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Crystallization and preliminary crystallographic analysis of the cell-surface alginate-binding protein Algp7 isolated from *Sphingomonas* sp. A1

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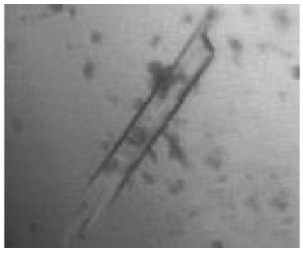
Sphingomonas sp. A1, a Gram-negative bacterium, directly internalizes alginate macromolecules through a mouth-like pit that is present on its cell surface. The alginate-binding protein Algp7, which was found to be localized on the cell surface, contributes to the accumulation of alginate in the pit. Algp7 was crystallized at 293 K by means of the sitting-drop vapour-diffusion method with polyethylene glycol 3350 as a crystallizing agent. Preliminary X-ray analysis showed that the Algp7 crystal belonged to space group $P2_12_12_1$, with unit-cell parameters a = 50.1, b = 98.0, c = 100.1 Å, and that it diffracted to 2.8 Å resolution.

1. Introduction

Sphingomonas sp. A1 directly internalizes alginate polysaccharides into the cytoplasm, crossing the outer and inner membranes simultaneously through a so-called super-channel (Murata *et al.*, 2008). This bacterium is therefore peculiar in that almost all other bacteria secrete polysaccharide-degrading enzymes extracellularly and then translocate the resulting oligosaccharides and/or monosaccharides into the cytoplasm. In the presence of alginate, *Sphingomonas* sp. A1 forms a mouth-like pit with a diameter of 0.02–0.1 µm on the cell surface *via* the rearrangement of pleat structures and accumulates the polymer in this pit (Hisano *et al.*, 1996). The transport of alginate from the pit to the inner membrane is mediated by periplasmic alginate-binding proteins, namely AlgQ1 (59 kDa) and AlgQ2 (59 kDa) (Momma *et al.*, 2005). The ABC transporter [AlgM1 (37 kDa)–AlgM2 (33 kDa)–AlgS (40 kDa)–AlgS] localized in the inner membrane then directly translocates alginate into the cytoplasm (Momma *et al.*, 2000). The alginate thus internalized is depolymerized to form the constituent monosaccharides through the action of four cytoplasmic alginate lyases: A1-I (65 kDa), A1-II (25 kDa), A1-III (40 kDa) and A1-IV (86 kDa) (Yoon *et al.*, 2000; Hashimoto *et al.*, 2000).

Using proteome analysis, Hashimoto *et al.* (2005) and He *et al.* (2008) found that alginate induced the expression of a cell-surface protein, Algp7 (accession No. AB211543), which then bound to the alginate polymer. This suggests that Algp7 plays a role in the accumulation of external alginate in the cell-surface pit. Algp7 and Algp7-homologous proteins (with approximately 60% identity) consist of approximately 300 amino-acid residues and contain a metallopeptidase motif (Pfam, peptidase_M75); however, their exact structure is unknown.

In this study, we crystallized Algp7 isolated from *Sphingomonas* sp. A1 and determined the crystal properties of this protein by X-ray crystallography.



2. Methods and results

2.1. Analytical methods

The protein content was determined either by the method of Bradford (1976) with bovine serum albumin as the standard or by measuring the absorbance at 280 nm using a cuvette with a path

length of 1 cm, assuming that an E_{280} of 0.569 (Algp7) corresponds to 1 mg ml^{-1}. The alginate-binding activity of Algp7, *i.e.* the dissociation constant (K_d), was determined by surface plasmon resonance biosensor analysis as described previously (He *et al.*, 2004, 2008); it was found to be 0.036 μ*M*.

2.2. Protein overproduction and purification

The overproduction and purification of Algp7 were carried out as described previously (He *et al.*, 2008) with a slight modification. Algp7 with a hexahistidine tag was purified from a cell extract of *Escherichia coli* BL21 (DE3) containing the plasmid pET44a-Algp7 by affinity chromatography (1.0 × 10 cm Talon column; Clontech) and ion-exchange chromatography (1.0 × 10 cm SuperQ-Toyopearl 650 M column; Tosoh). The eluted protein Algp7 was dialyzed against 20 m*M* Tris–HCl pH 7.5 overnight. The dialysate was used as purified Algp7.

The homogeneity of the purified protein was confirmed by SDS–PAGE (Laemmli, 1970); it was then concentrated to 4.6 mg ml^{-1} by ultrafiltration with a Centriprep (Millipore) for crystallization.

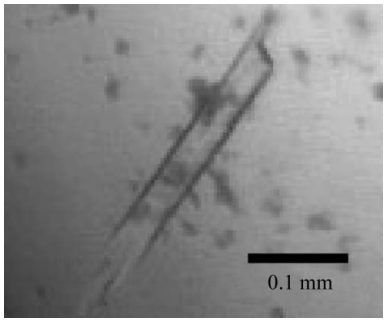


Figure 1
Algp7 crystal.

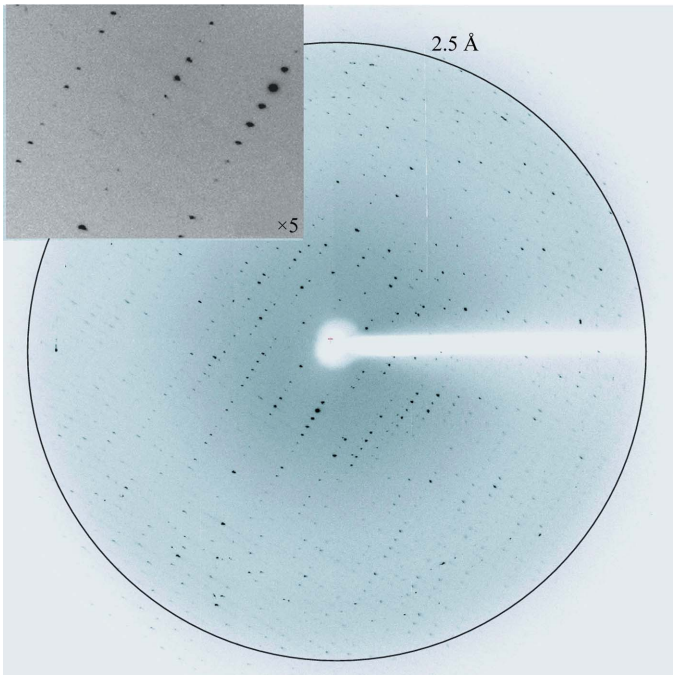


Figure 2
Diffraction image of the Algp7 crystal. A fivefold magnified image is shown at the top left.

Table 1
Data-collection statistics for the Algp7 crystal.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.00
Resolution (Å)	50.00–2.80 (2.90–2.80)
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 50.1, b = 98.0, c = 100.1
Total observations	42280
Independent reflections	12206 (1169)
Completeness (%)	96.5 (96.1)
$I/\sigma(I)$	9.4 (3.3)
Redundancy	3.5 (3.2)
R_{merge} (%)	8.9 (23.3)

2.3. Crystallization

Algp7 purified from *E. coli* cells was crystallized at 293 K using the sitting-drop vapour-diffusion method. The crystallization conditions were initially screened by sparse-matrix screening, which was conducted in a 96-well Intelli-Plate (Art Robbins Instruments) using commercial crystallization kits from Hampton Research. We attempted to crystallize the protein at a concentration of 4.6 mg ml^{-1} under a large number of conditions. The mother liquor (100 µl) was used as the reservoir solution and 1 µl Algp7 solution was mixed with 1 µl reservoir solution to form the drop. Rod-shaped Algp7 crystals were found in a droplet comprising 20% polyethylene glycol 3350 and 0.2 *M* ammonium acetate. In six months, the crystals in these droplets grew to a size larger than 0.2 mm at a temperature of 293 K (Fig. 1).

2.4. X-ray analysis

For cryoprotection, the Algp7 crystal was soaked in mother liquor (20% polyethylene glycol 3350 and 0.2 *M* ammonium acetate) containing 20% glycerol. The crystal was picked up from the soaking solution with a mounted nylon loop (Hampton Research) and placed directly in a cold nitrogen-gas stream at 100 K. X-ray diffraction images of the crystal were obtained at 100 K under the nitrogen-gas stream using a Jupiter 210 detector and synchrotron radiation of wavelength 1.0 Å at the BL-38B1 station of SPring-8, Japan (Fig. 2). The distance between the crystal and detector was set to 200 mm and 1° oscillation images were recorded with an exposure time of 10 s. The diffraction data for the crystal were collected to a resolution of 2.8 Å and were processed using *HKL*-2000 (*DENZO* and *SCALEPACK*; Otwinowski & Minor, 1997). The preliminary X-ray crystallographic properties of Algp7 are summarized in Table 1. The V_M value (Matthews, 1968), *i.e.* the crystal volume per unit of protein molecular mass, was calculated to be 2.20 Å^3 Da^{-1}, under the assumption that two molecules of the protein formed an asymmetric unit, and the solvent content was calculated to be 44.2%. The V_M value and solvent content lie within the ranges typically found for protein crystals.

A search for selenomethionine derivatives for phasing by the multiple-wavelength anomalous dispersion (MAD) method is currently in progress.

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