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Crystals of Fasciculin 2 from Green Mamba Snake Venom

PREPARATION AND PRELIMINARY X-RAY ANALYSIS*

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Fasciculin 2 from the venom of the green mamba, *Dendroaspis angusticeps*, has been crystallized. The crystals are tetragonal, with unit cell dimensions a = 48.9 Å and c = 82.0 Å, space group P 4_12_12 or P 4_32_12. Density measurements and pseudocentering of the *hk*0 zone indicate that there are 16 molecules in the unit cell.

Venoms from the *Elapidae* family of snakes contain a series of related small proteins ($M_r \sim 7000$) which display a variety of toxic actions. Of these, the best studied are the cardiotoxins and the α-neurotoxins; the three-dimensional structures of one cardiotoxin and several α-neurotoxins have already been determined by x-ray crystallographic methods (1–4). A third group of toxins, called fasciculins because of the muscular fasciculation they provoke when injected in the mouse (5), are known to be very potent inhibitors of several types of acetylcholinesterases ($K_i \sim 10^{-10}$ M) (6). Fasciculins are of considerable interest since they constitute the only known protein inhibitors of these enzymes.

Three fasciculins have been characterized to this date: fasciculins 1 and 2 from *Dendroaspis angusticeps* venom and toxin C from *D. polylepis* venom; the reported amino acid sequences of fasciculin 2 and toxin C indicate that fasciculins are structurally related to cardiotoxins and α-neurotoxins (7, 8). In this paper, we communicate the crystallization and preliminary x-ray analysis of fasciculin 2.

EXPERIMENTAL PROCEDURES

The toxin was purified in the following way: lyophilized *D. angusticeps* venom, purchased from Dr. C. Leakey, (Kenya) was fractionated by gel filtration chromatography on Sephadex G-50 (Pharmacia LKB Biotechnology Inc.) in 0.1 M ammonium acetate, pH 6.8. The fractions which induced inhibition of rat brain acetylcholinesterase activity were further separated by two successive ion exchange chromatography steps on CM-Sepharose CL-6B columns (IBF): 1) a linear gradient of 2 × 1 liters of 0.1 to 1 M ammonium acetate, pH 6.8, on a 2.5- × 22-cm column, and 2) a stepwise elution with 0.15 M ammonium acetate, pH 6.8, on a 1 × 200-cm column. The homogeneity of the purified fraction was controlled both by amino acid analysis and by analytical reversed-phase high performance liquid chromatography (9). Acetylcholinesterase activity was determined by the method of Ellman *et al.* (10) with acetylthiocholine as substrate and crude rat brain synaptosomal fraction (P2) as the enzyme source (11).

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RESULTS AND DISCUSSION

Crystals were originally grown by the hanging drop method (12) at 20 °C using tissue culture plates (Limbro, Catalogue No. FB-16-24-7C). Two μl of a 1-ml reservoir solution containing 35% saturation ammonium sulfate and 5 mM β-octyl glucoside buffered with 0.1 M MES,[1] pH 6.0, were mixed with the same volume of a 10 mg/ml protein solution in 0.05 M ammonium acetate. The resulting drop was equilibrated against 1 ml of reservoir solution. Medium sized (0.3 × 0.3 × 0.3 mm^3), bipyramidal crystals appeared after 2 to 5 days. Larger crystals (0.7 × 0.4 × 0.4 mm^3) were subsequently obtained using 12-μl sitting drops deposited on a spot plate and equilibrated against a 20-ml solution in a small plastic box. In this case, the protein and reservoir solutions were buffered at pH 6.5. Attempts to grow crystals in the absence of β-octyl glucoside under these conditions were completely unsuccessful.

X-ray precession photographs show that the crystals are tetragonal, with a = 48.9 Å and c = 82.0 Å. Systematic absences along the principal axes indicate that the space group is P 4_12_12 or P 4_32_12. Calculation of the V_M (13) for $n = 1$ and $n = 2$ (where n is the number of molecules in the asymmetric unit) gives values of 3.64 Å^3/dalton and 1.82 Å^3/dalton, respectively. Since both values are within the experimentally observed V_M range, the calculation of the actual number of molecules per asymmetric unit required the determination of the crystal density. Due to the instability of fasciculin crystals in Ficoll solutions (14), the procedure described by Stout and Jensen (15) was used instead: a mixture of *p*-xylene and carbon tetrachloride was carefully adjusted so that previously dried crystals immersed in it would neither sink nor float; the density value, obtained from the weight of a known volume of this mixture was 1.256 g cm^{-3}. Assuming that the cell parameters were not changed by this procedure and that the fasciculin molecule has a partial specific volume of 0.75 g cm^{-3}, which is typical for proteins in general (13), the calculated mass of protein per asymmetric unit is 15,160 daltons. Since fasciculin has a calculated molecular mass of 6,735 daltons, the density measurement indicates the presence of two molecules in the asymmetric unit.

Another indication of the number of molecules in the unit cell was obtained through examination of the precession photographs. At low resolution ($d > 12$ Å), the *hk*0 zone appears to be pseudocentered with reflections of the type $h + k = 2n + 1$ being systematically absent (Fig. 1). Since centering implies doubling of the number of equivalent positions, this observation is also consistent with the presence of 16 molecules in the unit cell. Furthermore, the pseudocentering im-

[1] The abbreviation used is: MES, 4-morpholineethanesulfonic acid.

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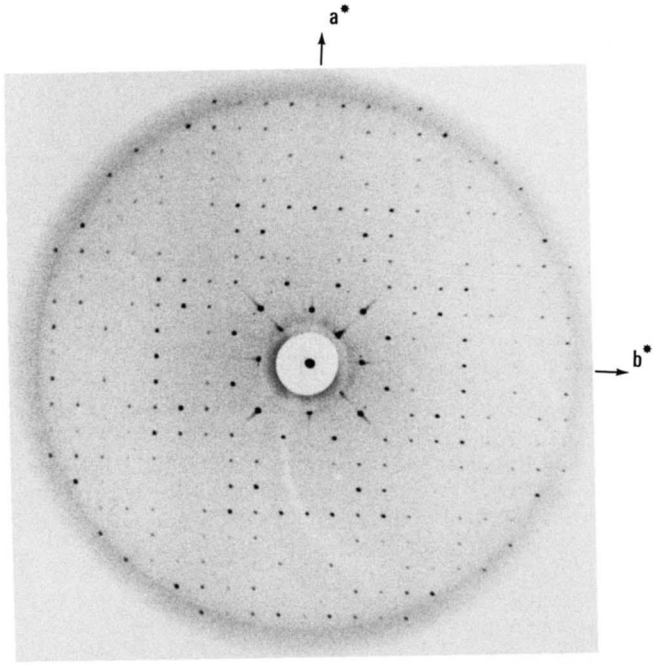

FIG. 1. **Precession photograph of the *hk*0 zone of a fasciculin 2 crystal (precession angle = 3°, crystal-to-film distance = 100 mm).** Reflections satisfying the condition $h + k = 2n + 1$ are systematically absent at low ($d > 12$ Å) resolution.

poses restrictions on the way the two crystallographically independent molecules are oriented relative to each other.

We have collected a complete native data set to 2.0 Å resolution on our Xentronics-Nicolet-Siemens area detector. Heavy atom searches are in progress.

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Note Added in Proof—Similar fasciculin crystals have been reported very recently by (Basu *et al.*) (Basu, S. P., Hannick, L. I., and Ward, K. B. (1989) *Toxicon* **27,** 832 (abstr.).

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