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Short sequence-paper

Crystallization and preliminary X-ray diffraction studies of a toxic phospholipase A_2 from the venom of *Vipera ammodytes meridionalis* complexed to a synthetic inhibitor

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Abstract

A toxic phospholipase A_2 (PLA_2) is isolated from the neurotoxic complex Vipoxin, the major lethal component of the venom of *Vipera ammodytes meridionalis*. The enzyme is complexed to the synthetic inhibitor elaidoylamide and crystallized. The crystals belong to the space group $P2_12_12_1$, with unit cell dimensions $a=46.57$ Å, $b=82.68$ Å, $c=119.47$ Å and $\beta=90°$. Initial diffraction data to 3.3 Å resolution are collected.

Keywords: Phospholipase A_2; Venom; X-ray analysis

Phospholipase A_2s (PLA_2, phosphatide 2-acylhydrolase, EC 3.1.1.4) are a fast expanding family of enzymes that specifically catalyze the hydrolysis of the *sn*-2 acyl ester bond of 1,2-diacyl-3-*sn*-phosphoglycerides releasing fatty acids and lysophospholipids [1]. The released arachidonic acid has an important physiological function as a precursor of eicosanoid mediators of inflammation: prostaglandins, leukotriens and tromboxanes. In this way, PLA_2 is involved in human diseases, such as rheumatoid arthritis, asthma and septic shock [2,3]. Lysophospholipids participate in phospholipids remodelling, cell signalling and membrane perturbation [1, and references therein]. Lypolitic enzymes influence a number of cellular functions: chemotaxis, cytotoxicity and cell differentiation [4].

PLA_2s are widely distributed in animal organs and physiological fluids: mammalian pancreas, snake venom, lung, gastric mucosa, liver, spleen, brain, heart, alveolar macrophages, intestine, membranes, placenta and synovial fluid. These enzymes are the major toxic components of the snake venom and display a wide variety of pharmacological activities such as neurotoxicity, cardiotoxicity, myotoxicity, anticoagulant, antiplatelet, hypotensive, haemolytic, hemorrhagic and edema-inducing effects [5]. The venomous snakes in Europe are representatives of the genera *Vipera* and three species, *Vipera ammodytes*, *Vipera aspis* and *Vipera berus* are of public health significance [6]. The snakes of the first species are the most toxic in Europe. Two subspecies of *V. ammodytes*, *V. ammodytes ammodytes* and *V. ammodytes meridionalis*, inhabit the Balkan peninsula. Envenomation of humans by these snakes is often lethal. The major toxic component of the venom from *V. a. meridionalis* is Vipoxin, a heterodimeric postsynaptic neurotoxin. It is a representative of a group of heterodimeric toxins found in the venom of snakes inhabiting widely separated regions of the world. Analogues of this neurotoxin were isolated from the venom of European vipers, *Vipera aspis zinnikeri* [7] and *Vipera aspis aspis* [6], as well as

Abbreviations: PLA_2, phospholipase A_2

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from the Taiwan (Asia) viper *Vipera russeli formosensis* [8]. Vipoxin is a non-covalent complex between two oppositely charged subunits: a basic, strongly toxic Asp49 PLA_2 with a *p*I of 10.4 and an acidic non-toxic and catalytically inactive PLA_2-like (Gln48 PLA_2) component with a *p*I of 4.6 [9]. Both subunits are closely related proteins with 62% sequence identity and the same length of the polypeptide chain, 122 residues [10]. Gln48 PLA_2 component lacks catalytic activity due to the substitution of the active site His48 by a glutamine residue and reduces both the catalytic activity and toxicity of Asp49 PLA_2. This is the first reported example of regulation of the toxic function generated by molecular evolution. Probably, the non-toxic subunit evolved from the toxic PLA_2 and acquired a chaperone function to avoid non-specific targeting of the toxic component. Vipoxin is an example of evolution of the toxic and phospholipolytic activities into non-toxic and inhibitory functions. In this paper, we report the crystallization and preliminary X-ray diffraction studies of the toxic Asp49 PLA_2 separated from the heterodimer and complexed to the synthetic inhibitor elaidoylamide (the amide of trans-9-octadecenoic acid).

The heterodimeric complex Vipoxin was dissociated to subunits and the two components, Asp49 PLA_2 and Gln48 PLA_2, were isolated in homogeneous state as described in Ref. [10]. A 10-fold molar excess of elaidoylamide inhibited almost all catalytic activity of the Vipoxin PLA_2 towards the major structural phospholipid in the brain phosphatidylcholine. The lyophilized toxic component was dissolved in distilled water at a concentration of 20 mg/ml. Crystallization conditions were screened by the hanging drop vapour-diffusion method using Jena Bioscience screening kits 1–5. Typically, a 2-μl drop of the protein solution was mixed with 2 μl of the screening agent. In condition D2 of kit 5 (20% PEG-10000, 50 mM HEPES, pH 7.5), small needle crystals were obtained. These conditions were further optimised and crystals suitable for X-ray analysis of the Vipoxin Asp49 PLA_2 in a complex with elaidoylamide were obtained as follows: 2 μl of the protein solution with a concentration of 20 mg/ml were mixed with 2 μl of elaidoylamide (10 mg/ml stock solution) and 2 μl of a precipitant solution containing 24% PEG-10000 and 50 mM HEPES, pH 7.5. The mixture was equilibrated against 1 ml of the same precipitant solution. Crystals were grown within 3 days at room temperature and for initial test experiments mounted in quartz capillaries. Diffraction data to 3.3 Å were obtained from a flash-frozen crystal at 100 K using synchrotron radiation ($\lambda = 0.802$ Å) at the consortiums beamline X13 at HASYLAB/DESY-Hamburg. A non-protein electron density corresponding to the inhibitor was observed. The preliminary X-ray data show that the crystal contains a non-covalently bound elaidoylamide. Evidently, the inhibitor possesses a high binding affinity towards the Vipoxin PLA_2.

The programs DENZO, SCALEPACK [11] were used for data processing and analysis. Autoindexing yielded unit-cell parameters of $a = 46.6$ Å, $b = 82.7$ Å, $c = 199.5$ Å, This crystal lattice is orthorhombic and belongs to the space group $P2_12_12_1$. Packing parameter calculations based on a molecular weight indicate the presence of 4 subunits of the inhibited PLA_2 in the asymmetric unit. This corresponds to a typical Matthews coefficient V_M of 2.1 $Å^3$ Da^{-1} [12] and a solvent content of 40%. The data collection parameters and statistics are summarised in Table 1.

Table 1
Data collection statistics

Space group	$P2_12_12_1$
Unit cell parameters	
a (Å)	46.57
b (Å)	82.68
c (Å)	119.47
Crystal volume per Dalton (V_M) ($Å^3$ Da^{-1})	2.13
Wavelength used (Å)	0.802
Resolution (Å)	20.0–3.3
Completeness (%)	99 (99)
Average I/σ	14 (3)
Number of images	127
Raw measurements used	69.923
Unique reflections	7.251
R^a_{merge}	0.117 (0.573)
Subunits per asymmetric unit	4

Values in parentheses are for the high resolution bin.
[a] $R_{merge} = \Sigma | I - <I> | / \Sigma I$, where $<I>$ is the average intensity and the summation is done for all reflections.

In conclusion, for the first time, an isolated toxic component of *Viperidae* snake venom heterodimeric neurotoxin, complexed to a synthetic inhibitor, has been crystallized and preliminary X-ray data are collected. The separated PLA_2 changes the target of the physiological attack and the type of toxicity: from the post- to the presynaptic membranes of the neuromuscular junctions. Most probably, Vipoxin exerts its toxicity through dissociation to subunits. The neurotoxic chain binds to the target membrane and the non-toxic component (chaperone subunit) remains in solution. The modulation of PLA_2 activity is of pharmacological interest and the 3-D structure of the enzyme–inhibitor complex can be used for structure-based drug design. Initial calculations to solve the phase problem by molecular replacement methods are in progress, as well as attempts to optimise the crystals for high-resolution data collection.

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