Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Expression, crystallization and preliminary X-ray diffraction analysis of a modification subunit of a putative type I restriction enzyme from *Vibrio vulnificus* YJ016

Hyun-Ju Lee, Kosuke Nishi, Jung-Mi Song and Jeong-Sun Kim*

Department of Chemistry, Chonnam National University, Gwangju 500-757, Republic of Korea

Correspondence e-mail: jsunkim@chonnam.ac.kr

Received 12 September 2009
Accepted 20 October 2009

Modification (HsdM) and specificity (HsdS) subunits are constituents of an active methyltransferase (MTase) of multifunctional type I restriction enzymes. To provide a molecular background on HsdM, a putative *hsdM* gene from *Vibrio vulnificus* YJ016 (HsdM_Vv) was cloned and the expressed protein was purified and crystallized from 22%(*w*/*v*) polyethylene glycol 8000, 0.02 *M* imidazole pH 7.5 and 5 m*M* β-mercaptoethanol. Diffraction data were collected to 1.86 Å resolution using synchrotron radiation. The crystal belonged to the tetragonal space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 78.9$, $c = 165.8$ Å. With one molecule in the asymmetric unit, the crystal volume per unit protein weight was 2.12 Å^3 Da^{-1}, with a solvent content of 42%.

1. Introduction

Methyltransferases (MTases; methylases) transfer a methyl moiety from a donor to an acceptor molecule and are widespread in prokaryotes and eukaryotes. While *S*-adenosyl-L-methionine frequently serves as the methyl-group donor, various molecules such as DNA, RNA, proteins or small molecules act as methyl-group acceptors. Bacterial and eukaryotic DNA MTases are involved in diverse and essential biological processes; for example, information transfer between generations and the regulation of normal or aberrant gene expression (Jeltsch, 2002*a*,*b*).

Restriction enzymes, which are representatives of DNA MTases, have multifunctional activities: the recognition of specific sequences, methylation and cleavage (restriction) (Murray, 2000). Upon the recognition of a hemimethylated sequence by HsdS, a methyl group is placed on the nonmethylated base of a cognate sequence and cleavage of the bacterial DNA is avoided. On the other hand, restriction activity is exerted when neither of the recognition sequences is methylated (Wilson & Murray, 1991).

Two crystal structures from *Methanococcus jannaschii* (Kim *et al.*, 2005) and *Mycoplasma genitalium* (Calisto *et al.*, 2005) have provided molecular information on HsdS subunits. Although crystal structures of HsdM subunits from two other organisms have been deposited in the Protein Data Bank (PDB) and released to the public [PDB codes 2okc (Joint Center for Structural Genomics, unpublished work) and 2ar0 (K. R. Rajashankar, R. Kniewel & C. D. Lima, unpublished work)], primary publications are not available to describe these structures. The recent solution structure of *Eco*KI MTase in complex with a DNA-mimicking protein Ocr has provided a more detailed structural model for the reaction mechanism of MTase (Kennaway *et al.*, 2009). In order to add structural information and to provide a molecular background on multifunctional type I restriction enzymes, we carried out the crystallization and preliminary X-ray crystallographic analysis of an HsdM subunit of a putative type I restriction enzyme from *Vibrio vulnificus* YJ016 (HsdM_Vv).

2. Methods

2.1. Cloning, expression and purification of HsdM_Vv

The *V. vulnificus* YJ016 gene coding for a putative HsdM subunit (gi:37678450; Met1–Lys530) was amplified from *V. vulnificus* YJ016 chromosomal DNA by the polymerase chain reaction (PCR). The PCR product was then cloned into pProExHTc (Invitrogen), which expresses 25 extra amino acids containing six consecutive His residues at the N-terminus. The expression construct was transformed into *Escherichia coli* B834 (DE3) and grown in LB medium containing 100 µg ml^{-1} ampicillin at 310 K. After induction with 1.0 m*M* IPTG for a further 8 h at 310 K, the culture was harvested by centrifugation at 5000*g* and 277 K. The cell pellet was resuspended in ice-cold buffer *A* (20 m*M* Tris–HCl pH 7.5 and 500 m*M* NaCl) and disrupted by ultrasonication. The cell debris was removed by centrifugation at 11 000*g* for 1 h. The HsdM_Vv fusion protein was purified using a 5 ml HisTrap chelating column (GE Healthcare, Uppsala, Sweden). After treatment with rTEV to cleave the six-His tag and the removal of salts by dialysis, the protein containing five additional amino acids (YFQGA) at the N-terminus was purified using a 5 ml HiTrap Q anion-exchange column (GE Healthcare, Uppsala, Sweden). The purified protein was >95% pure as judged by Coomassie Blue-stained SDS–PAGE (data not shown).

2.2. Crystallization

For crystallization, the purified HsdM_Vv protein was concentrated to 12 mg ml^{-1} in a buffer consisting of 20 m*M* Tris–HCl pH 7.5 and 300 m*M* NaCl. The initial crystallization condition for HsdM_Vv was obtained from sparse-matrix screening (Jancarik & Kim, 1991) using screens from Hampton Research and Emerald BioSystems. In order to obtain crystals suitable for X-ray diffraction, the precipitant and protein concentration, buffer pH and temperatures were changed systematically and various equilibrium strategies, for example the hanging-drop and sitting-drop vapour-diffusion methods, were tried.

2.3. X-ray data collection

For diffraction experiments, crystals were briefly immersed into a precipitant solution containing 10%(*v*/*v*) glycerol as a cryoprotectant and immediately placed into a 100 K nitrogen-gas stream. Native X-ray diffraction data were collected on MAX4A at Pohang Accelerator Laboratory (PAL, Republic of Korea) using 1° oscillation per image with a crystal-to-detector distance of 180 mm. The crystal was exposed for 10 s per image. A data set was collected to 1.86 Å resolution from a single crystal. The data were indexed and scaled with *HKL*-2000 (Otwinowski & Minor, 1997). The data-collection statistics are summarized in Table 1.

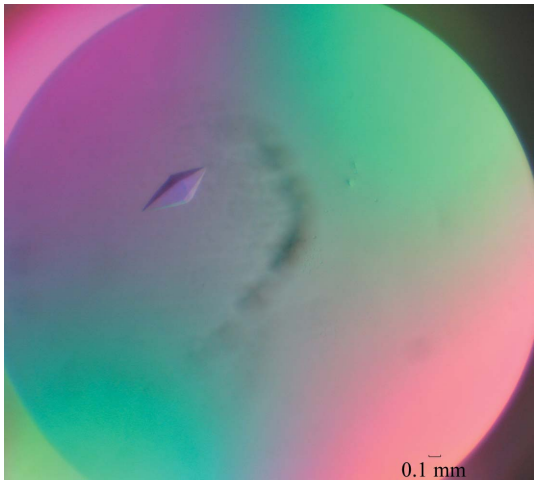

Figure 1
Crystals of HsdM_Vv. The crystals grew within one week at 291 K to maximum dimensions of approximately 0.4 × 0.2 × 0.2 mm.

Table 1
Data-collection statistics for HsdM_Vv.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.00
Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 78.9$, $c = 165.8$
Resolution range (Å)	50.0–1.86 (1.94–1.86)
Measured reflections	924644 (99076)
Unique reflections	44454 (4833)
Multiplicity	20.8 (20.5)
Temperature (K)	100
Matthews coefficient (Å^3 Da^{-1})	2.12
Solvent content (%)	42
No. of molecules in ASU	1
Completeness (%)	99.9 (100)
Mean $I/\sigma(I)$	29.1 (6.8)
$R_{\rm merge}$† (%)	9.0 (43.8)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity of an individual reflection and $\langle I(hkl)\rangle$ is the mean intensity of that reflection.

3. Results and discussion

Recombinant HsdM_Vv protein was successfully expressed and purified to homogeneity using sequential chromatographic steps. Crystals suitable for diffraction experiments were obtained using the sitting-drop vapour-diffusion method at 291 K within 7 d by mixing 2 µl protein solution and 2 µl reservoir solution and equilibrating against 200 µl reservoir solution, which consisted of 22%(*w*/*v*) polyethylene glycol 8000, 0.02 *M* imidazole pH 7.5 and 5 m*M* β-mercaptoethanol. The dimensions of the crystal used for data collection were

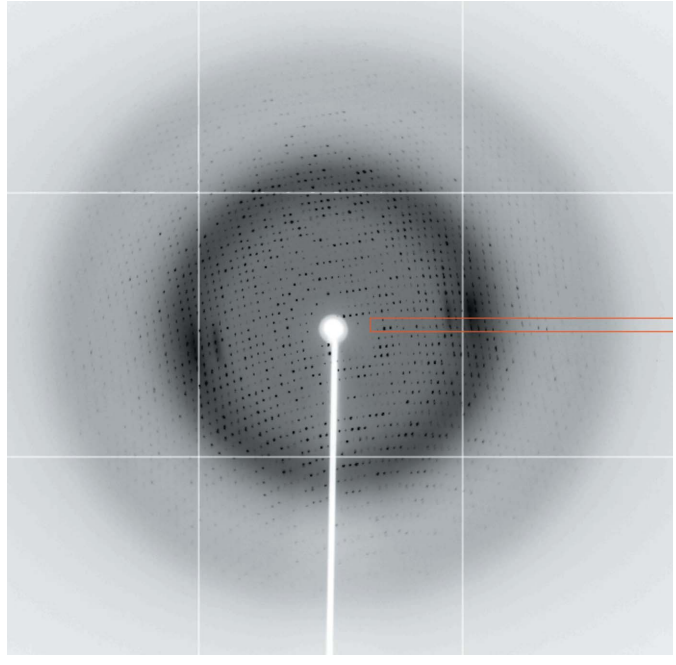

Figure 2
Representative X-ray diffraction image for HsdM_Vv. The crystal was exposed for 10 s over a 1° oscillation range. The edge of the detector corresponds to a resolution of 1.60 Å.

approximately 0.3 × 0.2 × 0.1 mm (Fig. 1) and it diffracted to 1.86 Å resolution (Fig. 2), which should be sufficient for structural studies of HsdM_Vv. The crystal belonged to the tetragonal space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 78.9$, $c = 165.8$ Å. The asymmetric unit contained one HsdM_Vv molecule of 60 kDa, resulting in a crystal volume per protein weight of 2.12 Å^3 Da^{-1} and a solvent content of 42% (Matthews, 1968). Elucidation of the crystal structure of HsdM_Vv is being attempted by molecular replacement using the two deposited HsdM structures, which have sequence identities of 31% and 30% for 420 and 284 aligned amino acids, respectively, as search models.

This work was partially supported by a Korea Research Foundation Grant funded by the Korean Government (MOEHRD; KRF-2007-331-C00209), in which the main calculations were performed by using the supercomputing resource of the Korea Institute of Science and Technology Information (KISTI), and partially by the CNU Grant funded by Chonnam National University (project administration No. 2005-0692).

References

Calisto, R. M., Pich, O. Q., Pinol, J., Fita, I. & Carpena, X. (2005). *J. Mol. Biol.* **351**, 749–762.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Jeltsch, A. (2002*a*). *ChemBioChem*, **3**, 274–293.
Jeltsch, A. (2002*b*). *ChemBioChem*, **3**, 382.
Kennaway, C. K., Obarska-Kosinska, A., White, J. H., Tuszynska, I., Cooper, L. P., Bujnicki, J. M., Trinick, J. & Dryden, D. T. (2009). *Nucleic Acids Res.* **37**, 762–770.
Kim, J. S., Giovanni, A. D., Jancarik, J., Adams, P. D., Yokota, H., Kim, R. & Kim, S.-H. (2005). *Proc. Natl Acad. Sci. USA*, **102**, 3248–3253.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Murray, N. E. (2000). *Microbiol. Mol. Biol. Rev.* **64**, 412–434.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Wilson, G. G. & Murray, N. E. (1991). *Annu. Rev. Genet.* **25**, 585–627.