Crystallization and Preliminary Diffraction Studies of the Lys-49 Phospholipase A₂ from Agkistrodon piscivorus piscivorus*

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Previous chemical and structural studies have proposed a major role for Asp-49 in the calcium-mediated activation of phospholipases A₂. Recently, a new class of phospholipases A₂ has been characterized with a lysine in the place of aspartate at position 49 (Maraganore, J. M., Merutka, G., Cho, W., Welch, W., Kezdy, F. J., and Heinrikson, R. L. (1984) J. Biol. Chem. 259, 13839–13843; Maraganore, J. M., and Heinrikson, R. L. (1986) J. Biol. Chem. 261, 4797–4804). Although both the Lys-49 and Asp-49 phospholipases require calcium for enzymatic activity, the Lys-49 enzymes appear to be unique in their ability to bind phospholipids prior to undergoing calcium-mediated activation. We have successfully crystallized the Lys-49 phospholipase A₂ from the venom of the American cottonmouth water moccasin (Agkistrodon piscivorus piscivorus). The crystals are tetragonal, the space group being P4₁2₁2 or P4₃2₁2 with unit cell dimensions a = b = 71.05 Å, and c = 57.76 Å. There is only one molecule in the asymmetric unit and the crystals provide good quality diffraction data to 2.2 Å.

All extracellular phospholipases A₂ hydrolyze the 2-ester bond of 1,2-diacyl-3-sn-phosphoglycerides in a calcium-dependent manner (1). A marked enhancement of enzymatic activity, sometimes on the order of 10⁵-fold, occurs when the substrate is condensed in micelles or lamellar aggregates (2, 3). Thus, phospholipase A₂ provides an ideal system for examining the structural basis of calcium’s role in modulating protein-membrane interactions.

Phospholipases A₂ have been isolated and characterized from a variety of sources and demonstrate a high degree of sequence homology (1, 3, 4). Refined crystallographic structures are available for the dimeric phospholipase A₂ from Crotalus atrox (Western diamondback rattlesnake) venom (5) as well as for the bovine (6) and porcine pancreatic phospholipases (7). Comparison of these structures has revealed a corresponding similarity on the tertiary level, particularly in those regions where the amino acid sequence is conserved (8). Several residues, including Asp-49 (believed to be associated with the critical calcium-binding domain (9)), had been considered invariant (10). Recently, a new class of phospholipases A₂ was reported which substitute lysine for aspartate in several regions where the amino acid sequence is conserved (10). Recently, a new class of phospholipases A₂ has been isolated from the venom of Agkistrodon piscivorus piscivorus (American cottonmouth water moccasin) and Bothrops atrox (fer-de-lance). In addition to the substitution at residue 49, major variations in sequence have been found in regions such as the N-terminal segment and the calcium binding loop. The full functional significance of these modifications remains to be established. Calcium and substrate binding experiments (11, 13) have suggested that the Lys-49 enzymes reverse the activation cycle employed by Asp-49 phospholipases; i.e. the Lys-49 phospholipases can bind to phospholipids in the absence of calcium though still requiring calcium for enzymatic hydrolysis.

As part of our laboratory’s continued interest in the mechanism of phospholipase A₂ action, we have crystallized the Lys-49 enzyme from A. piscivorus and obtained preliminary diffraction data.

EXPERIMENTAL PROCEDURES

Lyophilized venom from A. piscivorus was purchased from the Miami Serpentarium. The procedure described by Maraganore et al. (11) was employed to isolate pure Lys-49 phospholipase A₂. Large single crystals suitable for diffraction work were obtained by the method of vapor diffusion (Fig. 1) (12). Ten-μl droplets containing 10 mg/ml protein, 0.75 M ammonium sulfate, and 0.1 M Tris-H₂SO₄ (pH 8.0) were plated onto plastic coverslips and inverted over a 1-ml reservoir of 2.5 M ammonium sulfate buffered with 0.2 M Tris-H₂SO₄ (pH 8.0). Crystals that measured 0.4 × 0.3 × 0.2 mm were harvested after a period of approximately 3 weeks.

RESULTS AND DISCUSSION

Crystals provided “still” diffraction photographs with strong maxima at spacings less than 2 Å and well-defined oms of Agkistrodon piscivorus piscivorus (American cottonmouth water moccasin) and Bothrops atrox (fer-de-lance). In addition to the substitution at residue 49, major variations in sequence have been found in regions such as the N-terminal segment and the calcium binding loop. The full functional significance of these modifications remains to be established. Calcium and substrate binding experiments (11, 13) have suggested that the Lys-49 enzymes reverse the activation cycle employed by Asp-49 phospholipases; i.e. the Lys-49 phospholipases can bind to phospholipids in the absence of calcium though still requiring calcium for enzymatic hydrolysis.

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FIG. 1. Crystals of the Lys-49 phospholipase A₂ from A. piscivorus. This morphology is one of many, all of which were observed to possess identical diffraction patterns. The bar is 0.5 mm.
X-ray Quality Crystals of a Lys-49 Phospholipase A<sub>2</sub>

The space group is P4<sub>2</sub>,2 or its enantiomorph, P4<sub>2</sub>,2.

The unit cell dimensions derived from precession photographs are \( a = b = 71.05 \) Å, and \( c = 57.76 \) Å. Assuming a molecular weight of 13,947 and one molecule/asymmetric unit, \( V_n \) is approximately 2.26 Å<sup>3</sup>/dalton. This value appears near the center of the distribution of the volume parameter observed for protein crystals having similar molecular weights (14).

The goal of this study is to determine the three-dimensional structure of the Lys-49 phospholipase A<sub>2</sub> from <i>A. piscivorus</i> as a step towards understanding the mechanism by which this enzyme functions. The ability of this enzyme to bind phospholipids without subsequent hydrolysis affords an excellent opportunity for co-crystallization with substrate analogues. Additional comparisons between this new structure, and those previously described for the Asp-49 containing phospholipases, will help provide a more satisfactory explanation of the molecular events occurring during phospholipase A<sub>2</sub> catalysis.

Note Added in Proof—We have learned that T. J. Rydel, H. M. Einspahr, S. W. Muchmore, K. D. Watenpaugh, J. M. Maraganore, and R. L. Heinrikson of the Upjohn Company, Kalamazoo, MI have prepared and characterized crystallographically several crystal forms of the Lys-49 PLA<sub>2</sub> from the venom of <i>A. piscivorus</i>, one of which corresponds to the tetragonal form described here. Their work was presented at the annual meeting of the American Crystallographic Association, June 22–27, 1986, at McMaster University (Abstract PA62).

REFERENCES


FIG. 2. Precession photographs of the <i>A. piscivorus</i> Lys-49 phospholipase A<sub>2</sub>. Two orthogonal projections taken from the same crystal at room temperature using graphite monochromated CuKα from an Elliott GX6 rotating anode run at 40 mA and 40 kV. Both are 30-h exposures (\( \mu = 16^\circ \)). The spacing of the outermost reflection is 2.8 Å, crystal to film distance = 75 mm. a, (hk0) reciprocal lattice plane; b, (h0l) reciprocal lattice plane.