AR-NW12A/2013G084

Crystal structure of α-amylase from rice (*Oryza sativa*)

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1 Introduction

α-Amylase (EC.3.2.1.1) is an enzyme that catalyzes the hydrolysis of an α-1,4 glycoside linkage in large α-linked polysaccharides, such as starch and glycogen, to yield maltose and maltodextrin products. α-Amylase from rice (AmyI-1) plays a crucial role in degrading starch in various tissues and at various growth stages. We previously reported that AmyI-1 also functions as potential growth inhibitor against human pathogenic bacteria [1]. This enzyme is a glycoprotein with an *N*-glycosylated carbohydrate chain, a unique characteristic among plant α-amylases. In this study, we determined the crystal structure of AmyI-1, lacking the *N*-glycosylated carbohydrate chain characteristic of plant α-amylases, and described the structures of the catalytic domain, the carbohydrate-binding site, and the N-linked glycosylation site of AmyI-1.

2 Experiment

Escherichia coli expression system was used for the preparation of recombinant AmyI-1. Overexpressed AmyI-1 was purified by Ni affinity, size exclusion, and anion-exchange chromatography. After concentration, purified AmyI-1 (8.6 mg/mL) was crystallized at 20°C by sitting-drop vapor diffusion method. A crystal suitable for X-ray analysis was obtained by using a reservoir solution consisting of 20% w/v polyethylene glycol (PEG) 3350, 2% v/v tacsimate (pH 6.0), and 0.1 M Bis-Tris (pH 6.5). The AmyI-1 crystal belongs to the $P2_1$ space group with the following unit cell parameters: a = 70.9 Å, b = 125.3 Å, c = 96.6 Å, and β = 90.2°. Four molecules are present in an asymmetric unit. X-ray diffraction images of the crystal were collected at −168°C with synchrotron radiation of 1.000-Å wavelength at the AR-NW12A station of Photon Factory (Tsukuba, Japan). The initial phase was determined by molecular replacement with the structure of α-amylase AMY2 from barley (PDB ID, 1BG9), which was used as a reference model. The atomic coordinate and structure factor of AmyI-1 (PDB ID, 3WN6) have been deposited in the Protein Data Bank Japan (PDBj) (http://pdbj.org/).

3 Results and Discussion

Crystal structure of AmyI-1 was determined at 2.2-Å resolution (Fig. 1) [2]. The structure consists of a typical $(\beta/\alpha)_8$-barrel, which is well-conserved among most α-amylases in the glycoside hydrolase family-13. Structural superimposition indicated small variations in the catalytic domain and carbohydrate-binding sites between AmyI-1 and barley α-amylases (AMY1 and AMY2). By contrast, regions around the N-linked glycosylation sites displayed lower conservation of amino acid residues, including Asn-263, Asn-265, Thr-307, Asn-342, Pro-373, and Ala-374 in AmyI-1, which are not conserved in barley α-amylases, suggesting that these residues may contribute to the construction of the structure of glycosylated AmyI-1. The results of this study will contribute to the understanding of the molecular physiology and biological functions of AmyI-1, which is expressed as a major isozyme in the scutellar epithelium and the aleurone layers during the germination process of rice seeds.

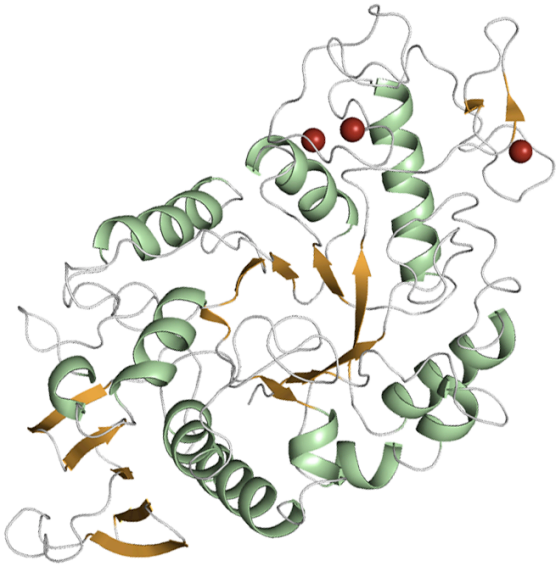

Fig. 1: Overall crystal structure of AmyI-1

Acknowledgement

We thank the beamline staff at the AR-NW12A station of the Photon Factory for their assistance with data collection.

References

[1] A. Ochiai *et al*., *J. Periodontal. Res*. **49**, 62 (2014).
[2] A. Ochiai *et al*., *Biosci. Biotechnol. Biochem*. **78**, 989 (2014).

Award

The Excellent Paper Award Published in Bioscience, Biotechnology, & Biochemistry in 2014. (2015)

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