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Crystal structure of 1,4-β-mannanase from *Eisenia fetida*

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1 Introduction

Endo-1,4-β-mannanase have been used for various bioprocesses such as bleaching of softwood pulps, declining viscosity of feeds and foods, and clarifying beverages. 1,4-β-mannanase has been isolated from bacteria, fungi, higher plants, mollusks, and arthropods, but it has not been reported about mannanase from annelida earthworm.

We have found a high mannanase activity in the body wall extract of the earthworm *Eisenia fetida*. We have cloned and expressed 1,4-β-mannanase from *Eisenia fetida* (Ef-Man). To understand the structure and function of Ef-Man, we have determined crystal structures of Ef-Man.

2 Experiment

Ef-Man crystals were obtained under the conditions containing ammonium citrate (crystal-1) or sodium potassium phosphate (crystal-2) as precipitants. Crystals (crystal-2) were soaked in 10 mM mannotriose (Man3) for 1 day. The crystal-1 and crystal-2 were transferred to the cryo-protectant solution consisting of the mother liquor with 20% glycerol. The crystals were cooled in a nitrogen-gas stream at 100 K during X-ray data collection.

Diffraction data sets were collected on the BL-17A beamline. The wavelength of X-rays was set to 0.98 Å and diffraction intensities were measured with Pilatus3 S6M detector. Crystal structures of Ef-Man were determined by the molecular replacement method with the crystal structure of 1,4-β-mannanase from *Mytilus edulis* (PDB 2C0H) [1] as a search model.

3 Results and Discussion

Thin plate crystals were obtained in both crystallization conditions. The crystal-1 and crystal-2 were diffracted to 1.7 Å and 1.6 Å resolutions, respectively (Table 1). Both crystals belong to the space group $P2_12_12_1$ with unit cell parameters of a = 50.1 Å, b = 69.5 Å, c = 86.6 Å (crystal-1) and a = 50.0 Å, b = 69.0 Å, c = 85.5 Å (crystal-2).

The final models of Ef-Man consist of Gln17-Gln377. The overall structures of crystal-1 and crystal-2 show high similarities each other. The superposition of the two structures indicated the root-mean-square distance of 0.18 Å for all Cα atoms. The crystal structure of Ef-Man also shows high similarities to the crystal structures of 1,4-β-mannanase from other species. Superposition of the Ef-Man structure to the mannanase structures shows rms distances of 1.04 Å for 347 Cα atoms (*M. edulis*, 2C0H), 0.87 Å for 357 Cα atoms (*Cryptococcus antaricticus*, 4OOU) [2], and 0.90 Å for 329 Cα atoms (*Aplysia kurodai*, 3VUP) [3].

In the crystal-1 structure of Ef-Man, one Tris molecule that included in the crystallization solution was bound to the active site. Although crystal-2 was soaked in the Man3 solution, there are no clear density of Man3 at the active site of the crystal-2 structure. Instead of Man3, water molecules were bound to the active site (Fig. 1). To obtain the structure complexed with Man3, we will modify soaking condition or perform co-crystallization with substrates.

Table 1: Diffraction data statistics

	crystal-1	crystal-2
Beamline	BL-17A	BL-17A
Wavelength (Å)	0.98	0.98
Resolution (Å)	50-1.70 (1.81-1.70)	50-1.60 (1.70-1.60)
Unique reflections	62 976	74 549
Completeness (%)	98.4 (97.1)	99.4 (98.6)
R_{sym} (%)	7.4 (46.4)	9.7 (52.7)
$\langle I \rangle / \langle \sigma(I) \rangle$	13.1 (2.7)	10.3 (3.0)

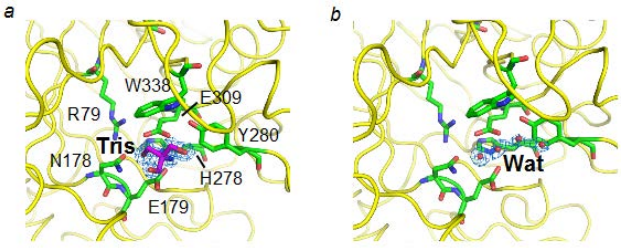

Fig. 1: Electron density at the active site of Ef-Man. $2F_o$-F_c map (blue mesh) of (a) crystal-1, (b) crystal-2.

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References

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