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Crystallization and preliminary X-ray crystallographic analysis of peptide deformylase from *Thermus thermophilus* HB8

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Peptide deformylase (PDF) is responsible for cleaving the formyl group at the N-terminus of nascent polypeptide chains in eubacteria and is essential to bacterial cell viability. A recombinant PDF of the thermophilic bacterium *Thermus thermophilus* HB8 has been crystallized by the hanging-drop vapour-diffusion method using PEG 4000 as a precipitant. The crystals belonged to the tetragonal space group $P4_1$ or $P4_3$, with unit-cell parameters $a = b = 62.58$, $c = 105.27$ Å, and are most likely to contain two molecules in an asymmetric unit, giving a crystal volume per protein weight (V_M) of 2.3 Å^3 Da^{-1} and a solvent content of 46.7%.

1. Introduction

A conserved and distinctive feature of the translation system in prokaryotes, chloroplasts and mitochondria is that every nascent polypeptide bears an N-terminal formyl group. Peptide deformylase (PDF; EC 3.5.1.88) catalyzes the hydrolytic removal of the N-terminal formyl group from such nascent polypeptides (Meinnel *et al.*, 1993). PDF is essential for bacterial cell growth (Meinnel & Blanquet, 1994; Margolis *et al.*, 2000). PDF is a unique and highly labile metallopeptidase which utilizes an Fe^{2+} ion to catalyze amide-bond hydrolysis (Rajagopalan *et al.*, 1997). Substitution of Ni^{2+} or Co^{2+} for the Fe^{2+} cofactor leads to the retention of almost full catalytic activity, whereas substitution of Zn^{2+} reduces the activity by over two orders of magnitude (Groche *et al.*, 1998; Rajagopalan *et al.*, 2000).

Several high-resolution structures of the bacterial enzyme are available from *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Thermotoga maritima*, *Streptococcus pneumoniae* and *Bacillus stearothermophilus* (Baldwin *et al.*, 2002; Becker *et al.*, 1998; Chan *et al.*, 1997; Guilloteau *et al.*, 2002; Kreusch *et al.*, 2003). The structure of PDF from the eukaryotic parasite *Plasmodium falciparum* has also been elucidated (Kumar *et al.*, 2002; Robien *et al.*, 2004). The PDF family is divided into two classes (Meinnel, 2000): Gram-negative [G(−)] and Gram-positive [G(+)] types. The G(−) PDFs share ∼30% sequence identity with one another within the class. *Thermus thermophilus* PDF (*Tth*PDF, 22 kDa) belongs to the G(−) type and shows 36% sequence identity to *E. coli* PDF (*Eco*PDF). *T. thermophilus* can survive at up to 358 K. The structure of the PDF from the thermophilic bacterium *T. maritima* (*Tma*PDF) was recently solved, but its C-terminal portion was not modelled owing to disorder (Kreusch *et al.*, 2003).

To gain additional insight into the molecular basis of adaptation of PDF to high temperature, we have carried out a crystallographic study of *Tth*PDF. Here, we report the results of our crystallization and preliminary X-ray crystallographic analysis of *Tth*PDF.

2. Methods and results

2.1. Cloning, overexpression and purification

Recombinant *Tth*PDF was overexpressed in *E. coli* strain BL21(DE3)pLysS using the pET system (Novagen). Expression of *Tth*PDF was induced by adding 1 m*M* IPTG. After induction, the *E. coli* culture was incubated at 310 K for an additional 3 h and then harvested by centrifugation. The cells were suspended in 10 m*M* sodium phosphate pH 7.0 and then disrupted by sonication on ice. The cell lysate was incubated at 333 K for 10 min. After centrifugation at 40 000*g* for 60 min at 277 K, the supernatant was applied onto a TSKgel Blue 5pw column (21.5 × 150 mm; Tosoh, Tokyo, Japan) and eluted with a linear gradient of 0–1.0 *M* NaCl in 20 m*M* MES pH 6.0. *Tth*PDF-containing fractions were applied onto a Mono Q 10/10 column (Amersham Biotech) and eluted with a linear gradient of 0–1.0 *M* NaCl in 20 m*M* Tris–HCl pH 8.0. *Tth*PDF-containing fractions were applied onto a Bio-Scale CHT10-I column (BioRad) and eluted with a linear gradient of 10–500 m*M* sodium phosphate pH 7.0. The fractions containing *Tth*PDF were then pooled and concentrated with an Apollo protein-concentration apparatus (Orbital Biosciences). Protein concentrations were determined by the BioRad protein assay kit using BSA as a standard. The protein solution was quickly

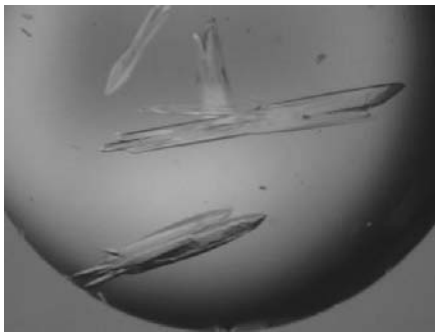

Figure 1
Crystals of *Tth*PDF grown by the hanging-drop vapour-diffusion method at 288 K.

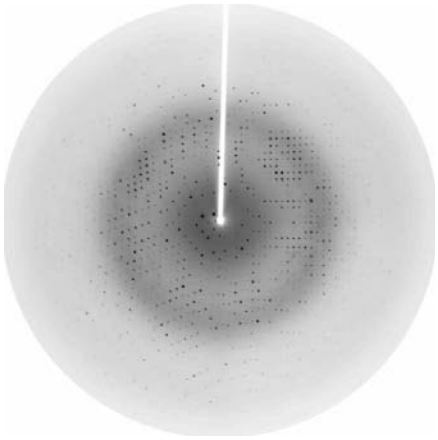

Figure 2
X-ray diffraction image from a *Tth*PDF crystal. The edge of the detector corresponds to a resolution of 1.79 Å.

frozen with liquid nitrogen and stored at 193 K.

2.2. Crystallization and X-ray data collection

Crystals suitable for X-ray analysis were obtained in a week by mixing 2 µl protein solution (20 mg ml^{-1} in 10 m*M* Tris–HCl pH 8.0) and 2.0 µl of a reservoir solution consisting of 20%(*w*/*v*) PEG 4000, 0.1 *M* Tris–HCl pH 8.0 and 0.2 *M* sodium acetate. Drops were equilibrated against 500 µl reservoir solution at 288 K. Fig. 1 shows typical crystals (0.05 × 0.05 × 1.0 mm).

Native X-ray diffraction data sets were collected using cryocooled (100 K) crystals at BL41XU at SPring-8 (Harima, Japan). The diffraction data were collected using a 1.0° oscillation with a crystal-to-detector distance of 130 mm. The crystals diffracted X-rays to 1.81 Å (Fig. 2). The data were indexed and scaled with *HKL*2000 and *SCALEPACK* (Otwinowski & Minor, 1997). The statistics of data collection are summarized in Table 1. The systematic absences showed that the crystals belonged to the space group $P4_1$ or $P4_3$. The unit-cell parameters were $a = b = 62.58$, $c = 105.27$ Å. The asymmetric unit was most likely to contain two molecules of *Tth*PDF, giving a crystal volume per protein weight of 2.3 Å^3 Da^{-1} and a solvent content of 46.7% (Matthews, 1968).

Table 1
Crystal parameters of *Tth*PDF.

Values in parentheses are for the highest resolution shell.

X-ray source	SPring-8 BL41XU
Wavelength (Å)	1.000
Unit-cell parameters (Å)	$a = 62.58$, $b = 62.58$, $c = 105.27$
Resolution range (Å)	50.0–1.81 (1.87–1.81)
Observed reflections	274721
Unique reflections	36620
Data completeness (%)	99.3 (96.3)
Redundancy	7.5 (6.4)
$R_{\rm merge}$†	0.040 (0.217)
$\langle I\rangle/\langle\sigma(I)\rangle$	53.1 (6.4)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl, including symmetry-related reflections, and $\langle I(hkl)\rangle$ is its average.

Our initial attempts to solve the structure of *Tth*PDF by molecular-replacement procedures using *Eco*PDF as a model with *MOLREP* and *EPMR* failed. The quality of the electron-density maps did not allow tracing of the polypeptide chain. The selenomethionine-substituted crystals only diffracted X-rays to 10 Å and we were not able to find any isomorphous derivatives. The structure of *Tth*PDF has been solved by the molecular-replacement method using the atomic coordinates of *Tma*PDF (PDB code 1lme; Kreusch *et al.*, 2003) as a search model (Collaborative Computational Project, Number 4, 1994), which shares ∼40% amino-acid sequence identity with *Tth*PDF. Compared with the structure of *Eco*PDF, global structural differences occurred in the C-terminal region. The C-terminal helix of *Tth*PDF was kinked, whereas that of *Eco*PDF was straight. This may be one of the reasons why *Tth*PDF is thermostable. The structural details will be described elsewhere.

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