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

Crystallization and preliminary crystallographic analysis of the (cytosine-5)-DNA methyltransferase NlaX from *Neisseria lactamica*

Galina S. Kachalova,[a,b]* Rimma I. Artyukh,[b] Natalia V. Lavrova,[c] Elena M. Ryazanova,[c] Anna S. Karyagina,[c] Elena A. Kubareva[d] and Hans D. Bartunik[a]

[a]Max-Planck Unit for Structural Molecular Biology, Protein Dynamics Group, Hamburg 22607, Germany, [b]Institute of Theoretical and Experimental Biophysics, Poushchino 142292, Russian Federation, [c]Institute of Agricultural Biotechnology, Moscow 127550, Russian Federation, and [d]A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119992, Russian Federation

Correspondence e-mail: galina@mpghdb.desy.de

Received 30 June 2005
Accepted 22 August 2005
Online 31 August 2005

Crystals of the (cytosine-5)-DNA methyltransferase NlaX from *Neisseria lactamica* (molecular weight 36.5 kDa) have been grown at 291 K using 2.5 *M* NaCl as precipitant. The crystals diffract to 3.0 Å resolution at 100 K. The crystals belong to space group *P*321, with unit-cell parameters a = 121.98, b = 121.98, c = 56.71 Å. There is one molecule in the asymmetric unit and the solvent content is estimated to be 62.1% by volume.

1. Introduction

DNA methylation has an almost ubiquitous occurrence in biology as a general tool for gene regulation (Jeltsch, 2002; Dryden, 1999; Vertino, 1999). In prokaryotes, the major roles of DNA methylation include the distinction between self and nonself DNA, the direction of post-replicative mismatch repair and the control of DNA replication and of the cell cycle. In mammals, DNA methylation in particular contributes to the modulation of gene expression and parental imprinting; aberrant DNA methylation is often involved in carcinogenesis.

S-Adenosyl-L-methionine (AdoMet) is the most commonly used methyl-donor molecule and AdoMet-dependent methyltransferases (MTases) act on a wide variety of target molecules (Cheng & Roberts, 2001). (Cytosine-5)-DNA methyltransferases (C5-MTases) catalyze the transfer of a methyl group from AdoMet to a DNA cytosine-ring carbon. The crystal structures of two prokaryotic C5-MTases, HhaI (Cheng *et al.*, 1993) and HaeIII (Reinisch *et al.*, 1995), and a human homologue, DNMT2 (Dong *et al.*, 2001), have been solved previously. DNMT2, which exhibits strong sequence and structural similarity to the prokaryotic C5-MTases but only weak *in vitro* methyltransferase activity, modifies DNA at CG sites in a loose ttnCGga(g/a) consensus sequence (Hermann *et al.*, 2003). The C5-MTases HhaI and HaeIII recognize and methylate the central cytosines of the canonical sites GCGC and GGCC, respectively (Wu & Santi, 1987; Slatko *et al.*, 1988). The crystal structures of the C5-MTases HhaI, HhaIII and DNMT2 all contain the enzyme in complexed form. These include binary complexes of the enzyme with AdoMet (Cheng *et al.*, 1993; O'Gara *et al.*, 1999), the AdoMet analogue AdoHcy (*S*-adenosyl-L-homocysteine; Dong *et al.*, 2001) or DNA (Reinisch *et al.*, 1995) and a tertiary complex of the enzyme with AdoHcy and DNA (Klimasauskas *et al.*, 1994; Kumar *et al.*, 1997; O'Gara, Klimasauskas *et al.*, 1996; O'Gara, Roberts *et al.*, 1996; O'Gara *et al.*, 1998; Sheikhnejad *et al.*, 1999). In all structures, the C5-MTases consist of one small and one large domain, with the DNA-binding cleft between the two domains. The AdoMet-binding pocket and the catalytic centre of each enzyme are located in the large domain. The small domains form sequence-specific contacts to the DNA, which mediate the target recognition (Jeltsch, 2002).

The object of our research is a prokaryotic C5-MTase from *Neisseria lactamica*, NlaX (Labbe *et al.*, 1990). NlaX is an AdoMet-dependent enzyme which recognizes a five-base-pair site (5′-CC*N*GG-3′, where *N* = A, T, C, G) in double-stranded DNA and methylates the second cytosine, forming 5′-methyl-2′-deoxycytidine (Kubareva *et al.*, 2002). The present paper describes the crystallization of NlaX and the preliminary results of diffraction data

collection. This is the first time that a C5-MTase has been crystallized as an apo enzyme. A crystal structure showing the open conformation could provide a valuable basis for investigating the mechanism of the formation of the active centre and the conformational transitions required for DNA binding.

2. Expression and purification

The plasmid carrying the gene for NlaX MTase was a kind gift from Professor P. C. K. Lau (Montreal, Canada). The gene for NlaX MTase was cloned into a pQE16 vector and the pQMNlaX plasmid was constructed (Karyagina *et al.*, 1995). Recombinant NlaX MTase contained 327 amino acids, including the additional sequence MetArgGlySer(His)$_6$ThrAspProLeu at its N-terminus compared with the native enzyme sequence (SwissProt Accession No. P24581). The molecular weight of the recombinant enzyme is 36.5 kDa. The NlaX MTase used in the present work was expressed in *Escherichia coli* M15 (pREP4, pQMNlaX). The cells were grown in 8.0 l LB medium [1%(*w*/*v*) Bacto tryptone, 0.5%(*w*/*v*) yeast extract, 0.5% NaCl, pH of the final medium 7.3] in the presence of kanamycin (25 mg ml^{-1}) and carbenicillin (100 mg ml^{-1}). The NlaX MTase expression was induced by IPTG (0.7 m*M*). After incubation for 22 h (180 rev min^{-1}, 293 K), the cells (6 g) were collected by centrifugation and suspended in 12 ml cold buffer *A* (20 m*M* Tris–HCl pH 8.6, 200 m*M* NaCl). The suspension was sonicated with an ultrasound disintegrator. The cell debris was removed by centrifugation. The supernatant was applied onto a Ni–NTA agarose column (15 mm width, 8 cm length) equilibrated with buffer *A* (20 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl). The column was then washed with buffer *B* (20 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl, 20 m*M* imidazole). The protein was eluted from the column using an imidazole gradient (0.02–0.5 *M*) in buffer *A*. SDS–PAGE analysis (Laemmli, 1970) indicated that NlaX MTase was eluted at 0.15–0.20 *M* imidazole. Fractions containing NlaX MTase were pooled, concentrated and loaded onto a Superdex 200 column (16 mm width, 60 cm length) equilibrated with 20 m*M* Tris–HCl buffer pH 8.0. The protein was eluted with the same buffer. The fractions containing NlaX MTase were applied onto a Heparin Sepharose 6 Fast Flow column (9 mm width, 2 cm length) equilibrated with buffer *A*. The protein was eluted from the column using an NaCl gradient (0.2–1.0 *M*) in buffer *A*. Fractions in which the NlaX MTase purity exceeded 95% (SDS–PAGE data) were pooled, dialyzed against 20 m*M* Tris–HCl buffer pH 8.0 containing 10%(*v*/*v*) glycerol and used for crystallization.

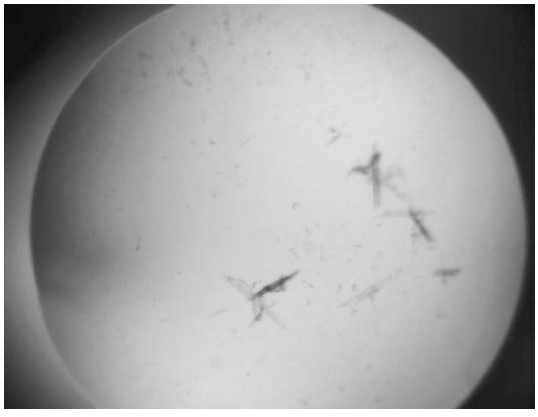

Figure 1
The crystals of NlaX MTase used for diffraction studies. Crystal dimensions are 0.05 × 0.07 × 0.15 mm.

Table 1
Data-collection statistics for DNA methyltransferase NlaX.

Values in parentheses are for data in the highest resolution shell.

Space group	*P*321
Unit-cell parameters (Å)	a = 121.98, b = 121.98, c = 56.71
Temperature (K)	100
Wavelength (Å)	1.05
Oscillation angle per image (°)	0.5
Crystal-to-dector distance (mm)	210
Resolution limits (Å)	20–3.00 (3.07–3.00)
Exposure time per image (s)	150
Total observations	30124
Unique reflections	9449
Completeness (%)	95.0 (94.7)
Average $I/\sigma(I)$	7.4 (2.2)
$R_{\rm merge}$†	17.2 (48.6)
Protomers per AU	1
$V_{\rm M}$ (Å^3 Da^{-1})	3.3

† $R_{\rm merge} = \sum_h \sum_j |I_{h,j} - \langle I_h \rangle| / \sum_h \sum_j I_{h,j}$.

3. Crystallization

The protein concentration was 3.6 mg ml^{-1}, as determined from its absorbance in 6.0 *M* guanidine–HCl at 280 nm assuming an ε_{280} of 0.648 for a 1.0 mg ml^{-1} solution. Initial crystallization conditions were screened using Crystal Screen (Hampton Research), Wizard I and Wizard II (deCODE Biostructures) and Crystal Screen Cryo (Hampton Research) reagent kits, applying the sitting-drop vapour-diffusion method. Crystals of good quality were obtained at pH 7.0 using 0.1 *M* Tris–HCl buffer, 0.2 *M* $MgCl_2$ and 2.5 *M* NaCl as precipitant. The crystallization drops comprised 1 µl protein solution and 1 µl reservoir solution. The mixture was equilibrated against 100 µl reservoir solution at 291 K. The crystals grew over several weeks as needles (Fig. 1). For diffraction experiments, crystals were flash-cooled directly from the drop solution.

4. Results and discussion

Diffraction data sets were measured for native crystals. All data were collected at 100 K using a MAR CCD detector on beamline BW6 at DORIS (DESY, Hamburg, Germany) using an X-ray wavelength of 1.05 Å. The images were processed and scaled using the *HKL* package (Otwinowski & Minor, 1997). The results are summarized in Table 1. The crystals belong to the trigonal space group *P*321, with unit-cell parameters a = 121.98, b = 121.98, c = 56.71 Å. A Matthews coefficient (Matthews, 1968) of 3.3 Å^3 Da^{-1} was calculated assuming the presence of one molecule in the asymmetric unit, corresponding to a solvent content of 62.1% by volume. This value agrees well with the Matthews coefficient of 3.08 Å^3 Da^{-1} determined as an average for protein–nucleic acid complexes (Kantardjiff & Rupp, 2003). Despite the 40% sequence identity between NlaX and HhaI MTases (PDB code 1svu), molecular replacement using the 1svu coordinates as a search model has not been successful, possibly as a consequence of large conformational differences caused by the absence of cofactor and substrate.

This work was supported by the Russian Foundation for Basic Research (Grant Nos. 04-04-48714 and 03-04-48476). We are grateful to Professor P. C. K. Lau (Biotechnology Research Institute, National Research Council Canada, Montreal, Quebec H4P 2R2, Canada) for the gift of the plasmid carrying the NlaX MTase gene.

References

Cheng, X., Kumar, S., Posfai, J., Pflugrath, J. W. & Roberts, R. J. (1993). *Cell*, **74**, 299–307.
Cheng, X. & Roberts, R. J. (2001). *Nucleic Acids Res.* **29**, 3784–3795.
Dong, A., Yoder, J. A., Zhang, X., Zhou, L., Bestor, H. & Cheng, X. (2001). *Nucleic Acids Res.* **29**, 439–448.
Dryden, D. T. F. (1999). *S-Adenosylmethionine-dependent Methyltransferases: Structures and Functions*, pp. 283–340. Singapore: World Scientific.
Hermann, A., Schmitt, S. & Jeltsch, A. (2003). *J. Biol. Chem.* **278**, 13717–13721.
Jeltsch, A. (2002). *ChemBioChem*, **3**, 274–293.
Kantardjiff, K. A. & Rupp, B. (2003). *Protein Sci.* **12**, 1865–1871.
Karyagina, A. S., Lunin, V. G., Levtchenko, I. Y., Labbe, D., Brousseau, R., Lau, P. C. & Nikolskaya, I. I. (1995). *Gene*, **157**, 93–96.
Klimasauskas, S., Kumar, S., Roberts, R. J. & Cheng, X. (1994). *Cell.* **76**, 357–369.
Kubareva, E. A., Walter, J., Karyagina, A. S., Vorob'eva, O. V., Lau, P. C. K. & Trautner, T. (2002). *BioTechniques*, **33**, 526–531.
Kumar, S., Horton, J. R., Jones, G. D., Walker, R. T., Roberts, R. J. & Cheng, X. (1997). *Nucleic Acids Res.* **25**, 2773–2783.
Labbe, D., Hoeltke, H. J. & Lau, P. C. K. (1990). *Mol. Gen. Genet.* **224**, 101–110.
Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
O'Gara, M., Horton, J. R., Roberts, R. J. & Cheng, X. (1998). *Nature Struct. Biol.* **5**, 872–877.
O'Gara, M., Klimasauskas, S., Roberts, R. J. & Cheng, X. (1996). *J. Mol. Biol.* **261**, 634–645.
O'Gara, M., Roberts, R. J. & Cheng, X. (1996). *J. Mol. Biol.* **263**, 597–606.
O'Gara, M., Zhang, X., Roberts, R. J. & Cheng, X. (1999). *J. Mol. Biol.* **287**, 201–209.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Reinisch, K. M., Chen, L., Verdine, G. L. & Lipscomb, W. N. (1995). *Cell*, **82**, 143–153.
Sheikhnejad, G., Brank, A., Christman, J. K., Goddard, A., Alvarez, E., Ford, H. Jr, Marquez, V. E., Marasco, C. J., Sufrin, J. R., O'Gara, M. & Cheng, X. (1999). *J. Mol. Biol.* **285**, 2021–2034.
Slatko, B. E., Croft, R., Moran, L. S. & Wilson, G. G. (1988). *Gene*, **74**, 45–50.
Vertino, P. M. (1999). *S-Adenosylmethionine-dependent Methyltransferases: Structures and Functions*, pp. 341–372. Singapore: World Scientific.
Wu, J. C. & Santi, D. V. (1987). *J. Biol. Chem.* **262**, 4778–4786.