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Crystallization and preliminary X-ray diffraction analysis of phospholipase A_1 isolated from hornet (*Vespa basalis*) venom

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Phospholipase A_1 (PLA_1) isolated from the black-bellied hornet (*Vespa basalis*) catalyzes the hydrolysis of emulsified phospholipids in addition to the potent haemolytic activity responsible for its lethal effect. In this study, the crystallization and preliminary crystallographic analysis of PLA_1 from hornet venom with a molecular weight of 32 kDa are reported. PLA_1 was crystallized at 277 K using PEG 4000 as precipitant and a 96.5% complete native data set was collected from a frozen crystal to 2.5 Å resolution at 100 K with an overall R_{merge} of 6.8%. The crystal belongs to the triclinic space group $P1$, with unit-cell parameters $a = 57.2$, $b = 70.2$, $c = 81.6$ Å, $\alpha = 107.0$, $\beta = 109.9$, $\gamma = 100.9°$. In each asymmetric unit, three or four subunits of PLA_1 are present according to the calculation of the solvent content.

1. Introduction

The black-bellied hornet (*Vespa basalis*) found in the mountainous area of Taiwan is considered to be the most dangerous of all the species of vespine wasps (Habermann, 1972). The venom of this insect causes local oedema, severe haemolysis and circulatory failure. The high incidence of deaths arising from stings is a consequence of the aggressive action and high toxicity of the venom (Ho & Ko, 1988). According to previous immunochemical studies, the most important allergenic toxins of vespid venoms are phospholipases, hyaluronidases, antigens 5 and acid phosphatases (King *et al.*, 1984; King, 1987).

Previous studies of the local effects of the hornet venom have shown that phospholipase A_1 (PLA_1) is one of the main components involved in the local inflammatory effects caused by *V. basalis* venom (Ho *et al.*, 1993; King *et al.*, 2003). In addition to playing an allergic role, PLA_1 of *V. basalis* venom is also capable of hydrolyzing the *sn*-1 fatty acids from phospholipids and converting these substrates into their corresponding lyso compounds with the release of fatty acids (Ho & Ko, 1988; Aoki *et al.*, 2002). Accordingly, PLA_1 is believed to be able to disrupt the phospholipid packing of several kinds of biological membranes, causing severe haemolysis which leads to the cardiac dysfunction that is responsible for lethality in animals (Ho *et al.*, 1993; Ho & Hwang, 1991; Yang *et al.*, 2008; Santos *et al.*, 2007). In order to further clarify the mechanism by which PLA_1 causes haemolysis, we have undertaken the determination of the crystal structure of PLA_1 isolated from *V. basalis* venom. The results presented in this paper mainly concern the crystallization and preliminary X-ray structural analysis of PLA_1.

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2. Experimental methods

2.1. Extraction and purification of PLA_1 from hornet venom

Crude hornet venom was obtained by pressing venom sacs dissected from hornet workers (Ho *et al.*, 1993). The methods of PLA_1 purification have been described previously (Ho *et al.*, 1993). Fractionation of the venom components was carried out on a size-exclusion column (Fractogel TSK HW 50) eluted with 50 m*M* ammonium acetate buffer pH 5.5. The lethal protein was further purified on a CM cation-exchange column eluted with a linear

gradient of ammonium acetate from 0.05 *M* at pH 5.5 to 1.0 *M* at pH 6.8. PLA_1 was then purified by repeated passage through a C_4 reverse-phase HPLC column using a linear gradient from 20 to 70% acetonitrile in 6 m*M* trifluoroacetic acid, with the OD of the elute being monitored at 280 nm. Finally, the organic solvent in which the PLA_1 was dissolved was removed by lyophilization. The purified PLA_1 was dissolved at 10 mg ml^{-1} in 50 m*M* Tris–HCl pH 7.0 prior to crystallization.

2.2. Crystallization

Initial crystallization conditions were obtained with Hampton Crystal Screens 1 and 2 using the sitting-drop vapour-diffusion method. Each of the solutions (1 µl) from the crystal screening kits was mixed with an equal volume of purified protein solution (10 mg ml^{-1}) at room temperature (~298 K) and equilibrated against 500 µl solution in the well. The conditions were refined and crystals were grown from well solution containing 0.2 *M* sodium acetate trihydrate, 0.1 *M* Tris–HCl pH 8.0, 25% PEG 4000 equilibrated at 277 K against 500 µl precipitation solution using the sitting-drop vapour-diffusion method. Crystals appeared within one month and the largest crystal grew to dimensions of approximately 300 × 150 × 100 µm (Fig. 1).

2.3. X-ray data collection and processing

Crystals were soaked in reservoir solution containing 15%(*v*/*v*) glycerol as a cryoprotectant prior to flash-cooling in a nitrogen-gas stream at 100 K. Diffraction data from the PLA_1 crystal were collected at 110 K on an in-house MicroMax002 X-ray generator using an R-AXIS IV^{++} image-plate system. The crystal-to-detector distance was 160 mm. The oscillation width and exposure time for each frame were 1° and 8 min, respectively. Crystallographic data integration and reduction were carried out using the *HKL*-2000 program package (Otwinowski & Minor, 1997). The crystallographic statistics concerning the data collection of PLA_1 are listed in Table 1.

3. Results and discussion

The PLA_1 crystal chosen for this study was shown to diffract X-rays to 2.5 Å resolution (Fig. 2) and to belong to space group *P*1, with unit-cell parameters $a = 57.2$, $b = 70.2$, $c = 81.6$ Å, $\alpha = 107.0$, $\beta = 109.9$, $\gamma = 100.9°$. The Matthews coefficient of 2.68 or 2.01 $Å^3\,Da^{-1}$ calculated using *MATTHEWS_COEF* (Collaborative Computational Project, Number 4, 1994; Terwilliger & Berendzen, 1999) indicated there were three or four molecules in the asymmetric unit, with a solvent content of 54.10 or 38.80%, respectively. The self-rotation map suggested that noncrystallographic rotational symmetry does not exist at $\kappa = 120°$ or 90° and the native Patterson map also confirmed that there was no additional translation symmetry between the molecules in the unit cell. A homology search of the PLA_1 structure took place using the *Phyre* server (http://www.sbg.bio.ic.ac.uk/~phyre/). The model-selection criterion was based on the *E* value and the estimated precision value. The sequence-alignment search indicated that PLA_1 shares 25–30% identity to the pancreatic lipase fold family (Soldatova *et al.*, 1993). The N-terminal domain from guinea pig pancreatic lipase (PDB code 1gpl) was chosen as the initial model since its *E* value was found to be the lowest (*E* value = 8.528989 × 10^{-36}) and a total of 286 residues were modelled (Carriere *et al.*, 1997). The core of the model consisted of a tightly packed β-sheet surrounded by five α-helices. The model was then used in the molecular-replacement method. The polyalanine model and other truncated models were also considered, but no significant solution was obtained. Heavy-atom derivates were also prepared for the

Table 1
Data-collection statistics for PLA_1 crystals.

Values in parentheses refer to the highest resolution shell.

X-ray source	MicroMax002
Wavelength (Å)	1.5418
Space group	*P*1
Unit-cell parameters (Å)	$a = 57.2$, $b = 70.2$, $c = 81.6$, $\alpha = 107.0$, $\beta = 109.9$, $\gamma = 100.9$
Resolution limits (Å)	50–2.5 (2.59–2.5)
Total reflections	91549
Unique reflections	35849
Completeness (%)	96.5 (95.0)
Redundancy	2.6 (2.6)
R_{merge}† (%)	6.8 (30.3)
$\langle I \rangle / \langle \sigma(I) \rangle$	13.8 (4.4)

† $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$.

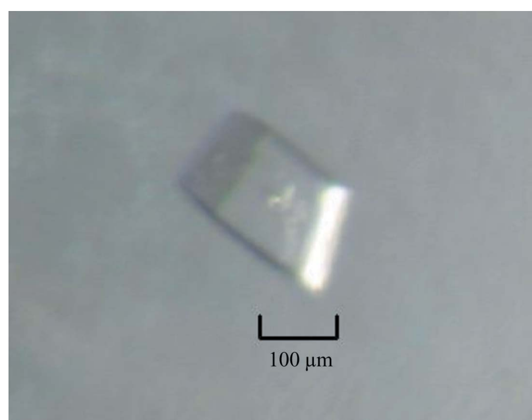

Figure 1
Crystals of PLA_1 obtained with 25% PEG 4000 as precipitant at pH 8.0 using the sitting-drop vapour-diffusion method. The approximate dimensions of the crystal are 300 × 150 × 100 µm.

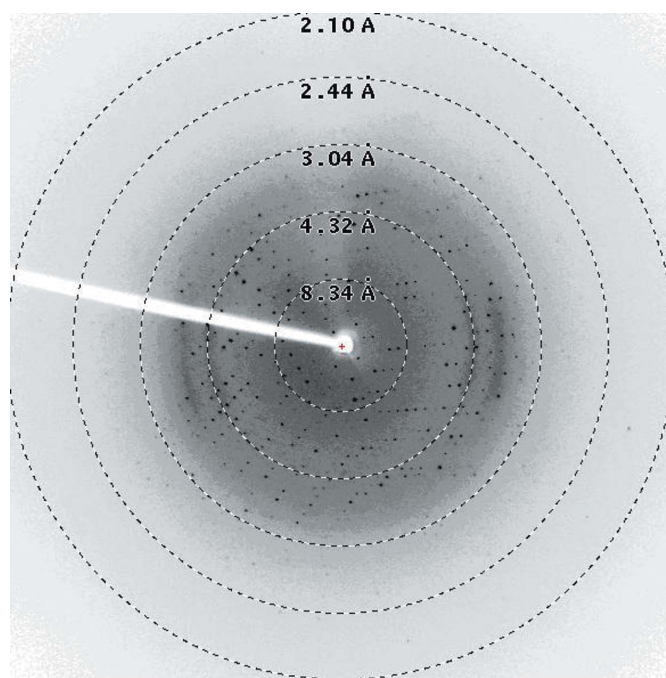

Figure 2
Typical X-ray diffraction pattern of PLA_1.

multiple isomorphous replacement (MIR) and multiwavelength anomalous dispersion (MAD) methods (Walsh *et al.*, 1999). The X-ray fluorescence scanning and anomalous data collection were carried out on the BL17B2 beamline, National Synchrotron Radiation Research Center (NSRRC), Taiwan. Attempts to determine the structure of the PLA_1 protein from these data sets are currently under way.

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