Expression, crystallization and preliminary X-ray crystallographic analysis of cystathionine β -lyase from *Acinetobacter baumannii* OXA-23

Diem-Quynh Nguyen,^{a,†}
Ho-Phuong-Thuy Ngo,^{a,†}
Yeh-Jin Ahn,^b Sang Hee Lee^c and
Lin-Woo Kang^{a,*}

^aDepartment of Biological Sciences, Konkuk University, 1 Hwayang dong, Gwangjin-gu, Seoul 143-701, Republic of Korea, ^bDepartment of Life Science, College of Natural Sciences, Sangmyung University, 7 Hongji-dong, Jongno-gu, Seoul 110-743, Republic of Korea, and ^cNational Leading Research Laboratory of Drug Resistance Proteomics, Department of Biological Sciences, Myongji University, 116 Myongji-ro, Yongin, Gyeonggi-do 449-728, Republic of Korea

† These authors contributed equally to this work.

Correspondence e-mail: lkang@konkuk.ac.kr

Received 17 June 2014
Accepted 5 August 2014

Multidrug-resistant *Acinetobacter baumannii* (Ab) has emerged as a leading nosocomial pathogen because of its resistance to most currently available antibiotics. Cystathionine β -lyase (CBL), a pyridoxal 5'-phosphate (PLP)-dependent enzyme, catalyzes the second step in the transsulfuration pathway, which is essential for the metabolic interconversion of the sulfur-containing amino acids homocysteine and methionine. The enzymes of the transsulfuration pathway are considered to be attractive drug targets owing to their specificity to microbes and plants. As a potential target for the development of novel antibacterial drugs, the AbCBL protein was expressed, purified and crystallized. An AbCBL crystal diffracted to 1.57 Å resolution and belonged to the trigonal space group $P3_12$, with unit-cell parameters $a = b = 102.9$, $c = 136.5$ Å. The asymmetric unit contained two monomers, with a corresponding V_M of $2.3 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 46.9%.

1. Introduction

Multidrug-resistant (MDR) *Acinetobacter baumannii* (Ab) causes nosocomial infections such as pneumonia, bacteraemia, urinary-tract infections and surgical infections in approximately 50% of patients admitted to intensive-care units (Roca *et al.*, 2012). Ab shows resistance to many classes of antibiotics, including β -lactams, aminoglycosides, quinolones, tetracyclines and polymyxins (Perez *et al.*, 2007). Owing to its multiple drug resistance, Ab is an emerging nosocomial pathogen, with reported outbreaks in several countries around the world (Peleg *et al.*, 2008). In order to discover new treatments for MDR Ab, enzymes that are essential for its growth or survival have been chosen as drug targets. Two of these enzymes have previously been reported (Huynh *et al.*, 2014; Nguyen *et al.*, 2013). Cystathionine β -lyase from Ab (AbCBL) is another drug target and is currently enzymatically and structurally uncharacterized.

Cystathionine β -lyase (CBL; EC 4.4.1.8) is a pyridoxal 5'-phosphate (PLP)-dependent enzyme that catalyzes the second step of the transsulfuration pathway, in which cystathionine is converted into homocysteine, thereby producing pyruvate and ammonia as side products during a β -elimination reaction. The transsulfuration pathway includes cystathionine γ -synthase (CGS), cystathionine β -lyase (CBL), cystathionine γ -lyase (CGL) and cystathionine β -synthase (CBS) enzymes. CGS and CBL belong to the forward direction of the transsulfuration pathway, which is specific to microbes and plants and does not exist in humans, while CBS and CGL belong to the reverse direction of the transsulfuration pathway, which exists in bacteria, fungi and mammals (Steegborn & Clausen, 2000). The lack of CGS and CBL in humans makes these enzymes attractive drug targets. CBL evolved from the same ancestor protein and is structurally related to CGS, an enzyme that catalyzes the first step of the transsulfuration pathway, in which cystathionine is formed from homoserine and cysteine (Belfaiza *et al.*, 1986; Clausen *et al.*, 1998; Steegborn *et al.*, 1999). To date, structures of *Escherichia coli* CBL (Clausen *et al.*, 1996), *Arabidopsis thaliana* CBL (Breitinger *et al.*, 2001), *E. coli* CGS (Clausen *et al.*, 1998), *Nicotiana tabacum* CGS (Steegborn *et al.*, 1999), *Mycobacterium ulcerans* CGS (Clifton *et al.*, 2011) and *Helicobacter pylori* CGS (PDB entry 4l0o; K. F. Tarique, S. A. A. Rehman, E. Ahmed & S. Gourinath, unpublished work) have been solved and their sequences share 29, 25, 26, 28, 25 and 30%

Table 1

Macromolecule-production information.

Source organism	<i>A. baumannii</i> OXA-23
DNA source	Genomic DNA
Forward primer†	5'-CCCCCGGATCCCATGAAAAAGCATAACAATTTTC-3'
Reverse primer†	5'-CCCCCCTCGAGTTACATTTGTTTCAAAGCATT-3'
Cloning vector	pETDuet-1 vector (Novagen)
Expression vector	pETDuet-1 vector (Novagen)
Expression host	<i>E. coli</i> BL21 (DE3)
Complete amino-acid sequence of the construct produced	MGSSHHHHHSGAPMKHNNFQTQLIHAPRKAPQFIETVQPP-LFRASTIIFKSTSHLFDRLHWTDPYDYSYGTGTPPTFTLGDNIAQVEGGLYCLLAPSGLSA INLVNSAVLATGDEVWVPDNIYGPNIHLNLYLKQYGINVQVYNPIDVSSFQPSDKTKL-LWLEAAGSVTLFEPDLKALVKKAKAHGVLTA LNTWAGL-AFNAFDSDHLSVDLTIHALTKYPSGGGDILMGSVVTRD-EKLHRLFRMHAILGISVSGDDTAQIQRSLAHMSLRYEQQ-SNNAKTLLTWLKEQPQFAQVLHPSDKAAPGHQYWQEICST-GKSAGLVSVVFKSDYDISAVRRFCDA LKLFKIGFSWGGPVS-LVMYLDLKMRLKLENTLQQGLLVRFICGLEDPQDLIQD-IENALKQM

† *Bam*HI and *Xho*I restriction sites are underlined.**Table 2**

Crystallization.

Method	Hanging-drop vapour diffusion
Plate type	24-well NeXtal crystallization plate
Temperature (K)	287
Protein concentration (mg ml ⁻¹)	10
Buffer composition of protein solution	25 mM Tris-HCl pH 7.5, 30 mM NaCl, 10% (v/v) glycerol
Composition of reservoir solution	0.1 M sodium citrate/citric acid pH 4.5, 19.8% PEG 3350
Volume and ratio of drop	1.8 µl (0.9 µl protein solution: 0.9 µl reservoir solution)
Volume of reservoir (µl)	1000

sequence identity with AbCBL. All of them exist as homotetramers formed by monomers related by a crystallographic twofold axis.

Previous reports have demonstrated that CBL is necessary for virulence in *Salmonella enterica* and *Xanthomonas oryzae* pv. *oryzae* (Ejim *et al.*, 2004; Wang *et al.*, 2008). We set out to determine the crystal structure of AbCBL as a potential drug target. In this study, we report the cloning, expression, crystallization and preliminary diffraction analysis of CBL from MDR Ab. With this study, we hope to offer guidelines for future investigations into the catalytic role of this enzyme, which could facilitate the development of novel anti-bacterial drugs against MDR Ab.

2. Materials and methods

2.1. Macromolecule production

The genomic DNA for carbapenem-resistant Ab OXA-23 (Espinal *et al.*, 2013) was isolated from a urine specimen from a patient hospitalized in Busan, Republic of Korea and was used as template for amplifying the *AbCBL* coding sequence of cystathionine-β-lyase by PCR (Table 1). The PCR reaction was performed using amfiE-COPCR Premix (GenDEPOT, USA). Amplified DNA fragments were purified using a QIAquick gel extraction kit (Qiagen, Germany), double-digested with *Bam*HI and *Xho*I, cloned into pETDuet-1 vector (Novagen) containing a 6×His tag at the N-terminus and finally transformed into *E. coli* BL21 (DE3) cells.

E. coli BL21 (DE3) cells harbouring pETDuet1-AbCBL were grown at 310 K in Luria-Bertani (LB) medium containing 50 µg ml⁻¹ ampicillin until they reached an OD₆₀₀ of 0.6. Protein expression was induced by the addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The cell pellet was collected by centrifugation at 3000g for 30 min at 277 K (Hanil Supra 30K A1000S-4 rotor),

Table 3

Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	5A SBII, PLS
Wavelength (Å)	0.97949
Temperature (K)	100
Detector	ADSC 315r
Crystal-to-detector distance (mm)	200
Rotation range per image (°)	1
Total rotation range (°)	360
Exposure time per image (s)	1
Space group	<i>P</i> 3 ₁ 12
Unit-cell parameters (Å, °)	<i>a</i> = <i>b</i> = 102.9, <i>c</i> = 136.5, $\alpha = \beta = 90, \gamma = 120$
Mosaicity (°)	0.2
Resolution range (Å)	50.0–1.57 (1.68–1.57)
Total No. of reflections	1812882
No. of unique reflections	114594
<i>R</i> _{merge} † (%)	25.2 (82.5)
<i>R</i> _{meas} ‡ (%)	26.0 (86.1)
<i>R</i> _{p.i.m.} § (%)	6.4 (24.1)
CC _{1/2} § (%)	99.5 (53.2)
Completeness (%)	99.9 (99.5)
Multiplicity	15.8 (12.2)
<i>I</i> / <i>σ</i> (<i>I</i>)	52.8 (8.1)

† $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity and $\langle I(hkl) \rangle$ is the average intensity of multiple observations of symmetry-related reflections. ‡ $R_{\text{meas}} = \sum_{hkl} [N(hkl) / (N(hkl) - 1)]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$. § CC_{1/2} is the half-set correlation coefficient as described by Karplus & Diederichs (2012).

resuspended in ice-cold lysis buffer [25 mM Tris-HCl pH 7.5, 300 mM NaCl, 15 mM imidazole, 10% (v/v) glycerol, 3 mM β-mercaptoethanol] and homogenized by ultrasonication on ice (Sonomasher). The crude cell extract was centrifuged for 45 min at 19 960g (Hanil) and 277 K to remove cell debris. The supernatant was applied onto Ni-NTA His-Bind resin (Novagen) and affinity purification was performed according to the manufacturer's protocol at 277 K. The column was washed with buffer *B* consisting of 25 mM Tris-HCl pH 7.5, 1 M NaCl, 25 mM imidazole. The AbCBL was then eluted using buffer *C* consisting of 25 mM Tris-HCl pH 7.5, 300 mM NaCl, 250 mM imidazole. The eluted AbCBL was further purified using a HiTrap Q ion-exchange column (GE Healthcare) at 277 K with a 90 ml linear gradient of 0.0–1.0 M NaCl in a buffer consisting of 25 mM Tris-HCl pH 7.5, 10% (v/v) glycerol at a flow rate of

Figure 1

Purified AbCBL (lane *P*) is shown on a 10% SDS-PAGE gel. Lane *M* contains molecular-mass marker (labelled in kDa).

1 ml min⁻¹. The AbCBL protein eluted at 280 mM NaCl. Approximately 40 mg of AbCBL was purified from 2 l of cell culture.

2.2. Crystallization

For crystallization, the protein solution was concentrated using Amicon spin concentrators (10 kDa cutoff, Millipore) to achieve a final concentration of 10 mg ml⁻¹ in a buffer consisting of 25 mM Tris-HCl pH 7.5, 30 mM NaCl, 10% (v/v) glycerol. Initial crystallization was carried out at 287 K by the sitting-drop vapour-diffusion method in 96-well Intelli-Plates (Art Robbins) using a Hydra II e-drop automated pipetting system (Matrix) and screening kits from Hampton Research (Index, Crystal Screen, Crystal Screen Cryo, Crystal Screen Lite, PEG/Ion and PEG/Ion 2) and Emerald Bio (Wizard Classic 1 and 2 and Wizard Precipitant Synergy). 0.5 µl protein solution was mixed with 0.5 µl reservoir solution and subsequently equilibrated against 70 µl reservoir solution. AbCBL crystals of various shapes (small sphere-shaped, droplet-shaped, small and irregular rod-shaped and large rod-shaped) were obtained after 1 d

Figure 2
Crystals of AbCBL obtained from 0.1 M sodium citrate/citric acid buffer pH 4.5 containing 19.8% (w/v) PEG 3350. The scale bar represents 0.02 mm.

under several conditions. The crystals were reproduced and optimized starting from condition A6 of Wizard Classic 1 [20% (w/v) polyethylene glycol (PEG) 3000, 100 mM sodium citrate/citric acid pH 5.5]. Large rod-shaped crystals were obtained using the hanging-drop method. Each drop, consisting of 0.9 µl protein solution and 0.9 µl reservoir solution, was positioned over 1 ml reservoir solution. Optimization was achieved by varying the sodium citrate pH (4.5–6.0) and the concentration of PEG 3000 [10–30% (w/v)]. After 2 d, rod-shaped AbCBL crystals with adequate dimensions were obtained using a reservoir solution consisting of 0.1 M sodium citrate/citric acid pH 4.5, 19.8% PEG 3350 (Table 2).

2.3. Data collection and processing

The fully grown crystals (0.2 × 0.02 × 0.01 mm) were flash-cooled at 100 K in liquid nitrogen using 20% (v/v) glycerol in the reservoir as a cryoprotectant. X-ray diffraction data were collected from the cryoprotected crystals (at 100 K) on an ADSC 315r detector using 1° oscillations with a crystal-to-detector distance of 200 mm on beam-line 5C SBII at the Pohang Light Source (PLS), Republic of Korea. The crystals diffracted to a resolution of 1.57 Å. Diffraction data were integrated and scaled using the *HKL-2000* program package (Otwinowski & Minor, 1997). Data-collection and processing statistics are given in Table 3.

3. Results and discussion

The homogeneity of the purified protein was analyzed by SDS-PAGE (Fig. 1). The molecular weight of purified AbCBL (which includes 14 residues preceding the N-terminus of wild-type AbCBL) is 44.0 kDa. The optimized crystals obtained using condition A6 of Wizard Classic 1 (Fig. 2) were finally chosen for X-ray diffraction. The AbCBL crystal belonged to the trigonal space group *P*3₁12, with unit-cell

Figure 3
Self-rotation functions calculated by *MOLREP* (Vagin & Teplyakov, 2010) using data from 50 to 4.0 Å resolution. (a) $\chi = 180^\circ$, (b) $\chi = 120^\circ$.

parameters $a = b = 102.9$, $c = 136.5$ Å. The diffraction image did not show any signs of anisotropic diffraction; however, the R_{merge} is quite high. This could result from decay of the crystals induced by X-rays or from an inadequate use of cryoprotectant. As per the calculated Matthews coefficient (Matthews, 1968), there are probably two monomers in the asymmetric unit, with a corresponding V_M of $2.3 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 46.9%. Self-rotation functions were calculated by *MOLREP* (Vagin & Teplyakov, 2010) using data in the resolution range 50–4 Å at $\chi = 180, 120, 90$ and 60° to detect twofold, threefold, fourfold and sixfold symmetry, respectively. Significant peaks were only found in the $\chi = 180$ and 120° sections and confirmed three twofold and one threefold symmetries (Fig. 3). In addition to the crystallographic symmetry, the $\chi = 180$ and 120° sections also showed weak peaks of noncrystallographic symmetry. Thus, the self-rotation function provides no conclusive information concerning the presence or location of noncrystallographic symmetry. The phase of the AbCBL structure is being determined by molecular replacement (MR) using cystathionine γ -synthase from *N. tabacum* (PDB entry 1qgn; Steegborn *et al.*, 1999) with 28% sequence identity as the search model.

We are grateful to staff members at beamline 5C SBII of Pohang Light Source, Republic of Korea. This research was supported by research grants from the Next-Generation BioGreen 21 Program (PJ009500), Rural Development Administration, Republic of Korea; the 2014 KU Brain Pool of Konkuk University; and the National Research Laboratory Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (No. 2011-0027928).

References

- Belfaiza, J., Parsot, C., Martel, A., de la Tour, C. B., Margarita, D., Cohen, G. N. & Saint-Girons, I. (1986). *Proc. Natl Acad. Sci. USA*, **83**, 867–871.
- Breiting, U., Clausen, T., Ehlert, S., Huber, R., Laber, B., Schmidt, F., Pohl, E. & Messerschmidt, A. (2001). *Plant Physiol.* **126**, 631–642.
- Clausen, T., Huber, R., Laber, B., Pohlenz, H. D. & Messerschmidt, A. (1996). *J. Mol. Biol.* **262**, 202–224.
- Clausen, T., Huber, R., Prade, L., Wahl, M. C. & Messerschmidt, A. (1998). *EMBO J.* **17**, 6827–6838.
- Clifton, M. C., Abendroth, J., Edwards, T. E., Leibly, D. J., Gillespie, A. K., Ferrell, M., Dieterich, S. H., Exley, I., Staker, B. L., Myler, P. J., Van Voorhis, W. C. & Stewart, L. J. (2011). *Acta Cryst.* **F67**, 1154–1158.
- Ejim, L. J., D'Costa, V. M., Elowe, N. H., Loredó-Ostí, J. C., Malo, D. & Wright, G. D. (2004). *Infect. Immun.* **72**, 3310–3314.
- Espinal, P., Macià, M. D., Roca, I., Gato, E., Ruíz, E., Fernández-Cuenca, F., Oliver, A., Rodríguez-Baño, J., Bou, G., Tomás, M. & Vila, J. (2013). *Antimicrob. Agents Chemother.* **57**, 589–591.
- Huynh, K. H., Tran, H.-T., Pham, T.-V., Ngo, H.-P.-T., Cha, S.-S., Chung, K. M., Lee, S. H. & Kang, L.-W. (2014). *Acta Cryst.* **F70**, 505–508.
- Karplus, P. A. & Diederichs, K. (2012). *Science*, **336**, 1030–1033.
- Matthews, B. W. (1968). *J. Mol. Biol.* **32**, 491–497.
- Nguyen, D.-D., Ngo, H.-P.-T., Hong, M., Pham, T.-V., Lee, J. H., Lee, J. J., Kwon, D. B., Lee, S. H. & Kang, L.-W. (2013). *Acta Cryst.* **F69**, 1041–1044.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **277**, 307–326.
- Peleg, A. Y., Seifert, H. & Paterson, D. L. (2008). *Clin. Microbiol. Rev.* **21**, 538–582.
- Perez, F., Hujer, A. M., Hujer, K. M., Decker, B. K., Rather, P. N. & Bonomo, R. A. (2007). *Antimicrob. Agents Chemother.* **51**, 3471–3484.
- Roca, I., Espinal, P., Vila-Farrés, X. & Vila, J. (2012). *Front. Microbiol.* **3**, 148.
- Steegborn, C. & Clausen, T. (2000). *Rec. Res. Dev. Biochem.* **2**, 191–207.
- Steegborn, C., Messerschmidt, A., Laber, B., Streber, W., Huber, R. & Clausen, T. (1999). *J. Mol. Biol.* **290**, 983–996.
- Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* **D66**, 22–25.
- Wang, J. C., So, B. H., Kim, J. H., Park, Y. J., Lee, B. M. & Kang, H. W. (2008). *Plant Pathol.* **57**, 1136–1145.