Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction studies of a novel bacterial esterase

Philip C. Bourne, Michail N. Isupov and Jennifer A. Littlechild*

Departments of Chemistry and Biological Sciences, University of Exeter, Stocker Road, Exeter EX4 4QD, England

Correspondence e-mail: j.a.littlechild@ex.ac.uk

Received 21 September 1998
Accepted 24 December 1998

A novel bacterial esterase has been crystallized in two forms suitable for X-ray diffraction studies. Crystals have been obtained by vapour-phase diffusion at 290 K using ammonium sulfate as precipitant. The first crystals grew in space group $C2$ with unit-cell parameters $a = 134.7$, $b = 55.8$, $c = 110.3$ Å, $\beta = 125.1^\circ$. A monoclinic data set has been collected to 2.0 Å resolution. Microseeding yielded a second crystal form which grew in space group $P2_12_12_1$ with unit-cell parameters $a = 57.1$, $b = 115.4$, $c = 130.4$ Å. Native data from these crystals have been collected to 1.6 Å resolution. A molecular envelope has been determined using an uranyl acetate derivative for phase calculation.

1. Introduction

Esterases are part of the large family of hydrolase enzymes which are responsible for the degradation of amide- and ester-type bonds in a wide range of substrate molecules. Hydrolase enzymes can utilize a wide variety of natural and unnatural substrates, and this has led them to be used extensively as stereoselective catalysts in the synthesis of optically pure molecules for the pharmaceutical and agrochemical industries.

Esterases are ubiquitous enzymes, having been identified in eukaryotes, eubacteria and archaeal species. Structural studies of hydrolase enzymes have shown most to have a conserved tertiary fold known as the α/β-hydrolase fold (Ollis *et al.*, 1992). These enzymes are usually found as either monomers or homodimers with subunit molecular weights varying between 25 and 60 kDa. They possess low sequence homology; however, most have been found to have a conserved pentapeptide (Gly–X_1–Ser–X_2–Gly) sequence around the catalytic serine residue (Brenner, 1988). The other members of the catalytic triad are histidine and either glutamic acid or aspartic acid. There are no other conserved residues within the hydrolase superfamily of enzymes.

A novel bacterium was isolated from a soil sample and identified as a member of the β subclass of the proteobacteria. An esterase was cloned from this strain, gene sequenced and overexpressed (Gledhill, manuscript in preparation). The amino-acid sequence deduced from the nucleotide sequence of the gene (1062 bp) corresponded to a protein of 354 amino-acid residues with a molecular weight of 35 528 Da. The recombinant enzyme is active as a dimer, as shown from gel-filtration studies of the purified protein. The esterase shows no significant sequence homology with any other known proteins. It does not contain the lipase/esterase consensus Gly–X–Ser–X–Gly motif or the variation of this found by Lawson *et al.* (1994). Here, we describe the crystallization of the enzyme in two crystal forms which diffract to medium and high resolution and their preliminary X-ray analysis.

2. Experimental

2.1. Expression and purification

The gene coding for the esterase was cloned from a proteobacterial source and overexpressed in *Agrobacterium* (Gledhill, manuscript in preparation). The cells were disrupted by continuous homogenization. The homogenate containing the recombinant protein was purified by absorption to WK10 ion-exchange resin (Mitsubishi Kasei Corp., Chiyoda-Ku, Tokyo, Japan). The esterase activity was eluted with 1 *M* NaCl. Further purification was carried out by blue Sepharose chromatography. A final gel-filtration step (Superose 12, Pharmacia) was required to purify the enzyme to homogeneity, as shown by polyacrylamide gel electrophoresis under denaturing conditions.

2.2. Crystallization and data collection

The first crystal form was obtained using aliquots of 9 µl protein solution (10 mg ml^{-1}) in 10 m*M* Tris–HCl pH 7.0 mixed with 1 µl of saturated ammonium sulfate and slowly equilibrated using the hanging-drop method of vapour-phase diffusion over a reservoir of 1.5–

© 1999 International Union of Crystallography
Printed in Denmark – all rights reserved

1.6 *M* ammonium sulfate. Plate-like crystals with maximum dimensions 1.5 × 0.2 × 0.1 mm were obtained after two weeks (Fig. 1). The crystals were harvested into a mother liquor containing 10 m*M* Tris–HCl pH 7.0, 2 *M* ammonium sulfate. For cryocooling, 30% glycerol was added to the mother liquor. These crystals showed diffraction to 1.8 Å resolution at Daresbury Synchrotron station 7.2 at 100 K; however, time restraints limited data collection to 2.0 Å. The space group was identified as *C*2 with unit-cell parameters $a = 134.7$, $b = 55.8$, $c = 110.3$ Å, $\beta = 125.1^\circ$. Frames were indexed and data processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The data were merged with an *R* factor of 6.5% and were 97.7% complete (Table 1).

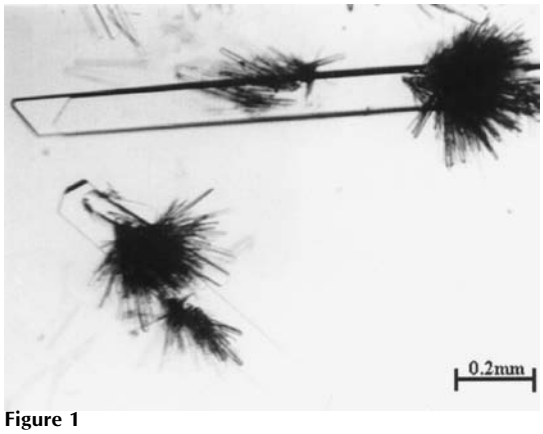

Figure 1
Monoclinic crystals of the esterase, which grow up to 1.5 mm in length.

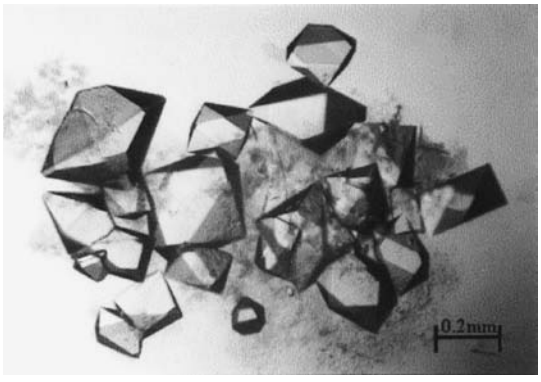

Figure 2
Orthorhombic crystals of the esterase grown after microseeding. The longest dimension of the crystals is 0.5 mm.

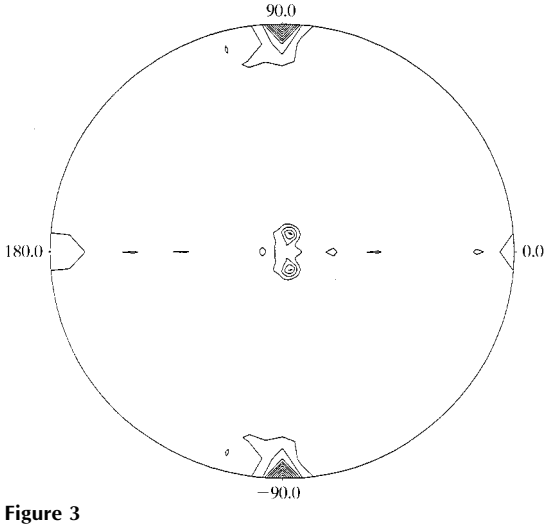

Figure 3
Self-rotation function calculated using monoclinic crystal data (section $\kappa = 180^\circ$, integration radius of 29 Å, calculated at 10–2.4 Å resolution).

Since the monoclinic crystals were too small for in-house data collection, crystal fragments were used for microseeding into fresh protein droplets. This produced a second crystal morphology, which was orthorhombic with unit-cell parameters $a = 58.5$, $b = 117.5$, $c = 132.2$ Å. On cryocooling, the unit cell contracted to $a = 57.1$, $b = 115.4$, $c = 130.4$ Å. Examination of the systematic absences suggested a space-group symmetry of $P2_12_12_1$. These crystals grew to maximum dimensions of 0.5 × 0.4 × 0.4 mm in two weeks (Fig. 2). A 1.6 Å resolution native data set was collected for the orthorhombic crystals on synchrotron station BW7A at EMBL, Hamburg. The data were merged with an *R* factor of 7.3% and were 88.0% complete (Table 2).

Data were also collected from the orthorhombic crystals in-house at room temperature using a Siemens Hi-Star area detector with graphite-monochromated Cu $K\alpha$ radiation from a Siemens rotating-anode generator operating at 50 kV and 100 mA. Frames were indexed and processed using the programs *FRAMBO* and *SAINT* (1993). Several native and derivative data sets were collected from these crystals. A comparison of the in-house native data sets showed the crystals to be non-isomorphous, even for crystals harvested from the same droplet, which showed a 1–3% variation in cell parameters. Cutting a single crystal into four fragments allowed collection of 2.6 Å resolution native data (21631 unique reflections; $R_{sym} = 8.6\%$) and 2.6 Å uranyl acetate heavy-atom derivative data (5 m*M*, 24 h soak).

Table 1
Data-processing statistics for synchrotron data collected from the monoclinic crystals.

Space group	*C*2
Unit-cell parameters (Å, °)	$a = 134.7$, $b = 55.8$, $c = 110.3$ $\beta = 125.1$
Resolution limit (Å)	2.0
Number of observed reflections	147688
Number of unique reflections	43220
R_{merge}† (%)	6.5
R_{merge}† (highest resolution shell) (%)	15.6
Completeness of data (overall) (%)	97.7
Completeness of data (highest resolution shell) (%)	99.0
$\langle I/\sigma I\rangle$	15.96

† $R_{merge} = (\sum_h \sum_i |I_h - I_{hi}|)/(\sum_h \sum_i I_{hi})$, where I_h is the mean intensity of the i observations of reflection h.

Table 2
Data-processing statistics for synchrotron data collected from the orthorhombic crystals.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	$a = 56.7$, $b = 115.2$, $c = 131.5$
Resolution limit (Å)	1.57
Number of observed reflections	381884
Number of unique reflections	111089
R_{merge}† (%)	7.3
R_{merge}† (highest resolution shell) (%)	44.1
Completeness of data (overall) (%)	88.0
Completeness of data (highest resolution shell) (%)	89.8
$\langle I/\sigma I\rangle$	18.9

† $R_{merge} = (\sum_h \sum_i |I_h - I_{hi}|)/(\sum_h \sum_i I_{hi})$, where I_h is the mean intensity of the i observations of reflection h.

3. Results and discussion

Calculation of the solvent content for the monoclinic crystal form suggests that there are one or two subunits per asymmetric unit (a.u.). V_m is 2.39 Å^3 Da^{-1} (Matthews, 1968) with a solvent content of 48% for two subunits per a.u. For one subunit per a.u., V_m is 4.77 Å^3 Da^{-1} with a solvent content of 74%. Self-rotation functions (Collaborative Computational Project, Number 4, 1994) calculated from the monoclinic data show strong features at a κ value of 180° (Fig. 3). This suggests the presence of a twofold molecular axis, supporting the assumption that the asymmetric unit contains a dimer.

Calculation of the solvent content for the orthorhombic form suggests one, two or three subunits per asymmetric unit. One subunit per a.u. gives a V_m of 6.04 with a solvent content of 80%, two subunits per a.u. gives a V_m of 3.02 with a solvent content

of 59% and three subunits per a.u. gives a V_m of 2.01 with a solvent content of 39%. Provided the esterase molecule is still a dimer in the crystal, two subunits per asymmetric unit seems to be plausible since there are no proper rotational crystallographic axes in the space group $P2_12_12_1$. Self-rotation functions calculated for the orthorhombic data were inconclusive.

For the uranyl acetate derivative data, eight heavy-atom sites were located using a Patterson search within *SHELX* at 10–6 Å resolution (Sheldrick *et al.*, 1993). Heavy-atom positions, occupancies and anomalous occupancies were refined using *MLPHARE* (Collaborative Computational Project, Number 4, 1994). The uranyl acetate derivative gave phases with a figure of merit of 0.49 at 10–3 Å resolution (phasing power of 1.90 and 1.42 for acentric and centric reflections, respectively; centric $R_{\rm Cullis}$ = 0.64). A molecular envelope has been calculated for the esterase; however, at this stage secondary-structure elements cannot be resolved. Three pairs of heavy atoms seem to be related by the twofold NCS axis which supports the suggestion that the asymmetric unit contains a dimer. However, the other two heavy-atom sites, one of which has the highest occupancy, do not obey this NCS. All the heavy atoms are located within the molecular envelope; however, the two atoms that do not have NCS mates seem to be located on the molecular interface. Further heavy-atom derivatives are currently being investigated and should result in the solution of this esterase structure to high resolution.

Dr Fred Antson is thanked for useful discussion. PCB and MNI are supported by grants from the Biotechnology and Biological Sciences Research Council. PCB is a CASE Student with SmithKline Beecham Pharmaceuticals. We thank the European Union for support of the work at EMBL Hamburg through the HCMP to Large Installations Project, Contract No. CHGE-CT93-0040.

References

Brenner, S. (1988). *Nature (London)*, **334**, 528–530.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Lawson, D. M., Derewenda, U., Serre, L., Ferri, S., Szittner, R., Wei, Y., Meighen, E. A. & Derewenda, Z. S. (1994). *Biochemistry*, **33**, 9382–9388.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Ollis, D. L., Cheah, E., Cygler, M., Dikstra, B., Frolow, F., Franken, S. M., Harel, M., Remington, S. J., Silman, I., Schrag, J., Sussman, J. L., Verschueren, K. H. G. & Goldman, A. (1992). *Protein Eng.* **5**, 197–211.
Otwinowski, A. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
SAINT (1993). *SAINT Software Reference Manual.* Siemens Analytical Instruments, Madison, Wisconsin, USA.
Sheldrick, G. M., Dauter, Z., Wilson, K. S., Hope, H. & Sieker, L. C. (1993). *Acta Cryst.* D**49**, 18–23.