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Crystallization and preliminary X-ray diffraction analysis of YisP protein from *Bacillus subtilis* subsp. *subtilis* strain 168

YisP is an enzyme involved in the pathway of biofilm formation in bacteria and is predicted to possess squalene synthase activity. A *BlastP* search using the YisP protein sequence from *Bacillus subtilis* subsp. *subtilis* strain 168 shows that it shares 23% identity with the dehydrosqualene synthase from *Staphylococcus aureus*. The YisP from *B. subtilis* 168 was expressed in *Escherichia coli* and the recombinant protein was purified and crystallized. The crystals, which belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 43.966$, $b = 77.576$, $c = 91.378$ Å, were obtained by the sitting-drop vapour-diffusion method and diffracted to 1.92 Å resolution. Structure determination using MAD and MIR methods is in progress.

1. Introduction

Eukaryotic cells possess microdomains called lipid rafts on the cell surface, regions where signalling and transport proteins are enriched to enhance their functions. Recently, it has been found that bacteria also contain such microdomains that are functionally similar to those of eukaryotic cells, and a squalene synthase inhibitor, zaragozic acid, can interfere with lipid raft formation. Through bioinformatics analysis of the *Bacillus subtilis* genome, a nonessential gene, *yisP* (GenBank accession No. NC_000964.3), has been found to participate in squalene synthesis (López & Kolter, 2010). The gene product, YisP (274 residues; Pfam ID PF00494), has sequence similarity to phytoene synthase or squalene synthase and is also inhibited by zaragozic acid (Kobayashi *et al.*, 2003). The deletion of *yisP* resulted in a complete loss of pellicle formation ability.

The dehydrosqualene synthase from *Staphylococcus aureus* (SaCrtM) and human squalene synthase (hSQS) have been purified and characterized (Liu *et al.*, 2008; Pandit *et al.*, 2000). CrtM catalyses the initial step in the biosynthesis of staphyloxanthin in most staphylococci strains and SQS is involved in the biosynthesis of sterols in eukaryotes. SaCrtM and hSQS share similar structures and both are inhibited by zaragozic acid (Liu *et al.*, 2012). YisP is predicted to have squalene synthase activity and to play a role in the bacteria biofilm formation pathway, which is also inhibited by zaragozic acid. Most YisP have two conserved DXXXD motifs, which interact with the substrate through a metal-mediated interaction (Liu *et al.*, 2008, 2012). However, YisP from *B. subtilis* subsp. *subtilis* strain 168 (BsYisP) only possesses the second DXXXD motif and does not have the first one (Fig. 1). BsYisP shares 27% and 11% amino-acid identity with SaCrtM and hSQS, respectively. Because of the lack of the first DXXXD motif, it will be very interesting to investigate the effects of this missing motif on the squalene synthase activity of BsYisP. Furthermore, structural information is of great importance to further understand YisP function and the mechanism of bacterial biofilm synthesis.

2. Materials and methods

2.1. Protein preparation

The gene encoding BsYisP (GenBank accession No. NC_000964.3) from *B. subtilis* 168 was amplified by polymerase chain reaction

(PCR) with the forward primer 5'-GGTATTGAGGGTCGCGCTG-GTGCTGGTGTATGATACATAGGAGTGAGAAAATG-3' and the reverse primer 5'-AGAGGAGAGTTAGAGCCGTTAGATAT-CAGACAGAATCTGCTT-3', and then cloned into the pET32 Xa/LIC vector. The recombinant plasmid was transformed into *Escherichia coli* BL21\beta-D-1-thiogalactopyranoside (IPTG) at 293 K for 24 h.

Cell pellets were harvested by centrifugation at 6000g and resuspended in lysis buffer consisting of 25 mM Tris-HCl pH 8.5, 150 mM NaCl, 20 mM imidazole. The cell lysate was prepared with a French press (JNBIO JN-3000PLUS) and then centrifuged at 17 000g to remove cell debris. The protein was purified with an ÄKTApurifier 10 (GE Healthcare Life Sciences) using an Ni-NTA column. The buffer used for the Ni-NTA column was 25 mM Tris-HCl pH 8.5, 150 mM NaCl, 20 mM imidazole. BsYisP protein was eluted at about 150 mM imidazole when using a 20–250 mM imidazole gradient. The protein was dialysed against buffer consisting of 25 mM Tris-HCl pH 8.5, 150 mM NaCl and then subjected to factor Xa digestion at 277 K for 48 h to remove the tag. The buffer for digestion contained 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 5 mM CaCl₂ and the ratio of factor Xa to BsYisP was 0.2 U mg⁻¹. The vector-derived peptide sequence AGAGA was left at the N-terminus after digestion. The mixture was then passed through another Ni-NTA column and subsequently untagged BsYisP was eluted with 5 mM imidazole-containing buffer and then dialysed twice against 5 l buffer (25 mM Tris-HCl pH 8.5, 150 mM NaCl) for storage. All purification procedures were performed at 293 K and the yield of BsYisP was about 3 mg l⁻¹. SDS-PAGE analysis was used to check the purity (>95%) of BsYisP. Expression of selenomethionine-substituted (SeMet) BsYisP was conducted based on the method of Van Duyne *et al.* (1993). The purification procedures and the factor Xa cleavage were the same as those described for the wild-type enzyme. The yield of SeMet BsYisP was about 2 mg l⁻¹ after purification. The native and SeMet proteins (31.8 kDa; amino acids 1–279 with AGAGA remaining at the N-terminus from the His-tag) were concentrated to 10 mg ml⁻¹ in

25 mM Tris-HCl pH 8.5, 150 mM NaCl using an Amicon Ultra-15 Centricon (Millipore).

2.2. Crystallization and data collection

Initial crystallization screening was performed manually using 768 different reservoir conditions from Hampton Research (Laguna Niguel, California, USA) kits, including Crystal Screen, Crystal Screen 2, Crystal Screen Cryo, Crystal Screen Lite, MembFac, Natrix, Index, SaltRx, SaltRx 2, PEG/Ion, PEG/Ion 2, Quik Screen and Grid Screens (ammonium sulfate, MPD, sodium chloride, sodium malonate, PEG 6000 and PEG/LiCl) with the sitting-drop vapour-diffusion method. In general, 1 μ l of BsYisP-containing solution (25 mM Tris-HCl pH 8.5, 150 mM NaCl) was mixed with 1 μ l reservoir solution in 24-well Cryschem Plates (Hampton Research) and equilibrated against 300 μ l reservoir solution at 278 K. The initial crystals of BsYisP were obtained within 2 d using Grid Screen PEG/LiCl condition C6 [1 M LiCl, 0.1 M Bicine pH 9.0, 20% (w/v) polyethylene glycol 6000]. The optimized crystallization condition for all crystals mentioned here was modified to 1 M LiCl, 0.1 M Bicine pH 9.0, 18–20% (w/v) polyethylene glycol 6000. An X-ray diffraction data set was collected to 1.92 Å resolution (based on the criterion of keeping the merging *R* factor below 0.5 in the highest-resolution shell) on beamline BL13B1 of the National Synchrotron Radiation Research Center (NSRRRC; Hsinchu, Taiwan). The diffraction images were processed using the program *HKL-2000* (Otwinowski & Minor, 1997). Data-collection statistics are shown in Table 1.

3. Results and discussion

As shown in Fig. 2, the BsYisP crystal reached dimensions of about 0.1 $\times$ 0.1 $\times$ 0.3 mm within 2–3 d. Prior to data collection at 100 K, the crystal was mounted in a cryoloop and flash-cooled in liquid nitrogen with a slightly modified cryoprotectant, which consisted of 0.1 M Bicine, pH 9.0, 1 M LiCl, 23% (w/v) polyethylene glycol 6000 and 12% glycerol. Based on the diffraction pattern (Fig. 3), the BsYisP

Figure 1
Sequence alignment. The amino-acid sequence of BsYisP is aligned with those of SaCrtM and hSQS. The conserved DXXXD motifs are shown in blue boxes.

Table 1

Data-collection statistics for the BsYisP crystal.

Values in parentheses are for the highest resolution shell.

Beamline	BL-13B1, NSRRC
Wavelength (Å)	0.97622
Resolution (Å)	25–1.92 (1.99–1.92)
Space group	$P2_12_12_1$
Unit-cell parameters	
<i>a</i> (Å)	43.966
<i>b</i> (Å)	77.576
<i>c</i> (Å)	91.378
No. of measured reflections	166594 (16746)
No. of unique reflections	24603 (2427)
Completeness (%)	99.9 (100)
$R_{\text{merge}}^{\dagger}$ (%)	6.9 (48.2)
Mean $I/\sigma(I)$	32.7 (5.5)
Multiplicity	6.8 (6.9)
Detector	MX300HE
Oscillation range (°)	1
Exposure time (s)	1
Crystal-to-detector distance (mm)	250

$$^{\dagger} R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}.$$

Figure 2

A crystal of BsYisP. The crystal reached approximate dimensions of $0.1 \times 0.1 \times 0.3$ mm in 3 d.

crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 43.966$, $b = 77.576$, $c = 91.378$ Å. Assuming the presence of one molecule per asymmetric unit, the Matthews coefficient V_M (Matthews, 1968) is $2.47 \text{ Å}^3 \text{ Da}^{-1}$ and the estimated solvent content is 50.3%.

A *BlastP* search of BsYisP against the Protein Data Bank (PDB) shows that BsYisP shares 23% identity with the dehydrosqualene synthase (SaCrtM; PDB entry 2zco; Liu *et al.*, 2008) from *S. aureus*. Molecular replacement (MR) with *Phaser* (McCoy *et al.*, 2007) failed using the SaCrtM structure as a search model. Currently, solution of the phase problem using the SeMet protein for multiple-wavelength

Figure 3

A diffraction pattern of the BsYisP crystal.

anomalous diffraction (MAD) and preparation of crystals of heavy-atom derivatives for the multiple isomorphous replacement (MIR) method are in progress.

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