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Crystallization and preliminary X-ray diffraction analysis of lectin-1 from *Pseudomonas aeruginosa*

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Carbohydrate recognition plays a role in the pathogenesis of *Pseudomonas aeruginosa*, a common cause of opportunistic infection in humans. Crystals of a carbohydrate-binding protein from *P. aeruginosa*, lectin PA-1, have now been obtained. The crystals belong to space group *I*222, with unit-cell parameters a = 40.25, b = 72.30, c = 133.82 Å, and diffract to beyond 1.9 Å resolution on a rotating-anode X-ray source. Details of crystal-growth conditions, diffraction data collection and processing are reported.

1. Introduction

The Gram-negative bacterium *Pseudomonas aeruginosa* is a significant cause of opportunistic infection in humans. The ubiquitous occurrence of the organism and its pathological effect on diverse tissues have recently been reviewed (Lyczak *et al.*, 2000). Colonization of the airways in 40–80% of adult individuals suffering from cystic fibrosis (Robinson, 2001) is a prominent example of a disease process specifically attributed to *P. aeruginosa.*

Recognition of specific carbohydrates plays an important role in *P. aeruginosa* infection (Avichezer *et al.*, 1992, and references therein). The pathogen produces two cytoplasmic lectins called PA-1 and PA-2. Both lectins have been cloned and sequenced (Avichezer *et al.*, 1992). A purified lectin preparation from *P. aeruginosa* was used in the production of a vaccine that protects mice from infection with the bacterium (Sudakevitz & Gilboa-Garber, 1987). PA-1 has been classified as a galactophilic lectin (Gilboa-Garber, 1982) and is composed of 121 amino acids with a broad hydrophilic stretch of amino acids sandwiched between N- and C-terminal hydrophobic domains. As would be expected, secondary-structure prediction indicates a protein rich in β-sheet (Avichezer *et al.*, 1992). Very little is known about the tertiary structure of the lectin, the mode of aggregation (if any) or the location of the galactose- and adenine-binding sites (Stoitsova *et al.*, 2003). In order to answer these questions, a crystallographic analysis of PA-1 was initiated. Here, we report the preliminary details of the X-ray diffraction analysis.

2. Materials and methods

Lectin PA-1 was purchased as a lyophilized powder from Sigma-Aldrich Corp. (catalogue No. L9895). The protein was reconstituted to a concentration of 20 mg ml^{-1} in 10 m*M* K_2HPO_4, 10 m*M* KH_2PO_4 pH 7.4, 150 m*M* NaCl, 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$ (Gilboa-Garber, 1982) and 0.25 *M* NDSB-201 (pyridinium propane sulfonate). The solution was passed through a 0.45 µm filter membrane. Crystallization experiments were conducted using the hanging-drop vapor-diffusion method: 2 µl of protein solution was mixed with 2 µl of precipitant solution on a microscope cover slip. The slip was inverted and incubated at 291 K over a well containing 0.5 ml of the same precipitant solution. Crystallization was attempted using ammonium sulfate solutions with concentrations ranging from 1.0 to 2.0 *M* as precipitant solution. Diffraction-quality crystals were obtained using 1.7–1.8 *M* ammonium sulfate as precipitant.

After 2 d, a crystal measuring approximately 0.2 × 0.2 × 0.4 mm (Fig. 1) was harvested and briefly soaked in a cryoprotectant solution that contained a 1:4(*v*:*v*) mixture of glycerol and well solution. After soaking, the crystal was mounted on the goniometer using a nylon fiber

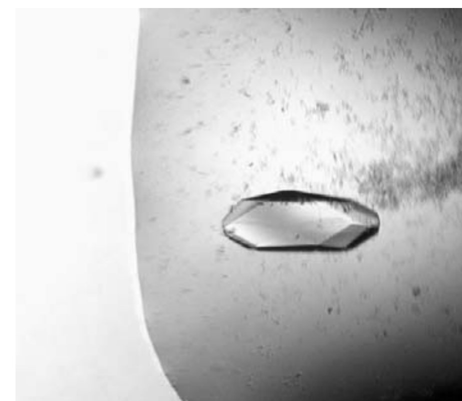

Figure 1
Crystal of lectin PA-1.

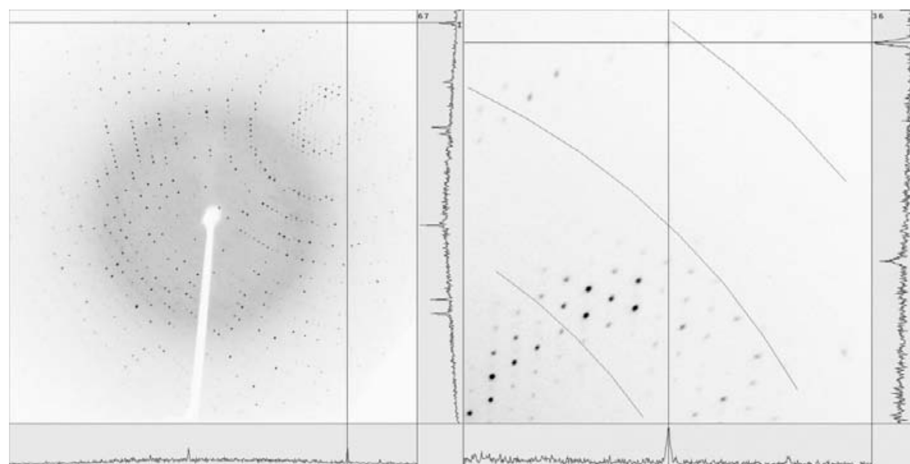

Figure 2
Typical 0.5° oscillation diffraction image of PA-1: crosshairs highlight a reflection at 1.85 Å with $I/\sigma(I)$ = 6.5.

loop (Teng, 1990) and flash-cooled (Hope, 1988) to 100 K in a nitrogen-gas cold stream. Diffraction data were recorded on a Rigaku R-AXIS IV image-plate detector with a Rigaku FR-D generator running at 4.75 kW equipped with Rigaku/MSC Max-Flux confocal optics. The crystal-to-detector distance was set to 150.0 mm. The data set consisted of 360 0.5° oscillation images recorded from a single crystal using an exposure time of 5 min per image. Data were indexed, integrated and scaled using the *HKL* suite (Otwinowski & Minor, 1997). Self-rotation and anomalous Patterson map analysis were performed using the *SHELXTL* suite of programs (v. 6.12, Bruker AXS).

3. Results and discussion

Crystallization in the presence of ammonium sulfate was attempted without preceding sparse-matrix trials. Initial crystals were observed after 1 d. While the presence of NDSB-201 in the protein solution was not strictly required for the crystallization of PA-1, it is noteworthy that the inclusion of NDSB-201 prevented precipitation during storage of the protein stock solution at 277 K.

Diffraction of the PA-1 crystal extended beyond 1.9 Å (Table 1, Fig. 2). Indexing of the diffraction pattern indicated that the crystal has a body-centered orthorhombic lattice, with unit-cell parameters a = 40.25, b = 72.30, c = 133.82 Å. Assuming a single molecule per asymmetric unit, in either space group $I222$ or $I2_12_12_1$, the calculated Matthews coefficient V_M (Matthews, 1968) is 3.78 $Å^3\,Da^{-1}$, which corresponds to a solvent content of 67%. Assuming two molecules per asymmetric unit, V_M is calculated to be 1.89 $Å^3\,Da^{-1}$, with a corresponding solvent content of 35%. A self-rotation search did not produce significant peaks on the κ = 180° section. However, this finding alone does not rule out or support the presence of a homodimer in the asymmetric unit.

Attempts to solve the structure using the sulfur anomalous scattering signal recorded in-house from native crystals have been initiated. Four putative sulfur sites have been identified with high probability using the program *XM* (Bruker *SHELXTL* package; Table 2) using a 3 Å resolution cutoff in space group $I222$. The same analysis in space group $I2_12_12_1$ failed to give a convincing solution. In addition, analysis of the 'sulfur' substructure did not show a consistent set of twofold-related sites as would be expected for a homodimer in the asymmetric unit. Thus, based on the *XM* analysis, the most probable space group is $I222$ with one molecule per asymmetric unit. The structure determination of PA-1 is in progress.

Table 1
Data-processing statistics.

Values in parentheses are for the highest resolution shell.

Resolution range (Å)	30.00–1.90 (1.97–1.90)
Unique observed reflections	15710 (1410)
Completeness (%)	99.0 (91.0)
$\langle I\rangle/\langle\sigma(I)\rangle$	43.9 (14.7)
Redundancy of unique reflections (%)	
More than 3 observations	92.9 (60.5)
More than 6 observations	65.4 (8.9)
R_{sym}† (%)	3.5 (7.0)

† $R_{sym} = \sum|I - \langle I\rangle|/\sum I$.

Table 2
XM heavy-atom statistics.

Space group	$I222$	$I2_12_12_1$
Correlation coefficient (all)	31.59	16.15
Correlation coefficient (weak)	21.13	7.52
Patterson FOM	30.75	18.69
No. of sites	4	4
No. of inter-site pairs	0	0

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