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Crystallographic study of rice α-galactosidase

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Introduction

In plants, galactomannnan is major storage polysaccharides in seeds, and α-Galactosidase (α-Gal; EC 3.2.1.22) is one of the key enzymes in the degradation of cell wall galactomannnan during germination. α-Gals catalyze the hydrolysis of α-1,6-linked α-galactosyl residues from galacto-oligosaccharides and polymeric galacto-(gluco)mannnans. α-Gals are widely distributed in animals, plants, and microorganisms. Recently we have succeeded in the cDNA cloning, expression, purification and crystallization of the glycosyl hydrolase family 27 α-Gal from rice cell cultured suspension. To know the structural bases, we conducted the X-ray crystallographic analysis of rice α-Gal.

Experimental

The needle and/or rod-shaped crystals of rice α-Gal were obtained within two weeks on mixing 5 µl of protein solution (15 mg/ml) with 5 µl of reservoir solution (5% 2-propanol, 0.1 M ammonium sulfate, 0.1 M acetate buffer pH4.5 with 5% D-galactose) at 293 K employing the hanging-drop vapor diffusion method. Diffraction experiments on the native crystals were first carried out at beamline BL6A, Photon Factory, Tsukuba, Japan. α-Gal crystals were flash-frozen in a nitrogen stream at 100 K using 20 % glycerol as a cryo-protectant. Diffraction data were collected using a Quantum CCD X-ray detector. Structure determination was conducted by the multiple isomorphous replacement (MIR) method using Hg and Au derivatives. The structure was refined to an R-factor of 16.0 % and an R_{free}-factor of 17.8% (Table 1).

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Table 1: Structure refinement statistics of rice α-galactosidase

Cell parameter ($P2_12_12_1$)	
a (Å)	63.7
b (Å)	71.4
c (Å)	84.2
Resolution (Å)	30-1.5
No. of reflections in refinement	61,697
Completeness (%)	97.0
R-factor (%)	16.0
R_{free}-factor (%)	17.8

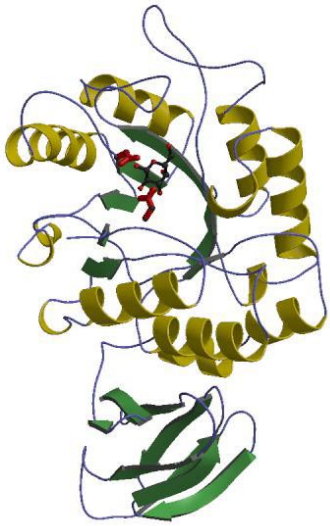

Figure 1 Ribbon model of rice α-Gal.

Results

The final model of rice α-Gal is composed of a single chain of 362 amino acids and can be divided into a catalytic domain, and a C-terminal domain (Fig. 1). The catalytic domain of rice α-Gal comprised a $(\beta/\alpha)_8$-barrel. The active site pocket was located on the C-terminal side of the central β-barrel of the catalytic domain, where a D-galactose molecule, which was added in the crystallization condition, was identified as a binding ligand. The C-terminal domain comprises eight β-strands containing a Greek key motif.

We made structural comparisons of rice α-Gal with chicken α-N-acetylgalactosaminidase, which belongs to the same family 27 of glycosyl hydrolases and folds into the similar overall structure, with a view toward understanding the substrate recognition mechanism in these enzymes.

References

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