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Crystallization and Preliminary X-ray Crystallographic Analysis of Peptide Deformylase from *Staphylococcus aureus*

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Abstract

The peptide deformylase from the pathogenic bacterium *Staphylococcus aureus* has been over-expressed in *Escherichia coli* and crystallized in the presence of the inhibitor actinonin at 297 K using polyethylene glycol 20000 as a precipitant. X-ray diffraction data have been collected to 2.2 Å resolution. The crystal is trigonal, belonging to the space group $P3_121$ (or its enantiomorph $P3_221$), with unit cell parameters of $a = b = 62.70$ Å, $c = 108.23$ Å, $\alpha = \beta = 90°$, and $\gamma = 120°$. An asymmetric unit contains a monomer of the recombinant enzyme, giving a V_M of 2.84 Å^3 Da^{-1} and a solvent content of 56.7%.

1. Introduction

Peptide deformylase (PDF; EC 3.5.1.31), a metallo-enzyme, is regarded as an attractive target for developing new antibacterial agents (Giglione *et al.*, 2000; Clements *et al.*, 2001), because it is essential in prokaryotes and is conserved throughout the eubacteria. It catalyzes the removal of the formyl group from the N-terminal methionine residue of newly synthesized polypeptides. PDFs have also been discovered in mitochondria and chloroplasts but relatively little is known about the role of deformylation in eukaryotes (Serero *et al.*, 2001; Bracchi-Richard *et al.*, 2001). Peptide deformylases have been grouped into at least two types (Giglione *et al.*, 2000; Guilloteau *et al.*, 2002). Compared to class I, members of class II PDF have two sequence insertions in the N-terminal part and have many hydrophobic amino acid residues at the C-terminus (Giglione *et al.*, 2000).

Staphylococcus aureus, a Gram-positive bacterium, is a major cause of hospital- and community-acquired infections. Development of methicillin-resistance and, more recently, vancomycin-resistance in *S. aureus* poses a serious health problem. The *def* gene of *S. aureus* encodes an active PDF comprising a 183-residue polypeptide chain of 20 559 Da. *S. aureus* PDF belongs to class II; it shows 56% sequence identity with *Bacillus subtilis* PDF (class II) and 35% against *E. coli* PDF (class I). The structure of *S. aureus* PDF in the inhibitor-unbound state has been reported recently (Baldwin *et al.*, 2002; Guilloteau *et al.*, 2002). One of the most prominent differences from the structure of class I PDFs is the absence of a long C-terminal helix. Since structure-based discovery of new antibacterial agents against *S. aureus* requires detailed structural information on the mode of inhibitor binding to its active site, we have undertaken structural determination of the inhibitor complex of *S. aureus* PDF. As the first step toward its structure elucidation, we over-expressed *S. aureus* PDF and crystallized it in the presence of actinonin, a naturally occurring hydroxamic acid pseudopeptide inhibitor (Chen *et al.*, 2000). And we report here crystallization conditions and preliminary X-ray crystallographic data.

2. Experimental

2-1. Protein expression and purification

The *def* gene encoding peptide deformylase from

S. aureus was amplified by the polymerase chain reaction (PCR) using the genomic DNA of *S. aureus* strain SA113 as template. All DNA manipulations were carried out using standard procedures (Sambrook & Russell, 2001). The forward and reverse oligonucleotide primers designed using the unpublished genome sequence of *S. aureus* strain COL (locus SA1100 in http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gsa) were 5'-G GAA TTC CAT ATG TTA ACA ATG AAA GAC ATC ATT AGC G-3' and 5'-CCG CCG CTC GAG AAC TTC TAC TGC ATC TGT ATG TGG-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 hexa-histidine containing tag to the C-terminus of the recombinant protein. The protein was over-expressed in *E. coli* C41(DE3) cells (Miroux and Walker, 1996). The cells were grown in Luria-Bertani medium up to OD_{600} of 0.5 at 310 K and the expression of the recombinant enzyme was induced by 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) at 293 K. Cells were continued to grow at 293 K for 18 h after IPTG induction and were harvested by centrifugation at 4 200 g (6000 rev min^{-1}; Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was resuspended in an ice-cold lysis buffer (20 mM Tris-HCl, pH 7.9, 0.5 M NaCl, 50 mM imidazole) and was 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 Ni^{2+}-chelated Hi-Trap chelating HP column (Amersham Biosciences). The next final step was gel filtration on a HiLoad 16/60 Superdex-200 prep-grade column (Amersham Biosciences) with an elution buffer of 20 mM Tris-HCl, pH 7.5, 120 mM NaCl, and 1 mM EDTA. The purified protein was concentrated to 30 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 7680 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/). The dynamic light scattering experiment was performed on a Model DynaPro-801 instrument from Protein Solutions (Lakewood, New Jersey).

2-2. Crystallization

Crystallization trials were carried out using the hanging-drop vapor-diffusion method using 24-well VDX tissue-culture plates (Hampton Research) at 297 K. 50 μl of the protein solution (~1.4 mM monomer concentration) and 100 μl of the actinonin aqueous solution (5.0 mM) were mixed, resulting in a molar ratio of 6.6 : 1 for actinonin over the PDF monomer. The protein sample was incubated on ice for 1 h before being used for crystallization. Each hanging drop was prepared by mixing 2 μl each of the protein solution and the reservoir solution and was placed over 1 ml of the reservoir solution. An initial crystallization trial was performed using Crystal Screen I and II kits (Hampton Research). When various additives were added to the hanging drop to optimize the crystallization conditions, each hanging drop was prepared by mixing 2 μl of the protein solution, 2 μl of the reservoir solution, and 0.4 μl of the additive solution.

2-3. X-ray diffraction experiment

The crystals were mounted in thin-walled glass capillaries and both ends of the capillary were filled with the mother liquor and then sealed with wax. The X-ray diffraction images were collected on a Bruker Hi-Star multiwire area detector at 293 K, using Cu $K\alpha$ radiation generated by a MAC Science M18XHF rotating anode generator, and were processed with the SADIE and SAINT software packages (Siemens).

3. Results

The recombinant def protein from *S. aureus* with a C-terminal tag containing hexa-histidine was over-produced in soluble form with a yield of ~7.5 mg of homogeneous protein from a liter culture. Metal analysis by Inductively Coupled Plasma Atomic Emission Spectrometry indicated that the recombinant enzyme is bound with mostly zinc-ions. The recombinant protein exists as a dimer in solution, as judged by the molecular mass estimated by the

Fig. 1. A crystal of *Staphylococcus aureus* peptide deformylase grown in the presence of the inhibitor actinonin. The largest crystals have approximate dimensions of 0.2 × 0.15 × 0.1 mm.

dynamic light scattering measurement (45.0 kDa, 14% polydispersity). Microcrystals were grown with the reservoir solution of 12% (*w/v*) polyethylene glycol 20000 and 100 mM MES buffer, pH 6.5 (Crystal Screen II, No. 22). Optimization of this crystallization condition was achieved by adding various additives to the hanging drop. The best crystals were obtained when calcium chloride was added to a final concentration of 20 mM. Trigonal crystals grew up to approximately 0.2 × 0.15 × 0.1 mm in size within 3 d (Fig. 1) and were reasonably stable against mechanical treatment and against X-ray irradiation. Since it was difficult to find a suitable cryoprotectant solution for flash-freezing the crystals, X-ray data were collected at 293 K using conventional X-rays. The space group was determined to be $P3_121$ (or its enantiomorph $P3_221$) with unit cell parameters of $a = b = 62.70$ Å, $c = 108.23$ Å, $\alpha = \beta = 90^{\circ}$, and $\gamma = 120^{\circ}$. The presence of one monomer of the recombinant protein in the asymmetric unit gives a crystal volume per protein mass (V_M) of 2.84 Å^3 Da^{-1}, with a corresponding solvent content of 56.7% (Matthews, 1968). The statistics for data collection are summarized in Table 1.

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Table 1. Data collection statistics. Values in parentheses refer to the highest resolution shell (2.27~2.20 Å)

X-ray wavelength (Å)	1.5418
Temperature (K)	293
Resolution range (Å)	24.3~2.20
Space group	$P3_121$ (or $P3_221$)
Unit cell parameters (Å, °)	$a = b = 62.70$, $c = 108.23$, $\alpha = \beta = 90$, $\gamma = 120$
Total/unique reflections	132 013/12 640
Completeness (%)	96.6 (71.5)
Mean $I/\sigma(I)$	19.6 (3.3)
R_{merge} (%)[a]	5.8 (28.2)

[a] $R_{merge} = \Sigma_h\Sigma_i|I(h, i) - \langle I(h)\rangle|/\Sigma_h\Sigma_i I(h, i)$, 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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