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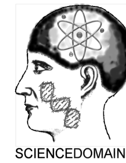

Sequence Homology Studies of Phospholipase A_2-like Gene from Bloodstream form of *Trypanosoma brucei*

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Authors' contributions

This work was carried out in collaboration between all authors. Author AJN designed the study and wrote the protocol. Author IYL managed the analyses of the study and wrote the first draft of the manuscript. Authors HMI and IAU performed the Bioinformatics analysis and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This work focused on the sequence homology studies of the enzyme, phospholipase A2 (PLA_2), in *Trypanosoma brucei* obtained from the blood of bull in Federe, Plateau State, Nigeria, West Africa

Place and Duration of Study: Department of Biochemistry, University of Jos, Nigeria; Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria, Department of Biotechnology, NVRI, Vom, Nigeria; between June 2009 and September 2011.

Methodology: *T. brucei* grown in rats were harvested and separated using diethyl amino ethyl (DEAE) cellulose chromatography. From the parasites' genomic DNA the PLA_2-like gene was amplified using consensus primers. The amplicon was cloned unto pMal-2cE vector and confirmed using direct PCR and restriction enzyme analyses. The PLA_2 gene and translated protein

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sequences were studied using National Center for Biotechnology Information (NCBI) Conserved Domain Search Tool and Conserved Domain Architectural Retrieval Tool
Results: Analyses of the 1344bp gene sequence using bioinformatics tools showed that it is very closely related to PLA_2 sequences of *T. brucei* (TREU 927) and *T. b. gambiense*. Motifs that are unique to PLA_2 (FSHGL) and lipases (GHSFG) were found to be present in the query sequence. The domains present in the studied sequence agreed closely with those of the human platelet activating factor acetyl hydrolase (PAF-AH). There was also a good sequence resemblance with PLA_2s from *T. cruzi, Metarhizium amisop, Metarphizium acridu* and PAF-AH in terms of architecture.
Conclusion: The PLA_2-like gene isolated from the blood stream form of *Trypanosoma brucei* and studied was found to posses the domains and motifs unique to PLA_2s and lipases and so homology was established among the proteins.

Keywords: Trypanosoma brucei; Phospholipase A_2; gene; motif; domain.

1. INTRODUCTION

Phospholipase A_2 (3.1.1.4) comprises of a diverse family of enzymes that hydrolyze glycerophoshospholipids at the sn-2 position giving rise to free fatty acids and lysophospholipids. The membership of the PLA_2 super family is ever expanding [1]. The enzymes have been classed into 15 groups [2]. These groups were regrouped into two – those utilizing a catalytic histidine and those using a catalytic serine. All the members of the PLA_2 super family carry a consensus sequence GXSXG which is common to many other lipases [3] despite some differences that are the basis for the various patterns of classification. The enzymes are classed based on their source, amino acid sequence, chain length and disulphide bond patterns [4] as well as based on the guiding information that the enzyme must catalyze the hydrolysis of the sn-2 ester bond of a natural phospholipid substrate. In addition, the enzyme must have complete protein sequence of the mature protein established; have homologous enzymes distinguished and the spliced variant established within subgroups; have the sequence homology and catalytic activity established in order to be classed along with others [1].

The physiological role of PLA_2 gene expressed as PLA_2 protein includes the hydrolysis of phospholipids yielding free fatty acids (arachidonic acid and oleic acid) and lysophospholipid. The fatty acids are important stores of energy. Arachidonic acid is an important metabolic intermediate for producing eicosanoids, which are regulatory factors implicated in a wide range of physiological and pathological states serve as potent mediators of inflammation and signal transduction [5]. The other product, lysophospholipid, is important in cell signaling, phospholipid remodeling and membrane perturbation [3]. This enzyme is also reported to regulate the entry of calcium ions into *Trypanosoma brucei* and help the parasite modulate the host-parasite interaction [6] thereby implicating PLA_2 in the pathogenesis of the *T. brucei* The PLA_2 family has become a major drug target for many different diseases [7,8].

PLA_2 has been reported in different species of trypanosomes to various degrees. Some reports show that phospholipase A_2 has been identified to have orthologs in *T. brucei*, *T. cruzi*, and humans and the *Leishmania* PLA_2/PAF-AH (LmjF.35.3020) contains a predicted N-terminal signal peptide sequence and transmembrane domain and a predicted lipase/platelet-activating factor acetylhydrolase sequence [9]. This group of enzymes has been isolated from various sources such as animal toxins like snake venom [10]; insect venom [9] and mammalian organs [11,12]. Some other reports show that the enzyme has also been isolated and purified to electrophoretic homogeneity in *T. congolense* [13]; that activity and kinetics of PLA_2 from *T. brucei gambiense* and *T. brucei* have been detected and studied [14]. The *Trypanosoma brucei* (Tb09.211.3650) Gene Data Base on TritrypDB hosted by the Sanger Institute, revealed a protein encoding gene was present having a product described as phospholipase A_2-like protein, putative. However, reports on PLA_2 in *T. brucei* distributed around the endemic regions are quite scarce. Equally, the PLA_2 from *Trypanosoma species* has not found inclusion in the several classes of the enzymes reported in literature possibly due to the scarcity of literature on its studies. Therefore, this work was designed to study the gene sequence of PLA_2 obtained from *T. brucei* in Nigeria, West Africa, for the first time, in comparison with those already

characterized. This may contribute in the understanding of the nature of the enzyme and so in subsequent classification. As part of the effort, Phospholipase A_2-like gene from blood stream form *Trypanosoma brucei brucei* had been reported to conserve domains and motifs peculiar to characterized PLA_2s and lipases [15]. Literatures have revealed that genome sequence analysis have helped in the rcharacterization of a PLA_2 cDNA from *Arabidopsis thaliana* [16] genes encoding heterodimeric phospholipases A_2 from the scorpion *Anuroctonus phaiodactylushav* [17] sequences and structural organization of phospholipase A_2 genes from *Vipera aspis aspis*, *V. aspis zinnikeri* and *Vipera berus berus* venom [18] plasmid pLA1 present in *N. pentaromativorans* US6-1 [19]; this approach has therefore found application in Metagenomics [20].

2. MATERIALS AND METHODS

2.1 Reagents and Equipment

Chemicals used were of analytical grade and purchased from Sigma and Pharmacia Fine chemicals. DNA extraction kit was purchased from Bio Basic Inc. Markham Ontario, Canada. *Taq* polymerase and High Fidelity Polymerase Enzyme Mix were bought from Promega, USA and Fermentas, respectively. High Pure polymerase chain reaction (PCR) Clean-Up Kit used to purify PCR products was purchased from Fermentas and 100 bp DNA molecular size marker was purchased from Roche, Mannheim Germany. Products from Fermentas and oligonucleotide primers were supplied and synthesized by Inqaba biotec Industry®, Pretoria South Africa. GeneAmp PCR System9700 used for amplification was obtained from Applied Biosystems, Indonesia and the Gel Documentation System was obtained from Synegene® Inc. Indonesia. Sequencing analyses were performed by Inqaba Biotec Industries®, Pretoria South Africa.

2.2 Parasites Isolation

An isolate of *T. b. brucei* from cows (Federe isolates) were obtained from the Parasitology Department, NITR, Vom. Adult albino rats were infected through intra peritoneal route with 0.2ml *Trypanosoma brucei* infected blood diluted with 2ml phosphate saline glucose (PSG) buffer pH 8.0 to give cell density of ≈ 1 x 10^6 cells/ml. Parasitemia was monitored by wet smear via tail snip. At peak parasitemia (≈ 1 x 10^8 cells/ml), the rats were euthanized and blood collected. Parasites were purified on DEAE-cellulose (pre-swollen whatman DE-52-Pharmacia Fine Chemicals) as previously described [21].

2.3 DNA Isolation

Genomic DNA was extracted from 200µl (≈ 1 x 10^8 cells/ml) of isolated *T.b. brucei* suspended in PSG buffer (0.6g NaH_2PO_4; 0.71g Na_2HPO_4 and 14.61g NaCl in 450ml distilled water adjusted to pH 8.0 with orthophosphoric acid) using ZR Genomic DNA Tissue Minipreps Kit (Zymo Research) according to the manufacturer's instructions. Briefly, cell lysis was with 500 µl lysis buffer (which consisted of 10 mM phosphate buffer containing and protease inhibitor) and proteinase K (3 µl of 10mg/ml) incubated at 55° C for 30 minutes. DNA was precipitated with 260 µl absolute ethanol. Precipitated DNA was captured in EZ-10 column by centrifuging at 12,000 x g for 1minute. The flow through was discarded while the EZ-10 column placed in a fresh vial and centrifuged again at 12,000 x g for 1minute to remove residual wash buffer. DNA was eluted into 1.5 ml microcentrifuge tubes with 50 µl elution buffer after incubation at 50°C for 2 minutes by centrifuging at 14,000 x g for 1 minute.

2.4 PCR Amplification of Phospholipase A_2 Like Gene

Phospholipase A_2 like gene was detected and amplified by Polymerase Chain Reaction (PCR) with primers designed based on the gene sequence of PLA_2 like gene in the GeneBank Data Base (Tb 09.211.3650, Phospholipase A_2-like protein, putative, *T. brucei*, chr 9). The primer designed was done in Inqaba Biotech Industry, Pretoria, South Africa. The primers used were as follows: Sense primer 5'-ATGGTAACGTGGGC GCTGAA GTAT- 3'carrying *BamHI* site and Anti-sense primer 5'-CTAACACGTTGAACACACTTC GGTA-3'carrying *PstI* site. High Fidelity Taq DNA Polymerase Enzyme kit (Fermentas) was used to amplify the gene from the genomic DNA according to the manufacturer's instructions. The optimum reaction mix in 50 µl volume was as follows: nuclease free water (37.6µl); 10 x PCR buffer (5.0 µl); dNTP mix (1.0 µl); each primer (1.0 µl); High Fidelity Taq enzyme mix (0.4 µl); Genomic DNA (5.0 µl of 50µg DNA/ml). The thermal cycling was carried out with the following process profile: initial denaturation at 94°C for 2minutes, elongation 94°C for 30 seconds, 56°C for 30 seconds, 68°C for 2

minutes running for 30 cycles, and final extension at 68ºC for 10 minutes; then ending/waiting at 4ºC for ∞. Ten microliters (10 µl) of the product was separated on 1.0% agarose gel to check the success of the process and the results documented using Gel Documentation System (Synegene®).

2.5 Cloning and Sequencing

The PLA_2 gene amplified by PCR from *T. b. brucei* was purified using High Pure PCR Product Clean-Up Kit (Fermentas) and ligated into *pMal-c2E* vector in a 20 µl ligation reaction. The ligation reaction mixture was incubated at 16ºC over night and subsequently used for transformation. The recombinant plasmid was designated *pMal-PLA₂* and transformed into *Escherichia coli* DH5α competent cells. Transformants were placed on LB agar containing 100µg/ml ampicillin. PCR Cloning Kit (Fermentas) was used to clone the purified PLA_2 from *T. b. brucei.* Colonies were randomly selected for screening of positive clones by PCR and restriction endonucleases digestion of plasmids using *BamHI* and *Pst I.* The sequence was submitted to the Gen Bank Data Base.

2.6 Transformation of Clone into *E. coli* (BL-21 (DE3)) and Expression

One micro liter of the ligated DNA construct was transformed into *E. coli* expression grade BL-21 (DE3) competent cells (Lucigen) according to the manufacturers' instruction. The colonies of the BL-21 (DE3) cells were picked from the agar plates and inoculated in 30ml broth medium (SOC plus 100µl/ml ampicillin) and incubated for 3 hours at 37ºC. The cells were then harvested by centrifugation at 5000g for 15 minutes at 4ºC. The medium was discarded from control and induced cells. The cell pellets were re-suspended in 5 ml lysis buffer and sonicated for short pulses of 10 seconds for 4 times on ice. The lysates were centrifuged at 10, 000g for 20 minutes at 4ºC. The supernatant which had the protein was collected. The pellet was re-suspended in 5 ml lysis buffer. All supernatants were then analyzed on 10 % SDS-PAGE.

2.7 Bioinformatics Sequence Analysis

The Finch TV® programmes (GeoPiza) was used to analyze the PLA_2 gene while NCBI BLAST programmes such as NCBI Conserved Domain Search Tool (CDD) and NCBI Conserved Domain Architectural Retrieval Tool (CDART) were used to study the PLA_2 gene and translated protein sequences in order to establish the possibility of homology or similarity.

3. RESULTS

The PCR amplification gave a 1.3kb PLA_2 like band from the gene from genomic DNA of *Trypanosoma brucei*. The amplicon cloned unto pMal-c2E vector between the restriction sites of *BamH1* and *Pst1* yielded pMal-PLA_2 of about 7.9kb which is approximately the size of the pMal-c2E vector (6.6kb) and PLA_2 (1.3kb) put together. Direct PCR analysis done to confirm the quality of the insert DNA gave amplicon of about 1.3kb which was similar in size with the PLA_2 like gene amplified from the genome DNA of bloodstream form *T. b. brucei*. The digestion of the purified pMal-PLA_2 clone and separation on agarose gel electrophoresis equally revealed the pMal-2cE vector with about 6.6kb as well as the PLA_2 like gene (1.3kb). These confirmed the cloning of the PLA_2 like gene into pMal-2cE to form pMal-PLA_2 clone. The heterologous expression of *T. brucei* PLA_2 in pMal-c2E plasmid recorded a success in the transformation process of the competent *E. coli* cells. On the other hand, the heterologous expression of the recombinant PLA_2 in BL-21 (DE3) competent *E. coli* cells was not successful. The corresponding fractions obtained from the colonies were resolved on SDS-PAGE. This put a limit to biochemical characterization of protein in order to confirm the finding from bioinformatics analysis.

The gene sequence with 1, 344bp nucleotides primary sequence obtained has been assigned the accession number *JN603736*. The BLASTN Alignment view programme compared the PLA_2 like gene sequence with gene sequences were from *T. brucei* TREU927 PLA_2 like protein (Gene ID: Tb 3661014 Tb 09.211, 3650; XM 822413.1) and *T. brucei gambiense* (DAL 972, FN 554972.1).

The detail NCBI BLASTN alignment view (Fig. 1) showed that no deletion occurred in the PLA_2 like gene (query) except eight substitutions distributed at positions 51, 61, 178, 282, 530, 810, 888 and 970 as compared with the two gene sequences in the Gene Bank DB. The substitutions in positions 51, 61, 282, 530, and 888 do not change the amino acid in those respective positions because the new codons still coded for the same amino acids. On the other hand, the substitutions in positions 178, 810 and

970 caused changes in amino acids in those positions i.e. at position 78 [TTG changed to TTC]: TTG codes for Leucine while TTC codes for Phenylalanine; at position 810 [AGT changed to ATT]: AGT codes for Serine while ATT codes Isoleucine and at position 970 [GTA changed to CTA]: GTA codes Valine while CTA codes for Leucine.

The parasite Genomes WU-BLAST2 analysis of the sequence gave 99% identity and similarity to the *T. brucei* (TREU 927) PLA_2 like sequence from partial mRNA, chromosome 9 and *T. brucei gambiense* DAL 972 Chr 9, complete sequence PLA_2 (Table 1). The result also revealed that there was 100% coverage, no gaps, 99% identity and zero E – values in each comparison.

The translated PLA_2 primary structure of 447 amino acids was compared with GeneBank PLA_2 sequence Databases in NCBI using blastx established homology with those of other putative PLA_2 proteins from *T. brucei* (TREU927), *T. b gambiense* (DAL972), *T. cruzi*, *Leishmannia major* and characterized human Platelet – Activating Factor Acetyl Hydrolase (Fig. 2). The alignment showed that the protein carries a conserved *GHSFG* lipase motif and an *FSHGL* motif peculiar to PLA_2s which are also conserved in the query. These significantly elaborated the query sequence similarity with the PLA_2 family.

```
Query          1      ATGGTAACGTGGGCGCTGAAGTATTTTGTTCGCGTAGTCCGATGGTCGACAGAAGCATTC  60
TREU927:      61      ..................................................A.........  60
DAL972:1656744        ..................................................G.........  1656803
Query         61      CTAATTTGGCCCACACGGCCACTTTTTGACTATGCCACCTCATTGCATTGTGTTCCCATA  120
TREU927       61      C ..........................................................  120
DAL972:1656804        T...........................................................  1656863
Query        121      AGCGGCACATTTATTACTTCGGTTCTGCTCTGCTTCTACGGGTTCCCACTTTTGTGGTCT  180
TREU927      121      .....................................................C......  180
DAL972:1656864        .....................................................G......  1656923
Query        241      CCACTATTGAAACCCATTGGCGGTCGCTATAGCGTGGGCCTCGTGCATATGAACGGCTGC  300
TREU927      241      .........................................T..................  300
DAL972:1656984        .........................................C..................  1657043
Query        481      AACGCGGTGCCCGCCGCTTTGCTCAACCAATATGAGAGAGTGCCGCCTATTGTTGTGTTC  540
TREU927      481      ............................................A...............  540
DAL972:1657224        ............................................G...............  1657283
Query        781      AAGGACTTTTGGACAACTTTGGCTACAGTAATTCAGATATTGACAAGTTTCTTAGCAAA  840
TREU927      781      ...........................T................................  840
DAL972:1657524        ...........................G................................  1657583
Query        841      CCGTTGCAGGTACATCTTGCGGGTCATTCATTTGGCGGTGCCACTGTACTCGCGGCTGCA  900
TREU927      841      ...............................................A............  900
DAL972:1657584        ...............................................G............  1657643
Query        961      CCATGATGGTACCAATACAAAATGAACATTTTTGCAACCCGCTTTCTGATGGCCGTAAA  1020
TREU927      961      ........G...................................................  1020
DAL972:1657704        ........C...................................................  1657763
Query       1321      ..............................ACCGAAGTGTGTTCAACGTGTTAG  1344
TREU927     1321      ..............................ACCGAAGTGTGTTCAACGTGTTAG  1344
DAL972:1658064        ..............................ACCGAAGTGTGTTCAACGTGTTAG  1658087
```

Fig. 1. NCBI BLASTN Alignment View of PLA_2 like gene sequence with *T. brucei* TREU927 PLA_2 like protein from partial mRNA length = 1344(Gene ID: Tb 3661014 Tb09.211.3650; XM 822413.1) and *T.brucei gambiense* DAL972, chromosome 9, complete sequence length = 2,160,261 (FN554972.1)

Table 1. Comparison of PLA_2-like gene sequence with those of two sequences in data base (DB) using NCBI and Parasite Genomes Washington University Basic Local Alignment Search Tool 2 (WU-BLAST2)

DB- AN	Source	Max score	Total score	Length(bp)	Query coverage (%)	Identity (%)	Gaps (%)	E.value
FN554972.1	*T. brucei gambiense*	2640	2640	2,160,261	100	99	0	0
XM841212.1	*T.brucei* TREU927	2640	2640	1,344	100	99	0	0

Each source parasite is identified by its data base accession number (DB-AN) and the sequence length given in base pairs (bp)

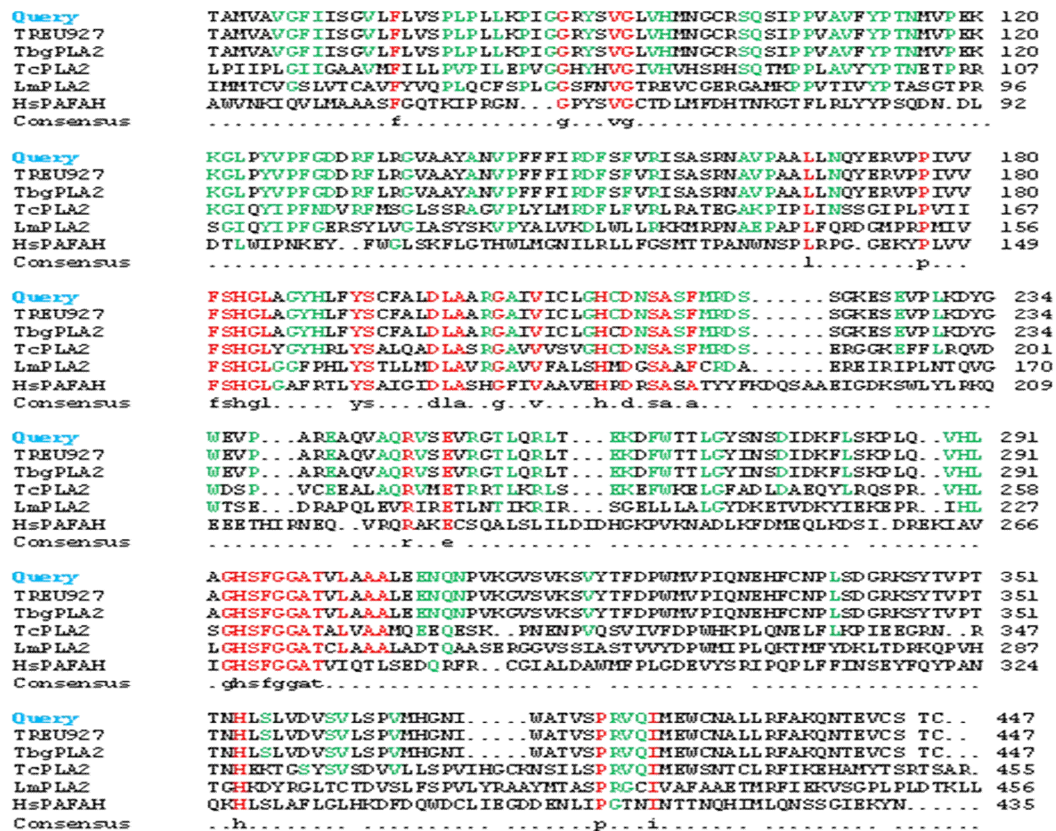

Fig. 2. Amino acid sequence alignment of PLA_2-like protein with PLA_2 from *T. brucei* (TRUE927), *T. b. gambiense* ($TbgPLA_2$), *T. congolense* ($TcPLA_2$), *Leishmania major* ($LmPLA_2$) and PAF-AH. Conserved motifs and lipase consensus motif were marked with red colour

The NCBI conserved Domain search Tool analysis of the protein sequence predicted the Conserved domains (Fig. 3). The result showed that the PLA_2 translated protein sequence which had 447 amino acid residues was mapped to PAF-AH super family, a sub-family of PLA_2 super family. The conserved domains were marked in red ink. Equally predicted was the Conserved Domain Architecture of the protein sequence using the Conserved Domain Architecture Retrieval Tool (CDART).

The results (Fig. 4) showed the graphic view of conserved domains architecture on PLA_2 like protein. Proteins with similar architectures were PLA_2 from *T. cruzi*, *Metarhizium amisop, Metarphizium acridu* and PAF-AH.

4. DISCUSSION

The gene sequence (1,344 bp) presented and analyzed using some Bioinformatics Tools revealed some interesting high lights. The

revealed 99% identity and similarity between the PLA_2 – like gene from *T. b. brucei* and that of *T. brucei* (TREU 927) PLA_2 – like sequence from partial mRNA, chromosome 9 and *T. b gambiense* (DAL 972 chromosome 9, PLA_2 complete sequence) as shown (Table 1) signify that they are homologous. Also the translated protein sequence of the PLA_2-like gene from *T. b. brucei* (Fig. 2) revealed identities and similarities in the conserved domains region with members of the Platelet Activity Factor Acetyl Hydrolase (PAF-AH) super family. These findings agree with previous reports [9]. This further substantiated by the report that PAF-AH is a subfamily of the PLA_2 super family responsible for inactivation of platelet-activating factor through the cleavage of an acetyl group [22,23]. The conserved domains among the protein sequences were presented (Fig. 3) revealing that large portions of the sequence were conserved. The sequential order of the conserved domains in the protein sequence was also conserved as revealed by the CDART analysis. The sequence of PLA_2 like protein from *T. b. brucei* was similar in architecture (Fig. 4) to the sequences of PLA_2 from *T. cruzi, M. anisop, M. acridu* and PAF-AH in the Genebank DB. This implies that the sequences are similar in architecture and not just ordinary sequence similarity. Since the conserved Domain Database brings together several collections of multiple sequence alignment with conserved domains [22] and the CDART performs similarity searches based on the sequential order of the conserved domains, get them grouped and scored by architecture [21], then the PLA_2 like gene (JN603736) used in this study was a PLA_2 gene of the *T. b. brucei*. Other important features conserved in the studied sequence were the lipase motif (*GHSHG*) and the motif (*FSHGL*) peculiar to PLA_2s. The sequences of PLA_2 from *T. brucei* (TREU 927), *T. b. gambiense*, *T. cruzi, Leishmania major* and characterized PAF-AH shared consensus sequences along with the conserved motifs with the query protein as produced by the NCBI BLASTX sequence alignment. This further elaborated the sequence similarity of the PLA_2 like protein from *T. b. brucei* with the PLA_2 super family as a justification that it was a PLA_2 gene that was amplified and studied. This is another case of homology which had been reported to be common among the members of the PLA_2 super family [1,24]. These conserved domains and motifs are similar to those reported in a study using *T. brucei* (strain EATRO 427 clone MITat 1.2) [25]. Also, the use of Trans-sialidase-like gene from the bloodstream form of *Trypanosoma evansi* that conserved most of the active siteresidues and motifs found in *Trypanosomal sialidases* and trans-sialidases has been reported as a basis for homology [26].

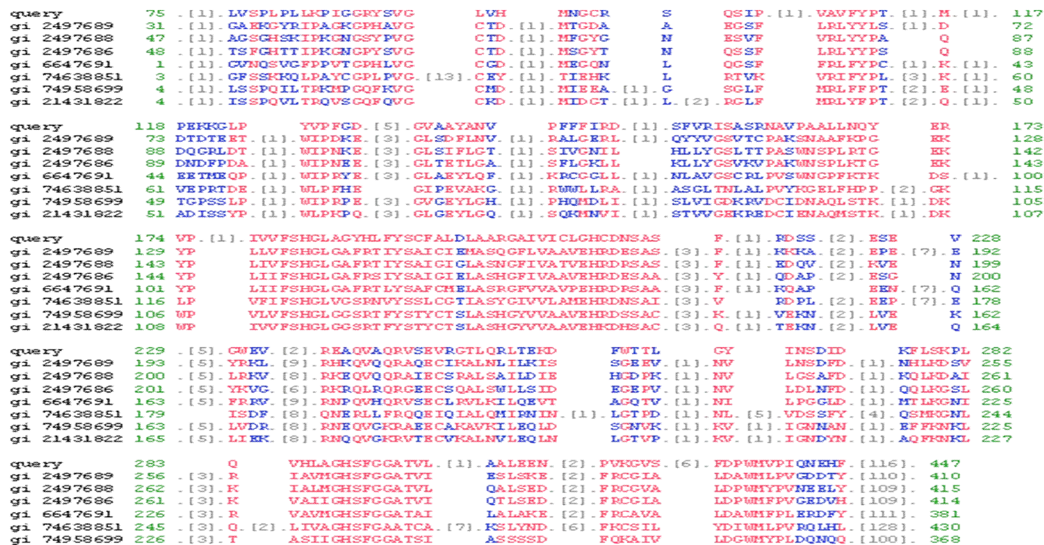

Fig. 3 Conserved Domains in PLA_2-like protein as aligned to members of PAF-AH super family. This was shown in red colour signifying high relatedness while domains that were weakly related were indicated in blue colour. Unaligned portions were left and indicated by numbers in brackets. The members of the PAF-AH super family used in the alignment were represented by their respective identification numbers

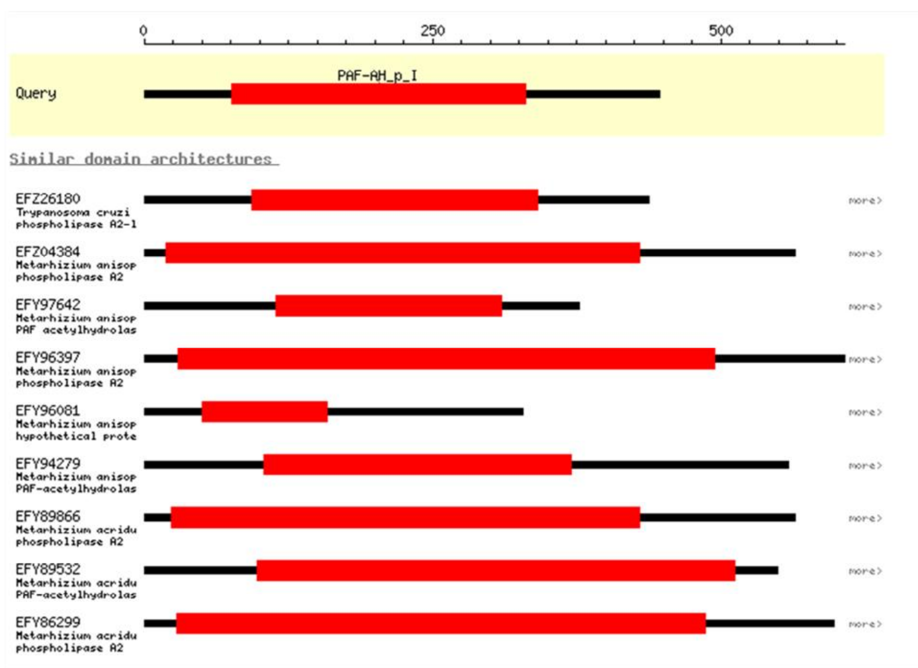

Fig. 4. Conserved domains architecture on PLA_2 like protein. Proteins with similar architectures include PLA_2 from *T.cruzi, Metarhizium anisop, Metarhizium acridu* and PAF-AH

The Alignment view (Fig. 1) revealed only substitutions in the PLA_2 like gene at positions 178, 810 and 970 that caused changes in the amino acids at the respective positions. These alterations did not appear in the active center (GHSFG) of the enzyme and so the activity of this enzyme may remain unaltered. This revelation agrees with conclusions that the nucleotide substitution can change the triplet sequence (codons) and hence can cause redundancy of the protein especially when it occurs on the catalytic domain [27]. However, the changes in such protein sequences may also occur at positions irrelevant for enzymatic activity [28]. These findings may provide ground for considering the enzyme a possible member of one of the classes in the superfamily.

5. CONCLUSION

Conclusively, the presence of PLA_2 and lipase motifs, PLA_2 conserved domains and the high percentage identity and similarity of the PLA_2 -like sequence to some characterized PLA_2 primary sequences indicate that it is a PLA_2 homologue of the Platelet-activating factor acetylhydrolase superfamily. The unsuccessful expression of the recombinant PLA_2 in BL-21 (DE3) competent *E. coli* cells as displayed on fractions obtained from the colonies resolved on SDS-PAGE suggests that alternative ways for the expression need to sought in order to biochemically characterize the gene product.

ETHICAL APPROVAL

Animal experiments were carried out in accordance with the instructions for the care and use provided by the university of Jos, Nigeria where the animal experiments were carried out. The experiments were examined and approved by the university of Jos ethics committee

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Six DA, Dennis EA. The expanding superfamily of phospholipase A_2 enzymes: Classification and characterization. Biochem. Biophy. Acta. 2000;1488:1-19.
2. Schaloske RH, Dennis EA. The phospholipase A_2 superfamily and its group numbering system. Biochem. Biophy. Acta. 2006;1761:1246-59.
3. Balsinde J, Balbao MA, Insel PA, Dennis EA. Regulation and inhibition of phospholipase A_2. Annu. Rev. Pharmacol. Toxicol. 1999;39:175-89.
4. Modesto, JC, Spencer PJ, Fritzen M, Valenca RC, Oliva MLV, de Silva MB, et al. BE-I-PLA_2, a novel acidic phospholipase A_2 from Bothrops erythromelas venom: Isolation, cloning and characterization as potent anti-platelet and inductor of prostaglandin I_2 release by endothelial cells. Biochem. Pharm. 2006;72:377-84.
5. Vadas P, Pruzanski W. Role of Secretory PLA_2 in the pathobiology of disease. Laboratory Investigation. 1986;55:391–398.
6. Eintracht J, Maathai R, Mellors A Ruben L. Calcium entry in *Trypanosoma brucei* is regulated by phospholipase A_2 and arachidonic acid. The Biochemistry Journal. 1998;336:659-666.
7. Gururao H, Manoj K, Punit K, Tej PS, Ramaswamy PK. Human group III PLA_2 as a drug target: Structural analysis and inhibitor binding studies. International Journal Of Biological Macromolecules. 2010;47(4):496-501.
8. Burke JE, Dennis EA. Phospholipase A_2 structure/function, mechanism, and signaling. Journal of Lipid Research. 2009;237-242.
9. Pawlowic M. Phospholipid Metabolism of *Leishmania* Parasites. A PhD Dissertation In Biology. Texas Tech University, USA; 2013.
10. Abubakar MS, Nok AJ, Abdurahman AK, Haruna AK, Shok M. Purification of two Phospholipase enzymes from *Naja n. nigricolis Reinhardt* venom. J. Biochem. Mol. Toxicol. 2003;17(1):53-58.
11. Prenner C, Mach L, Glossl J, Marz L. The antigenicity of the carbohydrate moiety of an insect glycoprotein, honey-bee (*Apis mellifera*) venom phospholipase A_2: The role of a 1,3-fucosylation of the asparagine-bound N-acetylglucosamine. Biochem. J. 1992;284:377-380.
12. Valentin E, Ghomashchi F, Gelb MH, Lasdunski M, Lambeau G. On the diversity of Secreted PLA_2s. J. Biol. Chem. 1999;274(29):31195-31202.
13. Nok AJ, Esievo KAN, Ibrahim S, Ukoha AI, Ikediobi CO. Phospholipase A_2 from Trypanosoma congolense: Charaterization and haematological properties. Cell Biochem. Function. 1993;11:125-130.
14. Shuaibu MN, Kanbara H, Yangai T, Ameh DA, Bonire JJ, Nok AJ. PLA_2 from *T.brucei gambiense and T. b. brucei*: inhibition by organotis. J. Enzyme Inhib. 2001;16:433-441.
15. Longdet IY. Phospholipase A_2-like gene from blood stream form *Trypanosoma brucei brucei* conserves domains and motifs peculiar to characterized PLA_2s and lipases. 3rd International Conference on Proteomics and Bioinformatics (OMICS Group Conferences) July 15 – 17, 2013; Philadelphia, USA: http://omicsgroup.com/conferences/proteomics-bioinformatics-2013/
16. Ryu SB, Leeb HY, Doellinga JH, Paltaa JP. Characterization of a cDNA encoding Arabidopsis secretory phospholipase A_2: an enzyme that generates bioactive lysophospholipids and free fatty acids. Biochim. Biophys. Acta. 2005;1736:144 – 151.
17. Valdez-Cruz NA, Segovia L, Corona M. Sequence analysis and phylogenetic relationship of genes encoding heterodimeric phospholipases A_2 from the venom of the scorpion *Anuroctonus phaiodactylus*. Gene. 2007;396(1):149-58.
18. Guillemin I, Bouchier C, Garrigues T, Wisner A, Choumet V. Sequences and structural organization of phospholipase A_2 genes from *Vipera aspis aspis*, *V. aspis zinnikeri* and *Vipera berus berus* venom: Identification of the origin of a new viper population based on ammodytin I1 heterogeneity. Euro. J. Biochem. 2003;270(13):2697–2706.
19. Yun SH, Choi C-W, Lee S-Y, Lee YG, Kwon J, et al. Proteomic Characterization of Plasmid pLA1 for Biodegradation of Polycyclic Aromatic Hydrocarbons in the Marine Bacterium, *Novosphingobium pentaromativorans* US6-1. PLoS ONE. 2014;9(3):e90812. DOI:10.1371/journal.pone.0090812.

20. Wood DE, Salzberg SL. Method Kraken: Ultrafast metagenomic sequence classification using exact alignments. Genome Biology. 2014;15:R46. DOI:10.1186/gb-2014-15-3-r46
21. Lanham SM, Godfrey DG. Isolation of Normal plasma lactose concentrations and kinetics of salivarian trypanosomes from man and mammals intravenously infused lactose in cattle: Research in using DEAE cellulose. Experimental Parasitology and Veterinary Science. 1970;65:1-4.
22. Derewenda ZS, Derewenda U. The Structure and Function of PAF-AH. Cell Mol. Life Sci. 1998;54(5):446 – 55.
23. Marchler-Bauer A, Lu S, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C, et al. CDD: a conserved domain database for the functional 207 annotation of proteins. Nuc. Acids Res. 2011;39:225-9.
24. Geer LY, Domrachev M, Lipman DJ, Bryant SH. CDART: Protein homology by domain architecture. Genome Res. 2002;12(10):1619-23.
25. Muhammad K. Identification and characterization of phospholipase A_2 from *Trypanosoma brucei. A PhD* Dissertation. Faculty of Chemistry and Pharmacy of the University of Tübingen; 2009.
26. Yakubu B, Nok AJ, Sanni I, Inuwa HM. Trans-sialidase-like gene from the bloodstream form of *Trypanosoma evansi* conserves most of the active site residues and motifs found in *Trypanosomal sialidases* and trans-sialidases. African J. Biotech. 2011;10(13):2388-2398.
27. Granner DK. Protein Synthesis and the Genetic Code. In: Murray RK, Granner DK, Mayes PA, Rodwell VW, editors. Herper's Illustrated Biochemisstry 26th Edition, USA: McGraw-Hill. 2003;368 -73.
28. Motagna G, Cremona LM, Paris G, Amaya FM, Bushiazzo A, Alzari, M, Frasch CCA.. Transialidase from the African trypanosome, *Trypanosoma brucei.* Eur. J. Biochem. 2002;269:2941–50.

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