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RESEARCH ARTICLE

Posttranslational features of Phospholipase A_2 like Protein from blood stream form *Trypanosoma brucei brucei*

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Abstract

Posttranslational modifications (PTMs) of proteins are crucial for understanding their dynamics and the various signaling pathways or networks in cells. The interest in phospholipase A_2 was because of its biotechnological potentials and involvement in the pathogenicity of *Trypanosoma species*. Bloodstream rat adapted strain of *T. brucei brucei* was grown in rats and separated using DEAE cellulose chromatography. Genomic DNA of the parasites was isolated and the PLA_2-like gene amplified. The gene sequence gave a 1344bp length. The protein translation of the PLA_2 like gene using BLASTX gave a 447 peptide sequence. This translated protein sequence analysed using bioinformatics tools for posttranslational modifications revealed transmembrane helices; 13 phosphorylation sites; 6 methylation sites and an O-glycosylation site. The electronically detected features in this report agree with those characterized and their potentials in the sequence were discussed.

INTRODUCTION

Phospholipase A_2 (3.1.1.4) belongs to a superfamily of enzymes that hydrolyse glycerophoshospholipids at the sn-2 position giving rise to free fatty acids and lysophospholipids. This group of enzymes has been described in a variety of organisms such as vertebrates (mammals, lizards and snake venoms), insects (bees, wasp and scorpions) and parasites (*Trypanosoma spp)*. We had reported the presence and sequence homology studies of PLA_2 like gene from *Trypanosoma brucei* (Longdet and Yakubu, 2014; Longdet et al., 2014). All the members of the PLA_2 superfamily carry a consensus sequence GXSXG which is common to many other lipases (Balsinde et al., 1999) despite some differences that are the basis for the various patterns of classification. 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 enzyme, PLA_2, in blood stream form of *T. b. brucei.* During protein biosynthesis, the polypeptide chains created undergo a series of activities, such as cutting, folding, and posttranslational modification (PTM) (Qiu et al., 2014). Since understanding PTMs in proteins is crucial for dynamic proteome analysis of various diseases and epigenetic inheritance, the analyses of posttranslational features of phospholipase A_2 like Protein was considered imperative.

MATERIALS AND METHODS

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. Oligonucleotide primers were 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.

Trypanosomes

Trypanosoma brucei brucei infected blood from infected bulls was collected from Federe, Plateau State, Nigeria during a *Trypanosoma* surveillance study by the Nigerian Institute of Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. The *Trypanosoma brucei brucei* infected bloods were supplied by the Parasitology Department, Nigerian Institute of Trypanosomiasis Research (NITR), Vom, Nigeria.

Parasites isolation

Adult albino rats were infected intraperitionally with 0.2ml infected blood diluted with 2ml phosphate saline glucose (PSG) buffer pH 8.0 to give cell density of $\approx 1 \times 10^6$ cells/ml. Parasitemia was monitored by wet smear via tail snip. At peak parasitemia ($\approx 1 \times 10^8$ cells/ml), the rats were euthanized and blood collected. Parasites were purified on DEAE-cellulose (pre-swollen whatman DE-52-Pharmacia Fine Chemicals) according to the method of (Lanham and Godfrey, 1970): about 25g DEAE-cellulose was suspended in 200ml separation buffer and equilibrated to pH 8.0 with orthophosphoric acid. Column was prepared by pouring the DEAE-cellulose slurry to 100ml volume and equilibrated with 5 volumes of separation buffer. The blood was loaded onto the equilibrated DEAE-cellulose column (3 x 20cm) with ratio 1:4 of blood to cellulose column. The parasites were chromatographically eluted with PSG buffer pH 8.0 into test tubes and the elution monitored by microscopy. Parasites were concentrated by centrifugation at 2,700 x g for 10 minutes and re-suspended in 2 ml PSG buffer pH 8.0.

DNA Isolation

Genomic DNA was extracted from 200µl of isolated *T.b. brucei* suspended in PSG buffer pH 8.0 using ZR Genomic DNA Tissue Minipreps Kit (Zymo Research) according to the manufacturer's instructions. Briefly, cell lysis was with 500 µl lysis buffer (TBM) and proteinase K (3 µl of 10mg/ml) incubated at 55^o C for 30 minutes. DNA was precipitated with 260 µl absolute ethanol. Precipitated DNA was captured in EZ-10 column by spinning 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 ^{o}C for 2 minutes by spinning at 14,000 x g for 1 minute.

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'-ATGGTAACGT GGGCGCTGAAGTAT- 3'carrying *BamHI* site and Anti-sense primer 5'-CTAACACGTTGAACACACTTCGGTA-3'carrying *PstI* site. High Fidelity Taq DNA Polymerase Enzyme Mix (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 with $MgC1_2$ (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). The thermal cycling was carried out with the following process profile: initial denaturation at 94^0C for 2minutes, elongation 94^0C for 30 seconds, 56^0C for 30 seconds, 68^0C for 2 minutes running for 30 cycles, and final extension at 68 ^{0}C for 10 minutes; then ending/waiting at 4^0C 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®).

Bioinformatics Sequence and Posttranslational Features Analyses

The Finch TV® programmes (GeoPiza) was used to analyse the PLA_2 gene while NCBI BLASTX programme, Transmembrane Helix Topology Prediction Method (TMHMM) server version 2.0, O – glycosylation Prediction Electronic Tool (O-GPET) version 1.0, Phosphorylation Sites Prediction Tool (Kinase Phos) and protein methylation modification prediction (MeMo) web server were used to study the PLA_2 gene and translated protein sequences.

RESULTS

Transmembrane Helix Topology Prediction Method (TMHMM) analysis of the translated protein sequence (447 amino acids) showed presence of transmembrane helices. The amino acid sequences of these transmembrane helices were between positions 31 and 53 (YATSLHCVPISGTFITSVLLCFY) and 55 and 77 (FPLFWS TAMVAVGFIISGVLFLV). The other portions were predicted to be either outside (1 and 30 and positions 78 and 447) or inside (54) the cell.

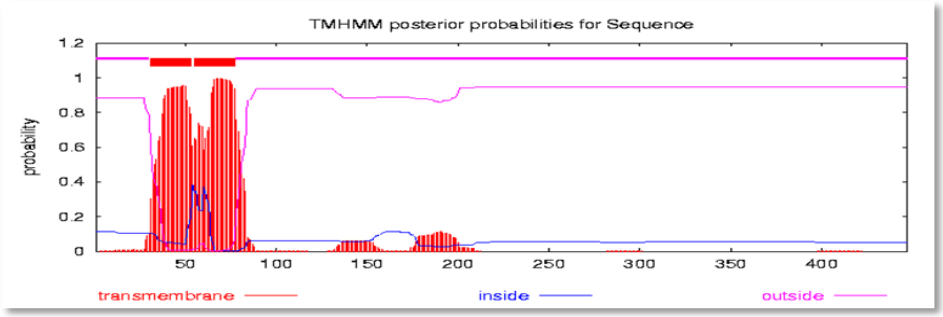

Figure 1 Predicted Transmembrane helices in PLA_2 like protein from *T.b.brucei*

```
Sequence Length: 447
Sequence Number of predicted TMHs:  2
Possible N-terminal signal sequence
Sequence    TMHMM2.0    outside          1     30
Sequence    TMHMM2.0    TMhelix         31     53
Sequence    TMHMM2.0    inside          54     54
Sequence    TMHMM2.0    TMhelix         55     77
Sequence    TMHMM2.0    outside         78    447
```

Phosphorylation Sites Prediction of the translated protein using the Bioinformatics Tool KinasPhos showed the sequence has 13 possible phosphorylation sites as shown in Figure 2 and Table 1 on serine, threonine and tyrosine residues. The protein kinases predicted to catalyse the phosphorylation reactions included Protein kinase C, Protein kinase A, Protein kinase G, Insulin Receptor kinase and calmodulin kinase.

```
MVTWALKYFV RVVRWSTEAF LIWPTRPLFD YATSLHCVPI SGTFITSVLL CFYGFPLFWS  60
----------- -------T--- ----------- ----------- ----------- ----------- PKC
----------- -------T--- ----------- ----------- ----------- ----------- PKA

TAMVAVGFII SGVLFLVSPL PLLKPIGGRY SVGLVHMNGC RSQSIPPVAV FYPTNMVPEK 120

KGLPYVPFGD DRFLRGVAAY ANVPFFFIRD FSFVRISASR NAVPAALLNQ YERVPPIVVF 180
----Y------ ----------- ----------- ----------- ----------- ----------- Jak
----------- ----------- ----------- ------S---- ----------- ----------- PKG

SHGLAGYHLF YSCFALDLAA RGAIVICLGH CDNSASFMRD SSGKESEVPL KDYGWEVPAR 240
----------- ----------- ----------- ----------- S---------- ----------- PKG
----------- ----------- ----------- ----------- -S--------- ----------- ATM
----------- ----------- ----------- ----------- -S--------- ----------- IKK
----------- ----------- ----------- ----------- -----S---- ----------- PKG

EAQVAQRVSE VRGTLQRLTE KDFWTTLGYI NSDIDKFLSK PLQVHLAGHS FGGATVLAAA 300
----------- ---T------- ----------- ----------- ----------- ----------- PKC
----------- ----------- --------Y- ----------- ----------- ----------- INSR
----------- ----------- --------Y- ----------- ----------- ----------- Jak

LEENQNPVKG VSVKSVYTFD PWMVPIQNEH FCNPLSDGRK SYTVPTVTVH SDDWVKDSES 360
----------- ----------- ----------- ----------- S---------- ----------- PKG
----------- ----------- ----------- ----------- -Y--------- ----------- INSR

WEFFKRMKAL VLEQSAYASL NEVEKQALFG IVVTKNTNHL SLVDVSVLSP VMHGNIWATV 420
----------- -------Y--- ----------- ----------- ----------- ----------- INSR

SPRVQIMEWC NALLRFAKQN TEVCSTC 447
S---------- ----------- ------- CDK
----------- ----------- T------ PKC
```

Figure 2 Phosphorylation sites on PLA_2 like protein from *T.b.brucei* PKC (Protein kinase C); PKA (Protein kinase A); PKG (Protein kinase G); INSR (Insulin Receptor kinase); CDK (calmodulin kinase)

Table 1 Predicted Phosphorylation Sites

Summary Result			
Protein Name	Predicted Phosphorylated Sites		
	Serine(S)	Threonine(T)	Tyrosine(Y)
Query (PLA_2-like)	6	3	4

The PLA_2 translated protein sequence analysed with O-glycosylation prediction electronic tool (O-GPET version 1.0) predicted O-glycosylation residue (Threonine) at position 266.
Signal site for the translated protein query was predicted using Signal P 3.0 server. The result revealed 3 probable scores for each amino acid position. The scores were S-score, C-score and Y-score. The point of convergence of the 3 different scores gives the location of a signal peptide at position 20 on the protein sequence as indicated in Figure 3.

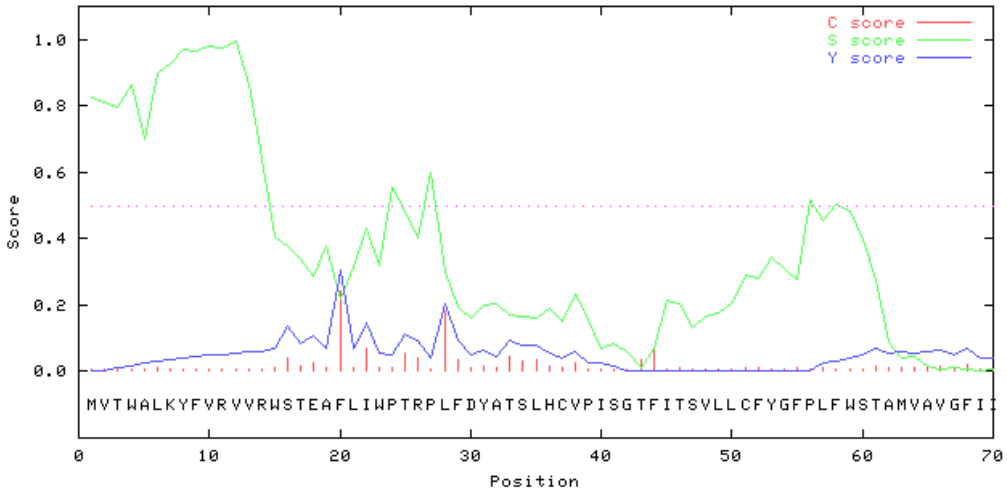

Figure 3 Predicted Signal Peptides Residues on PLA_2 like protein sequence from *T.b.brucei*
S-score for the signal peptide prediction was reported for every single amino acid position; *C-score* is the ``cleavage site'' score; Y-score where the slope of the S-score is steep and a significant C-score is found

The protein methylation modification prediction results showed six sites (3 lysine, K and 3 arginine, R) as presented in Table 2.

Table 2 Protein methylation sites on PLA_2 like protein sequence from *T.b.brucei*

Residue	**Position**	**Flanking sequences**
R	135	FGDDRFLRGVAAYAN
R	201	FALDLAARGAIVICL
R	252	AQRVSEVRGTLQRLT
K	261	TLQRLTEKDFWTTLG
K	314	PVKGVSVKSVYTFDP
K	340	NPLSDGRKSYTVPTV

DISCUSSION

The results of the sequence structure studies of the translated protein revealed some significant features. The protein was predicted to have transmembrane helices. This implies that the protein associates itself with either the cell or organelle membrane. This is consistent with earlier reports on the location of PLA_2 in trypanosomes. This protein had been described as extracellelluar in *T. brucei* (Eintracht et al., 1998) and *T. cruzi* (Connelly et al., 1885). Additionally, the transmembrane sequence suggests that the protein contains amino acids on its N- Terminus which

serve as a signal sequence used for targeted secretion after synthesis in the endoplasmic reticulum. This was corroborated by the finding of this work that the PLA_2 from *T.b.brucei* has a signal peptide at position 20. This peptide serves as a signal position needed to govern the protein as it is transported to and across the membrane (Heijne et al., 1986; Chou et al., 2002).

The translated protein sequence was shown to have 13 phosphorylation sites. This agrees with the understanding that some proteins have specific phosphorylation sites which may be significant for their catalytic action**. C**ommon modification sites are on seryl, threonyl, or tyrosyl residues of proteins (Rodwell et al., 2003). The process is usually catalyzed by protein kinases in a thermodynamically spontaneous way.

The translated PLA_2 sequence also revealed an O – glycosylation residue (Threonine) at position 266. This means the protein may have some sugar units added to it to build up glycan chains. O – glycosylation is a Post Translational Modification, which occurs in vast majority of proteins and are described to be essential for function (Hamby and Hirst et al., 2008). This may occur on Serine or Threonine residues and the accurate prediction of the sequence location of PTMs enhances functional characterization of proteins (Spiro et al., 2002). Glycosylation plays a role in protein folding, transport and half life, as well as being involved in cell-cell interactions and antigenicity (Spiro et al., 2002; Hart, 1992; Seitz et al., 2000). It was also shown to promote and stabilize the extended conformations of the whole peptide and specifically the conformations of the back bone fragment (Rubinstein et al., 2004).

The presence of the methylation sites on lysine and arginine in the PLA_2 protein sequence from blood stream form of *T.b.brucei*, agrees with reports in literature (Chen et al., 2006; Shi et al., 2012) and methylation sites have been implicated in the pathological processes (Shao-Ping et al., 2015). Protein methylation is an important and reversible post-translational modification of proteins (PTMs), which governs cellular dynamics and plasticity. Conclusively, PLA_2 protein sequence from blood stream form of *T.b.brucei*, is richly modified and exposing the potentials of the protein post translational modification process as possible therapeutic targets.

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REFERENCES

1. Longdet, I.Y. and Yakubu,B. (2014): Molecular Detection of Phospholipase A_2–Like Gene in some Infectious Parasites and Venomous Insects. J. Med. Biol. Sc., 4(1): 43 – 51.
2. Longdet, I.Y., Inuwa, H.M., Umar, I.A., Nok, A.J. (2014): Sequence Homology Studies of Phospholipase A_2-like Gene from Bloodstream form of *Trypanosoma brucei*. British Biotech. J., 5(3): 156-165.
3. Balsinde, J., Balbao, M.A., Insel, P.A. and Dennis, E.A. (1999): Regulation and inhibition of phospholipase A_2. Annu. Rev. Pharmacol. Toxicol., 39: 175-189.
4. Qiu, W,R. , Xiao, X., Wei-Zhong, L. and Kuo-Chen, C. (2014): Methyl-PseAAC: Identification of Protein Methylation Sites via a Pseudo Amino Acid Composition Approach, BioMed Res. International (2014), 12 pages http://dx.doi.org/10.1155/20 14/9 47416
5. Lanham, S.M. and Godfrey, D.G. (1970): 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. Exp. Parasit. and Vet. Sc., 65: 1-4.
6. Eintracht, J., Maathai, R., Mellors, A. and Ruben, L. (1998): Calcium entry in *Trypanosoma brucei* is regulated by phospholipase A_2 and arachidonic acid. Biochem. J., 336: 659-666.
7. Connelly, M.C. and Kierszenbaum, F. (1985): Increased host cell-Trypanosoma cruzi interaction following phospholipase D treatment of the parasite surface. Mol. Biochem. Parasitol., 17(2): 191-202.
8. Heijne, G. (1986): A new method for predicting signal sequence cleavage sites. Nucleic Acids Research. 14: 4683-4690. J. Biol.Chem,. 269 (18):13057-13060.
9. K. Chou, Prediction of Protein Signal Squences. Current Protein and Peptide Science, 3 (2002) 615 – 622.
10. Rodwell, V.W. and Kenelly, P.J. (2003): Enzymes: Regulation of Activities. In: Murray RK, Granner DK, Mayes PA, Rodwell VW (eds) Herper's Illustrated Biochemistry 26th Edition. USA: McGraw-Hill, pp. l72 -179,.
11. Hamby, S.E. and Hirst, J. (2008): Prediction of glycosylation sites using random forests. BMC Bioinformatics, **9,**500.

12. Spiro, R.J. (2002): Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. Glycobiology, 12(4): 43-56**.**
13. Hart, G. W. (1992): Glycosylation. Curr. Opin. Cell Biol**.,** 4: 1017-1023.
14. Seitz, O. (2000): Synthesis and the effects of glycosylation on protein structure and activity. Chem BioChem., 1: 214-246.
15. Rubinstein, A., Kinarsky, L. and Sherman, S. (2004): Molecular Dynamics Simulations of the O-glycosylated 21-residue MUC1 Peptides. Int. J. Mol. Sci., *5*: 119-128
16. Chen, H., Xue, Y., Huang, N., Yao, X. and Sun, Z. (2006): MeMo: a web tool for prediction of protein methylation modifications. Nucleic Acids Res., 1(34): 249-253
17. Shi, S.P., Qiu, J.D., Sun, X.Y., Suo, S.B., Huang, S.Y., Liang, R.P. (2012): PMeS: Prediction of Methylation Sites Based on Enhanced Feature Encoding Scheme. PLoS ONE 7(6) (2012): e38772. doi:10.1371/journal.pone.0038772
18. Shao-Ping, S., Hao-Dong, X.., Ping-Ping, W. and Jian-Ding, Q. (2015): PMeS: Prediction of Methylation Sites Based on Enhanced Feature Encoding Scheme. Mol. BioSyst**.,** 11: 2610-2619