Crystallization and preliminary X-ray analysis of a highly stable novel SGNH hydrolase (Est24) from *Sinorhizobium meliloti*

Bum Han Ryu,^{a‡} Duy Duc Nguyen,^{b‡} Tri Duc Ngo,^b Changsuk Oh,^b Ramesh Pandian,^b Kyeong Kyu Kim^{b*} and T. Doohun Kim^{a*}

^aDepartment of Applied Chemistry and Biological Engineering, College of Engineering, Ajou University, Suwon 443-749, Republic of Korea, and ^bDepartment of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, Republic of Korea

‡ These authors contributed equally to this work.

Correspondence e-mail: kyeongkyu@skku.edu, doohunkim@ajou.ac.kr

Received 10 September 2013

Accepted 16 December 2013

The SGNH hydrolase family includes enzymes that catalyze the hydrolysis of a broad range of substrates. Here, the crystallization and preliminary X-ray crystallographic studies of a novel SGNH hydrolase (Est24) from *Sinorhizobium meliloti* were performed. Recombinant Est24 protein containing an N-terminal His tag was expressed in *Escherichia coli* and purified to homogeneity. Est24 was then crystallized using a solution consisting of 0.2 M ammonium phosphate pH 4.6, 20% polyethylene glycol 3350. X-ray diffraction data were collected to a resolution of 1.45 Å with an R_{merge} of 9.4%. The Est24 crystals belonged to space group C2, with unit-cell parameters $a = 129.09$, $b = 88.63$, $c = 86.15$ Å, $\alpha = 90.00$, $\beta = 114.30$, $\gamma = 90.00^\circ$. A molecular-replacement solution was obtained using the crystal structure of *Mycobacterium smegmatis* arylesterase as a template and structure refinement of Est24 is in progress.

1. Introduction

The SGNH hydrolase superfamily of enzymes [Pfam Clan SGNH_hydrolase (CL0264)] has been identified based on the presence of serine, glycine, asparagine and histidine residues in four highly conserved motifs around the active site (Akoh *et al.*, 2004; Arpigny & Jaeger, 1999). Members of the SGNH hydrolase superfamily also exhibit a nucleophilic GDSL sequence motif in the N-terminal region, which forms the catalytic triad together with an aspartic acid and a histidine (DXXH motif). Interestingly, the members of the SGNH hydrolase superfamily exhibit multifunctional activities and broad substrate specificities owing to their flexible substrate-binding pocket (Chepyshko *et al.*, 2012; Lo *et al.*, 2003; Mølgaard *et al.*, 2000). Although current knowledge of the SGNH hydrolase family is very limited, SGNH hydrolases are commonly used in many fields of biotechnology such as the food, cosmetics, textiles and pharmaceutical industries (Wilhelm *et al.*, 2011; Panda & Gowrishankar, 2005).

Recently, a number of bacterial SGNH hydrolases have been identified from *Butyrivibrio proteoclasticus* (Till *et al.*, 2013), *Neisseria gonorrhoeae* (Pfeffer *et al.*, 2013), *Sinorhizobium meliloti* (Bae *et al.*, 2013; Hwang *et al.*, 2010), *Pseudomonas aeruginosa* (Sánchez *et al.*, 2012), *Listeria innocua* (Kim *et al.*, 2012) and metagenomic libraries (Navarro-Fernández *et al.*, 2011; Wang *et al.*, 2011). Furthermore, several SGNH family hydrolases from higher plants have been isolated, cloned and characterized (Muralidharan *et al.*, 2013; Teutschbein *et al.*, 2010; Abdelkafi *et al.*, 2009). In addition, it has been reported that the SGNH family consists of 108 members and 114 members in the genomes of *Arabidopsis thaliana* and *Oryza sativa*, respectively (Chepyshko *et al.*, 2012; Volokita *et al.*, 2010). To date, the crystal structures of only certain SGNH hydrolases from *P. aeruginosa* (Kovačić *et al.*, 2013; PDB entry 4jgg), *B. proteoclasticus* (Till *et al.*, 2013; PDB entry 4dev), *Streptomyces albidoflavus* (Murayama *et al.*, 2013; PDB entry 4hyq), *Streptomyces* sp. SF2575 (Pickens *et al.*, 2011; PDB entry 3skv), *Saccharomyces cerevisiae* (Ma *et al.*, 2011; PDB entry 3mil), *Pseudoalteromonas* sp. 643A (Brzuszkiewicz *et al.*, 2009; PDB entry 3hp4), *Mycobacterium smegmatis* (Mathews *et al.*, 2007; PDB entry 2q0s), *Arabidopsis thaliana* (Bitto

© 2014 International Union of Crystallography
All rights reserved

et al., 2005; PDB entry 1xfi), *Pseudomonas fluorescens* (Cheeseman *et al.*, 2004; PDB entry 3hea), *Escherichia coli* (Lo *et al.*, 2003; PDB entry 1jrl) and *Aspergillus aculeatus* (Mølgaard *et al.*, 2000; PDB entry 3cl1u) are known. Although interest in SGNH family proteins is increasing, knowledge of their structure, physiological roles and reaction mechanisms remains very limited.

In a previous study, a highly stable, novel SGNH hydrolase (Est24) from *S. meliloti* was identified, actively expressed in *Escherichia coli*, characterized and immobilized for operational stability (Bae *et al.*, 2013). This enzyme was highly reactive towards α -naphthyl acetate and (*R*)-methyl- β -3-hydroxy-2-methylpropionate. In addition, Est24 was able to hydrolyze short-chain esters such as *p*-nitrophenyl acetate (C_2), butyrate (C_4) and valerate (C_5). No significant enzyme activity was observed towards *p*-nitrophenyl esters with acyl chains longer than C_8 . The broad specificity of Est24 increases the potential utility of these proteins as biocatalysts in environmental and industrial applications such as fibre degradation, carbohydrate manipulation and drug-intermediate preparation. Moreover, cross-linked enzyme aggregates (CLEAs) of Est24 retained 83% of their initial activity after 1 h of incubation at 333 K. Here, we performed crystallization and preliminary crystallographic analysis of Est24. The crystal structure of Est24 will add to the existing pool of data pertaining to the SGNH hydrolase family and can be used as a molecular basis for potential industrial applications.

2. Experimental procedures

2.1. Expression and purification of Est24

The cloning of the Est24 gene from the chromosomal DNA of *S. meliloti* [KCTC; Korean Collection for Type Cultures (WDCM 597), Seoul, Republic of Korea] has been described previously (Bae *et al.*, 2013). After DNA sequencing, the recombinant plasmid (pQE30-Est24) was used to express Est24 in *E. coli* XL1-Blue (Stratagene, La Jolla, California, USA). Transformed *E. coli* cells were grown in LB medium containing 100 $\mu\text{g ml}^{-1}$ ampicillin at 310 K and were induced with 1 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) when the OD_{600} reached ~ 0.45 . After induction for 3 h, the cells were harvested, resuspended and sonicated in lysis buffer (50 mM sodium phosphate pH 8.0, 100 mM NaCl, 10 mM imidazole). Following sonication, the crude cell extract was centrifuged at 15 000 rev min^{-1} for 20 min and the supernatant was loaded onto Ni-IDA resin and washed with 20 mM imidazole. Est24 was then eluted in a high

imidazole concentration buffer (50 mM sodium phosphate pH 8.0, 100 mM NaCl, 250 mM imidazole) and desalted on a PD-10 column (GE Healthcare, Waukesha, Wisconsin, USA) with 20 mM Tris-HCl pH 8.0. The protein concentration was determined using a Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, California, USA) and the purity of Est24 was confirmed by SDS-PAGE. The recombinant protein thus produced was a fusion protein of full-length Est24 with 12 additional residues (MRGSHHHHHHGS) at the N-terminus. MALDI-TOF-MS was conducted to further confirm the molecular weight of Est24, which gave the predicted value (m/z) of 24 813 Da. The final proteins were stored without further modification at 253 K.

2.2. Crystallization

Primary crystallization screening of Est24 was performed using purified Est24 in 20 mM Tris-HCl pH 8.0 buffer *via* the microbatch method (Chayen *et al.*, 1990) under Al's Oil using Wizard I and II (Emerald BioSystems, Washington, USA) as crystallization screening solutions at 298 K. A drop consisting of 1.5 μl screening solution and 1.5 μl protein solution at a concentration of 6 mg ml^{-1} was placed into each well of a Nunc 96-well Mini Tray (Nalge Nunc International, New York, USA).

2.3. X-ray data collection and data processing

Crystals were transferred into a cryosolution consisting of the screening solution supplemented with 25% glycerol and flash-cooled in a cold nitrogen-gas stream at 100 K prior to data collection (Ryu *et al.*, 2012). Diffraction data were collected on an ADSC Quantum 315 CCD detector (beamline 7A, Pohang Accelerator Laboratory, Republic of Korea) at 100 K. The wavelength of the synchrotron X-rays was 0.9793 Å and the crystal-to-detector distance was 220 mm. The exposure time was 3 s per frame and the crystals were rotated through a total of 180° with a 0.5° oscillation range per frame. The data were processed, integrated and scaled using the *HKL*-2000 package (Otwinowski & Minor, 1997). The structure solution was

Figure 1
Est24 crystals were obtained using PEG 3350 as precipitant. The crystal dimensions are 0.05 $\times$ 0.03 $\times$ 0.01 mm.

Figure 2
A representative X-ray diffraction image of the Est24 crystal. The oscillation range is 0.5°. The resolution of each circle is indicated.

Table 1

X-ray data-collection and processing statistics.

Values in parentheses are for the outermost resolution shell.

Space group	C2
Unit-cell parameters (Å, °)	$a = 129.09$, $b = 88.63$, $c = 86.15$, $\beta = 114.30$
Wavelength (Å)	0.9793
Resolution (Å)	50.0–1.45 (1.50–1.45)
Total reflections	416944
Unique reflections	145944
Completeness (%)	93.4 (71.4)
$R_{\text{merge}}^{\dagger}$ (%)	9.4 (38.9)
Mean $I/\sigma(I)$	20.3 (2.0)
Wilson B factor (Å ²)	14.3
Mosaicity (°)	0.97

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

obtained by the molecular-replacement (MR) method using the crystal structure of arylesterase from *M. smegmatis* (PDB entry 2q0s; Mathews *et al.*, 2007) as a template after stripping ligands and waters. The *MOLREP* program (Vagin & Teplyakov, 2010) from the *CCP4* suite (Winn *et al.*, 2011) was used for MR.

3. Results and discussion

Recombinant SGNH hydrolase (Est24) from *S. meliloti* was expressed in *E. coli* XL1-Blue cells and purified to homogeneity for crystallization. Diffraction-quality crystals were obtained using PEG/Ion screen condition No. 43 (0.2 M ammonium phosphate monobasic pH 4.6, 20% polyethylene glycol 3350). Trigonal-shaped crystals appeared within 6 d and grew to final dimensions of approximately $0.05 \times 0.03 \times 0.01$ mm after two weeks (Fig. 1). The crystal belonged to space group C2, with unit-cell parameters $a = 129.09$, $b = 88.63$, $c = 86.15$ Å, $\alpha = 90.00$, $\beta = 114.30$, $\gamma = 90.00^\circ$. The diffraction data set was processed to 1.45 Å resolution with an R_{merge} of 9.4% (Table 1, Fig. 2). The data-collection statistics are given in Table 1. With the assumption of four molecules per asymmetric unit, the Matthews coefficient (V_M) was calculated to be $2.11 \text{ Å}^3 \text{ Da}^{-1}$ (Matthews, 1968). This V_M value is within the range commonly observed for protein crystals and corresponds to 41.57% solvent content. MR at 1.45 Å resolution yielded the best result, with a correlation coefficient of 52.4% and an initial R factor of 41.6% when chain A from the crystal structure of *M. smegmatis* arylesterase, which has 64% sequence identity to Est24, was used as a template. The model obtained by MR showed good crystal packing without clashes between symmetry-related molecules. The crystal structure of Est24 will be useful for further applications of Est24 as well as to understand protein–ligand complexes in the SGNH hydrolase family. The downstream applications of Est24 such as industrial catalysts for the degradation of fish and olive oils are in progress.

This work was supported by grants from the National Research Foundation of Korea (NRF-2012S1A2A1A01028907, NRF-2013K2A1A2053659 and NRF-2012R1A1A2000910) to TDK. KKK is supported by the Next-Generation BioGreen 21 Program (SSAC PJ008107), Korea Healthcare Technology R&D Project (A092006)

and the National Research Foundation of Korea (NRF) (2011-0028878).

References

- Abdelkafi, S., Ogata, H., Barouh, N., Fouquet, B., Lebrun, R., Pina, M., Scheirlinckx, F., Villeneuve, P. & Carrière, F. (2009). *Biochim. Biophys. Acta*, **1791**, 1048–1056.
- Akoh, C. C., Lee, G.-C., Liaw, Y.-C., Huang, T.-H. & Shaw, J.-F. (2004). *Prog. Lipid Res.* **43**, 534–552.
- Arpigny, J. L. & Jaeger, K.-E. (1999). *Biochem. J.* **343**, 177–183.
- Bae, S. Y., Ryu, B. H., Jang, E., Kim, S. & Kim, T. D. (2013). *Appl. Microbiol. Biotechnol.* **97**, 1637–1647.
- Bitto, E., Bingman, C. A., McCoy, J. G., Allard, S. T. M., Wesenberg, G. E. & Phillips, G. N. (2005). *Acta Cryst.* **D61**, 1655–1661.
- Brzuszkiewicz, A., Nowak, E., Dauter, Z., Dauter, M., Cieśliński, H., Długolecka, A. & Kur, J. (2009). *Acta Cryst.* **F65**, 862–865.
- Chayen, N. E., Shaw Stewart, P. D., Maeder, D. L. & Blow, D. M. (1990). *J. Appl. Cryst.* **23**, 297–302.
- Cheeseman, J. D., Tocilj, A., Park, S., Schrag, J. D. & Kazlauskas, R. J. (2004). *Acta Cryst.* **D60**, 1237–1243.
- Chepyshko, H., Lai, C. P., Huang, L. M., Liu, J. H. & Shaw, J. F. (2012). *BMC Genomics*, **13**, 309.
- Hwang, H., Kim, S., Yoon, S., Ryu, Y., Lee, S. Y. & Kim, T. D. (2010). *Int. J. Biol. Macromol.* **46**, 145–152.
- Kim, S., Bae, S. Y., Kim, S. J., Ngo, T. D., Kim, K. K. & Kim, T. D. (2012). *Int. J. Biol. Macromol.* **50**, 103–111.
- Kovačić, F., Granzin, J., Wilhelm, S., Kojić-Prodić, B., Batra-Safferling, R. & Jaeger, K. E. (2013). *PLoS One*, **8**, e69125.
- Lo, Y.-C., Lin, S.-C., Shaw, J.-F. & Liaw, Y.-C. (2003). *J. Mol. Biol.* **330**, 539–551.
- Ma, J., Lu, Q., Yuan, Y., Ge, H., Li, K., Zhao, W., Gao, Y., Niu, L. & Teng, M. (2011). *Proteins*, **79**, 662–668.
- Mathews, I., Soltis, M., Saldajeno, M., Ganshaw, G., Sala, R., Weyler, W., Cervin, M. A., Whited, G. & Bott, R. (2007). *Biochemistry*, **46**, 8969–8979.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Mølgaard, A., Kauppinen, S. & Larsen, S. (2000). *Structure*, **8**, 373–383.
- Muralidharan, M., Buss, K., Larrimore, K. E., Segerson, N. A., Kannan, L. & Mor, T. S. (2013). *Plant Mol. Biol.* **81**, 565–576.
- Murayama, K., Kano, K., Matsumoto, Y. & Sugimori, D. (2013). *J. Struct. Biol.* **182**, 192–196.
- Navarro-Fernández, J., Nechitaylo, T. Y., Guerrero, J. A., Golyshina, O. V., García-Carmona, F., Sánchez-Ferrer, A. & Golyshin, P. N. (2011). *Environ. Microbiol.* **13**, 3036–3046.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Panda, T. & Gowrishankar, B. S. (2005). *Appl. Microbiol. Biotechnol.* **67**, 160–169.
- Pfeffer, J. M., Weadge, J. T. & Clarke, A. J. (2013). *J. Biol. Chem.* **288**, 2605–2613.
- Pickens, L. B., Sawaya, M. R., Rasool, H., Pashkov, I., Yeates, T. O. & Tang, Y. (2011). *J. Biol. Chem.* **286**, 41539–41551.
- Ryu, B. H., Ngo, T. D., Jang, E., Kim, S., Ju, H., Kim, K. K. & Kim, T. D. (2012). *Acta Cryst.* **F68**, 560–564.
- Sánchez, D. G., Otero, L. H., Hernández, C. M., Serra, A. L., Encarnación, S., Domenech, C. E. & Lisa, A. T. (2012). *Microbiol. Res.* **167**, 317–325.
- Teutschbein, J., Gross, W., Nimtz, M., Milkowski, C., Hause, B. & Strack, D. (2010). *J. Biol. Chem.* **285**, 38374–38381.
- Till, M., Goldstone, D. C., Attwood, G. T., Moon, C. D., Kelly, W. J. & Arcus, V. L. (2013). *Proteins*, **81**, 911–917.
- Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* **D66**, 22–25.
- Volokita, M., Rosilio-Brami, T., Rivkin, N. & Zik, M. (2010). *Mol. Biol. Evol.* **28**, 551–565.
- Wang, G., Meng, K., Luo, H., Wang, Y., Huang, H., Shi, P., Pan, X., Yang, P. & Yao, B. (2011). *Appl. Microbiol. Biotechnol.* **91**, 1561–1570.
- Wilhelm, S., Rosenau, F., Kolmar, H. & Jaeger, K. E. (2011). *ChemBiochem*, **12**, 1476–1485.
- Winn, M. D. *et al.* (2011). *Acta Cryst.* **D67**, 235–242.