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Crystallization and preliminary X-ray crystallographic analysis of peptide deformylase from *Pseudomonas aeruginosa*

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Peptide deformylase (PDF) from the pathogenic bacterium *Pseudomonas aeruginosa* has been overexpressed in *Escherichia coli* and crystallized in the presence of its inhibitor actinonin at 297 K using polyethylene glycol (PEG) 4000 as a precipitant. The diffraction limit and the spot shape of the crystals could be slightly improved by the crystal annealing/dehydration procedure. X-ray diffraction data to 1.85 Å have been collected using synchrotron radiation. The crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 68.75, b = 74.46, c = 77.18 Å. The asymmetric unit contains two subunits of peptide deformylase, with a corresponding crystal volume per protein mass (V_M) of 2.45 Å^3 Da^{-1} and a solvent content of 49.8%.

1. Introduction

Peptide deformylase (PDF; EC 3.5.1.31) is an essential bacterial metalloenzyme which deformylates the *N*-formylmethionine residue of newly synthesized polypeptides. Removal of the *N*-formyl group from a nascent polypeptide chain in bacteria is required for the proper folding and function of proteins, whereas mitochondrial peptide deformylase does not appear to be essential in humans. Therefore, PDF represents a novel target for antibacterial chemotherapy (Clements *et al.*, 2001). The gene encoding PDF is present in all sequenced bacterial genomes. Recently, PDFs have also been discovered in organelles of eukaryotes, but relatively little is known about the role of deformylation in eukaryotes (Serero *et al.*, 2001; Bracchi-Richard *et al.*, 2001). Structures of PDFs from *E. coli* (Clements *et al.*, 2001; Becker *et al.*, 1998) and the pathogenic protozoa *Plasmodium falciparum* (Kumar *et al.*, 2002) as well as the crystallization of PDF from the pathogenic bacterium *Leptospira interrogans* (Li *et al.*, 2002) have been reported.

Pseudomonas aeruginosa is a ubiquitous environmental Gram-negative bacterium that is one of the top three causes of opportunistic human infections. A major factor in its prominence as a pathogen is its intrinsic resistance to antibiotics and disinfectants. Cystic fibrosis is related to infection by this pathogen. The complete genome sequence of the strain PAO1 has been reported (Stover *et al.*, 2000). PDF from *P. aeruginosa*, encoded by the *def* gene, is a homodimer, with each subunit comprising 168 amino-acid residues (subunit M_r = 19 365). It shows an amino-acid sequence identity of 56% with PDF from *E. coli*. As the first step toward its structure elucidation, we overexpressed *P. aeruginosa* PDF and crystallized it in the presence of its inhibitor actinonin. The crystallization conditions and preliminary X-ray crystallographic data are reported here.

2. Experimental

2.1. Overexpression and purification

The *def* gene encoding peptide deformylase from *P. aeruginosa* strain PAO1 (PA0019) was amplified by the polymerase chain reaction (PCR) using the chromosomal DNA as template. The forward and reverse oligonucleotide primers designed using the published genome sequence (Stover *et al.*, 2000) were 5′-G GAA TTC CAT ATG GCC ATC CTG AAC ATT CTC GA-3′ and 5′-CCG CTC GAG CGC CTG CTG TCG ATG CTG CTT-3′, respectively. The PCR product was digested with *Nde*I and *Xho*I and was then inserted into the *Nde*I/*Xho*I-digested expression vector pET-21a (Novagen). This construction added a hexahistidine tag to the C-terminus of the recombinant protein.

The protein was overexpressed in *E. coli* B834(DE3) cells. The cells were grown in Luria–Bertani medium to an OD_{600} of 0.5 at 310 K and the expression of the recombinant enzyme was induced by 0.5 m*M* isopropyl β-D-thiogalactopyranoside (IPTG) at 293 K. Cell growth continued at 293 K for 12 h after IPTG induction and cells were harvested by centrifugation at 4200*g* (6000 rev min^{-1}; Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.9, 0.5 *M* NaCl, 50 m*M* imidazole) and homogenized by sonication. The crude lysate was centrifuged at 70 400*g* (30 000 rev min^{-1}; Beckman 45Ti rotor) for 1 h at 277 K and the cell debris was

discarded. The first purification step utilized the C-terminal histidine tag by using an Ni^{2+}-chelated Hi-Trap chelating HP column (Amersham Biosciences). The next step was gel filtration on a HiLoad 16/60 Superdex-200 prep-grade column (Amersham Biosciences) with an elution buffer of 25 m*M* Tris–HCl pH 7.5, 200 m*M* NaCl. The purified protein was concentrated to 17 mg ml^{-1} using an YM10 membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated extinction coefficient of 5120 M^{-1} cm^{-1} (SWISS–PROT; http://www.expasy.ch/).

2.2. Crystallization and X-ray data collection

Equal volumes of the protein solution (~0.85 m*M* monomer concentration) and aqueous actinonin solution (5.0 m*M*) were mixed, resulting in an approximately 1:5.9 molar ratio of PDF monomer to actinonin. The protein sample was incubated on ice for 1 h before being used for crystallization. Crystallization was performed by the hanging-drop vapour-diffusion method using 24-well tissue-culture VDX plates (Hampton Research) at 297 K. Each hanging drop was prepared by mixing 2 µl each of the protein solution and the reservoir solution. It was placed over 0.5 ml of the reservoir solution in each well. Initial crystallization conditions were established using Crystal Screen I (Jancarik & Kim, 1991), Crystal Screen II and MembFac screening solutions (Hampton Research). Rod-shaped crystals obtained under PEG 4000 conditions were further optimized.

X-ray diffraction data were collected at 100 K with a MacScience 2030 image-plate detector at the BL-6B experimental station, Pohang Light Source, Korea. A crystal was flash-frozen in liquid nitrogen using a cryoprotectant solution consisting of 100 m*M* ADA pH 6.5, 100 m*M* lithium sulfate, 2%(*v*/*v*) 2-propanol, 20%(*w*/*v*) PEG 4000 and 10%(*w*/*v*) PEG 400. The wavelength of the synchrotron X-rays was 0.9796 Å. The crystal was rotated through a total of 150°, with 1.0° oscillation per frame. The raw data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

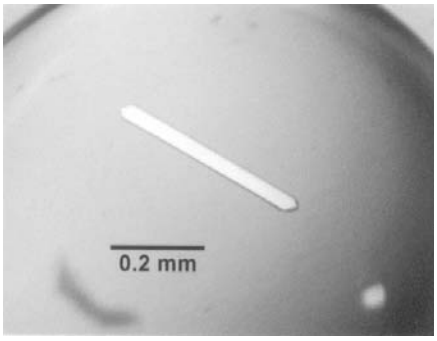

Figure 1
A crystal of *P. aeruginosa* peptide deformylase grown in the presence of actinonin. Its approximate dimensions are 0.04 × 0.02 × 0.4 mm.

3. Results

Recombinant *P. aeruginosa* PDF with a C-terminal hexahistidine tag was overexpressed as a soluble form in *E. coli* with a yield of ~10 mg of homogeneous protein per litre of culture. The crystals grew to dimensions of 0.04 × 0.02 × 0.4 mm within 2 d (Fig. 1) using the optimized reservoir solution of 100 m*M* ADA pH 6.5, 100 m*M* lithium sulfate, 2%(*v*/*v*) 2-propanol and 12%(*w*/*v*) PEG 4000. The flash-frozen crystals diffracted initially to ~2.0 Å, but the shape of the reflections was poor, particularly at high resolution. Since we and others have previously experienced a significant improvement in the resolution limit and the spot shape of the protein crystals after crystal annealing/dehydration (Harp *et al.*, 1998; Yang *et al.*, 2002), the crystal was removed from the cold nitrogen-gas stream, placed in a 200 µl drop of the cryoprotectant solution without sealing for 30 min and flash-frozen again. After crystal annealing/dehydration, the diffraction limit was slightly improved to 1.85 Å and the spot shape was also ameliorated. A total of 161 692 measured reflections were merged into 32 782 unique reflections, with an $R_{\rm merge}$ (on intensity) of 4.1%. The merged data set is 95% complete to 1.85 Å. The crystal belongs to the primitive orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 68.75 (6), b = 74.46 (6), c = 77.18 (11) Å, where the estimated standard deviations are given in parentheses. The presence of two subunits of PDF in an asymmetric unit gives a crystal volume per protein mass ($V_{\rm M}$) of 2.45 Å^3 Da^{-1} and a corresponding solvent content of 49.8% (Matthews, 1968). The statistics for data collection are summarized in Table 1. The structure of *P. aeruginosa* PDF has been solved by the molecular-replacement method using the model of *E. coli* PDF (PDB code 1bs4) as a search model. The rotation and translation solution gave an R factor of 46.1% for the 20–4.0 Å data after rigid-body refinement. After several rounds of refinement of individual atomic parameters and manual model rebuilding, the model was refined to an R factor of 18.1% for the 20–1.85 Å data. Actinonin and a metal ion bound at the active site are clearly defined by the electron density and this structural information will be useful in structure-based inhibitor design. The structural details will be described in a separate paper.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (1.92–1.85 Å).

X-ray wavelength (Å)	0.9796 (Pohang Light Source, BL-6B)
Temperature (K)	100
Resolution range (Å)	30.0–1.85
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 68.75, b = 74.46, c = 77.18
Total/unique reflections	161692/32782
Completeness (%)	95.0 (91.2)
Mean $I/\sigma(I)$	19.3 (8.4)
$R_{\rm merge}$† (%)	4.1 (12.9)

† $R_{\rm merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h, i)$ is the intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean value of $I(h, i)$ for all i measurements.

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