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Full Length Research Paper

Characterization of phospholipase A $_2$ (PLA$_2$) from *Echis ocellatus* venom

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Phospholipase A$_2$ (EC. 3.1.1.4) was isolated and partially characterized from the venom of *Echis ocellatus*. The enzyme was purified 13.5-fold with a yield of 86.69% on DEAE-Sephadex G-75 column. The PLA$_2$ from *E. ocellatus* venom had broad pH and temperature ranges with optima of 7.5 and 40°C respectively. Initial velocity studies for the determination of kinetic constants with L- - lecithin as substrate revealed a Km and Vmax of 1mg/ml and 0.35 µmoles/min respectively. The enzyme activity was enhanced by Ca^{2+} and strongly inhibited by Mg^{2+} and Co^{2+}. Cu^{2+} was fairly inhibitory to the enzyme. The relevance of these findings towards understanding the biochemistry of *E. ocellatus* envenomation and development of antivenom for *E. ocellatus* venom is discussed.

Key words: *Echis ocellatus*, PLA$_2$, venom.

INTRODUCTION

The saw-scaled viper *Echis ocellatus* is among the commonest cause of envenomation in West Africa, being responsible for approximately 95% of the reported cases in northern Nigeria causing several hundreds of deaths annually (Bharati et al., 2003; Hasson et al., 2003). Snake venoms are composed of complex mixture of active substances, mainly peptide and proteins which are able to interfere with the course of several biological processes including thrombosis by affecting platelet aggregation and blood coagulation (Rossing and Tans, 1992). Some of these proteins include enzymes like phospholipase A$_2$ and metalloproteases (Sallau et al., 2005).

Phospholipase A$_2$ (PLA$_2$) are enzymes found to catalyze the hydrolysis of fatty ester in the 2-position of 3-sn-phospholipid to release fatty acid and lysophos-pholipid; the fatty acid so formed may act as either a second messenger or a precursor of eicosanoids (Fuly et al*.,* 2002; Huang and Mackessy, 2004). The enzyme from snake venoms is primarily used for trophic and defense functions in most species but show wide range of phar-macological activities such as neurotoxicity, myotoxicity, cardiotoxicity, but with a greater impact on platelet aggre-gation and blood coagulation (Bharati et al., 2003; Hasson et al*.,* 2003; Shashidharamurthy and Kemparaju, 2006; Higuchi et al., 2007). The enzyme is therefore a highly interesting molecule to venom researchers because in addition to digesting the prey, it mimics the pathological action of the whole venom poisoning (Shashidharamurthy and Kemparaju, 2006).

PLA$_2$ have been purified and characterized from *Lachesis muta* , *Naja naja* and *Bothrops leucurus* snake venoms (Fuly et al., 2002; Shashidharamurthy and Kemparaju, 2006; Higuchi, et al., 2007) and its inhibition in *Echis carinatum* venom by *Guiera senegalensis* extract has been demonstrated (Sallau et al., 2005). However, information concerning the counterpart enzyme in *E. ocellatus* that could be important in antivenom design is scanty. Therefore, in this study, we report on the purification and characterization of PLA$_2$ from *E. ocellatus* venom with the view to obtain kinetic data for the enzyme and establish other characteristics that would be impor-tant in achieving the said objective.

MATERIALS AND METHODS

Reagents were purchased from Sigma Chemical Company, St. Louis, USA. Freeze dried *E. ocellatus* venom was a gift from Dr. Y.P. Ofemile of the Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

Partial purification of phospholipase A$_2$

Two ml of 10 mg/ml of crude *E. ocellatus* venom was loaded onto DEAE cellulose column (1.5 x 33 cm) pre-equilibrated with 50 mM

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Table 1. Purification profile of phospholipase A_2 isolated from *Echis ocellatus* venom.

Purification step	Protein (mg)	Total activity (μmol/min)	Specific activity (μmol/min/mg)	Purification fold	Yield (%)
Crude	6.70	16.00	2.39	1.00	1.00
Ion Exchange on DEAE- cellulose	1.20	8.00	6.67	2.80	50.00
Gel filtration on Sephadex G-75	0.43	13.87	32.26	13.50	86.69

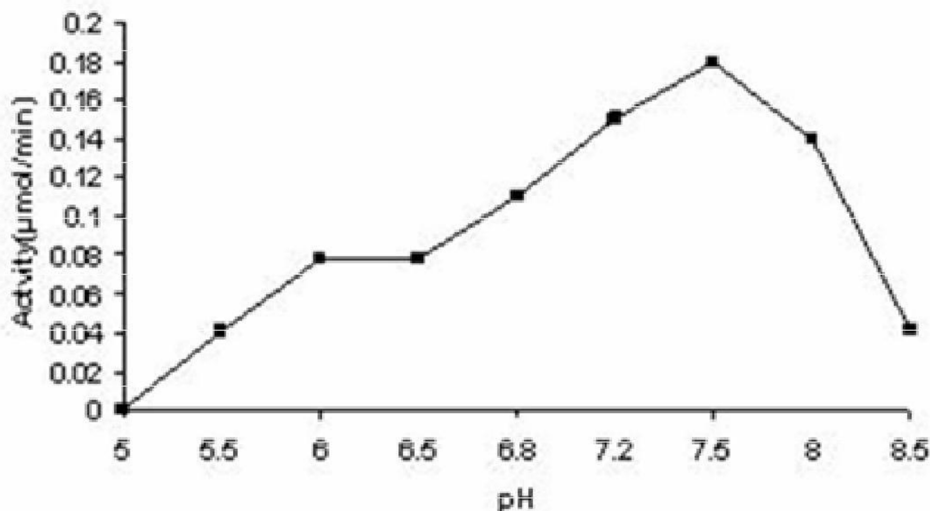

Figure 1. Effect of pH on PLA_2 activity.

phosphate buffer pH 6.8. The column was eluted stepwise with NaCl gradient (0.01 – 0.1 M) at a flow rate of 1ml/min. Twenty, 2 ml fractions were collected and assayed for Phospholipase A_2 activity and total protein. The PLA_2 active fractions were pooled together and loaded on DEAE Sephadex G-75 column equilibrated with phosphate buffer (pH 6.5). The column was eluted with the same buffer, maintaining a flow rate of 2ml/min. Fractions were collected and assayed for PLA_2 and total protein concentration.

PLA_2 Assay

The PLA_2 activity was assayed as described by (Bhat and Gowda, 1989). Briefly, 25 l of 1 mg/ml L -lecithin substrate was incubated with 10 l of the enzyme for 10 min at 37^oC. The reaction was then terminated by immersing the tube in a boiling water bath for 2min and the amount of released free fatty acid measured titrimetrically at pH 8.0. The activity of Phospholipase A_2 was defined as the amount of enzyme that hydrolyzes 1 μmole of fatty acids from L- α-Lecithin per minute under standard conditions.

Effect of pH and temperature on PLA_2 activity

A pH-dependent assay of the partially purified enzyme was performed using 50mM acetate buffer pH 5.0- 6.5, 50mM phosphate buffer pH 6.8 - 7.2 and Tris HCl buffer pH 7.5 - 8.5. Also, the effect of temperature was determined by assaying the enzyme activity at varying temperatures ranging from (25 - 70^oC).

Initial velocity studies: The enzyme was assayed with varying concentrations of the substrate (0.25 – 1 mg/ml) L- -lecithin. Initial velocity values obtained were then used to plot Lineweaver-Burk's plot to determine the Km and Vmax.

Effects of some divalent cations on PLA_2 activity: The enzyme assay described above was performed in the presence of chloride salts of the following divalent ions; Ca^{2+}, Co^{2+}, Mg^{2+}, and Cu^{2+} at 10mM final concentrations.

RESULTS

The purification profile of PLA_2 from *E. ocellatus* venom is summarized in Table 1. The *E. ocellatus* venom was found to possess PLA_2 activity. The crude enzyme had a specific activity of 2.39 mol/min/mg, which was increased to 6.67 and 32.26 mol/min/mg after passing through DEAE cellulose and DEAE sephadex 75 columns respectively.

The partially purified PLA_2 enzyme was most active at pH 7.5 (Figure 1) with complete cessation of activity below pH 5.0 and above pH 9.0 while maximum activity was observed at 40^oC (Figure 2) . Initial velocity data were used to compute the kinetic parameters of the enzyme. The Km of 1mg/ml and Vmax of 0.35 μmoles/min were obtained (Figure 3). Ca^{2+} was found to increase PLA_2 activity two-fold while Cu^{2+}, Mg^{2+} and Co^{2+} were inhibitory to the enzyme (Figure 4).

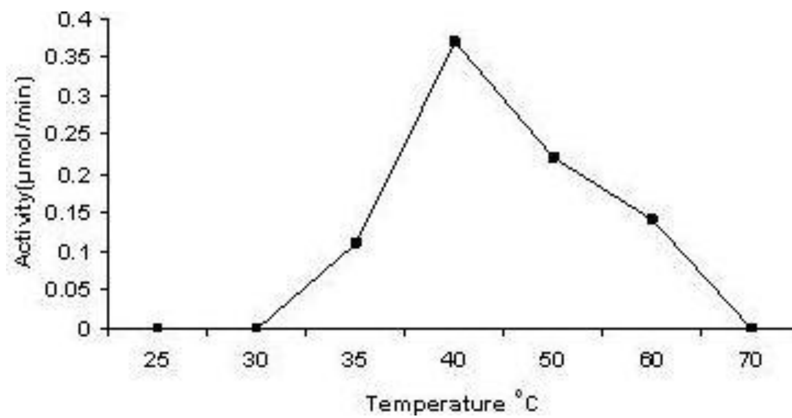

Figure 2. Effect of temperature on PLA_2 activity

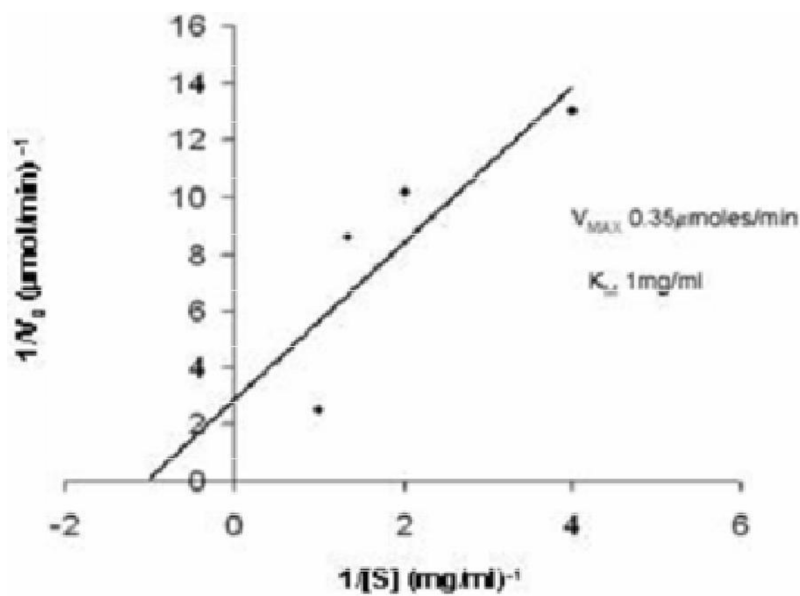

Figure 3. Lineweaver Burk's plot of PLA_2 from *Echis oceliatus* venom.

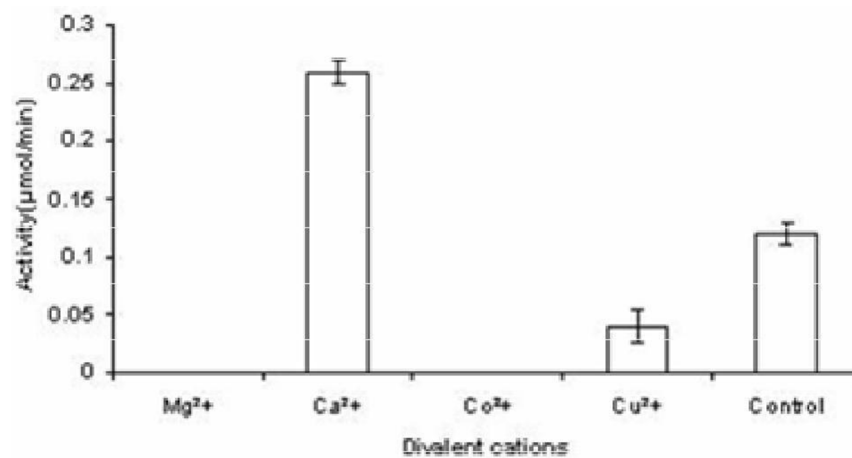

Figure 4. Effects of some divalent cations on PLA_2 activity.

DISCUSSION

Understanding the characteristics of PLA_2 from snake venoms is a serious concern for venom researchers, as it would help the production of effective therapeutic anti-venoms, considering the role of the enzyme in envenom-mations. This report reveals the isolation and partial characterization of PLA_2 from *E. ocellatus* venom which belongs to the Class II PLA_2 predominantly found in viperid snakes (Yang, 1994; Scott and Sigler, 1994).

The increase in specific activity of the crude PLA_2 after the two purification steps could be due to the removal of other synergistically interacting components of the ve-nom. The very high percentage yield obtained was higher than those obtained from ostrich pancreatic PLA_2 and dromedary PLA_2 (Bacha et al., 2007) which could mean that the purification steps used here were more appro-priate for this enzyme.

The *E. ocellatus* venom PLA_2 optimal activity at pH 7.5 was found to be similar to that reported for *L. muta* and *N. naja* venoms PLA_2 (Fuly et al., 2002; Shashidara-murthy and Kemparaju, 2006) and could be attributed to the pathological role of the enzyme in envenomations as basic pH contributes to the enhancement of the enzyme-tic reaction by general base catalytic mechanism. Gene-ral base-mediated attack on productively bound sub-strates has been described by Scott and Sigler (1994) to be the first out of the three essential steps in catalysis of PLA_2 enzymes as deduced from crystallographic studies on the enzyme. The PLA_2 optimum temperature agrees with other findings by Shashidaramurthy and Kemparaju, (2006) ; Bacha et al., (2007). The increase in body tempe-rature of snake bitten victims above the physiological temperature could also make the condition favorable for PLA_2 to exert its hydrolytic function effectively.

The fairly low Km value is an indication of moderately high affinity of the enzyme for phospholipids which further substantiates the observed toxicities in viperid snakes as a result of PLA_2. Furthermore, the Vmax obtained implies that at the end of 1min. post *E. ocellatus* envenomation, at least 0.35 moles of free fatty acids would have been excised from the victim's red blood cells leading to haemolysis of red blood cells observed after envenoma-tions (Rosenberg, 1979). It could also lead to liberation of free fatty acids from other membrane phospholipids making the milieu highly acidic with the lysophospholipids generated exhibiting a detergent-like action that leads to degeneration of muscle fibres (myotoxicity) and/or inhibit-ing the release of neurotransmitters (neurotoxicity), both of which are major forms of viperid PLA_2 toxicity (Caratsch et al., 1985). The increase in activity of *E. ocellatus* PLA_2 in the presence of Ca^{2+} shows that the enzyme is Ca^{2+} dependent. Studies have shown that snake venoms PLA_2 need submicromolar concentrations of Ca^{2+} to be catalytically active and also increase in Ca^{2+} is needed for both enzyme binding and catalysis (Shashidaramurthy and Kemparaju, 2006; Higuchi et al., 2007). Yang (1994) has described the calcium binding loop in myotoxicity of PLA_2 and that calcium binds to His48 of PLA_2 located at the catalytic site of the enzyme activating it towards its toxic function.. The observed effect of copper, magnesium and cobalt, although diva-lent as calcium, may suggest a probable modification of enzyme protein conformation after binding differently from the pattern calcium does, and hence causing a de-crease in the enzyme action. The observation could also give a guide towards inclusion of effective quantity of these inhibiting divalent cations in antivenom design for *E. ocellatus* venom.

It was therefore concluded that *E. ocellatus* venom contains PLA_2 with biochemical characters similar to other venoms PLA_2.

REFERENCES

Bacha BA, Gargouri Y, Bezzine S, Mosbah H, Mejdoub H (2007). Ostrich pancreatic phospholipase A_2: Purification and biochemical characterization. J. Chromatography. 857:108-114.

Bharati K, Hasson SS, Oliver J, Laing GD, Theakston RDG, Harrison RA (2003). Molecular cloning of phospholipase A_2 from venom gland of *Echis* carpet vipers. Toxicon. 41: 941-947.

Bhat MK, Gowda TV (1989). Purification and characterization of myotoxic phospholipase A_2 from Indian cobra (*Naja naja naja*) venom. Biochem. Int. 25:1023-1034.

Caratsch CG, Miledi R, Strong PN (1985). Influence of divalent cations on phospholipase-dependent action of bungarotoxin at frog neuromuscular. J. Physiol. 319:179-191.

Fuly AL, Miranda AP, Zingali RB, Guimares JA (2002). Purification and characterization of a phospholipase A_2 isoenzyme isolated from *Lachesis muta* snake venom. Biochem. Pharmacol. 63: 1589-1597.

Hasson SS, Theakston RDG, Harrison RA (2003). Cloning of a prothrombin activator-like metalloproteinase from the West African saw-scaled viper, *Echis ocellatus.* Toxicon. 42: 629-634.

Higuchi DA, Barbosa CMV, Bincoletto C, Chagas JR, Magalhaes A, Richardson M, Sanchez EF, Pesquero JB, Araujo RC, Pesquero JL (2007). Purification and partial characterization of two phospholipases A_2 from *Bothrops leucurus* (white tailed-jararaca) snake venom. Biochemie. 89: 319-328.

Huang P, Mackessy SP (2004). Biochemical characterization of phospholipase A_2 (trimorphin) from the venom of the *Sonoran Lyre Snake Trimorphodon biscutatus lambda* (family Colubridae). Toxicon. 44: 27-36.

Rosing J, Tans G (1992). Structural and functional properties of snake venom prothrombin activators. Toxicon. 30:1515-1527.

Rosenberg P (1979). Pharmacology of phospholipase A_2 from snake venom. In: Lec, C.Y.V., (ed.), Snake venom. Berlin, springer. pP.403.

Sallau AB, Njoku GC, Olabisi AR, Wurocheke AU, Abdulkadir AA, Isah S, Abubakar MS, Ibrahim S (2005). Effect of *Guiera senegalensis* leaf extract on some *Echis carinatus* venom enzymes. J. Med. Sci. 5(4): 280-283.

Scott DL, Sigler PB (1994). Structure and catalytic mechanism of secretory PLA_2. Adv. Prot. Chem. 43:53-58.

Shashidharamurthy R, Kemparaju K (2006). A neurotoxic phospholipase A_2 variant: Isolation and characterization from eastern regional Indian cobra (*Naja naja*). Toxicon. 47:727-733.

Yang CC (1994). Structure-function relationship of PLA_2 from snake venoms. J. Toxicol. Toxin Rev. 13:125-127.