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Crystallization and preliminary X-ray crystallographic studies of phosphopantetheine adenylyltransferase from *Helicobacter pylori*

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Phosphopantetheine adenylyltransferase (PPAT; EC 2.7.7.3) is an essential enzyme in the coenzyme A (CoA) biosynthetic pathway and catalyzes the reversible transfer of an adenylyl group from ATP to 4′-phosphopantetheine to form 3′-dephospho-CoA. PPAT from *Helicobacter pylori* has been overexpressed in *Escherichia coli* and crystallized at 296 K using sodium chloride as a precipitant by the hanging-drop vapour-diffusion method. X-ray diffraction data have been collected to 2.00 Å resolution at 100 K using synchrotron radiation. The crystals belong to the trigonal space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 80.50$, $c = 143.05$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. Six monomers of PPAT are likely to be present in the asymmetric unit, giving a V_M of 2.39 Å^3 Da^{-1} and a solvent content of 49%.

1. Introduction

Coenzyme A (CoA) is an essential cofactor in numerous biosynthetic, degradative and energy-yielding metabolic pathways and is required in several key reactions in intermediary metabolism (Geerlof *et al.*, 1999). It is synthesized in five steps from pantothenate (vitamin B_5), cysteine and ATP (Robishaw & Neely, 1985). Phosphopantetheine adenylyltransferase (PPAT), a member of the nucleotidyltransferase superfamily (Bork *et al.*, 1995), catalyzes the penultimate step in this biosynthetic pathway. It reversibly transfers an adenylyl group from ATP to 4′-phosphopantetheine to yield 3′-dephospho-CoA and pyrophosphate. The rate of CoA biosynthesis is regulated by feedback inhibition of the first enzyme of the pathway, pantothenate kinase (Harvolsen & Skrede, 1982). Studies of the intermediates of CoA biosynthesis have shown that both pantothenate and 4′-phosphopantetheine can accumulate in the cell (Jackowski & Rock, 1984), suggesting that PPAT catalyzes an additional rate-limiting step in the pathway.

At present, structural information on PPAT is limited to that of *Escherichia coli* (Izard & Geerlof, 1999; Izard, 2002). *E. coli* PPAT is a homohexamer; it has a dinucleotide-binding fold and the conserved His18 of the sequence motif TNGH plays an essential role in transition-state stabilization (Izard, 2002). PPAT represents an attractive antibacterial target, since it catalyzes a key regulatory step in the CoA biosynthetic pathway and the human PPAT is very dissimilar from its bacterial counterparts (Gerdes *et al.*, 2002). However, no three-dimensional structure of PPAT from any human pathogen has yet been reported. Therefore, structural data on PPATs from bacterial pathogens will be valuable in structure-based inhibitor discovery. The *coaD* (previously *kdtB*) gene of *Helicobacter pylori* encodes PPAT, a 157-residue protein (calculated $M_r = 17\,668$). A significant level (47%) of amino-acid sequence identity exists between PPATs from *H. pylori* and *E. coli*. As a first step toward structure determination of *H. pylori* PPAT, we report here its overexpression, crystallization and preliminary X-ray crystallographic data.

2. Experimental

2.1. Protein expression and purification

The *coaD* gene (previously *kdtB*; HP1475) encoding PPAT was amplified by the polymerase chain reaction using the genomic DNA of *H. pylori* 26695 strain as a template. The forward and reverse oligonucleotide primers designed using the published genome sequence (Tomb *et al.*, 1997) were 5′-G GAA TTC **CAT ATG** CAA AAA ATC GGC ATT TAC CCG-3′ and 5′-CCG CCG **CTC GAG** AGC CTT TGA AAT CAA AGG ATA AAT TT-3′, respectively. The bases in bold represent the *Nde*I and *Xho*I restriction-enzyme cleavage sites, respectively. The amplified DNA was inserted into the *Nde*I/*Xho*I-digested expression vector pET-21a(+). This vector construction adds an eight-residue tag containing six histidine residues to the C-terminus of the gene product to facilitate protein purification. The protein was overexpressed in *E. coli* strain B834(DE3) cells. The cells were grown at 303 K in Luria–Bertani medium containing

50 µg ml^{-1} ampicillin to an OD_{600} of 0.6 and expression of the recombinant enzyme was induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 m*M*. Cell growth continued at 303 K for 6 h after IPTG induction and cells were harvested by centrifugation at 4200*g* (6000 rev min^{-1}; Hanil Supra 21K rotor) for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer *A* (20 m*M* Tris–HCl pH 7.9, 500 m*M* sodium chloride) containing 5 m*M* imidazole, 2 m*M* phenylmethylsulfonyl fluoride (PMSF) and 1 m*M* β-mercaptoethanol and was then homogenized by ultrasonication. The crude lysate was centrifugated at 70 400*g* (30 000 rev min^{-1}; Beckman 45 Ti rotor) for 1 h at 277 K. The supernatant was loaded onto a Hi-Trap chelating HP column (Amersham-Pharmacia), which was previously charged with Ni^{2+} and equilibrated with buffer *A*. After washing the column with buffer *A* containing 60 m*M* imidazole, the bound protein was eluted with a linear gradient of 0–1.0 *M* imidazole. Next, gel filtration was perfomed on a HiLoad XK 16 Superdex 200 prep-grade column (Amersham-Pharmacia), which was previously equilibrated with buffer *B* (50 m*M* potassium phosphate pH 6.4, 200 m*M* sodium chloride, 1 m*M* β-mercaptoethanol, 1 m*M* EDTA). The homogeneity of the protein was assessed by SDS–PAGE (Laemmli, 1970). Finally, the purified enzyme was concentrated to 24.3 mg ml^{-1} using an YM10 ultrafiltration membrane (Amicon) and was stored at 203 K. 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/).

2.2. Crystallization and X-ray data collection

Crystallization was performed by the hanging-drop vapour-diffusion method at 296 K using 24-well VDX plates (Hampton Research). Each hanging drop was prepared by mixing 2 µl of the reservoir solution and 2 µl of the protein solution at 24.3 mg ml^{-1} and was placed over 1 ml of the reservoir solution. Initial crystallization conditions were established using Screen I, Screen II, MembFac (Hampton Research), Wizard I and Wizard II (Emerald BioStructures, Inc.) screening kits.

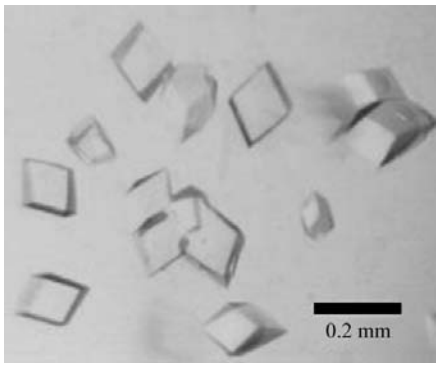

Figure 1
Crystals of phosphopantetheine adenylyltransferase from *H. pylori*. Their approximate dimensions are 0.1 × 0.1 × 0.1 mm.

A crystal of *H. pylori* PPAT was flash-frozen using a solution comprising 0.1 *M* Tris–HCl pH 7.0, 2.5 *M* sodium chloride, 0.2 *M* lithium sulfate and 15%(*v*/*v*) glycerol as a cryoprotectant. X-ray diffraction data were collected at 100 K on a DIP-2030 image-plate detector (MacScience Co.) at beamline BL-6B of Pohang Light Source, Korea. The crystal was rotated through a total of 120°, with a 1.0° oscillation range per frame. The wavelength of the synchrotron radiation was 1.000 Å. The raw data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results

H. pylori PPAT was highly overexpressed in soluble form as a fusion with a C-terminal eight-residue tag (LEHHHHHH), with a yield of ∼50 mg of homogeneous protein per litre of culture. Well diffracting crystals of the recombinant PPAT were obtained with reservoir solution comprising 0.1 *M* Tris–HCl pH 7.0, 2.5 *M* sodium chloride and 0.2 *M* lithium sulfate. The crystals grew to approximate dimensions of 0.1 × 0.1 × 0.1 mm within 3 d (Fig. 1). A set of X-ray diffraction data has been collected to 2.00 Å resolution at 100 K using synchrotron radiation. A total of 638 724 measured reflections were merged into 34 377 unique reflections with an R_{merge} of 4.2%. The merged data set is 92.9% complete to 2.00 Å resolution. The space group was determined to be $P3_121$ or $P3_221$ on the basis of systematic absences and the symmetry of the diffraction intensities. The unit-cell parameters are $a = b = 80.50$, $c = 143.05$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. Table 1 summarizes the data-collection statistics. The presence of six monomers of PPAT in each asymmetric unit gives a crystal volume per protein mass (V_M) of 2.39 Å^3 Da^{-1} and a solvent content of 49% (Matthews, 1968).

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (2.09–2.00 Å).

X-ray wavelength (Å)	1.000
Temperature (K)	100
Space group	$P3_121$ or $P3_221$
Unit-cell parameters (Å)	$a = 80.50$, $b = 80.50$, $c = 143.05$
Resolution range (Å)	30.0–2.00
Total/unique reflections	638724/34377
R_{merge}† (%)	4.2 (20.2)
Data completeness (%)	92.9 (85.8)
Average $I/\sigma(I)$	34.9 (4.6)

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

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