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Crystallization and preliminary X-ray crystallographic analysis of archaeal O^6-methylguanine–DNA methyltransferase

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Abstract

Crystals of archaeal O^6-methylguanine–DNA methyltransferase (MGMT) from hyperthermophilic archaeon *Pyrococcus kodakaraensis* strain KOD1 have been grown at room temperature using polyethylene glycol as a precipitant. The diffraction pattern of the crystal extends to 2.0 Å resolution at room temperature upon exposure to Cu $K\alpha$ radiation. The crystal belongs to the space group $P2_12_12_1$ with unit-cell dimensions of $a = 52.8$, $b = 86.6$ and $c = 39.9$ Å. The presence of one molecule per asymmetric unit gives a crystal volume per protein mass (V_m) of 2.3 Å^3 Da^{-1} and a solvent content of 48% by volume. A full set of X-ray diffraction data was collected to 2.0 Å Bragg spacings from the native crystal.

1. Introduction

Alkylating agents, for example, *N*-methyl-*N*′,*N*-nitrosoguanidine, produce alkylated purine and pyrimidine bases in DNA. Among them, O^6-methylguanine appears to be most responsible for induction of mutations and cancers (Strauss *et al.*, 1975). O^6-Methylguanine can pair with thymine to induce G·C to A·T base-pair transitions (Coulondre & Miller, 1977). These transitions are blocked by the action of suicidal O^6-methylguanine–DNA methyltransferase (MGMT). This protein transfers an alkyl group substituted at the guanine O^6 to one of its own cysteine residues. Methylated MGMT is inactive in further O^6-methylguanine–DNA repair, because active-site cysteine is blocked by covalent methylation.

O^6-Methylguanine–DNA methyltransferase is present in various organisms ranging from bacteria to human cells. Recently, the crystal structure of the 178-amino-acid C-terminal domain (Ada-C) of Ada protein, coded for by the *ada* gene, a bacterial MGMT from *E. coli*, was determined (Moore *et al.*, 1994). However, no structural information on eukaryotic or archaeal MGMT has been reported.

P. kodakaraensis strain KOD1 was isolated from a solfataric hot spring of Kodakara Island, Kagoshima, Japan (Morikawa *et al.*, 1994). This strain belongs to archaea which constitutes a third primary kingdom of living organisms sharing characteristics with prokaryotic and eukaryotic cells (Woese & Fox, 1977; Woese *et al.*, 1990). The growth temperature and pH were 368 K and 7.0, respectively. Enzymes produced in the strain have been shown to be extremely thermostable and to have eukaryotic characteristics (Fujiwara *et al.*, 1996).

2. Materials and methods

2.1. *Crystallization*

The archaeal MGMT was overexpressed in *E. coli* strain HMS174(DE3)pLysS, purified (Leclere *et al.*, 1998), and dialyzed against 50 m*M* Tris–HCl buffer at pH 8.0 containing 0.1 m*M* EDTA. The dialyzed protein was concentrated to

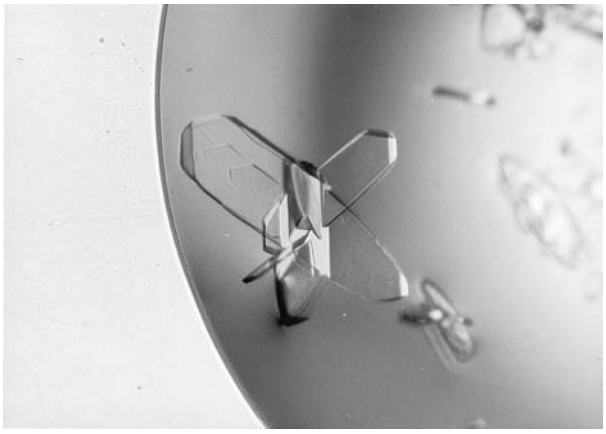

Fig. 1. Form I crystals of MGMT with the maximum dimensions 0.2 × 0.1 × 0.05 mm.

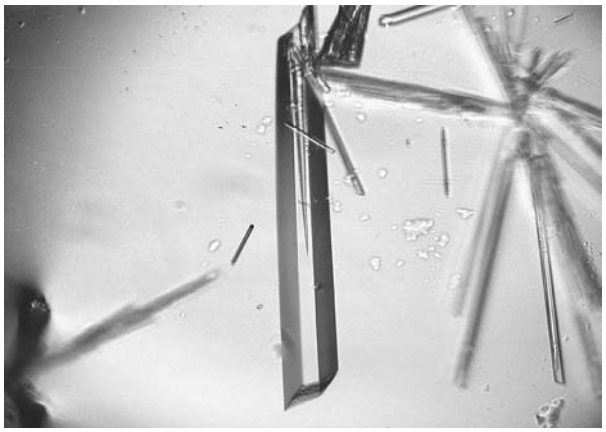

Fig. 2. Form II crystals of MGMT with the maximum dimensions 0.8 × 0.1 × 0.1 mm.

Table 1. *Diffraction data statistics of form II crystal*

Resolution (Å)	No. of reflections (unique)	Completeness (%)	R_{merge}
20.00–4.30	1335	94.1	0.042
4.30–3.42	1285	97.5	0.064
3.42–2.99	1286	98.5	0.080
2.99–2.71	1285	98.8	0.101
2.71–2.52	1252	98.4	0.119
2.52–2.37	1264	98.4	0.144
2.37–2.25	1254	97.6	0.162
2.25–2.15	1252	98.5	0.192
2.15–2.07	1240	98.7	0.230
2.07–2.00	1239	98.2	0.286
Overall	12692	97.8	0.086

10 mg ml^{-1}. All the crystallization experiments were carried out using the hanging-drop vapor-diffusion technique. The droplet (typically 4 µl) was prepared by mixing equal volumes (2 µl) of the protein and reservoir solutions. A number of precipitants, including salts, polyethylene glycol solutions, and organic solvents, were explored over a broad pH range (4.0–10.0). As a result, two forms of crystals appeared.

Form I crystals (Fig. 1) were obtained by the following procedure. The reservoir solution was prepared by mixing 400 µl of 50%(*w*/*w*) PEG 8000, 200 µl of 1.0 *M* Zn acetate, 100 µl of 1.0 *M* sodium cacodylate pH 6.5 and 300 µl distilled water, giving the final concentrations of 20% PEG 8000, 200 m*M* Zn acetate and 100 m*M* sodium cacodylate. Form I crystals were thin plates with a maximum size of 0.2 × 0.1 × 0.05 mm.

Form II crystals were obtained as follows. First, needle-shaped microcrystals were grown against reservoir solutions of 15% PEG 8000 and 200 m*M* ammonium sulfate. When 15%(*w*/*w*) PEG 20000 [300 µl of 50%(*w*/*w*) PEG 20000] was used instead of PEG 8000, rod-shaped crystals appeared. When the concentration of PEG 20000 was decreased to 12%(*w*/*w*), crystals reached dimensions of 0.8 × 0.1 × 0.1 mm in a few weeks.

2.2. *X-ray crystallographic studies*

All the X-ray diffraction measurements were made at room temperature on a Rigaku R-AXIS IIc imaging-plate detector system coupled to a Rigaku RU-300 fine-focused rotating-anode X-ray generator with a Cu target. The Laue symmetry and unit-cell parameters were determined from three still images by the *PROCESS* program package (Higashi, 1989; Sato *et al.*, 1992). The full X-ray diffraction data were processed and scaled to give a unique set of data using *DENZO* and *SCALEPACK* (Otwinowski, 1993) and systematic extinctions in the intensity data were checked by *HKLPLOT* (Eleanor Dodson, unpublished data; Collaborative Computational Project, Number 4, 1994).

3. Results

When form I crystals (Fig. 1) were exposed to X-rays, the diffraction spots were observed to Bragg spacings of at least 2.0 Å. The space group was determined to be *P*1 with unit-cell parameters of a = 43.8, b = 54.2 and c = 43.8 Å, α = 75.4, β = 79.8 and γ = 85.5°. The asymmetric unit contains two molecules with a mass of 19 500, giving a crystal volume per protein mass (V_m) of 2.5 Å^3 Da^{-1} (Matthews, 1968). As the mosaicity of the crystals was estimated at more than 1° by *SCALEPACK*, the structure determination using form I crystals was abandoned.

Diffraction intensities from form II crystals (Fig. 2) were also observed to at least 2.0 Å Bragg spacings. Unit-cell parameters were determined as a = 52.8, b = 86.6 and c = 39.9 Å, in space group $P2_12_12_1$. The asymmetric unit contains a single molecule with a mass of 19 500, giving a crystal volume per protein mass (V_m) of 2.3 Å^3 Da^{-1} and the solvent content of 48% by volume. The mosaicity of the crystals was estimated to be about 0.2°; form II crystals were found to be suitable for X-ray studies. As listed in Table 1, a data set was collected. It consists of 73 204 measurements of 12 692 unique reflections with an overall R_{merge} of 8.6% ($R_{merge} = \sum |I - \langle I \rangle| / \sum I$) and overall $I/\sigma(I)$ of 9.9. This represents 97.8% of theoretically observable reflections at 2.0 Å resolution. The outermost shell of data between 2.07 and 2.00 Å is 98.2% complete. Attempts were made to solve the MGMT structure by molecular replacement with *AMoRe* (Navaza, 1994; Collaborative Computational Project, Number 4, 1994) and *X-PLOR* (Brünger, 1990) using Ada-C from *E. coli* as a probe structure; however no consistent set of rotation-function solutions could be obtained. MGMT from KOD1 has a low amino-acid sequence homology with Ada-C except around the active site; homology with Ada-C is about 13%. For this reason, the molecular-replacement technique may not be successful in this case. Structure determination of the MGMT by multiple isomorphous replacement methods is under way. To date, one heavy-atom derivative has been identified.

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