Purification, crystallization and X-ray crystallographic studies on a putative methyltransferase, YtqB, from *Bacillus subtilis*

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S-Adenosyl-L-methionine (SAM)-dependent methyltransferases (MTases) catalyze the transfer of a methyl group from a SAM cofactor to specific substrate molecules, including small chemicals, proteins, DNAs and RNAs, and are required for various cellular functions, such as regulation of gene expression and biosynthesis of metabolites. *Bacillus subtilis* YtqB is a putative SAM-dependent MTase whose biological function has not been characterized. To provide biochemical and structural insights into the role of YtqB in bacteria, the recombinant YtqB protein was overexpressed in the *Escherichia coli* expression system and purified by chromatographic methods. YtqB crystals were obtained in PEG-containing conditions and diffracted to 1.68 Å resolution. The YtqB crystals belonged to space group $P2_12_12_1$, with two molecules in the asymmetric unit.

1. Introduction

Methylation takes place on diverse biological molecules, including small chemicals, nucleic acids and proteins, and participates in a variety of biological processes in all three phylogenetic domains of life: archaea, bacteria and eukarya (Liscombe *et al.*, 2012). Methylation of DNA or histone is involved in epigenetic regulation of gene expression and aberrant methylation patterns have been implicated in cancer (Varier & Timmers, 2011; Cheng & Blumenthal, 2008; Del Rizzo & Trievel, 2011). tRNA and rRNA are post-transcriptionally methylated mainly at N and C atoms of the base moiety and at O atoms of the ribosyl group in a specific nucleotide position, and their methylation has been shown to modulate RNA stability, translation initiation, mRNA decoding and antibiotic resistance (Motorin & Helm, 2010, 2011). Methylation also plays key roles in the biosynthesis of primary and secondary metabolites in cells and can be applied to the production of pharmaceuticals in the pharmaceutical industry (Struck *et al.*, 2012).

Methylation is catalyzed most frequently by methyltransferases (MTases) in cells. MTases generally deliver the methyl group of S-adenosyl-L-methionine (SAM) cofactor to oxygen-centred, nitrogen-centred, carbon-centred or metal-centred nucleophiles of substrates (Martin & McMillan, 2002). Based on the tertiary structure of the core fold, SAM-dependent MTases are classified into five main structural families (I–V; Schubert *et al.*, 2003). Each family possesses structurally unique folds and SAM-binding pocket, suggesting that the five MTase families evolved independently. For example, class I MTases have the Rossmann-like fold in which a β -sheet is flanked by α -helices, and accommodate a SAM molecule in the structurally similar position that is generated in part by the highly conserved acidic residue and GxG motif (Gana *et al.*, 2013; Liscombe *et al.*, 2012; Schluckebier *et al.*, 1999). However, class V MTases contain an SET domain structure, which is folded into three discrete β -sheets that surround a knot-like structure (Del Rizzo & Trievel, 2011; Qian & Zhou, 2006; Xiao *et al.*, 2003). The class V MTases form a hydrophobic channel that places SAM and substrate molecules at its opposite ends.

Bacillus subtilis expresses YtqB, a putative MTase (Kunst *et al.*, 1997). Secondary-structure prediction proposes that YtqB possesses

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the Rossmann-like fold of class I MTases. Comparative sequence analyses of YtqB with other class I MTases suggest that YtqB contains the canonical GxG motif and the acidic residue potentially as a part of the SAM-binding site. However, to reveal the exact SAM-binding mode of YtqB, complete structural characterization of the YtqB–SAM interaction is required. Moreover, because YtqB lacks additional domains or N-terminal extensions that generally provide substrate specificity in class I MTases, it is unclear how YtqB recruits its cognate substrate for methylation. As a first step in defining the methylation mechanism and the biological function of YtqB, we have carried out overexpression, purification, crystallization and X-ray diffraction studies on YtqB as described below.

2. Materials and methods

2.1. Construction of the YtqB expression vector

The genomic DNAs of *B. subtilis* were purified by the Exgene Cell SV kit (GeneAll). The YtqB gene (UniProt E0TY72; residues 1–194) was amplified by PCR from the genomic DNAs using forward (5'-TAAGGATCCGATGATTTTGAAAAAATTCTTCCTTAC-AGC-3'; *Bam*HI restriction-enzyme site in bold) and reverse (5'-GCCGATGTCGACTCATTTGCTGATTTGAGCTTTTTTTTCG-3'; *Sal*I restriction-enzyme site in bold) primers. The PCR products and a modified pET49b vector (pET49bm) that contains the N-terminal His₆ tag and thrombin cleavage site were digested using *Bam*HI and *Sal*I, and ligated to construct the YtqB expression vector (Hong *et al.*, 2012; Song & Yoon, 2014). The ligation products were transformed into *Escherichia coli* strain DH5 α and a correct clone was selected by restriction-enzyme digestion and DNA sequencing.

2.2. Expression of the YtqB protein

The YtqB expression vector DNAs were transformed into *E. coli* strain BL21 (DE3). Cells were grown at 37°C in LB medium containing 50 μ g ml⁻¹ kanamycin until the OD₆₀₀ (optical density at 600 nm) reached ~0.8. IPTG was then added to the culture to a final concentration of 1 mM and the YtqB protein expression was induced for ~17 h at 18°C.

Figure 1
SDS-PAGE analysis of the purified YtqB protein (lane 2) and protein standards (lane 1; labelled in kDa).

2.3. Purification of the YtqB protein

Cells were collected by centrifugation and sonicated in 50 mM Tris pH 8.0, 200 mM NaCl, 5 mM β -mercaptoethanol (β ME), 1 mM PMSF. The cell lysate was cleared by centrifugation (~25 000g). The supernatant was incubated with Ni-NTA resin (Qiagen) in the presence of 10 mM imidazole at 4°C for 2 h. The resins were applied onto an Econo-Column (Bio-Rad) and were washed using 10 mM imidazole, 50 mM Tris pH 8.0, 200 mM NaCl, 5 mM β ME. The YtqB protein was eluted using 250 mM imidazole, 50 mM Tris pH 8.0, 200 mM NaCl, 5 mM β ME and dialyzed against 20 mM Tris pH 8.0, 5 mM β ME. The His₆ tag that is appended to the YtqB protein was removed by thrombin at 18°C for ~3 h. The YtqB protein was further purified by anion-exchange chromatography using a Mono Q 10/100 column. The column was equilibrated with 20 mM Tris pH 8.0, 5 mM β ME and the thrombin-digested YtqB in 20 mM Tris pH 8.0, 5 mM β ME was injected into the column. YtqB was eluted using a 0.0–0.5 M NaCl gradient. The YtqB eluate was concentrated to ~15 mg ml⁻¹ for crystallization.

2.4. Crystallization of the YtqB protein

Crystallization was carried out by the sitting-drop vapour-diffusion method. 0.5 μ l of ~15 mg ml⁻¹ YtqB protein solution was mixed with 0.5 μ l well solution in 96-well crystallization plates (Molecular Dimensions) for initial crystallization screening or in 24-well Crychem plates (Hampton Research) for crystallization optimization and the 1 μ l drop was equilibrated against well solution at 18°C. YtqB crystals were initially obtained using the JCSG Core Suite kit (Qiagen) in 30% PEG 200, 5% PEG 3000, 0.1 M MES pH 6.0. The crystallization condition was optimized and modified to 30–36% PEG 200, 5% PEG 2000, 0.1 M sodium cacodylate pH 6.5 or 30–36% PEG 200, 5% PEG 2000, 0.1 M HEPES pH 7.0.

2.5. X-ray diffraction of YtqB crystals

A single crystal in a crystallization drop was directly mounted and flash-cooled under a cryo-stream at –173°C. A total of 360 frames of X-ray diffraction images were collected with a 0.5° oscillation, an exposure time of 1 s per frame and a crystal-to-detector distance of

Figure 2
YtqB crystals (~50 × 50 × 400 μ m) obtained in 30% PEG 200, 5% PEG 2000, 0.1 M HEPES pH 7.0.

Table 1

Data-collection statistics for YtqB.

Values in parentheses are for the highest resolution shell.

X-ray source	BL-7A, PAL
Wavelength (Å)	0.9796
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	$a = 48.87$, $b = 76.47$, $c = 100.67$
Resolution range (Å)	20.00–1.68 (1.74–1.68)
No. of observations	222065
No. of unique reflections	43606
Multiplicity	5.1 (5.2)
Completeness (%)	99.1 (99.0)
$R_{\text{merge}}^{\dagger}$ (%)	4.8 (48.5)
$R_{\text{meas}}^{\ddagger}$ (%)	5.4 (54.0)
$\langle I/\sigma(I) \rangle$	41.6 (4.5)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$. $\ddagger R_{\text{meas}}$ was estimated by multiplying the conventional R_{merge} value by the factor $[N/(N-1)]^{1/2}$, where N is the data multiplicity.

180 mm using an ADSC Quantum 270 detector on beamline 7A of the Pohang Accelerator Laboratory, Republic of Korea (Fig. 3). Diffraction data were indexed, integrated and scaled using the *HKL-2000* package (Otwinowski & Minor, 1997).

3. Results and discussion

B. subtilis YtqB was overexpressed in an *E. coli* expression system using BL21 (DE3) strain and was purified to homogeneity in a soluble form by two purification steps of Ni-NTA affinity chromatography and anion-exchange chromatography, yielding ~4 mg from 1 l culture. In SDS-PAGE analysis, the purified YtqB (calculated molecular weight 21.8 kDa) migrated as a single band between the 15 and 27 kDa standards with a purity of greater than 95% (Fig. 1).

YtqB was crystallized in PEG solutions at pH 6.5–7.0 (Fig. 2). Crystals appeared in ~8 h and were fully grown in ~24 h. X-ray diffraction data were collected from a single crystal to 1.68 Å resolution (Fig. 2). A summary of the data-processing statistics is shown in Table 1. 222 065 observed reflections were merged to produce 43 606 unique reflections with a multiplicity of 5.1, a cumulative merging R factor of 4.8% and a completeness of 99.1%. Indexing, scaling and analysis of systematic absences revealed that the YtqB crystal belonged to the primitive orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 48.87$, $b = 76.47$, $c = 100.67$ Å. Matthews coefficient calculation indicates that the asymmetric unit contains two copies of YtqB (Matthews coefficient V_M of $2.16 \text{ Å}^3 \text{ Da}^{-1}$; 42.9% solvent content; Matthews, 1968; Kantardjieff & Rupp, 2003). Analysis of the native Patterson map did not identify any large off-origin peaks, suggesting that there is no translational pseudosymmetry in the crystal. The crystal structures of YtqB alone and in complex with SAM will be described elsewhere. We anticipate that our structural study of YtqB will make a significant contribution to the elucidation of the biological function of YtqB in bacteria.

X-ray diffraction data sets were collected on beamline 7A of the Pohang Accelerator Laboratory, Republic of Korea. This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the

Figure 3

An X-ray diffraction image of the YtqB crystal shown with a 1.7 Å resolution ring.

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