Crystallization and preliminary X-ray characterization of MutT2, MSMEG_5148 from *Mycobacterium smegmatis*

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Crystallization of MutT2, MSMEG_5148 from *Mycobacterium smegmatis*, has been carried out and the crystals have been characterized using X-ray diffraction. Matthews coefficient calculation suggests the possibility of one protein molecule in the asymmetric unit of the orthorhombic unit cell, space group $P2_12_12$ or $P2_12_2$. Solution of the structure of the protein by molecular replacement using the known three-dimensional structure of a bacterial Nudix hydrolase is envisaged.

1. Introduction

Excessive accumulation of non-canonical nucleotides such as oxidatively damaged nucleoside triphosphates (NTPs) and deoxynucleoside triphosphates (dNTPs) in the nucleotide pool impairs the fidelity of DNA replication and RNA transcription (Nakabeppu *et al.*, 2010). These non-canonical nucleotides are generated by the reactive oxygen species and reactive nitrogen intermediates produced during cellular metabolism (Wink *et al.*, 1991; Cooke *et al.*, 2003; Kurthkoti *et al.*, 2008). If not sanitized, they may be incorporated into DNA during replication, resulting in ambiguous base pairing, leading to the subsequent accumulation of mutations.

In addition to DNA-repair mechanisms, cells have also developed an elaborate enzymatic system that sanitizes non-canonical nucleoside triphosphates from the nucleotide pool by hydrolyzing them to the corresponding diphosphates or monophosphates and hence avoiding their misincorporation into DNA or RNA (Zhang *et al.*, 2013). MutT-like proteins which perform this function have been extensively studied (Bessman *et al.*, 1996). They belong to the Nudix hydrolases, a superfamily of Mg^{2+} -requiring enzymes that are ubiquitous in nature and are found in all classes of organisms and catalyze the hydrolysis of nucleoside diphosphate linked to other moieties, X (Mildvan *et al.*, 2005; McLennan, 2006). It has been reported that the genome of *Mycobacterium smegmatis* encodes four putative Nudix hydrolases, MutT1, MutT2, MutT3 and MutT4, that correspond to *Escherichia coli* MutT (Cole *et al.*, 1998; Dos Vultos *et al.*, 2006; Davis & Forse, 2009). Previous studies have shown that MutT1 and MutT2 have 8-oxo-guanosine triphosphatase activity (Dos Vultos *et al.*, 2006; Patil *et al.*, 2013). However, recent studies indicate that *M. smegmatis* MutT2 (*MsMutT2*) hydrolyzes dCTP more efficiently than 8-oxo-dGTP and 8-oxo-GTP (Sang & Varshney, 2013). It also hydrolyzes 5-methyl-dCTP (Sang & Varshney, 2013). Thus, inside mycobacteria *MsMutT2* plays a role in hydrolyzing non-canonical nucleotides such as 8-oxo-dGTP, 8-oxo-GTP and 5-methyl dCTP and may have a regulatory role in maintaining the physiological levels of canonical dCTP. This dual functionality of the enzyme is still unexplained and the regulation of the two enzymatic activities has yet to be explored. Here, we report the crystallization and preliminary X-ray studies of MutT2 from *M. smegmatis* (*MsMutT2*) in a continuation of our ongoing structural studies of various mycobacterial proteins (Vijayan, 2005; Krishna *et al.*, 2007; Selvaraj *et al.*, 2007; Roy *et al.*, 2008; Kaushal *et al.*, 2008; Prabu *et al.*, 2009; Chetnani *et al.*, 2010; Arif *et al.*, 2012; Abhinav *et al.*, 2013).

2. Materials and methods

2.1. Crystallization

MsMutT2 was cloned, overproduced and purified as reported previously (Sang & Varshney, 2013). Crystallization conditions were explored using kits commercially available from Hampton Research employing the microbatch-under-oil method. Diffraction-quality thin rectangular crystals (Fig. 1) of the protein grew in about 5 d in a drop consisting of 2 μ l of 25 mg ml⁻¹ protein solution in 0.020 M Tris-HCl pH 8.0, 0.150 M NaCl, 10% (v/v) glycerol, 0.002 M β -mercaptoethanol and 2 μ l crystallant solution consisting of 0.1 M MES monohydrate pH 6.0, 20% (v/v) Jeffamine M-600 pH 7.0.

2.2. X-ray data collection and processing

Initially, intensity data were collected at a home source to a resolution of 1.76 Å using a MAR345 detector mounted on a Bruker MICROSTAR ULTRA II Cu $K\alpha$ rotating-anode X-ray generator. Subsequently, high-resolution diffraction data were collected from a single crystal with dimensions of around $0.7 \times 0.05 \times 0.01$ mm on synchrotron X-ray beamline BM14 (tuned for maximum flux at 0.8266 Å with a 150 μ m beam aperture and equipped with a CCD detector) at the European Synchrotron Radiation Facility, Grenoble, France. No cryoprotectant was required for data collection at 100 K. The intensity data were processed and merged using *iMosflm* (Battye *et al.*, 2011) 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* program suite. The crystal parameters, data-collection parameters and processing statistics are summarized in Table 1.

3. Results and discussion

MutT2 from *M. smegmatis* has been crystallized and the crystals were characterized using X-ray diffraction. The crystals belonged to space group $P2_12_12$ or $P2_122$ as indicated by symmetry and systematic absences, with unit-cell parameters $a = 65.84$, $b = 59.87$, $c = 31.50$ Å. A value of $1.94 \text{ Å}^3 \text{ Da}^{-1}$ for the Matthews coefficient suggests the possibility of one protein molecule in the asymmetric unit of the orthorhombic unit cell (Matthews, 1968).

Among proteins of known three-dimensional structure, the *E. coli* Orf135 protein (PDB entry 2rrk; K. Kawasaki & M. Mishima, unpublished work) exhibits the maximum sequence identity to the sequence of *MsMutT2*. The values of identity and similarity are

Figure 1
Crystals of *MsMutT2*.

Table 1

Data-collection and processing statistics.

Values in parentheses are for the outermost resolution shell.

No. of crystals	1
X-ray source	BM14, ESRF
Wavelength (Å)	0.8266
Detector	MAR 225 CCD
Crystal-to-detector distance (mm)	101.7
Rotation range per image (°)	1
Total rotation range (°)	240
Exposure time per image (s)	3
Resolution range (Å)	16.46–1.10 (1.16–1.10)
Space group	$P2_12_12$ or $P2_122$
Unit-cell parameters (Å)	$a = 65.84$, $b = 59.87$, $c = 31.50$
Average mosaicity (°)	0.70
Total No. of measured intensities	443305 (60456)
Total No. of unique reflections	49097 (6891)
Multiplicity	9.0 (8.8)
Mean $I/\sigma(I)$	16.0 (3.0)
Completeness (%)	95.9 (93.4)
$R_{\text{merge}}^{\dagger}$ (%)	6.2 (77.0)
$R_{\text{meas}}^{\ddagger}$ (%)	6.6 (81.7)

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

37.5% and 51.4%, respectively. Structure solution of *MsMutT2* using molecular replacement with the known solution structure of *E. coli* Orf135 protein, a CTP diphosphatase (PDB entry 2rrk), as the search model is envisaged.

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