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Crystallization and X-ray diffraction analyses of the outer membrane pyochelin receptor FptA from *Pseudomonas aeruginosa*

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FptA, the pyochelin outer membrane receptor from *Pseudomonas aeruginosa*, is a siderophore receptor involved in iron uptake when the bacterium grows under iron limitation. Two crystal forms of the FptA–pyochelin complex were obtained under different crystallization conditions. They belong to space groups $P1$ and $P2_12_12_1$ and data sets were collected for both crystal forms. The triclinic crystals diffract to 3.2 Å resolution and the orthorhombic crystals show a 1.9 Å resolution limit. A data set at the peak of the iron K edge was also collected at 3.1 Å resolution.

1. Introduction

Pseudomonas aeruginosa, a Gram-negative bacterium, is an opportunistic pathogen that infects a large range of host organisms (He *et al.*, 2004). *P. aeruginosa* can infect injured, immunodeficient and otherwise compromised patients and iron is a crucial element for its virulence. Under aerobic conditions iron availability is very low since it forms ferric oxyhydroxide which is insoluble (Neilands, 1995). In cells, it is stored as an inorganic complex by bacterrioferritin, ferritin, transferrin or lactoferrin and its concentration in fluids is very low ($\sim 10^{-18}$ M; Posey & Gherardini, 2000), whereas micromolar concentrations are required for bacterial growth (Poole & McKay, 2003). When grown under iron limitation, many bacteria synthesize and release into the external medium small molecules called siderophores (MW < 2000 Da) that solubilize iron and deliver it to the iron-deficient cells. The small molecules of less than 600 Da can diffuse across the outer membrane *via* a non-energy-consuming mechanism through non-specific porins or substrate-specific porins. The iron–siderophore complexes and vitamin B_{12} are transported across the outer membrane by TonB-dependent receptors. This mechanism uses the energy derived from the proton motive force of the cytoplasmic membrane *via* specific interactions between the N-terminal part of the receptor and the C-terminal part of the TonB protein of the energy-transducing TonB–ExbB–ExbD complex located in the inner membrane (Moeck & Coulton, 1998). To date, four crystal structures of siderophore receptors from *Escherichia coli* have been solved (Ferguson *et al.*, 1998, 2002; Buchanan *et al.*, 1999; Chimento *et al.*, 2003). Recently, we solved the first three-dimensional structure of a siderophore receptor from another bacterium, *P. aeruginosa*: FpvA bound to pyoverdine (Cobessi *et al.*, 2004). The five structures show a similar overall fold composed of a transmembrane β-barrel of 22 antiparallel β-strands filled by a small domain called the cork domain containing a mixed four-stranded β-sheet.

P. aeruginosa synthesizes and releases into the external medium a major fluorescent siderophore called pyoverdine, which is transported across the outer membrane by FpvA (MW 86 500 Da; Poole *et al.*, 1993), and also pyochelin (Pch), which is transported by another siderophore receptor, FptA (MW 76 000 Da; Ankenbauer & Quan, 1994). FpvA and FptA belong to two different classes of siderophore receptors, the former containing an N-terminal extension involved in signal transduction. In contrast to pyoverdine, which consists of a family of strain-specific molecules with little cross-species recognition, Pch is unique and recognized by its cognate receptor whatever the *P. aeruginosa* strain (Poole & McKay, 2003). Therefore, its outer membrane receptor could be an interesting target for antibiotic drugs. Owing to the importance of *P. aeruginosa* in human infections and its growing resistance to antibiotics, we undertook the crystallographic studies of the pyochelin receptor FptA. We describe here the crystallization and preliminary X-ray data analyses of the FptA–Pch complex.

2. Materials and methods

2.1. Purification

FptA was purified from the *P. aeruginosa* CDC5 (pVR2) used for the overexpression of FpvA grown in a minimal medium (Demange *et al.*, 1990) in the presence of carbenicillin. FptA was purified using the same protocol as for FpvA (Schalk *et al.*, 1999).

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2.2. Crystallization

Crystallization experiments were carried out at 295 K in Linbro plates using the sitting-drop vapour-diffusion method. Several initial crystallization conditions were found using the Screen Lite sparse-matrix screen from Hampton Research and the Classics Lite from Nextal. 1 µl of protein solution was mixed with an equal volume of reservoir solution and equilibrated by vapour diffusion with the same precipitant.

From these preliminary results, crystallization solutions incorporating a cryoprotecting agent were set up.

2.3. Data collection and processing

X-ray diffraction experiments were performed at 100 K from crystals mounted in cryoloops and flash-frozen in liquid nitrogen. The data sets were collected on beamline BM30A at ESRF and on beamline BL1 at BESSY using a MAR CCD detector. A single anomalous data set was also collected at the peak of the iron *K* edge on beamline BL1 at BESSY. All data sets were processed and scaled using *DENZO* and *SCALEPACK* (Otwinoski & Minor, 1997) and *XDS* (Kabsch, 1993).

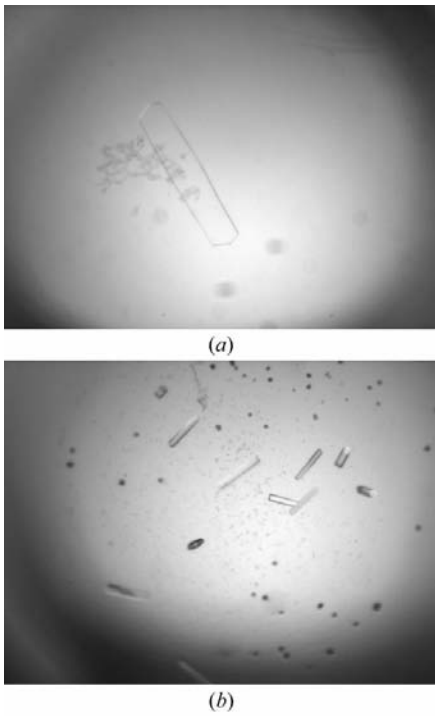

Figure 1
FptA–Pch crystals. (*a*) FptA–Pch triclinic crystal grown in 1.0 *M* $MgSO_4$ and 20% glycerol. The crystal reached typical dimensions of 0.1 mm in width and 1.0 mm in length. (*b*) Fpta–Pch orthorhombic crystals. The crystals reached typical dimensions of 0.05 × 0.05 × 0.3 mm.

Table 1
Crystallographic data statistics.

Values in parentheses are for the highest resolution shell.

Crystal system	Triclinic	Orthorhombic	Orthorhombic
Wavelength (Å)	0.979762	0.97870	1.73886
Resolution (Å)	29.7–3.55 (3.63–3.55)	29.4–2.05 (2.1–2.05)	19.9–3.1 (3.2–3.1)
Total observations	33472	529902	156242
Unique reflections	25073	67188	37203
Completeness (%)	86.6 (82.4)	99.8 (99.7)	99.9 (100.0)
$I/\sigma(I)$	4.6 (2.1)	19.0 (5.1)	11.1 (3.6)
R_{sym} (%)	10.1 (22.6)	7.0 (44.3)	11.2 (43.3)

3. Results and discussion

All crystallization experiments were performed using a 1 mg ml^{-1} protein solution in 0.05% lauryl dimethylamine oxide (Anatrace) and 10 m*M*Tris–HCl pH 8.0.

The first crystal form was grown in salt solutions containing 0.8–1.0 *M* $(NH_4)_2SO_4$ or 0.8–1.0 *M* $MgSO_4$ and 20% glycerol as cryoprotecting agent with or without 0.1 *M* sodium acetate buffer pH 4.6. The crystals were very thin long plates reaching up to 1.0 mm in length and approximately 100 µm in width (Fig. 1). The diffraction data were collected on beamline BM30A at ESRF from one crystal diffracting to 3.2 Å resolution (Table 1). The data were processed and scaled at 3.55 Å resolution using *DENZO* and *SCALEPACK* (Otwinoski & Minor, 1997). The crystal belongs to space group *P*1, with unit-cell parameters a = 85.17, b = 98.80, c = 94.58 Å, α = 62.32, β = 63.10, γ = 69.93°. Based on only the Matthews coefficient calculation (Matthews, 1968), two (V_M = 4.1 Å^3 Da^{-1}, solvent content 69.6%) or three (V_M = 2.7 Å^3 Da^{-1}, solvent content 54.4%) molecules can be accommodated in an asymmetric unit. Analysis of the self-rotation function calculated using *GLRF* (Tong & Rossmann, 1990) between 12 and 5 Å resolution shows a peak in the section κ = 180° revealing the presence of a non-crystallographic twofold axis (φ = 0, ψ = 90°), which suggests the presence of two molecules in the asymmetric unit (Fig. 2).

The second crystal form was obtained by mixing polyethylene glycol (PEG 6K, 8K or 10K; Fluka) at a 4.5–15% concentration with either Li_2SO_4, $MgSO_4$ or $(NH_4)_2SO_4$ at a 0.1–0.3 *M* concentration in 0.1 *M* sodium acetate buffer pH 4.6. The best crystals were grown in a solution containing 4.5–9% PEG 8K or 10K, 150 m*M* $(NH_4)_2SO_4$, 25% ethylene glycol as cryoprotecting agent and 0.1 *M* sodium acetate buffer pH 4.6. Crystals

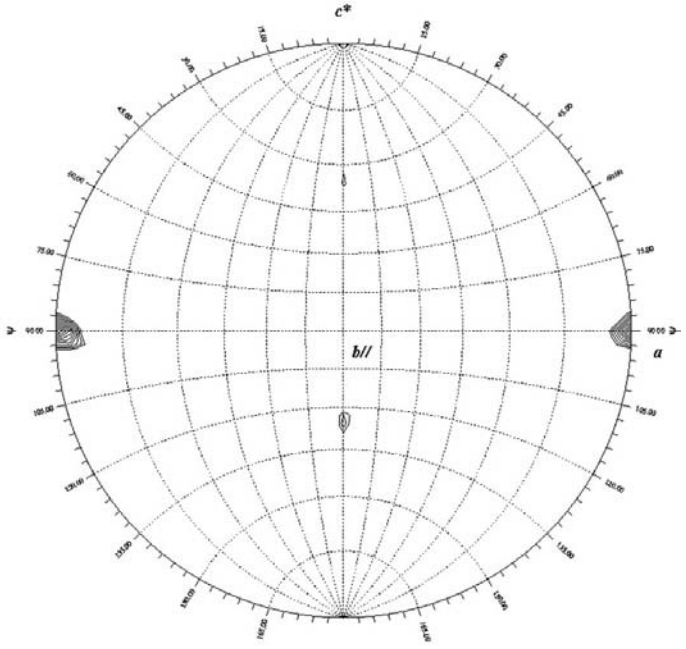

Figure 2
Self-rotation function map plotted for the κ = 180° section calculated between 12 and 5 Å resolution using *GLRF* and the data collected from one triclinic crystal.

appear within a few days and reach typical dimensions of 0.05 × 0.05 × 0.3 mm (Fig. 1). They diffract to 1.9 Å resolution and a complete data set was collected from one crystal on beamline BL1 at BESSY. The data were processed and scaled at 2.05 Å resolution using *XDS* (Kabsch, 1993). Systematic absences ($2n + 1$) were observed for the $h00$, $0k0$ and $00l$ reflection classes resulting from the presence of 2_1 axes. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters a = 76.38, b = 84.88, c = 163.12 Å (Table 1). On the basis of the Matthews coefficient calculation (Matthews, 1968), the asymmetric unit contains one molecule with a corresponding V_M of 3.5 Å^3 Da^{-1} and a solvent content of 64.4%.

Since FptA is expressed and purified from *P. aeruginosa*, the crystals might contain FptA bound to ferric pyochelin. A fluorescence-scan experiment around the Fe *K* edge was therefore performed and its analysis showed a peak at 7130 eV (λ = 1.73886 Å), suggesting the presence of iron in the crystals. In order to find the iron position, an single anomalous data set at the peak of the Fe *K* edge was also collected at 3.1 Å resolution from an orthorhombic crystal on beamline BL1 at BESSY (Table 1). The data set was processed and scaled using *XDS* (Kabsch, 1993). The completeness of the data is 99.9 between 19.9 and 3.1 Å resolution (Table 1). The R_{sym} and R_{merge} values are 11.2 and 11.9% (46.2% between 3.2 and 3.1 Å resolution), respectively.

Trials to solve the FptA structure by molecular replacement in both space groups were undertaken using *AMoRe* (Navaza, 1994) and the atomic coordinates of FpvA–Pvd (Cobessi *et al.*, 2004) as a search model (32.5% sequence identity and 65.6% similarity). The non-conserved residues in the sequence alignment were replaced by alanine residues. For both space groups, the cross-rotation and translation functions were calculated using the data between 12 and 5 Å resolution and a 30 Å integration radius. The *R* factor and correlation coefficient using two molecules in the asymmetric unit are 50.5 and 37.6% for the triclinic crystal. These values are 49.9 and 36.8% for the orthorhombic crystal, which contains one molecule in the asymmetric unit. The solutions to the phase problem were confirmed by crystal-packing analyses. Model rebuilding and refinement are in progress using the data collected at 2.05 Å resolution and investigations of crystallization conditions for FptA bound to ferric pyochelin are also under way.

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