BL-1A, BL-17A, NE-3A/2020G521

X-ray structures of *Burkholderia stabilis* cholesterol esterase

Yoshiaki YASUTAKE[1,2,*], Kenji KONISHI[3,4], Shuji MURAMATSU[3], Keitaro YOSHIDA[1], Sachiyo ABURATANI[1,2,5], Shin-ichi SAKASEGAWA[3], Tomohiro TAMURA[1,2,4]

[1]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo 062-8517, Japan

[2]Computational Bio Big-Data Open Innovation Laboratory, AIST, Tokyo 169-8555, Japan

[3]Asahi Kasei Pharma Corporation, Shizuoka 410-2321, Japan

[4]Laboratory of Molecular Environmental Microbiology, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

[5]Cellular and Molecular Biotechnology Research Institute, AIST, Tokyo 135-0064, Japan

1 Introduction

Cholesterol esterase (Che; EC 3.1.1.13) is an enzyme that catalyzes the hydrolysis of cholesterol esters to produce cholesterol and fatty acids. Che is widely distributed in various organisms, ranging from prokaryotes to humans. A highly active and thermostable Che enzyme has been identified in the gram-negative bacterium, *Burkholderia stabilis* strain FERMP-21014, and this enzyme (BsChe) is currently in practical use as a diagnostic enzyme for determining the serum cholesterol levels in clinical settings [1]. BsChe is a homolog of the well-characterized bacterial triacylglycerol lipases (Lip; EC 3.1.1.3) such as *Burkholderia cepacia* lipase (BcLip) and *Burkholderia glumae* lipase (BgLip), with amino acid sequence identity of ~90%. Thus, BsChe exhibits both Lip activity as well as Che activity. However, to date, Che activities have not been reported for typical bacterial Lips, except for BsChe. To understand the structural basis for such high Che activity of BsChe, we performed a crystallographic analysis of BsChe.

2 Experiment

Commercially available lyophilized BsChe (Asahi Kasei Pharma Corporation, Japan) was used in this study. After anion exchange chromatography, the sample was concentrated to 12 mg/mL and used for crystallization screening with sitting-drop vapor diffusion. Two distinct morphologies of BsChe crystals were identified. Form I crystals were obtained using a reservoir solution consisting of 0.1 M imidazole (pH 6.5), 15% polyethylene glycol (PEG) 3350, and 40% 2-propanol. Form II crystals were obtained using a solution comprising 0.5 M sodium citrate (pH 6.0), 8% PEG 4000, and 30% 2-propanol. Both crystals were cryoprotected using 10% 2-propanol and 20% glycerol. X-ray diffraction data for the crystal forms I and II were collected at the Photon Factory (PF), using the beamlines BL-17A and AR-NE3A to a resolution of 1.08 and 2.10 Å, respectively. The data were processed using the programs XDS/AIMLESS, and the structures were determined using the molecular replacement method in MOLREP with the BcLip atomic model as a search probe. Model refinement was performed using the programs REFMAC and PHENIX.

3 Results and Discussion

The X-ray diffraction data and refinement statistics for the two crystals (Form I and II) of BsChe are summarized in Table 1.

Table 1: X-ray diffraction data and model refinement statistics

Crystals	Form I	Form II
BL	BL-17A	AR NE-3A
Space group	$C222_1$	$P2_1$
Unit cell (Å, deg)	a = 58.2, b = 61.4, c = 147.2	a = 186.3, b = 47.1, c = 70.1, β = 90.1
Resolution (Å)	1.08 (1.15-1.08)	2.10 (2.23-2.10)
R_{meas}	0.040 (0.220)	0.122 (0.552)
I/σ	28.9 (7.1)	8.7 (2.3)
Completeness (%)	93.0 (62.0)	99.5 (98.7)
Multiplicity	6.0	3.4
R_{work}/R_{free}	0.098/0.118	0.219/0.252

The asymmetric unit of the form I crystal contains one BsChe monomer (chain AI), while the asymmetric unit of form II crystal contains four unique monomers (chains AII, BII, CII, and DII). Among the five structures of BsChe monomers determined in this study, only chain CII exhibited an open conformation with a destroyed active site cleft, which has not yet been reported in the structural studies of bacterial Lips. The remaining four structures showed a closed conformation very similar to that of well-characterized BcLip and BgLip structures. The open conformation of BsChe suggests a possible conformational change related to substrate binding and egress [2].

However, we could not obtain the crystals of BsChe in complex with the substrate/product; thus, an in silico docking study was conducted to determine the sterol-binding mechanism of BsChe. As shown in Figure 1, all of the reasonable docking poses that exhibit inter-atomic distances of ≤4.0 Å between the side-chain hydroxyl group of BsChe Ser87 (the catalytic nucleophile) and the sterol ester carbon 28 (C28) (the target carbon atom attacked by Ser87 for ester-bond cleavage) occupy the active site cleft in the same orientation, and the sterol moiety is located at

the side chain of Leu266 and Ile287. These two residues are not conserved in typical bacterial Lips. Therefore, we performed a site-directed mutagenesis study to investigate whether these two residues are important for Che activity. We found that all the BsChe mutants, namely L266V, I287L, and L266V/I287L, significantly decreased Che activity while retaining Lip activity. In addition, we found that some bacterial Lips, such as BcLip, acquire Che activity with V266L and L287I mutations [2].

In summary, our findings provide important insights into the substrate binding and selectivity mechanisms of evolutionarily-related bacterial Lips.

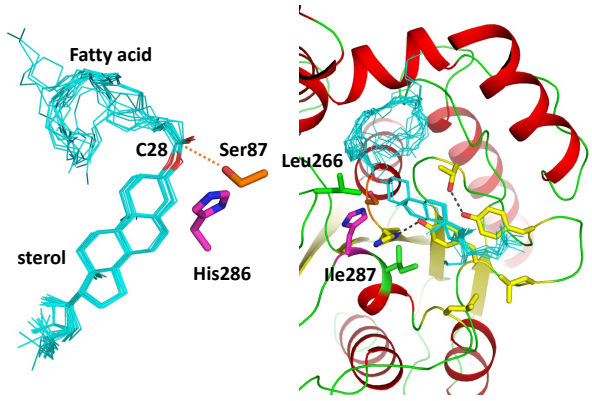

Fig. 1: Results of the molecular docking calculations using the closed structure of *Burkholderia stabilis* strain FERMP-21014 cholesterol esterase (BsChe) and cholesterol linoleate, the most favorable substrate for BsChe. A total of 17 reasonable docking poses are shown in the thin stick model (see main text).

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* y-yasutake@aist.go.jp