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Crystallization and preliminary X-ray analysis of the subclass B3 metallo-β-lactamase SMB-1 that confers carbapenem resistance

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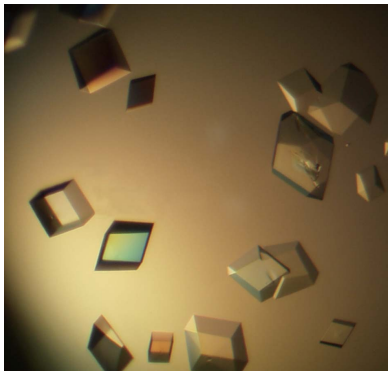


The carbapenem-hydrolyzing subclass B3 metallo-β-lactamase SMB-1 was expressed in *Escherichia coli* and purified. Diffraction data were collected from two types of SMB-1 crystals that were obtained under different conditions. One crystal (SMB-1a) belonged to the trigonal space group $P3_1$ with unit-cell parameters $a = b = 67.83$, $c = 48.67$ Å, while the other crystal (SMB-1b) also belonged to space group $P3_1$ but with unit-cell parameters $a = b = 67.25$, $c = 46.83$ Å. Both crystals contained one molecule per asymmetric unit. Initial phases were determined by molecular replacement; further refinement and model building are in progress.

1. Introduction

Metallo-β-lactamases (MBLs) catalyze the hydrolysis of amide bonds in a variety of β-lactam antibiotics, penicillins, cephalosporins and carbapenems, resulting in inactivation of the antimicrobial activity of β-lactam antibiotics. The MBLs hydrolyzing β-lactams are classified into three major groups based on their primary amino-acid sequence: subclasses B1, B2 and B3 (Garau *et al.*, 2004). MBLs commonly contain one or two zinc ions that are bound by histidine, aspartic acid and cysteine residues in the active site (PDB entries 1sml and 1x8g; Garau *et al.*, 2004). Recently, the emergence of carbapenem-resistant *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae via* the production of MBLs is becoming a great concern in clinical settings because carbapenem antibiotics remain important agents for the treatment of infectious diseases such as bacteraemia as well as infections of the respiratory tract and urinary tract caused by *Enterobacteriaceae*.

The most prevalent MBLs among the *Enterobacteriaceae* are the subclass B1 MBLs, which include IMP-type, VIM-type, NDM-type and SPM-type MBLs. The responsible genes are often procured with a mobile genetic element such as a transposon and are further embedded with transferable plasmids (Cornaglia *et al.*, 2011). Therefore, these MBLs can easily be spread among different bacterial species. The genes of several subclass B1 MBLs such as the IND-type and BlaB-type MBLs are intrinsically presented on the chromosome of human opportunistic pathogens (Cricco & Vila, 1999). The genes of subclass B3 MBLs identified to date are from human opportunistic pathogens and environmental microbial genomes (Cricco & Vila, 1999; Stoczko *et al.*, 2006). We recently identified a novel subclass B3 MBL named SMB-1 from the human opportunistic pathogen *Serratia marcescens*, which was isolated from a urine sample of an inpatient (Wachino *et al.*, 2011). The gene for SMB-1 was flanked by two copies of an IS*CR1* element, which is known to mediate the translocation of a variety of antibiotic-resistance genes in Gram-negative bacteria. As such, we hypothesized that the SMB-1 gene was integrated into the chromosome of *S. marcescens* from another bacterial species through a genetic recombination event caused by IS*CR1* (Toleman & Walsh, 2011; Wachino *et al.*, 2011).

Structure-based design of a potent specific inhibitor that can repress MBL activity could be one solution to overcome β-lactam resistance conferred by MBLs. To date, the X-ray crystal structures of subclass B1 MBLs in complex with various inhibitory agents have been reported, including IMP-1–mercaptocarboxylate derivatives

(Concha *et al.*, 2000), IMP-1–dansylC4SH (Kurosaki *et al.*, 2006), VIM-2–phenylC3SH (Yamaguchi *et al.*, 2007), BlaB–D-captopril (García-Saez *et al.*, 2003) and CcrA–biphenyltetrazoles (Toney *et al.*, 1998). In addition, the crystal structures of subclass B3 MBLs in complex with inhibitory agents have been reported, including L1–D-captopril (Nauton *et al.*, 2008) and BJP-1–4-nitrobenzenesulfonamide (Docquier *et al.*, 2010). To develop potent MBL inhibitors, further information on the three-dimensional structure of MBLs, especially subclass B3 MBLs, will be required. As such, we report here the crystallization and preliminary X-ray analysis of the subclass B3 MBL SMB-1.

2. Methods and results

2.1. Protein expression, purification and crystallization

The expression of SMB-1 (GenBank accession No. AB636283) in *E. coli* and purification were performed according to a previously described method (Wachino *et al.*, 2011). In brief, full-length SMB-1 protein (residues 1–280) including the predicted signal peptide (residues 1–18) was overexpressed using a pET30a vector and *E. coli* strain BL21(DE3)pLysS. The expressed protein was purified by three chromatographic steps using a HiTrap SP HP column (GE Healthcare), a HiLoad 16/60 Superdex 200 pg column (GE Healthcare) and a HiPrep 16/60 Sephacryl S-200 HR column (GE Healthcare) (Fig. 1). The purified protein was then exchanged into 20 m*M* HEPES–NaOH buffer pH 7.5 by diafiltration using an Amicon Ultra-15 Centricon (Millipore) and finally concentrated to 15 mg ml^{-1}. The N-terminal sequence of mature SMB-1 was determined to be QDRDW (residues 19–23), which corresponds to the sequence after cleavage of the predicted signal peptide described in our previous study (Wachino *et al.*, 2011). Mature SMB-1 was calculated to have a molecular mass of 27.7 kDa.

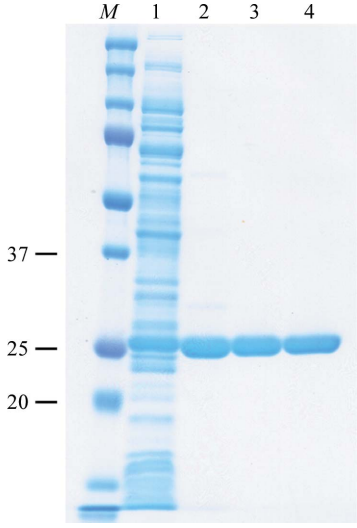
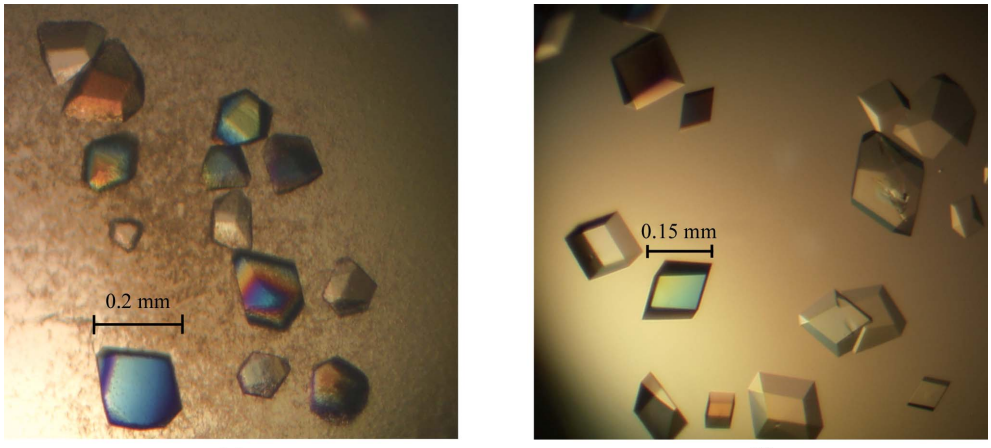


Figure 1
Purification of SMB-1. Lane *M*, protein size markers (labelled in kDa); lane 1, soluble cell extract; lane 2, fraction after cation-exchange chromatography; lane 3, fraction after size-exclusion chromatography with a HiLoad 16/60 Superdex 200 pg column; lane 4, fraction after size-exclusion chromatography with a HiPrep 16/60 Sephacryl S-200 HR column. The protein was subjected to SDS–PAGE on 12.5% polyacrylamide gel and stained with Coomassie Brilliant Blue.

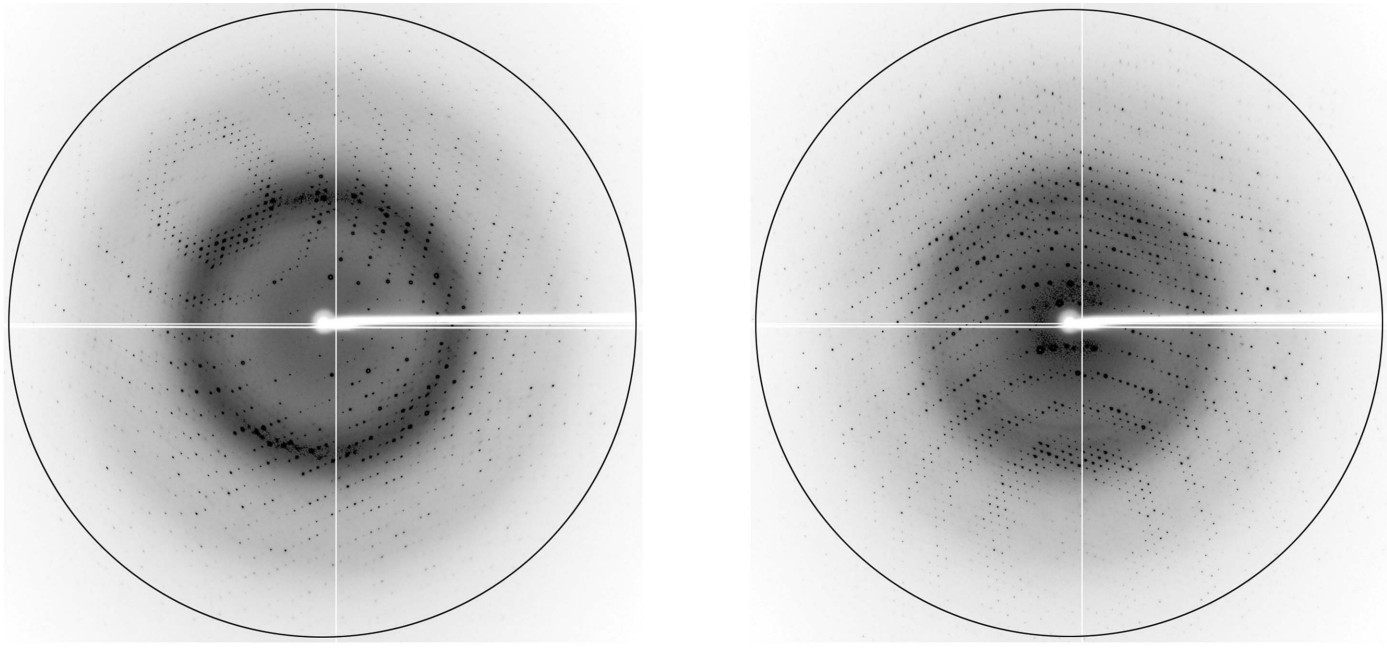
Initial screening for crystallization was performed manually using 290 different reservoir conditions from commercially available screening kits, including Crystal Screen, Crystal Screen 2, PEG/Ion Screen and Grid Screens (PEG/LiCl, Sodium Chloride, Ammonium Sulfate and PEG 6000) from Hampton Research and the Wizard III random sparse-matrix crystallization screen from Emerald BioSystems. All initial crystallization experiments were performed at 293 K using the sitting-drop vapour-diffusion method. 1 µl purified SMB-1 protein solution and 1 µl reservoir solution were mixed in 96-well Intelli-Plates (Art Robbins Instruments) and equilibrated against 100 µl reservoir solution. Crystals were obtained within one week using several reservoir solutions, including Crystal Screen 2 condition No. 13 [0.2 *M* ammonium sulfate, 0.1 *M* sodium acetate trihydrate pH 4.6, 30%(*w*/*v*) PEG monomethyl ether (MME) 2000] and Grid Screen PEG/LiCl condition D3 [1.0 *M* lithium chloride, 0.1 *M* MES pH 6.0, 30%(*w*/*v*) PEG 6000]. The pH and the salt and precipitant concentrations in the reservoir solution were further optimized manually using the hanging-drop vapour-diffusion method. Purified protein (3 µl) and reservoir solution (3 µl) were mixed and equilibrated against 500 µl reservoir solution.

As a result, two types of crystals that were suitable for the collection of diffraction data were obtained under different conditions. One crystal (SMB-1a) was obtained using a reservoir solution consisting of 0.2 *M* ammonium sulfate, 0.1 *M* sodium acetate trihydrate pH 5.3, 28%(*w*/*v*) PEG MME 2000. The crystals grew to dimensions of approximately 0.2 × 0.2 × 0.1 mm after one week (Fig. 2*a*). A second crystal (SMB-1b) was obtained using a reservoir solution consisting of 1.0 *M* lithium chloride, 0.1 *M* MES pH 5.8,



(*a*) (*b*)

Figure 2
Optimized crystals: (*a*) SMB-1a and (*b*) SMB-1b.



(*a*) (*b*)

Figure 3
X-ray diffraction images of (*a*) SMB-1a and (*b*) SMB-1b crystals. The black circles indicate a resolution of 1.65 Å.

Table 1
Data-collection statistics.

Values in parentheses are for the last shell.

Crystal	SMB-1a	SMB-1b
Beamline	BL-5A, PF	BL-5A, PF
Wavelength (Å)	1.00000	1.00000
Oscillation angle (°)	1	1
Crystal-to-detector distance (mm)	142.3	142.3
Exposure time (s)	1.0	1.0
Temperature (K)	100	100
Resolution range (Å)	50.0–1.60 (1.63–1.60)	50.0–1.60 (1.63–1.60)
V_M (Å^3 Da^{-1})	2.33	2.21
Solvent content (%)	47.3	44.2
Space group	$P3_1$	$P3_1$
Unit-cell parameters		
a (Å)	67.83	67.25
b (Å)	67.83	67.25
c (Å)	48.67	46.83
No. of observed reflections	367548	338253
No. of unique reflections	32909 (1654)	31322 (1567)
Completeness (%)	99.4 (100.0)	99.7 (100.0)
Multiplicity	11.2 (11.0)	10.8 (10.2)
R_{merge}† (%)	5.1 (21.6)	5.8 (23.3)
Mean $I/\sigma(I)$	73.4 (17.3)	71.1 (15.4)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity for reflection hkl and $\langle I(hkl)\rangle$ is the average intensity calculated for reflection hkl from replicate data.

26%(*w*/*v*) PEG 6000. The crystals grew to dimensions of approximately 0.15 × 0.15 × 0.1 mm after one week (Fig. 2*b*).

2.2. X-ray diffraction, data collection and analysis

Prior to data collection, the SMB-1a crystals were soaked in a cryoprotectant solution consisting of 0.2 *M* ammonium sulfate, 0.1 *M* sodium acetate trihydrate pH 5.3, 28%(*w*/*v*) PEG MME 2000, 20%(*v*/*v*) glycerol for a few seconds. X-ray diffraction data were collected at 100 K using a Quantum 315 CCD detector (Area Detector Systems Corp.) on beamline BL-5A at the Photon Factory (PF; Tsukuba, Japan; Fig. 3*a*). The collected diffraction data were processed and scaled at 1.60 Å resolution using *DENZO* and *SCALEPACK* from the *HKL*-2000 program package (Otwinowski & Minor, 1997). The space group was determined to be $P3_1$ or $P3_2$, with unit-cell parameters $a = b = 67.83$, $c = 48.67$ Å. The number of molecules in each asymmetric unit was estimated to be one from the Matthews coefficient (2.33 Å^3 Da^{-1}), corresponding to a solvent content of 47.3%. Scaling and merging of the crystallographic data showed an overall R_{merge} of 5.1%. The SMB-1b crystals were soaked for cryoprotection in a solution consisting of 1.0 *M* lithium chloride, 0.1 *M* MES pH 5.8, 26%(*w*/*v*) PEG 6000, 20%(*v*/*v*) glycerol for a few seconds. The diffraction data were collected (Fig. 3*b*), processed and scaled at 1.60 Å resolution. The SMB-1b crystals belonged to space group $P3_1$ or $P3_2$, with unit-cell parameters $a = b = 67.25$, $c = 46.83$ Å. The asymmetric unit of the SMB-1b crystal was estimated to contain one molecule from the Matthews coefficient (2.21 Å^3 Da^{-1}), corresponding to a solvent content of 44.2%. Scaling and merging of the crystallographic data showed an overall R_{merge} of 5.8%. A summary of the data statistics is shown in Table 1.

The structure of SMB-1 was solved by molecular replacement using the *MOLREP* program (Vagin & Teplyakov, 2010) from the *CCP*4 suite (Winn *et al.*, 2011). The L1 MBL from *Stenotrophomonas maltophilia*, which has 31% amino-acid identity to SMB-1, was used as the search model (Ullah *et al.*, 1998). The structure of L1 solved using 1.7 Å resolution diffraction data (Nauton *et al.*, 2008; PDB entry 1sml) was modified by the deletion of water molecules, other ions and several loop structures; mismatched amino acids were replaced by alanine. The molecular replacement was performed using the modified L1 structure as the search model and it was confirmed that both the SMB-1a and the SMB-1b crystals belonged to space group $P3_1$. Further refinement and model building are in progress.

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