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Crystallization and preliminary X-ray analysis of cardiotoxic phospholipase A_2 from *Ophiophagus hannah* (king cobra)

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An acidic phospholipase A_2 exhibiting cardiotoxicity, myotoxicity and anti-platelet activity was isolated from *Ophiophagus hannah* (king cobra) from Guangxi, China. It contains an unusual 'pancreatic loop'. The enzyme was purified to homogeneity and crystallized using polyethylene glycol and ethylene glycol as precipitants. The crystal belongs to space group *C*2, with unit-cell parameters a = 117.92, b = 62.94, c = 57.16 Å, β = 100.93°. Diffraction data were collected to 2.6 Å.

1. Introduction

Phospholipase A_2 (PLA_2; E.C. 3.1.1.4) enzymes are esterolytic hydrolases catalyzing the hydrolysis of *sn*-3 phosphoglycerides at their 2-position. Snake venoms contain abundant PLA_2s. In addition to common catalytic properties, venom PLA_2 enzymes show a wide variety of pharmacological and biological activities, such as anti-platelet aggregation, oedema formation, inflammatory reaction, neurotoxicity, myotoxicity, cardiotoxicity, haemolytic and anticoagulant activities (Kini & Evans, 1989; Arni & Ward, 1996). Studies of venom PLA_2s have therefore received wide attention. Several PLA_2 enzymes with cardiotoxicity have been reported; they are all basic (Lee *et al.*, 1977; Chang *et al.*, 1983; Liu *et al.*, 1990). *O. hannah* (king cobra) is the largest venomous snake in the world and has a distribution range limited to South China and Southeast Asia. Several PLA_2 enzymes have been isolated and characterized from the venom of this snake (Tan & Saifuddin, 1990; Chiu *et al.*, 1995). Huang *et al.* (1997) described an acidic PLA_2 (OHV-$APLA_2$) from king cobra from China with a molecular weight of 13 719 Da and an isoelectric point of 5.7. The enzyme exhibits activities inhibiting platelet aggregation and inducing myotoxic and cardiotoxic effects (Huang & Gopalakrishnakone, 1996). This is the first acidic PLA_2 which induces cardiotoxicity. We also isolated an acidic PLA_2 with an isoelectric point of 5.5 from king cobra collected in the Guangxi province of China, which was designated OH-$APLA_2$. The pharmacological and biological property studies showed that OH-$APLA_2$ also induced cardiotoxicity and myotoxicity in experimental models and had an inhibitory effect on human platelet aggregation as measured by nephelometry methods (Wang, 1999). The enzyme was found to be the same as that reported by Huang *et al.* (1997), as the sequence of OH-$APLA_2$ deduced from cDNA (Q. Y. Wang, *et al.*, unpublished results) is identical to that of OHV-$APLA_2$. Unlike most snake-venom PLA_2s, OH-$APLA_2$ or OHV-$APLA_2$ contains a five amino-acid insertion at residues 62–66, which usually exists only in mammalian pancreatic PLA_2s and is known as the 'pancreatic loop' (Arni & Ward, 1996). The 'pancreatic loop' may be an important characteristic of PLA_2 from *O. hannah* venom and may be related to its biological activities and evolution (Alape-Giron *et al.*, 1999). Several structures of venom PLA_2s with pharmacological activities have been determined (for reviews, see Ownby *et al.*, 1999; Lin *et al.*, 2000); however, no cardiotoxic PLA_2 structure has yet been reported. As part of our laboratory's continued interest in the structure–function relationship of venom PLA_2 enzymes, we report here preliminary crystallographic studies on OH-$APLA_2$.

2. Experimental and results

OH-$APLA_2$ used in the present studies was purified from the venom of *O. hannah* from the Guangxi province of China as described by Wang (1999). The crude venom was fractionated on a Sephadex G-75 column. The peak with PLA_2 activity was then collected and further purified by ion exchange on a CM-Sephadex CL-6B and a DEAE-Sephadex A-50 column. The OH-$APLA_2$ fraction was selected and its purity was confirmed by PAGE, SDS–PAGE and HPLC. Crystallization trials were performed by the hanging-drop vapour-diffusion method at 291 K. After an initial search using Crystal Screen methods (Jancarik & Kim, 1991; Hampton Research), it was found that the enzyme was readily crystallized from alcoholic solutions. The crystallization

conditions were then refined by altering the concentration and molecular weight of the polyethylene glycol and the concentration of the protein. The optimal crystallization conditions for growing crystals suitable for X-ray analysis were as follows: a droplet containing 5 mg ml^{-1} protein, 5% PEG 4000, 4% ethylene glycol and 0.1 *M* HEPES as buffer (pH 7.5) was equilibrated over reservoir solution containing 10% PEG 4000 and 8% ethylene glycol. Single crystals appeared within one week with maximum dimensions of 0.5 × 0.3 × 0.25 mm (Fig. 1). The crystals diffract to 2.6 Å resolution.

Diffraction data were collected using a MAR 345 image plate. 180° of data to 2.6 Å were collected from a single crystal (1° oscillation and 480 s exposure time per frame). During the collection no radiation damage was observed. The data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1999). The crystal belongs to the monoclinic system, space group *C*2, with unit-cell parameters $a = 117.92$, $b = 62.94$, $c = 57.16$ Å, $\beta = 100.93°$. The data set in the resolution range 20.0–2.6 Å contains 32 758 observations of 11 965 unique reflections and has an $R_{\rm merge}$ of 8.7% and a completeness of 93.8% (75.0% for the last shell).[1] Analysis of the packing density shows that 2–4 molecules in the asymmetric unit would yield a reasonable solvent content, with three molecules being most likely ($V_{\rm M}$ = 2.53 Å^3 Da^{-1}; $V_{\rm solv}$ = 51.38%; Matthews, 1968). The crystal structure determination by molecular replacement (Rossmann & Blow, 1962) using the *AMoRe* program package (Navaza, 1994) is in progress.

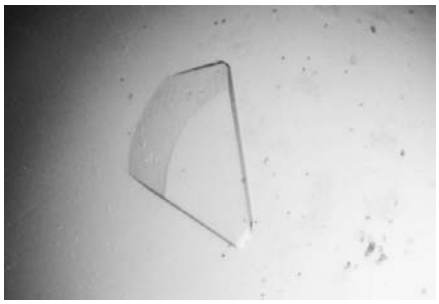

Figure 1
Crystal of cardiotoxic phospholipase A_2 from *O. hannah* (king cobra).

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[1] Supplementary data are available from the IUCr electronic archive (Reference: gr2120). Services for accessing these data are described at the back of the journal.

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