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Crystallization and preliminary X-ray analysis of reducing-end xylose-releasing exo-oligoxylanase from *Bacillus halodurans* C-125

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The reducing-end xylose-releasing exo-oligoxylanase (Rex) from *Bacillus halodurans* C-125, a novel family GH8 glycoside hydrolase, was crystallized by the hanging-drop vapour-diffusion method using 13.6 mg ml^{-1} purified Rex, 5.6%(*v*/*v*) polyethylene glycol 4000, 70 m*M* sodium acetate pH 4.6 and 30%(*v*/*v*) glycerol. Suitable crystals grew after incubation for 5 d at 293 K. The crystals belonged to space group $P2_12_12_1$, with unit-cell parameters a = 52.69, b = 86.02, c = 87.92 Å. X-ray diffraction data were collected at a resolution of 1.35 Å.

1. Introduction

Glycoside hydrolases (GHs) are classified into more than 90 families based on amino-acid sequence identity (Henrissat, 1991; Bourne & Henrissat, 2001; http://afmb.cnrs-mrs.fr/CAZY/). The GH8 family includes various inverting endo-hydrolytic enzymes, such as cellulase (EC 3.2.1.4), endo-1,4-β-xylanase (EC 3.2.1.8), licheninase (EC 3.2.1.73) and chitosanase (EC 3.2.1.132). Recently, we demonstrated that a GH8 protein from *Bacillus halodurans* C-125 (BH2105 protein), which was annotated as an endo-xylanase owing to its similarity to GH8 endo-xylanases (Kim *et al.*, 1995; Yoon *et al.*, 1998; Collins *et al.*, 2002), is a unique exo-oligoxylanase that hydrolyzes xylo-oligosaccharides at their reducing ends to release xylose (Honda & Kitaoka, 2004). Therefore, we named the enzyme reducing-end xylose-releasing exo-oligoxylanase (Rex). To our knowledge, Rex is the only completely exo-hydrolytic enzyme that releases a monosaccharide from the reducing end. Of the members of the GH8 family, the three-dimensional structures of chitosanase from *Bacillus* sp. K-17 (Adachi *et al.*, 2004), cellulase from *Clostridium thermocellum* (Alzari *et al.*, 1996; Guerin *et al.*, 2002) and endo-1,4-β-xylanase from *Pseudoalteromonas haloplanktis* (Petegem *et al.*, 2003) have been determined. These molecules have been shown to adopt $(\alpha/\alpha)_6$-barrel structures belonging to clan GH-M (Guimaraes *et al.*, 2002). Comparison of these three structures (Adachi *et al.*, 2004) reveals differences in the substrate specificities of polymer recognition, as all the substrates (chitosan, cellulose and xylan, respectively) have an identical backbone structure consisting of β-1,4 glycosyl polymers. Thus, structural comparison of Rex with other GH8 enzymes, especially with the endo-β-1,4 xylanase from *P. haloplanktis* (Petegem *et al.*, 2003), will reveal the mechanism of the exo-splitting reaction. Here, we describe the crystallization and results of preliminary X-ray analysis of Rex from *B. halodurans* C-125.

2. Experimental procedures and results

2.1. Crystallization

Rex was purified as described previously (Honda & Kitaoka, 2004). The purified enzyme, which had a molecular weight of 46 075 Da, was concentrated to 13.6 mg ml^{-1} using a Microcon centrifugal filter device (Millipore, Billerica, MA, USA) after dialysis against distilled water. Protein concentrations were determined from the absorbance at 280 nm based on theoretical molar absorption coefficients (106 210 M^{-1} cm^{-1}) determined from the amino-acid composition of BH2105 (Pace *et al.*, 1995). The crystallization

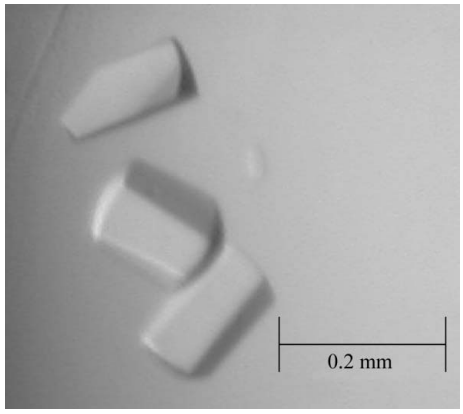

Figure 1
Crystals of Rex obtained under the optimized conditions. See text for experimental details.

procedure was performed using the sitting-drop vapour-diffusion method. To determine the initial conditions, each drop was prepared by mixing 2 µl purified enzyme solution with the same volume of each reservoir solution of Crystal Screen Cryo and Crystal Screen 2 crystallization kits (Hampton Research, CA, USA) and equilibrated against 100 µl of the same reservoir solution at 293 K; crystals of BH2105 were grown from drops of solution No. 37 [5.6%(*v*/*v*) polyethylene glycol 4000, 70 m*M* sodium acetate pH 4.6, 30%(*v*/*v*) glycerol] and solution No. 20 [20%(*v*/*v*) polyethylene glycol, 80 m*M* sodium acetate pH 4.6, 160 m*M* ammonium sulfate, 20%(*v*/*v*) glycerol] from Crystal Screen Cryo. Finally, the optimal crystallization conditions were obtained with solution No. 37 of Crystal Screen Cryo at 293 K using the hanging-drop vapour-diffusion method for 5 d to give the crystals as shown in Fig. 1.

2.2. Data collection

Diffraction data were collected using a charge-coupled device (CCD) camera (ADSC Quantum 315) at the BL-5A station of the Photon Factory, High Energy Accelerator Research Organization (KEK), Tsukuba, Japan (λ = 1.000 Å). Rex crystals were flash-cooled in a stream of nitrogen maintained at 100 K. A total of 220 frames were collected with oscillations of 0.5° and exposure times of 10 s. Diffraction images were indexed, integrated and scaled using the *HKL*2000 program suite (Otwinowski & Minor, 1997).

The crystals of Rex belong to the orthorhombic space group $P2_12_12_1$ and diffract to a resolution of 1.35 Å. The data-collection statistics are presented in Table 1. Assuming the presence of one molecule of Rex per asymmetric unit, the calculated V_M value (Kantardjieff & Rupp, 2003) and solvent content were 2.21 Å^3 Da^{-1} and 44.3%, respectively.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 52.69, b = 86.02, c = 87.92
Resolution range (Å)	62.02–1.35 (1.40–1.35)
Measured reflections ($I > 3\sigma$)	166098 (15930)
Unique reflections	87069 (8400)
Completeness (%)	98.4 (96.4)
Redundancy	1.9 (1.9)
Mean $I/\sigma(I)$	36.0 (4.7†)
$R_{\rm merge}$‡ (%)	5.3 (28.5)

† Reflections in the last shell with $I/\sigma(I) > 3$ = 47.8%. ‡ $R_{\rm merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h, i)$ is the intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean value of $I(h, i)$ for all i measurements.

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