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Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of HP1352, a putative DNA methyltransferase in *Helicobacter pylori*

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The putative methyltransferase gene HP1352 from *Helicobacter pylori* strain 26695 was heterologously expressed in *Escherichia coli*. The 359-amino-acid gene product was purified and crystallized. The crystals belong to space group $I2_12_12_1$ and show diffraction to at least 2.5 Å resolution. The unit-cell parameters are a = 69.6, b = 86.6, c = 140.0 Å. A greater than 90% complete native data set has been collected and structure determination using the molecular-replacement method is ongoing.

1. Introduction

The gastric pathogen *Helicobacter pylori* is the second most common cause of chronic infection worldwide (Everhart, 2000). In addition to causing gastritis and stomach ulcers, this bacterium is also strongly associated with both intestinal-type and diffuse-type adenocarcinomas (Nomura *et al.*, 1991; Parsonnet *et al.*, 1991). Of all cancers, stomach cancer is the second most common to cause death (Parkin *et al.*, 2001).

Having a small genome and high medical relevance, *H. pylori* is a suitable target for structural and functional genomics studies. To date, two strains have been sequenced (Alm *et al.*, 1999; Tomb *et al.*, 1997) and several whole-genome sequence comparisons have been described (Alm *et al.*, 1999, 2000; Doig *et al.*, 1999; Hancock *et al.*, 1998; Janssen *et al.*, 2001).

In prokaryotic organisms, DNA methyltransferases are best known for their role in restriction-modification systems (Kobayashi *et al.*, 1999). However, these enzymes also have regulatory function in the bacterial cell, *e.g.* in regulation of transcription (Nou *et al.*, 1993), DNA-mismatch repair (Schlagman *et al.*, 1986) and control of DNA replication and cell division (Boye & Lobner-Olesen, 1990; Stephens *et al.*, 1996). In the sequenced strains, J99 and 26695, there is an abundance of restriction-modification genes (Alm *et al.*, 1999). Many of the putative methyltransferases lack cognate restriction enzymes and can therefore be hypothesized to have functions analogous to DNA adenine methylase (Dam) in *Escherichia coli* and cell-cycle regulated methyltransferase (CcrM) in *Caulobacter crescentus*. The HP1352 methyltransferase in *H. pylori*, numbered according to the TIGR nomenclature, recognizes the sequence GANTC (Lin *et al.*, 2001) and the predicted amino-acid sequence has 45.9% identity to the CcrM protein from *C. crescentus*. In *Caulobacter*, this enzyme is essential for viability (Stephens *et al.*, 1996). In mutants expressing CcrM throughout the cell cycle the cells have abnormal morphology, suggesting that differential CcrM methylation helps to regulate replication and cell division (Zweiger *et al.*, 1994). The presence of the HP1352 gene in clinical *H. pylori* isolates was associated with induction of a more robust host response in gnotobiotic transgenic mice, suggesting that this protein could be indirectly or directly involved in gene regulation associated with a more virulent genotype (Björkholm *et al.*, 2002).

This research project was initiated with the intention of setting up an efficient production line for crystallographic structure determination of proteins from *H. pylori*. This paper reports the first successful crystallization of a protein resulting from this collaboration.

2. Methods

2.1. Cloning

The 1077-base-pair gene encoding the HP1352 protein was PCR amplified using genomic DNA from strain 26695 as template and cloned into pTrcHis2 vector (Invitrogen, Karlsruhe, Germany) *Nco*I/*Xho*I for tag-free expression in Rosetta cells (Novagen, WI, USA).

Sequencing of the recombinant DNA, performed by MWG-Biotech (Ebersberg, Germany), confirmed the integrity of the cloned DNA.

2.2. Expression and purification

Luria broth medium containing 0.2% glucose, 50 µg ml^{-1} ampicillin and 34 µg ml^{-1} chloramphenicol was inoculated with a preculture of the Rosetta strain containing the HP1352 construct. Bacterial growth was performed at 310 K to an OD_{580} of 0.6. Expression was induced with 1 m*M* isopropyl-

β-D-1-thiogalactopyranoside for 20 h at 293 K. The cells were harvested by centrifugation and washed with 150 m*M* NaCl, 2 m*M* KH_2PO_4, 8 m*M* K_2HPO_4. The cell pellet was stored frozen at 253 K.

At the time of purification, the cell pellet was thawed and the cells were resuspended in buffer *A* [20 m*M* bis-tris propane (BTP) pH 7.8, 300 m*M* NaCl, 5 m*M* EDTA pH 7.0, 2 m*M* tris(2-carboxyethyl)phosphine hydrochloride (TCEP)] supplemented with Protease Inhibitor Cocktail and DNaseI (both from Boehringer Roche, Mannheim) and with lysozyme.

The slurry was sonicated to disrupt any remaining intact cells and cell debris was removed by centrifugation. The supernatant was loaded onto a HiTrap heparin-affinity column. The proteins were eluted by application of a linear 40 column volume (CV) gradient to buffer *B* (20 m*M* BTP pH 7.8, 1 *M* NaCl, 5 m*M* EDTA pH 7.0, 2 m*M* TCEP). The fractions containing the second peak were diluted and loaded onto a HiTrap SP cation-exchange column, washed with buffer *C* (20 m*M* BTP pH 7.8, 150 m*M* NaCl, 5 m*M* EDTA pH 7.0, 2 m*M* TCEP) and eluted in a linear 30 CV gradient to buffer *B*.

The HP1352 protein was concentrated and loaded onto a Superdex 200 16/60 size-exclusion column. Homogeneous protein was purified from this column by isocratic elution with buffer *D* (20 m*M* BTP pH 7.8, 150 m*M* NaCl, 2 m*M* TCEP).

The entire purification was carried out at 277 K and all buffers were flushed with nitrogen before use to prevent protein oxidation.

All chemicals were purchased from Sigma–Aldrich (Taufkirchen, Germany) unless indicated otherwise. All chromatographic columns where purchased from Amersham Biosciences (Freiburg, Germany).

The protein was identified as adenine-specific DNA methyltransferase from *H. pylori* by matrix-assisted laser desorption ionization (MALDI) mass peptide fingerprint analysis of the expressed protein. The sequence coverage was 57%.

2.3. Crystallization

Initial crystals were obtained with the hanging-drop vapour-diffusion method using Cryo Screen I (Hampton Research, Laguna Niguel, CA, USA). Crystals were obtained under several conditions, of which No. 17 was deemed to be the most promising for further screening. The crystallization conditions were refined using a sitting-drop technique. The best crystals were obtained using 1.5 µl of protein sample at 0.5 mg ml^{-1} in buffer *D*, which was mixed with 3.5 µl mother liquor containing 0.17 *M* $LiSO_4$, 85 m*M* Tris–HCl pH 8.5, 25.5%(*w*/*v*) PEG 4000 and 15.5%(*w*/*v*) glycerol. Crystal growth was initiated by streak seeding with a cat's whisker, using crystals grown in the same mother liquor but with a tenfold higher protein concentration.

2.4. X-ray diffraction data collection

A crystal was mounted in a nylon loop (Hampton Research, Laguna Niguel, CA, USA) and cooled to 100 K in an N_2 gas stream (Oxford Cryosystems, UK). A diffraction data set of high quality was collected at beamline X11 (EMBL Hamburg Outstation, Germany) using a CCD detector (marUSA Inc. Evanston, IL, USA). A wavelength of 0.8111 Å and a crystal-to-detector distance of 220 mm were used. Data integration was performed with *DENZO* and scaling and merging were performed with *SCALEPACK*; both programs are from the *HKL* program package (Otwinowski & Minor, 1997).

3. Results & discussion

Crystals of dimensions 0.3 × 0.2 × 0.2 mm were obtained (Fig. 1) after 2–3 d. These crystals belong to space group $I2_12_12_1$, diffract X-rays to better than 2.5 Å resolution and are suitable for structure determination. The unit-cell parameters are $a = 69.6$, $b = 86.6$, $c = 140.0$ Å. Assuming the presence of one subunit in the asymmetric unit, the solvent content is approximately 48%. The relevant data-collection statistics are presented in Table 1. Structure solution with the molecular-replacement method is in progress and we are also preparing for expression of a SeMet-enriched protein suitable for phase determination by the multiwavelength anomalous diffraction (MAD) method.

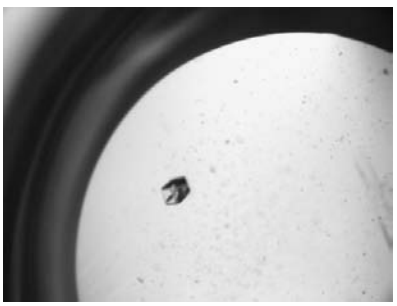

Figure 1
An orthorhombic crystal of a putative DNA methyltransferase from *H. pylori* (gene HP1352). The crystal dimensions are 0.3 × 0.2 × 0.2 mm. It diffracts X-rays to at least 2.5 Å resolution.

Table 1
Data-collection and processing statistics.

Values in parentheses refer to the highest resolution shell.

Space group	$I2_12_12_1$
Unit-cell parameters (Å)	$a = 69.6$, $b = 86.6$, $c = 140.0$
Resolution limits (Å)	20–2.5 (2.54–2.5)
No. of unique reflections	15099 (716)
Completeness (%)	97.1 (95.1)
R factor† (%)	4.1 (25.0)
$I/\sigma(I)$	31.4 (4.7)
Solvent content‡ (%)	48

† As defined in *SCALEPACK* (Otwinowski, 1997). ‡ Assuming one subunit per asymmetric unit.

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