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SHORT COMMUNICATION

CRYSTALLIZATION AND PRELIMINARY DIFFRACTION ANALYSIS OF CHOLESTEROL ESTERASE FROM *CANDIDA CYLINDRACEA*

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Summary—Cholesterol esterase (EC 3.1.1.13) from the microorganism *Candida cylindracea* has been crystallized in two forms. Crystals, typically 0.30 × 0.15 × 0.10 mm in size, diffract rotating anode generated x-rays to beyond 3 Å are suitable for data collection for an x-ray crystallographic investigation. A monoclinic crystal form in the space group $P2_1$ was found to have cell dimensions of a = 122.9 Å, b = 101.0 Å, c = 95.2 Å and $\beta = 108.3°$. The asymmetric unit of the cell contains two dimers of 129 kDa each. A second crystal form, in the triclinic space group P1, has cell dimensions of a = 58.6 Å, b = 88.7 Å, c = 58.6 Å, $\alpha = 93.3°$, $\beta = 113.8°$ and $\gamma = 96.0°$, and has one dimer per asymmetric unit.

Cholesterol esterase (EC 3.1.1.13) from *Candida cylindracea* is a dimeric enzyme having a molecular weight of 129,000. The enzyme catalyzes the following biochemical reaction:

$$\text{Fatty acid ester of cholesterol} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{Fatty acid} + \text{cholesterol}$$

Unsaturated fatty acid esters in the C_{18}-series are preferred over saturated fatty acids as substrates for the enzyme [1]. Of all the cholesterol esters, the rate of hydrolysis is highest for linoleate [2]. Cholesterol and cholesterol esters make up 50% of the mass of atherosclerotic lesions in the intima of major arteries [3]. Cholesterol linoleate constitutes a major fraction of the esters that form fibrous plaque. The growth of atherosclerotic lesions is influenced by the interconversion of cholesterol and cholesterol esters.

We report here the crystallization of this enzyme and also the results from the preliminary diffraction experiment for crystal characterization and feasibility study. Two crystal forms have been obtained, both of which diffract reasonably well and could be used for x-ray crystallographic determination of the 3-D structure of the enzyme.

MATERIALS AND METHODS

Commercially available cholesterol esterase with bovine serum albumin contamination was purchased from Boehringer–Mannheim, Indianapolis, Ind., U.S.A. All other reagents were of the best grade from one or more of these suppliers: Sigma Chemical Co, St Louis, Mo.; Fisher Scientific, Rochester, N.Y.; Steraloids Inc., Wilton, N.H.; Boehringer–Mannheim.

Purification

The enzyme, having an initial specific activity of 46 Units/mg, was passed through a P-6DG desalting column, 2.5 cm in diameter and 20 cm long, to eliminate the NaCl in which the sample was dissolved. The input volume was 4 ml and the volume of the pooled fractions 25 ml. The sample was then equilibrated with 50 mM phosphate buffer at pH 7.3 and placed on a gel filtration column, 2.5 cm in diameter and 10 cm long, packed with Bio-gel P-100 in 50 mM phosphate buffer at pH 7.3. Cholesterol esterase eluted as two baseline-resolved peaks, separate from the serum albumin peak. The peaks at volumes 131 and 165 ml had apparent molecular weights corresponding to a dimer and a monomer of cholesterol esterase, respectively. Active fractions from both peaks were pooled and concentrated using Amicon concentrator to 10–15 mg/ml of protein. The entire purification experiment was performed at 4°C. Sodium Dodecyl Sulfate (SDS)–gel electrophoresis was performed under denaturing condition with the starting material as well as with the final products.

Assays

The protein assay was performed according to the method of Lowry *et al.* [4] using bovine serum albumin as a standard. The enzyme assay was performed according to the method described by Uwajima and Terada [1]. The increase in absorbance at 500 nm was recorded. The substrate solution was prepared by dissolving 70 mg of cholesterol linoleate in 2.5 ml of Thesit (Boehringer–Mannheim) and adding to it 23 ml of 1 M NaCl. The reaction mixture also contained roughly 10 mM of cholic acid, which enhances the activity of the enzyme [1].

Crystallization

The crystallization experiments were performed by the vapor diffusion of hanging drop technique [5] at room temperature. The droplets of protein solution were stabilized with PEG-3350 and then diffused against sealed wells containing reservoirs of 8–25% PEG solution.

X-ray diffraction

The diffraction experiments were conducted on a precession camera using x-rays from a Rigaku rotating anode generator having a 0.3 mm focal spot and operating at 4.0 kW. The crystals, stabilized with a synthetic mother liquor of 25% PEG solution in phosphate buffer at pH 7.3, were mounted in glass capillaries.

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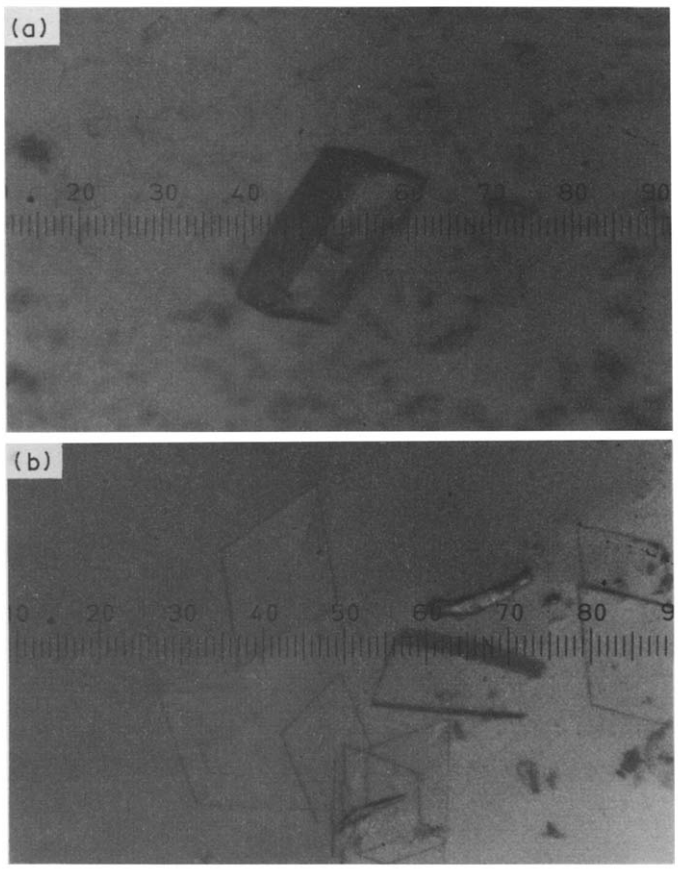

Fig. 1. Two forms of crystals of cholesterol esterase: (a) Form I, and (b) Form II. The smallest division of the scale is 0.016 mm.

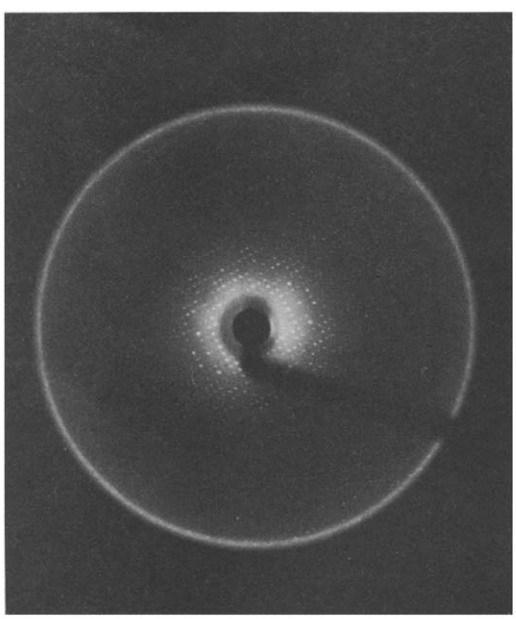

Fig. 2. Precession photograph of Form I showing the Okl layer. The photograph was taken with CuK_α radiation from a Rigaku rotating anode generator operating at 4 kW. $\mu = 15°$, Crystal-to-film distance = 75 mm, Exposure time = 7 h.

Table 1. Summary of crystal data

Crystal form	Form I Monoclinic	Form II Triclinic
Space group	$P2_1$	P1
a	122.9 Å	58.6 Å
b	101.0 Å	88.7 Å
c	95.2 Å	58.6 Å
α	90.0°	93.3°
β	108.3°	113.8°
γ	90.0°	96.0°
Cell volume (Å^3)	1,121,944	275,188
No. of dimer/a.u.*	2	1
Specific volume (Å^3/Da)	2.2	2.1
Mass/a.u. (Da)	258,000	129,000

*a.u. = asymmetric unit.

RESULTS

The elution volumes of the two active peaks were larger than expected, suggesting that the mobility of the enzyme was retarded by interaction with the column. Similar behavior has been reported for porcine cholesterol esterase, which contains 2% carbohydrate by weight and elutes as a dimeric and a monomeric enzyme upon gel filtration [6]. The carbohydrate content of the present microbial enzyme is unknown. The final specific activities of the two peaks were 161 Units/mg and 96 Units/mg, respectively. The SDS-gel electrophoresis of both peaks showed identical clean bands of molecular weight 63 ± 2 kDa, unlike the porcine enzyme where SDS-gel showed that two different isozymes were responsible for the dimer and the monomer peaks [6]. Crystals appeared within 4–6 days from a wide range of PEG concentrations. Two crystal forms were characterized.

Form I obtained from Peak 2, were shaped like parallelepipeds and are shown in Fig. 1(a). Peak 1 produced flat plate like crystals (Form II). Morphologically similar flat plates were also obtained from Peak 2 enzyme in presence of 0.04 mM cholesterol linoleate; however, characterization of this third crystal form is incomplete. The diffraction pattern from crystal Form I is shown in Fig. 2. The unit cell dimensions, unit cell volume and specific volume for the two crystal forms are given in Table 1. The data are consistent with the presence of one dimer in the triclinic cell and two dimers in the asymmetric unit of the monoclinic cell.

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