Crystallization and Preliminary X-Ray Crystallographic Studies of *Thermus thermophilus* HB8 MutM Protein Involved in Repairs of Oxidative DNA Damage

Mitsuaki Sugahara,*,† Tsutomu Mikawa,*,[1] Ryuichi Kato,* Keiichi Fukuyama,* Takashi Kumasaka,† Masaki Yamamoto,† Yorinao Inoue,† and Seiki Kuramitsu*,‡,[2]

**Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560–0043; ‡Genomic Sciences Center (RIKEN), 2–1 Hirosawa, Wako, Saitama 351–0198; and †RIKEN Harima Institute, 1–1–1 Kouto, Mikazuki, Sayo-gun, Hyogo 679–5148*

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MutM protein, which removes the oxidatively damaged DNA base product, 8-oxoguanine (GO), has been crystallized by means of a hanging-drop vapor-diffusion procedure using polyethyleneglycol monomethylether 2000 as a precipitant in 2-(cyclohexylamino) ethanesulfonic acid (CHES) buffer, pH 9.8. The diffraction data derived from oscillation photographs indicate that the crystals belong to the monoclinic system and space group $P2_1$. The crystals have unit-cell dimensions of $a = 45.4$ Å, $b = 62.0$ Å, $c = 99.7$ Å, and $\beta = 90.8°$. Assuming that the asymmetric unit contains two molecules, the V_m value was calculated to be 2.35 Å^3•Da^{-1}. The crystals diffracted X-rays to at least 2.1 Å resolution and were suitable for high-resolution X-ray crystal structure determination.

Key words: crystallization, DNA repair enzyme, MutM protein, oxidative DNA damage, X-ray diffraction, *Thermus thermophilus* HB8.

DNA is always damaged by reactive oxygen species produced on aerobic energy metabolism or oxidative stress in aerobic organisms, including human beings. One of the most general products of oxidative DNA damage is the 8-oxoguanine (GO) lesion. As GO is able to pair with cytosine (C) as well as adenine (A), it causes transversion from guanine (G) to thymine (T). This G-to-T transversion accelerates the rate of spontaneous mutation, and therefore has been implicated in aging and the promotion of diseases, including cancer (*1*). To prevent mutation, aerobic organisms have DNA repair enzymes that remove GO bases from GO:C pairs in DNA. In yeast, mouse and human, Oggl protein and its homologues show this activity, and their genes have recently been cloned (*2–6*). In *Escherichia coli*, MutM protein exhibits DNA glycosylase activity and aprinic/apyrimidinic (AP) nicking activity, by which GO lesions are efficiently removed from GO:C pairs (*7, 8*). Recently, it was demonstrated that the amino group of the N-terminal proline of MutM protein acts as a nucleophile in these reactions (*9*). An experiment involving site-directed mutagenesis of *E. coli* MutM protein suggested that Lys155 of the protein directly interacts with the C8 oxygen of GO (*10*).

Recently, we cloned and sequenced the *mutM* gene from an extremely thermophilic bacterium, *Thermus thermophilus* HB8 (*11*). The deduced amino acid sequence of the *T. thermophilus* MutM protein exhibit 42% homology with the corresponding protein of *E. coli*. Thus, the functions as well as the three-dimensional structures of these proteins are assumed to be similar in these organisms.

MutM protein of *T. thermophilus* HB8 was overproduced in *E. coli*, and its GO-specific DNA glycosylase activity has been characterized (*11*). The purified MutM protein is a single polypeptide chain consisting of 266 amino-acid residues. As expected, this protein is stable in wide temperature (up to 75°C at pH 7.5) and pH (pH 5–11 at 25°C) ranges. The results of limited proteolysis and urea denaturation of the MutM protein suggested that it consists of at least two domains (Sugahara, M., unpublished observations). The N-terminal domain has two residues (Pro1 and Lys155 in *E. coli* MutM protein), which directly interact with GO lesions. The C-terminal domain has a zinc-finger motif, which participates in the DNA binding. However, the detailed mechanisms by which MutM protein recognizes and removes GO lesions remained to be elucidated. In order to understand the structure-function relationships of this protein at the atomic level, we started the analysis of its three-dimensional structure by X-ray crystallography. Here we describe the crystallization and preliminary X-ray diffraction analysis of the *T. thermophilus* MutM protein.

MutM protein was purified as described previously (*11*). The purified enzyme in 50 mM Tris-HCl buffer (pH 7.6), containing 5 mM 2-mercaptoethanol, was filtered and concentrated by ultrafiltration up to about 10 mg•ml^{-1}. Crystallization of the MutM protein was performed by the hanging-drop vapor-diffusion method at 20°C. The screening for initial crystallization conditions was performed with 50 stock solutions (pH 4.6, 5.5, 6.5, 7.5, 8.5, and 9.5) including precipitants of purified polyethyleneglycols (PEG-400,

[1] Present address: Cellular & Molecular Biology Laboratory, The Institute of Physical and Chemical Research (RIKEN), 2–1 Hirosawa, Wako, Saitama 351–0198.

[2] To whom correspondence should be addressed. Tel: +81-6-6850-5433, Fax: +81-6-6850-5442, E-mail: kuramitu@bio.sci.osaka-u.ac.jp

Abbreviations: GO, 8-oxoguanine; PEG, polyethyleneglycol; CHES, 2-(cyclohexylamino) ethanesulfonic acid.

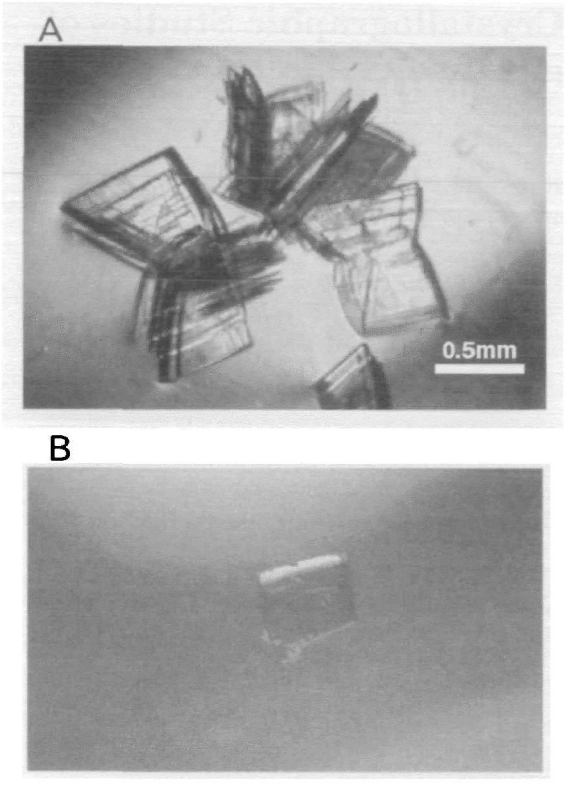

Fig. 1. **Typical crystals of the MutM protein from *T. thermophilus* HB8.** The crystal consists of many thin plates (A). A single plate crystal (B) extruding from these blocks was used for the X-ray diffraction experiment. The approximate size of the plate crystal was 0.5 × 0.3 × 0.02 mm³.

2000, 4000, and 8000), salts (ammonium sulfate, sodium chloride, sodium formate, and lithium sulfate), and organic solvents (2-methyl-2, 4-pentanediol and 2-propanol). MutM protein formed crystals in a few days in solutions containing PEG and sodium formate at pH9.5. The crystals, however, consisted of many thin plates (Fig. 1A). By optimizing the crystallization conditions, we were able to grow single crystal regions large enough for an X-ray diffraction experiment (Fig. 1B). The reservoir solution that gave the best crystals contained 5% (w/v) polyethyleneglycol monomethylether 2000, 100 mM 2-(cyclohexylamino) ethanesulfonic acid (CHES) buffer (pH9.8), and 5 mM 2-mercaptoethanol.

Initial intensity data were collected with a laboratory X-ray source at room temperature. A crystal was sealed in a glass capillary tube with a small amount of the mother liquid. X-ray diffraction studies were carried out with a Rigaku R-AXIS IV imaging-plate system using Ni-filtered Cu Kα radiation. The X-rays, generated with a Rigaku rotating anode at 40 kV and 100 mA, were focused with Ni-coated double-bent mirrors. Diffraction data recorded on the imaging plates were processed and scaled with DENZO and SCALEPACK (*12*). The crystals belong to the monoclinic system, with unit-cell dimensions of a = 45.4 Å, b = 62.0 Å, c = 99.7 Å, and β = 90.8°. Assuming that two MutM protein molecules are present in an asymmetric unit, the V_m value and solvent content were calculated to be 2.35 Å³•Da⁻¹ and 48.8%, respectively (*13*). The systematic absence of diffraction data is compatible with space group $P2_1$. We collected the data set up to 3.2 Å resolution (crystal

TABLE I. **Intensity measurements of MutM protein crystals.**

	Crystal 1	Crystal 2	Crystal 3
Temperature (K)	296	100	100
Wavelength (Å)	1.5418	1.5418	1.04
Oscillation angle (°)	1.5	1.5	2.0
Resolution limit (Å)	3.2	3.0	2.1
Measured reflections	33,991	47,474	301,427
Independent reflections	9,319	10,715	31,112
Completeness (%)†	87.0 (75.3)	72.6 (53.8)	91.5 (85.8)
Mean I/σ (I)†	7.9 (2.5)	6.9 (2.3)	32.3 (8.3)
R_{merge} (%)†‡	9.5 (24.5)	8.7 (25.8)	5.8 (23.6)

†Reflections with $F<\sigma$ (F) were rejected. Numerals in parentheses indicate the data in the highest-resolution shell. ‡$R_{merge} = \Sigma_h\Sigma_i|I_{hi} - \langle I_h\rangle|/\Sigma_h\Sigma_i I_{hi}$.

irradiation, it was mounted in the loop and flash-cooled to 100 K using a Cryostream Cooler (Oxford Cryosystems). Before flash-cooling, the crystal was soaked for several seconds in a cryoprotectant solution prepared by the addition of glycerol to the mother liquid to a final concentration of 20% (v/v). Upon freezing of the crystal, the cell dimensions changed slightly to a = 44.4 Å, b = 61.3 Å, c = 98.3 Å, and β = 91.7°. We collected the diffraction data set for the frozen crystals up to 3.0 Å resolution (crystal 2 in Table I).

We achieved higher-resolution data collection for frozen crystals using synchrotron radiation at the BL45XU of the SPring-8 (*14*). The diffraction intensities recorded on IP were collected with a Rigaku R-AXIS IV imaging-plate system, and then processed and scaled with DENZO and SCALEPACK (*12*). The intensity measurement results are presented in Table I (see crystal 3). Table I shows that the present crystals yield diffraction data up to 2.1 Å resolution and are suitable for X-ray analysis at atomic resolution. We are now conducting structural analysis by means of the multi-wavelength anomalous dispersion method, utilizing an intrinsic zinc atom of the MutM protein.

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