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Crystal structure of α-amylase from *Eisenia fetida*

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1 Introduction

We have already reported that the earthworm *Eisenia fetida* has some cold-adapted enzymes, and furthermore, 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 novel raw-starch-digesting α-amylase from *E. fetida* (Ef-Amy I).

We have purified two α-amylases (Ef-Amy I and II), which are electrophoretically homogeneous states. Ef-Amy I and II are composed of similar amino acids (89% sequence identity), however, the cold adaptability and the starch-digesting activity are different between Ef-Amy I and II. To understand those differences at atomic level, we have determined crystal structure of Ef-Amy I.

2 Experiment

Ef-Amy I crystals were obtained under the conditions containing lithium sulphate (crystal-1), PEG1000 (crystal-2), ammonium citrate (crystal-3), or PEG3350 (crystal-4) as precipitants. 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-Amy I were determined by the molecular replacement method with the crystal structure of porcine pancreatic α-amylase (PDB 1KXQ) [3] as a search model.

3 Results and Discussion

Prismatic crystals were obtained in every crystallization condition. The diffraction data sets of all crystals were obtained higher than 1.9 Å resolution (Table 1), especially crystal-1 and 4 were diffracted to 1.3 Å and 1.4 Å resolutions, respectively. All crystals belong to the space group $P3_221$ with unit cell parameters of $a = b =$ 96~97 Å, $c =$ 121~122 Å.

The final model of Ef-Amy I (crystal-1) consists of Ala17-Ala513, which includes all mature peptides (Gln18-Val510). The final R_{work} and $R_{free\ (5\%)}$ factors were 14.7% and 16.8%, respectively. Overall structure of Ef-Amy I comprises a TIM barrel, which is the common fold in GH13 family proteins, and C-terminal domain (including Greek key motif) (Fig. 1a), and shows high similarities to the crystal structures of α-amylase from other species. Superposition of the Ef-Amy I structure to the amylase structures shows rms distances of 0.89 Å for 450 Cα atoms (Porcine pancreatic, 1PIG) [4], 0.87 Å for 450 Cα atoms (Human pancreatic, 4W93) [5], and 0.91 Å for 429 Cα atoms (Tenebrio molitor, 1JAE) [6].

The molecular surface of Ef-Amy I is negatively charged as well as that of Ef-EG2 and Ef-Man (Fig. 1b). In the crystal-1 structure of Ef-Amy I, one acetate ion that included in the crystallization solution was bound to the active site. To obtain the structure of Ef-Amy II, we continue crystallization experiment.

Table 1: Diffraction data statistics

	crystal-1	crystal-2	crystal-3	crystal-4
Resolution (Å)	50-1.30 (1.38-1.30)	50-1.90 (2.02-1.90)	50-1.70 (1.81-1.70)	50-1.40 (1.49-1.40)
Unit Cell *a, c* (Å)	96.2, 122.0	97.1, 121.4	96.1, 121.1	96.4, 121.2
Completeness (%)	99.8 (99.0)	99.9 (99.7)	99.8 (98.7)	99.9 (99.4)
R_{sym} (%)	5.1 (67.3)	8.1 (55.8)	7.0 (46.9)	6.2 (49.6)
$\langle I \rangle / \langle \sigma(I) \rangle$	15.1 (2.1)	12.3 (2.3)	14.0 (3.4)	14.1 (2.7)

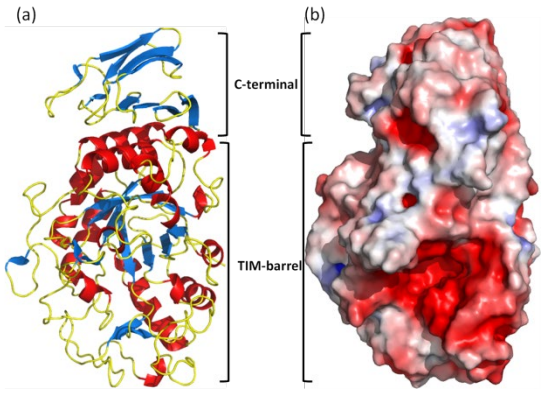

Fig. 1: Crystal structure of Ef-Amy I.
(a) Ribbon diagram. (b) Electrostatic potentials.

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References

[1] T. Arimori *et al.*, *J. Synchrotron Rad.* **20**, 884 (2013).
[2] M. Ueda *et al.*, *Enzyme Microb. Technol.* **117**, 15 (2018).
[3] A. Desmyter *et al.*, *J. Biol. Chem.* **277**, 23645 (2002).
[4] M. Machius *et al.*, *J. Mol. Biol.* **260**, 409 (1996).
[5] L. K. Williams *et al.*, *Nat. Chem. Biol.* **11**, 691 (2015).
[6] S. Strobi *et al.*, *J. Mol. Biol.* **278**, 617 (1998).

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