

Structural and Functional Studies of Rice Beta-Glucosidase (II)

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Plants contain a large amount of polysaccharides and glycosides that perform many functions, from structural components of the cell wall to metabolic intermediates to defense compound and phytohormone storage forms. In order to utilize these compounds efficiently, plants produce a number of enzymes, including cellulases and endoglucanases that cut in the middle of large polysaccharides and beta-glucosidases /exoglucanases that release glucose molecules from the nonreducing ends of the released oligosaccharides and from glycosides. These enzymes belong to a number of related families, including glycosyl hydrolase families 1 (GH1), 3 (GH3), and 5 (GH5).

In rice, which is the most important crop plant in Asia and the monocot model for genomic studies, the genome has been determined to contain 34 GH1 genes likely to produce active enzymes (*BMC Plant Biol.* 6, 33, 2006). Of these, *Os3Bglu7*, which produces the BGLu1 protein, is the most widely expressed. This protein has been found to have high activity toward short 1,3-linked and long 1,4-linked oligosaccharides, while it has lower activity toward cellobiose and beta-mannoside compared to the closely related barley BGQ60 beta-glucosidase. In order to determine the structural basis for oligosaccharide binding of BGLu1, which may be critical to cell wall remodeling in rice growth and development, and for the selectivity towards cellotriose compared to cellobiose and beta-glucoside compared to beta-mannoside, we have determined the structure of this enzyme and its covalent intermediate with 2-deoxy-2-F-glucoside (G2F).

The structure of the free BGLu1 protein could be solved to 2.2 Å resolution, while the structure of BGLu1 in complex with 2-F-glucoside, which forms a stable covalent reaction intermediate, was solved at 1.55 Å resolution based on data collected at the NSRRC 13B beamline (Fig. 1). The complex structure showed that the sugar was in a relaxed 4C_1 chair conformation, typical of a stable intermediate, but not of docked substrates and also showed an interesting interaction between the BGLu1-G2F molecule and a glycerol molecule from the solvent (Fig. 2). The glycerol appeared to be positioned to form to make a nucleophilic attack on the anomeric carbon with its 2-hydroxyl group. Thus, this structure appears to represent a trapped intermediate in a transglycosylation reaction, the first such intermediate reported, to our knowledge.

The determination of the structure of rice BGLu1 and its covalent intermediate with G2F, together with the subsequent structures of a BGLu1 mutant in complex with cellopentaose and of a rice GH5 β -glucosidase which are now in progress, provide critical information on the recognition of sugars by plant β -glucosidases/exoglucanases.

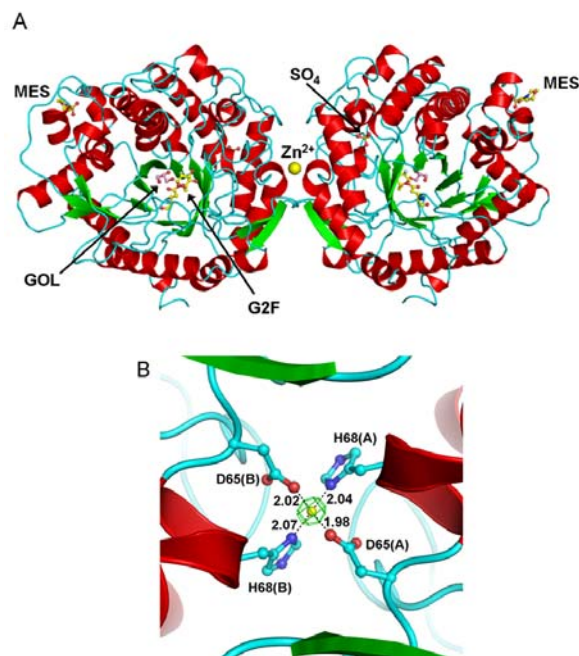


Figure 1. The structure of rice BGLu1 beta-glucosidase in complex with 2-deoxy-2-fluoroglucoside (G2F). A. The asymmetric unit of the BGLu1-G2F complex is shown with the various small molecules found in the structure identified. The structure of this covalent reaction intermediate was solved to 1.55 Å. B. A Zn ion was found to be chelated between the two BGLu1 molecules in the asymmetric unit. *J. Mol. Biol.* . (under review).

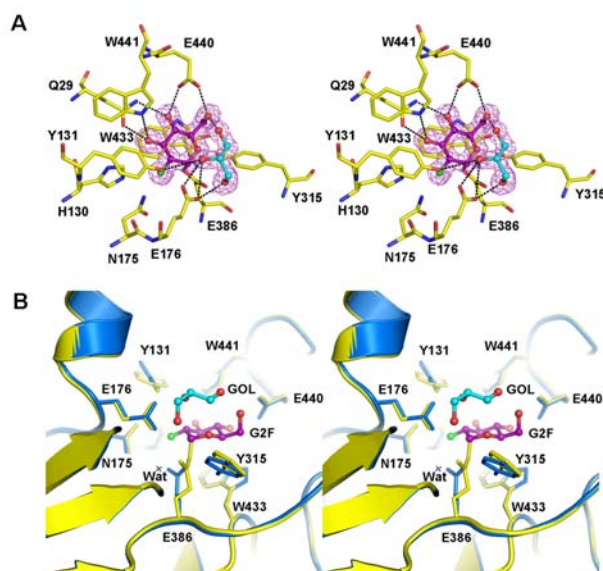


Figure 2. The structure of the apparent transglycosylation intermediate. A. The interaction between the covalent G2F (purple), the surrounding residues and the glycerol (cyan) are shown with the $||F_o|-|F_c||$ map for the ligands at 3 sigma. B. The BGLu1 (blue) and BGLu1-G2F complex (yellow) structures are superimposed for comparison.