Middle-East Journal of Scientific Research 3 (2): 49-52, 2008
ISSN 1990-9233

Bloodstream Form of *Trypanosoma evansi* Contains β-Galactosidase

A.B. Sallau, M.A. Ibrahim, A. Salihu and I.A. Yusuf

Department Of Biochemistry, Ahmadu Bello University, Zaria, Nigeria

Abstract: β-Galactosidase (EC 3.2.1.23) was isolated and characterized from the bloodstream form of *Trypanosoma evansi.* The galactose hydrolase was purified 6.98 fold with a yield of 33.33% by anion exchange chromatography using DEAE- cellulose and size exclusion procedure on Sephadex G- 75 column. The *T. evansi* β-Galactosidase had broad pH and temperature ranges with an optimum of 6.0 and 37°C respectively. Initial velocity studies for the determination of kinetic parameters using 4-methylumbelliferyl-β-D-galactoside (MU-Gal) as substrate revealed a K_M and V_{max} of 0.08mM and 0.07 μmol/min respectively. The enzyme activity was enhanced by cations: Fe^{2+}, Co^{2+} and Mg^{2+} while Ca^{2+} was found to inhibit it.

Key words: β-Galactosidase · Enzyme · Galactosides · *T. evansi*

INTRODUCTION

Trypanosoma evansi, the causative agent of *surra,* is a heamoflagellate protozoan parasites that infects domestic animals causing animal trypanosomiasis. It has the broadest geographical and host range among the pathogenic animal trypanosomes. The mechanism of pathogenesis is in part due to the action of sialidase, which cleaves erythrocytes surface sialic acids and expose β-Galactosyl residues. These residues are then recognized by β-D- galactose specific lectins on macrophages leading to erythrophagocytosis and subsequently anemia [1-3].

β-Galactosidase is an enzyme that catalyses the hydrolysis of β-Galactosides from lectins, glycoproteins and gangliosides. The enzyme has been purified and characterized in *Tritrichomonas foetus* [4] where it is required for mucin breakdown during invasion process [5]. So far, among all the species of trypanosomes, β-Galactosidase has only been described in *T. congolense* [6, 7] and *T. cruzi* [8, 9] but has never been characterized from any of the African trypanosomes. In this paper, we report for the first time the purification and some biochemical characterization of β galactosidase from *T. evansi*. β-Galactosidase from *T. evansi* may play some significant role in the infection biology of the parasite which could be exploited as a target for deciphering a novel and more potent chemotherapeutic agent than the contemporary antitrypanosomal drugs which have been found resistant by the trypanosomes. Moreso, understanding the key agents involved in the pathogenesis and pathophysiology of the *T. evansi* infection will improve the current strategies in place for the development of vaccine against trypanosomiasis.

MATERIALS AND METHODS

Five experimentally *T. evansi* (IL-1392) infected rats were obtained from Department Of Parasitology and Entomology, Faculty Of Veterinary Medicine, Ahmadu Bello University, Zaria- Nigeria. All other reagents were purchased from Sigma Chemical Company, USA.

Treatment of Experimental Animals

Isolation of β-Galactosidase from Bloodstream Form of *T. evansi*: Bloodstream *T. evansi* parasites were isolated from the rats at a cell density of 10^8 trypanosomes/ml of blood. The trypanosomes were separated from the red blood cells (RBC) using the Lanham and Godfrey method [10], centrifuged at 10000xg for 10minutes and washed in phosphate buffered saline (PBS), pH7.2. Freezing and thawing with gentle agitation and treatment with 2% Triton X-100 solubilized the parasites and released the enzyme.

Purification of β Galactosidase from Bloodstream Form of *T. evansi*: The purification entailed first a micropurification procedure involving the loading of two mls of the crude enzyme onto a pre-equilibrated DEAE cellulose column (2.5cm X 30cm), pH 7.2 and eluted with a linear gradient of NaCl (0.01-0.1M). Twelve, 5ml fractions were collected and assayed for β-Galactosidase and total

Corresponding Author: M.A. Ibrahim, Department Of Biochemistry, Ahmadu Bello University, Zaria, Nigeria

Table 1: Summary of Purification protocols of β galactosidase from *T. evansi*

Purification Step	Protein (mg)	Total activity (μmol/min)x10^{-3}	Specific activity (μmol/min/mg)x10^{-3}	Purification fold	Yield (%)
Crude	0.90	84	93.33	1	100
Ion-exchange chromatography (DEAE-cellulose)	0.12	30	250	2.70	35.70
Gel filtration on Sephadex G 75	0.043	28	651.20	6.98	33.33

proteins. The β-Galactosidase active fractions were pooled and loaded on another 0.2M phosphate buffer (pH 7.4) pre- equilibrated column containing G-75 Sephadex followed by elution with the same buffer to obtain twenty five fractions and assayed for β-Galactosidase activity and total protein.

Assay of β-Galactosidase and Total Proteins: β-Galactosidase activity was assayed by monitoring the release (hydrolysis) of methylumbelliferone (MU) from 4-methylumbelliferyl- β -D-galactosides (MU-gal) in the presence of the enzyme. The assay protocol involves incubating 25μl of 1mM MU-gal and 100μl of the enzyme for 60 minutes at 37°C and the mixture adjusted to pH 10 with 0.1M NaOH. The absorbance was then read at 360nm. β-Galactosidase activity was defined as the amount of enzyme required to hydrolyse one (1.0) μmol of MU from MU-gal per minute under the described assay condition. Total proteins were determined by the Biuret method using Bovine serum albumin as standard.

Temperature and pH Dependent Studies: A pH dependent assay of the enzyme was performed using 50mM acetate buffer pH 4.0-6.0 and 50mM phosphate buffer pH 6.5-8.0. The temperature- dependent study was conducted by assaying the enzyme activity at varying temperatures between 5 and 80°C.

Initial Velocity Studies: Kinetic experiments were performed by assaying the enzyme activity with varying concentrations (0.05-1.0mM) of the MU-gal substrate. Initial velocity values obtained were then used to plot Lineweaver-Burk's plot to determine the Km and Vmax of the enzyme towards the substrate used.

Effect of Some Divalent Cations: The enzyme assay described above was conducted in the presence of the following divalent cations; Ca^{2+}, Co^{2+}, Mg^{2+} and Fe^{2+} at 10mM final concentrations using their chlorides salts.

RESULTS

Table 1 summarizes the results of the purification profile of β-Galactosidase from *T. evansi*. No enzyme activity was detected before the addition of the Triton X-100. The enzyme was purified 6.9 fold with a 33.33% yield after the gel filteration on Sephadex G-75. The pH activity profile of *T. evansi* β-Galactosidase was a typical bell shaped curve with optimal activity at pH 6.0 (Fig. 1) while the relationship between temperature and enzyme activity was broad with optimal activity at 37°C (Fig. 2).

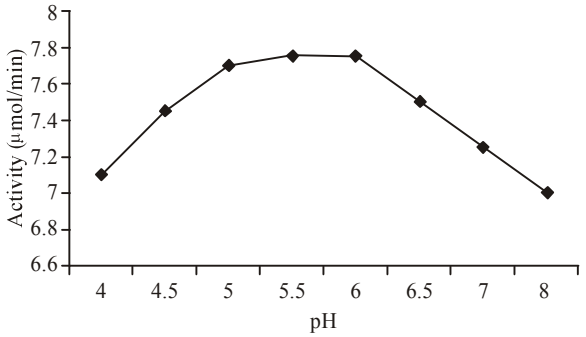

Fig. 1: Effect of pH on *T. evansi* β-galactosidase activity

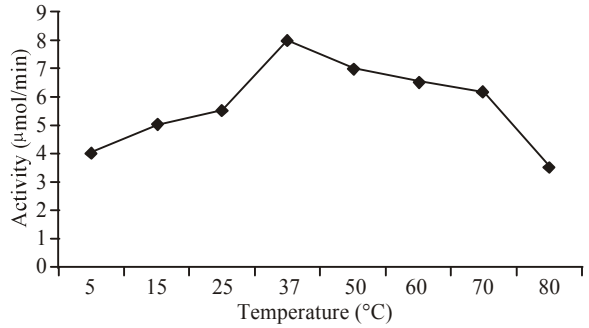

Fig. 2: Effect of temperature on *T. evansi* β-galactosidase

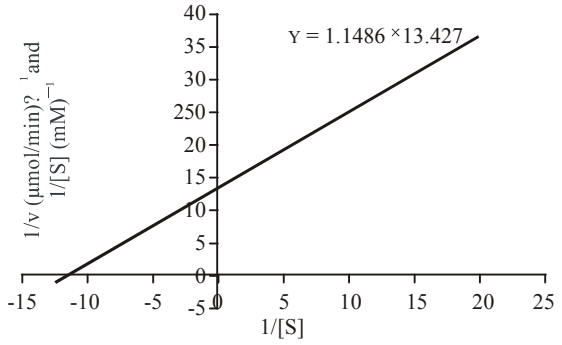

Fig. 3: Lineweaver Burk's plot for *T. evansi* β-galactosidase

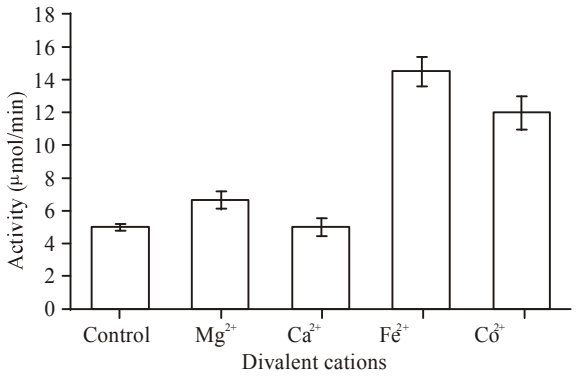

Fig. 4: Effect of divalent cations on β-galactosidase activity

The computed kinetic parameters of the enzyme using Lineweaver- Burk's plot revealed a Km of 0.08mM and Vmax of 0.07 µmol/min with MU-gal as substrate (Fig. 3). In the analysis of the effects of some divalent metal cations on the activity of the enzyme, Fe^{2+} and Co^{2+} were found to activate the enzyme by about 3 folds and a slight activation by Mg^{2+} while Ca^{2+} caused a mild decrease in the activity of the *T. evansi* enzyme (Fig. 4).

DISCUSSION

β-Galactosidases have been reported in bacteria, viruses and some protozoans [4, 11-13]. So far only *T. cruzi* [8, 9] and *T. congolense* [6, 7] are known to have β-Galactosidase but is yet to be characterized. This report is the first to attempt the purification and characterization of β-Galactosidase from trypanosomes.

The addition of Triton X-100 had effect on *T. evansi* β-Galactosidase activity, indicating that a membrane solubilization is required for the catalytic activity of the enzyme; thus *T. evansi* β-Galactosidase could be a glycosylphosphatidylinositol-linked (GPI) membrane bound enzyme. Sialidase (neuraminidase) from *T. evansi* have been reported by Nok *et al*. [3] to be a GPI linked protein. It will not be surprising that both enzymes because of this common ground exhibit some sort of synergistic role in the pathogenesis of *T. evansi* infection as the sialidase hydrolyse sialic acids from erythrocyte surfaces during the course of the parasites infection exposing β-Galactosyl epitopes on the asialo-erythrocytes surfaces. The increase in specific activity of the crude β-Galactosidase after the two purification steps could be attributed to the removal of other synergistically interacting proteins.

The pH dependent activity profile revealed optimum activity at 6.0. An acidic pH optimum region contributes to the enhancement of the enzymatic reaction of the *T. evansi* β-Galactosidase by general acid catalytic mechanism. The control of intracellular concentration of H^+ is a very essential pathological character of *T. evansi*. Indeed, the parasite has a very sensitive pH intracellular pool which is released by ionophore nigericin [14]. *Tritrichomonas foetus* β-Galactosidase has pH optimum at 5.8 [4] whereas both *Propionibacterium acidipropioni* and *Bifidobacterium longum* β-Galactosidases have been shown to have pH optimum at 7.0 [12, 15]. Thus, the contrasting pH profile could be organism related. The *T. evansi* β-Galactosidase was optimally active at 37°C which is a favorable temperature for parasitic life in the mammalian host. Optimal activities for *P. acidipropioni, B. longum* and *Tilapia nilotica* intestine β-Galactosidases [12, 15, 16] have been reported to be within the range 37-50°C. It therefore appears that optimal activity at 37-50°C is a common property of β-Galactosidases.

The fairly low Km value of the enzyme is an indication of moderately high affinity of the enzyme for the substrate. The methylumbelliferone component could contribute to the enhanced activity of the enzyme by general acid catalysis and proximity of the participating species. Moreover, the Vmax of 0.07µmol/min may imply that at the end of 24 hours post *T. evansi* infection, at least 100 µmoles of β-Galactosides would have been exiced from the host RBC and/or other glycoconjugates. Cleavage of β galactosides which links sialic acids and RBC membrane could lead to the liberation of both sialic acid and the β-Galactosides which consequently accelerate the death of such erythrocytes leading to anemia observed in the course of the disease [3]. Fe^{2+} and Co^{2+} were found to activate the enzyme by about 3 folds whereas Mg^{2+} slightly activated the enzyme. The presence of these ions could modulate the activity of the enzyme during the course of *T. evansi* infection.

We have therefore concluded that the bloodstream form of *T. evansi* expresses β galactosidase and was characterized herein. We are presently working on the role (if any) of *T. evansi* β-Galactosidase in causing anemia.

REFERENCES

1. Muller R., N.W. Franco and R. Schauer, 1981. Involvement of membrane galactose in the in vitro sequestration of desialylated erythrocytes, Hoppe-seylers Zeitschrift fur Physiologische Chemie, 364: 1615-1620.

2. Umar, I.A., D.A. Ameh and K.A.N. Esievo, 1998. Normal plasma lactose concentrations and kinetics of intravenously infused lactose in cattle. Research in Veterinary Science, 65: 1-4.
3. Nok, A.J., H.C. Nzelibe and S.K. Yako, 2003. *Trypanosoma evansi* sialidase: surface localization, properties and Hydrolysis of Ghost Red Blood Cells and brains cells-implication in Trypanosomiasis. Zietschrift fur Naturforschung, 58: 594-601.
4. Vella, M. and P. Greenwell, 1997. Purification and partial characterization of β-Galactosidase from *Tritrichomonas foetus*. Glycoconjugate Journal, 14(7): 883-887.
5. Connaris, S. and P. Greenwell, 1997. Glycosidases in mucin-dwelling protozoans. Glycoconjugate Journal, 14(7): 879-882.
6. Nok, A.J. and E.O. Balogun 2003. A Bloodstream *Trypanosoma congolense* Sialidase could be involved in anemia during experimental trypanosomiasis. J. Biochem., 133: 725-730
7. Buza, J. and J. Nassena, 1999. Trypanosome non specific IgM antibodies detected in serum of *Trypanosoma congolense*-infected cattle are polyreactive. Veterinary Immunology and Immunopathology, 69(1): 1-9.
8. Buckner, F.S., J.A. Wilson and W.C. Vanvoohirs, 1999. Detection of live *T. cruzi* in tissues of infected mice by using histochemical stain for β-Galactosidase. Infection Immunity, 67(1): 403-409.
9. Lesenne, A., S. Mueles-serrano, P.C. Fernandez, J.A. Escario and A.G. Barrio, 2002. Biological characterization of a β-Galactosidase expressing clone of *Trypanosoma cruzi* strain. Memorias do Instituto Oswaldo Cruz., 97(8): 1101-1105.
10. Lanham, S.M. and D.G. Godfrey, 1970. Isolation of salivarian trypanosomes from man and mammals using DEAE cellulose. Experimental Parasitology, 28: 521-534.
11. He, T., M.G. Preibe, R.G. Vonk and G.W. Welling, 2005. Identification of bacteria with β-Galactosidase activity in faeces from lactase non persistent subjects. Federation of European Microbiology Society Microbial Ecology, 54(3): 463-469.
12. Zerate, G., A.P. Chaia and G. Oliver, 2002. Some characteristics of practical relevance of the β-Galactosidase from potential strains of probiotic strains of *Propionibacterium acidipropioninci.* Anaerobe, 8(5): 259-267.
13. Vera, A., A. Aris, X. Daura, M.A. Marin and A. Villverde, 2005. Engineering the E. coli β-Galactosidase for the screening of antiviral protease inhibitors. Biochemistry and Biophysics Research Communication, 329: 453-456.
14. Mendoza, M., A. Mijares, H. Rojas, M. Ramos and R. DiPolo, 2001. *Trypanosoma evansi:* a convenient model for studying homeostasis using fluorometric ratio imaging from single parasites. Experimental Parasitolgy, 99: 213-219.
15. Hsu, C.A., R. Chui Yu and C. Chunchou, 2006. Purification and characterization of a sodium stimulated β-Galactosidase from *Bifidobacterium longum* CCRC 15708. World J. Microbiol. Biotechnol., 22(4): 355-361.
16. Taniguchi, Y.A. and K. Takano, 2004. Purification and properties of β-Galactosidase from Tilapia intestines: Digestive enzyme of Tilapia-X. Fisheries Science, 70(4): 688-694.