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Crystallization and preliminary X-ray diffraction studies of the carboxylesterase EST2 from *Alicyclobacillus acidocaldarius*

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EST2, a thermophilic carboxylesterase from *Alicyclobacillus acidocaldarius*, belonging to the HSL group of the esterase/lipase superfamily, has been crystallized for the first time. Ammonium sulfate was used as a precipitant and the crystallization proceeded at pH 7.8. The crystals belong to space group $P4_12_12$ or its enantiomorph $P4_32_12$, with unit-cell parameters $a = b = 78.8$, $c = 106.4$ Å. A complete data set has been collected at the synchrotron source Elettra in Trieste to 2.4 Å resolution, using a single frozen crystal.

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1. Introduction

Esterases, lipases and cholinesterases belong to a large family of phylogenetically related proteins with representatives in both the eukaryote and prokaryote kingdoms and among proteins which lack enzymatic activity (Krejei *et al.*, 1991; Hemilä *et al.*, 1994).

Three subfamilies have been identified: (i) the C group, comprising cholinesterases from vertebrates and invertebrates, lipases from fungi, a number of esterases and some non-enzymatic proteins, (ii) the L group, comprising lipases from vertebrates and bacteria, lipoprotein lipases, lecithin–cholesterol acyltransferases and related non-enzymatic vitellogenins from flies, (iii) the H group, comprising protein ORF3 from *A. acidocaldarius*, *N*-acetylphosphino-thricintripeptide deacetylase from *Streptomyces hygroscopicus*, acetylhydrolase from *S. viridochromogenes*, lipase 2 from *Moraxella* TA144 (MOL), an esterase from *Acinetobacter calcoaceticus* (ACE), esterase AES encoded by the ORF203 of *Escherichia coli*, Vsh5 from the slime mould *Dictiostelium discoideum* and the recently sequenced human liver arylacetamide deacetylase (HDAC).

The H group was named hormone-sensitive lipases (HSL) by Hemilä *et al.* (1994). These authors first reported the cloning and sequencing of a gene from *A. acidocaldarius* encoding a protein of unknown function (ORF3) homologous to the HSL from human and rat.

The three-dimensional structures of several proteins belonging to the C and L groups have been determined recently. They revealed a common topological fold, named the α/β-hydrolase fold, independent of the sequence similarity.

The sequence similarity of proteins of the H group with those of the other groups is not very high, and no structural characterization for any protein of this group is available to date.

It is also worth noting that esterases and lipases from thermophilic bacteria have been investigated little. In fact, only carboxylesterases from *Sulfolobus acidocaldarius* (Sobek & Gorish, 1989), *Bacillus stearothermophilus* (Matsunaga *et al.*, 1974; Owusu & Cowan, 1991; Wood *et al.*, 1995) and *A. acidocaldarius* (Manco *et al.*, 1994) have been purified and partially characterized to date. Therefore, structural studies on new enzymes from thermophilic bacteria could provide fundamental insights into evolutionary relationships in the esterase/lipase family, as well as into the basis of the stability of this class of proteins. They may also provide useful information for future applications in biotransformations.

Recently, Manco *et al.* (1997) reported the purification of a new esterase (EST2) from *A. acidocaldarius* and demonstrated its identity with the aforementioned H-group protein ORF3. On the basis of a secondary-structure-driven multisequence alignment, they predicted the α/β-hydrolase topological fold for this enzyme and identified Ser155, Asp252 and His282 as putative members of the catalytic triad. A structure–function relationship study on this representative protein of the HSL group was undertaken. The gene was over-expressed in *E. coli*, purified and partially characterized (Manco *et al.*, 1998).

This paper provides the first crystallization information and preliminary crystallographic studies of a member of the HSL family of proteins: the thermophilic EST2.

2. Materials and methods

The purified protein in 25 m*M* Tris–HCl pH 8.3, 0.1 *M* NaCl, 2.5 m*M* $MgCl_2$ was concen-

Table 1
Crystallographic data and data-collection statistics.

Temperature (K)	100
Space group	$P4_12_12$ or $P4_32_12$
Unit-cell dimensions (Å)	
a	78.8
b	78.8
c	106.4
Mosaicity (°)	0.64
Total observations	290245
Unique reflections	13530
Multiplicity	21.4
Complete resolution range (Å)	20.0–2.4
Outermost resolution shell (Å)	2.49–2.40
Overall completeness (%)	98.8
Outermost shell completeness (%)	99.6
Overall $I/\sigma(I)$	39.0
Outermost shell $I/\sigma(I)$	8.3
Overall $I/\sigma(I) > 3$ (%)	89.8
Outermost shell $I/\sigma(I) > 3$ (%)	67.7
Overall R_{merge} (%)	4.2
Outermost shell R_{merge} (%)	18.3

trated to 4 mg ml^{-1} and crystallized at 295 K. Crystal screening was performed using the sparse-matrix method (Hampton Research kit) coupled with the hanging-drop vapour-diffusion technique (Jancarik & Kim, 1991).

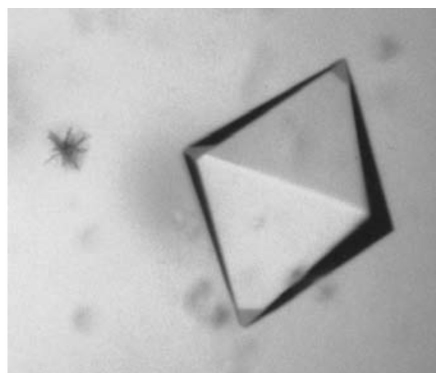

Figure 1
Tetragonal crystals of EST2 exhibiting a characteristic bipyramidal shape. Actual dimensions of the largest crystal are 0.2 × 0.2 × 0.4 mm.

Crystals suitable for data collection were grown from drops containing 4 µl protein solution mixed with 4 µl precipitating solution (2 *M* ammonium sulfate, 100 m*M* HEPES pH 7.8, 2% PEG 400). Crystals of dimensions 0.2 × 0.2 × 0.4 mm grew in one week.

For data collection, crystals were briefly washed in solutions of 2 *M* ammonium sulfate, 100 m*M* HEPES pH 7.8, 2% PEG 400 and 10% glycerol, retrieved with a 0.3 mm nylon loop and flash-frozen in the nitrogen stream of an Oxford Cryosystems Cryostream cooler operated at a temperature of 100 K. Data were collected at the Elettra synchrotron source in Trieste and were processed using the *HKL* crystallographic data-reduction package (Otwinowski & Minor, 1997).

3. Results

Large well formed crystals of EST2 grew in one week using the hanging-drop method. The crystals exhibited tetragonal morphology (see Fig. 1), and examination of systematic absences allowed the identification of the space group as either $P4_12_12$ or its enantiomorph $P4_32_12$. At room temperature, diffraction from crystals under investigation was found to fade rapidly; however, it was possible to collect a complete data set at 100 K to 2.4 Å resolution using a flash-cooled crystal. The scaling gave a final R_{merge} of 4.2% for 290 245 reflections, of which 13 530 were unique corresponding to 98.8% completeness (99.6% complete in the shell 2.49–2.40 Å). The unit-cell dimensions at 100 K are $a = b = 78.8$, $c = 106.4$ Å. A solvent content of 48.8% was calculated using the method of Matthews (1968), assuming the crystals to have one subunit per asymmetric unit (V_m = 2.4 Å^3 Da^{-1}). The value of the crystal-packing parameter V_m is well within the accepted range for proteins. Table 1 reports the crystallographic data and the data-collection statistics.

A selenomethionine derivative is being crystallized, and structure determination of the protein will proceed by multiple wavelength anomalous diffraction techniques (Hendrickson, 1991).

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