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X-ray crystal structures of three mutants of endo-1,4-β-glucanase from *Eisenia fetida*

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1 Introduction

The earthworm *Eisenia fetida* has some cold-adapted enzymes. We have reported tertiary structures of enzymes from *E. fetida*, endo-1,4-β-glucanase (Ef-EG2) [1], endo-1,4-β-mannanase [2], and α-amylase [3]. Here, we report X-ray crystallographic studies of three mutants of Ef-EG2.

Salt-bridges have been reported to affect the catalytic properties and thermostabilities of enzymes. We have purified three mutants of Ef-EG2 (D43R, N372D, and Q387E) that have been designed to introduce an additional salt-bridge on the surface of the protein. The mutation sites are located away from the catalytic cleft. Among three mutants, only the D43R mutant increases relative activity compared to the wildtype. We have determined the crystal structures of D43R, N372D, Q387E mutants of Ef-EG2 to understand the molecular properties that influence the catalytic activity.

2 Experiment

Crystallization experiments of three mutants of Ef-EG2 were performed by the hanging-drop vapor-diffusion method at 293 K. Crystals were obtained with the reservoir solution containing polyethylene glycol 3350 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-5A (D43R) and BL-17A (N372D, Q387E) beamlines. The wavelengths of X-rays were set to 1.0 Å (D43R) and 0.98 Å (N372D, Q387E). The crystal structures of three mutants of Ef-EG2 were determined by the molecular replacement method with the crystal structure of wildtype (PDB ID: 3WC3) as a search model.

3 Results and Discussion

The diffraction data sets were obtained at 1.7 Å (D43R), 1.6 Å (N372D), 1.6 Å (Q387E) resolutions (Table 1). The crystals belong to the space group $P2_1$ with unit cell parameters of a = 53.3 Å, b = 71.7 Å, c = 55.4 Å, β = 114.2 (D43R; N372D and Q387E show similar unit cell parameters).

The crystal structures of three mutants consist of amino acid residues Tyr23-Asp454. The R_{work}/R_{free} factors after structure refinement were 13.7%/17.7% (D43R), 14.1%/17.2% (N372D), 14.6%/17.5% (Q387E). The overall structures show high similarities among three mutants and wildtype, and the superposition of wildtype and each mutant indicates the r.m.s.d. of 0.31-0.33 Å for 410 Cα atoms.

All three mutants do not make an effective salt-bridge, within hydrogen-bonding distance, at the mutation sites (Fig. 1). In wildtype, Asp43 is involved in the coordination of a Na^+ ion together with Asp55, which is located close to Asp43. In D43R mutant, Arg43 does not form a hydrogen bond with Asp55, but the side chains of Arg43 and Asp55 are located within an electrostatic interaction distance. In N372D and Q387E, the hydrogen bonds around the mutation sites do not show significant differences compared to wildtype. These results suggest that the structural flexibility on the surface of protein affects the catalytic activity of Ef-EG2.

Table 1: Diffraction data statistics

	D43R	N372D	Q387E
Resolution (Å)	50-1.7 (1.73-1.70)	50-1.6 (1.63-1.60)	50-1.6 (1.63-1.60)
Unit cell a, b, c (Å), β (°)	53.3, 71.7, 55.4, 114.2	53.3, 71.6, 55.1, 113.8	53.4, 71.6, 55.2, 114.0
Completeness (%)	99.9 (99.2)	99.5 (96.6)	99.4 (97.9)
R_{meas} (%)	13.4 (49.9)	8.6 (49.1)	11.2 (52.3)
$I/\sigma(I)$	11.6 (3.6)	10.0 (2.7)	8.3 (2.5)

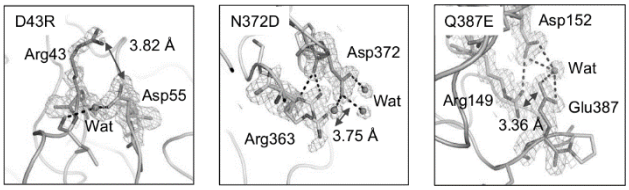

Fig. 1: Structures of the mutation sites of D43R (left), N372 (center), and Q387E (right). $2F_o$-F_c electron density map contoured at 1.0σ level is shown as gray mesh. The distances between the side chains of Arg43-Asp55, Arg363-Asp372, and Arg149-Glu378 are indicated in each panel.

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References

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