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Crystallization and preliminary X-ray crystallographic studies of monoacylglycerol lipase of the moderately thermophilic *Bacillus* sp. H-257

Kazunari Yoneda,[a] Tomohiro Nishimura,[a] Nobuhiko Katunuma,[a] Shigeyuki Imamura,[b] Katsutoshi Nitta[c] and Hideaki Tsuge[a]*

[a]Institute for Health Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8054, Japan, [b]Diagnostic Research and Development, Diagnostic Division, ASAHI KASEI Corporation, 632-1 Mifuku, Ohito, Shizuoka 410-2321, Japan, and [c]Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

Correspondence e-mail: tsuge@tokushima.bunri-u.ac.jp

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Thermostable monoacylglycerol lipase (MGLP; EC 3.1.1.23) from the moderately thermophilic *Bacillus* sp. H-257 has a unique substrate specificity. It hydrolyzes monoacylglycerols but does not hydrolyze di- or triacylglycerols. Crystals of the enzyme were obtained by the hanging-drop vapour-diffusion method using ammonium sulfate as a precipitant and benzamidine as an additive. The orthorhombic crystals belong to the space group $P2_12_12_1$, with unit-cell parameters a = 43.53, b = 100.82, c = 108.17 Å. The crystals diffract to at least 2.3 Å resolution and a native data set has been collected to 2.6 Å resolution on a CCD detector using synchrotron radiation.

1. Introduction

Lipase (triacylglycerol lipase; EC 3.1.1.3) hydrolyzes triacylglycerols to diacylglycerols, monoacylglycerols, fatty acids and glycerol. Lipase, which is widely distributed in animals, plants and microorganisms, is important and is useful in various industrial fields as a biocatalyst. There are many studies of di- and triacylglycerol lipases (Chahinian *et al.*, 2000; Moriyama *et al.*, 1999; Lee & Severson, 1994); however, only limited information on a specific monoacylglycerol lipase is available. We have purified the MGLP consisting of 250 amino acids produced by the moderately thermophilic *Bacillus* sp. strain H-257 and reported its enzymatic properties (Imamura & Kitaura, 2000), molecular cloning, sequencing and expression in *Escherichia coli* (Kitaura *et al.*, 2001). This MGLP hydrolyzes monoacylglycerols; however, it does not hydrolyze di- and triacylglycerols to an appreciable extent. The enzyme is thermostable up to 333 K and should be useful for analytical purposes such as the measurement of serum lipase levels and the analysis of monoacylglycerols in food.

Several lipase structures have been elucidated (Jaeger *et al.*, 1999) and all have an α/β hydrolase fold, with most lipases containing a helical segment called the lid that covers the active site. Bacterial lipases and esterases are currently classified into eight families based on their amino-acid sequences and fundamental biological properties (Arpigny & Jaeger, 1999). It appears that MGLP could not be assigned to any of these families of lipases because of its lack of sequence similarity. Here, we report the crystallization and preliminary X-ray crystallographic analysis of monoacylglycerol lipase from the moderately thermophilic *Bacillus* sp. H-257, with the aim of determining its three-dimensional structure in order to clarify the molecular mechanism of monoacylglycerol lipase and its molecular evolution.

2. Materials and methods

2.1. Crystallization and preliminary X-ray diffraction analysis

Production and purification of recombinant MGLP was carried out as described previously (Imamura & Kitaura, 2000). The enzyme solution (12 mg ml^{-1}) from freeze-dried enzyme including mannitol was dialyzed against 10 m*M* Tris buffer pH 7.5. Crystallization was carried out by the hanging-drop vapour-diffusion method. A sample solution was prepared by mixing 90 µl of 12 mg ml^{-1} protein with 18 µl of 20% benzamidine. 2 µl of the sample solution was mixed with 2 µl of a precipitant solution containing 1.5 *M* ammonium sulfate, 100 m*M* citrate pH 5.8 and 0.17 *M* tartrate. The diffraction data were measured at room temperature from a single crystal placed in a thin-walled glass capillary tube using an ADSC Quantum4R CCD detector system (Area Detector Systems, Poway, CA) on the BL-6A beamline at the Photon Factory. The data sets were processed using *DPS*/*MOSFLM* (Rossmann & van Beek, 1999) and *SCALA* (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

3.1. Crystallization

Commercially available screens were used to determine the initial crystallization conditions (Hampton Research, USA). An oil-like precipitate was observed using 2.0 *M* ammonium sulfate and 5%(*v*/*v*) 2-propanol as a precipitant

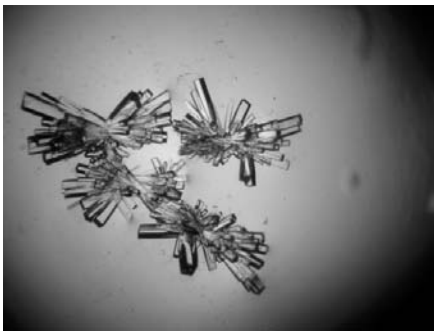

Figure 1
Aggregates of MGLP crystals. A single crystal of good diffraction quality was cut from the aggregate.

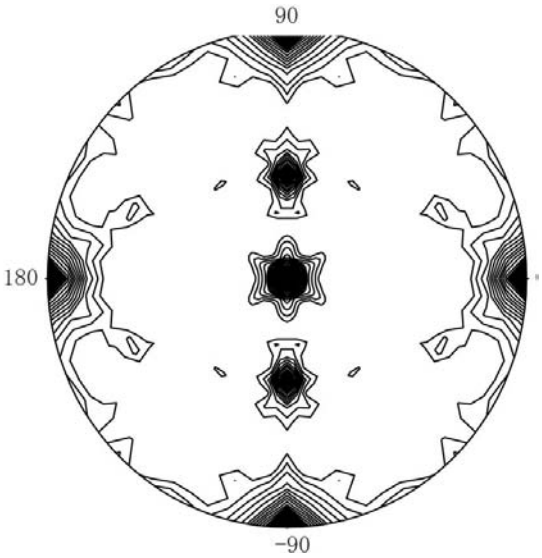

Figure 2
Self-rotation function using native data of MGLP. Two large peaks corresponding to a non-crystallographic twofold axis were observed.

solution. Needle-shaped crystals, which were subsequently optimized, were observed using 1.5 *M* ammonium sulfate, 100 m*M* citrate pH 5.8 and 0.17 *M* tartrate as a precipitant solution and 20% benzamidine hydrochloride as an additive. Crystals were obtained at 277 K and took two weeks to reach maximal size. As the crystals grew as aggregates (Fig. 1), a single crystal was cut from the aggregates to obtain a diffraction-quality crystal. The usual size of a single crystal was 0.05 × 0.1 × 0.1 mm. Benzamidine was essential for crystallization. It can be hypothesized that benzamidine binds the MGLP as well as a serine protease (Brady *et al.*, 1990). However, it did not inhibit the enzyme activity.

3.2. Preliminary X-ray diffraction analysis

The diffraction data sets were collected at 279 K using synchrotron radiation (λ = 1.00 Å). One crystal was used to collect a native data set to 2.6 Å. These crystals belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 43.53, b = 100.82, c = 108.17 Å. A summary of data statistics is shown in Table 1. The native data were analyzed *via* a self-rotation function search using the program *POLARRFN* from the *CCP*4 program package. The strongest peak (peak value 59.8) apart from the origin peak on the twofold section (κ = 180°) was observed at ω = 45 or 135, φ = 90° (Fig. 2) and implies the presence of a dimer of MGLP which has a non-crystallographic twofold axis. Assuming the presence of two molecules in the asymmetric unit, V_M is 2.09 Å^3 Da^{-1} and the solvent content is 40.7% (Matthews, 1968).

A search for heavy-atom derivatives is under way for use in multiple isomorphous replacement phasing. Sequence similarity was found with a carboxyesterase precursor of *Bacillus stearothermophilus* and a putative esterase/lipase of *Streptomyces coelicor* as described elsewhere (Kitaura *et al.*, 2001). The Ser residue, one of the active-site residues, is located within a semi-conserved pentapeptide, either G(A)-*X*-S-*X*-G or G-*X*-S-*X*-S, where *X* indicates any amino acid. The conserved pentapeptide was found in MGLP (95-GLSMG-99); however, two other active-site residues (His and Asp) have not been identified because of the low sequence similarity between MGLP and other lipases. A detailed overview of the α/β hydrolase fold in lipase has been given by Schrag & Cygler (1997). All bacterial lipase structures known to date demonstrate the α/β hydrolase fold, with some variation. The MGLP structure may also have a similar α/β hydrolase fold; however, we expect that elucidation of the structure will provide a new insight into the unique substrate specificity of this enzyme.

Table 1
Data-collection statistics of MGLP.

Beam source	Photon Factory BL6A
Detector	ADSC CCD
Wavelength (Å)	1.0
Number of crystals	1
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 43.53, b = 100.82, c = 108.17
Total No. of observations	85371
No. of unique reflections	21446
Resolution range (Å)	40–2.6
Completeness (%)	98.4
R_{merge} (%)	12.1
Multiplicity	4.0

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