Crystallization and preliminary X-ray crystallographic analysis of a putative acetylxyln esterase from *Talaromyces cellulolyticus*

Masahiro Watanabe and
Kazuhiko Ishikawa*

Biomass Refinery Research Center, National
Institute of Advanced Industrial Science, 3-11-32
Kagamiyama, Higashi-Hiroshima 739-0046,
Japan

Correspondence e-mail:
kazu-ishikawa@aist.go.jp

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Acetylxyln esterase (AXE) catalyzes the hydrolytic cleavage of the ester bond between acetic acid and hemicellulose in plant cell walls. A putative AXE gene exhibiting high homology to carbohydrate esterase family 3 was found in the genome database of the fungus *Talaromyces cellulolyticus* (formerly known as *Acremonium cellulolyticus*). A truncated form of the protein, the catalytic domain of the enzyme, was prepared and crystallized. The best crystal was obtained at 293 K using 0.17 M ammonium sulfate, 28% PEG 4000, 5% (v/v) glycerol, 0.5% (w/v) *n*-octyl- β -D-glucoside. X-ray diffraction data were collected to 1.50 Å resolution. The crystal belonged to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = 70.90$, $b = 70.90$, $c = 87.09$ Å. One enzyme molecule per asymmetric unit gave a crystal volume per protein mass (V_M) of $2.62 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 53.0% (v/v).

1. Introduction

Lignocellulosic biomass in the plant cell wall, which is the most abundant carbon source on Earth, is a highly complicated macromolecular structure comprising various carbohydrate polymers (Sunna & Antranikian, 1997). Lignocellulose is effectively degraded by microbial biological processes (Sunna & Antranikian, 1997), which is of increasing industrial significance for the production of biofuels and biomaterials (Christov & Prior, 1993). However, most cell-wall polysaccharides, including xylan, mannan and glucomannan, are largely acetylated or acylated, and thus sterically inhibit access of glycoside hydrolases and prevent their attack on the glycosidic linkages in the polysaccharides. Removal of the acetylated moieties by carbohydrate esterases could therefore accelerate the degradation of polysaccharides by glycoside hydrolases (Chávez *et al.*, 2006; van den Brink & Vries, 2011).

The fungus *Talaromyces cellulolyticus* (formerly known as *Acremonium cellulolyticus*) produces large quantities of various cellulases. A putative acetylxyln esterase gene (*axe*; Gene ID AB937137) belonging to carbohydrate esterase family 3 (CE3) (acetylxyln esterase; AXE; EC 3.1.1.72; Correia *et al.*, 2008) was identified in *T. cellulolyticus* by an amino-acid sequence homology search. *T. cellulolyticus* AXE (TcAXE) comprises 283 amino acids that fold to provide four structural components: a putative N-terminal signal peptide, a catalytic core domain, a serine/threonine-rich linker region and a carbohydrate-binding module (CBM-1) at the C-terminus. However, we have not yet identified the activity of TcAXE. In this study, we expressed, prepared and crystallized the catalytic domain (amino acids 21–226, molecular weight 22.5 kDa) of the enzyme, which was named TcAXE206. No crystallographic data have been reported for any fungal AXE (CE3) and little structural information is available for this enzyme class. Here, we report the first crystals of TcAXE206 suitable for X-ray analysis at atomic resolution.

2. Materials and methods

2.1. Macromolecule production

cDNA encoding the *axe* gene was amplified by reverse transcription PCR from *T. cellulolyticus* CF-2612 genomic DNA. The amplified full-length cDNA was subcloned and the catalytic domain was

Table 1

Macromolecule-production information.

Source organism	<i>T. cellulolyticus</i>
DNA source	<i>T. cellulolyticus</i> CF-2612
Forward primer	AATCATATGGTCAAAGTCATGCTGCTCG
Reverse primer	ATTGGATCCTTAAGAGACGTCCTTGATGAG
Cloning vector	pUC19
Expression vector	pET-11a
Expression host	<i>E. coli</i> BL21(DE3)
Complete amino-acid sequence of the construct produced	MVKVMLLGDSITEITCWRPLVWEQITSAGLAGSVDFVGSMD-LQPNCSRPGFDPDEHSGWQAYDIARNNIAGVQNTKP-DIVQFMLGTDVNIHGRNADSIIGSYITMLNAMRAANPRV-KVIVDKIIPTSWSDATIEAVNTAIPGWVQQTTAESPVVI-ADCSRAAGFTNDMLRDDGVHPNSKGDQFIAGQIGPKLIQL-IKDV

truncated by PCR. The truncated gene encoding TcAXE206 was ligated into the pET-11a vector (Novagen, Madison, Wisconsin, USA) with *NdeI/BamHI* sites (underlined in Table 1). The gene was transformed into *Escherichia coli* BL21(DE3) cells (Invitrogen, Carlsbad, California, USA). The harvested cells were resuspended in a buffer consisting of 20 mM Tris–HCl pH 8.0, 50 mM NaCl and then lysed by sonication, followed by centrifugation at 35 870g for 30 min at 4°C. The supernatant was applied onto a HiTrap Q HP column (GE Healthcare, Little Chalfont, England) equilibrated with 20 mM Tris–HCl pH 8.0, 50 mM NaCl. The protein was eluted using an increasing linear gradient of NaCl. Fractions containing the target protein were pooled, dialyzed into 20 mM Tris–HCl pH 8.0, 2 M NaCl and applied onto a HiTrap Butyl HP column (GE Healthcare) equilibrated with the same buffer. The protein was eluted using a decreasing linear gradient of NaCl. Finally, the eluted protein was applied onto a Superdex 200 16/60 gel-filtration column (GE Healthcare) equilibrated with 20 mM Tris–HCl pH 8.0, 50 mM NaCl. The purity of the enzyme was confirmed by SDS–PAGE. The protein concentration was determined using the molar extinction coefficient of 36 105 M^{−1} cm^{−1} calculated from the amino-acid sequence (Gill & von Hippel, 1989). Detailed information on enzyme production is given in Table 1.

2.2. Crystallization

The purified protein was concentrated to 10 mg ml^{−1} using an Amicon Centricon YM-10 (Millipore, Billerica, Massachusetts, USA). Initial crystallization screening was performed manually using Wizard Classic 1 and 2 and Wizard Classic 3 and 4 (Emerald Bio, Bainbridge Island, Washington, USA) by the sitting-drop vapour-diffusion method at 293 K. Each drop comprised 0.5 µl protein solution and 0.5 µl reservoir solution and was equilibrated against 60 µl reservoir solution. After one week, small crystals were obtained using several conditions, of which No. 30 from Wizard Classic 3 and 4 [170 mM ammonium sulfate, 25.5%(w/v) PEG 4000, 15%(v/v) glycerol] was the most promising. The best-quality crystals of the enzyme were obtained when using a final concentration of 0.5%(w/v) *n*-octyl-β-D-glucoside (βOG) as an additive. Details of the method of crystallization are given in Table 2.

2.3. Data collection and processing

Selected crystals were harvested and immersed in a cryoprotectant with the same composition as the mother liquor. The soaked crystal was immediately flash-cooled under a stream of nitrogen gas at 100 K. X-ray diffraction data for TcAXE206 were collected on the BL44XU beamline at SPring-8, Hyogo, Japan. The data were processed to 1.5 Å resolution using HKL-2000 (DENZO and

Table 2

Crystallization.

Method	Hanging-drop vapour diffusion
Plate type	TPP
Temperature (K)	293
Protein concentration (mg ml ^{−1})	10
Buffer composition of protein solution	20 mM Tris–HCl pH 8.0, 50 mM NaCl
Composition of reservoir solution	170 mM ammonium sulfate, 28%(w/v) PEG 4000, 5%(v/v) glycerol, 0.5%(w/v) βOG
Volume and ratio of drop	4 µl (1:1)
Volume of reservoir (ml)	1

Table 3

Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	BL44XU, SPring-8
Wavelength (Å)	0.9
Temperature (K)	100
Detector	Rayonix MX225-HE
Crystal-to-detector distance (mm)	180
Rotation range per image (°)	1.0
Total rotation range (°)	150
Exposure time per image (s)	1.0
Space group	<i>P</i> ₄ ₁ ₂ ₁ ₂ or <i>P</i> ₄ ₃ ₂ ₁ ₂
<i>a</i> , <i>b</i> , <i>c</i> (Å)	70.90, 70.90, 87.09
α , β , γ (°)	90, 90, 90
Mosaicity (°)	0.3
Resolution range (Å)	50.0–1.5 (1.53–1.50)
Total No. of reflections	248943
No. of unique reflections	35924
Completeness (%)	99.0 (95.8)
Multiplicity	6.9 (3.7)
$\langle I/\sigma(I) \rangle$	26.5 (2.3)
$R_{\text{merge}}^{\dagger}$ (%)	6.9 (42.0)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th measurement of reflection *hkl* and $\langle I(hkl) \rangle$ is the mean value of $I_i(hkl)$ for all *i* measurements.

SCALEPACK; Otwinowski & Minor, 1997). Detailed information on data collection and processing is given in Table 3.

3. Results and discussion

Tetragonal bipyramidal crystals were obtained after two weeks. The average dimensions of the crystals were about 0.15 × 0.1 × 0.1 mm. Diffraction data were collected to a resolution limit of 1.50 Å. The data consisted of 248 943 measurements and 35 924 unique reflections. Data collection and analysis revealed that the crystal belonged to space group *P*₄₁₂₁₂ or *P*₄₃₂₁₂, with unit-cell parameters *a* = 70.90, *b* = 70.90, *c* = 87.09 Å. Scaling and merging of the crystallographic data resulted in an overall R_{merge} of 6.9%. The data set had an overall completeness of 99.0%. The data-collection and refinement statistics are given in Table 3. There was one enzyme molecule per asymmetric unit, giving a crystal volume per protein mass (V_M) of 2.62 Å³ Da^{−1} and a solvent content of 53.0%(v/v) (Matthews, 1968). Determination of the structure of TcAXE206 is in progress and the structural information obtained will be used for functional analysis.

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