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Analyses of *Trypanosoma brucei* Phospholipase A_2 Structure and Function Using Bioinformatics Approach

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Author's contribution

The study was designed, analyzed and discussed by the author. The author takes full responsibility for the whole study including data collation, manuscript drafting and editing.

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ABSTRACT

Aim: Studying Phospholipase A_2 is increasingly needful because of the enzyme's biotechnological potentials and involvement in the pathogenicity of *Trypanosoma species*. This work was therefore designed to study some of the structural features of putative PLA_2 from *T. brucei.*

Place and Duration of Study: Department of Biotechnology, National Veterinary research Institute, Vom and Department of Parasitology, National Institute of Trypanosomiasis Research, Vom, Nigeria, between July 2015 and May 2016.

Methodology: Bloodstream rat adapted strain of *T. brucei* was grown in rats and separated using DEAE cellulose chromatography. Genomic DNA of the parasites was isolated and the PLA_2 gene amplified and sequenced (GenBank DB accession number: JN603736). The translated protein structure prediction server PSIPRED, Phyre2 web portal for protein modeling, prediction and analysis, (ProSA) -web, RAMPAGE server, 3DLigandSite and PSI-Blast tool were used to analyse the *T. brucei* PLA_2 translated protein sequence.

Results: The analyses of the PLA_2 protein with 447 peptide sequence revealed its secondary structure; 3D structure showing amino acid residues that lie in a helix, strand or coil; ProSA-web z-

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scores with an overall model quality value of -7.01; the Ramachandran plot that among the 447 residues, 433 (96.86%) were in favoured region; ligand binding site at position 235 (Trp) and its transmembrane helices and membrane topology.

Conclusion: A good quality structural model of *Trypanosoma brucei* PLA_2 was determined and found to be closely related to that of Platelet-activating factor acetylhydrolase.

Keywords: Trypanosoma brucei; phospholipase A_2; 3D structure; bioinformatics.

1. INTRODUCTION

Phospholipase A_2 (3.1.1.4) enzyme superfamily that hydrolyse glycerophospholipids at the sn-2 position releasing free fatty acids and lysophospholipids is attracting much attention recently. The potentials of this group of enzymes for application in biotechnological researches and industries have been reported [1,2,3,4]. In an attempt to situate the PLA_2 from *T. brucei* in the superfamily classification, we studied and reported its sequence homology to characterized PLA_2 [5] and the protein posttranslational features [6]. Although this group of enzymes has been reported in a variety of organisms and some placed in appropriate classes, the PLA_2 from *T. brucei* has not been classed yet.

The information needed for classifying the enzymes are: their source, amino acid sequence, chain length and disulphide bond patterns [7]. Other information needed include: established complete protein sequence of the mature protein, established spliced variant within subgroups and established sequence homology and catalytic activity [8]. This necessitates studies in the structure of the PLA_2 from *T. brucei.* The structure of the PLA_2 protein from *T. brucei* has not been solved. Therefore, this study was designed to undertake prediction studies of the structure and functions of putative PLA_2 from bloodstream form *T. brucei.*

2. MATERIALS AND METHODS

2.1 Parasites Isolation

The *Trypanosoma brucei* infected blood was supplied by the Parasitology Department, Nigerian Institute of Trypanosomiasis Research (NITR), Vom, Nigeria.

Adult albino rats were infected intraperitionally with 0.2 ml infected blood diluted with 2 ml 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) according to the method already described [9]: about 25 g DEAE-cellulose was suspended in 200 ml separation buffer and equilibrated to pH 8.0 with orthophosphoric acid. Column was prepared by pouring the DEAE-cellulose slurry to 100 ml volume and equilibrated with 5 volumes of separation buffer. The blood was loaded onto the equilibrated DEAE-cellulose column (3 x 20 cm) with ratio 1:4 of blood to cellulose column. The parasites were chromatographically eluted with phosphate saline glucose (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.

2.2 DNA Isolation

Genomic DNA was extracted from 200 µl of isolated *T. 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° 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 1 minute. The flow through was discarded while the EZ-10 column placed in a fresh vial and centrifuged again at 12,000 x g for 1 minute to remove residual wash buffer. DNA was eluted into 1.5 ml microcentrifuge tubes with 50 µl elution buffer after incubation at 50ºC for 2 minutes by spinning at 14,000 x g for 1 minute.

2.3 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 GGGCGCTGAA GTAT-3' and Anti-sense primer 5'-CTAACACGTTGAACACACTTCG GTA-3'. 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 (37.6 µl); 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 94ºC for 2 minutes, elongation 94ºC for 30 seconds, 56ºC for 30 seconds, 68ºC 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®).

2.4 Sequencing and Bioinformatics Analyses

The PLA_2 gene amplified by PCR from *T. brucei* was purified using High Pure PCR Product Clean-Up Kit (Fermentas) Taq-polymerase catalysed cycle sequencing using fluorescent labelled dye terminator reaction protocol and analysis was done on a 3130XL Genetic Analyzer (ABI). BigDye® V3.1 (ABI) Kit was used according to the manufacturer's instructions. The Finch TV® programmes (GeoPiza) was used to read and edit the PLA_2 gene sequence which was submitted to the Gen Bank Data Base.

NCBI BLAST programmes were used to analyse the sequence.

2.5 Secondary Structure Prediction

The secondary structure prediction of the translated PLA_2 protein was carried out using protein structure prediction server PSIPRED to identify the similarities among the protein structures [10].

2.6 3D Structure Prediction

The Phyre2 web portal for protein modeling, prediction and analysis was used to study the PLA_2 translated protein sequences. 'Normal' mode modelling by Phyre2 produces a set of potential 3D models of the protein based on alignment to known protein structures. The pipeline involves constructing 3D models of the protein based on the alignments between the (Hidden Markov Models) HMM of the sequence and the HMMs of known structure [11].

2.7 3D Structure Validation

3D structure of *T. brucei* PLA_2 obtained through Phyre2 web portal was checked for validity using Protein Structure Analysis (ProSA) -web, which is frequently employed in protein structure validation [12]. The output presents overall model quality displayed in a plot that contains the *z*-scores of all experimentally determined protein chains in current PDB.

2.8 Ramachandran Plot

The Ramachandran plot [13] which is the 2D plot of the φ-ψ torsion angles of the protein backbone and provides a simple view of the conformation of a protein was used to study the predicted 3D model of *T. brucei* PLA_2. In the analysis, the φ-ψ angles cluster into distinct regions in the Ramachandran plot determined using RAMPAGE server [14] where each region corresponds to a particular secondary structure.

2.9 Ligand Binding Site

The model with closest similarity was submitted for binding site prediction by 3DLigandSite. This performed predicting ligand-binding sites using similar structures [15].

2.10 Transmembrane Helix Prediction

The sequence and the set of homologues detected by PSI-Blast were processed by a Support Vector Machine (a powerful machine learning tool) in order to determine whether the sequence contains transmembrane helices and to predict their topology in the membrane. For this purpose Phyre2 uses memsat-sym. The extracellular and cytoplasmic sides of the membrane were labeled and the beginning and end of each transmembrane helix illustrated with a number indicating the residue index [11].

3. RESULTS AND DISCUSSION

3.1 Secondary Structure Prediction

The secondary structure prediction for *T. brucei* PLA_2, putative, protein carried out using protein structure prediction server PSEPRED to identify the similarities among the protein structures is presented in Fig. 1. The secondary structure shows the distribution of the amino acids within the different segments (helix, strand or coil) of the protein [16,17]. Most of the amino acid residues reside in the coil rather than the helix or strand. The Secondary structure of a protein plays primordial role in bioinformatics for drug research and development [18]. This requires that the secondary structures of all proteins be predicted.

3.2 3D Structure Prediction

The Phyre2 web portal for protein modeling, prediction and analysis used to predict the 3D structure of the PLA_2 translated protein sequence from *T. brucei* gave a structure shown in Fig. 2. The structure prediction used 72% of the sequence for modeling giving 100.0% confidence by the single highest scoring template of platelet-activating factor acetylhydrolase as shown in Table 1. This places the *T. brucei* PLA_2 among the hydrolases and closely related to platelet-activating factor acetylhydrolase in structure. This structure shows the spiral conformations or α-helixes, β- sheets or strands and thin random coils in a protein. 3D structure is found to be essential for docking the protein with other ligands thereby allowing the affinity of a structure towards other structures to be deduced [18]. This is important in understanding the function of the protein in the course of interaction with other compounds. Protein structures have informed drug design and have illuminated the mechanism of inhibition [19].

3.3 3D Model Validation

The 3D structure of *T. brucei* PLA_2 subjected to ProSA-web z-scores shows all protein chains in PDB determined by X-ray crystallography in light blue colour while those by NMR spectroscopy appeared in dark blue. The plot shows only chains with less than 1000 residues and a z-score ≤ 10. The z-score of PLA_2 was highlighted as large black dot with an overall model quality value of -7.01 (Fig. 3). The Z score reveals that the structure of *T. brucei* PLA_2 possesses good quality [20].

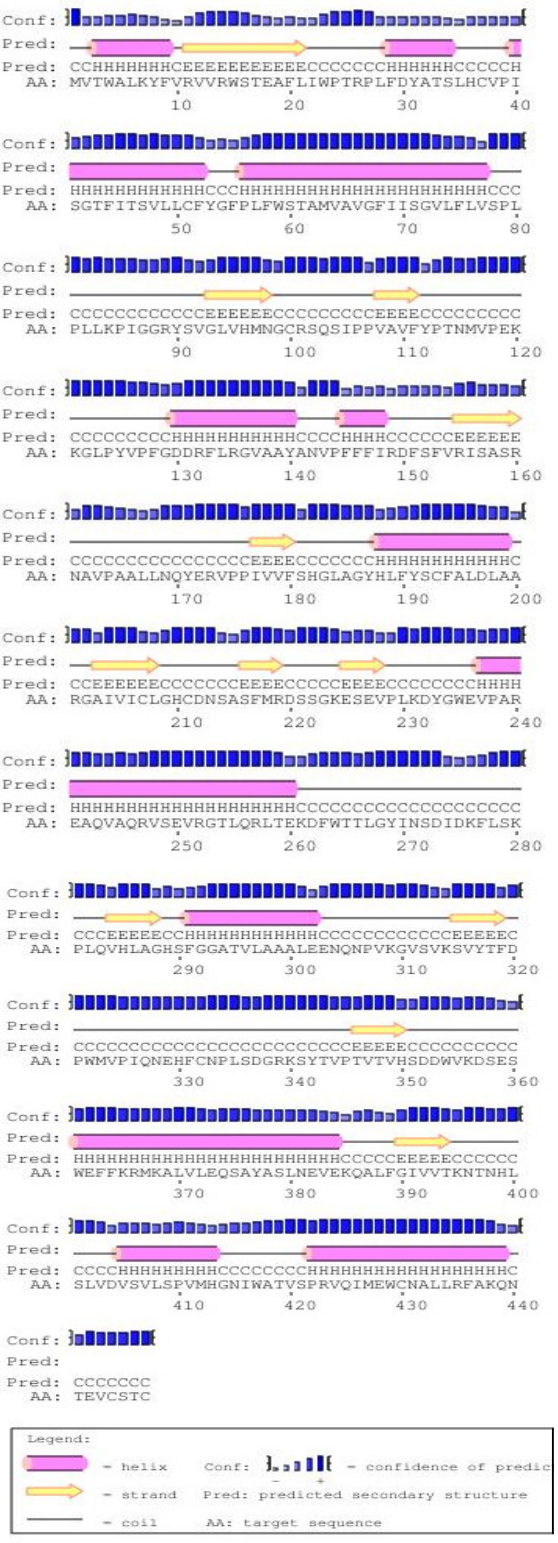


Fig. 1. The predicted secondary structure of *T. brucei* PLA_2 using protein structure prediction server PSEPRED

Table 1. Summary of properties of predicted model of *T. brucei brucei* PLA_2

PDB template	PDB header	PDB molecule	Confidence	Coverage
c3d59B	Hydrolase	Platelet-activating factor acetylhydrolase	100%	72%

Fig. 2. Predicted 3D structure of the PLA_2 translated protein sequence from *T. b. brucei*

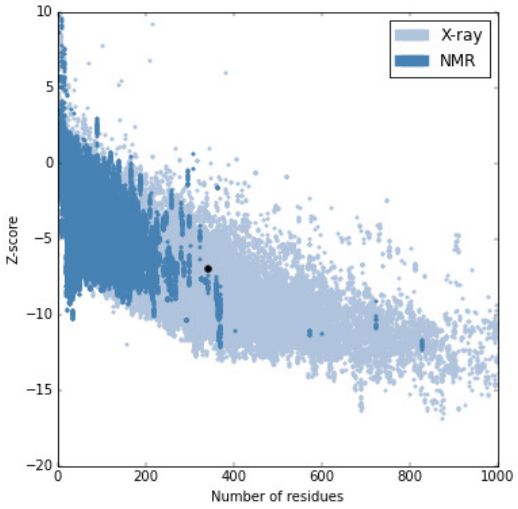


Fig. 3. PROSA plot of the PLA_2 translated protein structure from *T. brucei*

3.4 Ramachandran Plot

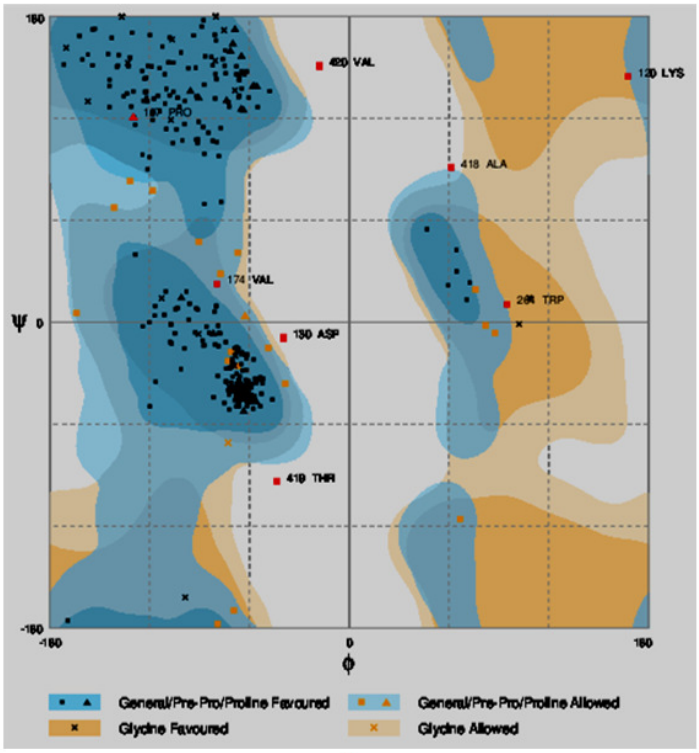
The Ramachandran plot of *T. brucei* PLA_2 appeared in usual four different plots [21]. The plots are elucidating the position of general amino acids, glycine pre proline and proline. These plots are comprehensively viewed as a single Ramachandran plot. The colouring/shading on the plot represents the different regions [22]. The darkest areas or the "core" regions represent the most favourable combinations of phi-psi values and the percentage residues in the "core" regions give a better guide to the stereochemical quality [23]. The Ramachandran plot reveals that among the 447amino acid residues, 433 (96.86%) were in favoured region while only about 3.13% were in the allowed and/or outlier regions (Fig. 4A). The Ramachandran plot also showed that glycine, pre-Pro and proline of *T. brucei* PLA_2 fall in the allowed region (Fig. 4B). The results confirm that the predicted 3-Dimenrsional structure of *T. brucei* PLA_2 is acceptable and of good quality.

3.5 Ligand Binding Site

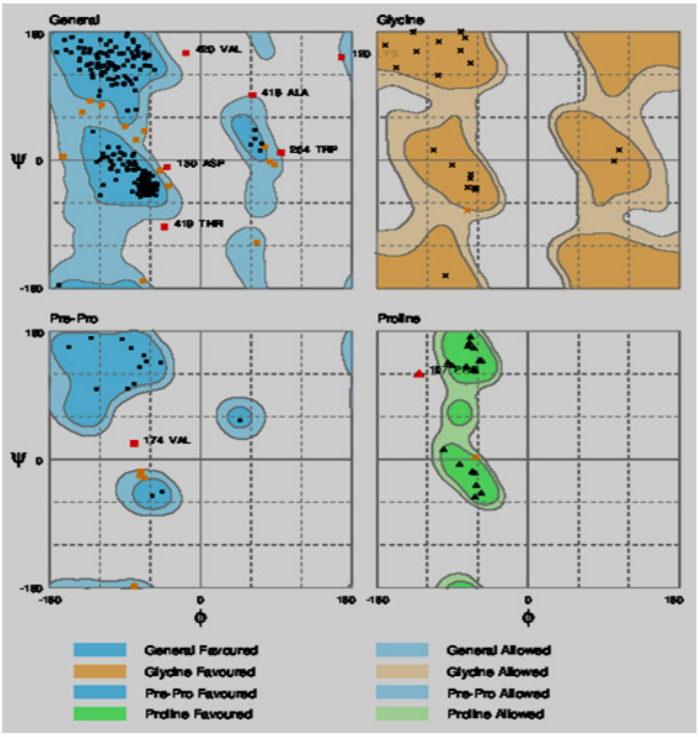
The *T. brucei* PLA_2 protein sequence was predicted to have a tryptophan residue at position 235 where a ligand (N-acetyl-D-glucosamine (NAG)) binds. Enzymes make a most important group of drug targets, and identifying possible ligand-enzyme interactions is of major importance in many drug discovery processes [24,25]. Therefore, identifying this possible ligand-enzyme interaction in PLA_2 may open an opportunity in drug discovery against *Trypanosoma spp* infection.

3.6 Transmembrane Helix Prediction

The sequence and the set of homologues detected by PSI-Blast and processed by Phyre2 using memsat-sym determined transmembrane helices and predicted their topology in the membrane as shown in Fig. 5. This implies that the protein associates itself with either the cell or organelle membrane and so it is a secretory form. PLA_2 had been described as extracellelluar in *T. brucei* [26]. The transmembrane sequence contains amino acids on its N- terminus which serve as a signal sequence used for targeted secretion after synthesis in the endoplasmic reticulum. This peptide serves as a signal position needed to govern the protein as it is transported to and across the membrane [27]. This predicted transmembrane domains pattern might be different in the mature protein without signal peptides.



A



B

Fig. 4. The Ramachandran plot of the predicted *T. brucei* PLA_2 structure

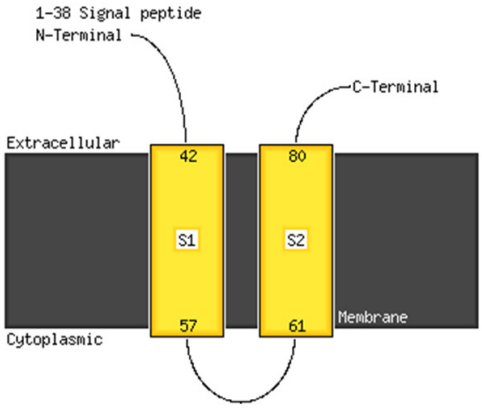


Fig. 5. Transmembrane helix prediction and membrane topology of the PLA_2 translated protein sequence from *T. brucei*

4. CONCLUSION

The structural features of the *Trypanosoma brucei* PLA_2 protein placed it among the hydrolases as well as closely related to platelet-activating factor acetylhydrolase in structure. Therefore, both *Trypanosoma brucei* PLA_2 protein and platelet-activating factor acetylhydrolase can be classed together since they appear to be homologues.

ETHICAL APPROVAL

The author declares that the rats used in this study were handled according to the prescribed law of the Animal Utilization Study Committee, in the University of Jos, Nigeria and from which approval was obtained.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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