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Crystal structure of vespid phospholipase A_1 reveals insights into the mechanism for cause of membrane dysfunction

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ABSTRACT

Vespid phospholipase A_1 ($vPLA_1$) from the black-bellied hornet (*Vespa basalis*) catalyzes the hydrolysis of emulsified phospholipids and shows potent hemolytic activity that is responsible for its lethal effect. To investigate the mechanism of $vPLA_1$ towards its function such as hemolysis and emulsification, we isolated $vPLA_1$ from *V. basalis* venom and determined its crystal structure at 2.5 Å resolution. $vPLA_1$ belongs to the α/β hydrolase fold family. It contains a tightly packed β-sheet surrounded by ten α-helices and a Gly-X-Ser-X-Gly motif, characteristic of a serine hydrolyase active site. A bound phospholipid was modeled into the active site adjacent to the catalytic Ser-His-Asp triad indicating that Gln95 is located at hydrogen-bonding distance from the substrate's phosphate group. Moreover, a hydrophobic surface comprised by the side chains of Phe53, Phe62, Met91, Tyr99, Leu197, Ala167 and Pro169 may serve as the acyl chain-binding site. $vPLA_1$ shows global similarity to the N-terminal domain of human pancreatic lipase (HPL), but with some local differences. The lid domain and the β9 loop responsible for substrate selectivity in $vPLA_1$ are shorter than in HPL. Thus, solvent-exposed hydrophilic residues can easily accommodate the polar head groups of phospholipids, thereby accounting for the high activity level of $vPLA_1$. Our result provides a potential explanation for the ability of $vPLA_1$ to hydrolyze phospholipids of cell membrane.

1. Introduction

The venom of the black-bellied hornet (*Vespa basalis*), one of the most dangerous vespine species in Taiwan (Ho and Hwang, 1991; Ho et al., 1993), is highly toxic and causes local edema, severe hemolysis and circulatory failure. A high incidence of death is caused by the aggressive action of this insect (Ho et al., 1993). Previous studies showed that the most important allergenic toxins in vespid venoms are phospholipases, hyaluronidases, antigen 5 and acid phosphatases (Einarsson et al., 1985; Monsalve et al., 2012; Mulfinger et al., 1986).

Vespid phospholipase A_1 ($vPLA_1$) is one of the primary *V. basalis* venom components with local inflammatory effects (Ho et al., 1993). In addition to causing allergic reactions, $vPLA_1$ can hydrolyze the sn-1 fatty acids in phospholipids and convert them into their corresponding lyso compounds (Fig. 1A) (Santos et al., 2007; Sukprasert et al., 2013). Accordingly, $vPLA_1$ may disrupt the phospholipid packing of biological membranes, causing severe hemolysis and leading to cardiac dysfunction and death in animals.

The $vPLA_1$ in vespid venoms was identified as a member of the pancreatic lipase gene family. $vPLA_1$s share approximately 20–30% homology with the N-terminal catalytic domain of pancreatic lipases including human pancreatic lipase (HPL) and human pancreatic lipase-related protein 2 (HPLRP2) and contain the typical catalytic triad residues Ser-His-Asp. In addition to the catalytic core domain, this α/β hydrolase family also features a frequently amphipathic lid domain that interacts with the hydrocarbon chains of the substrate. However, the lipase activity of $vPLA_1$ is very low (Arima et al., 2012; Yang and Lowe, 2000).

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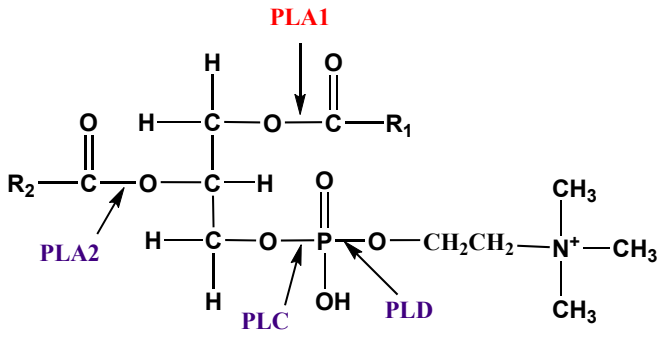

Fig. 1. Chemical structure of the phospholipid, arrows indicates the cleavage sites of PLA.

To clarify the mechanism by which $vPLA_1$ causes hemolysis, we performed a structural determination of $vPLA_1$ at a 2.5 Å resolution by using the molecular replacement method. We found that the lid domain and β9 loop responsible for substrate selectivity are shorter in $vPLA_1$ compared with HPL, which suggests that the vespid enzyme exhibits only phospholipase A1 activity. Furthermore, a hydrophobic surface area adjacent to the catalytic site was proposed as a putative binding site for the acyl chain in phospholipase A1 structure. Finally, the positional hydrolytic selectivity was discussed on the basis of these structural characteristics.

2. Material and methods

2.1. Purification of $vPLA_1$ from black-bellied tiger hornet (V. basalis) venom

The $vPLA_1$ purification methods were described previously (Chou and Hou, 2008). Fractionated venom components were purified by using a size-exclusion column (Fractogel TSK HW 50) and eluted with 50 m*M* ammonium acetate buffer at pH 5.5. $vPLA_1$ purification was accomplished with a CM ion-exchange column and the purified protein was eluted with a linear gradient of ammonium acetate ranging from 0.05 *M* at pH 5.5 to 1.0 *M* at pH 6.8 with repeated passages through a C_4 reverse-phase HPLC column. These passages were performed by using a linear acetonitrile gradient ranging from 20 to 70% in 6 m*M* trifluoroacetic acid with monitoring at OD 280 nm. Finally, the organic solvent dissolved in the $vPLA_1$ was removed by lyophilization. The purified $vPLA_1$ was dissolved to a concentration of 10 mg ml^{-1} in 50 m*M* Tris–HCl (pH 7.0) prior to crystallization.

2.2. Crystallization and X-ray data collection and processing

$vPLA_1$ crystallization and data collection were described previously (Chou and Hou, 2008; Ho and Ko, 1988). The initial crystallization conditions were obtained with Hampton Crystal Screen Kits 1 and 2 by sitting-drop vapor-diffusion method. The conditions were refined, and the crystals were grown from well solutions containing 0.2 M sodium acetate trihydrate, 0.1 M Tris–HCl (pH 8.0), and 25% PEG 4000 that had been equilibrated at 277 K against 500 μl of precipitation solution by sitting-drop vapor-diffusion method. Crystals appeared within one month and the largest crystal grew to dimensions of approximately 300 × 150 × 100 μm. The crystals were soaked in a 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 $vPLA_1$ crystal were collected at 110 K on an in-house MicroMax002 X-ray generator with 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 performed with the *HKL*-2000 program package.

2.3. Structure determination and refinement

The three dimensional structure was determined by using the N-terminal domain (NTD) from guinea pig pancreatic lipase (PDBID: 1GPL) (Withers-Martinez et al., 1996). Iterative cycles of model building with Mifit (http://code.google.com/p/mifit/) and computational refinement with CNS were performed for each structure (Brunger et al., 1998); 5% of the total reflections were set aside for R_{free} calculations (Brunger, 1993). The stereochemical quality of structures was assessed with the *PROCHECK* program (Morris et al., 1992). Molecular figures were visualized and depicted with PyMOL (The PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC.) (Baugh et al., 2011). The crystallographic statistics concerning the data collection and refinement parameters are summarized in Table 1.

2.4. Molecular modeling

The crystal structure of $vPLA_1$ (PDBID: 4QNN) lacks a substrate in its active site. We used the complete structure of HPL (PDBID: 1LPA) with phosphatidylcholine (PC) as a template to construct a plausible $vPLA_1$–PC complex with the molecular modeling programs Discovery Studio 2.5 (Accelrys Inc.) (Chen et al., 2013; Lin et al., 2014). The quality of the model geometry was evaluated by using the RMSD derivation of the bond length and bond angle. The quality of the modeled structure was tested with PROCHECK (Laskowski et al., 1996).

3. Results

3.1. The overall $vPLA_1$ structure

A homology search of the $vPLA_1$ structure was performed by

Table 1
Data collection and refinement statistics for the $vPLA_1$ crystal.

Crystal	vPLA1
λ for data collection (Å)	1.5418
Data collection	
Space group	*P*1
Cell dimensions (Å)	
a, b, c (Å)	57.2, 70.2, 81.6
α, β, γ (°)	107.0, 109.9, 100.9°
Resolution	50–2.50
R_{merge} (%)	6.8 (30.3)
Average $I/\sigma(I)$	13.8 (4.4)
Completeness (%)	96.5 (95.0)
Redundancy	2.6 (2.6)
Z	4
Refinement	
Resolution	25.0–2.5 Å
No. of reflections	35,849
R_{factor}/R_{free} (5% data)	0.2042/0.2789
RMSD length (Å)	1.270
RMSD angle (°)	2.166
No. of atom	
Protein	9168
Cl	24
Hg	3
Water	477
Ramachandran plot (%)	
Most favored	82.2
Additionally allowed	15.2
Other	2.6