African Journal of Biotechnology Vol. 10(13), pp. 2388-2398, 28 March, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.835
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

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

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Accepted 25 February, 2011

Trans-sialidase (TS) is a unique enzyme with glycosyltransferase and sialidase (SA) activities. Although many authors reported that TS is not found in the blood stream form (BSF) of African trypanosomes, SA activity has been observed in the sera of infected animals and the BSF parasites, contributing to initial and continuous anaemia. In this study, a polymerase chain reaction (PCR) based approach was used to detect a trans-sialidase gene from the bloodstream form of *Trypanosoma evansi* (*Te*) obtained from the blood of infected camels. Sequence analysis of the cloned *TeTS* gene indicated 99% identity to some African trypanosomes trans-sialidase genes. Unique amino acids motifs found to occur in all African trypanosomes *TS* genes were identified in the *TeTS* gene. Catalytic site residues common to *SA* and *TS* genes were identified on the catalytic region of the gene. These results indicate that *TeTS* is homologous to *TS* gene sequences and thus strongly suggests the occurrence of TS in the BSF of *T. evansi*.

Key words: Active-site, gene, motifs, trans-sialidase, *Trypanosoma evansi*.

INTRODUCTION

Trans-sialidase (TS) is an enzyme that catalyses the transfer of α-2→3 linked sialic acid to another carbohydrate forming a new α-2→3 glycosidic linkage to galactose or N-acetylgalactosamine. It acts like sialidase (SA) in the absence of an appropriate acceptor, thus both activities can be exhibited by the same enzyme (Pontes de Carvalho et al., 1993; Tiralongo et al., 2003). Sialidase has been defined as an enzyme that catalyses the hydrolysis or trans-glycosylation (trans-sialidases) of sialic acid residues from various glycoconjugates (Watts et al., 2006). Sialylated glycoconjugates such as glycoproteins, glycopeptides, glycolipids and cell-surface oligosaccharides have been shown to be involved in a wide range of biological processes for example cell-cell communication, signal transduction and host-pathogen interactions (Schauer, 2000; Fujimura et al., 2001). Trans-sialidase is involved in the sialylation of the procyclic acidic repetitive protein (PARP) of *Trypanosoma brucei* and the glutamic acid-alanine rich protein (GARP) of *Trypanosoma conglonse* which protect them from digestive conditions in the fly gut (Engstler et al., 1995). In *Trypanosoma cruzi* infections, it is known to sialylate host cell glycoconjugates to generate receptors, which are used for parasite adherence and entry into host cells

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Abbreviations: TeTS, *Trypanosoma evansi* trans-sialidase; **aa,** amino acid; **PARP,** procyclic acidic repetitive protein; **GARP,** glutamic acid-alanine rich protein; **BSF,** bloodstream form; **PCF,** procyclic form; **SA,** sialidase; **TS,** trans-sialidase; **VSG,** variable surface glycoproteins; **SAPA,** shade acute phase antigen; **CGA,** citrate glucose anticoagulant; **ITS,** internal transcribed spacer1; **CAP,** catabolite activator protein; **NCBI,** National Center for Biotechnology Information; **BLAST,** Basic Local Alignment Search Tool; **PCR,** polymerase chain reaction.

(Schenkman et al., 1994). TS have been shown to have biotechnological applications for instance, in the development of synthetic carbohydrates based anticancer vaccines (Holmberg et al., 2000).

The involvement of TS in trypanosomal pathogenecity has been established (Tiralongo et al., 2003; Schenkman et al., 1994). However; their existence in the bloodstream form (BSF) of African trypanosomes is still controversial. It is believed that the procyclic culture form (PCF) African trypanosomes need it to sialylate cell surface glycoconjugates to protect them from digestive conditions in the mid-guts of their vectors. Bloodstream form African trypanosomes on the other hand do not need it as they have variable surface glycoprotein (VSG) to protect them against the immune system of the host and hence do not require TS activity. Earlier studies by some authors (Engstler et al., 1995; Schenkman et al., 1994) showed that sialidase and trans-sialidase are not found in the BSF of some African trypanosomes. SA activity however, has been observed in the sera of infected animals (Nok and Balogun, 2003) and the BSF parasites (Nok et al., 2003; Brutai et al., 2006) and finally contribute to initial and continuous anaemia. These results suggest the importance of SA/or TS molecules in BSF African trypanosomes. A study has shown that *T. brucei* trypanosome possess SA and TS enzymes activities but exhibited by separate proteins (Montagna et al., 2006). These are new findings which are likely to shade light on the involvement of SA/or TS in the pathogenecity of African trypanosomes. Tiralongo et al. (2003) also showed that *T. congolense* possesses both enzyme activities and it is likely that the same protein exhibited both activities as the situation or need of the moment arises. These results suggest that Africa trypanosomes could modulate differential expression of each of these enzymes as the need arises. Although the expression of VSGs is the single most important mechanism of protection against the immune system of the host for the BSF trypanosomes, SA/TS is believed to have a significant role in their survival and pathogenicity evidenced by initial and continuous anaemia in African trypanosomiasis. The non detection of TS activity in the bloodstream form of African trypanosomes may be as a result of low expression of the protein, due to developmental regulation as reported by some authors in *T. cruzi* (Engstler et al., 1995; Schenhman et al., 1994; Montagna et al., 2002). This may also be because the protein is expressed only when there is need for it at a particular moment within the infection period. This suggests that glycosyltransferase activity may be very low at certain stages as SA activity dominates. There is need for more information on TS/ SA in African trypanosomes than is currently available to be able to understand and fully explain the involvement or non-involvement of TS in the pathogenesis of African trypanosomes.

Due to the controversy in the involvement of TS/or SA in the pathogenecity of African trypanosomes and due to its important biotechnological capability, studying trans-sialidase has become very attractive. So far, only few trans-sialidases have been studied in detail: the American *T. cruzi* trans-sialidase (Scudder et al., 1993; Schenkman and Eichinger, 1993; Pereira et al., 1991) and the African *T. brucei* trans-sialidase (Engstler et al., 1992; Engstler et al., 1993; Uemura et al., 1992; Montagna et al., 2002; Tiralongo et al., 2003). The availability of further trans-sialidase sequences will ensure a better understanding of its involvement in pathogenecity of African trypanosomes. This can also provide the opportunity to develop highly specific structure based trans-sialidase inhibitors as a therapy or vaccine for trypanosomiasis. In this study, we sought to detect and characterise trans-sialidase gene in the bloodstream form of *Trypanosoma evansi*. This study will provide sequence information from an additional African trypanosome species and may be of significance to elucidate the mechanism of TS activity as well as confirm or reveal findings about essential residues required for transfer activity. The study also revealed the presence of *TS* gene in the bloodstream form of African trypanosome (*T. evansi*) which hitherto was not investigated.

MATERIALS AND METHODS

Reagents and equipment

All chemicals were analytical grade and purchased from Sigma. 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 ABgene® Epsom Surry, U.K., respectively. High Pure polymerase chain reaction (PCR) Clean-Up Kit used to purify PCR products and 100 bp DNA molecular size marker were purchased from Roche, Mannheim Germany and oligonucleotide primers were synthesized by Inqaba biotec®, Pretoria South Africa. GeneAmp PCR System9700 used for amplification was obtained from Applied Biosystems, Indonesia and the Gel Documentation System (Synegene®) obtained from Synegene® Inc. Indonesia. Sequencing analyses were performed by Inqaba biotec®, Pretoria South Africa.

Trypanosomes

T. evansi infected bloods from infected camels were collected from Kano Central Abattoir, Kano State, Nigeria and Sokoto Central Abattoir, Sokoto State, Nigeria during a *Trypanosoma* surveillance study. The *T. evansi* infected bloods were supplied by the Parasitology Department, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria.

PCR detection and amplification of trans-sialidase like gene

Total DNA was extracted from 200µl of isolated *T. evansi* suspended in PBS pH8.0 using Spin Column Blood Genomic DNA Minipreps Kit (Bio Basic Inc. Markham Ontario, Canada) according to the manufacturer's instructions. Briefly, cell lysis was done with 500 µl lysis buffer and proteinase K (3 µl of 10mg/ml) incubated at 55°C for 30 min. DNA was precipitated with 260 µl absolute ethanol. Precipitated DNA was captured in EZ-10 column by spin-

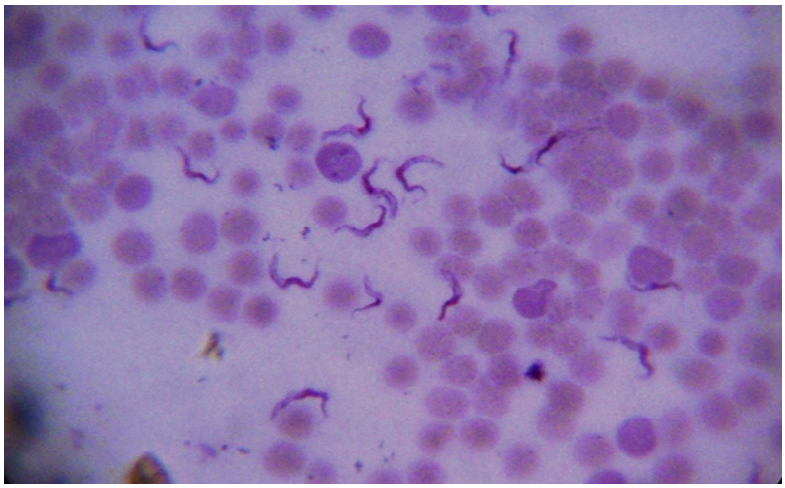

Figure 1. Blood stream form *T. evansi* stained with Giemsa and viewed under light microscope (x100). Parasites were long, slender forms.

ning at 8,000 rpm for 1 min.The flow through was discarded and centrifuged again at 8,000 rpm for 1 min to remove residual wash buffer. DNA was eluted into 1.5 ml microcentifuge tubes with 50 µl elution buffer after incubation at 50°C for 2 min by spinning at 10,000 rpm for 1min.

We used primers designed by Montagna et al. (2002) to detect trans-sialidase gene in the bloodstream form of *T.evansi*. Total DNA was extracted as described above. High Fidelity Polymerase Enzyme Mix (ABgene®, Epsom, and Surrey, U.K.) was used according to the manufacturers' instructions. The optimum reaction mix in 50 µl volume was as follows: mix_1; 15 µl nuclease free water; 5.0 µl of 10 x PCR buffer with $MgCl_2$; 1.0 µl of enzyme mix. Mix_2; 15 µl nuclease free water; 4.0 µl dNTPs; 1.0 µl of each primer (20 pmoles/µl) and 8.0 µl (0.0221 µg) of DNA. The thermal cycling profile was as follows: initial denaturation, 94°C for 2 min; 10 cycles of 94°C for 10 s; 55 °C for 30 s; 68°C for 2 min followed by 20 cycles of 94°C for 10 s; 55°C for 30 s; 68°C for 2 min and final extension at 68°C for 7 min. 10 µl of PCR amplicons was separated on 1.0% agarose gel containing 5 µl of ethidium bromide (10 mg/ml) and the results documented using Gel Documentation System (Synegne®).

Cloning and sequencing

The PCR-amplified *TeTS* gene from *T. evansi* was purified using High Pure PCR Product Clean-Up Kit (Roche, Mannheim Germany) and ligated into *pCAPs* vector in a 20 µl ligation reaction. The ligation reaction mixture was incubated at 25°C for 30 min and subsequently used for transformation. The recombinant plasmid was designated *pCAPs-TeTS* and transformed into *Escherichia coli* DH5α competent cells. Transformants were placed on LB agar containing 100 µg/ml ampicillin. PCR Cloning Kit (Roche, Mannheim Germany) was used to clone the purified TS from *T. evansi*. The vector *pCAPs* is a specially designed suicide vector containing the lethal mutant gene of the catabolite activator protein (CAP). Ligation of the blunt ended DNA fragment generated from PCR into *MluN1* restriction site disrupts the expression of the *CAP* gene. Only positive recombinants grow after transformation as cells that harbour the non-recombinant lethal gene are killed during transformation event. Colonies were randomly selected for screening of positive clones by PCR and restriction endonucleases digestion of plasmids using *Nhe* and *BglII*.

Taq-polymerase catalysed cycle sequencing using fluorescent-labelled dye terminator reaction protocol and analysis was on a 3130XL Genetic Analyzer (ABI). BigDye® V3.1 (ABI) Kit was used according to the manufacturer's instructions. The sequence was submitted to the GenBank and assigned the accession number FJ597949.

RESULTS

Trypanosome species

T. evansi infected blood of camels were collected in two abattoirs (Sokoto and Kano abattoirs) in the North-Western region of Nigeria where camels are slaughtered for meat. The infected blood samples were collected during a surveillance study by the Parasitology Department of the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. Buffycoats were prepared from the infected blood for microscopical identification and PCR amplification of the TeTS gene. The buffycoat microscopical examination identified the parasites as *T. evansi* (Figure 1); the parasites were slender (long) forms (monomorphic). The bloodstream form of *T. brucei* is pleomorphic (short stumpy forms and slender forms).

PCR detection and amplification of trans-sialidase like gene

We sought to detect trans-sialidase gene in the blood-stream form of *T.evansi* using trans-sialidase gene primers designed by Montagna et al. (2002). They assembled an open reading frame of 2316 bp after performing a Basic Local Alignment Search Tool (BLAST) search using sequences corresponding to the catalytic domain of *T. cruzi* (L26499) a member of family 1 of *T.*

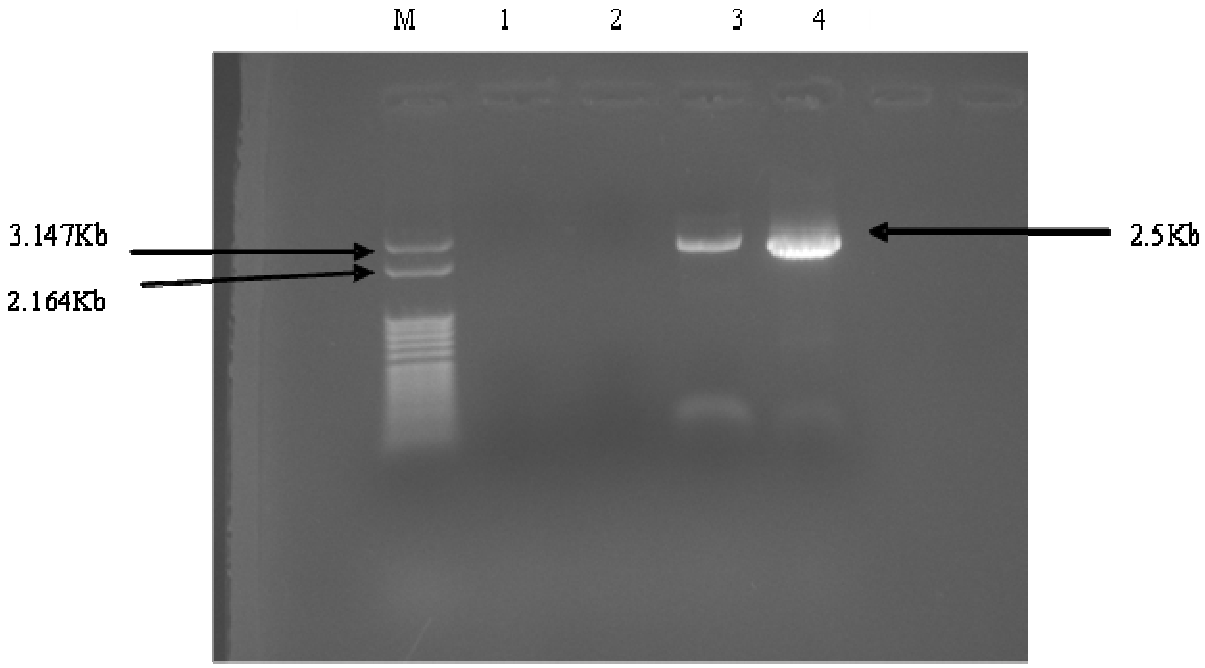

Figure 2. Trans sialidase gene PCR. M –50 bp DNA marker (Sigma); Lanes 1 and 2 – *T.evansi* (Sokoto and Kano isolates) amplified with *Taq* polymerase; Lanes 3 and 4 – *T.evansi* (Sokoto and Kano isolates) amplified with High Fidelity Polymerase Enzyme Mix (Roche).

cruzi trans-sialidase/sialidase super family. The PCR amplification using genomic DNA extracted from isolated *T.evansi* from camels infected blood of camels infected blood resulted in the amplification of 2.5 kb trans-sialidase like gene (Figure 2). Montagna et al. (2002) amplified a 2.109 kb fragment from the procyclic form of *T. brucei* which is about 0.39 kb shorter than the bloodstream form of *T. evansi* trans-sialidase (*TeTS*) gene. The difference in size may not be readily explained as the same primers were designed and used in both amplifications. However, some sialidases and trans-sialidases have been shown to be multidomain proteins and hence have longer gene lengths. The catalytic sites of *TeTS* and *T. brucei* trans-sialidase reported by Montagna et al. (2002) are likely similar but not identical. Tiralongo et al. (2003) amplified a 1.491 kb *T. congolense* partial TS1 gene for putative trans-sialidase, while Uemura et al. (1992) amplified 2.895 kb TS from *T. cruzi.* The *T.congolense* sequence obtained by Tiralongo et al. (2003) was a partial sequence and hence was not the full length of the gene. *T. cruzi TS* gene is expectedly longer as the C-terminal is known to be a tendemly repeated sequences named shade acute phase antigen (SAPA) which is not found in African trypanosomes TS genes so far studied.

The gene was amplified from the two isolates of *T. evansi* (Sokoto and Kano isolates; so called because they were obtained from Sokoto and Kano abattoirs) and the results were reproducible when the amplification was repeated five times with different genomic DNA extracts. The same size (2.5 kb) trans-sialidase-like gene was also amplified from the BSF form *T. brucei* isolate was obtained from an infected cow (results not shown). Amplification was not achieved when *Taq* polymerase was used for the amplification of the gene (Figure 2), showing that the gene is indeed well above 2 kb requiring the use of high fidelity polymerase enzyme mix. High fidelity polymerase enzyme mix (Roche, Mannheim Germany) is an enzyme mix which contains the 5'-3' polymerase activity and the 3'-5' proofreading activity. It is worth noting that the amplification reagents mixture and cycling programme required only slight optimization adjustment. This is indicative of the abundance and availability of the gene in the BSF African trypanosomes studied and suggests its production most likely as an adaptation or housekeeping gene.

The amplification of 2.5 kb trans-sialidase-like gene from the BSF of *T. evansi* indicates the presence of this gene in the BSF African trypanosomes. Montagna et al. (2002) postulated that the presence of trans-sialidase activity is unique to a few trypanosomes and are developmentally regulated. The amplification of a trans-sialidase-like gene in the bloodstream form of *T. evansi* supports the developmental regulation of *TS* gene as trans-sialidase activity has not been detected in the bloodstream forms of African trypanosomes (Engstler et al., 1995; Schenkman et al., 1994; Montagna et al., 2002). However, accumulated results have implicated sialidase to contribute to initial and continuous anaemia in African trypanosome infections, suggestive of sialidase/trans-sialidase activity

5’-

TGCGCTCTGACTTCCAAGGCTGCGGGCAAGGGGACGACGCGTGAGGCATTTCTGTCCGGCGGTGCGTGGGCTTTGA
GAAAGAAGCTGAGCGAGAAGGATGGTGAAGTGTGGTGGTGGCAGGATGGACCCGATTGGAAGGATAAGTATGAT
AAGGAATGGGAGAGATGGTTCAAAGAAGAGAAAGGTCCCTGGGGAGGGTCTGAGAAGCGTAGCGAATGGTTCGC
TCGAATGACAGGTGGGTACATAACGCTCGGCAAAACGAAGATACTTTCATCTGCTATTGAGGGTAGTGATAAGGT
AGAGCGCACTGTGCATTCCTTTCGTATTCCTTCGTTTGTTGAGGTTGATGGGGTGCTGATGGGTATTGGTGATGCCC
GGTATCTTACCTCCACGGATTACTTCTTCACCGACACCGTTGCTAAATACAGTGCGGACGGGGGCAAAACATGGAA
AACAGAGGTCATCATTGAAAATGGTCGCGTGGATCCCACATACTCTCGTGTTGTGGATCCTACTGTCGTTGCTAAG
GCGGACAGTGTCTTTGTGCTTGTGGCAAGATACAATGTCACGAAGGGGTATTGGCACAACGAAAACAACGCAGCG
GGTATAGCGGATTGGGAACCTTTCATGTACAAGGGTGTAGTGACTAAGGGTGCCGACGGTAAAACCAGTGATGTG
CGGATCTCTTGGACAAAGACACCACTGAAACCCCTCTACGACTTCACTGTTGCAGGAAGCAAGGGCACGCAGTTC
ATTGGAGGAGCTGGTAACGGCGTTGTAACATTGAACGGTACAATATTGTTTCCGGTGCAGGCGAGGAATGAAGAC
AATGCCGTTGTAAGCATGGTTATGTACTCTGTTGACGATGGTGTGAGTTGGCATTTTGCCCGTGGTGAAACGGCGC
TTCTCACATCGGAAGCTTCTCTTACTGAGTGGAATGGGAAACTGCTGATGAGCGCGCGGACAGACACTTCTGGCGT
TAACGTAGAAGGTGGGTTCCGCAAGGTGTTCGAATCTAGCAACCTTGGGGCAACGTGGGAGGAATCACTCGGAAC
GATTTCCCGCGTAATTGGGAACTCACCGGACCGTACGAAACCGTCTCCAACGGCCAACTATCCCGGTAGTTCGGGG
GCTCTTATTACTGTGACGCTTGGGGATGTGCCTGTGATGTTGATTACCCACCCGAAAAACACAAAGGGGGCATGGA
GCCGGGACCGTCTACAGCTGTGGATGACAGATGGTAACCGTATGTGGCTTGTTGGCCAGATATCGGAGGGCGACG
ATAACAGCGCTTACAGTTCTTTGCTGTTGGCCCGTGATGGACTGCTTTATTGTCTGCACGAGCAGAACATTGACGA
AGTGTATAGTCTTCATCTTGTGCACCTTGTGGATGAGTTGGAGAAGGTTAACGCGACGGTGCGGAAATGGAAGGCC
CAGGACGCCTTACTTGCTGGCCTTTGCTCTTCATCACGGAAAAAGAACGACCCCACCTGCTCCGGTGTCCCTACCG
ATGGCCTTGTTGGTTTACTCGCCGGCCCTGTTGGTGCGAGTGTGTGGGCTGATGTGTACGACTGCGTGAATGCCAG
TATCTCTGATGGTGTGAAGGTTTCGGAAGGTGTGCAGCTGGGAGGCAAAAGAAACAGCCGTGTGCTGTGGCCTGT
GAGCGAGCAGGGACAGGACCAGAGGTATTACTTCGCCAACACGCACTTTACGCTGCTTGCCACCGTTCGGTTCGCG
GGGGAACCGAAGGCGGAGGCACCGCTGATGGGATTTTCAAACGCAGAGGGGAAAACGAGCGAAACTTTGAGTCT
CACAGTTGGCGGCAAGAAGTGGGTCCTAACGTACGGCTCCGTCCGTAAAGAGGGCCCAACCACGTCGATGGATTG
GAATCAAACCCATCAGATTGCGCTCACACTGCGTGATGGTAAAGTGGATGCTCATGTCAATGGAGAGCTCATAATA
AAAGAAGTGAGTGTAGGCGCTTCTGAATCTTCTGCACATCTACATCTCTCACACTTTTTTATTGGAGCGCCGGTAAA
TGACAGTGGGGAGGGAGGCAATAATGTGATCGTCAGAAATGTTCTTCTGTACAATCGAAAGCTCGATGAGGACGA
ACTGCAAGTGCTGTATAGCAACAGGGAAAAGATACAACCGGTCGTCAGTGCAGTTGGTATCCCCGAAGGTATGAG
CGCACCTCGGTTATGTTGTCTGCTGATCTTAATGTATGTGTTGGCGATTTGAAGATCT-3’

Figure 3. Primary nucleotide sequence of TeTS gene; a 2248 bp FinchTV (GeoPiza) edited sequence from 4 cycles sequencing reactions with 4 primers: primer1; Int: 1; Int: 2; Int: 3 was obtained.

in the BSF of these parasites (Nok and Balogun 2003; Brutai et al., 2006; Banks, 1980; Esievo, 1982). In contrast, TS activity in the PCF as a protective strategy from digestion in the midgut of tsetsefly has been established (Engstler et al., 1995; Schenkman and Eichinger 1993).

T. evansi trans-sialidase (TeTS) gene sequence

The service of Inqaba Biotech®, Pretoria, South Africa was employed in the sequencing of the primary sequence of the trans-sialidase-like gene from the bloodstream form of *T. evansi*. *Taq*-polymerase catalysed cycle sequencing using fluorescent-labelled dye terminator reaction protocol was employed and analysis was on 3130XL Genetic Analyzer (ABI). A 2,248bp nucleotides primary sequence was obtained after editing using Finch TV® programme (GeoPiza) as shown in Figure 3. Sequence analysis in the non redundant (NR) sequence data base at NCBI by using BLASTN and TBLASTX (Altschul et al., 1990; Altschul, et al., 1997) and Parasite

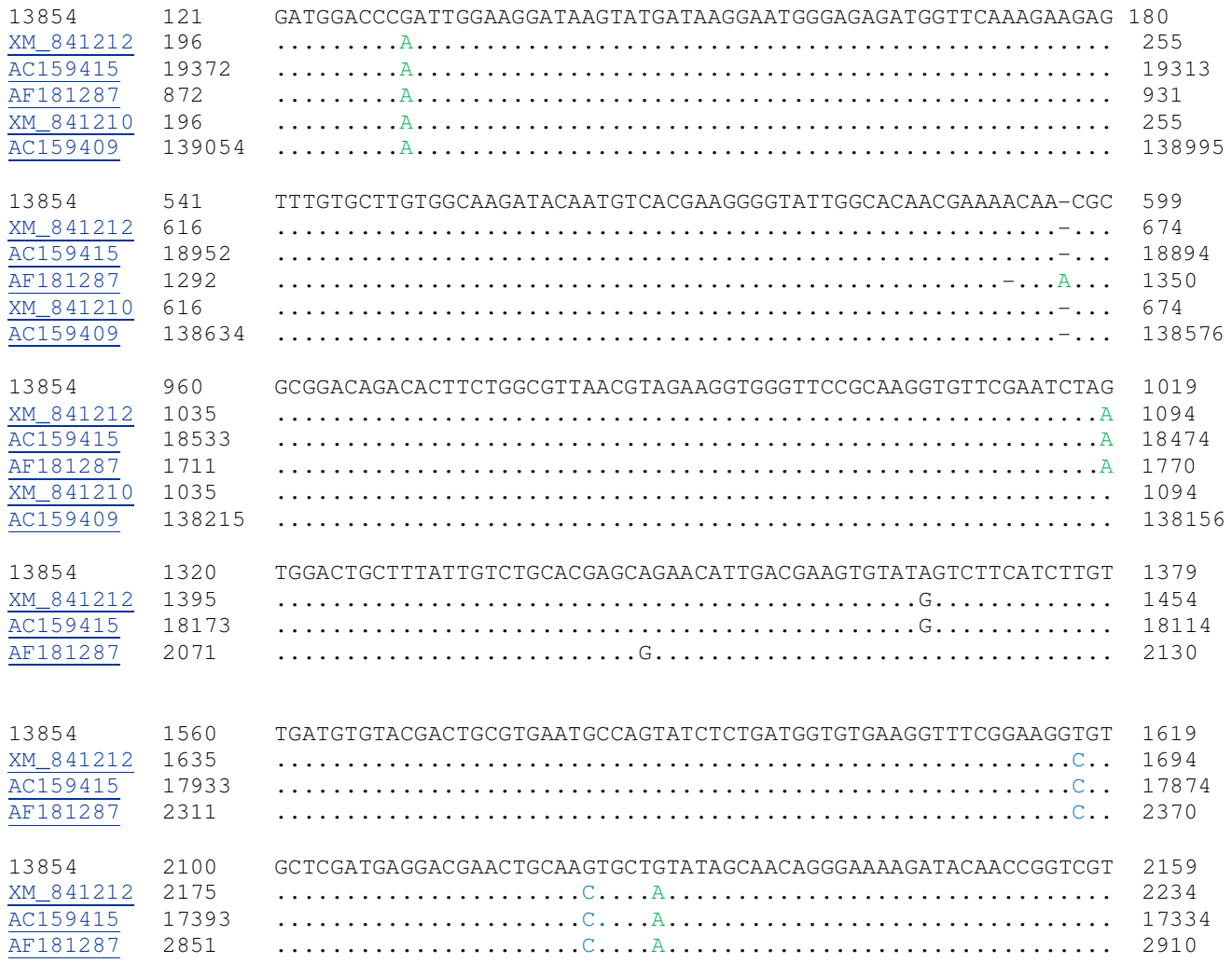

Figure 4. . NCBI BLAST alignment view of TeTS gene (13854); XM841212 T.b. (TREU917 TS partial mRNA); AC159415 (T.b. Chromosome7 clone RPC193-25D22); AF181287 (T.b. TSI 1p); XM841210 (T.b. putative mRNA); AC159409 (T.b. Chromosome7 Clone RPC19315M23).

Genomes WU-BLAST2 indicated 99% maximum identity and similarity to *T. brucei* (TREU927) trans-sialidase partial mRNA (XM841212), *T. brucei* chromosome7 clone RPC193-25D22 complete sequence (AC159416), *T. brucei* putative trans-sialidase B38 gene complete cds (AF181287). However, the maximum identity and similarity to *T. congolense* partial trans-sialidase gene putative trans-sialidase (AJ535487) was 67%; 58% for *T. cruzi* TCTS-154 gene trans-sialidase (D50684) and 59% for *Trypanosoma rangeli* sialidase gene partial cds (U46073).

The detailed NR alignment view (Figure 4) showed that *TeTS* gene had six substitutions (changes) at positions 131, 1019, 1080, 1366, 1617 and 2122 when compared with *T. brucei* TREU927 TS partial mRNA (XM841212), *T. brucei* chromosome 7 clone RPCI93-25D22 complete sequence (AC159415), *T. brucei* TSI1P gene complete cds (AF181287), *T. brucei* TREU927 TS putative gene (TB927.7.6830) (XM841210) and *T. brucei* chromosome 7 clone RPCI93-15M23 complete sequence (AC159409) in the NCBI data base. A deletion occurred at position 596 just like for *T.brucei* TREU927 TS partial mRNA (XM841212), *T.brucei* chromosome7 clone RPCI93-25D22 complete sequence (AC159415),l *T. brucei* TREU927 TS putative gene (TB927.7.6830) (XM841210) and *T. brucei* chromosome 7 clone RPCI93-15M23 complete sequence (AC159409). A single nucleotide substitution or deletion could change the triplet sequence (codons) and hence can cause redundancy of the protein especially when it occurs on the catalytic domain. However, the changes may occur at positions irrelevant for the enzymatic activity as earlier reported by Montagna et al. (2002) on *TbTS* gene as the recombinant proteins displayed both sialidase and trans-sialidase activity. These results suggest that the primary nucleotides sequence of TeTS (FJ597949) is that of a trans-sialidase

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TeTS   1   CALTSKAAGKGTTREAFLSGGAWALRKKLSEKDGEVWWWQDGPDWKDKYDKEWERWFKEE  60
TbTS  26   ....................S....................N.................  85

TeTS  61   KGPWGGSEKRSEWFARMTGGYITLGKTKILSSAIEGSDKVERTVHSFRIPSFVEVDGVLM  120
TbTS  86   ..............................................................  145

TeTS 121   GIGDARYLTSTDYFFTDTVAKYSADGGKTWKTEVIIENGRVDPTYSRVVDPTVVAKADSV  180
TbTS 146   ................................A.............................  205

TeTS 181   FVLVARYNVTKGYWHNENNAAGIADWEPFMYKGVVTKGADGKTSDVRISWTKTPLKPLYD  240
TbTS 206   ............................V.................................  265

TeTS 241   FTVAGSKGTQFIGGAGNGVVTLNGTILFPVQARNEDNAVVSMVMYSVDDGVSWHFARGET  300
TbTS 266   ..............................................................  325

TeTS 301   ALLTSEASLTEWNGKLLMSARTDTSGVNVEGGFRKVFESSNLGATWEESLGTISRVIGNS  360
TbTS 326   ....................................L.........................  385

TeTS 361   PDRTKPSPTANYPGSSGALITVTLGDVPVMLITHPKNTKGAWSRDRLQLWMTDGNRMWLV  420
TbTS 386   ............................................................  445

TeTS 421   GQISEGDDNSAYSSLLLARDGLLYCLHEQNIDEVYSLHLVHLVDELEKVNATVRKWKAQD  480
TbTS 446   ............................................................  505

TeTS 481   ALLAGLCSSSRKKNDPTCSGVPTDGLVGLLAGPVGASVWADVYDCVNASISDGVKVSEGV  540
TbTS 506   ..............................................................  565

TeTS 541   QLGGKRNSRVLWPVSEQGQDQRYYFANTHFTLLATVRFAGEPKAEAPLMGFSNAEGKTSE  600
TbTS 566   ........PL....................................................  625

TeTS 601   TLSLTVGGKKWVLTYGSVRKEGPTTSMDWNQTHQIALTLRDGKVDAHVNGELIIKEVSVG  660
TbTS 626   ..............................................A.............  685

TeTS 661   ASESSAHLHLSHFFIGAPVNDSGEGGNNVIVRNVLLYNRKLDEDELQVLYSNREKIQPVV  720
TbTS 686   ..............................................................  745

TeTS 721   SAVGIPEGMSAPRLCCLLILMYVLAI  746
TbTS 746   ..........................  771
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Figure 5. Comparison of protein structure and sequence between TeTS and TbTS (Putative TS B38p gene). The positions of the identified sialidase/trans-sialidase superfamily motifs are coloured. Red, mismatches; green, FRIP motif; purple, ISRVIGNS motif; olive green, Asp box; orange accent, VPVMLITHP motif; light blue, LYCLHE motif.

gene.

TeTS primary protein structure of 748 amino acids was compared at National Center for Biotechnology Information (NCBI) by using TbLASTX [32, 33]. TeTS protein had 99% identity (99% similarity) with *T. brucei* TREU927 trans-sialidase (XP8463051), 98% identity (98% similarity) with *T. brucei* putative TS B38P gene (AF3102321). However, TeTS had 40% identity (57% similarity) with *T. cruzi* trans-sialidase (ACF17927) and 61% identity (73% similarity) with *T. congolense* putative TS (AD59552.1). BLAST2 sequences alignment of TeTS with *T. brucei* putative TS B38p gene (AF310232) showed 8 points of mismatch or substitutions, three of which were within the catalytic domain (Figure 5). The substitutions were V154 in TeTS to A179 in TbTS, M210 in TeTS to V235 in TbTS and E337 in TeTS to L362 in

Table 1. TeTS active site residues and their postulated effects in trypanosomal SA and TS.

S/N	TeTS	T.b.br.TS	1EUS	1KIT	1NSCB	2SLI	Postulated Effect
1	R86	R133	R15	R195	R24	R201	Binds the CO^- group of sia (Taylor et al., 1992; Crennel et al., 1994; Montagna et al., 2002)
2	D105	E331	D34	D214	D43	D220	Possible proton donor (Chong et al., 1992)
3	E292	R346	E205	E570	E186	E502	Stablizes a putative sialosyl cation intermediate (Burmeister et al., 1993; Crennel et al., 1994)
4	R330	R431	R238	R622	R220	E533	Binds the CO- group of sia (Taylor et al., 1992; Crennel et al., 1994; Montagna et al., 2002)
5	R409	R431	R307	R695	R309	R610	Binds the CO- group of sia (Taylor et al., 1992; Crennel et al., 1994; Montagna et al., 2002)
6	Y428	Y457	Y326	Y714	Y328	Y629	Stablizes a putative sialosyl cation intermediate (Burmeister et al., 1993; Crennel et al., 1994)
7	E469	E473	E354	E743	E372	E670	Stablizes one of the arginine side chains (Taylor et al., 1992; Crennel et al., 1994)

Sia, Sialic acid; SA, sialidase; TS, trans-sialidase.

TbTS.

Conserved motifs identified and common to bacterial and trypanosomal SA and TS

TeTS gene sequence contained 6 of the residues that compose the consensus sequence, called Asp-box (Figures 5) which contains aspartate at a central position in a stretch of 8 amino acids (S-X-D-X-G-X-T-W) and it is repeated 2-5 times in sialidase sequences (Buschiazzo et al*.,* 2000; Roggentin et al*.,* 1989; Chuenkova et al., 1999). FRIP is another conserved motif found near the N-terminus region, which is also common to bacterial and trypanosomal SA and TS. The stretch of amino acids ISRVIGNS and VPVMLITHP (Figure 5) now found to occur in all African trypanosomes TS genes so far studied was identified in TeTS gene. The motif LYCHE common to all known trypanosomal TS was also found in TeTS gene.

Conservation of active site residues in the TeTS gene sequence

We sought to identify the catalytic site and the critical active site residues on the TeTS gene that are common to SA and TS gene families. Native TS is formed by 2 separate domains, a conserved segment in the N-terminus containing the catalytic region and a heterogeneous region in the C terminus required for enzyme oligomerization or aggregation (Schenkman et al., 1994). A specialized BLAST search for conserved domains at the NCBI using blatp2.2.14 revealed the conservation of seven catalytic site residues on the catalytic region (86-469 aa) of TeTS gene (Table 1). TeTS belongs to the sialidase sequence cluster and superfamily like most trypanosome TS genes studied so far, which function to bind and hydrolyse sialic acid residues from various glycoconjugates as well as playing roles in pathogenesis, nutrition and cellular recognition.

The arginine triad (R86, R330, and R409) which binds to the carboxylate group common to all sialic acid derivatives has been found to be conserved in TeTS gene. Other active catalytic site residues that have also been found to be conserved in the TeTS structure are E469, E292, Y428 and D105. The glutamic acid residue (E469) is known to stabilize one of the arginine side chains, while E292 and Y428 are known to stabilize a putative sialosyl cation intermediate. The negatively charged group, aspartic acid (D105) was proposed as a proton donor in hydrolytic reactions in bacterial and protozoan sialidases and trans-sialidases. The rest of the active site residues found to be conserved in *TeTS* gene are shown in Table 1.

DISCUSSION

We show for the first time the amplification of TS gene in the bloodstream form of *T. evansi* and the first report of the amplification of *TS g*ene in the BSF of African trypanosomes. Montagna et al. (2002, 2006) and Tiralongo et al. (2003) reported the detection of TS activity in the PCFs of African trypanosomes. Although the existence of trans-sialidase in the bloodstream form African trypanosomes is under controversy, several studies have shown the detection of sialidase activity in the serum of African trypanosome infected animals and the BSF African trypanosomes (Nok and Balogun, 2003; Nok et al., 2003; Brutai et al., 2006; Esievo, 1982). The results of this study coupled with recent findings on the occurrence of both TS and SA activities in some African trypanosomes (Tiralongo et al., 2003; Montagna et al., 2006) and the exhibition of the 2 enzymes activities by the same protein (Tiralongo et al., 2003) suggests that

African trypanosomes could modulate differential expression of each of these enzymes as the need arises. While the expression of VSGs is now believed to be the single most important mechanism of protection against the immune system of the host for the BSF trypanosomes, it is evident that SA/TS seem to have a significant role in their survival and pathogenicity evidenced by initial and continuous anaemia in African trypanosomiasis. Anaemia as a result of extravascular haemolysis has been shown to be due to increased red cell damage as a result of sialidase activity among other factors (Banks, 1980; Esievo, 1982). We strongly believe that the results of this study is thought provoking and will reorientate the minds of researchers in this field to direct the search light on the involvement of SA/TS in the pathogenecity of African trypanosomes. There is need for more information on TS/SA in African trypanosomes than is currently available, to be able to understand and fully explain the involvement or non-involvement of TS in the pathogenesis of BSF African trypanosomes.

A report by Montagna et al. (2002) showed a recombinant *T. brucei* TS protein was found to have both SA and TS activity, whose catalytic region was found to be identical to that of *T. cruzi* TS. They suggested the existence of distinct donor and acceptor binding sites to account for sialyltransferase activity of TS as earlier modelled by Schenkman et al. (1994). They explained that a sialidase that binds sialic acid α-linked to galactose could function as TS. Hence the same enzyme can exhibit both activities either sialidase or glycosyltransferase activity as the situation or need of the moment arises. It is really thought provoking to suggest that trans-sialidase activity is present in BSF African trypanosomes as evidenced by the results of this study. However, this was not substantiated by TS activity tests to confirm these results. Also, mutagenesis studies like this study reveal that trypanosomal sialidases and trans-sialidases share a similar active-site architecture in which several amino acids are conserved (Montagna et al., 2002; Cremona et al., 1995; Paris et al., 2005). The non detection of TS activity in the bloodstream form of African trypanosomes in earlier studies may be due to several factors such as low expression of the protein due to developmental regulation, need at a particular moment within the infection period. This suggests that glycosyltransferase activity may be very low at certain stages as SA activity dominates. There is need for more information on TS/SA in African trypanosomes than is currently available to be able to understand and fully explain the involvement or non-involvement of TS in the pathogenesis of African trypanosomes.

In protozoan parasites like *T. cruzi, Entamoaba histolytica, Trichomonas vaginalis*, the existence of pathogenic or adaptation genes have been established (Taylor et al., 1992). The detection of *TS* gene in the BSF of *T. evansi* suggests that BSF African trypanosomes also have sialidase or trans-sialidase genes *in vivo* which may be specific genes for adaptation and/or pathogenesis to their mammalian hosts. Highly sensitive and specific techniques such as real time RT-PCR and micro array may reveal the expression of *TS* gene and its protein levels in the BSF of African trypanosomes.

Further studies on TS activity should be conducted in the BSF of African trypanosomes especially *T. evansi* in order to ascertain the existence of expressed TS in African trypanosomes or sera of infected animals or humans.

The high degree of identity and similarity (≥99%) of *TeTS gene* to the *T. brucei* partial TS mRNA, putative TS gene complete cds and *T. brucei* chromosome7 clone RPC193-25D22 complete sequence indicates that it is homologues to the sequences of *T. brucei* TS genes studied. Interestingly, *TeTS* (FJ597949) possesses a comparable degree of similarity (67 and 58%, respectively) to the *T. congolense* partial gene putative TS and *T. cruzi* TCTS-154 gene. This emphasizes again that *TeTS gene* amplified is a *TS* gene which may be expressed by the BSF *T. evansi*. A comparison of translated protein structure of *TeTS gene* also showed comparable identity and similarity establishing homology of the *TeTS gene* protein primary structure to the trypanosome TS genes already known and characterised. Trans-sialidase and sialidase protein sequences also have common conserved motifs irrespective of the source or organism of origin. All the conserved motifs common to bacterial and trypanosomal SA and TS have been identified in the TeTS protein sequence. The stretch of amino acids ISRVIGNS, VPVMLITHP and ZYCHE now found to occur in all African trypanosomes TS gene so far studied was identified in *TeTS* gene indicative of homology to African trypanosome *TS* gene. However, the occurrence of these unique sequences has not been associated with any function. The Asp box in the TeTS is located at similar position in the fold of the protein for *T. brucei brucei* TS, *Micromonospora viridifaciens* SA, *Vibrio cholera* SA and human *Influenza A virus* SA (Schreier et al., 1998; Roggentin et al., 1993; Cremona et al., 1995). Asp box motifs have been shown to have no effect on the enzymatic activity because they are remote from the active site. They may however be involved in protein folding as they are located on the surface of the protein (Cremona et al., 1995).

Conclusions

In this study, we showed for the first time the amplification of TS gene in the bloodstream form of *T. evansi* and the first report of the amplification of *TS* gene in the BSF of African trypanosomes. The results of this study indicate that *TeTS* is homologous to *TS* gene sequences and thus strongly suggests the occurrence of TS in the BSF of African trypanosomes. The results of this study coupled with recent findings on the occurrence of both TS

and SA activities in some African trypanosomes (Tiralongo et al., 2003; Montagna et al., 2006) and the exhibition of the 2 enzymes activities by the same protein suggests that African trypanosomes could modulate differential expression of each of these enzymes activities as the need arises. The non detection of TS activity in the blood-stream form of African trypanosomes in earlier studies may be due to several factors such as low expression of the protein due to developmental regulation, need at a particular moment within the infection period. This suggests that glycosyltransferase activity may be very low at certain stages as SA activity dominates. However, since TS activity was not assayed in this study, the results could only show the presence of *TS* gene in BSF *T. evansi* and its sequence homology to *TS* genes so far studied. There is need for more information on TS/ SA in African trypanosomes than is currently available to be able to understand and fully explain the involvement or non-involvement of TS in the pathogenesis of African trypanosomes.

ACKNOWLEDGEMENTS

We thank the Executive Director, Dr L.H. Lombin and the Board of Management of the National Veterinary Research Institute, Vom Nigeria where this research work was conducted for the support received to carry out this work. We are particularly grateful to Dr Aminu Mohammed (a Ph. D student of the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria) for providing the *T. evansi* infected blood samples from camels obtained from abattoirs in Kano and Sokoto, Nigeria.

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