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Expression, crystallization and preliminary X-ray crystallographic analysis of peptide deformylase from *Campylobacter jejuni*

Campylobacter jejuni is one of the major foodborne pathogens causing human infection. Peptide deformylase, a metallohydrolase, catalyzes the deformylation of *N*-formylated methionine in newly synthesized polypeptides in prokaryotes and some eukaryotic organelles. The deformylation process is an essential step in protein synthesis and has attracted much attention as a potential target for the development of novel antibacterial agents. Here, the cloned codon-optimized *def* gene from *C. jejuni* was synthesized and the protein was expressed, purified and crystallized. *C. jejuni* peptide deformylase crystals obtained at pH 7.0 and pH 6.5 diffracted to 2.9 Å resolution and belonged to the trigonal space group *R*3, with unit-cell parameters $a = b = 105.7$, $c = 58.0$ Å. One monomer existed in the asymmetric unit, with a corresponding V_M of 3.1 Å³ Da⁻¹ and a solvent content of 60.4%.

1. Introduction

Campylobacter jejuni is one of the major foodborne pathogens causing human infection in the United States (Heres *et al.*, 2004; Scallan *et al.*, 2011). The infection mainly arises from the consumption of raw or undercooked poultry (Friedman *et al.*, 2004). Annually, approximately 0.8 million cases of *C. jejuni* infection are reported, resulting in more than 8000 hospitalizations and an economic loss of about \$1.5 billion in the United States (Scharff, 2012). We selected peptide deformylase from *C. jejuni* (CjPDF) as a drug target.

The increasing clinical cases of multidrug-resistant bacteria have become a major concern in the public health arena and the discovery of novel classes of antibiotics has become increasingly important (Theuretzbacher, 2012). Peptide deformylase is one target because it is an essential metalloenzyme and is common to all bacteria. Currently, many efforts have been made to develop novel antibacterials against bacterial peptide deformylases (Chen *et al.*, 2000). Peptide deformylase catalyzes the removal of a formyl group from the *N*-terminal methionine of ribosome-synthesized polypeptides. The methionyl aminopeptidase then removes the *N*-terminal methionine to produce mature proteins. Deformylation is part of the methionine cycle involved in protein synthesis and is essential to generate mature proteins in bacteria (Meinzel *et al.*, 1993).

2. Materials and methods

2.1. Cloning

The *C. jejuni* *def* gene encodes a total of 175 amino-acid residues. The codon-optimized *C. jejuni* *def* gene was synthesized (Macrogen, Korea) and used as a template for PCR to amplify the gene, yielding a 549 bp DNA fragment by using the primers 5'-CCC CCC **CAT ATG** GTG CGC AAA ATT ATT ACC TA-3' and 5'-CCC CCC **GGA TCC** TTA TTT TTC TTT TTT GTG GTT C-3'. The bases in bold designate the *Nde*I and *Bam*HI digestion sites. The fragment was double-digested with *Nde*I and *Bam*HI, and subsequently ligated into a pET11a-TEV-AAA expression vector, which was modified to contain an *N*-terminal 6×His tag followed by a *Tobacco etch virus* (TEV) protease cleavage site and three alanine residues before the *Nde*I site to facilitate purification of the expressed protein.

Table 1

Data-collection statistics.

Values in parentheses are for the outer shell.

Crystal	CjPDF, pH 7.0	CjPDF, pH 6.5
Synchrotron	5C, PAL	5C, PAL
Wavelength (Å)	0.97952	0.97952
Detector	ADSC Q315r	ADSC Q315r
Temperature of data collection (K)	100	100
Crystal-to-detector distance (mm)	250	250
Rotation range per image (°)	1	1
Total rotation range (°)	117	114
Exposure time per image (s)	3	3
Resolution range (Å)	50.00–2.90 (2.95–2.90)	50.00–2.90 (2.95–2.90)
Space group	R3	R3
Unit-cell parameters (Å)		
<i>a</i>	105.7	105.7
<i>b</i>	105.7	105.7
<i>c</i>	58.0	58.0
Total No. of reflections	19253	17871
No. of unique reflections	5403	5241
Completeness (%)	97.9 (100.0)	97.8 (100.0)
Molecules per asymmetric unit	1	1
<i>V_M</i> (Å ³ Da ^{−1})	3.1	3.1
Solvent content (%)	60.5	60.4
Average <i>I</i> / <i>σ</i> (<i>I</i>) (%)	33.8 (3.6)	36.1 (5.6)
<i>R_{merge}</i> [†] (%)	7.1 (65.8)	6.2 (34.2)
Multiplicity	3.6 (3.7)	3.4 (3.6)

[†] $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th measurement of reflection *hkl* and $\langle I(hkl) \rangle$ is the average intensity of reflection *hkl*.

2.2. Overexpression and purification

The resulting recombinant pET11a-TEV-AAA-CjPDF vector was transformed into *Escherichia coli* BL21(DE3) cells and CjPDF was overexpressed. The cells were grown in Luria–Bertani medium supplemented with 50 µg ml^{−1} ampicillin at 310 K until an OD₆₀₀ of 0.6 was reached. Protein expression was induced by the addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The cells were cultured at 298 K for an additional 16 h. Cells were harvested by centrifugation for 30 min at 3000g (Supra 30K A1000S-4 rotor; Hanil, Seoul, Republic of Korea) at 277 K. The cell pellet was then resuspended in ice-cold lysis buffer consisting of 25 mM Tris–HCl pH 7.5, 250 mM NaCl, 3 mM β-mercaptoethanol and homogenized on ice using ultrasonication (Sonomasher). The crude cell extract was centrifuged for 30 min at 19 960g (Vision VS24-SMTi V508A rotor) at 277 K to remove cell debris. The lysate was applied onto a Ni²⁺-

charged resin (Ni–NTA His-Bind Resin, Novagen) to purify CjPDF. Eluted fractions containing CjPDF were treated with TEV protease for 6 h at 293 K at a ratio of 30:1 (CjPDF:TEV protease) by weight to remove the 6×His tag and thereafter dialyzed against 20 mM Tris–HCl pH 8.0, 150 mM NaCl, 3 mM β-mercaptoethanol. After the TEV cleavage reaction, the purified CjPDF protein has the extra residues GHAAA at the N-terminus from the expression vector. The resulting solution was applied onto the Ni²⁺-charged resin again to remove uncleaved His-tagged CjPDF. The unbound flowthrough fraction was loaded onto a 5 ml HiTrap FF column (GE Healthcare), washed and eluted with a gradient of buffer A (20 mM Tris–HCl pH 8.0, 15 mM NaCl, 3 mM β-mercaptoethanol) to buffer B (20 mM Tris–HCl pH 8.0, 1 M NaCl, 3 mM β-mercaptoethanol). For crystallization, purified CjPDF was dialyzed against 20 mM Tris–HCl pH 8.0, 20 mM NaCl, 3 mM β-mercaptoethanol. Purified protein was concentrated using a Vivaspinn20 (3000 MWCO, Vivascience) to a final protein concentration of 6 mg ml^{−1}.

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 eDrop automated pipetting system (Matrix) and screening kits from Hampton Research and Emerald BioSystems. We obtained crystals of CjPDF in only one of the conditions. The small, thin, cubic-shaped crystals appeared in PEG/Ion (Hampton Research) condition No. 26 [0.2 M zinc acetate dihydrate, 20%(w/v) PEG 3350 pH 6.4; Fig. 1] and were reproduced in hanging drops, which consisted of 0.9 µl protein solution mixed with 0.9 µl reservoir solution. Each hanging drop was positioned over 1 ml reservoir solution. The well was sealed with a cover slip using vacuum grease. In order to obtain single protein crystals, the streak-seeding method was used (Stura & Wilson, 1991; Bergfors, 2003). Initial crystals of CjPDF were picked up using the tip of a human hair as a seeding wand and the tip was immediately streaked sequentially through the pre-equilibrated hanging drops. Drops were pre-equilibrated over the reservoir solution for 2 h before seeding. The seeding method was important to obtain single CjPDF crystals consistently. Optimization of the initial crystals was achieved by changing the buffer composition of the reservoir solution. After 4 d, single crystals with dimensions of 0.1 × 0.1 × 0.1 mm were obtained using a reservoir solution consisting of 20%(w/v) PEG 3350, 0.2 M zinc acetate dihydrate and one of four

Figure 1

Initial and optimized single crystals of CjPDF. (a) Initial crystal, (b) single crystals from streak-seeding, (c) optimized single crystals of CjPDF (0.1 × 0.1 × 0.1 mm). The scale bar is 0.1 mm in length.

Figure 2
Purified CjPDF protein on a 12% SDS-PAGE gel.

different buffers at 0.1 M: bis(2-hydroxyethyl)amino-tris(hydroxymethyl) methane, tris-aminomethane, MES monohydrate or sodium cacodylate trihydrate in the pH range between 6.0 and 7.0. The fully grown crystals were transferred briefly to a cryosolution consisting of the mother liquor solution with the addition of 20% (v/v) PEG 400, picked up with a loop and flash-cooled at 100 K in liquid nitrogen. X-ray diffraction data were collected from the cryocooled crystal using an ADSC Q315r CCD detector on beamline 5C at the Pohang Accelerator Laboratory (PAL), Republic of Korea. The crystals diffracted to 2.9 Å resolution. Data were integrated and scaled using *DENZO* and *SCALEPACK*, respectively (Otwinowski & Minor, 1997). The final statistics of data collection and processing are summarized in Table 1.

3. Results and discussion

The purification protocol for the recombinant form of CjPDF consistently yielded more than 25 mg of CjPDF per litre of culture medium and the protein exhibited a single band on SDS-PAGE at approximately 20 kDa (Fig. 2). CjPDF crystals grew in the condition 20% (w/v) PEG 3350, 0.2 M zinc acetate dihydrate with all of the four different buffers [bis(2-hydroxyethyl)amino-tris(hydroxymethyl) methane, tris-aminomethane, MES monohydrate and sodium cacodylate trihydrate] at pH values of 6.0, 6.5 and 7.0. Crystals with

bis(2-hydroxyethyl)amino-tris(hydroxymethyl) methane grew to the largest dimensions (0.1 × 0.1 × 0.1 mm) and were therefore selected for X-ray diffraction. Crystals from pH 6.5 and pH 7.0 diffracted to 2.9 Å resolution, whereas crystals grown at pH 6.0 cracked and dissolved when soaked in the cryoprotectant solution. The space group was selected using the auto-indexing program in *HKL-2000* (Otwinowski & Minor, 1997) and data-collection statistics are provided in Table 1. The CjPDF crystals belonged to the crystallographic space group *R*3, with unit-cell parameters $a = b = 105.7$, $c = 58.0$ Å. According to calculation of the Matthews coefficient (Matthews, 1968), there was one monomer in the asymmetric unit with a V_M of 3.1 Å³ Da⁻¹ and a solvent content of 60.5%.

We determined the structure of CjPDF by molecular replacement using *Phaser* (McCoy *et al.*, 2007) in the *CCP4* program package (Winn *et al.*, 2011) with peptide deformylase from *Helicobacter pylori* (PDB entry 2ew5; 57% sequence identity; Cai *et al.*, 2006) as a search model. The initial R_{free} value of the CjPDF structure at pH 6.5 was 0.38 and that of the structure at pH 7.0 was 0.34. The structural details of these two CjPDF structures will be described in a separate paper. Our structural data for CjPDF will be useful for developing antibacterial drugs against *C. jejuni*.

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