SHORT COMMUNICATIONS

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Crystallization and preliminary X-ray diffraction studies of the *Pseudomonas marginata* esterase EstB

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Abstract

Crystals of the esterase EstB were obtained at 277 K with the hanging-drop technique from polyethylene glycol 4000 solutions containing 2-propanol at pH 7.5. The crystals belong to the trigonal space group $P3_121$ (or $P3_221$) with cell dimensions $a = b = 82.9$ and $c = 193.4$ Å (at 100 K). The crystals diffract beyond a resolution of 2.0 Å.

1. Introduction

Esterases (E.C. 3.1.1) catalyze the hydrolysis of esters to the corresponding alcohol and carboxylic acid. They are of increasing importance for applications in biocatalysis (Turner, 1994; Azerad, 1995) due to the diverse range of properties concerning substrate specificity, regioselectivity and enantioselectivity available from various members of this class of enzymes.

Unlike lipases, esterases act preferentially on water-soluble esters with short-chain fatty acids (Walker & Mackness, 1983; Tsujita, Shirai, Saito & Okuda, 1990).

Pseudomonads are known to contain a broad variety of esterases, of which several have been identified, cloned and sequenced in recent years. Examples are the esterases from *Pseudomonas fluorescens* (Choi, Jeohn, Rhee & Yoo, 1990; Hong, Jang, Choi & Yoo, 1991; Kim, Lee, Choi, Park & Yoo, 1994), *P. putida* (Ozaki, Sakimae & Numazawa, 1994), and *Pseudomonas sp.* (McKay *et al.*, 1992; Shimada *et al.*, 1993).

In this paper, we describe the crystallization of a novel esterase (EstB) from *P. marginata*, which is a 'prototypical' esterase, *i.e.* it can hydrolyse triglycerides and *o*- or *p*-nitrophenyl esters with short-chain fatty-acid residues of up to six C atoms. However, it also has the specific ability to hydrolyze sterically hindered esters of tertiary alcohols in a stereoselective manner, which makes this enzyme a particularly interesting candidate for biotechnological applications.

The *estB* gene was cloned and expressed in *E. coli* and the DNA sequence was determined in our laboratory (Petersen, 1995; Petersen, Sölkner, Stanzer, Schlacher & Schwab, 1997). Two different motifs observed in serine hydrolases could be identified in the EstB sequence, a G-*X*-S-*X*-G hydrolase motif and a class-A β-lactamase consensus sequence S-*X*-*X*-K. Class A β-lactamases belong to a superfamily of serine β-lactamases (Bush, 1989; Joris *et al.*, 1988), which also includes a variety of penicillin-binding proteins. All these proteins contain a Ser-*X*-*X*-Lys motif, with the serine being the active-site residue. However, no significant homologies to further major sequence motifs of penicillin-recognizing enzymes were found in EstB.

The active-site serine in EstB could be assigned by site-specific mutagenesis to Ser75. Suprisingly, the active serine is located within the β-lactamase motif (Petersen *et al.*, 1997), but EstB does not show detectable β-lactamase activity. Thus, it is likely that this enzyme belongs to a novel type of esterases.

2. Experimental

EstB esterase was efficiently produced as a soluble cytoplasmic protein by cultivating the *E. coli* BL21[DE3] harbouring the expression plasmid pEP6EX in LB medium at 303 K for about 12 h. Induction of the *tac* promoter was performed with 0.1 m*M* IPTG. The size of the expressed product could be determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (10%) to be 42 kDa which corresponded very well to the molecular mass of 41 695 Da calculated from the deduced amino-acid sequence of the esterase. Esterase EstB was purified to homogeneity from lysates obtained by ultrasonic cell disruption in a three-step procedure including ammonium sulfate fractionation, hydrophobic interaction chromatography (Fractogel TSK Butyl 650) and ion-exchange chromatography on Q-Sepharose (Petersen, 1995).

Crystals of the esterase were grown at 277 K by the hanging-drop vapor-diffusion method (McPherson, 1976) using Linbro multi-well tissue plates. Drops containing 3 µl of 5.6 mg ml^{-1} protein solution and 3 µl precipitant buffer were equilibrated against 700 µl precipitant buffer. The reservoir contained 10%(*w*/*v*) PEG 4000 and 10%(*v*/*v*) 2-propanol in 0.05 *M* NaHepes buffer (pH = 7.5). Colourless crystals appeared after six days and grew to a final size of approximately 0.15 × 0.15 × 0.15 mm.

Since crystals mounted with mother liquor in capillaries deteriorated in the X-ray beam at room temperature after about 3 h, we applied cryotemperature data-collection techniques. Crystals were transferred to a mixture of reservoir solution with 20%(*v*/*v*) glycerol. After soaking for several seconds, the crystals were picked up with a loop made of polymeric fiber (Teng, 1990; Gamblin & Rodgers, 1993) and shock cooled by immersing in liquid nitrogen. The duration of exposure to cryoprotectant was found to be crucial, since crystals rapidly decayed in the cryoprotectant, as judged from an increase in mosaicity and, after longer exposure, by the appearance of cracks. At 100 K (the temperature of the nitrogen gas stream 1.5 cm away from the crystal) crystals were stable during data collection on a rotating-anode X-ray source (Cu $K\alpha$ radiation, 40 kV, 80 mA, 0.3 × 0.3 mm apparent focal spot size, graphite monochromator, collimator size 0.3 mm) equipped with a three-

circle goniometer, a Siemens X-1000 area detector and a locally constructed N_2 gas-stream cryostat. The crystal-to-detector distance was set to 15 cm to resolve the spots along the long c^* axis. A data set to a resolution of 2.5 Å was collected using scans of 0.1° framewidth. The collected data were indexed and processed with the program *XDS* (Blum, Metcalf, Harrison & Wiley, 1987; Kabsch, 1988*a*,*b*).

3. Results and discussion

The overexpressed esterase from *P. marginata* crystallizes with PEG 4000 and 2-propanol as the precipitating agents at pH 7.5. They diffract to better than 2.0 Å resolution. At cryogenic temperatures (about 100 K) the crystals are stable during collection of a complete data set.

The cell was identified to be trigonal with cell dimensions $a = b = 82.9$, $c = 193.4$ Å and systematic absences consistent with the symmetry of space group $P3_121$ or $P3_221$. The standard crystallographic merging residual (on intensities) was 4.8%. The space-group symmetry, the unit-cell volume of 11.6×10^5 Å^3 and the molecular mass of 40 kDa for the protein lead to a V_m of 2.4 Å^{3}Da^{-1} for a dimer in the asymmetric unit. This is in the expected range of 1.6–3.5 Å^{3}Da^{-1} for V_m (Matthews, 1968). A self-rotation function was calculated using the program *POLARRFN* (written by W. Kabsch, distributed in the *CCP*4 package; Collaborative Computational Project, Number 4, 1994). It showed the highest non-origin peak near the crystallographic dyad along x (at $\omega = 90$, $\varphi = 12.7$ and $\kappa = 180°$, height 30% of the origin peak, see Fig. 1). There is no other peak higher than 20%. Thus, although neither the observed V_m value nor the self-rotation function yield an unambiguous decision concerning the number of molecules per asymmetric unit, both observations hint at a non-crystallographic dimer, with the non-crystallographic dyad roughly along the x axis. Recently, we have also collected synchrotron data at the EMBL beamline X11 (DESY, Hamburg) to a resolution of 1.6 Å. An experimentally observed frame of these data is shown in Fig. 2.

Sequence alignment showed regions of higher homology (within the N-terminal region of the protein) with the β-lactamase from *Enterobacter cloacae*, whose crystal structure is known (Lobovsky *et al.*, 1993, PDB entry 2blt). Two blocks of 40 and 26% identity within 52 and 54 residues, respectively, were significant. However, attempts to solve the EstB structure with the 2blt coordinates were so far unsuccessful. Therefore, we are in the process of screening for suitable heavy-atom derivatives.

Table 1. *Data-collection statistics and completeness of the data set*

Number of measured reflections	150050
Number of unique reflections	28851
R_{sym}* (%)	4.8

	Completeness (%)	
Resolution (Å)	$I > 0\sigma(I)$	$I > 3\sigma(I)$
20.0–4.78	99.8	99.6
4.78–3.80	98.8	98.6
3.80–3.32	97.8	97.5
3.81–3.02	97.4	97.0
3.82–2.81	96.5	95.5
2.81–2.64	95.3	93.9
2.82–2.51	92.7	90.9
2.51–2.40	90.8	88.9

* $R_{sym} = \sum_h \sum_i |I_{ih} - \langle I_h \rangle| / \sum_h \sum_i \langle I_h \rangle$, where $\langle I_h \rangle$ is the mean intensity of the i observation of reflection h.

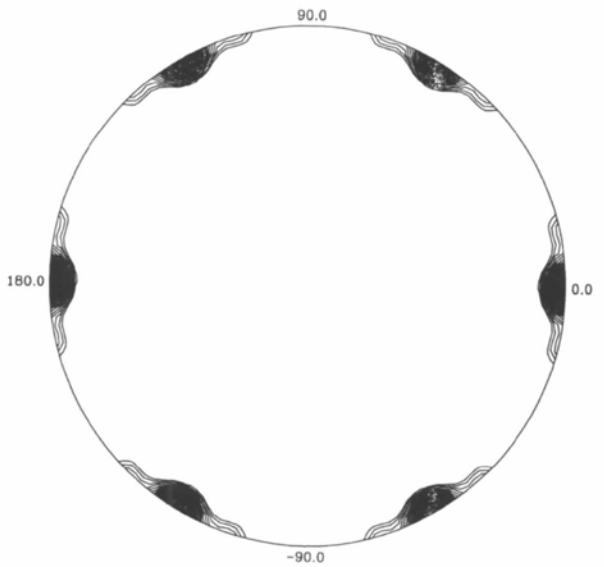

Fig. 1. Self-rotation function of EstB crystals from low-temperature diffraction data (calculated with the program *POLARRFN*). Radial coordinate ω, angular coordinate φ (counterclockwise). Contour lines are drawn starting at 20% of origin peak with intervals of 2.5%.

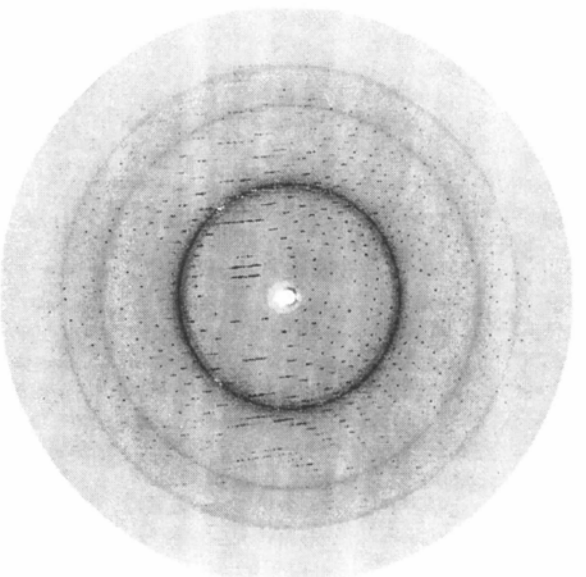

Fig. 2. Diffraction pattern of an EstB crystal, collected at the EMBL beamline X11 (DESY, Hamburg). A wavelength of 0.9118 Å, crystal-to-detector distance of 230 mm, an oscillation range of 0.5° and a MAR imaging plate with a diameter of 30 cm were used. Data were processed with the program *DENZO* (Otwinowski, 1993; Minor, 1993).

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