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Purification, crystallization and preliminary crystallographic analysis of a natural complex of phospholipase A_2 from *Echis carinatus* (saw-scaled viper)

Akanksha Nagpal, Vikas Chandra, Punit Kaur and T. P. Singh*

Department of Biophysics, All India Institute of Medical Sciences, New Delhi 110029, India

Correspondence e-mail: tps@medinst.ernet.in

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A novel complex of phospholipase A_2 complexed with another venom protein has been isolated and purified from saw-scaled viper (*Echis carinatus*) venom. The molecular weights of the two components are 16 and 14 kDa, respectively. The complex was purified using an Affigel blue column and an anion-exchange (DEAE Sephacel) column. Long diamond-shaped crystals were obtained by hanging-drop vapour diffusion. The protein complex was dissolved at a concentration of 10 mg ml^{-1} in 20 m*M* sodium cacodylate, 1 m*M* $CaCl_2$ and 2% dioxane at pH 6.0. The reservoir contained the same buffer with 7%(*w*/*v*) PEG 4000. Crystals appeared within 2–3 weeks. Native data to 2.9 Å resolution have been obtained at 291 K. The crystals belong to the monoclinic space group $P2_1$ with unit-cell parameters a = 74.47, b = 47.87, c = 106.39 Å, β = 104.5° and contain two molecules per asymmetric unit. Structure determination by molecular replacement is in progress.

1. Introduction

The phospholipase A_2 enzymes are important components of the cellular machinery which responds to inflammatory stimuli and maintains cell homeostasis by membrane remodelling. The phospholipase A_2 (E.C. 3.1.1.4; *sn*-2 acyl hydrolases) specifically hydrolyses the *sn*-2 ester bond of phospholipids in a Ca^{2+}-dependent manner, displaying enhanced activity towards lipids in lamellar aggregates both in membranes and at other lipid–water interfaces (Ramirez & Jain, 1991; Jain *et al.*, 1995). These enzymes are widely distributed in nature and have traditionally been classified as intracellular or extracellular. The intracellular PLA_2s, which have high molecular weights (31–110 kDa), are often membrane associated and are involved in phospholipid metabolism, signal transduction and other varied cellular functions (Mukherjee *et al.*, 1994). Extracellular PLA_2s (low molecular weights, 12–18 kDa) are abundant in mammalian pancreatic juices, in snake venoms (Verheij *et al.*, 1981; Van den Bergh *et al.*, 1989; Kini, 1997) and insect venoms. The extracellular PLA_2s occur in many isoforms and their molecular weights also vary along with their p*I* values. PLA_2s also exist as dimers and trimers (Keith *et al.*, 1981; Fremont *et al.*, 1993). In addition to these homopolymeric associations, the existence of heteropolymeric PLA_2s has long been established (Bon *et al.*, 1979; Pearson *et al.*, 1993). There have been reports of PLA_2 forming natural complexes with inhibitors (Tchorbanov & Aleksiev, 1981) and other protein molecules, such as β-kunitz protease inhibitor in the case of β-bungarotoxin (Kwong *et al.*, 1995). It has also been found that several PLA_2 species are present in a single venom source and these isozymes may differ broadly in physical, chemical, enzymatic and pharmacological features. The high degree of sequence homology and functional diversity of venom PLA_2s make their structure–function relationships subtle and complicated.

The PLA_2 complex from the venom of *Echis carinatus* has not been studied previously. Preliminary investigations suggest that all three forms of PLA_2 (acidic, neutral and basic) exist in this venom (Kemparaju *et al.*, 1994). It is observed that PLA_2 exists as a complex with another venom protein and that the isoelectric point of the complex is around 5. As the molecular weights of the two components are not equal, it may be a heterodimer.

2. Experimental

2.1. Purification

The lyophilized crude venom of *E. carinatus* was obtained from Irula snake farm, Mahabalipuram, India. Preliminary experiments indicated that the PLA_2 was complexed with another protein of a similar but not identical molecular weight. 250 mg of crude venom was dissolved in 50 m*M* ammonium acetate buffer pH 6.0 to obtain a solution of final concentration 10 mg ml^{-1}. The venom solution was then centrifuged at 10000*g* to remove insoluble material. The supernatant was diluted to 1:1

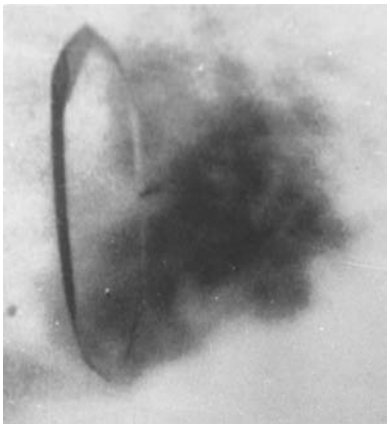

Figure 1
Crystals of PLA_2 complex from *E. carinatus* (dimensions 0.4 × 0.3 × 0.2 mm) grown at room temperature.

with 50 m*M* ammonium acetate buffer pH 6.0. This sample was loaded onto an Affigel blue column (12 × 2.5 cm) which had been pre-equilibrated with 50 m*M* ammonium acetate buffer pH 6.0. The flow was maintained at 0.5 ml min^{-1}. The column was washed with the same buffer to remove all unbound protein. The second buffer, 50 m*M* ammonium bicarbonate pH 8.0, was passed through the column to remove other bound proteins. A gradient of 10–70% dioxane was run in the same buffer to elute the natural PLA_2 complex, which eluted at 40% dioxane. The fractions containing PLA_2 activity (Marinetti, 1965) were pooled, dialyzed and concentrated on an Amicon ultrafiltration unit. The complex was loaded on a DEAE Sephacel anion-exchange column (6.0 × 2.5 cm) pre-equilibrated with 50 m*M* ammonium bicarbonate pH 8.0. A 0.0–0.5 *M* linear gradient of NaCl was run in the same buffer. The PLA_2 complex eluted at 0.3 *M* NaCl. The fractions showing PLA_2 activity were pooled, dialyzed and lyophilized. The homogenity of the complex was checked on SDS–PAGE, which showed two bands corresponding to 16 and 14 kDa. Under native conditions, only one single band was observed around 30 kDa. The N-terminal sequences of both components were determined using an automatic amino-acid sequencer (Biosystems). The N-terminal sequence of the 16 kDa protein is Ser–Val–Val–Glu–Leu–Gly–Lys–Ile–Glu–Glu– and that of the 14 kDa protein is Asp–Phe–Asp–Ser–Pro–Gly–Glu–Tyr–Ser–Glu–. Complete sequence determination is in progress.

2.2. Crystallization and data collection

Crystals of the PLA_2 complex were obtained by the sitting-drop vapour-diffusion method using microbridges at 298 K. The protein was dissolved in 20 m*M* cacodylate buffer pH 6.0 containing 1 m*M* $CaCl_2$ and 2% dioxane. The reservoir contained the same buffer with 7%(*w*/*v*) PEG 4000. The protein concentration was kept at 10 mg ml^{-1}. Crystals of dimensions 0.4 × 0.3 × 0.2 mm (Fig. 1) were obtained in three weeks at room temperature.

3. Results and discussion

A 3.01 Å data set with a completeness of 91% (84% in the resolution range 3.01–3.41 Å) and an R_{sym} of 8.3% was obtained using Cu $K\alpha$ radiation on a MAR Research imaging-plate scanner mounted on a Rigaku RU-200 rotating-anode X-ray generator operating at 40 kV and 100 mA. Using an autoindexing program provided with the package *DENZO* (Otwinowski, 1993) and examining the diffraction data set, it was found that the crystals belong to the monoclinic space group $P2_1$ with unit-cell parameters a = 74.47, b = 47.87, c = 106.39 Å, β = 104.5°. Assuming two molecules of the complex with a molecular weight of 30 kDa per asymmetric unit, the crystals have a calculated V_m of 3.06 $Å^3$ Da^{-1} (Matthews, 1968) and an estimated solvent content of 59%. The structure determination of the PLA_2 complex is in progress using molecular replacement and multiple isomorphous replacement methods.

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References

Bon, C., Changeux, J. P., Jeng, T. W. & Fraenkel-Conrat, H. (1979). *Eur. J. Biochem.* **99**, 471–481.
Fremont, D. H., Anderson, D. H., Wilson, J. A., Dennis, E. A. & Xuong, N. H. (1993). *Proc. Natl Acad. Sci. USA*, **90**, 342–346.
Jain, M. K., Gelb, M. H., Rogers, J. & Berg, O. (1995). *Methods Enzymol.* **249**, 567–614.
Keith, C., Feldman, D. S., Deganello, S., Glick, J., Ward, K. B., Jones, E. D. & Sigler, P. B. (1981). *J. Biol. Chem.* **256**, 8602–8607.
Kemparaju, K., Prasad, N. & Gowda, V. T. (1994). *Toxicon*, **32**(10), 1187–1196.
Kini, R. M. (1997). *Venom Phospholipase A_2 Enzymes.* Singapore: John Wiley.
Kwong, P. D., McDonald, N. Q., Sigler, P. B. & Hendrickson, W. A. (1995). *Structure*, **3**(10), 1109–1119.
Marinetti, G. V. (1965). *Biochim. Biophys. Acta*, **98**, 554–565.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mukherjee, A. B., Miele, L. & Pattabiraman, N. (1994). *Biochem. Pharmacol.* **48**, 1–10.
Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.
Pearson, J. A., Tyler, M. I., Retson, K. V. & Howden, M. E. H. (1993). *Biochim. Biophys. Acta*, **1161**, 223–229.
Ramirez, F. & Jain, M. K. (1991). *Proteins Struct. Funct. Genet.* **9**, 229–239.
Tchorbanov, B. & Aleksiev, B. (1981). *J. Appl. Biochem.* **3**, 558–56.
Van den Bergh, C. J., Slotboom, A. J., Verheij, H. M. & deHaas, G. H. (1989). *J. Cell Biochem.* **39**(4), 379–390.
Verheij, H. M., Slotboom, A. J. & deHaas, G. H. (1981). *Rev. Physiol. Biochem. Pharmacol.* **91**, 91–203.