Crystallization and preliminary X-ray analysis of the catalytic domains of *Paenibacillus* sp. strain FPU-7 cell-surface-expressed chitinase ChiW

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The polysaccharide chitin is effectively hydrolyzed and utilized as a carbon and nitrogen source by the Gram-positive bacterium *Paenibacillus* sp. strain FPU-7. ChiW is a unique cell-surface-expressed chitinase among the *Paenibacillus* sp. strain FPU-7-secreted chitinases. An N-terminally truncated ChiW protein, primarily comprised of the two catalytic domains of the full-length protein, was successfully overexpressed in *Escherichia coli*, purified as a functional recombinant protein with a molecular mass of approximately 98 kDa and crystallized. Preliminary X-ray analysis showed that the crystal diffracted to 1.93 Å resolution and belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 112.1$, $b = 128.2$, $c = 162.6$ Å, suggesting the presence of two molecules in an asymmetric unit.

1. Introduction

The Gram-positive soil bacterium *Paenibacillus* sp. strain FPU-7 was reported to have a high degradation activity towards chitin (Itoh *et al.*, 2013). It is noteworthy that ChiW was identified as a novel cell-surface-expressed multi-modular chitinase (Itoh *et al.*, 2013). ChiW contains a signal peptide (amino acids 1–30) and three repeated surface-layer homology (SLH; amino acids 31–197) domains anchored to the bacterial surface layer (Mesnage *et al.*, 2000; Schneewind & Missiakas, 2012; Fig. 1a). The SLH domains are followed by two homologous catalytic domains (54% amino-acid sequence identity) belonging to the glycoside hydrolase family GH-18 chitinases (first GH-18, amino acids 557–927; second GH-18, amino acids 1045–1418; Itoh *et al.*, 2014; <http://www.cazy.org/>; Henrissat & Bairoch, 1996; Fig. 1a). The remaining regions, in particular amino acids 198–556, showed no sequence similarity to other known proteins. Although several chitinases with two catalytic domains in a single polypeptide chain have been identified in a wide variety of organisms (Tanaka *et al.*, 1999; Howard *et al.*, 2004; Hiramatsu *et al.*, 1999), ChiW is the first chitinase that is expressed on the bacterial surface layer.

A recombinant ChiW-SLHd construct (amino acids 198–1418; Fig. 1a) lacking the SLH domains was purified from *Escherichia coli* cells and several of its enzymatic properties have been determined (Itoh *et al.*, 2013, 2014). The detailed crystal structures of the catalytic domains (TIM-barrel) of GH-18 chitinases have been investigated (http://www.cazy.org/GH18_structure.html). However, to our knowledge, no information is currently available on the structures of the cell-surface-expressed multi-modular chitinases in the Protein Data Bank (PDB). Since crystallizations of full-length recombinant ChiW-SLHd protein have thus far been unsuccessful, we prepared the truncated mutant protein ChiW-CD (amino acids 557–1418; Fig. 1a), which is primarily composed of two tandem catalytic GH-18 domains. Here, we report the crystallization and preliminary crystallographic analysis of ChiW-CD.

2. Materials and methods

2.1. Macromolecule production

The ChiW-SLHd gene (DDBJ/EMBL/GenBank database accession No. AB683965) was subcloned into the expression vector

Table 1
ChiW-CD production information.

Source organism	<i>Paenibacillus</i> sp. strain FPU-7
DNA source	pColdIII_ChiW-SLHd expression vector with C-terminal 6×His tag
Forward primer	5'-CTCGAGGGTACCGAGCTCCATATGCAC-3'
Reverse primer	5'-GTGCGAGGGCAAAATTGTTTCTTATATCCCGGCTTG-3'
Expression vector	pColdIII
Expression host	<i>E. coli</i> BL21

pColdIII (Takara, Otsu, Shiga, Japan) from *Paenibacillus* sp. strain FPU-7 genomic DNA as previously described (Itoh *et al.*, 2013). Inverse PCR was used for constructing the truncated mutant ChiW-CD (Fig. 1*a*) with a C-terminal 6×His tag using a KOD Plus mutagenesis kit (Toyobo, Osaka, Japan) and the expression vector for ChiW-SLHd as a template. Successful plasmid construction was confirmed by DNA sequencing with an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California, USA). The expression vector for ChiW-CD was transformed into competent *E. coli* BL21 cells (Novagen, Darmstadt, Germany; Table 1).

Recombinant ChiW-CD was purified from *E. coli* cells by the same procedure used for ChiW-SLHd with minor variations (Itoh *et al.*, 2014). In brief, the cultured cells were chemically disrupted and the cell extract was subsequently purified by Ni-immobilized metal-affinity chromatography (HisTrap HP column; GE Healthcare, Little Chalfont, England) and anion-exchange chromatography (Mono Q HR 10/10 HR column; GE Healthcare). Enzyme-containing fractions

Figure 1
Schematic representation of *Paenibacillus* sp. strain FPU-7 ChiW primary structure and SDS-PAGE analysis of purified ChiW-CD. (a) ChiW denotes full-length *Paenibacillus* sp. strain FPU-7 ChiW protein; ChiW-SLHd and ChiW-CD denote the truncated ChiW proteins. (b) SDS-PAGE protein profile of ChiW-CD. Lane M, molecular-weight standards (labelled in kDa); lane 1, purified ChiW-CD (1 µg).

Table 2
Crystallization conditions.

Method	Sitting-drop vapour diffusion
Plate type	24-well plate
Temperature (K)	293
Protein concentration (mg ml ⁻¹)	10
Buffer composition of protein solution	20 mM HEPES pH 7.0
Composition of reservoir solution	0.94 M ammonium phosphate, 0.1 M citrate-phosphate pH 5.5
Volume and ratio of drop	4 µl, 1:1 enzyme solution:reservoir solution
Volume of reservoir (µl)	500

Table 3
Data-collection statistics for ChiW-CD.

Values in parentheses are for the outer shell.

Diffraction source	BL-17A, Photon Factory
Wavelength (Å)	0.98
Temperature (K)	95
Detector	Quantum 315r CCD
Crystal-to-detector distance (mm)	256.1
Rotation range per image (°)	0.5
Total rotation range (°)	180
Exposure time per image (s)	5.0
Space group	<i>P</i> ₂ ₁ ₂ ₁
Unit-cell parameters (Å)	<i>a</i> = 112.1, <i>b</i> = 128.2, <i>c</i> = 162.6
Mosaicity (°)	0.453
Resolution range (Å)	50.0–1.93 (1.96–1.93)
Total No. of reflections	1215285
No. of unique reflections	174215 (8716)
Completeness (%)	98.9 (100)
Multiplicity	7.0 (6.8)
<i>I</i> /σ(<i>I</i>)	29.7 (2.8)
<i>R</i> _{meas} † (%)	8.6 (57.2)
Overall <i>B</i> factor from Wilson plot (Å ²)	25.5

† $R_{\text{meas}} = \sum_{hkl} \{N(hkl)/[N(hkl) - 1]\}^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the *i*th observation of reflection *hkl* and $\langle I(hkl) \rangle$ is the weighted average intensity for all *i* observations of reflection *hkl*.

were combined and dialyzed at 277 K overnight against 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer pH 7.0. The protein concentration was determined by UV spectrophotometry using the theoretical molar extinction coefficient $\epsilon_{280} = 193\,900\text{ M}^{-1}\text{ cm}^{-1}$ according to the ExPASy ProtParam tool server (<http://web.expasy.org/protparam/>; Gasteiger *et al.*, 2005). ChiW-CD was purified from the bacterial cells to homogeneity, as assessed by 12%(w/v) SDS-PAGE followed by Coomassie Brilliant Blue (CBB) R-250 staining.

2.2. Crystallization

Initial screening for ChiW-CD crystallization was performed using the commercially available crystallization kits Crystal Screen and Crystal Screen 2 (Hampton Research, Aliso Viejo, California, USA) and Wizard Classic Crystallization Screens 1 and 2 (Emerald BioSystems, Bedford, Massachusetts, USA) by the sitting-drop vapour-diffusion method in 96-well plates at 293 K. Crystallization conditions were further refined using a 24-well plate. Final crystal preparation was performed by the sitting-drop vapour-diffusion method. The drop (4 µl) consisted of 2 µl enzyme solution (10 mg ml⁻¹) and 2 µl reservoir solution (0.5 ml) comprising 0.94 M ammonium phosphate, 0.1 M citrate-phosphate buffer (0.1 M citric acid and 0.2 M dibasic sodium phosphate solutions were mixed to adjust the pH to 5.5) (Table 2).

2.3. Data collection and processing

Single crystals were soaked in a cryoprotectant solution consisting of 0.27 M ammonium phosphate, 0.029 M citrate-phosphate pH 5.5,

Figure 2
Crystals of ChiW-CD.

5 M sodium formate followed by cooling in a stream of cold nitrogen gas. The diffraction patterns were then examined on beamlines BL38B1 at SPring-8 and BL-17A at the Photon Factory. X-ray diffraction data for the ChiW-CD crystals were collected using Quantum 315r CCD detectors (Area Detector Systems Corporation, Poway, California, USA) and were indexed, integrated and scaled using *HKL-2000* (Otwinowski & Minor, 1997). Molecular-replacement trials were performed using *Phaser* (McCoy *et al.*, 2007) from the *CCP4* software package (Winn *et al.*, 2011) (Table 3).

3. Results and discussion

The overexpressed ChiW-CD in *E. coli* was successfully purified to homogeneity as observed by SDS-PAGE (Fig. 1*b*). Other characteristics of ChiW-CD, such as enzymatic activity, N-terminal amino-acid residues and secondary-structural information evaluated from the far-UV circular-dichroism spectrum, are described in the Supporting Information¹.

After incubation for 3 d, small crystals appeared in Wizard Classic 1 No. 9 condition consisting of 1.0 M ammonium phosphate, 0.1 M sodium acetate pH 4.5. Plate-like crystals were generated under the optimized condition of 0.94 M ammonium phosphate, 0.1 M citrate-phosphate buffer pH 5.5 (Table 2, Fig. 2). Several diffraction data sets were collected at the SPring-8 or Photon Factory synchrotron-radiation facilities. The highest quality X-ray diffraction data set was collected to 1.93 Å resolution on the BL-17A beamline of the Photon Factory (Fig. 3). A summary of the data-collection statistics is shown in Table 3. Based on a Matthews coefficient V_M of $2.99 \text{ Å}^3 \text{ Da}^{-1}$ (solvent content of 58.9%) we assume that two monomers exist in the asymmetric unit (Matthews, 1968). Initial molecular-replacement trials were performed with *Phaser* using the coordinates of *Bacillus circulans* WL-12 chitinase A1 catalytic domain (PDB entry 1itx; 42% sequence identity; Matsumoto *et al.*, 1999) as the search model. Since ChiW-CD consists of two GH-18 catalytic domains (amino acids 557–927 and 1045–141; Fig. 1*a*) and two molecules are proposed to exist in the asymmetric unit, four unambiguous solutions were generated (RFZ = 5.1, 4.2, 4.0 and 3.2; TFZ = 37.0; PAK = 40; LLG = 327). However, the entire polypeptide chain sequence could not be conclusively traced, except for the catalytic domains, because the electron densities of the remaining region (amino acids 928–1044; Fig. 1*a*) were too low to identify the amino-acid structures.

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Figure 3
X-ray diffraction image of ChiW-CD. The outer black circle corresponds to 1.8 Å resolution.

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References

- Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D. & Bairoch, A. (2005). *The Proteomics Protocols Handbook*, edited by J. M. Walker, pp. 571–607. Totowa: Humana Press.
- Henrissat, B. & Bairoch, A. (1996). *Biochem. J.* **316**, 695–696.
- Hiramatsu, S., Ishihara, M., Fujie, M., Usami, S. & Yamada, T. (1999). *Virology*, **260**, 308–315.
- Howard, M. B., Ekborg, N. A., Taylor, L. E. II, Weiner, R. M. & Hutcheson, S. W. (2004). *J. Bacteriol.* **186**, 1297–1303.
- Itoh, T., Hibi, T., Fujii, Y., Sugimoto, I., Fujiwara, A., Suzuki, F., Iwasaki, Y., Kim, J.-K., Taketo, A. & Kimoto, H. (2013). *Appl. Environ. Microbiol.* **79**, 7482–7490.
- Itoh, T., Sugimoto, I., Hibi, T., Suzuki, F., Taketo, A., Fujii, Y. & Kimoto, H. (2014). In the press.
- Matsumoto, T., Nonaka, T., Hashimoto, M., Watanabe, T. & Mitsui, Y. (1999). *Proc. Jpn. Acad. Ser. B*, **75**, 269–274.

¹ Supporting information has been deposited in the IUCr electronic archive (Reference: NO5046).

- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
- Mesnage, S., Fontaine, T., Mignot, T., Delepierre, M., Mock, M. & Fouet, A. (2000). *EMBO J.* **19**, 4473–4484.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Schneewind, O. & Missiakas, D. M. (2012). *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **367**, 1123–1139.
- Sreerama, N., Venyaminov, S. Y. & Woody, R. W. (1999). *Protein Sci.* **8**, 370–380.
- Tanaka, T., Fujiwara, S., Nishikori, S., Fukui, T., Takagi, M. & Imanaka, T. (1999). *Appl. Environ. Microbiol.* **65**, 5338–5344.
- Winn, M. D. *et al.* (2011). *Acta Cryst.* **D67**, 235–242.