SHORT COMMUNICATIONS

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Expression, purification and preliminary X-ray crystallographic analysis of PsaA, a putative metal-transporter protein of *Streptococcus pneumoniae*

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Abstract

The putative metal-transporter protein PsaA of *Streptococcus pneumoniae* is of potential interest both as a vaccine and also as a drug target. The overexpression of the protein in *E. coli*, and its subsequent purification and crystallization are described. The crystals are rectangular rods and diffract to beyond 2.7 Å resolution. The crystal space group is $P2_12_12_1$ with unit-cell dimensions a = 59.9, b = 66.5 and c = 69.9 Å.

1. Introduction

PsaA is a surface protein produced by all clinical isolates of *Streptococcus pneumoniae* (the pneumococcus; Russell *et al.*, 1990). This bacterium is an important human pathogen, and the recent observation that PsaA is a protective immunogen in a mouse model of invasive pneumococcal disease has identified it as a candidate vaccine antigen for use in humans (Talkington *et al.*, 1996). We have shown that a defined mutant of *S. pneumoniae* in which the *psaA* gene has been interrupted by insertion-duplication mutagenesis is massively less virulent than its otherwise isogenic parental strain (Berry & Paton, 1996). PsaA was initially thought to be a pneumococcal adhesin, on account of its high degree of deduced amino-acid sequence homology with putative fimbrial adhesins of several oral streptococci, and the fact that *psaA*$^-$ mutants display markedly reduced *in vitro* adherence to a human type II pneumocyte cell line (Berry & Paton, 1996). However, a recent study by Dintilhac *et al.* (1997) suggests that *psaA* is a member of a newly identified metal-binding sub-family (cluster 9) of extra-cytoplasmic bacterial receptors, with PsaA being a manganese (or possibly zinc) transporter. Whilst the extracellular solute-binding receptors of Gram-positive bacteria (Sutcliffe & Russell, 1995) are related to the periplasmic solute-binding receptors of Gram-negative bacteria (Tam & Saier, 1993), no detailed three-dimensional structure is available for any member of the Gram-positive superfamily. Of the periplasmic solute-binding receptors that have had their three-dimensional structures elucidated (Quiocho & Ledvina, 1996), none to date has been either a manganese or a zinc transporter.

The primary translation product of the *psaA* gene is predicted to be 309 amino acids long (Berry & Paton, 1996). It contains a 19 amino-acid signal peptide which includes the prolipoprotein-recognition sequence LXXC, and cleavage would be expected to result in a 290 amino-acid mature polypeptide linked to lipid *via* the resultant N-terminal Cys. As part of an ongoing study of the function of PsaA in the pathogenesis of pneumococcal disease and of its suitability as a vaccine or target for drugs, we have commenced determination of its three-dimensional structure. We describe here the expression, purification, crystallization and preliminary X-ray diffraction analysis of PsaA.

2. Materials and methods

2.1. *Expression and purification of PsaA*

The previously cloned *psaA* gene from *Streptococcus pneumoniae* D39 (NCTC7466; Berry & Paton, 1996) was used as a template for PCR with primers 5′-CATTCTTGTAGCATGCGCTAGCGGAAA-3′ and 5′-CGTTTTGGTACCGCTTATTTTGCCAATCC-3′. These primers incorporate *Sph*I and *Kpn*I restriction sites respectively (underlined), permitting cloning into the *Sph*I and *Kpn*I sites of the expression vector pQE-30 (QIAGEN, USA). This construct was transformed into *Escherichia coli* K-12 SG13009(pREP4) (QIAGEN, USA) and directed production of a fusion protein consisting of the mature 290 amino-acid PsaA polypeptide preceded by a vector-encoded peptide $MRGSH_6GSA$. *E. coli* cells were grown at 310 K to mid-logarithmic phase in LB broth (Maniatis *et al.*, 1982) supplemented with 100 µg ml^{-1} ampicillin and 25 µg ml^{-1} kanamycin, and then induced for 2 h with 0.2 m*M* IPTG. Cells were then harvested by centrifugation (15000*g*, 10 min, 277 K), resuspended in 10 m*M* sodium phosphate, pH 7.0, and lysed by treatment in an Aminco French pressure cell at 8.4×10^4 kPa. The lysate was then centrifuged (100000*g*, 60 min, 277 K) and the supernatant loaded onto a column of DEAE Sepharose CL-6B (Pharmacia Biotech, Sweden), which was then eluted with a linear gradient of 10–300 m*M* sodium phosphate, pH 7.0. PsaA-containing fractions were detected by immunoblot using mouse anti-PsaA serum. Peak fractions were pooled, equilibrated in wash buffer (50 m*M* sodium phosphate, 300 m*M* NaCl and 10% glycerol, pH 6.0), and loaded onto a Ni–NTA column (QIAGEN, USA), which was then washed with the same buffer. The PsaA fusion protein was eluted from the column with wash buffer supplemented with 0.1 *M* imidazole. The final material was greater than 95% pure, as judged by SDS–PAGE, with the yield of protein being approximately 12 mg per litre of culture. An aliquot of the final product was subjected to N-terminal sequencing using a G1000A protein sequencer (Hewlett Packard, USA); the sequence of the first 20 residues corresponded to that expected for the PsaA fusion protein.

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2.2. *Crystallization and preliminary X-ray analysis*

Initial investigations with crystal screens I and II (Hampton Research, USA) failed to identify any suitable crystallization conditions. For crystallization trials the protein was dialysed against 20 m*M* HEPES (pH 7.5), 0.02% azide and concentrated to either 1.9 or 15.6 mg ml^{-1} using Amicon Centricon and Micron tubes (molecular-weight cutoff 10 kDa). All trials were performed using the hanging-drop vapour-diffusion method (McPherson, 1982) at 291 K, with drops being mixed as 1 µl protein with 1 µl precipitant from a 1 ml precipitant well solution. Subsequent screening with a set of media containing high salt concentrations and protein at 12.7 mg ml^{-1} yielded clusters of thin rod-like crystals after 3 weeks in the presence of 3.6 *M* K_2HPO_4/NaH_2PO_4 (pH 7.0), 0.1 *M* MES (pH 6.5) and 0.02% azide. All subsequent crystals were grown by either multiple macroseeding or microseeding from these crystals as follows. Seed crystals were washed in 4.0 *M* K_2HPO_4/NaH_2PO_4 (pH 7.5) and introduced into drops consisting of 1 µl protein (concentrated to approximately 18 mg ml^{-1}) mixed with 1 µl of 3.0 *M* K_2HPO_4/NaH_2PO_4 (pH 7.5), 0.1 *M* MES (pH 6.5) and 0.1 *M* GuHCl. Prior to seed introduction the drops were pre-equilibrated for 12–24 h against 3.0 *M* K_2HPO_4/NaH_2PO_4 (pH 7.5), 0.1 *M* MES (pH 6.5) and 0.1 *M* GuHCl at 291 K. Seeded crystals (Fig. 1) typically grew to a maximum size of 0.5 × 0.04 × 0.04 mm over 4–7 days; however, the tendency to form clusters could not be overcome (Fig. 1).

2.3. *X-ray analysis and data collection*

Crystals were transferred briefly to a solution containing 3.0 *M* K_2HPO_4/NaH_2PO_4 (pH 7.5), 0.1 *M* MES (pH 6.5) and 15% glycerol and then flash-frozen in a rayon loop (Teng, 1990) mounted on a goniometer in a stream of cryo-cooled nitrogen gas at 108 K using an MSC low-temperature system (Molecular Sciences Corporation, USA). PsaA crystals diffracted to at least 2.7 Å resolution (Fig. 2), but were found to vary in quality and were often twinned in directions orthogonal to the *a** axis. Extensive attempts were made to overcome the twinning problem, including variation of the seeding technique, variation of the nature and pH of the buffer, and the introduction of additional reagents (salts, metal ions, solvents, chaotropes *etc.*) to the crystallization media. The twinning problem appeared to be more severe when the crystals grew in clusters (Fig. 1), but none of the above attempts succeeded in eliminating cluster formation. Diffraction quality was also found to vary along the length of the crystals, with the rod ends typically being better ordered than the rod centres. The introduction of 0.1 *M* GuHCl offered some improvement in crystal quality and was included in all subsequent seeding media.

A preliminary native data set was built up from the better crystals using an R-AXIS IV image-plate detector (Rigaku, Japan) equipped with a helium window and mounted on a MacScience M18XHF-SRA generator (MacScience, Japan) operating at 40 kV and 50 mA with a copper anode. Given the

Table 1. *Statistics of native data collection*

Number of frames	186
Number of measured reflections	29433
Number of rejected measurements	699†
Number of unique reflections	7717
Resolution range (Å)	20–2.7
Multiplicity	3.9 (3.3)‡
Completeness (%)	95.7 (88.6)
R_{sym} (%)§	12.0 (27.2)
$\langle I/\sigma(I)\rangle$	11.2 (4.6)

† Assuming that at a 50% rejection probability threshold, 1% of the observations would be rejected. ‡ Figures in parentheses refer to statistics in the outer resolution shell 2.8–2.7 Å. § $R_{sym} = \sum_h \sum_i |I_{ih} - \langle I_h\rangle| / \sum_h \sum_i I_{ih}$.

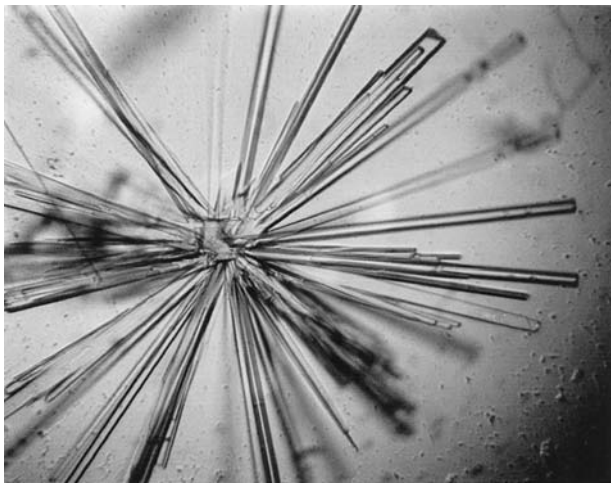

Fig. 1. A single cluster of PsaA crystals grown according to the seeding protocol described in the text. The typical size of the larger crystals is 0.5 × 0.03 × 0.03 mm; the variation in quality is readily apparent.

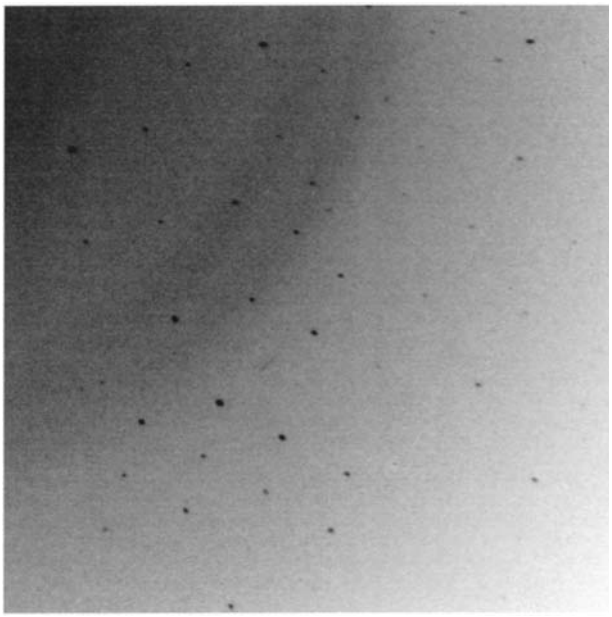

Fig. 2. A section of a 1.0° oscillation pattern obtained from a crystal of PsaA. The lower right-hand corner of the image corresponds to 2.5 Å resolution; diffraction spots are visible to ~2.7 Å. The exposure time was 2 h; the image was recorded at a distance of 120 mm on an R-AXIS IV detector, as described in the text.

small size of the crystals and the concomitantly low signal-to-noise ratio, a initial partial data set was combined with that obtained from a latter crystal to give the final native data set with improved $\langle I/\sigma(I)\rangle$. Care was taken to exclude frames that exhibited ice contamination or high background due to the plane of the cryo-loop being too close to the direction of the X-ray beam. All frames were collected as 1° oscillations at a crystal-to-detector distance of 120 mm with an exposure time of 2 h frame^{-1} for the first crystal (28 frames) and 1 h frame^{-1} for the second crystal (158 frames). Data processing was carried out using the *HKL* 1.6 software suite (Otwinowski & Minor, 1996).

3. Results and discussion

The crystals exhibited an orthorhombic lattice with dimensions a = 59.9, b = 66.5 and c = 69.9 Å, and inspection of the axial data revealed the space group to be $P2_12_12_1$. Native data-processing statistics are summarized in Table 1. The Matthews number (Matthews, 1968) is calculated to be 2.05 Å^3 Da^{-1} (assuming a molecular weight of 34000 Da for the fusion protein and one molecule per asymmetric unit). The corresponding solvent content is 40%. We are currently undertaking heavy-atom screening in an attempt to determine the structure of PsaA *via* multiple isomorphous replacement.

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References

Berry, A. M. & Paton, J. C. (1996). *Infect. Immun.* **64**, 5255–5262.
Dintilhac, A., Alloing, G., Granadel, C. & Claverys, J.-P. (1997). *Mol. Microbiol.* **25**, 727–739.
McPherson, A. (1982). *Preparation and Analysis of Protein Crystals.* New York: John Wiley.
Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982). *Molecular Cloning: a Laboratory Manual.* New York: Cold Spring Harbor Laboratory, Cold Spring Harbor.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1996). *Methods Enzymol.* **276**, 307–326.
Quiocho, F. A. & Ledvina, P. S. (1996). *Mol. Microbiol.* **20**, 17–25.
Russell, H., Tharpe, J. A., Wells, D. E., White, E. H. & Johnson, J. E. (1990). *J. Clin. Microbiol.* **28**, 2191–2195.
Sutcliffe, I. C. & Russell, R. R. B. (1995). *J. Bacteriol.* **177**, 1123–1128.
Talkington, D. F., Brown, B. G., Tharpe, J. A., Koenig, A. & Russell, H. (1996). *Microb. Pathogen.* **2**, 17–22.
Tam, R. & Saier, M. H. Jr (1993). *Microbiol. Rev.* **57**, 320–346.
Teng, T. Y. (1990). *J. Appl. Cryst.* **23**, 387–391.