Crystallization and preliminary X-ray diffraction analysis of Lin1840, a putative β -glucosidase from *Listeria innocua*

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Lin1840 is a putative β -glucosidase that is predicted to be involved in 1,2- β -glucan metabolism since the *lin1839* gene encoding a 1,2- β -oligoglucan phosphorylase and the *lin1840* gene are located in the same gene cluster. Here, Lin1840 was crystallized. The crystals of Lin1840 diffracted to beyond 1.8 Å resolution. The crystal belonged to space group *I*121, with unit-cell parameters $a = 89.75$, $b = 95.10$, $c = 215.00$ Å, $\alpha = 90.00$, $\beta = 96.34$, $\gamma = 90.00^\circ$.

1. Introduction

β -Glucosidases are universally distributed in almost all organisms to hydrolyze various kinds of aryl- β -glucosides, alkyl- β -glucosides and glycosyl- β -glucosides (Bhatia *et al.*, 2002). Many β -glucosidases from microorganisms play a role in the intracellular and extracellular degradation of β -glucosides to produce glucose, an easily metabolizable sugar (Sternberg *et al.*, 1977; Shewale, 1982). Some fungal β -glucosidases that show high activity on β -1,3-glucan are thought to be involved in fungal cell-wall rearrangement (Nakajima *et al.*, 2012a,b). In plants, β -glucosidases cleave β -glucosides and cellooligosaccharides not only for dissimilation but also for differentiation (Falk & Rask, 1995), growth (Leah *et al.*, 1995), lignification (Dharmawardhana *et al.*, 1995) and regulation of chemical defences against pathogen attack (Niemeyer, 1988; Poulton, 1990). Industrially, β -glucosidases are used for ethanol production from cellulose. Since cellulose is degraded mainly by cellobiohydrolase to produce cellobiose, cellobiose has to be cleaved by β -glucosidases to release glucose (Béguin, 1990).

β -Glucosidases are classified into glycoside hydrolase (GH) families 1, 3, 5, 9 and 30 according to amino-acid sequence (Henrissat & Davies, 1997). Although the catalytic mechanisms and the residues involved in the recognition of β -glucosides are highly conserved in each family, recognition of the aglycone moiety is widely diversified, resulting in the many EC numbers for enzymes cleaving β -glucosides (Ketudat Cairns & Esen, 2010).

Lin1840 from *Listeria innocua* is annotated as a putative GH3 β -glucosidase. Although Lin1840 has not yet been characterized, a gene cluster formed around the *lin1840* gene provides some indications about the function of Lin1840. Recently, we have revealed that the *lin1839* gene in the gene cluster encodes 1,2- β -oligoglucan phosphorylase (OGP_{Lin}), which phosphorylates 1,2- β -glucan with a degree of polymerization of greater than 2 (Nakajima *et al.*, 2014). Although phosphorylases catalyze a reversible reaction, they are thought to be involved in dissimilation of their substrates (Nakajima *et al.*, 2008, 2009a,b, 2010a,b). Thus, Lin1840 is expected to be involved in 1,2- β -glucan metabolism with OGP_{Lin}. Although the closest homologue of Lin1840 (42% amino-acid sequence identity) among known structures is a GH3 β -glucosidase obtained from a compost metagenome (JMB19063; PDB entry 3u4a), the catalytic properties of JMB19063 were only examined on cellooligosaccharides (McAndrew *et al.*, 2013). Barley β -glucosidase (PDB entry 1ex1) exhibits broad specificity on β -linked gluco-disaccharides (Hrmova *et al.*, 2002). β -Glucosidase from *Thermotoga neapolitana* (PDB entry 2x40) efficiently degrades alkyl- β -glucosides (Pozzo *et al.*, 2010). No enzyme specific to 1,2- β -glucooligosaccharides can be found among

Table 1

Lin1840r production information.

Source organism	<i>L. innocua</i> Clip 11262 (ATCC BAA-680)
Expression vector	pET-28a
Expression host	<i>E. coli</i> BL21(DE3)
Complete amino-acid sequence of the construct produced	MEQEKVQELVSQMTLDEKIAQCLQLSPFLFKGTNKAELTGP-LLQEMKLTDAHTENAGSVLGSSSALDMIGIQAAYLKTNRL-GIPLVFMADVIHGKTVFPPIPLALGCSFDRETRVMAEVS-ALEATADGHHVTFSPMLDLVRDPRWGRVMESTGEDPFLNS-ELGKAMVDGYQGDASKLNENLEQMAACVKHFAAYGAAEAG-LEYNTVMSTRELYQNYLPAYNAAIQAGAKLVMTAFNVVD-GIPATMNKWLNRDVLRGEMEFDGLISDWGAAEVINHG-ARNPKAAQFSMEAGVDLEMMTCYIHELKGLIEGKLSE-NLLDEAVLRMLNLKNDLGLFEDPYRGLKNDRDKDILTDE-SRGKARAAGVESAVLENKSRLLPLAKEAKIALVGPLATS-PDILGGWNVYGEKDGINVTGLREVFTVEVVSLEYTEL-SEEDKVAVKAQVQNMDDVVLALGEKNEWGGEAGSLATIRL-PEAQYQLAKFVQTLGKPVVITLFGNRPLEVKEALAESDAL-LELWFPGTAGRVTAADLLSGASNPSSGKLSMSFPQTGQIP-VYVNHRLTRGPQTPENKGERVYVSHYLDIPNEPFYFPGYK-SYSEFELKTSPLKELNLSHVEVTIKNISDIAGKEVI-QVYLQDVTASISRPVKELKAFKVALQAGEEKTVTFTLTS-EAFSFFYNHLEKQEPGLHRVFGTSSDVEDVFEVGVGGY-VLEHHHHH

the structurally determined GH3 enzymes. Thus, the structure of Lin1840 will also provide important clues to the relationship between its structure and function. In this report, we describe the crystallization and preliminary X-ray diffraction analysis of Lin1840.

2. Materials and methods

2.1. Cloning, expression and purification

L. innocua Clip 11262 (ATCC BAA-680) was purchased from the American Type Culture Collection (Manassas, Virginia, USA). The gene encoding Lin1840 (GenBank accession No. CAC97071) was amplified by PCR using Gflex DNA polymerase (Takara Bio, Shiga, Japan) and a colony of *L. innocua* cultured with BHC medium (Becton, Dickinson & Co., New Jersey, USA) as a template. Initially, amplification of the 5' and 3' regions of the gene was performed to delete the *Xho*I site in the gene using the silent mutations shown as lower-case letters in the primers. The primer pair 5'-GGAGGT-TCCCATGGAACAACAAGAAAAAG-3' (primer 1) and 5'-CAACGCCTGCTGCACGAGCTTTCCCGC-3' was used for amplification

Table 2

Crystallization conditions.

Method	Hanging drop
Plate type	24-well plate
Temperature (K)	298
Protein concentration (mg ml ⁻¹)	10
Buffer composition of protein solution	5 mM MOPS–NaOH pH 7.0
Composition of reservoir solution	85 mM Tris–HCl pH 9.0, 0.17 M LiSO ₄ , 25.5%(w/v) PEG 4000, 10%(v/v) glycerol
Volume and ratio of drop	2 µl, 1:1 protein solution:reservoir solution
Volume of reservoir (µl)	1000

of the 5' region of the gene and the primer pair 5'-GCGGGA-AAGCTCGTGCAGCAGGCGTTG-3' and 5'-TTTAACACTCGAG-CACATATCCAC-3' (primer 2) was used for the 3' region of the gene. Next, the two PCR products were combined into the whole *lin1840* gene by amplification using the products of the first PCR as templates and the primer pair primers 1 and 2. The amplified whole *lin1840* gene was treated with *Nco*I and *Xho*I (shown in bold in the primers) and was then inserted into the same site of pET-28a(+) (Novagen, Madison, Wisconsin, USA) to encode a His₆-tag fusion at the C-terminus. *Escherichia coli* BL21(DE3) cells were transformed using the constructed plasmid. The transformant was grown in 800 ml Luria–Bertani medium containing 30 µg ml⁻¹ kanamycin at 310 K until the OD₆₆₀ reached 0.6, followed by induction using 0.1 mM isopropyl β-D-1-thiogalactopyranoside at 293 K for 19 h. The cells were collected by centrifugation at 3900g for 5 min and then suspended in 50 mM MOPS–NaOH buffer pH 7.0 containing 150 mM NaCl (buffer A). The suspended cells were disrupted by sonication and centrifuged at 27 000g for 20 min. The obtained supernatant was loaded onto a HisTrap FF crude column (5 ml; GE Healthcare, Buckinghamshire, England) equilibrated with buffer A containing 10 mM imidazole using an ÄKTAprime plus (GE Healthcare). After unbound components had been washed out with buffer A containing 10 mM imidazole, the target protein was eluted using a linear gradient of 10–250 mM imidazole in buffer A. The sample concentrated to 1 ml was loaded onto Superdex 200 (HiLoad 16/60; GE Healthcare) equilibrated with buffer A at a flow rate of 1 ml min⁻¹. The major peak was collected for the following procedure. Macromolecule-production information is summarized in Table 1.

2.2. Crystallization

Recombinant Lin1840 (Lin1840r) was concentrated to 10 mg ml⁻¹ in 5 mM MOPS–NaOH pH 7.0. Initial crystallization trials were performed by the sitting-drop vapour-diffusion method at 298 K using PEG/Ion and Crystal Screen Cryo (Hampton Research, California, USA). Reservoir solution (1 µl) was added to 1 µl of 10 mg ml⁻¹ protein solution. Crystals were obtained after one week in condition No. 17 of Crystal Screen Cryo [15%(v/v) glycerol, 0.17 M LiSO₄, 0.085 M Tris–HCl pH 8.5, 25.5%(w/v) PEG 4000]. The

Figure 1

SDS–PAGE analysis of Lin1840r. Lane M, protein marker (labelled in kDa); lanes 1 and 2, Lin1840r purified by Ni–NTA affinity and size-exclusion chromatography, respectively.

Figure 2

A crystal of Lin1840r from an optimized condition.

optimized crystallization condition was 0.17 M LiSO₄, 0.085 M Tris–HCl pH 8.5–9.0, 25–30% (w/v) PEG 4000, 10–15% (v/v) glycerol with 10 mg ml^{−1} Lin1840r. Crystallization information is summarized in Table 2.

2.3. Data collection and processing

The crystals were cooled and then kept at 100 K in a nitrogen-gas stream during data collection. A set of X-ray diffraction data for Lin1840r was collected using a CCD detector (ADSC Quantum 210r) on beamline AR-NW12A at Photon Factory, Tsukuba, Japan. The diffraction data set was processed using *iMosflm* (Battye *et al.*, 2011).

3. Results and discussion

Lin1840r with a His₆ tag at the C-terminus was expressed and purified (Fig. 1). The estimated molecular weight of Lin1840r on SDS–PAGE is approximately 80 kDa, which is consistent with a theoretical molecular mass of 80 531 Da. The purified protein was crystallized in 85 mM Tris–HCl buffer pH 9.0 containing 0.17 M LiSO₄, 25.5% (w/v) PEG 4000, 10% (v/v) glycerol, which resulted in the formation of rhombic-shaped crystals with approximate dimensions of 0.5 × 0.2 × 0.03 mm (Fig. 2). A single crystal was used for X-ray diffraction experiments and a set of diffraction data was collected to 1.6 Å resolution (Fig. 3). Processing of the diffraction data to 1.8 Å resolution with *iMosflm* was performed based on the criterion that $\langle I/\sigma(I) \rangle$ for the highest shell was greater than 2. A summary of the data-collection statistics is given in Table 3. The crystal belonged to space group *I*121, with unit-cell parameters $a = 89.75$, $b = 95.10$, $c = 215.00$ Å, $\alpha = 90.00$, $\beta = 96.34$, $\gamma = 90.00^\circ$. The overall R_{merge} was 11.9% and the R_{merge} in the outermost shell was 60.5%. Assuming that the Matthews coefficient and solvent content are within the normal range for protein crystals (Matthews, 1968), there could be two or three Lin1840r molecules in the asymmetric unit. With two

Table 3

Data collection and processing.

Values in parentheses are for the outermost shell.

Beamline	AR-NW12A
Wavelength (Å)	1.0000
Temperature (K)	100
Rotation range per image (°)	1
Total rotation range (°)	270
Exposure time per image (s)	2
Space group	<i>I</i> 121
Unit-cell parameters (Å, °)	$a = 89.75$, $b = 95.10$, $c = 215.00$, $\beta = 96.34$
Resolution range (Å)	28.39–1.80 (1.83–1.80)
Total No. of reflections	802599 (33475)
No. of unique reflections	163230 (7868)
Completeness (%)	98.5 (95.9)
Multiplicity	4.9 (4.3)
$\langle I/\sigma(I) \rangle$	8.2 (2.2)
R_{merge} (%)	11.9 (60.5)

molecules in the asymmetric unit, the calculated Matthews coefficient and solvent content are 2.87 Å³ Da^{−1} and 57%, respectively. With three molecules in the asymmetric unit, the calculated Matthews coefficient and solvent content are 1.92 Å³ Da^{−1} and 36%, respectively. To find noncrystallographic symmetry (NCS) within the asymmetric unit, we calculated a self-rotation function. Two peaks corresponding to a twofold NCS axis were observed in the $\kappa = 180^\circ$ section, while no substantial peaks corresponding to a threefold NCS axis were observed in the $\kappa = 120^\circ$ section. These results suggest that there are two molecules in the asymmetric unit. Molecular replacement using JMB19063 as a model failed to determine the Lin1840r structure in spite of its 42% amino-acid sequence identity to Lin1840. Use of three structurally determined enzymes that were similar to Lin1840 according to a *BLAST* search as models was also unsuccessful, probably because of low amino-acid sequence identity [29% for PDB entry 2x40, 29% for PDB entry 1ex1 and 27% for PDB entry 3rrx (exo-1,3/1,4-β-glucanase from *Pseudoalteromonas* sp. BB1; Nakatani *et al.*, 2012)]. Multiwavelength anomalous diffraction using a selenomethionyl derivative might be necessary for phase solution.

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Figure 3

X-ray diffraction image from a Lin1840r crystal. The resolution at the edge of the detector is 1.6 Å.

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