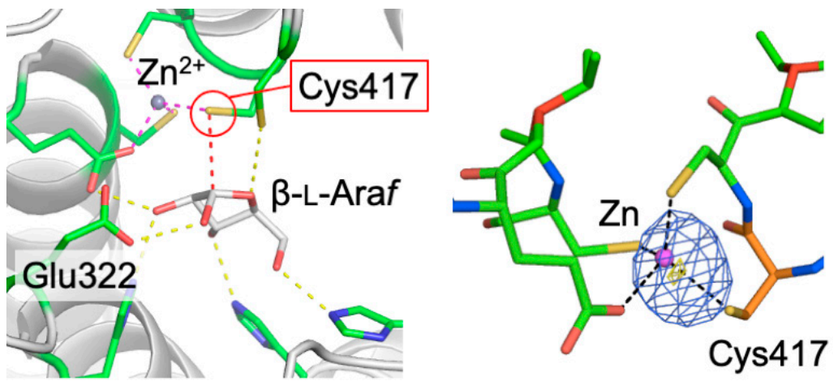

Figure 2: Left, the HypBA1 active site complexed with β-L-arabinofuranose (β-L-Araf). **Right**, anomalous scattering analysis, indicating that the metal coordinated by Cys417 is Zn.

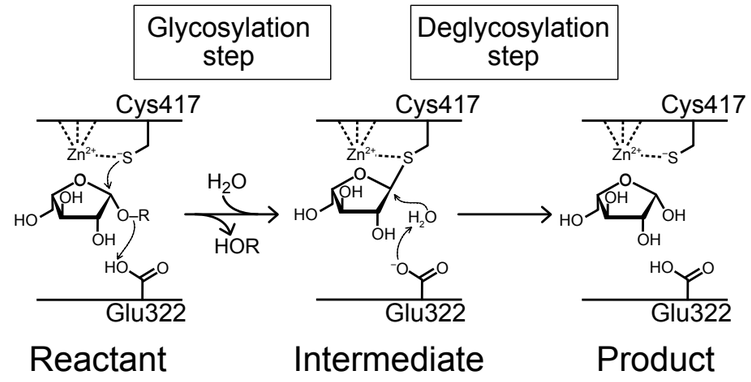

Figure 3: Proposed reaction mechanism of HypBA1.

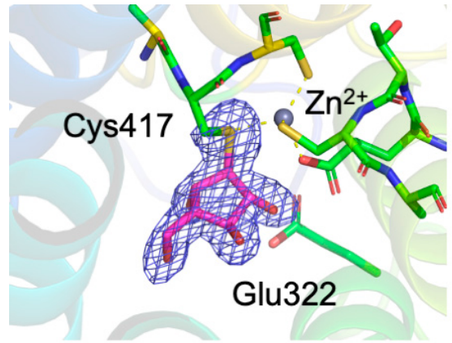

Figure 4: Covalent intermediate-like structure of HypBA1 complexed with a mechanistic inhibitor.

In 2020, we addressed this question in a collaborative study with research groups in Europe [2]. We used a novel inhibitor synthesized by Prof. Herman S. Overkleeft and his colleagues at Leiden University in the Netherlands. Mass spectroscopy analysis confirmed that this cyclophellitol-derived inhibitor specifically labeled the presumptive catalytic residue (Cys417) of HypBA1. Furthermore, high-quality crystallographic analysis at 1.75 Å resolution revealed that the three-dimensional structure of the covalent adduct is analogous to the expected transition state intermediate (**Fig. 4**). This high resolution was achieved by screening a large number of crystals using the automated data collection system of the SBRC beamlines. The covalent structure enabled detailed computational analysis of the deglycosylation step. Molecular dynamics and quantum mechanics/molecular mechanics were examined by Prof. Carme Rovira and her colleagues at the University of Barcelona in Spain. These results clearly indicate that the deglycosylation reaction can proceed with finely tuned energetics facilitated by the Zn^{2+}-Cys417 interaction. Prof. Gideon Davies and his colleagues at the University of York in the UK provided biochemical data and spearheaded this four-country international collaboration.

This study proved that glycoside hydrolases can utilize a cysteine-based catalytic mechanism, expanding the possibilities for engineering this class of enzymes. A large number of glycoside hydrolases, including amylases and cellulases, have been applied to advances in the food industry, large-scale biomass conversion, and medical research. This study will serve as a basis for developing carbohydrate-degrading enzymes with desired properties.

REFERENCES

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BEAMLINES

BL-1A, BL-5A, BL-17A, AR-NE3A and AR-NW12A

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