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## Overproduction, purification and preliminary X-ray diffraction analysis of a sulfotransferase from *Mycobacterium tuberculosis* H37Rv

Sulfotransferase STF1 from the *Mycobacterium tuberculosis* H37Rv genome was overproduced in *Escherichia coli*, purified and crystallized using the hanging-drop vapour-diffusion method. The crystals diffract to 1.5 Å resolution using synchrotron radiation at SPring-8. The crystals are monoclinic and belong to space group  $P2_1$ , with unit-cell parameters  $a = 40.86$ ,  $b = 95.76$ ,  $c = 48.04$  Å,  $\beta = 106.43^\circ$ . The calculated Matthews coefficient is approximately  $2.1 \text{ \AA}^3 \text{ Da}^{-1}$  assuming the presence of one molecule of STF1 in the asymmetric unit. A substrate-binding assay using a PAP-agarose column suggests that STF1 exhibits sulfotransferase activity.

### 1. Introduction

Sulfotransferases (STs) catalyze a transfer reaction of the sulfuryl group from the ubiquitous 3'-phosphoadenosine 5'-phosphosulfate (PAPS) donor to various acceptor substrates. A large number of STs have been isolated from mammals and characterized with respect to protein structures and enzymatic properties. STs are classified into Golgi-membrane-associated proteins and cytosolic proteins based on their cellular localization. The Golgi-membrane enzymes sulfonate carbohydrates and tyrosyl residues on proteins, whereas the cytosolic STs sulfonate low-molecular-weight molecules such as phenol and steroid hormones. Although the Golgi and cytosolic enzymes display little sequence similarity, they share highly conserved amino acids in the PAPS-binding sites (Kakuta *et al.*, 1998). Moreover, X-ray crystallographic studies of STs indicate a significant conformational similarity between enzymes belonging to the two types of STs (Negishi *et al.*, 2001).

Although a vast amount of information has been accumulated on mammalian STs, very few studies of bacterial STs have thus far been pursued. NodH, isolated from *Rhizobium* species, is involved in the sulfation of Nod factor, which plays an essential role in the recognition of a host plant and infection. In addition, it is known that STs are involved in the creation of unique sulfoglycolipids that are associated with *Mycobacterium* virulence. The sulfated glycolipids accumulated on the membrane surface can be correlated with the virulence of *M. tuberculosis* (Mougous *et al.*, 2004). Thus, the sulfoglycolipids are regarded as virulence factors. However, the mechanism of sulfoglycolipid synthesis and virulence are unknown.

Several putative genes encoding ST were identified in the genome of *M. tuberculosis* and related species (Mougous *et al.*, 2002). The *M. tuberculosis* genome encodes four putative STs (STF0, STF1, STF2 and STF3). One ST, referred to as STF2, has previously been overproduced in *Escherichia coli* (Rivera-Marrero *et al.*, 2002). STF2 can sulfonate commercial glycosphingolipids as well as glycolipids prepared from *M. smegmatis*. To gain further insight into the structure–function relationships of bacterial STs, we overproduced and crystallized a putative ST from *M. tuberculosis*, referred to as STF1. Furthermore, the potential for STF1 to act as a sulfotransferase was evaluated by a substrate-binding assay using a PAP-agarose column. In the present paper, we report the crystallization and preliminary X-ray diffraction analysis of STF1.



## 2. Materials and methods

### 2.1. Cloning and expression

PCR primers were designed based on the DNA sequence of the STF1 gene (Rv3529c) annotated from the *M. tuberculosis* H37Rv genome database. The primer sequences were 5'-primer, 5'-ggatcc-ATGACTCGGCGTCCCGATCGGAAA-3' (*Bam*HI site in lower case), and 3'-primer, 5'-gaattcTCACAGCCCAGCGCAACCGCTC-TTT-3' (*Eco*RI site in lower case). The target gene was amplified by PCR using the following profile for 30 cycles after a preheat at 369 K: 1 min at 369 K, 30 s at 328 K, 30 s at 345 K (Ex Taq DNA polymerase, Takara). The PCR product (approximately 1100 bp) was cloned into pGEM-T Easy (Promega) and subcloned into pGEX4T3 (Amersham Biosciences) with *Bam*HI and *Eco*RI sites to create a GST-STF1 fusion protein. The nucleotide sequence of the STF1 gene was confirmed using a CEQ 2000KL (Beckman). The resulting expression plasmid was transformed into *E. coli* BL21 (DE3) Codon Plus RIL cells. The transformant was cultured in 2×YT medium containing 100 µg ml<sup>-1</sup> ampicillin and grown at 310 K while shaking at 200 rev min<sup>-1</sup> without pre-culturing. After 5 h ( $A_{600} \approx 0.5$ ), the culture was cooled to 293 K, kept for 2 h with shaking and then supplemented with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for induction. After 15 h fermentation, the cells were harvested by centrifugation.

### 2.2. Purification

All purifications were performed at 283 K. Harvested cells were suspended in a sonication buffer consisting of 50 mM Tris-HCl pH 8.0 and 150 mM NaCl and disrupted by sonication. The lysate was placed in an ultracentrifuge (35 000 rev min<sup>-1</sup>) for 30 min at 277 K. The harvested supernatant was gently stirred on ice with 1 ml glutathione Sepharose 4B affinity resin (Amersham Biosciences) equilibrated with sonication buffer. The resin was washed using the batch method with three washes of sonication buffer, two washes with sonication buffer containing 0.5% Triton X-100 and a final wash with sonication buffer. Finally, the resin with bound fusion protein was suspended in 5 ml sonication buffer containing 20 units thrombin (Sigma) and digested for 12 h at 283 K. After digestion, the supernatant was passed through a Benzamidine Sepharose 6B (Amersham Biosciences) column to remove thrombin and concentrated to 0.6 mg ml<sup>-1</sup> by ultrafiltration (Amicon Ultra-4, 10 kDa cutoff, Millipore). The concentrated protein was then run through a Superose gel-filtration column (Amersham Biosciences) using the sonication buffer. The flowthrough containing the target protein was collected and equilibrated with water by ultrafiltration. The purity of the prepared sample was judged using 10% SDS-PAGE stained with Coomassie Brilliant Blue. The N-terminal sequence of the purified

protein was determined with a PSQ-1 gas-phase sequencer (Shimadzu).

The molecular weight was determined by gel-filtration and SDS-PAGE as described above. Chymotrypsinogen A (19.9 kDa) and ovalbumin (45.8 kDa) (Amersham Biosciences) were used as marker proteins.

### 2.3. Substrate-binding assay with PAP-agarose column

STF1 was loaded onto a PAP-agarose column (0.5 ml, adenosine 3',5'-diphosphate-agarose, Sigma) equilibrated with 50 mM Tris-HCl pH 7.4. The column was then washed with 2.5 ml buffer and STF1 was eluted with a stepwise gradient of NaCl (50 mM per step). The effluents were analyzed by SDS-PAGE as described. The protein was then dialyzed in water and concentrated to 1.1 mg ml<sup>-1</sup>.

### 2.4. Crystallization

Crystallization conditions were screened with the sitting-drop vapour-diffusion method using Index Screen Kits (Hampton Research) at 293 K. Drops were prepared by mixing 0.8 µl reservoir with 0.8 µl solution containing 1.1 mg ml<sup>-1</sup> protein in water and were equilibrated by vapour diffusion against reservoir solution. After one week, small crystals of STF1 were observed with reservoir conditions consisting of 0.1 M bis-Tris-HCl pH 6.5 and 25% (w/v) PEG 3350 or 20% (w/v) PEG MME 5000. Using the hanging-drop vapour-diffusion method, small crystal nuclei from these crystals were used to microseed fresh drops of the protein and reservoir solution (1 µl each). The drops were then equilibrated at 293 K. Finally, a single crystal suitable for X-ray diffraction measurement was obtained using a reservoir solution consisting of 0.1 M bis-Tris-HCl pH 7.1, 20% (w/v) PEG MME 5000.

### 2.5. X-ray diffraction analysis

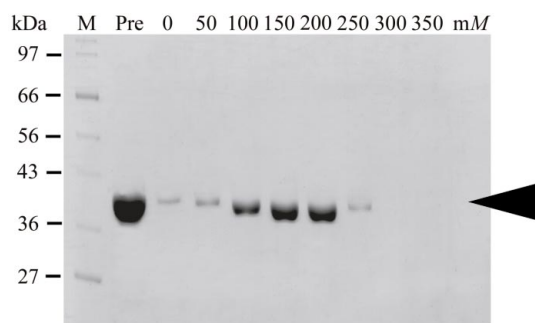
Crystals were harvested in cryoloops and soaked for 1 min in a solution consisting of 0.1 M bis-Tris-HCl pH 7.1 and 32.5% (w/v) PEG MME 5000. The crystal mounted in a cryoloop was flash-cooled in a nitrogen-gas stream at 93 K. X-ray diffraction data were collected using a MAR CCD system and synchrotron radiation (1.000 Å wavelength) at beamline BL41XU in SPring-8. The oscillation angle was 1.0° and the exposure time was 2.0 s per frame. A total of 180 diffraction images were recorded at a camera distance of 100 mm and were processed using *HKL2000* (Otwinowski, 1993).

## 3. Results and discussion

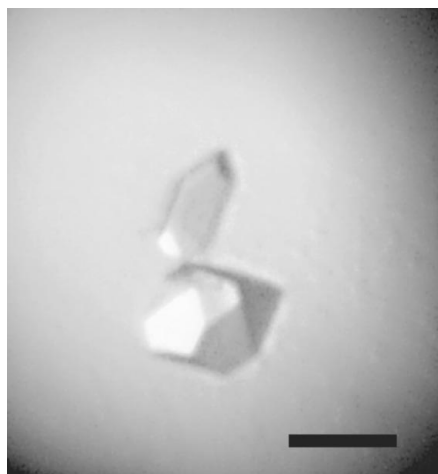
A putative sulfotransferase STF1 was overproduced in *E. coli* and purified. The molecular weight of STF1 (43.5 kDa from the primary structure) was determined to be 41.7 kDa by SDS-PAGE (Fig. 1) and 38.0 kDa by gel filtration (data not shown), suggesting that STF1 exists as a monomer in solution.

Based on the primary sequence, it appears that STF1 contains the PAPS-binding motifs conserved among known STs. The 5' PSB motif consisting of residues R<sup>101</sup>TGTTAL<sup>107</sup> and the 3' PB motif consisting of residues R<sup>256</sup>PVETIMAS<sup>264</sup> were detected. We performed substrate-binding assay with a PAP-agarose column. The column is generally used for affinity chromatography of STs. STF1 was adsorbed on the column and eluted with 200 mM NaCl (Fig. 1). This suggests that STF1 harbours sulfotransferase activity.

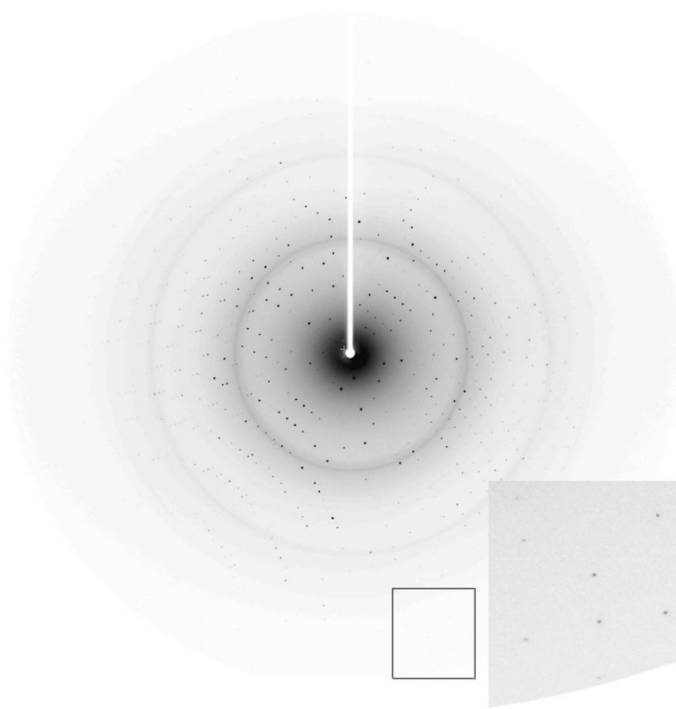
The crystallization conditions for overproduced STF1 were obtained by the sparse-matrix method and optimized to produce a single crystal suitable for X-ray analysis. Crystals suitable for X-ray



**Figure 1** SDS-PAGE of PAP-agarose assay. The arrow indicates the eluted STF1.



**Figure 2**  
Crystals of STF1. Scale bar: 0.1 mm.



**Figure 3**  
Diffraction pattern of the STF1 crystals. The frame edge is at 1.5 Å resolution.

analysis were obtained from the optimized crystallization conditions (Fig. 2;  $0.2 \times 0.3 \times 0.1$  mm). The crystals produced diffraction data at 1.5 Å resolution at beamline BL41XU, SPring-8 (Fig. 3). Processing of the diffraction data defined the crystals as belonging to the monoclinic space group  $P2_1$ , with unit-cell parameters  $a = 40.86$ ,

**Table 1**  
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.000
Space group	$P2_1$
Resolution (Å)	50.0–1.50 (1.55–1.50)
No. measured reflections	231636 (18612)
No. unique reflections	56164 (5474)
Completeness	99.3 (98.3)
$I/\sigma(I)$	26.1 (29.0)
$R_{\text{merge}}^\dagger$	0.034 (0.125)

$^\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$ , where  $I_i(hkl)$  is the  $i$ th intensity measurement of reflection  $hkl$ , including symmetry-related reflections, and  $\langle I(hkl) \rangle$  is its average.

$b = 95.76$ ,  $c = 48.04$  Å,  $\beta = 106.43^\circ$  (Table 1). The calculated Matthews coefficient was approximately  $2.1 \text{ \AA}^3 \text{ Da}^{-1}$  assuming the presence of one molecule of STF1 in the asymmetric unit. Recently, Mougous and coworkers reported the crystal structure of sulfotransferase (STF0) of *M. smegmatis* in relation to sulfolipid synthesis (Mougous *et al.*, 2004). STF1 has almost no sequence similarity to any of the sulfotransferases of known tertiary structures, including STF0, except in the PAPS-binding motif. We have tried molecular-replacement methods for phase determination, but have not succeeded. The crystal structure is now being solved by the MAD method with selenium as the anomalous scattering atom using synchrotron radiation.

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