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Crystallization and Preliminary X-ray Diffraction Study of a Novel Bacterial Homologue of Mammalian Hormone-Sensitive Lipase (halip1) from *Halocynthiibacter arcticus*

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Abstract: Hormone sensitive lipase is a central enzyme in triacylglycerol hydrolysis, lipid modification, and transformation of various lipids. Microbial hormone-sensitive lipases, which are highly similar to a catalytic domain of mammalian equivalents, have attracted strong attention due to their application potentials. Here, characterization and a preliminary X-ray crystallographic analysis of a novel bacterial homologue of hormone-sensitive lipase (*Ha*Lip1) from *Halocynthiibacter arcticus* is reported. Sequence analysis shows that *Ha*Lip1 has a conserved serine residue within the GDSAG motif. In addition, a characteristic HGGG motif for oxyanion formation was identified. The *Ha*Lip1 protein was overexpressed in *E. coli*. SDS-PAGE, overlay assay, and mass analysis were performed to confirm purity and activity of *Ha*Lip1 protein. Furthermore, *Ha*Lip1 was crystallized in a condtion consisting of 25% (*w*/*v*) PEG 3350, 0.1 M Hepes-KOH, pH 7.5, 0.2 M sodium chloride. Diffraction data were processed to 1.30 Å with an R_{merge} of 7.3%. The crystals of *Ha*Lip1 belong to the $P2_12_12_1$, with unit cell parameters of a = 54.6 Å, b = 59.5 Å, and c = 82.9 Å.

Keywords: hormone sensitive lipase; psychrophilic bacterium; crystallization

1. Introduction

Hormone-sensitive lipases (HSLs, E.C. 3.1.1.79), a subfamily of lipases/esterase, have functional roles in hydrolysis of triacylglycerol, lipid modification, and energy homeostasis [1–3]. In addition, these enzymes are involved in the chemical transformation of a wide range of chemical compounds including carbohydrates, fatty acids, and steroids. In mammals, HSLs consist of an N-terminal protein–protein interaction domain and a C-terminal catalytic functional domain [4,5]. In contrast, bacterial HSLs, which are highly homologous to mammalian C-terminal domains, have been identified [6]. These bacterial HSLs have a highly conserved catalytic triad of Ser–His–Asp, with the functional Ser located in a characteristic GD(T)SAG motif. These HSLs are classified into two subfamilies of GDSAG and GTSAG based on their sequence motif. In addition, a characteristic HGGG motif was suggested to be responsible for the formation of oxyanion hole [7–9].

Although several bacterial HSLs were identified from metagenomic DNA libraries and several bacteria [10–15], there is limited information available on HSLs from extremophiles. Here, we describe crystallization, and preliminary X-ray diffraction analysis of a novel hormone-sensitive lipase (*Ha*Lip1) from *Halocynthiibacter arcticus*, which was isolated from a marine sediment in the Arctic region [16]. Although the genome of *H. arcticus* is supposed to have 4675 protein-coding genes, information about its gene products is still largely unknown [17]. Specifically, structural information on HSLs from the genus *Halocynthiibacter* are largely unavailable. Therefore, structural studies of *Ha*Lip1 will provide molecular understanding on the catalytic mechanism of bacterial HSLs at molecular level. Furthermore, considering the fact that bacterial HSLs have attracted great interest due to their industrial potential [18,19], *Ha*Lip1 could be an invaluable biocatalyst with unique properties for biotechnological applications.

2. Materials and Methods

2.1. Materials

DNA modifying enzymes and restriction enzymes were obtained from New England BioLabs (Ipswich, MA, USA) or Takara Biomedical Korea (Seoul, Korea). DNA purification kits and other molecular biology kits were purchased from Qiagen Korea (Daejon, Korea). Protein columns and other reagents were obtained from GE Healthcare Korea (Seoul, Korea).

2.2. Cloning and Purification

Psychrophilic bacteria of *H. arcticus* (KCTC 42129) were grown and genomic DNA was purified as described previously [13]. The *Ha*Lip1 gene was amplified by polymerase chain reaction and final product was subcloned into pET-21a. No signal sequence was found in *Ha*Lip1. The following primers using *NheI* and *XhoI* were used (forward primer: 5′-ATGCTA GCTAGC ATGGCACAAGTCACC-3′, and reverse primer: 5′-GTACCG CTCGAG GGCAAGAAATGCCCG-3′). *E. coli* BL21(λDE3) cells transformed with a recombinant plasmid (pET21a-*Ha*Lip1) were cultured in LB medium. After 1 mM isopropyl-β-D-1-thiogalactoside (IPTG) induction for 18 h at 27 °C, bacterial cells were harvested and then suspended in cell lysis buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 50 mM imidazole). After sonication and centrifugation at 4 °C, the final supernatant fractions were loaded onto a His-tag column, followed by an imidazole gradient elution (from 50 to 200 mM). Finally, the pooled fractions were desalted using a PD-10 column and stored in a storage buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl).

2.3. Biochemical Characterization

Overlay assay of *Ha*Lip1with 4-methylumbelliferyl acetate was carried out as described previously [20,21]. The hydrolytic activity of *Ha*Lip1 was confirmed by observing the fluorescence of 4-methylumbelliferone. Determination of molecular mass was performed using a Voyager Bio-Spectrometry system in positive ion mode (NICEM, Seoul, Korea). Sequences of *Ha*Lip1 and its related hydrolases were obtained from SWISS = PROT. Multiple sequence alignments were performed with Clustal Omega and ESPript.

2.4. Crystallization

The crystallization screening trials were carried out by sitting-drop vapor-diffusion method using an automated crystallization robot (SPT Labtech, Boston, MA, USA) with commercial screening kits of MCSG 1T~4T (Anatrace, Maumee, OH, USA), JCSG-plus (Molecular Dimensions, Maumee, OH, USA), and PGA Screen (Molecular Dimensions, Maumee, OH, USA) [22]. The crystallization trials contained 300 nL of protein solution with an equal volume of reservoir solution in a 96-well plate. After 3 days, crystals of *Ha*Lip1 appeared in several well reservoirs. The single crystal of *Ha*Lip1 for X-ray analysis

was obtained under the MCSG1T #95 condition of 25% (*w*/*v*) PEG 3350, 0.1 M Hepes-NaOH, pH 7.5, and 0.2 M sodium chloride.

2.5. X-ray Diffraction Data Collection and Data Processing

The diffraction-quality crystals of *Ha*Lip1 were transferred to a paratone oil containing cryo-protectant solution (Hampton Research, Aliso Viejo, CA, USA). After brief and gentle soaking, these crystals were effectively mounted on a synchrotron facility. X-ray diffraction data were collected using an Eiger X 9M detector (Dectris, Baden, Switzerland) at beamline 5C of the Pohang Light Source (PAL, Pohang, Korea). The final crystals were rotated with 1.0° oscillation range per frame. Diffraction data were collected, processed, and finally indexed using HKL2000 (Table 1). The *Ha*Lip1 crystal belongs to a primitive orthorhombic space group. Further analysis of the integrated intensities showed that the space group of *Ha*Lip1 was $P2_12_12_1$ (see also Table S1).

Table 1. X-ray data collection statistics of *Ha*Lip1.

Space Group	$\mathbf{P2_12_12_1}$
Unit cell parameters (Å)	a = 54.6, b = 59.5, c = 82.9
Wavelength (Å)	0.9794
Resolution (Å)	50.00–1.30 (1.32–1.30)
Unique reflections	67,202 (3311)
Completeness (%)	99.4 (98.8)
Redundancy	12.3 (12.2)
R_{meas} † (%)	8.7 (37.6)
Mean I/σ(I)	57.0 (13.6)
CC1/2 (%)	99.9 (95.1)
Wilson B (Å^2)	35.4

† $R_{meas} = \sum_{hkl} \{N\,(hkl)/[N\,(hkl) - 1]\}^{1/2} \sum_i |I_i(hkl) - <I(hkl)>| / \sum_{hkl} \sum_i I(hkl)$. Values in parentheses refer to the highest resolution shells.

3. Results and Discussion

Multiple sequence alignments of *Ha*Lip1 with three homologs in protein data bank (PDB) showed that all of them share common sequence motifs, which are necessary for their function and regulation (Figure 1). Specifically, *Ha*Lip1 showed significant sequence identity with a slightly acidophilic carboxylesterase (EstFa_R) from *Ferroplasma acidiphilum* (3WJ2, 28.2%) [23], a chloramphenicol-metabolizing enzyme (EstDL136) from a metagenome (6AAE, 31.5%) [24], and an alkaline esterase (Est8) from a metagenomic source (4YPV, 25.5%) [25].

Three highly conserved amino acids of Ser^{147}, Asp^{242}, and His^{272} could form a catalytic triad, with Ser^{147} which is located in a characteristic GDSAG motif. In *Ha*Lip1 and related homologs, Asp, not Glu, is observed, although Glu is often used [13,26,27]. Furthermore, a highly conserved HGGG motif, which is suggested to be involved in oxyanion hole formation [6,28], was identified. In primary sequence analysis, *Ha*Lip1 has high percentages of small amino acids such as Gly (7.3%) and Ala (14.7%). Furthermore, the total number of acidic amino acids (Asp + Glu) was 41, while that of basic amino acids (Lys + Arg) was 31. Interestingly, this property is also frequently observed in psychrophilic enzymes [29].

The recombinant *Ha*Lip1 was overexpressed and purified to an electrophoretic homogeneity using an Ni^{2+}-affinity His-tag column (Figure 2A). The hydrolytic activity of *Ha*Lip1 was examined by an overlay assay with 4-methylumbelliferyl acetate [10,13]. As shown in Figure 2B, high fluorescence was observed at the same position where *Ha*Lip1 was located in native-page. The molecular mass of *Ha*Lip1 was determined using MALDI-TOF mass analysis, which indicated a main peak (m/z) at 33.3 kDa. This value is highly consistent with the calculated mass of *Ha*Lip1.

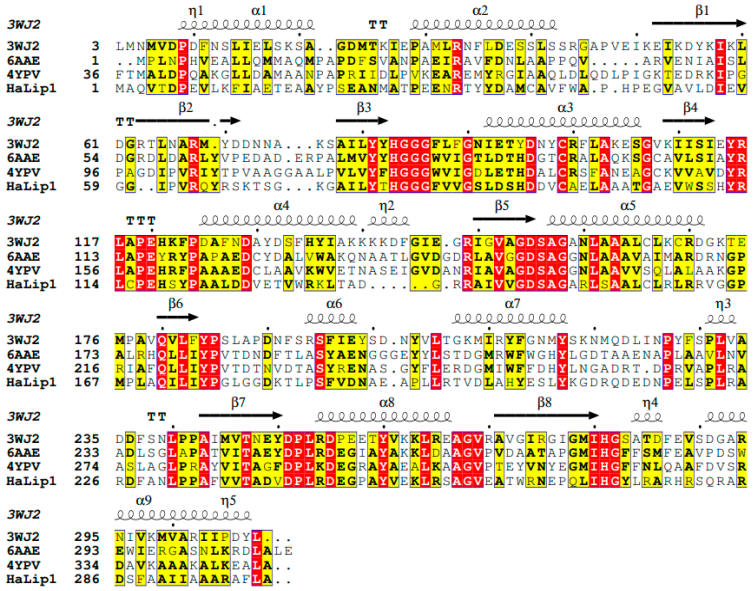

Figure 1. Multiple sequence alignments including *Ha*Lip1 and three related enzymes. Identical and highly conserved amino acids are shown in red and yellow, respectively.

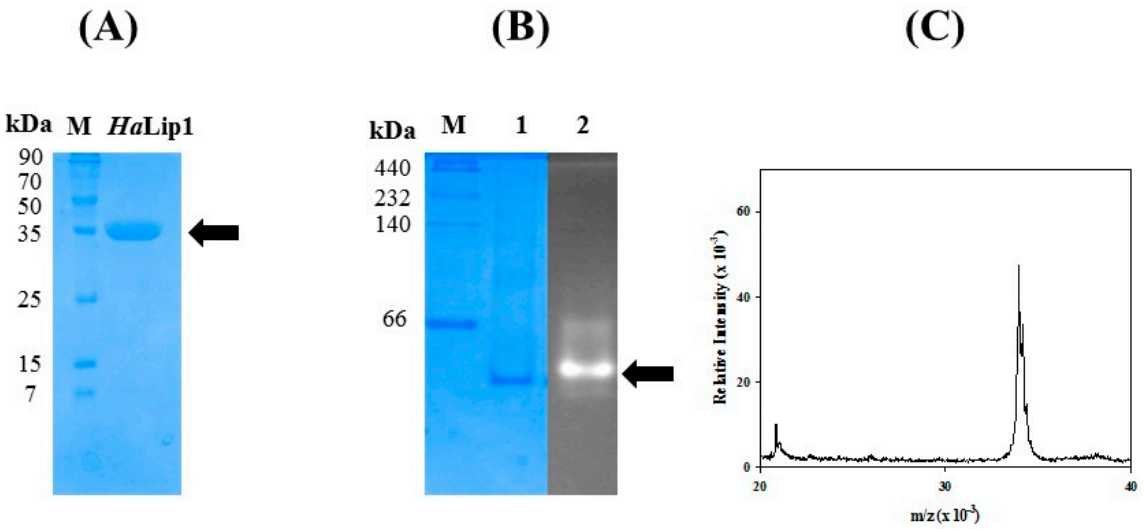

Figure 2. Biochemical characterization of *Ha*Lip1. (**A**) SDS-PAGE analysis of purified *Ha*Lip1. (**B**) Overlay hydrolytic analysis of *Ha*Lip1. 1: Molecular markers, 2: coomassie brilliant Blue staining in native-page, 3: fluorescence due to the formation of cleave product was shown. (**C**) Mass analysis of *Ha*Lip1.

For crystallization studies, purified *Ha*Lip1 (14.2 mg/mL) was screened with commercially available crystallization kits [22]. A diffraction-quality crystal of *Ha*Lip1 was obtained under the MCSG1T #95 condition of 25% (*w*/*v*) PEG 3350, 0.1 M Hepes-NaOH, pH 7.5, 0.2 M sodium chloride. The diffraction-quality crystal grew to final dimensions of 0.4 × 0.2 × 0.3 mm^3 (Figure 3). The X-ray radiation maintains isotropic diffraction throughout 360° rotation with 1° per each frame. The crystals of *Ha*Lip1 belonged to a $P2_12_12_1$ space group with cell parameter of a = 54.6 Å, b = 59.5 Å, and c = 82.9 Å. The diffraction data were collected and processed to 1.3 Å resolution with an R_{merge} value of 7.3% (Table 1). The data collection and processing statistics are summarized in Table 1. Considering one *Ha*Lip1 molecule per asymmetric unit, the Matthews coefficient (V_M) is 2.06 $Å^3$/Da. This value corresponds to 40.4% solvent content [30]. The structural determination of *Ha*Lip1 is in progress, and further structural studies will elucidate the catalytic mechanism of *Ha*Lip1 at molecular level.

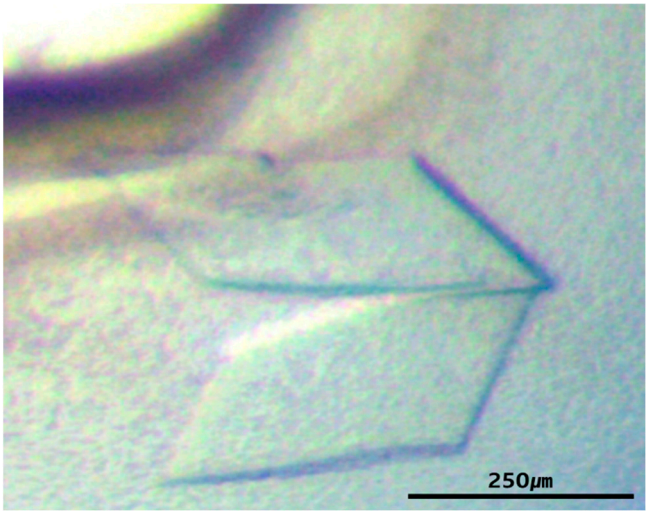

Figure 3. Crystals images of *Ha*Lip1. These crystals grew to final dimensions of $0.4 \times 0.2 \times 0.3$ mm^3. A scale bar is also shown in the image.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4352/10/11/963/s1, Figure S1: Statistics of X-ray diffraction data processing *Ha*Lip1 crystal

Author Contributions: S.J., W.Y., and T.D.K. identified, expressed, and purified *Ha*Lip1. J.H., H.D., H.-W.K., and J.H.L. crystallized and obtained x-ray diffraction data. K.K.K., J.H.L., and T.D.K. coordinated the whole project. J.H.L., and T.D.K. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of financial interests.

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