Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary diffraction studies of GIM-1, a class B carbapenem-hydrolyzing β-lactamase

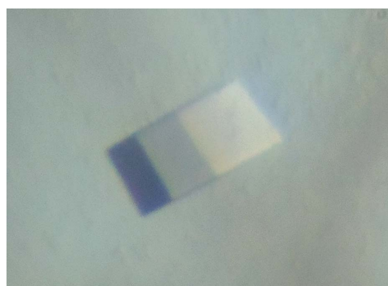
Myoung-Ki Hong,[a]‡ Jung Hun Lee,[b]‡ Dae Beom Kwon,[b] Jin-Kwang Kim,[c] Thi-Huyen Tran,[a] Dinh-Duc Nguyen,[a] Byeong Chul Jeong,[b] Sang Hee Lee[b]* and Lin-Woo Kang[c,d]*

[a]Department of Advanced Technology Fusion, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea, [b]Drug Resistance Proteomics Laboratory, Department of Biological Sciences, Myongji University, 116 Myongjiro, Yongin, Gyeonggido 449-728, Republic of Korea, [c]Department of Biological Sciences, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea, and [d]Center for Biotechnology Research in UBITA (CBRU), Seoul 143-701, Republic of Korea

‡ These authors contributed equally to this work.

Correspondence e-mail: sangheelee@mju.ac.kr, lkang@konkuk.ac.kr

Received 5 July 2012
Accepted 14 August 2012



© 2012 International Union of Crystallography
All rights reserved

GIM-1 is a member of the class B carbapenemases (metallo-β-lactamases; MBLs) and has a wide spectrum of activity against carbapenems, penicillins and extended-spectrum cephalosporins, but not aztreonam. GIM-1 presents an enormous challenge to infection control, particularly in the eradication of Gram-negative pathogens including Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and nonfermenters. There are presently few or no drugs in late-stage development for these pathogens and GIM-1 is a potential target for the development of antimicrobial agents against pathogens producing MBLs. In this study, GIM-1 was cloned, overexpressed and crystallized. The GIM-1 crystals diffracted to 1.4 Å resolution and belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 38.5, b = 67.6, c = 72.8 Å. One molecule is present in the asymmetric unit, with a corresponding V_M of 1.69 $Å^3$ Da^{-1} and a solvent content of 27.1%.

1. Introduction

Carbapenems are increasingly being utilized against a variety of infections owing to the emergence of bacteria that produce extended-spectrum β-lactamases (ESBLs) in the Enterobacteriaceae, in particular *Escherichia coli*, *Klebsiella pneumoniae* and other enteric bacteria (Lee *et al.*, 2009, 2012; Overturf, 2010). Carbapenems such as imipenem and meropenem are often the drugs of last resort for ESBL-producing pathogens (Overturf, 2010). Presently, β-lactamases are classified into four distinct classes based on structural similarity (classes A, B, C and D; Bush & Jacoby, 2010; Frère, 1995). Class B β-lactamases are zinc-dependent enzymes that are also known as metallo-β-lactamases (MBLs) and are able to inactivate almost all β-lactams, including carbapenems (Crowder *et al.*, 2006). Increasing numbers (more than 100 variants) of these β-lactamases are being found in the clinic; their genes are primarily located on plasmids (Papp-Wallace *et al.*, 2011; Queenan & Bush, 2007). Recently, the continued occurrence of GIM-1, a class B MBL, has been reported and understanding the molecular details of GIM-1 has become more important (Rieber, Frontzek & Pfeifer, 2012; Rieber, Frontzek, von Baum *et al.*, 2012). Their potential for wide dispersal together with their broad substrate specificity highlights the importance of investigating the structural details of these enzymes.

There are over 100 known MBL variants and numerous crystal structures have been reported, including those of IMP-1 (active on imipenem; Concha *et al.*, 2000), VIM-2 (Verona integron-encoded metallo-β-lactamase; Yamaguchi *et al.*, 2007) and SPM-1 (Sao Paulo metallo-β-lactamase; Murphy *et al.*, 2006). GIM-1 has 44.3, 27.2 and 22.8% sequence identity to mature IMP-1, VIM-1 and SPM-1, respectively. These three MBLs are clinically related to GIM-1 (German imipenemase; Castanheira *et al.*, 2004). Unlike the other MBLs, which showed a more significant activity towards imipenem than towards meropenem, GIM-1 demonstrated similar kinetic (k_{cat}/K_m) ratios with imipenem and meropenem (Castanheira *et al.*, 2004). In order to elucidate the differing kinetic properties of GIM-1, we expressed, purified, crystallized GIM-1 and performed a preliminary X-ray crystallographic analysis.

2. Materials and methods

2.1. Cloning

The class B carbapenemase GIM-1 was first isolated from *Pseudomonas aeruginosa* 73-5671 (Castanheira *et al.*, 2004). To express the GIM-1 gene ($bla_{GIM\text{-}1}$) in *E. coli*, a codon-optimized $bla_{GIM\text{-}1}$ gene was chemically synthesized using GenScript technology (GenScript, Piscataway, New Jersey, USA) and amplified by polymerase chain reaction (PCR). The sequences of the forward and reverse oligonucleotide primers designed from the $bla_{GIM\text{-}1}$ gene for PCR were 5′-ATA **CAT ATG** CAC CAT CAT CAT CAT CAT *GAC GAC GAC GAC AAG* CAG GGT CAT AAA CCG CTG GA-3′ (*Nde*I restriction site in bold) and 5′-GAG **CTC GAG** TTA GTC TGC AGA TGC TTC GGC A-3′ (*Xho*I restriction site in bold), respectively. The underlined bases represent a hexahistidine-tag site and the bases in italics indicate an enterokinase-recognition site. The amplified gene was double-digested with *Nde*I and *Xho*I and then inserted into the pET-30a expression vector (Novagen, Madison, Wisconsin, USA) which was digested with the same DNA restriction enzymes to produce the pET-30a/His$_6$-$bla_{GIM\text{-}1}$ plasmid. After verifying the DNA sequence, the plasmid DNA was transformed into *E. coli* strain BL21 (DE3) for overexpression of His$_6$-GIM-1.

2.2. Overexpression and purification

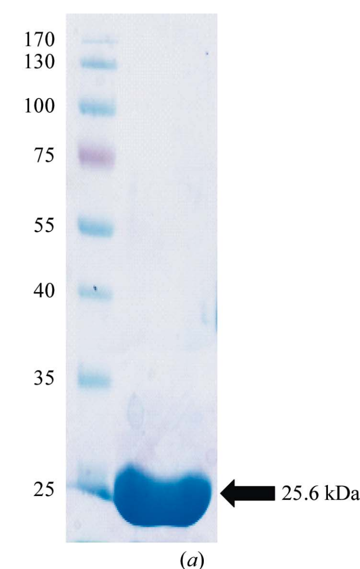
The transformed cells were grown to an OD_{600} of 0.5 at 310 K in Luria–Bertani medium (Difco, Detroit, Michigan, USA) containing 50 µg ml^{-1} kanamycin. Expression of His$_6$-GIM-1 was induced with 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16 h at 289 K. The cells were harvested by centrifugation at 3000*g* (Supra 30K A1000S-4 rotor, Hanil, Seoul, Republic of Korea) for 30 min at 277 K. The resulting cell pellet was resuspended in ice-cold lysis buffer (10 m*M* Tris–HCl pH 7.0) and homogenized by sonication (Sibata, Saitama, Japan). The crude lysate was centrifuged at 19 960*g* (Hanil) for 30 min at 277 K and the clarified supernatant was loaded onto a His-Bind column (Novagen, Wisconsin, USA) equilibrated with binding buffer (20 m*M* Tris–HCl, 10 m*M* imidazole, 500 m*M* NaCl pH 7.9). His$_6$-GIM-1 was eluted with binding buffer containing 1 *M* imidazole. Eluted fractions containing His$_6$-GIM-1 were pooled and concentrated to a volume of approximately 1 ml using a Vivaspin 20 concentrator (Sartorius, Göttingen, Germany). The His$_6$ tag was removed using enterokinase according to the manufacturer's instructions (Roche Molecular Biochemicals, Mannheim, Germany). The reaction mixture was desalted and concentrated using a Fast Desalting column (GE Healthcare Biosciences, Uppsala, Sweden) and then loaded onto a Superdex 200 prep-grade column (GE Healthcare) previously equilibrated with buffer *A* (10 m*M* MES pH 6.8) for further purification by gel filtration. The homogeneity of the purified protein was analyzed *via* SDS–PAGE (Fig. 1*a*). The purified GIM-1 without the His$_6$ tag was dialyzed against buffer *A* (10 m*M* MES pH 6.8) and was subsequently concentrated to 7 mg ml^{-1} for crystallization trials.

2.3. Crystallization

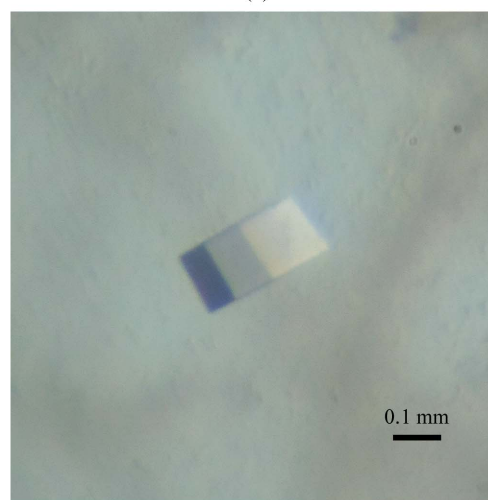
Crystallization conditions were initially screened by the sitting-drop vapour-diffusion method using a Hydra II eDrop automated pipetting system (Matrix) at 287 K. The initial crystallization conditions tested were from the Index kit (Hampton Research). After one week, crystals were observed in Index kit condition No. 96 [0.15 *M* potassium bromide, 30%(*w*/*v*) PEG MME 2000]. We optimized the initial crystals using the sitting-drop vapour-diffusion method in Cryschem plates (Hampton Research). Drops consisted of 0.5 µl protein solution (7 mg ml^{-1}) and 0.5 µl reservoir solution and were equilibrated against 70 µl reservoir solution at 287 K. Crystallization conditions were optimized by changing the concentrations of the major precipitant PEG MME 2000 and the salt potassium bromide from 27 to 32% and from 0.05 to 0.30 *M*, respectively. Various pH values from pH 5.5 to 8.0 were also used in the optimization. Only in 0.1 *M* bis-tris pH 5.5 were single crystals observed. Using a higher protein concentration of 18 mg ml^{-1}, larger crystals were obtained. The final well diffracting crystals were obtained from sitting drops consisting of 1 µl protein solution (18 mg ml^{-1}) and 1 µl reservoir solution [0.1 *M* bis-tris pH 5.5, 0.25 *M* potassium bromide, 27%(*w*/*v*) PEG MME 2000]. Crystals were cryoprotected in reservoir solution supplemented with 17–21%(*v*/*v*) PEG 400.

2.4. X-ray data collection

Crystals were mounted in a loop and transferred into cryoprotectant solution prior to cooling in liquid nitrogen. The cooled crystals were mounted on the goniometer in a stream of cold nitrogen at 100 K. X-ray diffraction data were collected using an ADSC Q315r



(*a*)



(*b*)

Figure 1
Purification and crystallization of GIM-1. (*a*) 12% SDS–PAGE of purified GIM-1. The left lane contains molecular-mass markers (labelled in kDa). (*b*) Crystal of GIM-1 with dimensions of 0.3 × 0.3 × 0.2 mm.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Beamline	5C SB II, PAL
Wavelength (Å)	0.97951
Resolution range (Å)	50.00–1.40 (1.42–1.40)
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 38.5, b = 67.6, c = 72.8
Total No. of reflections	439195
No. of unique reflections	37231
Completeness (%)	97.5 (82.7)
Molecules per asymmetric unit	1
Solvent content (%)	27.1
Average $I/\sigma(I)$	77.8 (16.0)
$R_{\rm merge}$† (%)	7.0 (21.6)
Multiplicity	11.8 (7.8)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I(hkl)$ is the intensity of reflection hkl, $\sum_{hkl}$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection hkl.

detector on beamline 5C SB II at the Pohang Light Source (PLS), Republic of Korea. X-ray diffraction data were collected to 1.4 Å resolution for the native protein. All data were collected with 1° oscillation per frame to give a total of 360° of data. All data were integrated and scaled using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

Initially, intergrown crystals were obtained by mixing 0.5 µl protein solution with 0.5 µl reservoir solution consisting of 0.15 *M* potassium bromide, 30%(*w*/*v*) PEG MME 2000. Although the intergrown crystals could be reproduced using the hanging-drop vapour-diffusion method, they could not be improved to single crystals. We switched the crystallization method from hanging drops to sitting drops, which led to the appearance of single crystals. After optimization, the best crystals were generated in sitting drops with a reservoir solution consisting of 0.1 *M* bis-tris pH 5.5, 0.25 *M* potassium bromide, 27%(*w*/*v*) PEG MME 2000. The crystals grew to maximum dimensions of approximately 0.3 × 0.3 × 0.2 mm in two weeks (Fig. 1*b*).

X-ray diffraction data were collected at the Pohang Light Source (PLS), Republic of Korea. Data-collection and processing statistics are given in Table 1. Auto-indexing was performed with *DENZO* and the results indicated that the crystals belonged to space group $P2_12_12_1$, with unit-cell parameters a = 38.5, b = 67.6, c = 72.8 Å. For space group $P2_12_12_1$ one monomer is present in the asymmetric unit with a corresponding $V_{\rm M}$ of 1.69 Å^3 Da^{-1} and a solvent content of 27.1%. Molecular replacement (MR) was performed with *MOLREP* (Vagin & Teplyakov, 2010) using the IMP-1 metallo-β-lactamase from *P. aeruginosa* (44.3% sequence identity; PDB entry 1dd6; Concha *et al.*, 2000) as the search model. The MR solution provided informative $2F_{\rm o} - F_{\rm c}$ and $F_{\rm o} - F_{\rm c}$ electron-density maps for model improvement. The initial R and $R_{\rm free}$ values from the MR solution were 29.4% and 32.1%, respectively, and the figure of merit was 77.2. The structural details will be described in a separate paper.

We warmly acknowledge the staff at beamline 5C SB II of the Pohang Light Source (PLS), Republic of Korea for their assistance. This study was supported by research grants from the National Research Foundation of Korea (NRF) funded by the Korean government (MEST; No. 2012-0008737), the Marine and Extreme Genome Research Center Program of the Ministry of Land, Transport and Maritime Affairs, Republic of Korea and the Next Generation BioGreen 21 Program (Nos. SA00005735 and PJ008174) of the Rural Development Administration of the Republic of Korea.

References

Bush, K. & Jacoby, G. A. (2010). *Antimicrob. Agents Chemother.* **54**, 969–976.
Castanheira, M., Toleman, M. A., Jones, R. N., Schmidt, F. J. & Walsh, T. R. (2004). *Antimicrob. Agents Chemother.* **48**, 4654–4661.
Concha, N. O., Janson, C. A., Rowling, P., Pearson, S., Cheever, C. A., Clarke, B. P., Lewis, C., Galleni, M., Frère, J.-M., Payne, D. J., Bateson, J. H. & Abdel-Meguid, S. S. (2000). *Biochemistry*, **39**, 4288–4298.
Crowder, M. W., Spencer, J. & Vila, A. J. (2006). *Acc. Chem. Res.* **39**, 721–728.
Frère, J.-M. (1995). *Mol. Microbiol.* **16**, 385–395.
Lee, J. H., Bae, I. K. & Lee, S. H. (2012). *Med. Res. Rev.* **32**, 216–232.
Lee, J. H., Jeong, S. H., Cha, S.-S. & Lee, S. H. (2009). *PLoS Pathog.* **5**, e1000221.
Murphy, T. A., Catto, L. E., Halford, S. E., Hadfield, A. T., Minor, W., Walsh, T. R. & Spencer, J. (2006). *J. Mol. Biol.* **357**, 890–903.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **277**, 307–326.
Overturf, G. D. (2010). *Pediatr. Infect. Dis. J.* **29**, 68–70.
Papp-Wallace, K. M., Endimiani, A., Taracila, M. A. & Bonomo, R. A. (2011). *Antimicrob. Agents Chemother.* **55**, 4943–4960.
Queenan, A. M. & Bush, K. (2007). *Clin. Microbiol. Rev.* **20**, 440–458.
Rieber, H., Frontzek, A. & Pfeifer, Y. (2012). *Antimicrob. Agents Chemother.* **56**, 4945–4947
Rieber, H., Frontzek, A., von Baum, H. & Pfeifer, Y. (2012). *J. Antimicrob. Chemother.* **67**, 1043–1045.
Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* D**66**, 22–25.
Yamaguchi, Y., Jin, W., Matsunaga, K., Ikemizu, S., Yamagata, Y., Wachino, J., Shibata, N., Arakawa, Y. & Kurosaki, H. (2007). *J. Med. Chem.* **50**, 6647–6653.