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Crystallization and preliminary X-ray studies of MutT1 (MSMEG_2390) from *Mycobacterium smegmatis*

MutT1 (MSMEG_2390) from *Mycobacterium smegmatis* has been crystallized and the crystals have been characterized using X-ray diffraction. The crystals belonged to space group $P2_12_12_1$. The Matthews coefficient suggested the possibility of one protein molecule in the asymmetric unit of the orthorhombic unit cell. Solution of the structure using the known three-dimensional structure of a bacterial MutT1 is anticipated.

1. Introduction

Reactive oxygen species produced during cellular metabolism or present as environmental pollutants cause severe damage to the integrity of DNA. Guanine, which is highly susceptible to oxidative stress, is converted to 7,8-dihydro-8-oxoguanine (8-oxo-G) or its derivatives by these reactive oxygen species. 8-Oxo-G is a potent mutagen because of its ambiguous pairing with cytosine and adenine during replication (Dos Vultos *et al.*, 2006). Eubacteria possess an elaborate 8-oxo-G (GO) repair system consisting of Fpg (MutM), MutY and MutT to deal with such threats to genomic integrity (Michaels & Miller, 1992; Kurthkoti & Varshney, 2011). Fpg is known to excise 8-oxo-G paired with cytosine or thymine, while MutY removes adenine or guanine incorporated against 8-oxo-G during replication (Au *et al.*, 1989; Lu & Chang, 1988; Tsai-Wu *et al.*, 1992; Kurthkoti *et al.*, 2010; Jain *et al.*, 2007). MutT hydrolyses 8-oxo-dGTP to 8-oxo-dGMP in the nucleotide pool, minimizing its direct incorporation into DNA (Michaels & Miller, 1992). Mycobacteria contain all of the proteins specific to the GO repair pathway (Cole *et al.*, 1998). MutT proteins are members of the Nudix hydrolases, a superfamily of Mg^{2+} -requiring enzymes that are found in all classes of organism, including viruses, bacteria, archaea and eukaryotes, that catalyze the hydrolysis of nucleoside diphosphates linked to other moieties, **X** (Mildvan *et al.*, 2005; McLennan, 2006). These enzymes have been shown to play a role in preventing the misincorporation of oxidized dGTPs (or dNTPs) by hydrolyzing them to nucleoside monophosphates and pyrophosphate. The *Mycobacterium smegmatis* genome encodes four putative Nudix hydrolases: MutT1, MutT2, MutT3 and MutT4 (Cole *et al.*, 1998; Dos Vultos *et al.*, 2006; Davis & Forse, 2009). It has been shown by knockout studies that both MutT1 and MutT4 have an antimutator role in *M. smegmatis* and that MutT1 and MutT2 have 8-oxoguanosine triphosphatase activity (Dos Vultos *et al.*, 2006). Here, we report the crystallization and preliminary X-ray studies of MutT1 from *M. smegmatis* (MsMutT1) as an extension of our ongoing structural studies of mycobacterial proteins (Vijayan, 2005; Krishna *et al.*, 2007; Kaushal *et al.*, 2008; Prabu *et al.*, 2009; Roy *et al.*, 2008; Selvaraj *et al.*, 2007; Chandran, Sharma *et al.*, 2010; Chetnani *et al.*, 2010; Chandran, Prabu *et al.*, 2010).

2. Materials and methods

2.1. Cloning, expression and purification

The MsMutT1 (MSMEG_2390) open reading frame was amplified by PCR from *M. smegmatis* mc²155 genomic DNA using the forward

primer *MsMutT1Fp* (5'-GATCCATATGTCGAAGGACACCGA-3') and the reverse primer *MsMutT1Rp* (5'-GTTGAAGCTTTACTTCTCGTC-3') containing *NdeI* and *HindIII* sites, respectively (indicated in bold). Subsequently, the 957 bp *NdeI*–*HindIII* fragment containing the *MsMutT1* open reading frame was cloned into a derivative of pTrc99C (pTrcHis) using *NdeI* and *HindIII* restriction endonucleases and was confirmed by DNA sequencing. During cloning, the peptide stretch MGSSHHHHHSSGLVPRGSH was appended to the N-terminus of the protein sequence (NCBI accession No. YP_886730; UniProt reference A0QUZ2; A. G. Patil & U. Varshney, unpublished work). The N-terminally hexa-His-tagged *MsMutT1* was expressed in *Escherichia coli* and was purified using Ni-NTA column chromatography following a standard protocol (Bornhorst & Falke, 2000).

2.2. Crystallization

Crystallization conditions were screened using commercially available screening kits from Hampton Research employing the microbatch-under-oil and hanging-drop vapour-diffusion methods. In both cases needle-shaped crystals (Fig. 1*a*) grew in about 7 d in a drop consisting of 2 µl 7 mg ml^{−1} protein solution in 0.025 M Tris–HCl buffer pH 7.5, 0.5 M NaCl, 10% (v/v) glycerol, 0.002 M β-mercaptoethanol and 2 µl precipitant solution consisting of 0.1 M MES, 0.2 M ammonium sulfate, 30% PEG monomethyl ether 5000, but these crystals diffracted poorly. Diffraction-quality thin rectangular crystals

(a)

(b)

Figure 1
Crystals of *MsMutT1* obtained (a) on initial screening and (b) after optimization.

Table 1

Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

No. of crystals	1
X-ray generator	Broker Microstar Ultra II rotating anode
Wavelength (Å)	1.54179
Detector	MAR 345
Crystal-to-detector distance (mm)	175
Rotation range per image (°)	1
Total rotation range (°)	270
Exposure time per image (s)	600
Resolution range (Å)	49.56–2.05 (2.16–2.05)
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
Unit-cell parameters (Å)	<i>a</i> = 39.09, <i>b</i> = 91.07, <i>c</i> = 99.12
Mosaicity (°)	0.59
Total No. of measured intensities	220566 (27677)
Total No. of unique reflections	22878 (3165)
Multiplicity	9.6 (8.7)
Mean <i>I</i> /σ(<i>I</i>)	16.1 (4.9)
Completeness (%)	99.5 (96.5)
<i>R</i> _{merge} [†] (%)	9.7 (47.7)
<i>R</i> _{meas} [‡] (%)	10.3 (50.6)
Overall <i>B</i> factor from Wilson plot (Å ²)	27.2

[†] $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where $I_i(hkl)$ is the *i*th intensity measurement of the reflection with indices *hkl* and $\langle I(hkl) \rangle$ is the weighted average intensity of all *i* intensity measurements of the reflection with indices *hkl*. [‡] $R_{\text{meas}} = \frac{\sum_{hkl} \{N(hkl)/[N(hkl) - 1]\}^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where *N* is the multiplicity.

(Fig. 1*b*) grew in about 15 d upon the addition of 0.01 M TCEP–HCl to the crystallization solution in the microbatch-under-oil method.

2.3. X-ray data collection and processing

Diffraction data were collected at 100 K using a Bruker Microstar Ultra II Cu Kα rotating-anode X-ray generator equipped with a MAR 345 detector. A single crystal with dimensions of around 0.5 × 0.1 × 0.01 mm was used for data collection. No separate cryoprotectant was required for data collection at 100 K. The intensity data were processed and merged using *MOSFLM* (Leslie, 2006) and *SCALA* (Evans, 1993) in the *CCP4* program suite (Winn *et al.*, 2011). Structure-factor amplitudes were obtained from intensities using *TRUNCATE* (French & Wilson, 1978) from the *CCP4* suite. The crystal parameters, data-collection parameters and processing statistics are summarized in Table 1.

2.4. Domain identification and sequence comparison

Domains were defined using the Conserved Domain Database (CDD) web server (Marchler-Bauer *et al.*, 2011). Sequence comparisons were carried out using *ClustalW* (Larkin *et al.*, 2007).

3. Results and discussion

MutT1 from *M. smegmatis* has successfully been crystallized and the crystals were characterized using X-ray diffraction. The symmetry and systematic absences indicated the space group to be *P*2₁2₁2₁, with unit-cell parameters *a* = 39.09, *b* = 91.07, *c* = 99.12 Å. A value of 2.32 Å³ Da^{−1} for the Matthews coefficient suggested the possibility of one protein molecule in the asymmetric unit of the orthorhombic unit cell (Matthews, 1968).

CDD (Marchler-Bauer *et al.*, 2011) identified an N-terminal Nudix hydrolase domain and a C-terminal histidine phosphatase domain in the sequence. Interestingly, the first domain corresponds to the single-domain MutT of *E. coli* (Nakamura *et al.*, 2010). As is the case for proteins in the histidine phosphatase superfamily, the C-terminal domain contains the conserved RHG motif (Rigden, 2008). Some of the proteins in this superfamily contain an extra subdomain which

covers the active site. This often serves to modulate the substrate specificity (Hamada *et al.*, 2005). Sequence comparisons do not indicate the presence of this subdomain in MsMutT1. Among proteins of known three-dimensional structure, the putative MutT1 from *Bifidobacterium adolescentis* (PDB entry 3fjy; New York SGX Research Center for Structural Genomics, unpublished work) exhibits the maximum sequence identity to the sequence of MsMutT1 involving both domains. The absolute value of the identity is somewhat low at 28%. However, attempts will be made to solve the structure by molecular replacement using the known structure of the putative MutT1 from *B. adolescentis* as the search model.

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