Science World Journal Vol 14(No 1) 2019
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Full Length Research Article

BIOCHEMICAL AND KINETIC PROPERTIES OF CRUDE PHOSPHOLIPASE A_2 FROM *NAJA NIGRICOLLIS* VENOM

[1]Laila Miftah Garba, [1]Abdullahi Balarabe Sallau,[*2]Mujaheed Abubakar, [2]Abdulrazak Muhammad Hussain [2]Zurki Ibrahim and [3]Zaharaddeen Muhammad

[1]Department of Biochemistry, Ahmadu Bello University, P.M.B. 1045, Zaria – Nigeria.
[2]Department of Biological Sciences, Sule Lamido University, P.M.B. 048, Kafin Hausa – Nigeria.
[3]Department of Chemistry, Sule Lamido University, P.M.B. 048, Kafin Hausa –Nigeria.

*Corresponding Author Email Address mujaheedabubakar88@gmail.com or mujahid.abubakar@jsu.edu.ng Phone: +2348039654644

ABSTRACT
Snake envenomation has been a source of concern worldwide due to potential mortality risks associated with it. This study was carried out to investigate the biochemical and kinetic properties of crude phospholipase A_2 (PLA_2) from *Naja nigricollis* venom. Biochemical and kinetic properties of the venom was determined using standard procedure. Though the results showed that the enzyme PLA_2 is stable at pH 4 and 6, the optimum pH of activity of the PLA_2 is 3, while the optimum temperature of activity of the enzyme is 70 ^oC. Of all the activators used ($CoCl_2$, $CaCl_2$, $MnCl_2$ and $MgCl_2$), $MgCl_2$ was the highest activator of the enzyme activity at 5 mM. The suggestive amino acids in the active site of the PLA_2 were aspartate and glutamate. The kinetic parameters, K_m and V_{max} of the PLA_2 were found to be 0.6 mg/ml and 3.3 µmol/min respectively. These findings could lend support to more understanding of biochemical and kinetic properties of crude *Naja nigricollis* PLA_2.

Keywords: Snake envenomation, *Naja nigiricollis,* Phosphalipase A_2, Biochemical properties.

INTRODUCTION

Naja nigricolis snake belongs to the Viperidae family and is one of the most poisonous snakes found in Nigeria (Nasidi, 2007). Their envenomations are characterized by conspicuous local tissues damage and in severe cases by systemic bleeding, haemorrhagic and cardiovascular shock (Nok, *et al.,* 2001). The venoms of viperidae snakes are rich sources of proteins with activity against various factors involved in coagulation and fibrinolysis (Faure *et al.*, 2000). Snake venoms are complex mixture of enzymatic and toxic proteins, which include phospholipase A_2 (PLA_2), myotoxins, hemorrhagic metallo-proteineases and other proteolytic enzymes, coagulant components, cardiotoxins, cytotoxins and neurotoxins (Aird, 2002; Soares *et al.,* 2005). The composition and effects of venom varies considerably between species to species. The venom leads to the disruption of normal cellular functions by these enzymes and toxins. The main enzymes of snake venom include the PLA_2, hydrolases, hyaluronidases, phosphatases, esterases, phosphodiesterase, neuclosidases and metalloproteases (Sallau *et al.*, 2008).

PLA_2 is the commonest enzyme found in snake venoms. It is a lipolytic enzyme that hydrolyses the fatty acyl ester bonds at the 2-sn position of membrane phospholipids producing equimolar amounts of free fatty acid (FFA) and lysophospholipid, mainly arachidonic acid (AA); these products then become available for conversion to potent pro inflammatory mediators, such as platelet activating factor and eicosanoids (Hasson *et al.,* 2003). The enzyme from snake venom is primarily use for trophic and defense functions in most species but show wide range of pharmacological activities such as neurotoxicity, myotocity, cardiotoxicity, with a greater impact on platelets aggregation and blood coagulation (Higuchi *et al.,* 2007). Some components of snake venom have great potential for medical use because of the specific actions of such components (Koh *et al.,* 2006). Snake venom components are also used in basic research in physiology, biochemistry and immunology. Venom components allow researchers to examine the operation of the process and to develop drugs to correct malfunctions due to diseases. For instance, venoms are currently being investigated for their potential use as antiviral and antibacterial agents (Petkovic *et al.,* 1991). Actions of different snake venom are broad and the understanding of their multiple poisoning processes is desirable in the formulation of a satisfactory antidote. In this study, we conducted a biochemical and kinetic studies of PLA_2 from *Naja nigricollis* venom.

MATERIALS AND METHODS

Chemicals and Reagent

The substrate (egg yolk), sodium hydroxide, calcium chloride, manganese chloride, magnesium chloride, conc. Hydrochloric acid, Tris base, cobalt chloride, phenolphthalein. All chemicals and reagent used were of analytically pure grade.

Snake Venom

Naja nigricollis venom crystals were a gift from Dr. Y. P. Ofemile of the department of Toxicology and pharmacology faculty of veterinary medicine Ahmadu Bello University Zaria. Stock solution of 10 mg/ml of the venom in a Tris/HCL buffer was prepared when needed for use.

PLA_2 Assay

The PLA_2 activity of the crude venom was determined by modified egg yolk coagulation method by Habermann and Neumann (1954). Briefly, fresh egg yolk (L-α-lecithin) was homogenized in distilled water to yield a concentration of 100mg/ml. The venom (10 µl) and 10 µl of 50 mM Tris/HCl buffer (pH 8.0) were incubated with 1000 µl substrate (L-α-lecithin) at 37±1 °C. At the end of 10 minutes incubation, the mixture was immersed in boiling water for 2 mins stop the reaction. The liberated fatty acids were titrated against 20 mM NaOH using phenolphthalein as indicator. The PLA_2 activity was determined as the amount of enzyme required to hydrolyze 1µmole of free fatty acids from lecithin under standard condition.

Science World Journal Vol 14(No 1) 2019
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Determination of the Effect of pH on the Activity of PLA_2
Optimum pH for activity of PLA_2 was determined by assaying for PLA_2 activity varying pH values: 3, 4, 5, 6, 7, 8 and 9. To test tubes arranged in duplicates for each 10 µl pH buffer, 10 µl of the crude venom and 1000 µl of the substrate (L-α-lecithin) of fibrinogen solution was added. The mixture was incubated for 10 minutes at 37 °C and the reaction is stopped by immersing the mixture in boiling water for 2 minutes. Free fatty acids (FFA) released was then titrated against NaOH solution.

Determination of the Effect of Temperature on the Activity of PLA_2
The effect of temperature on the activity of the enzyme was determined by assaying for PLA_2 activity at different temperatures: 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C and 100 °C. PLA_2 activity was determined by titrating FFA released.

Determination of the Kinetic Properties of Crude PLA_2
This was done via initial velocity studies according to the method described by Sallau *et al.* (2008) with some modifications. Briefly, the activity of PLA_2 on crude venom was determined by varying the concentration of α – Lecithin of 0.1 g/ml, 0.05 g/ml and 0.025 g/ml was incubated for 10 mins with the crude enzyme in the presence of buffer. After incubation, PLA_2 activity was then obtained by titration, FFA released using NaOH solution to obtain initial velocity data. The data was then used to plot the Lineweaver-Burk plot that was used to determine the kinetic parameters K_m and V_{max}.

Determination of the Amino Acids on the Active Site of the Enzyme (PLA_2)
This was done using Tris/HCL buffer of pH 3, 4 and 5 and varying the substrate concentration in each buffer (0.1 g/ml, 0.05 g/ml and 0.025 g/ml of the substrate) (Sallau *et al.,* 2008). The PLA_2 activity was determined in each case using the procedure stated earlier.

Determination of the Effect of Divalent Cat-ions on the Activity of PLA_2
10 mM, 5 mM and 1 mM concentrations of the divalent cat-ions; Mg^{2+}, Ca^{2+}, Co^{2+}, and Mn^{2+} were prepared using Tris-HCL buffer (pH 8.0). The PLA_2 activity was then determined using these buffers respectively

RESULTS

Effect of pH on the Activity of PLA_2
The effect of pH on crude *Naja nigricollis* PLA_2 activity was presented in Figure 1 which shows that the venom had a broad range of pH (from 3 to 9) with optimum pH at 3. The enzyme was highly active over the narrow pH range of 3 to 6. Low enzyme activities were observed in alkaline pH above 6.

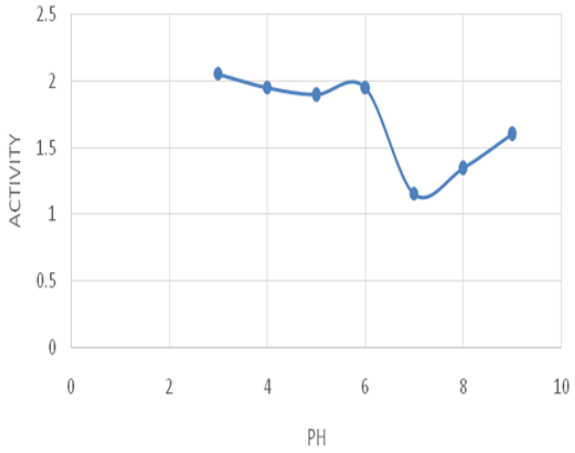

Figure 1: Effect of pH on PLA_2 activity

Effect of Temperature on the Activity of PLA_2
The effect of temperature on crude *Naja nigricollis* PLA_2 activity showed a broad temperature range of activity with an optimum value of 70 ºC (Figure 2). The enzyme continued to show some activity even at temperatures above 70 ºC but in a declined manner representing a drop of more than 40%.

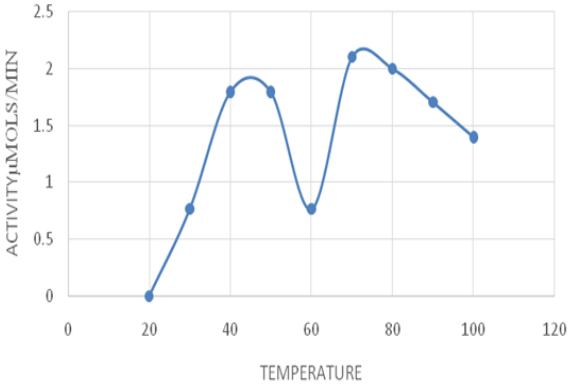

Figure 2: Effect of temperature on PLA_2 activity

Kinetic Properties of Crude PLA_2
The Lineweaver Burks plot (Figure 3) shows that the K_m and V_{max} values are 0.6 mg/ml and 3.3 µmol/min respectively.

Science World Journal Vol 14(No 1) 2019
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

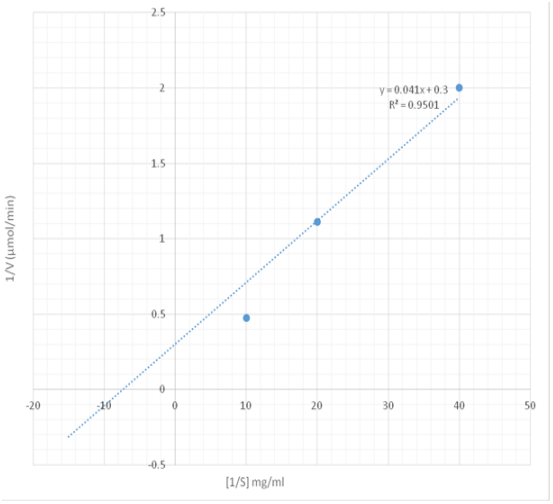

Figure 3: Lineweaver Burksplot for Crude PLA_2 using lecithin as a substrate

Amino Acids on the Active Site of the Enzyme (PLA_2)

Figure 4 shows the Amino Acids (aspartate and glutamate) could be possibly present on the active site of the PLA_2, because the pH optimum observed was acidic.

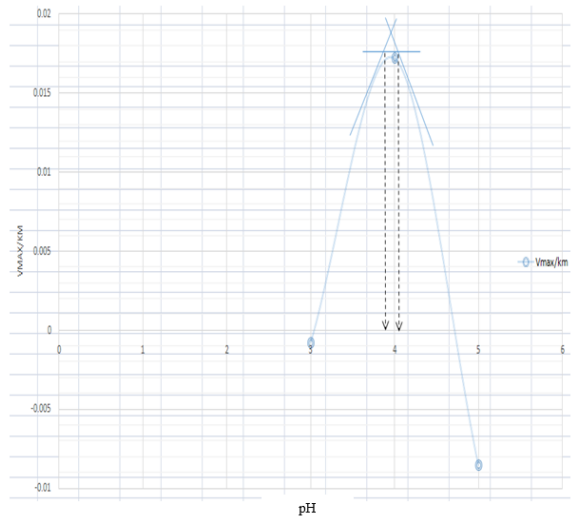

Figure 4: Determination of Amino Acid on the active site of the PLA

Effect of Divalent Cat-ions on the Activity of PLA_2

Figure 5 shows the effect of divalent cat-ions on the activity of crude PLA_2 from *Naja nigricollis* Venom, which indicates that $MgCl_2$ has the highest activator for the enzyme activity, followed by $CoCl_2$, $CaCl_2$ and $MnCl_2$.

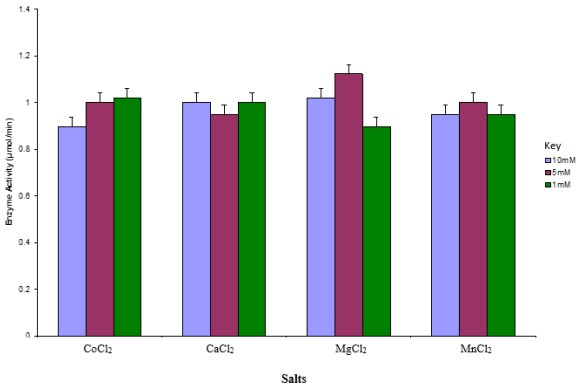

Figure 5: The Effect of Divalent Cations on the Activity of PLA_2

DISCUSSION

PLA_2 is an enzyme that catalyze the hydrolysis of fatty ester in the 2 position of 3- sn-phospholipid to release fatty acid and lysophospholipid; the fatty acid so formed may act as either a second messenger or a precursor of eicosanoids. The enzyme from snake venoms is primarily used for trophic and defense functions in most species but show wide range of pharmacological activities such as neurotoxicity, myotoxicity, cardiotoxicity, but with a greater impact on platelet aggregation and blood coagulation (Shao *et al*., 1993; Adamude *et al.,* 2016). 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 (Sallau *et al.,* 2008). Toxicological properties of snake bite are thought to be associated with enzymes especially PLA_2, which is found to be its most toxic component (Adamude *et al.,* 2016). Considering the role of PLA_2 in envenomation, understanding the characteristics of the enzyme from snake venom has raised concern for venom researchers, as it would help in the production of effective therapeutic antivenins (Shao *et al*., 1993). The enzyme displayed optimum activity at 70 ^oC and was active over a temperature range of 20 – 100 ^oC. This may be due to the fact that, *Naja nigricollis* is found mostly in sub Saharan Africa where temperature is usually high, hence this specie adapt to and tolerate higher temperatures. Interestingly most snake venom metalloproteases enzymes display optimum activity at 40 – 50 ^oC. The wide range of temperature and pH of activity for the enzyme shows that the enzyme is capable of withstanding extreme conditions which is important for venom toxicity.

As a result, the site of catalysis in the envenomed victim provides the optimum pH for the activity of the enzyme; blood too being alkaline in nature. The observed low K_m value of 0.6 mg/ml is a clear indication of the high kinetic efficiency of the enzyme. Previous studies by Torres *et al*. (2012) and Chernyshenko *et al*. (2010) reported Km values of 14.59 mg/ml and 8.5 µM from *Bothrops moojeni* and *Echis multisquamatis* venoms respectively. The observed property of low k_m value, though a disadvantage in terms of envenomation by *Naja nigricollis*, could be considered a great advantage in terms of the clinical usefulness of these enzymes in the treatment of human diseases and as diagnostic reagents. Divalent metal ions are involved in enzyme catalysis in a variety of ways which include activation of electrophiles or nucleophiles, bridging an enzyme with substrate together by means of coordinate bonds as well as holding reacting groups in

the required three dimensional orientations (Advani *et al*., 2010). Therefore, it was concluded that crude PLA_2 enzyme from *Naja nigricollis* venom revealed an optimum activity at 70 ^oC and an optimum pH of 3 with high kinetic efficiency. The Divalent Cations $MgCl_2$ showed the highest activator for the enzyme activity. The suggestive amino acids in the active site of the PLA_2 were aspartate and glutamate.

REFERENCES

Adamude, F. A., Nok, A. J., Aliyu, N. and Onyike E. (2016). Partial Purification and Characterization of Phospholipase A2 Inhibitor from *Echis ocellatus* Serum. *International Journal of Biochemistry Research and Review,* 13(1): 1-16. ISSN: 2231-086X, NLM ID: 101654445

Advani, S., Mishra, P., Dubey, S. and Thakur, S. (2010). Categoric prediction of metal ion mechanisms in the active sites of 17 select type II restriction endonucleases. *Biochemistry and Biophysics Research Communication, 402*: 177-179.

Aird, S. D. (2002). Ophidian envenomation strategies and the role of purines. *Toxicon, 40* (59): 35 – 393.

Chernyshenko, V. O., Gornytska, O. V., Platonova, T. M. and Sokolovska, L. I. (2010). A new fibrinogenase from *Echis multisquamatis* venom is a perspective agent for limited proteolysis and defibrinogenation. *Advances in Bioscience and Biotechnology, 1*: 91 - 96.

Faure, C., Villela, C., Perales, J. and Bon, C. (2000). Interaction of neurotoxic and nontoxic secretatory phospjholipase A2 with crotoxin inhibitor from crotalus serum. *Euro-Journal Biochemistry, 267*(15): 4799 - 808.

Habermann, E. and Neumann, W. (1954). Egg yolk coagulation method. *Physiol Chem*, *297*: 174 – 176.

Hasson, S. S., Al-Jabri, A. A., Sallam, T. A., Al-Balushi, M. S., and Mothana, R. A. A. (2010). Antisnake Venom Activity of *Hibiscus aethiopicus* L. against *Echis ocellatus* and *Naja nigricollis. Journal of Toxicology*, 8 pages. doi:10.1155/2010/837864.

Higuchi, D. A., Barbosa, C. M. V., Bincoletto, C., Chagas, J. R., Magalhaes, A., Richardson, M., … Pesquero, J. L. (2007). Purification and partial characterization of two phospholipases A2 from *Bothrops leucurus* (white tailed-jararaca) snake venom. *Biochemie. 89*: 319 - 328.

Koh, D.C.I., Armugam, A. and Jeyaseelan, K. (2006). Snake venom components and their applications in biomedicine. *Cellular and Molecular Life Sciences.* 63(24): 3030 - 3041. https://doi.org/10.1007/s00018-006-6315-0

Markfarlane, R. G. (1967). Russell's viper venom. *Br J Haematol, 13*: 437 – 451.

Nasidi, A. (2007). *Snakebite as a serious public health problem for Nigeria and Africa*.Available at:http://www.who.int/bloodproducts/snake_antivenoms/rabies_envenomings/en/index.ht ml. Accessed Jun 11.

Nok, J. A., Balogun, B., Lori, J. A. and Abubakar, M. S. (2001). Inhibition of *Naja nigricolis* venom acidic phospholipase A2 catalysed hydrolysis of Ghost red blood cells by columbin. *Journal of Enzyme inhibition*, *1*(4): 1 - 7.

Petkovic, D., Parloric, C. M., Joranovic, T., Panic Dizajic, V., Zaljelar, S., Matejavic, D. (1991). Neutralization of the activity of vipera ammmodytes snake venom on myocardium of rats by antitoxinum viperinus: a histopathological study. *J Toxicology Clinical. Exp,* H (6); 343 - 7.

Sallau, A. B., Ibrahim, M. A., Salihu, A. and Patrick, F. U. (2008). Characterization of phospholipase A2 (PLA2) from *Echis ocellatus* venom. *African Journal of Biochemistry Research, 2*(4): 098-101.

Shao, J., Shen, H., Havsteen, B. (1993). Purification, characterization and binding interactions of the Chinese cobra (*Naja naja* atra) serum antitoxic protein CSAP. *Biochem J, 293*: 559 - 566.

Soares, A. M., Ticli, F. K., Marcussi, S., Lourenco, M. V., Januario, A. H., Sampaio, S. V., … Paulo, S. (2005). Medicinal Plants with Inhibitory Properties Against Snake Venoms. *Current Medicinal Chemistry*, *12*(22): 2625 - 2641. **doi:** https://doi.org/10.2174/092986705774370655.

Torres, F. S., Rates, B., Gomes, M. T. R., Salas, C. E., Pimenta, A. M. C., Oliveira, F., … De Lima, M. E. (2012). Bmoo FIBMP-I: A New fibrinogenolytic Metalloproteinase from Bothropsmoojeni Snake Venom. *Toxicology*, Article ID 673941, 10 pages. https://doi.org/10.5402/2012/673941