

Preliminary crystallographic study of an acidic phospholipase A₂ from *Ophiophagus hannah* (king cobra)

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An acidic phospholipase A₂ (OH APLA₂-II) with an isoelectric point (pI) of 4.0 was recently isolated from *Ophiophagus hannah* (king cobra) from Guangxi province, China. Comparison of this enzyme to a previously reported homologous phospholipase A₂ from the same venom shows that it lacks toxicity and exhibits a greater phospholipase activity. OH APLA₂-II has been crystallized by the hanging-drop vapour-diffusion method using 1,6-hexanediol and magnesium chloride as precipitants. The crystal belongs to space group *P6₃*, with unit-cell parameters $a = b = 98.06$, $c = 132.39$ Å. The diffraction data were collected under cryoconditions (100 K) and reduced to 2.1 Å resolution. A molecular-replacement solution has been determined and shows that there are six molecules in one asymmetric unit.

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1. Introduction

Phospholipase A₂ (PLA₂; EC 3.1.1.4) catalyzes the hydrolysis of the fatty-acid ester at the *sn*-2 position of phospholipids, with Ca²⁺ as an obligatory cofactor. It is one of the most important enzymes for the metabolism of lipids. The secretory PLA₂s are classified into two main groups based on sequence and structural homology. In addition to being a catalyst for the hydrolysis of phospholipids, PLA₂s from snake venom possess a wide variety of pharmacological activities such as neurotoxicity, haemolytic activity, cardiotoxicity, myotoxicity and anticoagulant and antiplatelet activities (Kini, 1997; Huang *et al.*, 1997). The structural basis of various pharmacological effects such as cardiotoxicity and myotoxicity is still largely unknown.

Ophiophagus hannah (king cobra) is the largest venomous snake in the world and has a distribution range limited to South China and Southeast Asia. Several PLA₂ enzymes have been isolated and characterized from its venom (Tan & Saifuddin, 1990; Chiou *et al.*, 1995; Huang *et al.*, 1997). Two acidic PLA₂s were isolated from king cobra collected in the Guangxi province of China and were designated OH APLA₂ (pI = 5.5, MW = 13 719 Da; Wang, Zhuang *et al.*, 2001) and OH APLA₂-II (pI = 4.0, MW = 13 174 Da; Wang, 2001). Comparison of the amino-acid sequences deduced from cDNA showed an identity of 75%. Despite the high sequence homology, they have significant differences in their biological activities. OH APLA₂ causes myotoxicity and cardiotoxicity in experimental animals, while these were not observed on injection of OH APLA₂-II (Wang, 2001). In

addition, the phospholipase activity of OH APLA₂-II is greater than that of OH APLA₂ (Wang, 2001). Sequence comparison showed that OH APLA₂-II does not possess the 'pancreatic loop' (a five amino-acid insertion at residues 62–66; Arni & Ward, 1996) that is present in OH APLA₂ (Wang, Shu *et al.*, 2001). Thus, comparison of this pair of PLA₂s will elucidate the structural changes that may be implicated in cardiotoxicity, myotoxicity and increased catalytic activity. The crystal structure of OH APLA₂ has been determined to 2.6 Å resolution in our laboratory (Zhang *et al.*, 2002); here, we report the preliminary crystallographic study of OH APLA₂-II.

2. Purification and crystallization

The OH APLA₂-II used in the present studies was purified from the venom of *O. hannah* from Guangxi province, China. The crude venom was fractionated on a Sephadex G-75 column. The peak with PLA₂ 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 APLA₂-II fraction was selected and its purity was confirmed by PAGE, SDS-PAGE and HPLC. The experimental details have been described previously (Wang, 2001). Crystallization trials were performed by the hanging-drop vapour-diffusion method at 291 K. During the initial search, microcrystals of the enzyme were found in solutions containing 1,6-hexanediol as precipitant. The crystallization conditions were then refined by changing the concentrations of the protein, the precipitants and the reservoir solution. The optimal crystallization conditions

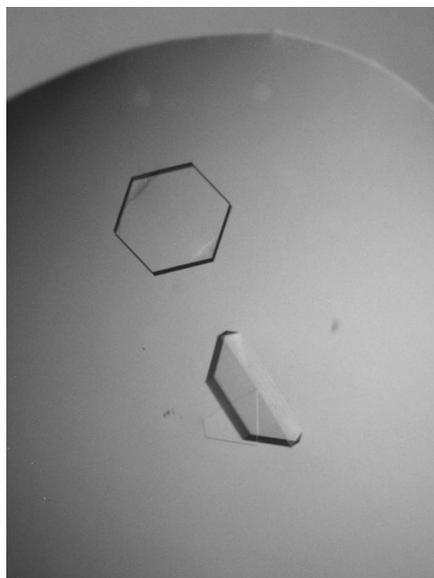


Figure 1
Crystals of phospholipase A₂ from *O. hannah* (OH APLA₂-II).

for growing crystals suitable for X-ray analysis were as follows: an 8 μ l droplet containing 10 mg ml⁻¹ protein, 3.4 M 1,6-hexandiol, 0.2 M magnesium chloride and 0.1 M Tris-HCl pH 8.5 as buffer was equilibrated against 0.5 ml reservoir solution containing 6 M 1,6-hexandiol and 0.01 M Tris-HCl pH 8.5 as buffer. Calcium ions were not added to the droplet. Single crystals appeared within two weeks and reached maximum dimensions after one month. The average crystal dimensions were 0.5 \times 0.5 \times 0.2 mm for hexagon-shaped crystals or 0.6 \times 0.3 \times 0.3 mm for hemihexagon-shaped crystals (Fig. 1). Diffraction data were collected using a hemihexagon-shaped crystal.

3. Data collection and processing

Diffraction data were collected on a MAR 345 image-plate detector (MAR Research) mounted on a Rigaku rotating copper-anode X-ray generator operated at 48 kV and 98 mA with a monochromator (Osmic Mirror; $\lambda = 1.5418$ Å). The crystal was mounted in a loop and data collection was performed at 100 K; no additional cryogen was needed because of the high 1,6-hexandiol concentration in the crystal mother solution. A total of 220 frames with

Table 1

Crystal parameters and X-ray diffraction data-collection statistics.

Values in parentheses are for the highest resolution shell (2.17–2.10 Å).

X-ray source	Cu K α
Wavelength (Å)	1.5418
Space group	<i>P</i> 6 ₃
Unit-cell parameters (Å, °)	<i>a</i> = <i>b</i> = 98.06, <i>c</i> = 132.39, $\alpha = \beta = 90$, $\gamma = 120$
Resolution range (Å)	17.0–2.1 (2.17–2.10)
<i>R</i> _{merge} (%)	7.3 (35.3)
Data completeness (%)	99.6 (96.2)
Average <i>I</i> / σ (<i>I</i>)	32.2 (6.6)
Total No. of observations	1002656
Total No. of independent reflections	41858

an oscillation angle of 1° and an exposure time of 60 s per frame were measured with the crystal-to-detector distance set to 185 mm. The crystal diffracted to 2.1 Å resolution and no appreciable radiation damage was observed during collection. The data were processed using the *HKL* program suite (Otwinowski & Minor, 1997). The space group was determined to be *P*6₃, with unit-cell parameters *a* = *b* = 98.06, *c* = 132.39 Å. The final merged data set consisted of 1 002 656 measurements of 41 858 unique reflections with an *R*_{merge} of 7.3% and a completeness of 99.6% in the range 17.0–2.1 Å. This data set showed an average *I*/ σ (*I*) of 32.2 and a high redundancy. Details of the data-collection statistics are summarized in Table 1. Analysis of the packing density showed that 4–7 molecules per asymmetric unit would yield a reasonable solvent content, with six molecules being the most likely (Matthews coefficient *V*_M = 2.19 Å³ Da⁻¹, estimated solvent content *V*_{solv} = 43.8%; Matthews, 1968).

4. Molecular replacement

An attempt was made to determine a molecular-replacement solution using *MOLREP* (Vagin & Teplyakov, 1997) with the structure of PLA₂ from the venom of *Naja atra*, which has 69% sequence identity (PDB code 1poa; Scott *et al.*, 1990), as a search model. The residues that differed between the two enzymes were treated as alanines. Six PLA₂ molecules were found in each asymmetric unit and rigid-body

refinement using the *CNS* program (Brünger *et al.*, 1998) resulted in an *R* factor of 44.3% (data used 17–3 Å). The crystal packing was reasonable. Six molecules in the asymmetric unit formed three similar molecular pairs. Two molecules within each pair were related by a dyad axis. All three dyad axes were perpendicular to both the crystallographic 6₃ screw axis and the crystallographic *a* axis, but one of them deviated from the other two by about 5 Å along the 6₃ screw axis. The unique molecular arrangement resulted in a pseudo-*P*6₃22 space group for the crystal. Crystal structure refinement is now under way.

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