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A Bloodstream *Trypanosoma congolense* Sialidase Could Be Involved in Anemia during Experimental Trypanosomiasis

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The release of Sialic acid (SA) into the serum by *Trypanosoma congolense* infected BalbC mice was investigated. A progressive increase in the level of serum SA corresponding to anemia and parasitemia was observed. At maximum parasitemia, the level of total SA from the red blood cells (RBC) dropped by about 45%. Solved polynomials revealed an association between free serum SA and RBC-SA. Positive roots of quadratics were used to predict complete cleavage of RBC-SA on day 7.01 and maximum accumulation of free serum SA on day 6.6. A steady rise in the level of serum sialidase (SD) activity and a low packed cell volume (PCV) with an increase in parasitemia were observed. Mice infused with galactose, methyl-β-gal, lactose, mannose, or L-arabinose and challenged by intraperitoneal inoculation with *Trypanosoma congolense* neither developed anemia nor secreted free SA above the control level even though there was detectable SD activity. Bloodstream *Trypanosoma congolense* parasites were isolated using DEAE cellulose from heparinized blood of experimentally infected BalbC mice. The parasites were lysed with 0.2% Triton-CF 54 to release membrane bound SD. The activity of the SD was proportional to the number of parasites. The enzyme was partially purified on Q-Sepharose and Fetuin agarose columns successively. The final active fraction from the latter column was used as the partially purified SD. The enzyme had an optimum pH of 6 and was maximally active at 37°C with a requirement for the divalent ions Ca^{2+} and Mg^{2+}. The enzyme was highly specific for NeuAc5α2,3 lac and Methylumbelliferyl-Neu5Ac (4-MU-Neu5Ac) with K_M values of 0.34 and 0.025 mM, respectively. It was inhibited competitively by 2,3-didehydroneuraminic acid (Neu5Ac2en) and para-nitro-phenyloxamic acid (pNPO) with inhibition binding constants K_i of 65 and 215 μM, respectively. In deviation from the procyclic trypanosomal SD, it lacked trans-sialidase (TS) activity. The possible role of a secreted bloodstream *Trypanosoma congolense* SD and the development of anemia in the pathogensesis of trypanosomiasis are discussed.

Key words: anemia, blood, sialidase, *Trypanosoma congolense*.

African trypanosomes are protozoan parasites that cause potentially fatal diseases in humans and domestic livestock. They have a life cycle that alternates between the tsetse fly and the mammal. In the bloodstream form, anemia is a clinical feature in human and animal trypanosomiasis (*1*). It also represents the main pathological feature in livestock infected with *T. vivax* and *T. congolense* (*2*, *3*). The mechanism of anemia in trypanosomiasis is still speculative, however, the predominance of hemolytic anemia in the disease is well documented (*4*) The role of phospholipases in the etiology of anemia in trypanosomiasis has been reported (*3*, *4*). Anemia occurring intravascularly at the beginning of the acute phase of trypanosomiasis in infected animals has in part been attributed to the activity of trypanosomal sialidase (*2*, *5*).

Sialidases [EC 3.2.1.18] are key-enzymes of sialic acid catabolism, which hydrolyse the glycosidic linkage between SA and the underlying sugars (*6*, *7*).

Unable to synthesize sialic acid (SA), trypanosomes use as a specific enzyme, the TS, to scavenge the monosaccharides from host glycoconjugate and sialylate acceptor molecules present on the surface of the parasite membrane (*8*). Indeed the presence of TS activity is unique to a few trypanosomes, being absent in all other cell types so far tested (*9*). While the role(s) of SD/TS have been adequately described in *T. cruzi* and Chagas disease (*10*, *11*), the current knowledge about the counterpart enzyme in the African trypanosome has only emphasized the developmental expression of SD and TS in procyclic forms of African trypanosomes with their roles unknown (*8*, *12*). However with the unusual surge in the level of circulating free serum SA in trypanosuceptible zebu cattle (*13*, *14*), it is logical to suppose that an enzymatic process should be in place rather than a mere chemical response. In the present study, we re-examined the development of anemia, free SA, and bound red blood cell SA (RBC-SA) during experimental infection of Balb C mice with *Trypanosoma congolense*. We also checked for the presence of serum SD and its origin and any possible role(s) played in the pathophysiology of trypanosomiasis. In this paper we report for the first time that *T. congolense* has a bloodstream SD that could be involved in anemia during experimental trypanosomiasis.

MATERIALS AND METHODS

Reagents were purchased from Sigma Chemical Company St Louis, USA. Partially purified bovine submandibular

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mucin was a gift from Prof. Dr R. Schauer (Kiel) FRG. It contained 70% Neu5Acα2,3 lac and 30% Neu5Ac α2,6 lac.

Trypanosoma congolense stb 212 was supplied by the National Institute for Trypanosomiasis Research Vom, Plateau State, Nigeria.

Sugar Infusion and Experimental Infection with T. congolense—In triplicates, the following saccharides: raffinose, melizitose, lactose, galactose, mannose, methyl-β-gal, L-arabinose, D-arabinose, D-fucose, at[5–200 mM, and methylumbelliferyl-β-D-galactoside (MU-β-D -gal) at 1–14 mM were administered to groups of four mice per saccharide. The mice were then infected by intraperitoneal inoculation with 10^4 *Trypanosoma congolense* cells. Two control groups were set up, one group was not infused but infected and the second control group was not infused and not infected. At different waves of parasitemia, representative members from each group were sacrificed and the levels of PCV, serum free sialic acid, and SD activity were determined. Also ghost erythrocytes were prepared from the mice and analyzed for bound SA (RBC-SA) at different levels of parasitemia.

Preparation of Haemoglobin-Free Erythrocyte Membranes (Ghosts)—The erythrocytes were washed by suspending them in about 3.0 ml of isotonic phosphate buffer (310 imOsm) with agitation and centrifuged at 1,000 ×*g* for 20 min. The supernatant was discarded and the erythrocytes re-suspended in the isotonic phosphate buffer and washed twice. The harvested erythrocytes were then haemolysed with 3-ml hypotonic phosphate buffer (20 imOsm) and agitated for 5 min. The haemolysate was centrifuged at 20,000 ×*g* for 40 min, and the recovered ghosts were washed in hypotonic buffer until a clear membrane was obtained.

Isolation and Partial Purification of SD from T. congolense—Bloodstream *T. congolense* was isolated from 4 BalbC mice experimentally infected with *T. congolense* stb212. At peak parasitemia (10^8 cells/ml), the mice were euthanised and their blood collected in a heparin coated 10 ml syringe. Parasites were separated using a DEAE-cellulose column as described (*15*). The separated parasites were suspended in 0.1 M phosphate buffer, pH 6.8, containing 0.2% Triton-CF 54 and kept at room temperature (25°C) for 30 min. The lysed cells were then centrifuged at 5000 ×*g* for 10 min. The supernatant was retained and the pellet re-extracted twice. The extracts were then pooled and loaded onto a Q-Sepharose column pre-equilibrated with 0.1 M phosphate buffer, pH 6.8. The SD flowed through without binding to the column and was immediately loaded to a Fetuin agarose column. After washing with 5 column volumes of phosphate buffer, the SD was eluted using 1 M lactose. The final preparation was active and used for all other experiments. Total protein was quantified as described by Bradford (*16*). Polyacrylamide gel electrophoresis was conducted as described by Laemli (*17*). Native PAGE was performed without SDS in 10% polyacrylamide gels impregnated with 1 mM MU-Neu5Ac. At the end of the run, the gel was incubated for 3 h at 37°C in 50 ml sodium acetate buffer, pH 5.5, and stained with 0.01% Calflour white M2R. After five washings, the gel was exposed to UV radiation for fluorescence of MU showing region of SD activity.

Haematological Analysis—Packed cell volume (PCV) for all the mice was determined at peak parasitemia by microhematocrit analysis.

Sialidase Assay—Sialidase activity was assayed as described (*18*). Briefly the substrate 4-MU-Neu5Ac at a final concentration of 0.7 mM in 50 mM acetate buffer, pH 5.5, was incubated with 50 μl of serum (or parasite lysate) at 37°C for 30 min. At the end of the incubation period, the reaction was terminated using 0.5 M Borate Buffer, pH 9.8. The released methylumbelliferone was then measured at 525 excitation and 490 emission with a multiplate Hitachi flouroskan meter.

For each determination, the spectrofluorimeter response was calibrated with pure methyllumbelliferone as standard and readings were corrected by subtracting serum and substrate blanks. The isolated, *T. congolense* SD was linear with incubation time up to 1 h and was directly proportional to the homogenate protein content. One unit of enzyme activity was defined as the amount of enzyme that hydrolyses 1 μmol 4-MU-Neu5Ac per minute under the described assay conditions.

Effect of pH and Temperature on the Activity of Bloodstream T. congolense SD—The pH profile was determined in the following buffers: 60-mM acetate pH 4–6; 66 mM phosphate, pH 6.5–7.2; and 0.1 M Tris-glycine, pH 7.5–9.5. The effect of temperature was measured after pre-equilibration of the enzyme at 5–50°C

Substrate Specificity—The following substrates were tested for specificity; Neu5Acα2,3lac, Neu5Acα2,6lac, 4-MU-Neu5Ac, Colomic acid, Fetuin, bovine submandibular mucin. The reaction assay mixture contained the following components in a total volume of 200 μl, 50 μl of 0.1 mM substrate solution, 100 μl of 50 mM acetate buffer, pH 6, and 50 μl of the partially purified enzyme (0.24 unit). The sialic acid contents of these substrates were measured by thiobarbituric (TBA) assay after mild hydrolysis with 0.1 M HCl at 80°C for 1 h. For the sialoglycoconjugates, each substrate was adjusted to contain 0.1 mM of bound sialic acid except for gangliosides (0.05 mM). In the case of gangliosides, Triton X-100 was added to the mixture to a final concentration of 0.1%. After incubation at 37°C for 30 min the sialic acid released was quantified by the TBA assay. For kinetic studies with the substrates Neu5Ac2, 3lac and 4-MU-Neu5Ac, a concentration range of (0.1–0.5 mM) was used. In the inhibition analysis with Neu5Ac2en and paranitro phenyl oxamic acid (pNPO), the concentration of the inhibitor was brought to 25 and 100 μM, respectively, in the total reaction mixture.

Transsialidase Assay—Osmotically lysed bloodstream *T. congolense* (8×10^8 cells) in 1 ml of 60 mM phosphate buffer, pH 6.8, containing 1 mM EDTA, 1 μM of pepstatin, 1 μM trasylol, and 1 μM leupeptin was assayed for the presence of *trans*-sialidase activity (*19*). Briefly, the reaction system contained 50 μl of 100 μM Neu5Ac2,3lac and 50 μl of 50 μM MU-Gal made up to 200 μl with 0.1 M phosphate buffer, pH 6.8 samples were incubated with 0.24 unit of the enzyme at 37°C for 2 h. The reaction was terminated by the addition of 800 μl of distilled water and the mixture passed through a 2-ml Q-Sepharose column and washed with 5 column volumes of water. The sialylated methylumbelliferyl galactoside (SA-Gal-MU) was then eluted with 200 μl 0.1 M HCl and incubated for 1 h

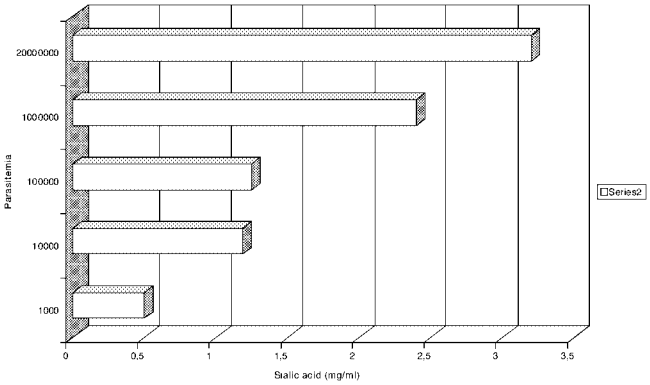

Fig. 1. **The effect of different levels of parasitemia on the release of free serum sialic acid in *Trypanosoma congolense* infected mice.** At each bout of parasitemia, exactly 100 μl of serum was recovered from the experimental mouse and used in the TBA assay for sialic acid as described in "MATERIALS AND METHODS." Averages of three experiments were used to construct the plot.

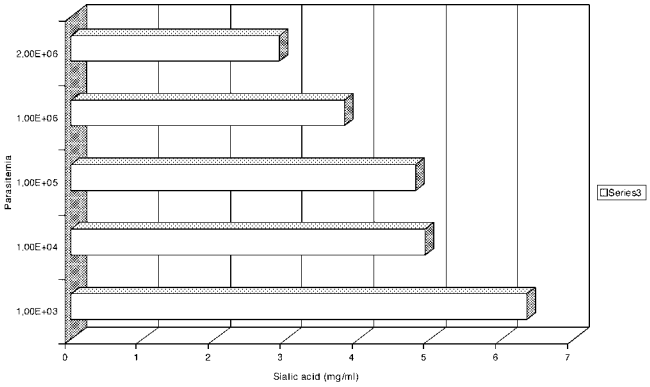

Fig. 2. **The effect of different levels of parasitemia on bound erythrocyte sialic acid (RBC-SA) of *Trypanosoma congolense* infected mice.** At each wave of parasitemia, representative members were sacrificed and erythrocytes was prepared from the recovered blood. The total RBC collected was incubated with 0.1 M HCl to liberate the bound sialic acid, which was then quantified as described in "MATERIALS AND METHODS." Averages of three experiments were used to construct the plot.

at 95°C. The reaction mixture was cooled on ice for 10 min, adjusted to pH 10 with 0.1 M NaOH, and the fluorescence of the released methylumbelliferone was then measured.

Sialic Acid Analysis—The free sialic acid concentration in serum was determined by the TBA assay using 100 μl of serum (*19*). Bound sialic acid was determined using 100 μl of serum incubated with 20 μl of 0.1 M HCl for 3 h to liberate the bound SA. The difference between the total and free SA represents the bound SA. The distribution of SA is thus; $[SA]_T = [SA]_B + [SA]_F$; $[SA]_F = [SA]_T - [SA]B$; (where T, B, and F stand for total, bound, and free sialic acids, respectively). The sialic acid content of freshly harvested *T. congolense* cells was also measured by thiobarbituric (TBA) assay (*12*). About 5×10^8 cells/ml were harvested at peak parasitemia from an experimental mouse and separated as described (*15*). The parasites were washed 5 times in ice-cold PBS, and resuspended in 500 μl of 0.1 M HCl, pH 1, at 80°C for 1 h.

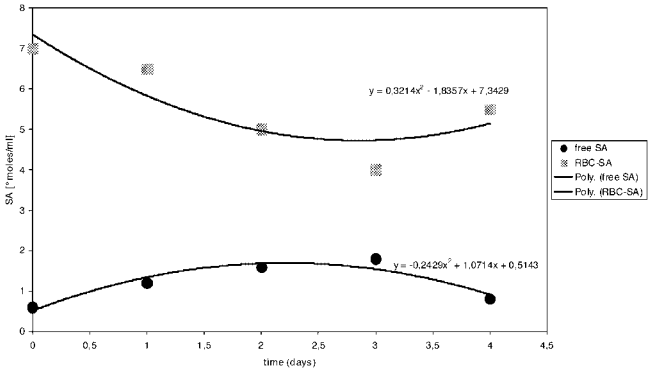

Fig. 3. **Polynomial relationships of free sialic acid (SA) and bound Sialic (RBC-SA) acid with days post-infection in mice infected with *Trypanosoma congolense*.**

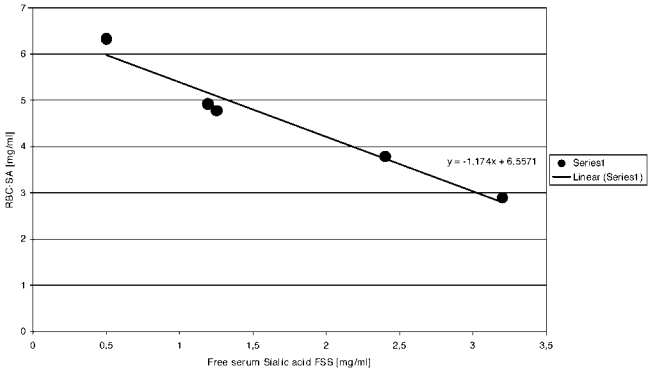

Fig. 4. **Plot showing the correlation between RBC-SA and free serum sialic acid (SA) at different levels of parasitemia in *Trypanosoma congolense* infected mice. Averages of three experiments were used to construct the plot.**

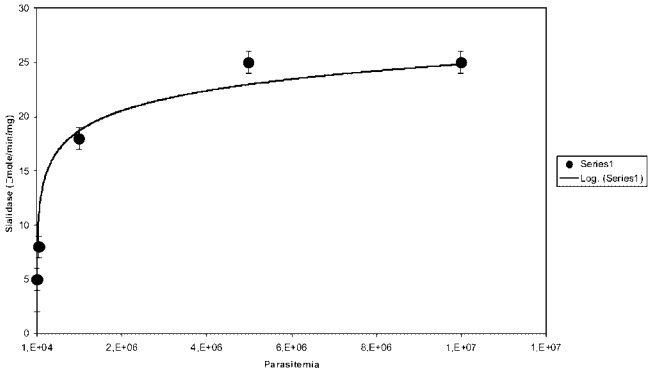

Fig. 5. **Effect of parasitemia on secreted serum sialidase in *Trypanosoma congolense* infected mice.**

After centrifugation at 10,000 ×*g* for 10 min, the sialic acid released was quantified by the TBA assay.

RESULTS

Changes in Serum and Erythrocyte Sialic Acid at Different Waves of Parasitemia—There was a progressive increase in the level of free sialic acid in the serum of infected mice (Fig. 1). The increase corresponded to a fall in the PCV by about 33% (not shown), which signals the

Table 1. **Purifcation profile of bloostream form Trypanosoma congolense SD.** Details are described in "MATERIALS AND METHODS." The results are expressed as averages of three experiments.

Purification Step	Specific activity (μmol/min/mg)	Protein (mg)	Activity (μmol/min)	Purification	Yield (%)
Crude cells	5	204	1020	1	100
Osmotic lysis	21	40	820	4	77
Q-Sepharose chromatography	140	4	560	28	54
Fetuin Sepharose chromatography	550	0.6	325	110	9

onset of anemia. At peak parasitemia, the level of the free SA increased by about 7 fold. In the same pattern the RBC-SA at different waves of parasitemia showed a gradual decrease in the level of mean surface RBC sialic acid (Fig. 2). Solved polynomials of the cleavage of RBC-SA and accumulation of serum SA showed that on day 7.02, the level of serum SA will reach 7 mg/ml. while on day 6.6, the RBC-SA will be completely cleaved by the enzyme (Fig. 3). The correlation between the free serum SA and the RBC-SA during the course of the *Trypanosoma congolense* infection is shown in (Fig. 4).

Sialidase (SD)—The level of serum SD also increased with parasitemia and remained constant at the fourth bout of parasitemia (Fig. 5). In the serum of control mice (uninfected), there was no detectable SD activity, neither was there any change in free serum SA and bound RBC-SA. Also the serum of infected mice treated with the trypanocidal drug suramin showed no detectable levels of SD. The summation of both observations strongly points to the parasite as the source of SD in infected mice. When the separated parasites were osmotically lysed and assayed for the enzyme, a very high SD activity was observed, thus confirming that the trypanosomes are the source of the serum SD during the course of infection. The purification profile of the enzyme is as shown in Table 1. It was purified about 170-fold with 18% recovery. When the native enzyme was electrophoresed in MU-Neu5Ac impregnated acrylamide gels, a strong fluorescence was observed upon exposure to UV-radiation indicative, of the MU released by the SD (not shown). The activity of the enzyme was optimal between pH 5–6 and at 37°C.

Substrate Specificity of Bloodstream T. congolense SD—The *T. congolense* SD showed strict preference for terminal α-linked sialic acids. Table 2 summarizes the results of substrate specificity analysis. Substrates possessing α-2,3-linked sialic acids, such as Neu5Acα2,3lac, fetuin, and bovine submandibular mucin, were readily hydrolyzed. Colomic acid, a homopolymer of α-2,8–linked sialic acid, and Neu5Acα2,6lac, were not suitable substrates. Detailed kinetic analysis from initial velocity data revealed K_m and V_{max} values of 0.025 mM and 820 μM/min, respectively, for Mu-Neu5Ac. The K_m and V_{max} values for Neu5Ac2, 3lac. were 0.34 mM and 1,900 μM/min.

Table 2. **Substrate specificity of bloodstream forms *Trypanosoma congolense* sialidase.** The substrates were adjusted as described in "MATERIAL AND METHODS" to contain 1 mM of bound sialic acid in 60 mM acetate buffer, pH 6. The results are averages of three readings.

Substrate	Product (Neu5Ac) (μmol/h/mg)
MU-α-Neu5Ac	706 ± 48
Neu5Ac2,3-α-lactose	608 ± 60
Neu5Ac2,6-α-lactose	58 ± 25
Colomic acid	50 ± 23
Fetuin	388 ± 65
Mucin	394 ± 45

The enzyme was strongly inhibited by Neu5Ac, 2en and weakly by pNPO. Analysis of the inhibition with Mu-Neu5Ac as substrate revealed competitive patterns for both Neu5Ac2en and pNP-oxamic acid with K_i values of 65 μM and 215 μM, respectively. The physiological index of efficiency (V_{max}/K_m) of the enzyme decreased from 32.8 min^{-1} to 24.6 min^{-1}, and 21.6 min^{-1} with Neu5Ac2en and pNPO, respectively, when Mu-Neu5Ac was used as substrate.

Effect of Sugar Infusion on Parasitemia, PCV, SD, and SA—Most of the saccharides at 5–50 mM improved the PCV and the level of SA to near those of the control group (Table 3). In all cases, the level of PCV and SA diminished when the level of sugar exceeded 50 mM. Lactose at 5 mM had the highest protective effect among the saccharides tested. It maintained the RBC-SA and PCV at about the same levels as those in the uninfected group. The activity of SD was unaffected by the sugars when compared with the uninfused group. Likewise, MU-β-D-gal, like the other saccahrides, protected RBC from hemolysis. In this group, the PCV and RBC- SA were maintained near the levels of the uninfected group. Results from the analysis of TS activity in the serum of the infected mice and from the isolated parasites at different waves of parasitemia showed no enzyme activity.

DISCUSSION

Sialidases have been reported in bacteria and viruses and their pathological significance has been fully elucidated (*21*, *22*). Also the role of SD in cell invasion in *T. cruzi* has been reported (*10*, *23*, *24*). So far, only procyclic forms of African trypanosomes are known to have SD, which also have an integral TS activity (*12*). This report is the first to reveal the presence of a bloodstream form of sialidase in *Trypanosoma congolense*. Moreover, the serum of the suramin-treated mice had no detectable SD, implicating the parasites as the source of the enzyme. We also show that the bloodstream *T. congolense* SD could be involved in the development of anemia during experimental trypanosomiasis in mice.

So far it is only the bloodstream forms of *T. vivax* that have been reported to have SD activity (*2*). Also *T. vivax* can by-pass the insect vector *Glossina* and be transmitted mechanically, a feature proposed to account for the possession of a SD at the bloodstream stage of the parasite life cycle (*19*). Our present finding on *T. congolense* implies that factors other than the absence of a vector stage may play key roles in bloodstream parasites expressing SD activity.

Table 3. **Effect of infusion of different sugars on bound SA (RBC-SA), packed cell volume (PCV) and sialidase (SD) in T congolense-infected mice.** The saccharides were dissolved in sterile phosphate-buffered saline. Details are described in "MATERIALS AND METHODS." The results are expressed as averages of three experiments.

Sugar	(mM)	SA (mM)	PCV	SD (IU)
Mannose	5	4.05 ± 0.1	32 ± 0.7	14 ± 3
Mannose	50	5.21 ± 0.1	39 ± 0.7	15 ± 3
Mannose	200	4.85 ± 0.2	34 ± 0.7	14 ± 3
Lactose	5	6.15 ± 0.1	35 ± 0.3	13 ± 4
Lactose	50	5.05 ± 0.1	31 ± 0.7	12 ± 3
Lactose	200	3.65 ± 0.1	29 ± 0.7	11 ± 4
Galactose	5	3.75 ± 0.1	33 ± 0.7	15 ± 3
Galactose	50	5.75 ± 0.1	37 ± 0.5	15 ± 4
Galactose	200	4.75 ± 0.1	32 ± 0.7	13 ± 4
MU-Gal	1	3.91 ± 0.1	32 ± 0.7	16 ± 4
MU-Gal	7	5.45 ± 0.1	36 ± 0.5	13 ± 4
MU-Gal	14	4.25 ± 0.1	30 ± 0.7	14 ± 3
Control (uninfected)	nil	5.85 ± 0.3	45 ± 0.7	3 ± 2
Control (infected)	nil	3.42 ± 0.2	29 ± 0.3	18 ± 4
Raffinose	5	4.35 ± 0.1	33 ± 0.5	16 ± 3
Raffinose	50	5.41 ± 0.1	37 ± 0.7	14 ± 4
mellizitose	5	4.25 ± 0.1	32 ± 0.7	15 ± 3
mellizitose	50	5.34 ± 0.1	36 ± 0.7	13 ± 4
Methyl-α-gal	5	3.81 ± 0.1	32 ± 0.7	15 ± 4
Methyl-α-gal	50	4.91 ± 0.1	37 ± 0.7	14 ± 3
Methyl-α-gal	200	4.31 ± 0.1	33 ± 0.4	16 ± 4
L-Arabinose	5	3.71 ± 0.1	32 ± 0.4	14 ± 4
L-Arabinose	50	4.91 ± 0.1	32 ± 0.7	13 ± 2
L-Arabinose	200	4.51 ± 0.1	32 ± 0.7	13 ± 3
D-Talose	**5**	**3.71 ± 0.1**	**32 ± 0.7**	**14 ± 2**
D-Talose	**50**	**5.71 ± 0.1**	**38 ± 0.7**	**15 ± 3**
D-Talose	**200**	**4.91 ± 0.1**	**32 ± 0.4**	**14 ± 3**
D-Fucose	**5**	**3.91 ± 0.1**	**32 ± 0.7**	**15 ± 4**
D-Fucose	50	3.61 ± 0.1	33 ± 0.7	18 ± 4

The kinetic properties of the enzyme resemble those from other protozoa such as *T. brucei, T. cruzi* and *T. vivax* (*10*, *18*, *24*, *25*) in their sensitivity to only α-2,3–linked sialic acids. In contrast however, the enzyme was strongly inhibited by Neu5Ac2en and weakly by pNPO. This difference could be a developmental stage-specific characteristic since the *T. brucei* and *T. congolense* enzymes are from procylic forms of the parasites (*19*, *26*). The pH optimum of the bloodstream *T. congolense* SD is similar to that of *T. vivax*, which is also broad between 5–6 (*19*). However in sharp contrast, the *T. congolense* SD is more stable than its *T. vivax* counterpart, retaining about 70% activity at room temperature (25°C) for 1 week. The contrast in stability could be linked to differences in tertiary structure.

The increase in the level of free serum SA accompanied by a decrease in bound erythrocyte surface SA suggests that the RBC is the source of the released free SA.

The increase in serum free SA to a stationary level followed by a gradual clearance at peak parasitemia may be due to autoinduction of pyruvate sialate lyase (PSL) production in the animal, leading to the hydrolysis of SA to pyruvate and the corresponding acyl-mannosamine. The predicted solved polynomials for positive roots of the quadratic equation showed that on day 6.18, all bound RBC will be cleaved by the trypanosomal SD, while the solution for the free serum SA equally showed that by day 7.01, the level of released SA will be maximal. Both solutions are close, suggesting that serum-SA is derived from RBC-SA.

A decrease in PCV, an index of anemia (*2*), was observed in the *T. congolense*–infected animals. The decline in PCV corresponded to a reduction in the RBC-SA, which, in part, is reported to cause anemia as a result of increased erythrophagocytosis and reduction in erythrocyte half-life (*27–29*). Anemia was significantly reduced in the mice infused with D-Gal, L-Arabinose, methyl-β-D-Gal, and lactose. The monosaccharides D-Gal and L-Arabinose are diastereoisomers of galactose at C-2 and C-3, respectively, suggesting that the alleviation of anemia could have configurational requirements for positions C-2 and C-3 of the saccharide.

The suppression of anemia by lactose infusion could be due to the terminal galactosyl residue, which binds galactose-specific lectins of macrophages. A galactose recognizing system of rat peritoneal macrophages has been reported to mediate in the binding and uptake of desialylated blood cells and glycoproteins (*19*, *26*). Since the trisaccahrides raffinose and mellizitose, which also contain galatosylated residues, failed to suppress anemia and had no effect on the cleavage of RBC-SA residues, it is possible that only free galactose can inhibit the devel-

opment of anemia. Indeed the trisaccharides raffinose and mellizitose are resistant to β-galactosidase activity, and therefore are unable to release free galactose for uptake by macrophages. The infused saccharide MU-Gal was easily hydrolysed, as observed by the strong fluorescence of the released MU when the serum was analysed for MU (not shown), affirming the role of endogenous β-galactosidase activity, which releases free galactose from the enzyme-susceptible substrate. The effect of the infusion of free galactose on anemia was less compared to galactose-containing oligosaccharides. This observation could be due to the anomerization of the galactose into a higher proportion of the β-anomeric form when administered as a monosaccharide.

The absence of SA and TS in the bloodstream forms of *T. congolense* is consistent with previous reports (*19*) on bloodstream forms of *T. brucei*. However in deviation from other African trypanosome bloodstream forms, the *T. congolense* exhibited SD activity that correlated with the development of parasitemia and anemia. The foregoing observations thus imply that during the developmental cycle of African trypanosomes from procyclic through metacyclic to bloodstream forms of the parasite, the expressions of SD/TS are not completely lost, but steadily disposed with respect to parasitic need of the trypanosome at the time. The TS could thus be dominant in the procyclic stage for the acquisition of SA to sialylate trypanosomal surface glycoprotein structures such as GARP and procyclin (*19*), thereby masking them from the hydrolytic effects of mid-gut proteases in the insect vector. The SD in the bloodstream forms could, on the other hand, play the role of de-sialylating RBC, thus subjecting them to clearance by macrophages and lead to anemia during the disease. The present level of information could have significance in the search for antidotes to anemia during trypanosomiasis.

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