FOR THE RECORD

Crystallization and preliminary X-ray crystallographic analysis of arylesterase from *Vibrio mimicus*

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Abstract: Single crystals of arylesterase (EC 3.1.1.2) from *Vibrio mimicus* have been obtained from ammonium sulfate as a precipitant at room temperature for 2 months. The present crystals diffract up to 2.2 Å resolution and belong to monoclinic space group P2₁. The cell dimensions are $a = 55.65(1)$ Å, $b = 53.46(1)$ Å, $c = 65.79(1)$ Å, and $\beta = 106.54(1)^\circ$. There are two molecules of molecular weight 22 kDa in an asymmetric unit with a solvent content of 43%.

Keywords: arylesterase; bacterial esterase; *Vibrio mimicus*; X-ray crystallography

Many esterases (ester hydrolase) (EC 3.1.1) that catalyze the hydrolysis of ester bonds were found to be important in biotechnology (Bjorkling et al., 1991). These enzymes show wide variety of substrate specificity, regioselectivity, and enantioselectivity that may be used to synthesize various ester compounds with very high substrate specificity (Tramper et al., 1985; Brooks et al., 1987; Tombo et al., 1987). They can effectively synthesize bioactive chiral compounds, such as drugs and pesticides. This class of enzymes has been subdivided into many subclasses according to substrate specificities. Arylesterases (EC 3.1.1.2), for example, show a preferential substrate specificity for aromatic esters. Their ability to hydrolyze several potent organophosphates plays an important role in the detoxification of organophosphorous compounds in mammalian systems (Mackness, 1989). However, very little is known about bacterial arylesterases. An arylesterase isolated from *Pseudomonas fluorescens* recently showed typical arylesterase activity without active-site serine (Choi et al., 1990). We have recently expressed, purified, and characterized a new arylesterase from *Vibrio mimicus* (Shaw et al., 1994). This arylesterase shows putative active-site serine in a hydrophobic domain. The mutations of serine-29 of this enzyme totally abolished its catalytic activity (Shaw et al., 1994). It contains a GDSLS

sequence that is homologous to the characteristic Gly-X-Ser-X-Gly conserved sequence that has been found in many serine hydrolases. A substitution of Ser-29 by alanine or cysteine abolished the esterase activity. Its optimum reaction temperature is about 50 °C and optimum pH is around 8.0 (Shaw et al., 1994).

Many crystal structures of related esterases that contain active-site serine have been reported (Brady et al., 1990; Winkler et al., 1990; Schrag et al., 1991; Sussman et al., 1991). They have a similar Ser-His-Glu or Ser-His-Asp catalytic site, which is common in serine protease (Brenner, 1988). Crystallization of other typical arylesterase has been reported (Kim et al., 1993). However, no X-ray crystal structure of arylesterase is available at this time. The structure and functional studies of this novel arylesterase from *V. mimicus* will not only illustrate the three-dimensional structure of bacterial arylesterase but also help us to understand the role of this enzyme and let us engineer it as a useful enzyme for the synthesis of drugs and chemicals. In this paper, we report the crystallization and preliminary studies of this novel arylesterase.

Homogeneous arylesterase can be expressed and purified as described previously (Shaw et al., 1994). In order to simplify the purification, the protein-coding region of DNA was subcloned into a pET20b (Novagen) expression vector (unpubl. result). The plasmid was transformed to host BL21(DE3) cells and cultured at 37 °C in a 500-mL flask (200 mL Luria Bertani medium containing 100 µg ampicillin per mL) at 250 rpm. After cells grew to 0.5–1.0 of OD₆₀₀, isopropyl-β-D-thiogalactosidase was added to a concentration of 1.0 µg/mL and incubation continued for 4 h. The cells were harvested by centrifugation, 16 mL binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9) was added and vortexed to suspension, and the solution was then frozen at –70 °C for 20 min. After thawing completely, the suspension was sonicated and then centrifuged. The supernatant (crude enzyme) was loaded on a His-Bind Resin (Novagen) column (1.0 mL resin), and the column was washed with 10 volumes of binding buffer, 6 volumes of washing buffer (60 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9), and finally the enzyme was eluted with elution buffer (1 M imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9). The purified enzyme was di-

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alyzed against water at 4 °C and concentrated. The final material appeared as a single band on SDS-PAGE. The arylesterase activity was checked as described previously (Shaw et al., 1994).

The conventional hanging drop vapor diffusion was used to screen the crystallization conditions (Ducruix & Giege, 1992). Initial crystallization conditions were screened by using the sparse matrix sampling techniques (Jancarik & Kim, 1991) with 1.5 μ L of protein solution equilibrated against an equal volume of the reservoir solutions. The protein was concentrated to a concentration of 12 mg/mL in 5 mM phosphate buffer at pH 6.8. Small needle crystals of arylesterase appeared after 2–3 weeks at 26 °C under conditions containing ammonium sulfate or PEG 4000 in buffer, pH between 5.6 and 7.5. To optimize the crystallization conditions, protein concentration was gradually decreased to about 6 mg/mL and three different buffer solutions were used. Both 20% PEG 4000 with 20% propanol and 1.6 M ammonium sulfate gave us suitable sized crystals; however, crystals from PEG did not diffract well. Only under 0.1 M citrate buffer at pH 5.6 did we obtained crystals with dimensions 0.35 mm $\times$ 0.2 mm $\times$ 0.1 mm after 2 months.

Data were collected on an R-axis II imaging plate detector system using CuK α radiation generated by a RIGAKU RU-300 rotating anode with voltage of 50 kV and current of 80 mA. The detector-to-image plate distance is set to 110 mm and a swing angle is set to 0.0. Three still photographs were recorded over 90° to a maximum of 2.2 Å resolution. The cell parameters were deduced from the peaks extracted from these images. Upon complete data set collection, the cell parameters were further refined using a software package supplied by Molecular Structure Corporation Company. The possible space group deduced from systematic absence is P2₁ and cell parameters are $a = 55.65(1)$ Å, $b = 53.46(1)$ Å, $c = 65.79(1)$ Å, and $\beta = 106.54(1)^\circ$. Though this crystal diffracts better than 2.2 Å, it decays severely on the last few frames. A total of 24,503 reflections were collected, 12,385 reflections being unique, which represents 92.2% of completeness in the resolution up to 2.5 Å. The R_{merge} for the reduced data set was 6.6% in this range.

The arylesterase crystal contains two molecules of molecular weight 22 kDa per asymmetric unit. The V_m value is calculated to be 2.15 by assuming four molecules per unit cell and indicates that the solvent content is approximately 43% according to the equation of Matthews (1968). The result of the self-rotation function shows one noncrystallographic two fold, consistent with the assumption that there are two molecules in an asymmetric unit (Fig. 1).

Amino acid sequence analyses indicate that arylesterase has GDSLS sequence that is homologous to the consensus G-X-S-X-G motif. A thioester bond formation has been proposed to be involved in the catalytic mechanism of this kind of enzyme. However, there is no cysteine in mature *V. mimicus* arylesterase and this rules out the possibility that the enzyme is a cysteine esterase. Detailed sequence comparison between arylesterase and other serine proteinases for which the crystal structures are available shows very low sequence homology, which is not justified for molecular replacement trials. Further sequence similarity searches of the Swiss protein sequence data bank and EMBL data bank revealed homology between the *V. mimicus* arylesterase and *Escherichia coli* acetyl CoA thioesterase I (Cho & Cronan, 1993). A sequence identity of 49% was found for these two proteins. Both *E. coli* acetyl CoA thioesterase I and our arylesterase have the G-X-S-X-S sequence and the first serine resi-

Fig. 1. Stereographic projection of the self-rotation function in spherical polar angles at $\kappa = 180^\circ$, contoured with the program Polarrfn from the CCP4 package (SERC Daresbury Laboratory, UK). It was obtained using data in the 15–6-Å resolution shell and Patterson function radius of 20 Å. The noncrystallographic peak is clearly seen for the spherical polar coordinates of $\omega = 90^\circ$, $\phi = 77^\circ$, and $\kappa = 180^\circ$.

due was proved to be active-site serine. The same pentapeptide GDSLS was also found in phospholipid-cholesterol acyltransferase from *Aeromonas hydrophila* (Thornton et al., 1988). However, there is no crystal structure available for these esterases.

Crystallographic structure studies of arylesterase are important to elucidate the structural differences between our arylesterase and other related esterases in order to understand the substrate specificity and stereoselectivity of these enzymes. The detailed three-dimensional structure is also essential for protein engineering and protein design on this enzyme. A search for suitable heavy atom derivatives is in progress.

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