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Purification, crystallization and preliminary X-ray analysis of a hexameric β-glucosidase from wheat

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Introduction

β-Glucosidase (EC. 3.2.1.21) is one of the largest groups in the glycoside hydrolase families, and is a member of families 1 or 3 [1]. These enzymes are responsible for cleavage of the β-glucosidic linkage between two (or more) sugars, or between a sugar and an aglycone. In plants, β-glucosidases are involved in lignification, regulation of the physiological activity of cytokinin, regulation of the biosynthesis of IAA, and chemical defense against pathogens and herbivores. Many secondary products in plants exist in a form of gluco-conjugate with one or two glucose units attaching to their hydroxy or thiol groups. Since glucosylation changes physiological activity, chemical stability, and solubility to water, β-glucosidases play important roles by cleaving glucosidic bonds to release monosaccharides and active aglycones such as cyanogenic compounds, flavonoids, and hydroxamic acids. The glucosidases involved in the hydrolysis of plant secondary metabolites are members of family 1 glycohydrolase.

Recently, we cloned three β-glucosidase genes from wheat seedlings and expressed them in *E. coli*. The recombinant glucosidases formed a hexamer, the active form, consisting of 64 kDa. The hexamer was easily dissociated into monomer, resulting in loss of activity. The crystal structures of family 1 β-glucosidases have been isolated from four plant species: white clover, white mustard, maize, and sorghum. The wheat enzyme, however, differs in its active oligomeric structure, since wheat glucosidase must be hexameric to be active as a hydrolase whereas the other plant glucosidases function as dimers. The crystal structures of family 1 β-glucosidase from some bacteria have been solved as octamers. The wheat enzyme, however, shares lower homologies (~35%) with bacterial ones than those with plants (~60%). Thus, it is of interest to compare the monomer association properties from quaternary structures. We therefore aimed to crystallize wheat β-glucosidase as a hexamer in order to clarify the relation between its structure and the activity.

Materials and Methods

The initial crystallization conditions were screened by Hampton Research Crystal Screens I and II; the final condition was 10 mM HEPES (pH 7.2), 1 M $LiSO_4$, and 150 mM NaCl as the reservoir buffer. All crystallization experiments were performed using the hanging-drop vapor diffusion method in a 24-well tissue-culture VDX plate at 293 K. Drops consisting of 1 μl protein (3.7 mg/ml) and 1 μl reservoir solution were used for the initial screening, and 1.5 μl and 1.5 μl were used for the final condition.

To obtain the complex of TaGlu1b and its substrate aglycone, DIMBOA, the crystals were soaked in the crystallization buffer with 0.5 mM DIMBOA and 30% glycerol as a cryoprotectant for 15 min, and were then cooled in a nitrogen stream at 100 K. The diffraction data were processed using the HKL2000 program.

Results and Discussion

From the initial screening, crystals were obtained from both 0.1 M HEPES pH 7.5, 2 M ammonium sulfate, 2% PEG400, and 10 mM HEPES pH 7.2, 1 M LiSO4, 150 mM NaCl. The first set of conditions produced only tiny crystals, so we proceeded to the latter one. The crystals were typically 0.2 mm in their longest dimension, with a maximum of around 0.3 mm; we used the largest crystals for data analysis. TaGlu1b crystals complexed with DIMBOA were obtained by soaking, and diffracted to 1.7 Å on beamline BL-6A at Photon Factory. However, crystals without the substrate diffracted less than 2.0 Å. The mosaicity of the crystals was very low (~0.12), and R_{merge} was 0.079 with high redundancy (20).

A molecular replacement solution, using the β-glucosidase from sorghum (PDB code 1v08) as the search model, has been obtained using the program Molrep. From this solution, the asymmetric unit contains one monomer, and the solvent content was 72.1%, which is relatively high in spite of the higher resolution [2]. Model building and refinement of this structure are under way.

Several structures of O-β-glucosidase from plants, including white clover, maize, and sorghum, have been inferred. The resolution obtained in the present study is better, however, and promises to give better understanding of the relation between structure and activity of O-β-glucosidase.

References

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