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Purification, crystallization and preliminary X-ray crystallographic analysis of a phospholipase A_2 from *Daboia russelli pulchella*

Vikas Chandra, Akanksha Nagpal, A. Srinivasan 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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Phospholipases are esterolytic enzymes which hydrolyze glycerophospholipids. The pharmacological efficiency of phospholipase A_2 (PLA_2) enzymes is reflected by their specificity towards a tissue or organ. The Russell's viper has been classified into two classes. Class 1 contains *Viper russelli russelli, Viper russelli siamensis* and *Viper russelli formosensis*, whereas class 2 contains *Daboia russelli pulchella*. The sequence identity between the PLA_2s from these two classes is 47%. The novel PLA_2 from *Daboia russelli pulchella* has been crystallized using the hanging-drop vapour-diffusion method with ammonium sulfate as precipitating agent. Crystals belong to the orthorhombic space group $C222_1$ with unit-cell parameters $a = 77.01$, $b = 92.29$, $c = 76.90$ Å and two molecules in the asymmetric unit. These crystals diffract to about 2.49 Å resolution using a rotating-anode source.

1. Introduction

Phospholipase A_2 (PLA_2, E.C. 3.1.1.4) enzymes hydrolyze phospholipids at the *sn*2 position of the glycerol backbone. The extracellular PLA_2s constitute a large family of homologous 13–18 kDa proteins which are major components of snake venoms (Verheij *et al.*, 1981; Van den Bergh *et al.*, 1989; Kini, 1997). The PLA_2 enzymes have been shown to possess different pharmacological functions such as neurotoxicity (Bon *et al.*, 1979), cardiotoxicity (Fletcher *et al.*, 1981), haemolyticity (Condrea *et al.*, 1981), haemorragicity (Gutierrez *et al.*, 1980), myotoxicity (Mebs, 1986; Gutierrez & Lomonte, 1995), platelet aggregation (Yuan *et al.*, 1993; Gerrard *et al.*, 1993), hypertensivity (Huang, 1984) and oedema-inducing action (Lloret & Moreno, 1993). It is remarkable that such diverse actions can arise from a similar structural scaffolding. The specific structural differences seem to be the key factors behind these actions. The small sequence differences might be responsible for the altered functions of PLA_2. Sometimes PLA_2s from a single species show specific sequence differences to give rise to various subgroups. In the case of Russell's viper, two types have been identified. One type contains *Viper russelli russelli, Viper russelli siamensis* and *Viper russelli formosensis*, whereas the second one contains *Daboia russelli pulchella* (Tsai *et al.*, 1996). There are several characteristic differences between these two subclasses, and the sequence identity between the two subclasses is 47%. One of the typical differences between the sequences of the two subclasses is that the sequence of subclass 1 contains Asn at the N-terminus, while subclass 2 has Ser. The sequence comparison of PLA_2 from *Daboia russelli pulchella* indicates that it shows a lower sequence homology with the PLA_2s of subclass 1 than it shows with PLA_2s from other snake venoms. There is no structural information available on the PLA_2s of subclass 2. Here, we report the isolation, purification and preliminary X-ray crystallographic data of the PLA_2 from the venom of *Daboia russelli*

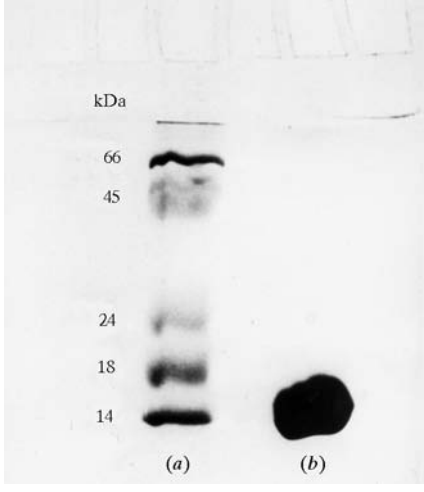

Figure 1
12.5% SDS–PAGE gel showing (*a*) protein standards with molecular weights 66, 45, 24, 18 and 14 kDa; (*b*) purified PLA_2 from *Daboia russelli pulchella.*

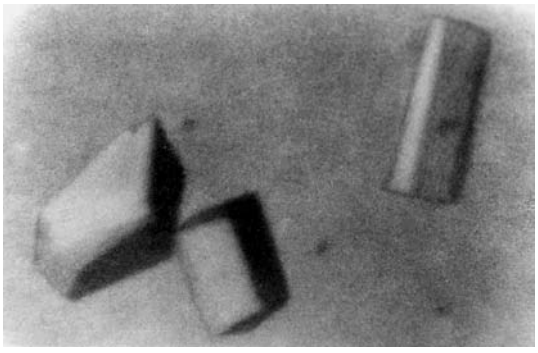

Figure 2
Crystals of PLA_2 from *Daboia russelli pulchella* (0.5 × 0.4 × 0.2 mm) grown at ambient temperature.

pulchella, which is found in south India and Sri Lanka.

2. Experimental

2.1. Purification

Crude venom of *Daboia russelli pulchella* was obtained from the Irula cooperative snake farm, Tamilnadu, India. 300 mg of venom was dissolved in deionized water to a concentration of 10 mg ml^{-1}. This was centrifuged at 8000*g* for 15 min to remove insoluble material. The supernatant was diluted to 1:1 with 20 m*M* ammonium acetate at pH 6.0. This sample was loaded on an Affigel blue column (15 × 2.5 cm) and the column was washed with 20 m*M* ammonium acetate at pH 6.0 with a flow rate of 0.3 ml min^{-1} to remove unbound proteins. 50 m*M* ammonium bicarbonate was used at the same flow rate to remove loosely bound proteins. Finally, a third buffer, 20 m*M* ammonium carbonate pH 10.5, was employed at the same flow rate to elute PLA_2, the presence of which was confirmed by an activity assay. The PLA_2 fractions were dialyzed against ammonium bicarbonate pH 8.0 and concentrated using an Amicon ultra-filtration unit. They were then loaded on to an SP Sephadex C-25 cation exchanger, which was pre-equilibrated with 20 m*M* ammonium bicarbonate pH 7.5 buffer. A linear gradient of NaCl in the same buffer was run between 0 and 0.5 *M*. The fractions having PLA_2 activity were eluted at 0.25 *M* and were pooled, dialyzed and lyophilized. The purity was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), which showed a single band with a molecular weight of 14.0 kDa (Fig. 1). The N-terminal sequence was determined using an Applied Biosystems 470A Protein Sequencer and was found to be Ser–Leu–Leu–Glu–Phe–Gly–Lys–Met–Ile–Leu–Glu–Phe–Tyr–Gly–Lys, which matched exactly the sequence of *Daboia russelli pulchella* (Gowda *et al.*, 1994).

2.2. Crystallization

Crystals of PLA_2 were obtained by the sitting-drop vapour-diffusion method. The protein was dissolved at 15 mg ml^{-1} in 20 m*M* sodium cacodylate buffer, 2 m*M* calcium chloride and 3% dioxane. It was equilibrated with the same buffer containing 1.2 *M* ammonium sulfate. Crystals of dimensions up to 0.5 × 0.4 × 0.2 mm were observed at 298 K within two months (Fig. 2).

3. Results

A 2.49 Å data set with 97.2% completeness for all data (92% completeness in the resolution range 2.59–2.49 Å) was obtained using Cu $K\alpha$ radiation on a MAR Research imaging-plate scanner mounted on a Rigaku RU-200 rotating-anode 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 belonged to the *C*-centered orthorhombic space group $C222_1$ with unit-cell parameters a = 77.01, b = 92.29, c = 76.90 Å and contained two molecules in the asymmetric unit. This corresponded to a crystal volume per unit molecular weight (V_m) of 2.44 Å^3 Da^{-1}, given the molecular weight of 14.0 kDa. The R_{sym} for 9887 unique reflections to 2.49 Å resolution was 8.9%. The R_{sym} for the highest resolution shell was 11.2%. The multiplicity for all the data was 2.8. $I/\sigma(I)$ in the last resolution shell was 10.7. The structure determination of the complex is in progress using molecular-replacement and multiple isomorphous replacement methods.

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