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A thermoactive secreted phospholipase A_2 purified from the venom glands of *Scorpio maurus*: Relation between the kinetic properties and the hemolytic activity

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ABSTRACT

A lipolytic activity was located in the scorpion venom glands (telsons), from which a phospholipase A_2 (*Sm*-PLVG) was purified. Like known phospholipases A_2 from scorpion venom, which are 14–18 kDa proteins, the purified *Scorpio maurus*-Phospholipase from Venom Glands (*Sm*-PLVG) has a molecular mass of 17 kDa containing long and short chains linked by disulfide bridge. It has a specific activity of 5500 U/mg measured at 47 °C and pH 8.5 using phosphatidylcholine as a substrate in presence of 8 mM NaTDC and 12 mM $CaCl_2$. The NH_2-terminal amino acid sequences of the purified *Sm*-PLVG showed similarities with those of long and short chains of some previously purified phospholipases from venom scorpions. Moreover, the *Sm*-PLVG exhibits hemolytic activity toward human, rabbit or rat erythrocytes. This hemolytic activity was related to its ability to interact with phospholipids' monolayer at high surface pressure. These properties are similar to those of phospholipases isolated from snake venoms.

1. Introduction

Venomous animals range within different phyla, including coelenterates, molluscs, arthropods, and chordates. Their venom glands elaborate a variety of potent polypeptide toxins which serve as weapons for both offensive (in getting prey for food) and defensive (in threatening predators) functions (Fry, 1999; Karalliedde, 1995; Kochva, 1987). Venom glands are also thought to have evolved from digestive glands, and the ancestral function of venom is probably in prey digestion. Venom glands thus also produce several water-soluble enzymes that contribute to the digestion of prey and may also facilitate the action of venom toxins. Interestingly, some venom enzymes have evolved into potent toxins. The best known example of venom enzymes endowed with toxic functions is probably that of secretory phospholipases A_2 (sPLA$_2$: EC 3.1.1.4). sPLA$_2$ are secreted proteins of 14–18 kDa (except for group III sPLA$_2$) that usually contain 6–8 disulfide bonds (Schaloske and Dennis, 2006). They need His/Asp dyad and require the presence of Ca^{2+} for catalytic activity. Various snake venom PLA$_2$s were sequenced and disulfide bond patterns were determined. The first non-venom PLA$_2$, named group-IB (GIB), was isolated from the pancreatic juices of cows and was also found in many other

Abbreviations: BSA, bovine serum albumin; EGTA, ethyleneglycoltetraacetic acid; EDTA, ethylenediaminetetraacetic acid; HPLC, high pressure liquid chromatography; GC, gas chromatography; NaTDC, sodium taurodeoxycholate; TLC, thin layer chromatography; kDa, kilodalton; *Sm*-PLVG, *Scorpio maurus* phospholipase from venom glands; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TEMED, N,N,N′,N′-tetramethyl-ethylenediamine; egg-PC, phosphatidylcholine from egg yolk; diC$_{12}$-PC, 1,2-dilauroyl-*sn*-Glycero-3-phosphocholine; diC$_{12}$-PE, 1,2-dilauroyl-*sn*-Glycero-3-phosphoethanolamine; PS, L-α-phosphatidyl-L-serine; PG, L-α-phosphatidyl-DL-glycerol; PL, phospholipase.

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mammals (Kramer et al., 1989). $sPLA_2$ display an important increase in activity when the substrate concentration is changed from monomers to aggregates, which are referred as "interfacial activation". Most $sPLA_2$s show higher activity on anionic phospholipids such as phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylserine (PS). Group-IA (GIA) and group-XIV (GXIV) sPLAs are more active against zwitterionic phosphatidylcholine (PC) vesicles. All $sPLA_2$s contain a highly conserved Ca^{2+} binding loop (XCGXGG) and a catalytic site motif (DXCCXXHD). Many $sPLA_2$s crystal structures are available such as the cobra venom GIA; human, porcine, and bovine pancreatic GIB; human GIIA; human GX; plant GXIB; and prokaryotic GXIV $sPLA_2$s. Although the amino acid identity level is low, $sPLA_2$s share common protein fold and feature the same catalytic His–Asp dyad (Dennis et al., 2011). These proteins are quite fascinating from both biological and structural interest.

$sPLA_2$ occur in venom from both vertebrate and invertebrate animals, are among the most abundant components, and can serve as digestive and toxic functions. They catalyze the hydrolysis of the *sn*-2 position of glycerophospholipids to generate fatty acid and lysophospholipid. $sPLA_2$ is a widely spread enzyme across the species and is classified into the ten groups on the basis of their sequence homology and disulfide pattern (Six and Dennis, 2000). Group I and group II $sPLA_2$ have been well characterized in terms of their structure and function. They share sequence and structural similarity. The other groups have not been so well characterized. Group III PLA_2s have been identified across the species: insect (Kuchler et al., 1989; Ryu et al., 2003; Scott et al., 1990), scorpion (Hariprasad et al., 2007; Incamnoi et al., 2013; Valdez-Cruz et al., 2004; Zamudio et al., 1997), gila monster (Gomez et al., 1989) and human (Valentin and Lambeau, 2000). They are involved in inflammation (Touqui and Alaoui-El-Azher, 2001; Murakami and Arni, 2003), apoptosis (Costa et al., 2008) and cancer (Murakami et al., 2005).

The scorpion venom consists of a number of proteins and peptides including the group III $sPLA_2$. The single translated polypeptide chain of the $sPLA_2$ from *Mesobuthus tamulus*, the Indian red scorpion, undergoes a furin type of enzymatic processing to attain a heterodimeric conformational state that comprise of a large enzymatic chain, covalently linked to a smaller non-enzymatic chain with the release of five residue peptide (Hariprasad et al., 2007). Moreover, in the venom of the scorpion *Pandinus imperator* a heterodimeric phospholipase A_2 (IpTxi) was described (Zamudio et al., 1997). This protein causes inhibition of ryanodine binding to the Ca^{2+} channels present in skeletal muscle, very likely due to an indirect effect caused by the fatty acid liberated by the PLA_2 activity of IpTxi.

Scorpio maurus belongs to the family Scorpionidae common in the Mediterranean, Middle East, Saudi Arabia and Jordon regions (Levy and Amitai, 1980). Reported studies were interested to explain pharmacological properties of identified toxins from this venom scorpion (Abdel-Rahman et al., 2009, 2010). However, to our knowledge, no phospholipase have been purified from the *S. maurus* venom glands so far. The aim of this work is to report the purification of a new heterodimeric phospholipase A_2 from the venom glands of the Tunisian scorpion *S. maurus*. This phospholipase tentatively named *Sm*-PLVG was characterized with respect to its biochemical properties.

2. Material and methods

2.1. Reagents and equipments

Bovine serum albumin (BSA), sodium taurodeoxycholate (NaTDC), ethyleneglycoltetraacetic acid (EGTA), ammonium sulfate, Triton X-100, egg-PC, diC_{12}-PC, diC_{12}-PE, PS, PG and protein markers for molecular masses were purchased from Sigma Chemical Co. (USA). Sodium dodecyl sulfate (SDS), acrylamide, ammonium persulfate, *N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine (TEMED) and Coomassie brilliant blue R-250 were from Bio-Rad laboratories (USA). Sephadex G-100, phenyl-Sepharose and Q-sepharose were from Pharmacia (Sweden). HPLC (Ultimate 3000, Dionex) system was from Dionex (Germany). TLC plates silica gel 60 F245 were purchased from Merck (Germany). Gas Chromatography (GC) was from Shimadzu (Japan). pH-Stat was from Metrohm (Switzerland). All other reagents were of analytical grade.

2.2. Animals

Scorpions (chelicerae, scorpionidae, *S. maurus*) (Fig. 1A) were collected alive from the area of Agareb (Sfax, Tunisia). The venom glands producing venom (the telsons) are situated in the last segment of metasoma (Fig. 1B). These glands are recuperated and stored at −20 °C.

2.3. Evaluation of the level of phospholipase activity in the scorpion telsons

Phospholipase activity was measured titrimetrically at pH 8.5 and at 47 °C with a pH-stat, under the standard conditions described previously (Six and Dennis, 2000), using egg-PC 1% as substrate in the presence of 8 mM NaTDC and 12 mM $CaCl_2$. One unit of phospholipase activity corresponds to 1 μmol of fatty acid liberated per minute under standard conditions.

2.4. Protein analysis

2.4.1. Determination of protein concentration

Protein concentration was measured spectrophotometrically according to the Bradford method (Bradford, 1976), using BSA as a standard.

2.4.2. HPLC analysis of Sm-PLVG

The enzyme *Sm*-PLVG, was injected onto a filtration HPLC system (Ultimate 3000, Dionex, Germany) equipped with a pump (LPG-3400SD), column oven and diode-array UV/VIS detector (DAD-3000RS). The output signal of the detector was recorded using Dionex Chromeleon™ chromatography Data System. The separation was executed on a Nucleogel® GFC 300-8 column (7.7 × 300 mm) maintained at 6 °C. The flow rate was 0.5 ml/min, the injection volume was 200 μl and the detection UV wavelength was set at 280 nm. The used mobile phase contains 100 mM NaCl,