# Purification and Characterization of Protein Tyrosine Phosphatase from *Trypanosoma Evansi*

**Dingwoke John[1], *Humphrey Nzelibe[2], Abdullahi Sallau[3], Suleiman Albaba[4]**

[1, 2, 3, 4] Department of Biochemistry, Ahmadu Bello University, Main Campus, P.M.B. 1054, Zaria, Kaduna State, Nigeria

**Abstract:** *Novel protein tyrosine phosphatase (PTPase) isolated from Trypanosoma evansi was purified and characterized, revealing the kinetic parameters to aid in the design of trypanocide that target the pathogenic activity of the enzyme in trypanosomiasis. Healthy albino rats of average weight 190g were inoculated intraperitoneally with 0.2ml of Trypanosoma evansi-infected host blood in normal saline. The parasites were harvested, and lysed following three cycles of freezing and thawing. PTPase was assayed with 10 mmol/L para-nitrophenylphosphate at 37 °C for 10 minutes. Assay specificity of PTPase was verified by adding sodium orthovanadate. The PTPase was purified following ammonium sulphate precipitation and Gel filtration on Sephadex G–75 column, characterized by determining the optimum temperature, temperature stability, optimum pH, pH stability, effects of divalent cations and initial velocity studies. Results revealed 13.23 purification fold and a yield of 41.45%. The PTPase had a molecular weight of 31.2 kDa, broad temperature and pH range with optima of 70 $^0C$ and 5.0 respectively. Initial velocity studies revealed a $K_M$ and $V_{max}$ of 3.44mM and 0.19μmol/min respectively. The PTPase activity was enhanced by $Ca^{2+}$ and inhibited by $Zn^{2+}$. These findings, especially the inclusion of $Zn^{2+}$ could aid in the design of trypanocides for attenuating trypanosome pandemic.*

**Keywords:** trypanosoma evansi, protein tyrosine phosphatase, regulatory mechanism, kinetic properties, trypanocide design.

## 1. Introduction

Animal trypanosomiasis is still a major problem retarding the production and growth of livestock in Africa [1]. The disease has undergone a dramatic and devastating resurgence in recent years, especially in sub-Saharan Africa and beyond [2]. *Trypanosoma evansi* is one of the pathogenic trypanosomes in animals. Surra, which means "rotten" is a disease condition caused by infection with *Trypanosoma evansi*. It causes significant mortality and production loss in livestock.

### 1.1 Literature Survey

Phosphorylation of proteins in specific tyrosyl residues is a major control mechanism for several processes such as normal cell growth, differentiation, metabolism, cell cycle, cell migration, and gene expression, among others [3]. The levels of cellular protein phosphorylation in tyrosine residues are controlled by the activities of both protein tyrosine kinases (PTKs) and phosphatases (PTPases).

Protein tyrosine phosphatases (EC 3.1.3.48) are enzymes that dephosphorylate proteins on tyrosine residues, and their protein tyrosine kinases counterparts, catalyze phosphorylation on tyrosine residues. Both enzymes are responsible for the regulation of signaling events that control fundamental biological processes. Protein tyrosine phosphatase catalyzes the removal of phosphate group attached to tyrosine residue, using cysteinyl-phosphate enzyme intermediate. Protein tyrosine phosphatase is implicated in the pathology of trypanosomiasis [4]. *Trypanosoma evansi* spends part of its life cycle in a tsetse vector and part in mammalian host. In mammalian host, the parasite exists in the stumpy forms which tsetse ingests after blood meal. In the tsetse midgut, the stumpy forms differentiate into procyclic forms and eventually the metacyclic forms in the salivary gland of the tsetse, which infect the hosts [5]. These stumpy forms (which lack immune evasion mechanisms that allow survival in the mammalian host) of the parasites are preserved and held ready for uptake into the tsetse fly by the dephosphorylation action of protein tyrosine phosphatase. This reveals protein tyrosine phosphatase as the life cycle regulator of *Trypanosoma evansi*. This molecular regulator could provide novel targets for therapeutic interference against trypanosomiasis [6]. In addition to protein tyrosine phosphatase, other phosphatases such as ectophosphatase, performing phosphatase activities have been reported in several microorganisms, including protozoa such as *Leishmania donovani* [7,8], *Trypanosoma Brucei, Trypanosoma cruzi, Trypanosoma rangeli* [9,10,11], and bacteria, such as *Mycobacterium bovis* [12]. In *Leishmani donovani,* the parasite induces phosphotyrosine phosphatase activity which helps the parasite to survive within its host without triggering an adequate immune response [13].

### 1.2 Problem definition

Protein tyrosine phosphatase from different trypanosome species have been purified and characterized [14], [15]. However, information concerning this enzyme with respect to *Trypanosoma evansi* is lacking. Therefore, in this study, we report the purification and characterization of protein tyrosine phosphatase isolated from *Trypanosoma evansi* for the first time with the view to establishing the kinetic properties of the enzyme. This would aid in the design of trypanocide that targets the life-cycle-pathogenic role of PTPase in trypanosome infections to ameliorate the scourge of the disease in livestock.

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## 2. Methodology/Approach

### 2.1 Inoculation and monitoring of parasitaemia

A total of five (5) healthy adult wistar albino rats weighing about 190g were used. The rats were obtained from the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. They were caged in animal house, fed with standard rats feed (vital feeds, Jos, Nigeria) and allowed access to water *ad libitum*. The rats were also allowed to acclimatize to the laboratory conditions for 14 days prior to the experiments. The rats were inoculated intraperitoneally with 0.2ml of trypanosoma evansi-infected host blood, contained in normal saline solution. The level of parasitaemia was monitored every 12 hours between the 6th and 7th day post inoculation; the animals were sacrificed at parasitemia level of 128 per field, which is equivalent to log 8.7 number of parasites per milliliter of blood [16]. *Trypanosoma evansi* was obtained from the Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

### 2.2 Harvesting of parasites from the blood

The parasites were harvested from the blood according to the method of [17]. The column was prepared by suspending about 5g of DE-Cellulose (DE-52) into 25ml of phosphate buffered saline glucose (PBSG) pH 8.0. The solution was allowed to equilibrate, forming slurry. The slurry was packed into a column (2×20cm) and further washed with phosphate buffered saline glucose. The blood collected in heparinized tubes was diluted in the ratio of 1:1 with phosphate buffered saline glucose and then loaded on the DE-52 column. The parasites were eluted with the same buffer, and each of the eluates was observed under a light microscope (×40 magnification) by wet film mount method to detect the presence of live trypanosomes. The eluates which contained the parasites were pooled together and centrifuged at 14,000×g for 30 min. The pellet was reconstituted in 10ml of the kept in phosphate buffered saline glucose until lysed.

## 3. Analytical Procedures

### 3.1 Protein concentration determination

Protein concentration was determined according to the method described by [18]. Coomassie Brilliant Blue G-250 (25mg) was dissolved in 12.5ml of 95% ethanol. To this solution, 25ml of 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 250ml. Protein solution (0.1ml) was pipetted in test tubes. 5mls of protein reagent was added to the test tube and the contents mixed by vortexing. The absorbance at 595 nm was measured after 2 minutes and before I hour against a reagent blank prepared from 0.1ml of the buffer and 5ml of protein reagent. The weight of protein was plotted against the corresponding absorbance resulting in a standard curve used to determine the protein in test samples.

### 3.2 Enzyme assay

Protein tyrosine phosphatase (PTPase) activity was assayed as described by [19]. The assay was performed with 10 mmol/L *para*-nitrophenylphosphate (*p*NPP) in buffer (10mM sodium phosphate, pH 8.0, 1 mmol/L Dithiothreitol, 1 mmol/L EDTA, and 10% glycerol) to a final reaction volume of 1ml (700µl assay buffer, 250µl *para*-nitrophenylphosphate, 50µl PTPase) at 37 °C for 10 minutes. The reaction was terminated after 10minutes, by adding 1ml of 0.2M NaOH. The *para*-nitrophenol released from *para*-nitrophenylphosphate was monitored by measuring the absorbance at 410 nm. The assay specificity for PTPase was verified by adding sodium orthovanadate (PTPase specific inhibitor) to the reaction mixture at a final concentration of 10µl, 15µl, and 25µl. Stock solution of Sodium orthovanadate was prepared by dissolving 100mg in water and stored at 4 $^0$C. Extinction coefficient of *para*-nitrophenol was 19.03 at 410 nm. One unit of activity was equivalent to 1 nmol/L *p*NPP hydrolyzed per min.

### 3.3 Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis

Electrophoresis of the purified enzyme protein under denaturing conditions was performed on 12% Acrylamide gel using a tris-glycine buffer, pH 8.3. The bands were visualized by staining with Coomassie Brilliant Blue [20]. The sample mixture was prepared by adding 20µl of the loading buffer (2.5ml of 1M Tris pH 6.8, 4ml Glycerol, 0.8g SDS, 2ml β-mercaptoethanol, Bromophenol blue, and 10ml distilled water) to 5µl of the enzyme protein, in a ratio of 1:4. The mixture was boiled for 10minutes and allowed to cool before loading. The separating gel (12%) was prepared to a final volume of 10 ml by adding; 3.35 ml deionized water, 2.5 ml of 1.5M Tris pH 8.8, 0.1% Sodium Dodecyl Sulphate (SDS), 4ml of 30% Bis-acrylamide solution, 50µl of 10% Ammonium persulphate (freshly prepared 0.1g/ml), and 15µl N, N, N', N'-tetramethylethylenediamine. The solution was swirled to mix properly, and the mixture was loaded immediately into the gel chamber of the machine before polymerization. The stacking gel (4%) was prepared to a final volume of 5ml by adding; 3ml deionized water, 1.25ml of 0.5M Tris pH 6.8, 50µl of 10% SDS, 665µl of 30% acrylamide, 25µl of 10% Ammonium persulphate (APS), and 10µl N, N, N', N'-tetramethylethylenediamine (TEMED). The tank was filled with 1500ml of running buffer working solution; 0.025M Tris (pH 8.3), 1.92M Glycine, and 0.1% SDS. Afterwards, the prepared sample (20µl) was loaded into the gel well; the electrophoresis was carried out at 150V for 50min with an ice-pack inserted in the tank to absorb heat. At the end of electrophoresis, the gel was stained overnight with Coomassie blue solution (0.1% Coomassie blue G-250 in 50% methanol, and 10% acetic acid) in a staining tray. This was followed by rinsing with destaining solution (10% methanol and 10% acetic acid) until the protein bands were visualized.

### 3.4 Determination of molecular weight of PTPase

The molecular weight of protein tyrosine phosphatase was estimated by Sodium Dodecylsulphate-Polyacrylamide Gel

Electrophoresis (SDS-PAGE) according to the method described by [21]. The standard marker-proteins used were trypsinogen PMSF (24kDa), carbonic anhydrase (29kDa) from BE for SDS-PAGE, α-lactabulmin (14.2kDa) from BE, trypsin inhibitor (20.1kDa) from glycine max, albumin (66kDa) for SDS-PAGE, albumin from chicken yolk (45kDa), and glyceraldehyde-3-phosphate dehydrogenase (36kDa).

## 4. Enzyme Purification

### 4.1 Cell lysis

After harvesting of parasites from the blood, they were lysed by modifying [22]. The parasites were suspended in 2000μl of homogenization buffer (10mM phosphate buffer, pH 8), containing 5% glycerol, 20mM NaCl, 2mM EDTA, 1μg/ml aprotinin, 1μg/ml sodium fluoride, 1μg/ml leupeptin, 1μg/ml sodium orthovanadate and 1μg/ml pepstatin A. The cells were lysed following a three cycle of freezing and thawing for disruption of the trypanosome membrane, resulting to a crude homogenate which was subjected to centrifugation ($4^{o}$C) at 14,000×g for 10 minutes. The pellet which contained protein tyrosine phosphatase bound to the plasma membrane was suspended in 2000μl of 50mM Tris-HCL pH 7.2, containing 1mM EDTA and 1% vol/vol triton X-100 for complete membrane solubilization and extraction of the protein tyrosine phosphatase. It was centrifuged at 14,000×g for 15 minutes, the supernatant was stored at -4 $^{o}$C and this served as the crude enzyme for this study.

### 4.2 Ammonium sulphate precipitation

Ammonium sulphate was added slowly to the crude enzyme with constant stirring starting from 40% to 70% saturation. The sample was allowed to stand overnight at 4$^{o}$C, thereafter, it was centrifuged at 14,000 ×g for 30 minutes; the pellet was reconstituted with phosphate buffer pH 8.0. Aliquots of the reconstituted pellet and supernatant were each suspended in the assay buffer (10mM sodium phosphate, pH 8.0, 1 mM Dithiothreitol, 1 mM EDTA, and 10% glycerol) and assayed for PTPase activity as described above. PTPase activity was evident in the pellet.

### 4.3 Gel filtration

A column of diameter 1.6 cm was packed with Sephadex G-75 to a length of 90cm and equilibrated at room temperature with 10mM phosphate buffer pH 8.0. The protein sample from ammonium sulphate precipitation was loaded on the column, and eluted with the same buffer. Twenty five fractions of 2ml each were collected at a flow rate of 14 minutes. Protein tyrosine phosphatase activity was then screened in each of the fractions. Fraction 11 and 13 with enzyme activity were pooled together. The protein concentration was estimated by the method described in section 3.1

## 5. Enzyme Characterization

### 5.1 Determination of optimum pH

The enzyme activity was determined as a function of pH at 37$^{o}$C. The assay mixture was incubated at different pH of 2, 3, 4, 5, 6, 7, using 10mM phosphate buffer. After 10 minutes of incubation, the reaction was terminated by adding 1ml of stop reagent (0.2M NaOH). The enzyme activity was determined.

### 5.2 Determination of optimum temperature

The optimal temperature of the enzyme was determined as a function of temperature at pH 5.0 in acetate buffer. The assay mixture was incubated at different temperatures of 20, 30, 40, 50, 60, 70 and 80$^{o}$C. The temperature of the water bath was monitored to ensure that desired temperature was maintained. After 10 minutes of incubation, the reaction was terminated using 1ml of 0.2M NaOH. The enzyme activity was determined as described in section 3.2.

### 5.3 Determination of pH stability

The pH stability of Protein tyrosine phosphatase was determined by pre-incubating the enzyme without substrate at different pH: 2, 3, 4, 5, 6, 7, 8, and 9 at room temperature for 10 minutes prior to substrate addition. Afterwards, the pH of each solution was adjusted to 8.0 and para-nitrophenylphosphate was added to the reaction mixture and incubated for additional 10 minutes before taking the reading at 410 nm.

### 5.4 Determination of temperature stability

The temperature stability of the purified enzyme was determined by pre-incubating 50μl of the enzyme in buffer at different temperatures: 20, 30, 40, 50, 60, 70, 80, and 90 $^{0}$C at pH of 8.0 for 10 minutes prior to addition of substrates. After 10 minutes of the pre-incubation, they were brought to room temperature and *para*-nitrophenylphosphate was added to the reaction mixture and then incubated for another 10 minutes before taking the reading at 410 nm.

### 5.5 Effects of divalent cations

The activity of protein tyrosine phosphatase was determined under the optimal conditions as obtained above, in the presence of 2mM of the following divalent ions: $CaCl_2$, $MgCl_2$, $MnCl_2$, $CuCl_2$ and $ZnCl_2$. The enzyme was pre-incubated for 10 minutes at room temperature with each of the cations prior to addition of substrate. The reaction was allowed to proceed for 10 minutes after the addition of substrate and terminated by adding 1ml of 0.2M NaOH. The enzyme activity was determined as described above. The activity without cations was taken as control.

### 5.6 Enzyme kinetics

The Michaelis-Menten constant ($K_M$) and maximum velocity ($V_{max}$) of the PTPase were determined using *para-*

nitrophenylphosphate as substrate. Different concentrations of the substrate, 1mM, 2mM, 3mM, 4mM and 5mM were prepared from a stock solution of 10mM and incubated with the enzyme for 10 minutes at the optimal temperature and pH. The absorbance was read at 410 nm. Initial velocity data obtained were used to determine the $K_M$ and $V_{max}$ [23].

## Results and Discussion

Results of the purified *Trypanosoma evansi* protein tyrosine phosphatase are summarized in Table 1. The crude protein contained 2.79mg/ml total protein with a specific activity of 3.86µmol/min/mg. Precipitation of the crude protein with ammonium sulphate resulted to increase in the purification fold to 2.78, increase of specific activity to 10.73 µmol/min/mg and 50.84% yield. The result obtained following gel filtration on Sephadex G-75 column revealed a total yield of 41.45% with a specific activity of 51.08µmol/min/mg. Gel filtration on Sephadex G-75 showed sequential reduction in the protein concentration of each purification step with corresponding increase of specific activity. Final purification fold of 13.23 and percentage yield of 41.45 were obtained. Figure 1 presents the elution profile of protein tyrosine phosphatase after gel filtration on Sephadex G-75 column. The plot resulted to a two-peak fraction of the enzyme. From the plot, the first peak has about 0.028 mg/ml of protein concentration with a corresponding enzyme activity of 1 µmol/min. The assay specificity of PTPase, as confirmed by the presence of sodium orthovanadate (specific PTPase inhibitor) in the reaction mixture inhibited about 90% of the dephosphorylation activity of protein tyrosine phosphatase in a dose-dependent fashion (result not shown). The second peak shows about 0.005 mg/ml of protein concentration and activity of 0.2 µmol/min.

**Table 1:** Purification summary of PTPase

| Purification Step | Total Protein (mg) | Total Activity (µmol/min) | Specific Activity (µmol/min/mg) | Purification Fold | Percentage Yield |
|---|---|---|---|---|---|
| Crude Protein | 2.79 | 10.76 | 3.86 | 1.00 | 100 |
| Ammonium sulphate precipitation | 0.51 | 5.47 | 10.73 | 2.78 | 50.84 |
| Gel Filtration | 0.13 | 4.64 | 51.08 | 13.23 | 41.45 |

One unit of activity was equivalent to the amount of enzyme required to hydrolyze 1 µmol *p*NPP per min
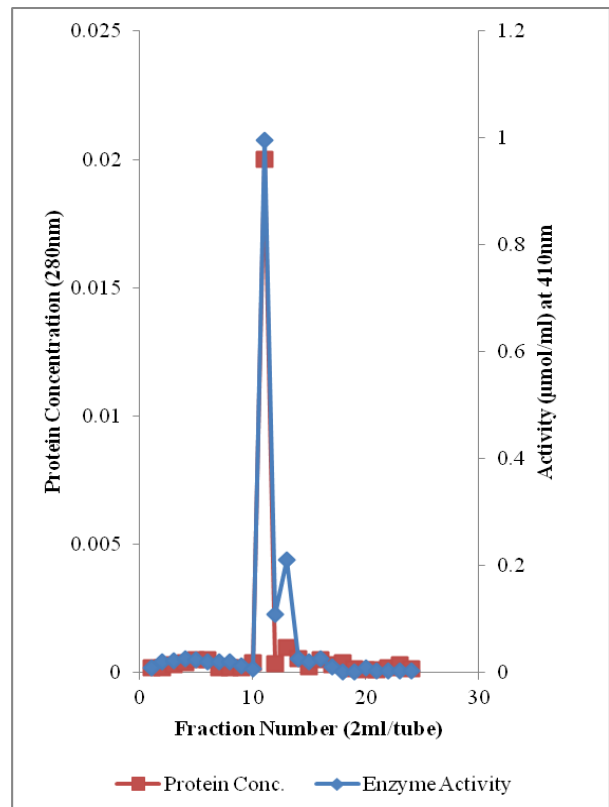


**Figure 1:** Elution Profile of *Trypanosoma evansi* Protein tyrosine phosphatase on Sephadex G-75 Column Chromatography (1.6 × 90cm)

### 6.1 Molecular weight of the PTPase

Electrophoretic result of the protein sample and the standard marker proteins is presented in figure 2. The PTPase molecular weight obtained was 31.2kDa, estimated from a plot of log of molecular weight of the standard marker proteins against their respective relative mobility ($R_f$ values).
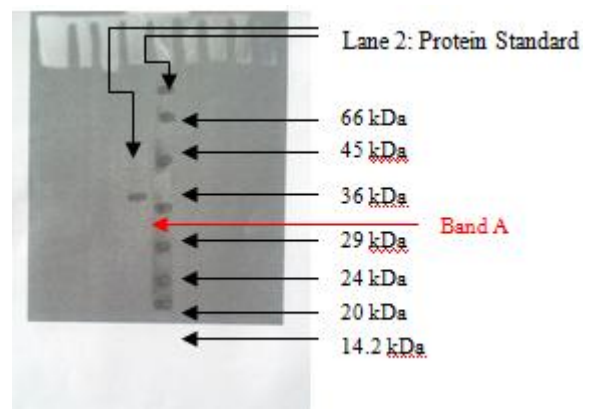


**Figure 2:** Electrophoregram of *Trypanosoma evansi* Protein Tyrosine Phosphatase (PTPase) on Polyacrylamide gel using Coomassie blue staining. Band A in lane 1, reveals the protein sample with 31.2kDa. The bands in lane 2 present the protein standards.

### 6.2 Effects of pH

The *Trypanosoma evansi* PTPase had optimum activity at pH 5.0 (figure 3). The activity decreased remarkably below pH 5.0 and above pH 5.0. However, the activity was stable after

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incubation of the enzyme for 30 minutes in a pH range 4.0 to 6.8 at 37 $^0$C (figure 4). These results indicate that the purified enzyme activity is stable through a wide range pH.
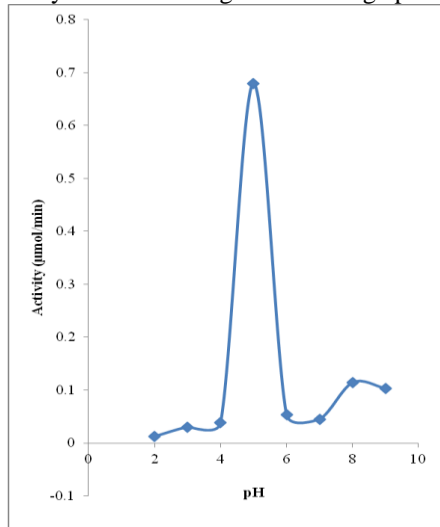


**Figure 3:** Effect of pH (with pH optimum of 5.0) on *Trypanosoma evansi* protein tyrosine phosphatase. The enzyme activity was determined as a function of pH at 37$^o$C
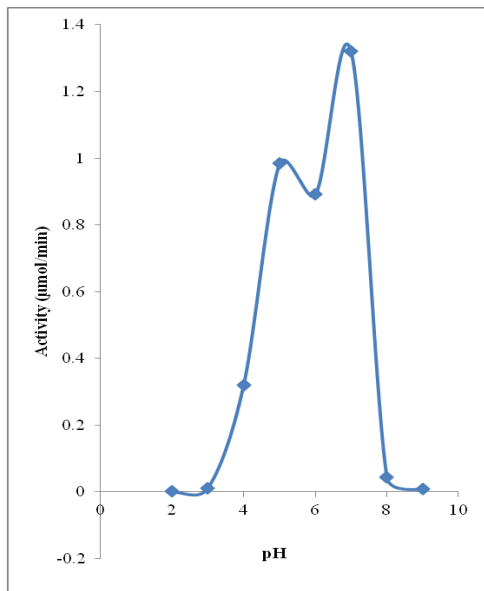


**Figure 4:** pH stability on *Trypanosoma evansi* Protein Tyrosine Phosphatase. PTPase activity was stable after incubation of the enzyme for 30 minutes in a pH range 4.0 to 6.8 at 37 $^0$C. Stability decreased markedly after incubation for 30 minutes at a pH below 3.0 at 37 $^0$C

### 6.3 Effect of temperature

The Enzyme activity was measured at different temperature and the optimum temperature for activity was observed at 70 $^0$C (figure 5). The thermostability of PTPase activity is shown in figure 6. Remarkably, the enzyme retained its full PTPase activity after heating at 70 $^0$C for 30 minutes, but lost activity after heating at 90 $^0$C for 30 minutes. Therefore, the activity of this enzyme is stable at heat treatment.
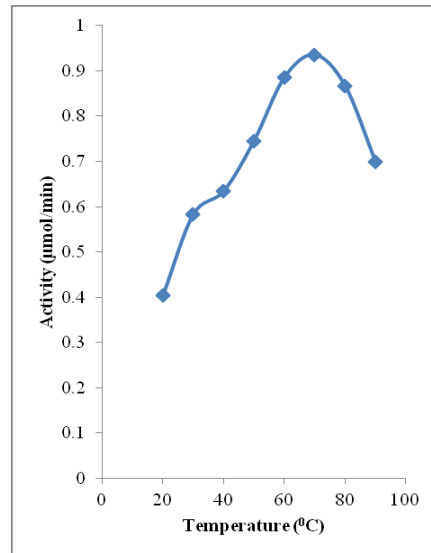


**Figure 5:** Effect of Temperature on *Trypanosoma evansi* Protein Tyrosine Phosphatase. The Optimal temperature (70 $^0$C) of the enzyme was determined as a function of temperature at pH 5.0 in acetate buffer.
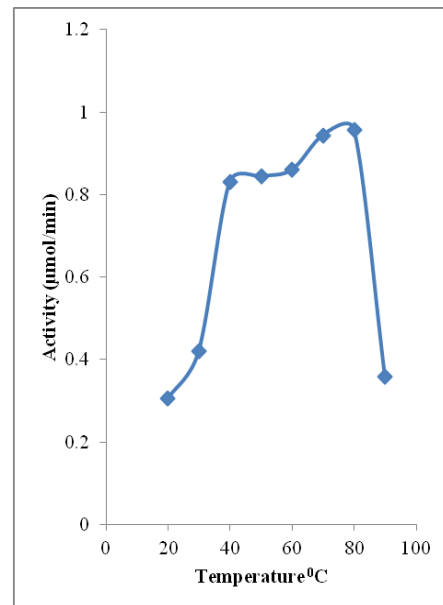


**Figure 6:** Temperature Stability of *Trypanosoma evansi* Protein Tyrosine Phosphatase. The temperature stability of the purified enzyme was determined by pre-incubating the enzyme in phosphate buffer at different temperatures

### 6.4 Effects of divalent cations

The effect of some divalent cations studied is shown in figure 7. It was revealed that PTPase activity was inhibited by $Zn^{2+}$, and $Cu^{2+}$. In addition, PTPase activity was activated by $Ca^{2+}$, $Mg^{2+}$, and $Mn^{2+}$.
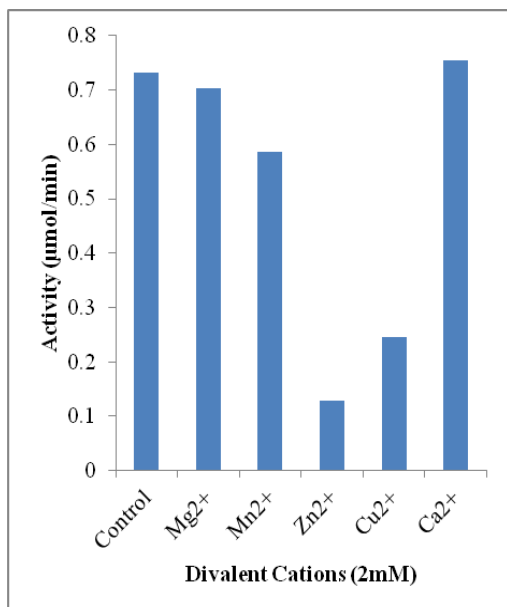
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**Figure 7:** Effects of some divalent cations on *Trypanosoma evansi* protein tyrosine phosphatase. The activity of protein tyrosine phosphatase was determined under the optimal conditions found above in the presence of 2mM of each cation.

## 6.5   Kinetic constant

The Michaelis-Menten constant ($K_M$) and maximum velocity ($V_{max}$) of the enzyme for the substrate hydrolysis presented by Lineweaver-Burk's plot (figure 8) revealed a $K_M$ of 3.44mM and $V_{max}$ of 0.19µmol/min respectively.
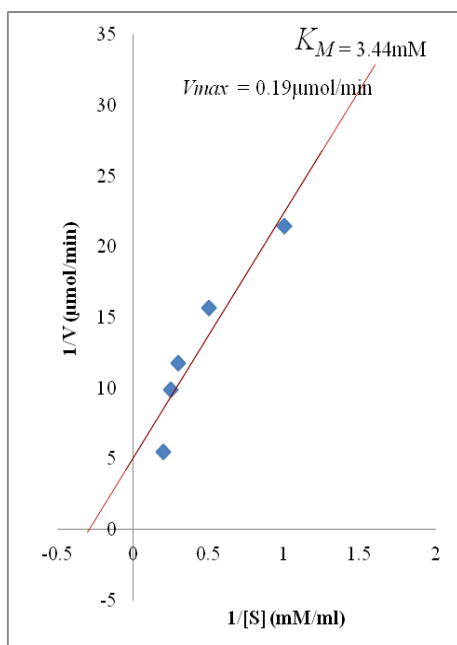


**Figure 8:** Lineweaver-Burk plot for the cleavage of *para*-nitrophenylphosphate by *Trypanosoma evansi* protein tyrosine phosphatase. The Michaelis-Menten constant ($K_M$) and maximum velocity ($V_{max}$) of the PTPase was determined under the optimal temperature and pH

Regulatory mechanism is the principle of every life processes such as cell growth and death, cell differentiation, metabolism, cell cycle among others. An understanding of

this mechanism would help in the control or target of certain regulatory pathways or biomolecules involved in such regulation, either by retarding, bursting or termination of its action. As such, protein tyrosine phosphatase (PTPase) is a major virulence factor of trypanosomiasis in the bloodstream. This work presents the purification and characterization of protein tyrosine phosphatase isolated from *trypanosoma evansi*; a member of the subgenus *Trypanozoon* known as the *brucei* group [24]. The purification profile revealed increase in specific activity of the enzyme at each purification step. Increase in the specific activity could be attributed to the removal of interacting protein components attached to the enzyme, which were interfering with the bonding of the enzyme to the substrate. It could also be as a result of the purification matrices in which the enzyme was subjected during the purification process.

Confirmation of the assay specificity was revealed by the presence of sodium orthovanadate (a PTPase inhibitor) in the reaction mixture which inhibited the dephosphorylation activity of protein tyrosine phosphatase in a dose dependent manner. The inhibition specificity exhibited by sodium orthovanadate was a strong indication of protein tyrosine phosphatase and the single band visualized on SDS-PADE reveals high degree of the enzyme purity. However, the estimated 31kDa of Protein tyrosine phosphatase from *Trypanosoma evansi* varies slightly compared to the 32kDa obtained from *Trypanosoma brucei brucei* as previously reported by [15]. This observed variation in the molecular weight could be ascribed to the sources of the enzyme which are different or due to different species of the parasite. The two peaks observed following gel filtration on Sephadex G-75 column behaved as a monomeric protein in solution when subjected to characterization suggesting that the enzyme could have subunits.

The *Trypanosoma evansi* PTPase optimal activity at pH 5.0 was found to be similar to that reported for entomopathogenic fungus *metarhizium anisopliae* and *T. brucei* PTPase [14], [15]. It was reported that protein tyrosine phosphatase has an optimum pH range of 2.8 to 4.8 using *para*-nitrophenyl phosphate substrate [25]. The result obtained is in agreement with the findings of Wang; that PTPase in general have optimal enzymatic activities at low pH values [25]. The probable reason for the observed optimal enzymatic activities at low pH values could be due to the configuration pattern of ions in the enzyme. It could also be due to the low molecular weight of the enzyme. The optimum temperature of 70 $^0$C is consistent with the report that protein tyrosine phosphatase from entomopathogenic fungus *metarhizium anisopliae* have optimum temperature of 70 $^0$C [14]. Our report suggests that protein tyrosine phosphatase is a heat-resistant enzyme and that this observed feature plays an important role in the adaptation of *Trypanosoma evansi* to extreme temperatures in-vivo. The heat-resistant characteristic could imply that the increase in body temperature of trypanosome-infected hosts makes the condition favorable for protein tyrosine phosphatase. The fairly low $K_M$ value of 3.4mM/min is an indication of moderate affinity of the enzyme for para-nitrophenylphosphate. More so, the $V_{max}$ value of 0.19µmol/min obtained implies that at the end of every

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minute of post infection, about 0.19µmoles of free phosphate groups would have been cleaved off, aiding the preservation of the stumpy forms of the parasite which is transmitted into another host. This shows that protein tyrosine phosphatase play important roles in the regulation of physiological activities of *trypanosoma evansi*.

The increase in activity of protein tyrosine phosphatase in the presence of $Ca^{2+}$, $Mg^{2+}$, and $Mn^{2+}$ shows that the enzyme is dependent on these divalent cations for activity. Studies have shown that the enzyme needs millimolar concentrations of the divalent cations to be more catalytically active [14]. The reduction in activity observed with $Zn^{2+}$ and $Cu^{2+}$, although divalent, could be as a result of probable modification of the enzyme conformation when bound; exhibiting a non similar binding pattern depicted in the previous divalent cations causing the reduction in enzyme activity. This observation implies that inclusion of these inhibiting divalent cations especially $Zn^{2+}$ could help in the design of trypanocides.

## Conclusion

Protein tyrosine phosphatase isolated from *Trypanosoma evansi* was purified to 13.23-fold and a yield of 41.45% on Sephadex G-75 column with a final specific activity of 51.08µmol/min. SDS-PAGE of the enzyme protein revealed a molecular weight of 31.2 kDa. The *T. evansi* PTP had broad temperature and pH ranges with optima of 70 $^0$C and 5.0 respectively. The inclusion of $Zn^{2+}$ and $Cu^{2+}$ could help in a trypanocide activity, especially $Zn^{2+}$. The relevance of these findings would be of utmost importance in the design of trypanocides against the dephosphorylation activity of PTPase.

## Future scope

The X-ray crystal structure of PTPase should be established, in order to find out where an inhibitor would interact with the enzyme, and explored scientifically for new potential in biotechnology applications.

## Acknowledgements

## References

[1] AU. Wurochekke, AJ. Nok. "In vitro anti trypanosomal activity of some medicinal plants used in the treatment of trypanosomosis in northern Nigeria," African Journal of Biotechnology. 3: 481-483. 2004.

[2] SC. Welburn, PG. Coleman, E. Fevre, I. Mandlin. "Sleeping sickness - a tale of two diseases," Trends in Parasitology. 17: 19 - 24. 2001.

[3] M. Karin, T. Hunter. "Transcriptional control by protein phosphorylation: signal transmission from the cell surface to the nucleus," Current Biology. 5: 747–757. 1995.

[4] S. Balazs, R. Irene, B. Richard, RM. Keith. "A novel phosphatase cascade regulates differentiation in trypanosome brucei via a glycosomal signaling pathway," Genes and Development. 24: 1306-1316. 2010.

[5] K. Fenn, KR. Matthews. "The cell biology of Trypanosoma brucei differentiation," Current Opinion in Microbiology. 10: 539–546. 2007.

[6] C. Seemay, C. Bryan, Jenson, P. Marilyn, A. Tom, G. Christoph. "The trypanosome brucei life cycle switch Tbtpt1 is structurally conserved and dephosphorylates the nucleolar protein NOPP44/46," Journal of Biological Chemistry. 285: 22075-22081. 2010.

[7] AT. Remaley, RH. Glew, DB. Kuhns, RE. Basford, AS. Waggoner, LA. Ernst, M. Pope. "*Leishmania donovani* Surface membrane acid phosphatase blocks neutrophils oxidative metabolite production," Experimental Parasitology. 60, 331–341. 1985

[8] EE. De Almeida-Amaral, R. Belmont-Firpo, MA. Vannier-Santos, JR. Meyer-Fernandes. "*Leishmania amazonensis*: Characterization of an ecto-phosphatase activity," Experimental Parasitology. 114, 334– 340. 2006.

[9] EC. Fernandes, FR. Meyer-Fernandes, MAC. Silva-Neto, AE. Vercesi. "*Trypanosoma brucei*: Ecto-phosphatase activity on the surface of intact procyclic forms," Zeitschrift fùr Naturforschung 52C, 351–358. 1997.

[10] JR. Meyer-Fernandes, MA. Da Silva-Neto, M. Dos Santo Soares, E. Fernandes, AE. Vercesi, MM. De Oliveira. "Ecto-phosphatase activities on the cell surface of the amastigote forms of *Trypanosoma cruzi*," Zeitschrift fùr Naturforschung. 54, 977–984. 1999.

[11] AL. Dos-Santos, CF. Dick, M. Alves-Bezerra, TS. Silveira, LS. Paes, KC. Gondim, JR. Meyer-Fernandes. "Interaction between *Trypanosoma rangeli* and the *Rhodnius prolixus* salivary gland depends on the phosphotyrosine ecto-phosphatase activity of the parasite," International Journal of Parasitology. 42, 819–827. 2012.

[12] M. Braibant, J. Content. "The cell surface associated phosphatase activity of *Mycobacterium bovis* BCG is not regulated by enviromental inorganic phosphate," FEMS Microbiology Letters. 195, 121– 126. 2001.

[13] JN. Blanchette, KA. Racette, R. Siminovitch, M. Faure, Olivier. "*Leishmania* -induced increases in activation of macrophage SHP-1 phosphatase are associated with impaired IFNγ-triggered JAK2 activation," European Journal of Immunology 29:3737. 1991.

[14] L. Zhenlum, W. Zhongkang, P. Guoxiong, Y. Youping, Z. Hua, C. Yueqing, X. Yuxian. "Purification and characterization of a novel thermo-stable extracellular protein tyrosine phosphatase from metarhizium anisophiae strain CQMa102. Journal of Bioscience, Biotechnology and Biochemistry," 70: 1961-1968. 2006.

[15] S. Szoor, W. Jude, H. mcElhinney, T. Lydia, R. Keith, Matthews. "Protein tyrosine phosphatase TbPTP1: a molecular switch controlling life cycle differentiation in trypanosomes," Journal of cell Biology. 175: 293-303. 2006.

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[16] WJ. Herbert, WHR. Lumsden. "A rapid matching method for estimating the host's Parasitemia," Experimental Parasitology. 40: 427-43. 1976.

[17] SM. Lanham, DG. Godfrey. "Isolation of salivarian trypanosomes from man and other mammals using DEAE-Cellulose," Experimental Parasitology. 28: 521-534. 1970.

[18] MM. Bradford. "A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding," Analytical Biochemistry. 7: 248-254. 1976.

[19] X. Zhang, S. Xing, X. Wu, F. Lin, X. Fu, W. Li. "Purification and characterization of protein tyrosine phosphatase MEG1 and preparation of anti-PTP MEG1 antibody," Chemical Research in Chinese Universities. 26 (4), 591-595. 2010

[20] UK. Laemmli. "Cleavage of structural protein during the assembly of the head of bacteriophage T4," Nature. 227(5259): 680-685. 1970.

[21] K. Weber, M. Osborn. "The reliability of molecular weight determination by Dodecyl sulfate-polyacrylamide gel electrophoresis," Journal of Biological Chemistry. 244 (16): 4406-4412. 1969.

[22] F. Genevieve, JG. David, AW. Lorie, O. Martin. "Role of host protein tyrosine phosphatase SHP-1 in leishmania donovani-induced inhibition of nitric oxide production," Infection and Immunity. 74(11):6272-6279. 2006.

[23] H. Lineweaver, D. Burk. "The determination of Enzyme dissociation constants," Journal of American Chemical Society. 56 (3): 658-666. 1934.

[24] OM. Radostits, DC. Blood, CC. Gay, Diseases caused by trypanosomes. In: veterinary medicine. Diseases of cattle, sheep, goats, pigs and horses. Baillière Tindall, London. 1994.

[25] Y. Wang, M. Fanguo, Y. Zhang. "Expression, purification and characterization of recombinant protein tyrosine phosphatase from Thermus Thermophilus HB27," Acta Biochimica et Biophysica Sinica. 689 – 698. 2009.

## Author Profile

**Dingwoke John Emeka Francis** received the B. Sc. degree (Biochemistry) from Nnamdi Azikiwe University, Awka and M. Sc. degree (Biochemistry) from Ahmadu Bello University, in 2008 and 2014 respectively. During his study period in Ahmadu Bello University, he was a graduate assistant (part-time) in Biochemistry Department. Currently, he awaits opportunity for his Doctorate degree studies.

**Humphrey C. Nzelibe** received the B. Sc. degree (Biochemistry) in 1979 from University of Lagos, M. Sc. and Ph. D degrees (Biochemistry) from Ahmadu Bello University Zaria in 1984 and 1993 respectively. He is currently a professor of biochemistry,consultant in enzymology and Industrial biotechnology at Ahmadu Bello University Zaria.

**Abdullahi B. Sallau** received the B. Sc. degree (Biochemistry) in 1996 from University of Maiduguri, M. Sc. and Ph. D degrees (Biochemistry) from Ahmadu Bello University Zaria in 2002 and 2009 respectively. He is a Senior Lecturer at Biochemistry Department Ahmadu Bello University Zaria.

**Suleiman Umar Albaba** received the B. Sc. degree (Biochemistry) in 2008 from Ahmadu Bello University Zaria. Currently, he awaits an oral external examination for the award of a masters degree (M. Sc. Biochemistry) in 2015 from Ahmadu Bello University Zaria. He had experience with the institution as a graduate assistant (part-time) in Biochemistry Department.

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