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Expression, crystallization and preliminary X-ray crystallographic analysis of peptide deformylase from *Xanthomonas oryzae* pv. *oryzae*

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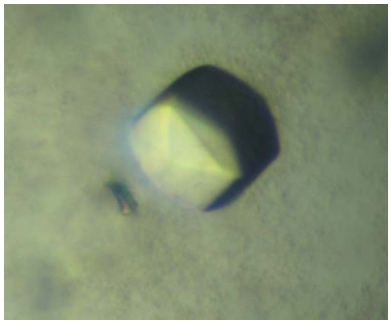


Peptide deformylase (PDF) catalyzes the removal of the *N*-formyl group from the N-terminus of newly synthesized polypeptides; this process is crucial for cell survival. As it is an antibacterial drug target against *Xanthomonas oryzae* pv. *oryzae* (Xoo), PDF from Xoo was cloned, expressed, purified and crystallized. Native PDF crystals diffracted to 2.7 Å resolution and belonged to the hexagonal space group $P6_122$, with unit-cell parameters $a = b = 59.0$, $c = 266.3$ Å. One monomer is present in the asymmetric unit, with a corresponding crystal volume per protein weight of 3.50 Å^3 Da^{-1} and a solvent content of 64.9%.

1. Introduction

In 2005, determination of the whole genome sequence of *Xanthomonas oryzae* pv. *oryzae* (Xoo; Lee *et al.*, 2005; Ochiai *et al.*, 2005) provided valuable information for developing antibacterial drugs against Xoo, which is a causal agent of bacterial blight in rice (*Oryza sativa* L.). Approximately 100 genes coding essential enzymes (Payne *et al.*, 2004, 2007) were selected from the 4538 putative genes of Xoo (Kim *et al.*, 2008; Lee *et al.*, 2005) as drug-target protein candidates. One of the target proteins is a peptide deformylase (PDF).

In bacteria, as well as in mitochondria and chloroplasts of eukaryotes, protein biosynthesis begins with *N*-formylmethionine, which results from the N-terminal formylation of methionine-tRNA by a formyltransferase (Becker *et al.*, 1998; Lucchini & Bianchetti, 1980). *N*-formylmethionine is often removed from the nascent protein chain (Waller, 1963). The first step of this process is catalyzed by PDF (EC 3.5.1.31), which removes the *N*-formyl group. The deformylated N-terminal methionine is then cleaved by methionine aminopeptidase (MAP; Adams & Capecchi, 1966; Fry & Lamborg, 1967). MAP (EC 3.4.11.18) specifically recognizes deformylated N-terminal methionine as a substrate and not *N*-formylmethionine. Therefore, inhibition of PDF will result in disruption of cellular processes and the death of bacterial cells (Chang *et al.*, 1989; Miller *et al.*, 1989) and this crucial role of PDF in bacterial cells makes it a promising drug target against bacteria.

PDF is a metalloproteinase that uses Fe^{2+} as the catalytic metal ion (Miller *et al.*, 1989). The essentiality of PDF has been shown for *Escherichia coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Mycobacterium tuberculosis* (Apfel *et al.*, 2001; Margolis *et al.*, 2000; Mazel *et al.*, 1994; Teo *et al.*, 2006). Sequence alignments from public data banks show that all sequenced pathogenic genomes contain the *pdf* gene and that the essential residues are highly conserved (Apfel *et al.*, 2001).

This study describes the cloning, expression, purification, crystallization and preliminary X-ray crystallographic studies of PDF. Three-dimensional structural studies will elucidate the molecular basis of the enzymatic reaction mechanism of PDF and will be useful for the design of a potential antibacterial drug against Xoo.

2. Materials and methods

2.1. Cloning

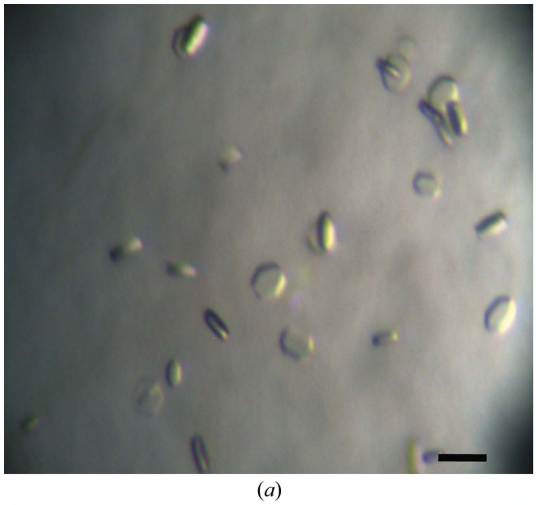
The *X. oryzae* pv. *oryzae pdf* gene encodes a total of 212 amino-acid residues. The portion of the gene coding for amino-acid residues 43–212 was amplified using the polymerase chain reaction using bacterial cells (Xoo KACC10331 strain) as template. The sequences of the forward and reverse oligonucleotide primers designed from the published genome sequence of Xoo1075 (Lee *et al.*, 2005) were 5′-GGG GGG **CAT ATG** ATT CGC GAC ATT ATC CGC ATG-3′ and 5′-GGG GGG **GGA TCC** CTA CAG ATC GTA AGA CAA GAC-3′, respectively. The bases in bold designate the *Nde*I and *Bam*HI digestion sites. The amplicon was double-digested with *Nde*I and *Bam*HI and ligated into a pET11a expression vector (Novagen).

2.2. Overexpression and purification

PDF was overexpressed in *E. coli* BL21 (DE3) cells. The cells were grown at 288 K to an OD_{600} of 0.6 in Luria–Bertani medium supplemented with 50 µg ml^{-1} ampicillin. Protein expression was induced by the addition of 0.5 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG). The cells were cultured at 288 K for an additional 16 h. Cells were then harvested by centrifugation for 30 min at 6000 rev min^{-1} (Vision VS24-SMTi V5006A rotor) at 277 K. The cell pellet was resuspended in ice-cold lysis buffer (25 m*M* Tris–HCl pH 7.5 and 3 m*M* *β*-mercaptoethanol) and homogenized by ultrasonication on ice (Sonomasher). The crude cell extract was centrifuged for 30 min at 15 000 rev min^{-1} and 277 K to remove cell debris. The supernatant was adjusted to 45% saturated ammonium sulfate. After 30 min stirring, the nonprecipitated material was removed by centrifugation for 30 min at 15 000 rev min^{-1} and 277 K. The precipitated protein was collected, dissolved in ice-cold lysis buffer and applied onto a Bio-Gel P60 column (2.5 × 50 cm; BioRad) equilibrated in lysis buffer containing 150 m*M* NaCl. The eluted fractions containing PDF were collected, diluted five times with ice-cold lysis buffer and loaded onto a UNO Q6 column (BioRad) for ion-exchange chromatography purification. The eluted PDF was further purified using a Bio-Gel P100 column (2.5 × 50 cm; BioRad). Purified PDF was obtained with a yield of 5–8 mg from 1 l cell culture. For crystallization, purified PDF was dialyzed for 4 h against a buffer consisting of 25 m*M* Tris, 15 m*M* NaCl, 3 m*M* *β*-mercaptoethanol and concentrated to a final concentration of 8 mg ml^{-1} in a Vivaspin20 with 10 000 Da molecular-weight cutoff (Vivascience).

2.3. Crystallization and X-ray data collection

Initial crystallization was carried out at 287 K by the sitting-drop vapour-diffusion method in 96-well Intelli-Plates (Art Robbins) using a Hydra II e-drop automated pipetting system (Matrix) and screening kits from Hampton Research and Emerald Biosystems. Initially, small and thin hexagonal-shaped crystals were observed from Hampton Crystal Screen condition No. 34 (0.05 *M* cadmium sulfate, 0.1 *M* HEPES pH 7.5 and 1.0 *M* sodium acetate trihydrate; Fig. 1*a*) and were reproduced as hanging drops (24-well plate from SPL) made up of 0.9 µl protein solution mixed with 0.9 µl reservoir solution. Each hanging drop was positioned over 1 ml reservoir solution and the cover slip was sealed with vacuum grease. Optimization was achieved by varying the concentration of sodium acetate trihydrate. After 1 d, hexagonal pillar-shaped crystals with adequate dimensions were obtained using a reservoir solution containing 0.05 *M* cadmium sulfate, 0.1 *M* HEPES pH 7.5 and 2.0 *M* sodium acetate trihydrate (Fig. 1*b*). The fully grown crystals were flash-cooled at 100 K in liquid



(*a*)

(*b*)

Figure 1
Crystals of PDF. (*a*) Initial crystals. (*b*) Optimized crystals of dimensions 0.6 × 0.4 × 0.25 mm. The scale bar represents 0.2 mm.

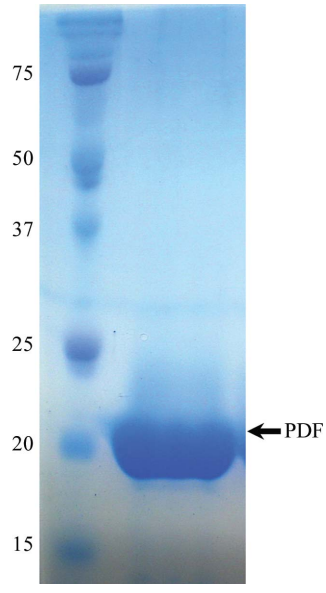


Figure 2
Purified PDF is shown on a 15% SDS–PAGE gel. Molecular-weight markers are labelled in kDa.

Table 1
Data-collection statistics.

Values in parentheses are for the outer shell.

X-ray source	Photon Factory BL-6A1
Wavelength (Å)	1.000
Unit-cell parameters (Å)	$a = b = 59.0$, $c = 266.3$
Space group	$P6_122$
Resolution	50.0–2.7 (2.8–2.7)
No. of observations	77654
No. of unique observations	8124
Completeness (%)	96.8 (98.2)
$R_{\rm merge}$† (%)	14.7 (38.2)
$\langle I/\sigma(I)\rangle$ (%)	14.2 (8.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I(hkl)$ is the intensity of reflection hkl, $\sum_{hkl}$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection hkl.

nitrogen with 20%(*v*/*v*) glycerol added to the reservoir solution as a cryoprotectant. X-ray diffraction data were collected with 1° oscillations and a crystal-to-detector distance of 220 mm using an ADSC Quantum 4R detector on beamline 6A1 of the Photon Factory, High Energy Accelerator Research Organization (KEK), Japan. The crystals diffracted to 2.7 Å resolution. Data were integrated and scaled using *DENZO* and *SCALEPACK*, respectively (Otwinowski & Minor, 1997).

3. Results and discussion

Initially, PDF was cloned, expressed and purified with an N-terminal 6×His tag and a TEV cleavage site using a modified pET11a vector (His-TEV-pET11a; Novagen) to facilitate the purification of the expressed protein. However, the PDF expressed with a 6×His/TEV cleavage tag appeared as two bands on an SDS–PAGE gel after affinity purification (data not shown). After cleavage by TEV protease, both bands still co-migrated in the ion-exchange chromatography procedure. Therefore, we recloned the gene into a pET11a vector without any tags and purified the protein by conventional means (as described in §2). This enabled us to successfully purify PDF as a single band of the expected molecular weight on SDS gels (Fig. 2)

Crystals of PDF belong to space group $P6_122$, with unit-cell parameters $a = b = 59.0$, $c = 266.3$ Å. The space group was derived by auto-indexing (Otwinowski & Minor, 1997) and data-collection statistics are provided in Table 1. According to the Matthews coefficient calculation (Matthews, 1968) there is one molecule in the asymmetric unit, with a corresponding crystal volume per protein weight of 3.50 Å^3 Da^{-1} and a solvent content of 64.9%. Molecular replacement (MR) was performed using *Phaser* from the *CCP*4 program package (McCoy *et al.*, 2007). Peptide deformylase from *Leptospira interrogans* (PDB code 1sv2; 42.3% sequence identity) was used as a search model. MR was successful and revealed one monomer in the asymmetric unit. The initial *R* factor from the molecular-replacement search was 49.8% and the resulting electron-density maps were of high quality. The structural details will be described in a separate paper. Our structural data for PDF will provide an insight into its enzymatic mechanisms and will be useful for developing a potential antibacterial drug against Xoo.

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References

Adams, J. M. & Capecchi, M. R. (1966). *Proc. Natl Acad. Sci. USA*, **55**, 147–155.
Apfel, C. M., Locher, H., Evers, S., Takacs, B., Hubschwerlen, C., Pirson, W., Page, M. G. & Keck, W. (2001). *Antimicrob. Agents Chemother.* **45**, 1058–1064.
Becker, A., Schlichting, I., Kabsch, W., Groche, D., Schultz, S. & Wagner, A. F. (1998). *Nature Struct. Biol.* **5**, 1053–1058.
Chang, S. Y., McGary, E. C. & Chang, S. (1989). *J. Bacteriol.* **171**, 4071–4072.
Fry, K. T. & Lamborg, M. R. (1967). *J. Mol. Biol.* **28**, 423–433.
Kim, J. G., Park, D., Kim, B. C., Cho, S. W., Kim, Y. T., Park, Y. J., Cho, H. J., Park, H., Kim, K. B., Yoon, K. O., Park, S. J., Lee, B. M. & Bhak, J. (2008). *BMC Bioinformatics*, **9**, 41.
Lee, B. M. *et al.* (2005). *Nucleic Acids Res.* **33**, 577–586.
Lucchini, G. & Bianchetti, R. (1980). *Biochim. Biophys. Acta*, **608**, 54–61.
Margolis, P. S., Hackbarth, C. J., Young, D. C., Wang, W., Chen, D., Yuan, Z., White, R. & Trias, J. (2000). *Antimicrob. Agents Chemother.* **44**, 1825–1831.
Matthews, B. W. (1968). *J. Mol. Biol.* **32**, 491–497.
Mazel, D., Pochet, S. & Marliere, P. (1994). *EMBO J.* **13**, 914–923.
McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
Miller, C. G., Kukral, A. M., Miller, J. L. & Movva, N. R. (1989). *J. Bacteriol.* **171**, 5215–5217.
Ochiai, H., Inoue, Y., Takeya, M., Sasaki, A. & Kaku, H. (2005). *Jpn Agric. Res. Q.* **39**, 275–287.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Payne, D. J., Gwynn, M. N., Holmes, D. J. & Pompliano, D. L. (2007). *Nature Rev. Drug Discov.* **6**, 29–40.
Payne, D. J., Gwynn, M. N., Holmes, D. J. & Rosenberg, M. (2004). *Methods Mol. Biol.* **266**, 231–259.
Teo, J. W. *et al.* (2006). *Antimicrob. Agents Chemother.* **50**, 3665–3673.
Waller, J. P. (1963). *J. Mol. Biol.* **7**, 483–496.