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Crystallization and initial X-ray diffraction analysis of the tellurite-resistance *S*-adenosyl-L-methionine transferase protein TehB from *Escherichia coli*

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TehB is an *S*-adenosyl-L-methionine (SAM) dependent methyltransferase that detoxifies tellurite in bacteria. The *Escherichia coli* TehB protein was purified and crystallized in the presence of both SAM and sinefungin. The TehB–SAM and TehB–sinefungin crystals both diffracted X-rays to 1.9 Å resolution. The TehB–SAM crystals belonged to space group $C2$, with unit-cell parameters $a = 60.0$, $b = 56.1$, $c = 130.6$ Å, $\beta = 97.9^\circ$. The TehB–sinefungin crystals belonged to space group $P2_1$, with unit-cell parameters $a = 59.1$, $b = 55.5$, $c = 129.7$ Å, $\beta = 95.9^\circ$.

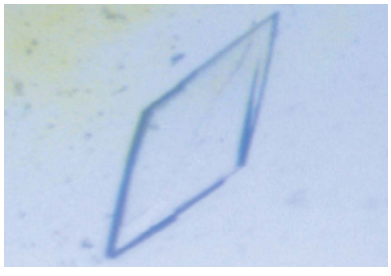
1. Introduction

Tellurite (TeO_3^{2-}) is found in low abundance in the environment (Taylor, 1999). It has been found to be very toxic to the majority of bacteria, with only small quantities being required for a detrimental effect on microorganisms (Silver, 1998; Taylor, 1999). Tellurite toxicity has been suggested to be a result of the generation of reactive oxygen species (ROS; Perez *et al.*, 2007) that act as strong oxidizing agents and can lead to the oxidation of many cellular thiols (Turner *et al.*, 1999), disrupting and causing the stoppage of protein/DNA synthesis and many reductases. Tellurite has also been suggested to replace sulfur in various biological reactions, with fatal effects on the cell (Taylor, 1999).

Bacteria have developed mechanisms that can detoxify tellurite either by reducing it to elemental tellurium (Te^0) or by methylation. Elemental tellurium is insoluble in water and appears as black deposits within cells (Borsetti *et al.*, 2003; Baesman *et al.*, 2007). In *Escherichia coli* the periplasmic nitrate reductase NapA has been associated with the reduction of tellurium to tellurite with the aid of a membrane-bound nitrate reductase (Avazeri *et al.*, 1997). Methylated tellurite has been detected as a volatile gas in GC/MS headspace gas analysis in bacteria that harbour plasmids that express methyltransferases (Cournoyer *et al.*, 1998; Ollivier *et al.*, 2008).

In *E. coli*, the membrane-bound protein TehA and the cytoplasmic methyltransferase TehB have been reported as the tellurite-resistance proteins (Turner *et al.*, 1995, 1997; Liu *et al.*, 2000; Dyllick-Brenzinger *et al.*, 2000). Liu *et al.* (2000) showed that TehB is a SAM-dependent methyltransferase and that tellurite can be methylated. In their study, they did not detect any volatile tellurite species. The modification and removal of tellurite by TehA and TehB occurs continuously.

In this study, TehB from *E. coli* has been purified and crystallized in the presence of SAM and of sinefungin (a SAM analogue) and data have been collected to 1.9 Å resolution for both complexes. We aim to determine the structure of the TehB enzyme and to shed light on the detailed mechanism of this methyltransferase.



2. Materials and methods

2.1. Production of native TehB

The *tehB* gene (gene identifier b1430) from *E. coli* MG1655 was cloned into the pEHis/TEV vector (Liu & Naismith, 2009), forming a *tehB* plasmid construct containing an N-terminal His_6-tag sequence followed by a TEV (tobacco etch mosaic virus) protease cleavage site between the His tag and TehB. The *tehB* plasmid was transformed and expressed in *E. coli* BL21 (DE3) PlysS host cells.

A freshly transformed colony was selected and used to inoculate 10 ml sterilized LB medium containing 34 µg ml^{-1} kanamycin. The cell culture was grown overnight with shaking at 200 rev min^{-1} at 310 K. The overnight culture was used to inoculate 1 l sterilized LB medium containing 34 µg ml^{-1} kanamycin in a 2.5 l baffled flask. The culture was shaken at 200 rev min^{-1} at 310 K until the OD_{600} reached 0.8 and overexpression was induced using 1 m*M* isopropyl *β*-D-1-thiogalactopyranoside for 4 h at 310 K. The cells were harvested at 6200*g* for 10 min at 277 K. The cell pellet was collected and stored at 213 K.

2.2. Protein purification

The cell pellet was resuspended in 200 ml 1× ice-cold phosphate-buffered saline (PBS) containing 0.1 mg ml^{-1} Pefabloc SC (Sigma), 20 U ml^{-1} DNase (Sigma) and 1 m*M* magnesium chloride. The cells were passed twice through a cell disruptor (Constant Systems) at 151 and 172 MPa. The residual unbroken cells, cellular debris and cell membranes were removed by centrifugation at 150 000*g* for 1 h. The supernatant containing the soluble protein fraction was collected. All subsequent steps were performed at 277 K.

The supernatant was brought to 20 m*M* imidazole in PBS and passed over a 5 ml His-Trap column HP (GE Healthcare) equilibrated with 20 m*M* imidazole in PBS. The column was then washed with five column volumes of 20 m*M* imidazole in PBS and finally with five column volumes of 30 m*M* imidazole in PBS. The protein was eluted with five column volumes of 500 m*M* imidazole in PBS. The protein was incubated with TEV protease at a 1:100 TEV protease: protein concentration ratio while being dialysed in 4 l gel-filtration buffer consisting of 20 m*M* Tris pH 7.5, 150 m*M* sodium chloride, 1 m*M* DTT and 5 m*M* ethylenediaminetetraacetic acid (EDTA) overnight.

The cleaved material was passed through a His-Trap column equilibrated with gel-filtration buffer to remove TEV protease, His_6 tag and any uncleaved protein. The flowthrough containing the cleaved protein was collected. The column was washed with a further 10 ml of buffer and collected. The flowthrough was concentrated to 500 µl using a 10 kDa cutoff concentrator (Millipore).

The concentrated protein was injected onto a Superdex 75 10/300 GL column (GE Healthcare) equilibrated with gel-filtration buffer without EDTA and DTT. A single peak containing TehB was observed on the chromatogram. The fraction containing TehB was analysed by SDS–PAGE and concentrated to 17 mg ml^{-1} using a 10 kDa cutoff concentrator (Millipore).

2.3. Crystallization

Initial crystallization screens were carried out with and without adding 1 m*M* SAM (Sigma) to the protein. Vapour diffusion in sitting drops was used to screen for initial conditions using a 96-well Innovadyne plate at 293 and 277 K. Conditions were screened using the PACT and JCSG+ (Qiagen) screens. The plates were set up using

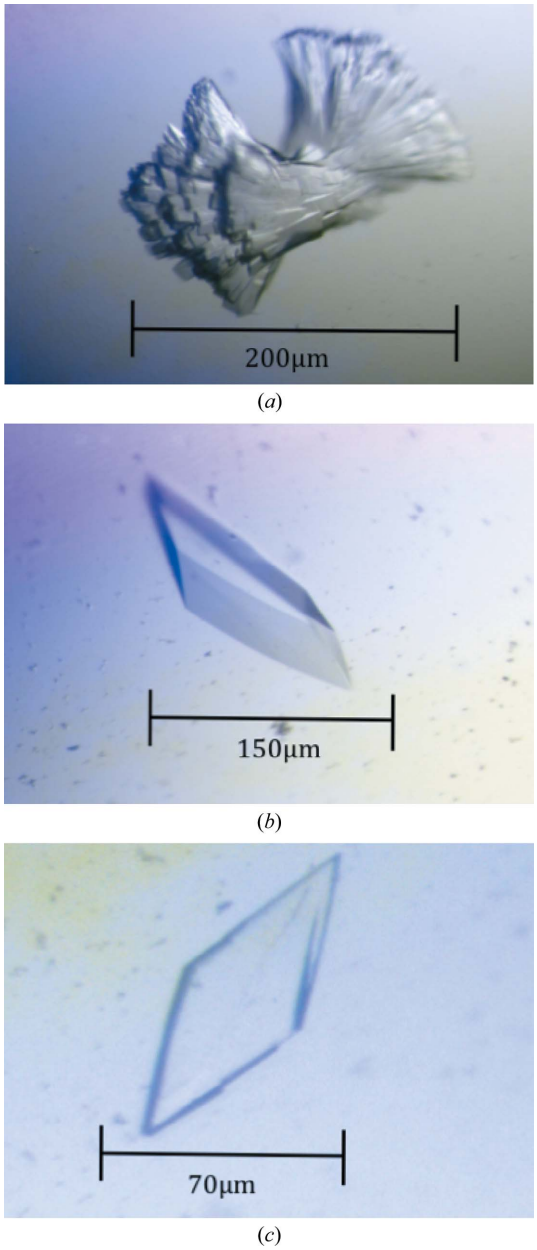


Figure 1
(*a*) Initial TehB crystals obtained in the presence of 1 m*M* SAM. (*b*) Single TehB crystal after microseeding. (*c*) TehB crystal obtained in the presence of 5 m*M* sinefungin after microseeding.

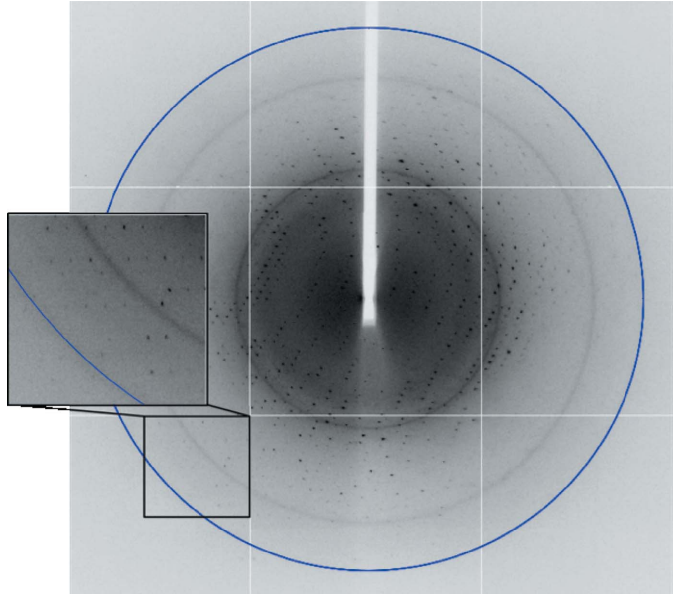


Figure 2
Diffraction pattern of TehB–SAM. The blue circle indicates the diffraction limit at 1.9 Å resolution. The inset shows the spots at high resolution.

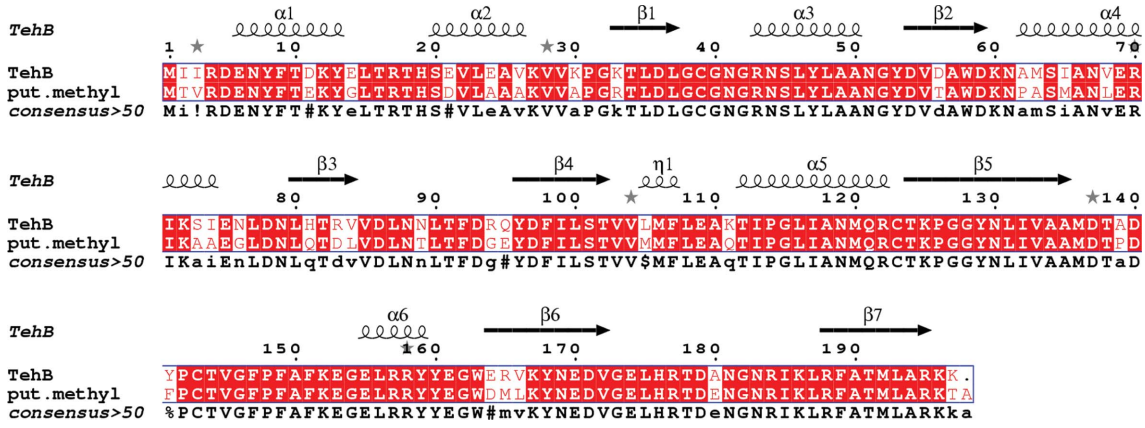


Figure 3
Sequence alignment of TehB from *E. coli* with the putative methyltransferase from *S. typhimurium* LT2. Conserved residues are shown in red boxes and the secondary structure is shown at the top of the sequence.

a Mosquito robot (TTP Labtech) to dispense drops consisting of 100 nl protein solution and 100 nl precipitant solution.

Initial crystals of TehB containing SAM (TehB–SAM) appeared after 4 d in 0.2 *M* NaF, 22% PEG 3350 at 293 K. Optimized crystallization condition screening was carried out by hanging-drop vapour diffusion using a 24-well Linbro plate (Hamilton Research), mixing 1 µl protein solution and 1 µl precipitant solution. Crystals appeared as multiple crystal clusters that were joined together (Fig. 1*a*). The addition of additives did not improve the crystal quality. Seeding was then carried out to try to obtain single crystals. A 2% lower PEG concentration than the optimal condition was used as the new precipitant condition (0.1 *M* NaF and 16% PEG 3350). Crystals from a single drop containing 0.1 *M* NaF and 18% PEG 3350 were taken, placed in 10 µl crystallization buffer and vortexed for a few minutes. 0.5 µl of this seed stock was added to a drop consisting of 1 µl protein solution mixed with 1 µl precipitant solution. Single large crystals were obtained overnight (Fig. 1*b*). TehB was also crystallized in the presence of 5 m*M* sinefungin (Sigma), a known cofactor analogue of SAM (Schluckebier *et al.*, 1997), under similar conditions as used for the TehB–SAM crystals: 0.1 *M* NaF and 16% PEG 3350. Microseeding was essential to obtain single crystals (Fig. 1*c*).

2.4. Data collection and processing

The crystals were transferred into paraffin oil for cryoprotection before being frozen in liquid nitrogen for storage and data collection. The TehB–SAM data were collected on beamline I04 with a wavelength of 1 Å (Fig. 2) and the TehB–sinefungin data were collected on beamline I03 with a wavelength of 0.97 Å at 100 K at Diamond Light Source. The data sets were collected using a CCD detector (ADSC Q315 CCD). All data were collected using a 0.5° oscillation range. The data were processed using *XDS* (Kabsch, 2010) and scaled using *SCALA* (Evans, 1993) under the *xia*2 interface (Winter, 2010). The data-collection statistics are summarized in Table 1.

3. Results and discussion

The crystals of TehB–SAM belonged to space group *C*2 and those of TehB–sinefungin belonged to space group $P2_1$. The asymmetric unit of the TehB–SAM crystals was calculated to contain two molecules (V_M = 2.16 Å^3 Da^{-1}), with an estimated solvent content of 43.2%. The asymmetric unit of the TehB–sinefungin crystals was calculated to contain four molecules (V_M = 2.39 Å^3 Da^{-1}), with an estimated solvent content of 48.5%. Molecular replacement of TehB–SAM using a putative methyltransferase from *Salmonella typhimurium* LT2 (PDB code 2i6g; Joint Centre for Structural Genomics, unpublished work) as a model was performed using *Phaser* (McCoy *et al.*, 2007). The two sequences share 91% homology and 84% identity. A sequence alignment of the two sequences is shown in Fig. 3. Molecular replacement located two copies of the putative methyltransferase within the asymmetric unit of TehB–SAM and four copies within the asymmetric unit of TehB–sinefungin, with *Z* scores of 33.4 and 39.5, respectively. Model building and refinement of both complexes is currently under way.

Table 1
Data-collection statistics for TehB–SAM and TehB–sinefungin.

Values in parentheses are for the highest resolution shell.

	TehB + 1 m*M* SAM	TehB + 5 m*M* sinefungin
Beamline	Diamond I04	Diamond I03
Wavelength (Å)	1.00	0.97
Resolution (Å)	41.0–1.9 (2.0–1.9)	42.1–1.9 (2.0–1.9)
Space group	*C*2	$P2_1$
Unit-cell parameters (Å, °)	*a* = 60.0, *b* = 56.1, *c* = 130.6, α = 90.0, β = 97.9, γ = 90.0	*a* = 59.1, *b* = 55.5, *c* = 129.7, α = 90.0, β = 95.9, γ = 90.0
Molecules in asymmetric unit	2	4
Measured reflections	95865 (13894)	120226 (17559)
Unique reflections	33256 (4901)	62310 (9309)
Completeness (%)	97.4 (98.9)	94.6 (97.2)
Mean $I/\sigma(I)$	6.3 (2.4)	7.4 (2.1)
Multiplicity	2.9 (2.8)	1.9 (1.9)
R_{merge}† (%)	11.3 (43.6)	8.3 (41.2)
Mosaicity (°)	0.89	0.48

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

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References

Avazeri, C., Turner, R. J., Pommier, J., Weiner, J. H., Giordano, G. & Vermeglio, A. (1997). *Microbiology*, **143**, 1181–1189.
Baesman, S. M., Bullen, T. D., Dewald, J., Zhang, D., Curran, S., Islam, F. S., Beveridge, T. J. & Oremland, R. S. (2007). *Appl. Environ. Microbiol.* **73**, 2135–2143.

Borsetti, F., Borghese, R., Francia, F., Randi, M. R., Fedi, S. & Zannoni, D. (2003). *Protoplasma*, **221**, 153–161.
Cournoyer, B., Watanabe, S. & Vivian, A. (1998). *Biochim. Biophys. Acta*, **1397**, 161–168.
Dyllick-Brenzinger, M., Liu, M., Winstone, T. L., Taylor, D. E. & Turner, R. J. (2000). *Biochem. Biophys. Res. Commun.* **277**, 394–400.
Evans, P. R. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 114–122. Warrington: Daresbury Laboratory.
Kabsch, W. (2010). *Acta Cryst.* D**66**, 125–132.
Liu, H. & Naismith, J. H. (2009). *Protein Expr. Purif.* **63**, 102–111.
Liu, M., Turner, R. J., Winstone, T. L., Saetre, A., Dyllick-Brenzinger, M., Jickling, G., Tari, L. W., Weiner, J. H. & Taylor, D. E. (2000). *J. Bacteriol.* **182**, 6509–6513.
McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
Ollivier, P. R., Bahrou, A. S., Marcus, S., Cox, T., Church, T. M. & Hanson, T. E. (2008). *Appl. Environ. Microbiol.* **74**, 7163–7173.
Perez, J. M., Calderon, I. L., Arenas, F. A., Fuentes, D. E., Pradenas, G. A., Fuentes, E. L., Sandoval, J. M., Castro, M. E., Elias, A. O. & Vasquez, C. C. (2007). *PLoS One*, **2**, e211.
Schluckebier, G., Kozak, M., Bleimling, N., Weinhold, E. & Saenger, W. (1997). *J. Mol. Biol.* **265**, 56–67.
Silver, S. (1998). *J. Ind. Microbiol. Biotechnol.* **20**, 1–12.
Taylor, D. E. (1999). *Trends Microbiol.* **7**, 111–115.
Turner, R. J., Taylor, D. E. & Weiner, J. H. (1997). *Antimicrob. Agents Chemother.* **41**, 440–444.
Turner, R. J., Weiner, J. H. & Taylor, D. E. (1995). *Microbiology*, **141**, 3133–3140.
Turner, R. J., Weiner, J. H. & Taylor, D. E. (1999). *Microbiology*, **145**, 2549–2557.
Winter, G. (2010). *J. Appl. Cryst.* **43**, 186–190.