Acta Cryst. (1994). D**50**, 225–227

Crystallization and Preliminary X-ray Analysis of a Lipase from *Chromobacterium viscosum*

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(*Received* 12 *July* 1993; *accepted* 17 *September* 1993)

Abstract

Lipase from *Chromobacterium viscosum* has been purified to homogeneity and crystallized in a form suitable for X-ray diffraction analysis from 10–14% polyethylene glycol 4000 and 10–14% 2-methyl-2,4-pentane diol at pH 6.4 in the presence of 0.25%(*w*/*v*) *n*-octyl-β-D-glucopyranoside. These crystals belong to space group $P2_12_12$ with refined lattice constants $a = 41.1$ Å, $b = 156.8$, $c = 43.6$ Å, indicating a cell content of one monomer per asymmetric unit of the crystal. The crystals diffract to a resolution of 2.2 Å.

Introduction

Triacylglycerol hydrolases (E.C. 3.1.1.3), commonly known as neutral lipases, are present in diverse organisms, including animals, plants, fungi and bacteria. They are surface-active enzymes, *i.e.* they preferably catalyse the hydrolysis of ester bonds at the lipid/water interface after activation by binding to substrate micelles. It is obvious, therefore, that the activity of lipases is considerably higher if the substrate is present in micelles so, consequently, the structure and sequence of events at the lipid/water interface is of interest. Recent advances in lipoprotein biochemistry have increased the industrial potential of lipase-catalysed reactions (Harwood, 1989). An understanding of the molecular architecture of lipases is sought, therefore, as a basis for protein engineering. The crystallization of lipases from diverse organisms has been reported, and the crystal structures of three fungal lipases (Brady *et al.*, 1990; Grochulski *et al.*, 1993; Schrag, Li, Wu & Cygler, 1991) and two human pancreatic lipases (van Tilbeurgh, Sarda, Verger & Cambillau, 1992; Winkler, D'Arcy & Hunziker, 1990) are known. However, no crystal structures of bacterial lipases are known. The crystal structures of the known lipases revealed the presence of a triad of Ser-His-Asp (or Ser-His-Glu) in the catalytic sites. These enzymes show a weak sequence homology in the active-site region only, but some similarity in their three-dimensional structures. This enzyme class shows an α/β hydrolase fold (Ollis *et al.*, 1992), which consists of a central β-pleated sheet connected by α-helices. The catalytic triad of all lipases is buried under a helical segment, called 'lid'.

The microbial lipase of *Chromobacterium viscosum* ATCC 6918 (CVL) (Sagai, Ohta, Suzuki & Imamura, 1989) exhibited sn 1,3-selectivity towards triolein and is highly active over wide ranges of pH (4–11) and temperature (298–343 K). Because of its extraordinary stability this lipase is ideally suited as a biocatalyst for the oil and fat industry. The mature enzyme consists of 319 amino acids with one disulfide bridge.

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ISSN 0907-4449

Materials and methods

CVL was purified by a three-step purification scheme including anion-exchange chromatography, hydrophobic interaction chromatography and gel filtration. Starting from 40 g crude enzyme preparation, 280 mg pure lipase (45% recovery) were obtained. Purified lipase was concentrated by ultrafiltration using a YM 10 membrane. Crystallization was achieved by the sitting-drop vapor-diffusion method using CRYSCHEM crystallization plates. Initial conditions were established by the factorial experiment (Jancarik & Kim, 1991) using a Biomek automated laboratory station (Beckman, Mannheim, Germany). In addition the effect of non-ionic detergents on crystallization was investigated (McPherson *et al.*, 1986). For X-ray analysis crystals were mounted in glass capillaries with a drop of mother liquor. Native data sets were collected using a Xentronics area detector and graphite-monochromated Cu $K\alpha$ radiation of a Rigaku RU-200 rotating anode operated at 50 kV and 100 mA. The diffraction limits were estimated from still frames with an exposure time of 60 s and detector swing-outs for 2.5 and 2.0 Å resolution (1 Å = 0.1 nm). The native data set was collected with a crystal-to-detector distance of 20 cm and a detector swing-out of 26° for 2.2 Å resolution. The exposure time for a frame width of 10 min arc was 4 min. For the native data set, three settings of 300 frames each were collected on a Siemens three-axis goniometer. Settings were separated by a 45° rotation in φ angle at constant χ = 45°. All measurements were carried out at room temperature. The crystals were stable in the X-ray beam for more than one week. The data set was processed using the *XENGEN*2.0 software (Howard *et al.*, 1987).

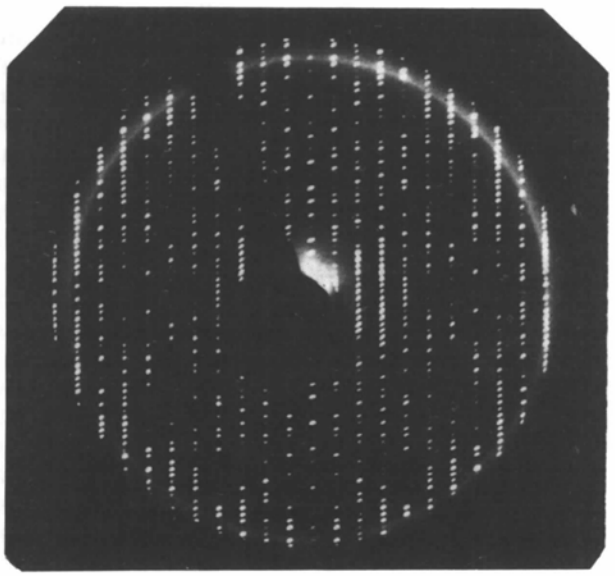

Fig. 1. 0*kl* precession photograph of a crystal of *Chromobacterium viscosum* lipase (μ = 11°, d = 100 mm).

Table 1. *Native data-set statistics of Chromobacterium viscosum lipase*

$R_{merge} = \sum_h \sum_i |I(h)_i - \langle I(h) \rangle| / \sum_h \sum_i I(h)_i$.

D (Å)	Unique reflections	Observed reflections	Percentage observed (%)	R_{merge} (%)
5.38	965	903	93.6	5.0
4.11	1335	1296	97.1	6.0
3.46	1623	1460	90.0	6.4
3.04	1856	1569	84.5	6.5
2.74	2020	1654	81.9	6.9
2.52	2271	1739	76.6	7.2
2.34	2433	1772	72.8	7.4
2.20	2545	1501	59.0	7.4
Observation	15 048	11 894	79.0	6.2

Results and discussion

Crystals could be grown from solutions containing polyethylene glycol (PEG) of different molecular weights as precipitant. The presence of *n*-octyl-β-D-glycopyranoside was found to be essential for growing large crystals. The optimized conditions are as follows: the reservoir solution consists of 10–14%(w/w) PEG 4000, 10–14%(v/v) 2-methyl-2,4-pentanediol (MPD) in 100 mM citrate–phosphate buffer pH 6.4, and the sitting-drop solution, before mixing with reservoir, contains 15 mg ml^{-1} lipase and 0.25%(w/v) *n*-octyl-β-D-glycopyranoside in 5 mM Tris/HCl buffer, pH 8. Crystals grow to their full size (0.6 × 0.4 × 0.3 mm) within one week at 292 K. The space group and preliminary lattice constants were determined by precession photographs (Fig. 1) using a Huber precession camera mounted on the same X-ray source. The photographs of the lipase crystals showed that the reciprocal lattice contained three mirror planes and that the reflections could be indexed on an orthorhombic lattice. Axis reflections of the class $h = 2n + 1$ and $k = 2n + 1$ were systematically absent, indicating the orthorhombic space group $P2_12_12$. Processing and scaling of the data yielded the refined lattice constants a = 41.1, b = 156.8, c = 43.6 Å and a symmetry R value of 6.2% [$R = \sum_h \sum_i |I(h)_i - \langle I(h) \rangle| / \sum_h \sum_i I(h)_i$] (Table 1). Assuming one monomeric enzyme of molecular mass 33 kDa in the asymmetric unit a crystal-packing parameter (Matthews, 1968) of V_M = 2.15 Å^3 Da^{-1} was calculated. The crystals diffract to 2.2 Å resolution. Further native data collection and a search for heavy-atom derivative data are in progress.

This work was financially supported by the BRIDGE Program of the Commission of the

European Communities (CEC), [BIOT-CT91-0258 (LNBE)].

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