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Crystal structure of α-amylase II from *Eisenia fetida*

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1 Introduction

The earthworm *Eisenia fetida* has some cold-adapted enzymes. We have reported two tertiary structures of enzymes from *E. fetida*, endo-1,4-β-glucanase (Ef-EG2) [1] and endo-1,4-β-mannanase (Ef-Man) [2]. Here, we report a raw-starch-digesting α-amylase from *E. fetida* (Ef-Amy II).

We have purified two α-amylases (Ef-Amy I and II), and they are composed of similar amino acids (89% sequence identity). However, the thermal stability and substrate specificity are different between Ef-Amy I and II. Previously, we have determined the crystal structure of Ef-Amy I at 1.3 Å resolution. The overall structure of Ef-Amy I comprises N-terminal TIM barrel and C-terminal β-barrel domains. To understand the differences in the properties of Ef-Amy I and II, we have determined crystal structure of Ef-Amy II.

2 Experiment

Crystallization experiments of Ef-Amy II were performed by the hanging-drop vapor-diffusion method at 293 K. Crystals were obtained with the reservoir solution containing poly (acrylic acid sodium salt) 5100 as a precipitant. Crystals were cooled in a nitrogen-gas stream at 100 K during X-ray data collection.

Diffraction data sets were collected at the BL-17A beamline. The wavelength of X-rays was set to 0.98 Å and diffraction intensities were measured with Eiger X 16M detector. The crystal structure of Ef-Amy II was determined by the molecular replacement method with the crystal structure of Ef-Amy I as a search model.

3 Results and Discussion

The diffraction data sets were obtained at 2.5 Å resolution (Table 1; the statistics for Ef-Amy I are shown as well). The crystal belongs to the space group $P2_12_12_1$ with unit cell parameters of a = 95.5 Å, b = 100.5 Å, c = 118.9 Å.

The crystal structure of Ef-Amy II consists of Ala17-Val506, which includes all mature peptides (Gln18-Val506). The R_{work} and R_{free} factors after structure refinement were 17.8% and 23.6%, respectively. The overall structure of Ef-Amy II shows high similarities to that of Ef-Amy I, and the superposition of the two structures indicates the r.m.s.d. of 0.64 Å for 484 Cα atoms (Fig. 1a).

The estimated catalytic residues D213, E249 and D316 show high structural similarities between Ef-Amy I and II (Fig. 1b). However, Ef-Amy II does not have the Gly-rich loop, which exists in Ef-Amy I and is considered to affect substrate binding in human salivary α-amylase [3]. This structural difference around the active site between Ef-Amy I and II seems to effect the substrate specificity. To confirm the roles of this loop, we continue structural studies of α-amylases from *E. fetida* in combination with biochemical studies.

Table 1: Diffraction data statistics

	Ef-Amy II	Ef-Amy I
Resolution (Å)	50-2.50 (2.60-2.50)	50-1.30 (1.38-1.30)
Space group	$P2_12_12_1$	$P3_221$
Unit Cell *a, b, c* (Å)	95.5, 100.5, 118.9	96.2, 96.2, 122.0
Completeness (%)	99.9 (99.8)	99.8 (99.0)
R_{sym} (%)	12.7 (54.6)	5.6 (78.8)
$\langle I \rangle / \langle \sigma(I) \rangle$	14.6 (4.0)	15.1 (2.1)

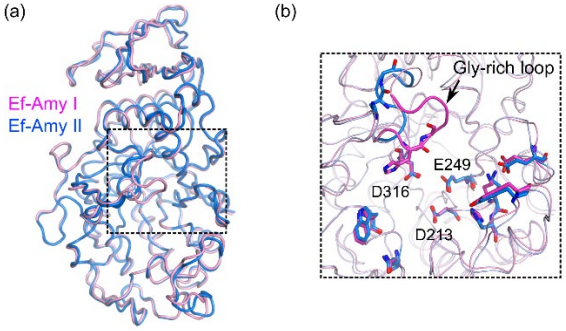

Fig. 1 Superposition between Ef-Amy II (blue) and I (pink). (a) The overall structures. (b) The structures around estimated active sites.

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References

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