ORIGINAL PAPER

E. O. Osir · L. Abubakar · M. O. Imbuga

Purification and characterization of a midgut lectin-trypsin complex from the tsetse fly *Glossina longipennis*

Received: 18 July 1994 / Accepted: 5 December 1994

Abstract A blood-meal-induced lectin (agglutinin) with proteolytic activity was isolated from midgut extracts of Glossina longipennis by a two-step procedure involving anion-exchange chromatography. It is a glycoprotein [native molecular weight (M_r, 61000±3000 da) composed of two noncovalently-linked subunits designated α (M_r, ~27000 da) and β (M_r, ~33000 da). The trypsin activity and the glycosyl residues were present on the α - and β -subunits, respectively. The native protein was capable of agglutinating both bloodstream-form and procyclic trypanosomes as well as rabbit red blood cells. This activity was strongly inhibited by D-glucosamine and weakly inhibited by N-acetyl-D-glucosamine. Similarly, soybean trypsin inhibitor abrogated agglutination of bloodstream-form parasites, whereas the procyclics were unaffected. The agglutination activity was sensitive to temperatures above 40° C but was unaffected by chelators of metal ions. Antibodies raised against the protein were used in immunoblotting experiments to show the presence of a similar protein in several members of the Glossina species. However, no cross-reactivity was detected with midgut extracts prepared from sandflies, mosquitoes, or stable flies. It is proposed that this molecule might play an important role in differentiation of bloodstream-form trypanosomes into procyclic (midgut) forms.

Introduction

Differentiation of bloodstream-form trypanosomes into procyclic (midgut) forms is accompanied by complex morphological and physiological changes that enable the parasites to adapt to a harsh environment within the fly midgut (Ghiotto et al. 1979; Roditi and Pearson 1990).

E. O. Osir (≥)¹ · L. Abubakar · M. O. Imbuga The International Centre of Insect Physiology and Ecology, P. O. Box 30772, Nairobi, Kenya

Present address:

¹ The Kenya Marine and Fisheries Research Institute, P. O. Box 81651, Mombasa, Kenya

The efficiency of the differentiation process appears to be crucial in determining the success of the establishment of an infection within the vector. Consequently, most studies on tsetse-trypanosome relationships have concentrated on trying to elucidate the fly midgut factors that influence differentiation of the parasites from blood-stream into procyclic forms (Maudlin 1991).

Initial work by Ibrahim et al. (1984) showed that Glossina austeni midgut extract was capable of agglutinating Trypanosoma brucei and that this activity was specifically inhibited by carbohydrates. This finding indicated the involvement of lectins or lectin-like molecules. It is now generally accepted that lectins mediate both lysis and differentiation of the trypanosomes (Maudlin and Welburn 1987, 1988a, b). According to a model proposed to explain the lectin-mediated establishment of trypanosomes, the action of an endochitinase produced by the relatively large numbers of Rickettsialike organisms (RLOs) degrades chitin and leads to an accumulation of glucosamine within the midguts of susceptible flies (Maudlin 1991). The glucosamine, in turn, blocks the lectin-mediated trypanocidal activity. On the other hand, refractory flies with few RLOs produce relatively less glucosamine and the parasites entering such flies are lysed by the lectins.

More recent work in our laboratory has provided evidence for the involvement of trypsin or trypsin-like enzymes in trypanosome differentiation and lysis (Imbuga et al. 1992). It was further noted that the lectins and trypsins are closely related (Osir et al. 1993). On the basis of these findings, it appeared that a modification to the lectin model in which the lectin was replaced with trypsin was necessary. The major drawback with the lectin model is that although the interaction between lectins and trypanosomes can be explained, it is not clear whether lysis of the parasites is a direct effect of lectin activity. To gain some insight into how the lectins or trypsins might recognize and lyse trypanosomes, we considered it pertinent to purify both molecules. This report deals with the purification and characterization of a bifunctional molecule that may play an important role in tsetse-trypanosome interactions.

Materials and methods

Experimental insects and animals

Male tsetse flies, Glossina longipennis Corti, were obtained from the Tsetse Vector Laboratory of the International Laboratory for Research on Animal Diseases (ILRAD), they were maintained at a temperature of 25°±0.5° C and a relative humidity of 80%–85%. Sandflies (Phlebotomus duboscqi), mosquitoes (Aedes aegypti), tsetse flies (G. morsitans morsitans, G. pallidipes) were supplied by the Insect and Animal Breeding Unit (IABU, ICIPE). Stable flies, Stomoxys calcitrans, were collected around an abattor located near Dagoreti, Nairobi. Male rats (Wistar strain) and New Zealand white rabbits were supplied by IABU.

Preparation of parasites and rabbit red blood cells

Rats were infected with a *Trypanosoma brucei brucei* stock derived from the East African Research Organization (EATRO) in 1969 (Otieno et al. 1983). The procedures for the preparation of bloodstream-form and procyclic parasites have been described elsewhere (Abubakar et al. 1994). Rabbit blood was collected in heparin and centrifuged (1000 g, 10 min) in a Heraeus 2 Minifuge (Osterode, Germany). After removal of the plasma and the "buffy coat," the pellet containing the red blood cells (RBCs) was washed five times in phosphate-buffered saline (PBS: 0.15 M sodium phosphate/0.15 M NaCl, pH 8.0) by centrifugation (2000 g, 10 min, 27° C).

Agglutination and enzyme assays

Doubling serial dilutions of the midgut extracts or the column fractions were prepared in PBS and placed into the wells of microtiter plate (Nunc, Denmark; see Tables 1–3 for details). Equal volumes of the parasite ($\sim 5.0 \times 10^6$ parasites/ml) or 2% RBC ($\sim 10^7$ cells/ml) suspensions were added to each dilution. The total reaction volume was maintained at 0.02 ml. After mixing, the plates were incubated (27° C, 2 h) and agglutination was scored using an inverted microscope (Leitz Dialux, Germany).

Agglutination titers were expressed as the reciprocals of the highest dilutions where complete agglutination of the parasites or RBCs was observed. The tests were carried out in triplicate, with the controls consisting only of parasites or rabbit RBCs and buffer. The requirement of divalent cations for parasite agglutination was assessed by incubating (12 h, 4° C) an agglutinin sample with 16 mM ethylenediaminetetraacetic acid (EDTA) or Ethylene bis (oxyethylenenitrilo)tetra acetic acid (EGTA) followed by overnight dialysis against PBS. To half of this sample was added MgCl₂ (0.1 mg/ml) and CaCl₂ (0.13 mg/ml), and the other half served as the control. Serial dilutions of both samples were separately used in the agglutination assays. The procedures for the assay of trypsin activity and estimation of protein concentrations have been described elsewhere (Imbuga et al. 1992).

Preparation of midgut extract and column chromatography

Teneral flies (24 h postemergence) were fed on rabbit blood and subsequently starved for 72 h. Midguts from these flies were dissected in ice-cold buffer (20 mM TRIS-HCl buffer, pH 8.0) and briefly homogenized using a Virtis homogenizer (Gardiner, USA). The homogenate was centrifuged (12000 g, 15 min, 4° C) in a Beckman Microfuge (Palo Alto, Calif., USA) and the supernatant solution (midgut extract) was stored at –70° C. The midgut extract (~13.5 mg) was first dialysed (24 h, 4° C) against buffer A (20 mM TRIS-HCl, pH 8.0), filtered through a 0.2-µm-pore filter (Nalge, Rochester, N.Y., USA), and then injected via a 500-µl loop into a Mono Q HR 5/% anion-exchange column (Pharmacia, Uppsala, Sweden) attached to a fast protein liquid chromatography (FPLC) system equipped with a model GP-250 gradient program-

mer. The flow rate was maintained at 0.5 ml min⁻¹ and the absorbance was continuously monitored at 280 nm. The column was washed with the same buffer and the bound proteins were eluted using a linear gradient (0–350 mM NaCl).

Fractions (1.0 ml) were collected and tested for both trypsin and agglutination activities. The active fractions were concentrated using polyethylene glycol (PEG-20000; Serva, Westbury, N.Y., USA), dialysed against buffer A (12 h, 4°C), and rechromatographed on the same column). The column was eluted using a linear gradient (150–300 mM NaCl). In another experiment, an agglutinin sample was tested for its ability to bind to a concanavalin A-Sepharose column (Nguu et al. 1992). Determination of the agglutinin molecular weight by gel-permeation chromatography was carried out in a Superose 6 HR/30 column (Pharmacia) attached on FPLC system (Osir et al. 1989).

Electrophoresis procedures

Polyacrylamide gel electrophoresis (PAGE) was performed according to Laemmli (1970). Gradients were cast using a gradient marker (BRL, Gaithersburg, Md., USA) and the gels were stained for protein with Coomassie brilliant blue. Isoelectric focusing (IEF) was performed as previously described (Osir et al. 1989). Staining for carbohydrates was carried out using the periodic acid-Schiff (PAS) reagent (Kapitany and Zebrowski 1973).

Visualization of trypsin activity by autoradiography

Midgut extracts and the agglutinin were separately incubated (18 h, 4° C) with tritiated diisopropylfluorophosphate ([1,3-³H]-DFP, 5 µCi; specific activity, 35 Ci/mmol; Amersham, England). In the case of crude midgut extract, 8.0 mM tosylamide-2-phenylethyl chloromethyl ketone (TPCK) was added to inhibit chymotrypsin activity prior to DFP labeling. The samples were subsequently separated by electrophoresis. To enhance the radioactivity signal, the gels were indubated (27° C, 30 min) in Amplify (Amersham, Buckinghamshire, England) and then dried using a model GSD-4 gel drier (Pharmacia, Uppsala, Sweden). Autoradiography was carried out using Fuji-RX film for 24 h at -70° C.

Effect of temperature on agglutinin

The thermal stability of the agglutinin was tested by separately incubating agglutinin samples in a water bath maintained at different temperatures. After incubation, the samples were left to attain room temperature (27° C) and the agglutination assays were carried out as described above.

Effect of carbohydrates and protease inhibitor on agglutination

Stock solutions (500 mM of different carbohydrates (listed in Table 2) were prepared in PBS and incubated (27° C, 30 min) with serial dilutions of the agglutinin sample (~0.20 mg/ml). Parasites or RBCs were then added and agglutination was scored. In another experiment, increasing concentrations (0–1 mg/ml) of soybean trypsin inhibitor (SBTI; Millipore Corp. Freehad, USA) were added to agglutinin samples (~0.20 mg/ml) and the residual enzyme activities were determined at each inhibitor concentration. Serial dilutions of each incubation mixture were then prepared, parasites or rabbit RBCs were added, and agglutinations scored.

Immunology procedures

Antibodies against the agglutinin were raised in a New Zealand white rabbit using a previously described protocol (Osir et al. 1986). The procedures for double radial immunodiffusion and immunoblotting have been described elsewhere (Ouchterlony 1968; Osir et al. 1989).

Results

Purification of the agglutinin

The first step in the purification of the agglutinin involved separation of the midgut extracts on a Mono Q anion-exchange column and elution of the bound proteins with a NaCl gradient. In the resulting profile, trypsin activity was detected in both the unbound and bound fractions, which had elution volumes (V_e) of 3.5 and 30 ml, respectively (Fig. 1A). However, agglutination activity was found only in the bound fractions, where it coeluted with trypsin activity at V_e=30 ml (Fig. 1A, arrow), In the second step, fractions with agglutination activity were rechromatographed on the same column but elution was carried out using a different NaCl gradient. This resulted in a single protein peak that eluted at 45% NaCl (Fig. 1B). The sample purity was ascertained by nondenaturing PAGE, which yielded a single protein band (Fig. 2). The protein had a specific activity of ~82 µmol min⁻¹ mg protein⁻¹ as determined by a specific trypsin substrate.

Agglutination properties

The ability of the isolated protein to agglutinate bloodstream-form/procyclic trypanosomes and RBCs was as-

0.2 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Fig. 1 A Separation of crude midgut extracts by anion-exchange chromatography. A sample of the midgut extract was loaded onto a Mono Q column equilibrated with buffer A. Elution was carried out using a linear gradient of 0–350 mM NaCl (0–0). Each fraction (1.0 ml) was assayed for agglutination and trypsin activities (shaded areas). The arrow shows the fractions with agglutination activities. B Peak II fractions (29–31) were rechromatographed on the same column. Elution was carried out using a 150–300 mM NaCl gradient (0–0)

sessed. As compared with bloodstream-form parasites, which gave an endpoint titer of 128, a lower protein concentration was required to agglutinate the procyclic culture forms (endpoint titer, 4096) and rabbit RBCs (endpoint titer, 2048). In a separate experiment, the agglutination activity was shown to be heat-labile since temperatures above 50° C reduced the titers by approximately 90% (Table 1).

Physical and chemical properties of the agglutinin

Electrophoresis of the agglutinin sample under non-denaturing conditions gave a single band with a native molecular weight (M_r of ~61000±3000 da (n=4; Fig. 2, lane 2). On the other hand, a calibrated Superose 6 HR 10/30 column gave a surprisingly low molecular-weight value of M_r , ~20000 da. However, as has previously been noted, gel-permeation chromatography often yields deceptively low molecular-weight estimates for some insect proteins (Telfer et al. 1983). The isoelectric point (pI) of the protein was estimated at 5.0. On SDS-PAGE, the agglutinin yielded two closely migrating subunits designated α (M_r , ~27000 da) and β (M_r , ~33000 da; Fig. 3, lane 3). Dissociation of the native protein did not require the disulfide-reducing agent β -mercaptoethanol, indicating

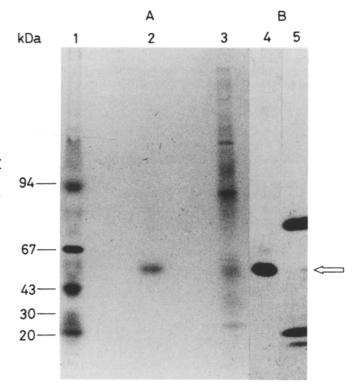


Fig. 2A, B Nondenaturing gel electrophoresis. Protein samples were separated by nondenaturing gradient PAGE (4%–15%). A Protein-stained gel (1 Low-molecular-weight standards from Pharmacia; 2 agglutinin \sim 30 μm ; 3 crude midgut extract \sim 100 μm). B Fluorogram showing the trypsins. Midgut extract and the agglutinin were preincubated with tritiated DFP before separation by nondenaturing PAGE (4 Labeled agglutinin \sim 8 μg , 5 labeled midgut extract \sim 15 μg). The arrow shows the position of the agglutinin

Table 1 Thermal stability of the agglutinin^a

Temperature (°C)	Agglutination titersb		
	Procyclic parasites	RBCs	
27	4096	2048	
37	4096	2048	
40	2048	1024	
50	4	8	
60	2	4	
70	2	2	

^a Agglutinin samples (~0.25 mg/ml) were incubated for 20 min at between 27° and 70° C and then left to attain room temperature. Serial dilutions were prepared and agglutination experiments were carried out using procyclic trypanosomes and rabbit RBCs. ^b End points expressed as reciprocals of the dilutions

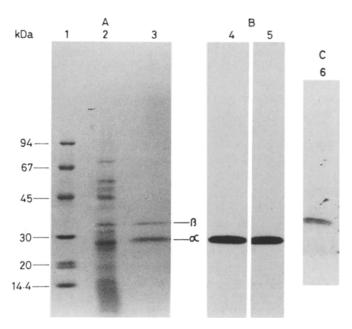


Fig. 3A–C Dissociating gel electrophoresis. Protein samples were separated by gradient SDS-PAGE (4%–15%). A Protein-stained gel (1 Low-molecular-weight standards from Bio-Rad, 2 crude midgut extract – ~40 mg, 3 agglutinin – ~15 μ g). B Fluorogram showing the trypsin band. Midgut extract and the agglutinin were labeled with tritiated DFP before separation by SDS-PAGE (4 Labeled agglutinin – ~8 μ g, 5 labeled crude midgut extract – ~10 μ g). C Carbohydrate staining. Samples were separated by SDS-PAGE and stained with PAS (6 Agglutinin – ~20 μ g)

that the two subunits are not covalently linked to each other. The presence of covalently bound carbohydrates on the β -subunit of the agglutinin was established by PAS staining of SDS-polyacrylamide gels (Fig. 3, lane 6). Lack of binding to concanavalin A indicated the absence of high mannose glycosyl residues (data not shown).

Labeling of the trypsins

When the crude midgut extract was labeled with tritiated DFP and separated by nondenaturing gel electrophoresis, at least four bands appeared (Fig. 2, lane 5). For reasons not yet clear, the lectin-trypsin complex was very faintly

Table 2 Effects of carbohydrates on agglutination^a

Type of sugar	Agglutination titersb		
	Procyclic parasites	RBCs	
Control	4096	2048	
Galactose	4096	2048	
Mannose	4096	2048	
Galactosamine	4096	2048	
Maltose	4096	2048	
Lactose	4096	2048	
Methyl α-D-glucopyranoside	4096	2048	
Methyl β-D-glucopyranoside	4096	2048	
Glucose	2048	2048	
Sucrose	1024	1024	
N-acetyl-D-glucosamine	512	256	
D-glucosamine	2	2	

^a A sample of the agglutinin (~0.20 mg/ml) was used to prepare serial dilutions in PBS. To each dilution was added 500 mM of the different sugars. Agglutination was assessed using procyclic trypanosomes and rabbit RBCs. Controls consisted of the reaction mixture in the absence of the sugars. ^b End points expressed as reciprocals of the dilutions

labeled with DFP in this case. However, strong labeling occurred when the isolated complex was used (Fig. 2, lane 4). Electrophoresis under denaturing conditions showed the trypsin activity to be localized on the α -subunit (Fig. 3, lane 4). Although four trypsins were present in the crude sample, these gave rise to a single band under denaturing conditions (Fig. 3, lane 5).

Effect of sugars on agglutination activity

Agglutination of the procyclic trypanosomes and rabbit RBCs was strongly inhibited by glucosamine (Table 2). At 500 mM, the agglutination titers were reduced by approximately 90% in both cases. On the other hand, the same concentration of *N*-acetyl-D-glucosamine reduced the titers by 27.3% and 25% in the case of rabbit RBCs and the procyclic trypanosomes, respectively. All the other sugars showed little, if any, inhibitory effect on agglutination.

Effect of SBTI on agglutination

Increasing concentrations of SBTI caused a marked inhibition of the agglutination of bloodstream-form parasites (Table 3). As compared with the control, SBTI at 1.0 mg/ml decreased agglutination of the parasites by approximately 97%. The same concentration of SBTI inhibited trypsin activity by 88%. In contrast, agglutination of procyclic parasites was unaffected by the inhibitor even at a concentration of 1.0 mg/ml. In the case of rabbit RBCs, SBTI caused only partial inhibition of the agglutination process (Table 3).

Immunology studies

In double radial immunodiffusion studies using rabbit antibodies against the isolated agglutinin, a single precipitin

Table 3 Effect of SBTI on agglutination (*pa* Partial agglutination)^a

Inhibitor	Agglutination titers ^b			Trypsin activity	
(mg/ml)	Bloodstream	Procyclic	RBCs	(µmol ml ⁻¹ min ⁻¹)	
0.0	128	4096	2048	12.3	
0.2	128	4096	pa	9.8	
0.4	64	4096	ра	6.3	
0.6	16	4096	pa	4.6	
0.8	8	4096	pa	3.2	
1.0	4	4096	pa	1.8	

^a Increasing concentrations of SBTI were incubated with samples of the isolated agglutinin (~0.20 mg/ml) and the residual trypsin activities were assayed at each inhibitor concentration. Serial dilutions of each mixture were mixed with bloodstream-form/procyclic parasites or rabbit RBCs and agglutination was assessed. ^b End points expressed as reciprocals of the dilutions

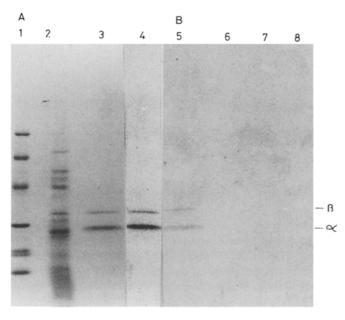


Fig. 4A, B Immunoblot analysis. Protein samples were first separated by SDS-PAGE and then transferred onto nitrocellulose paper. The blots were then reacted with the antiserum. A Proteinstained gel (1 Low-molecular-weight standards from Bio-Rad, 2 crude midgut extract – 40 μg, 3 agglutinin – ~30 μg). B Immunoblot (4 Agglutinin, 5 Glossina morsitans morsitans extract, 6 Stomoxys calcitrans extract, 7 Aedes aegypti extract, 8 Phlebotomus duboscqi extract)

band was observed with Glossina longipennis, G. morsitans morsitans, and G. pallidipes midgut extracts (data not shown). In contrast, Phlebotomus duboscqi, Stomoxys calcitrans, and Aedes aegypti midgut extracts showed no cross-reactivity. In immunoblotting experiments, the antiserum reacted with both of the agglutinin subunits (Fig. 4). No cross-reactivity was observed with any other proteins in the G. longipennis extract. Similar results were obtained with samples of midgut extracts from the other tsetse species. However, midgut samples prepared from the other blood-feeding insects showed no cross-reactivity.

Discussion

The initial discovery by Pereira et al. (1981) of lectins or lectin-like molecules in the crop, midgut, and haemo-

lymph of Rhodnius prolixus with the ability to agglutinate Trypanosome cruzi spurred the early interest in lectins in general and the roles they might play in parasitevector relationships. In tsetse flies, the presence of a glucosamine-binding lectin in the midgut was first demonstrated by Ibrahim et al. (1984). Evidence for the possible involvement of this lectin in the establishment of trypanosomes within the vector came from observations that high midgut infections resulted when glucosamine was incorporated into an infective blood meal (Maudlin and Welburn 1987). This observation led to the conclusion that the normal function of the lectins was to prevent the establishment of the parasites in the fly. The presence of glucosamine in the infective feed relieved the inhibition and, thus, facilitated the establishment of infection. However, the lectins were also needed for the parasites to differentiate (Maudlin 1991). Consequently, maintaining flies on a glucosamine-containing diet throughout their life led to the complete inhibition of lectin activity with a concomitant reduction in the number of parasites that established themselves within the fly (Maudlin and Welburn 1988a, b).

Although the involvement of lectins or lectin-like molecules in both lysis and differentiation of the parasites has become widely accepted, the mechanism of their action remains a matter of conjecture. For instance, it is unclear whether lysis and differentiation are the direct effects of the lectin activity. The whole picture is further complicated by the recent finding that trypsin or trypsin-like enzymes may also be involved in the same functions (Imbuga et al. 1992). The discovery in the present study of a bifunctional molecule with both trypsin and lectin activities, may be a reconciliation of the two views regarding the role of lectins or trypsins in trypanosome differentiation and lysis (Maudlin 1991; Imbuga et al. 1992; Osir et al. 1993).

The α -subunit was unequivocally shown to have trypsin activity since it bound tritiated DFP. Its molecular weight compares favorable with those of trypsins from other tsetse species such as *Glossina palpalis palpalis* (M_r , ~24000 and 26000 da) and *G. morsitans morsitans* (M_r , ~24000 da; Van den Abbelle and Decleir 1992). The presence of glycosyl residues on the β -subunit suggested that it was the lectin. Glycosyl residues have also been previously reported in other insect lectins (Komano et al.

1980). The molecular weight calculated for the lectin subunit was very close to that reported for hemolymph lectins of *Calliphora vomitoria* and *Sarcophaga peregrina* (M_r, ~30000–32000 da; Komano et al. 1980; McKennzie and Preston 1992) as well as *Periplaneta americana* (M_r, ~30000 da; Kubo and Natori 1987). Surprisingly, Stiles et al. (1990) have also reported the isolation of a midgut lectin in *G. palpalis* with a subunit molecular weight of M_r, ~67000 da. However, no mention was made of the native molecular weight in this case, and it is unclear how this lectin compares with the one described in the current report.

The discovery of a lectin in association with trypsin is interesting from a functional standpoint. High agglutination titers have previously been reported to result from trypsinization of RBCs (Goto et al. 1992). Similarly, Sharon and Lis (1989) have reported that agglutination of cells by lectins can be increased by mild proteolysis. In the case of the G. longipennis lectin-trypsin complex, it may be that the enzyme is required to cleave off specific surface molecule(s) from the bloodstream-form trypanosomes, a process that might expose the lectinbinding sites and, thus, facilitate agglutination. Assuming that such molecule(s) are lacking on procyclic parasites, their agglutination could occur even in the absence of trypsin activity. Stiles et al. (1990) also found that agglutination of procyclic T. brucei brucei and T. congolense was not affected by the protease inhibitor phenylmethylsulfonyl fluoride.

The specificity of the G. longipennis lectin-trypsin complex for glucosamine confirms previous reports (Ibrahim et al. 1984; Stiles et al. 1990). Interestingly, glucosamine has also been reported to be an inhibitor of midgut trypsin activity (Osir et al. 1993). It is presently unclear whether the interaction of glucosamine with the lectin-binding sites on the β -subunit interferes allosterically or sterically with the substrate-binding sites on the α -subunit. Further experiments will be needed to resolve this problem. An interesting observation was that the molecule appeared to be present only within members of Glossina species. The possibility that this might explain why tsetse flies are the only known vectors of trypanosomes might be worth exploring.

Acknowledgements We are grateful to Dr. S. K. Moloo (The International Laboratory for Research on Animal Diseases-ILRAD) for supplying *Glossina longipennis* and to Drs. L. H. Otieno and R. W. Vundla for useful discussions. This work was funded by a grant from the European Union.

References

- Abubakar L, Osir EO, Imbuga MO (1995) Properties of a blood-meal-induced midgut lectin from the tsetse fly *Glossina morsitans*. Parasitol Res 81:271–275
- Ghiotto V, Brun R, Jenni L, Hecker H (1979) Trypanosoma brucei: morphometric changes and loss of infectivity during transformation of bloodstream forms to procyclic forms in vitro. Exp Parasitol 48:447–456
- Goto R, Muramoto K, Yamazaki M, Kamiya H (1992) Purification and characterization of an agglutinin of the soft coral *Sinularia* species. Dev Comp Immunol 16:9–17

- Ibrahim EA, Ingram GA, Molyneux DH (1984) Haemagglutinins and parasite agglutinins in haemolymph and gut of *Glossina*. Trop Med Parasitol 35:151–156
- Imbuga MO, Osir EO, Labongo VL, Darji N, Otieno LH (1992) Studies on tsetse midgut factors that induce differentiation of bloodstream *Trypanosoma brucei brucei* in vitro. Parasitol Res 78:10–15
- Kapitany RA, Zebrowski EJ (1973) A high resolution PAS stain for polyacrylamide gel. Anal Biochem 56:361–369
- Komano H, Mizuno D, Natori S (1980) Purification of lectin induced in the haemolymph of *Sarcophaga peregrina* larvae on injury. J Biol Chem 255:2919–2924
- Kubo T, Natori S (1987) Purification and some properties of a lectin from the haemolymph of *Periplaneta americana* (American cockroach). Eur J Biochem 168:75–82
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
- Maudlin I (1991) Transmission of African trypanosomes: interaction among tsetse immune system, symbionts and parasites. Adv Dis Vector Res 7:117–147
- Maudlin I, Welburn SC (1987) Lectin mediated establishment of midgut infections of *Trypanosoma congolense* and *Trypanosoma brucei* in *Glossina morsitans*. Trop Med Parasitol 38:167–170
- Maudlin I, Welburn SC (1988a) The role of lectins and trypanosome genotype in the maturation of midgut infections in *Glossina morsitans*. Trop Med Parasitol 39:56–58
- Maudlin I, Welburn SČ (1988b) Tsetse immunity and the transmission of trypanosomiasis. Parasitol Today 4:109–111
- McKenzie ANJ, Preston TM (1992) Purification and characterization of a galactose-specific agglutinin from the haemolymph of the larval stages of the insect *Calliphora vomitoria*. Dev Comp Immunol 16:31–39
- Nguu EK, Osir EO, Ochanda JO, Olembo NK (1992) Isolation and properties of a 23-KD haemolymph protein from the tsetse fly, *Glossina morsitans morsitans*. Insect Sci Appl 13:189–197
- Osir EO, Labongo LV, Unnithan G (1989) A high molecular weight diapause-associated protein from the stem borer *Busse-ola fusca*: purification and properties. Arch Insect Biochem Physiol 11:173–187
- Osir EO, Wells MA, Law JH (1986) Studies on vitellogenin from the tobacco hornworm, *Manduca sexta*. Arch Insect Biochem Physiol 3:217–233
- Osir EO, Imbuga MO, Onyango P (1993) Inhibition of *Glossina* morsitans midgut trypsin activity by D-glucosamine. Parasitol Res 79:93–97
- Otieno LH, Darji N, Onyango P, Mpanga E (1983) Some observations on factors associated with the development of *Trypanosoma brucei brucei* infections in *Glossina morsitans morsitans*. Acta Trop (Basel) 40:113–120
- Ouchterlony O (1968) Handbook of immunodiffusion and immunoelectrophoresis. Ann Arbor Science, Ann Arbor, Michigan
- Pereira MEA, Andrade AFB, Ribeiro JMC (1981) Lectins of distinct specificity in *Rhodnius prolixus* interact selectively with *Trypanosoma cruzi*. Science 246:227–234
- Roditi I, Pearson TW (1990) The procyclin coat of African trypanosomes (or the not-so-naked trypanosomes). Parasitol Today 6:79–82
- Sharon N, Lis H (1989) Lectins as recognition molecules. Science 246:227-234
- Stiles JK, Ingram GA, Wallbanks KR, Molyneux DH, Maudlin I, Welburn S (1990) Identification of midgut trypanolysin and trypanoagglutinin in *Glossina palpalis* spp. (Diptera: Glossinidae). Parasitology 101:369–376
- Telfer WH, Keim PS, Law JH (1983) Arylphorin, a new protein from *Hyalophora cecropia*: comparisons with calliphorin and manducin. Insect Biochem 13:601–613
- Van den Abbelle VJ, Decleir W (1992) Study of vectorial capacity of Glossina sp. related to its digestive physiology and rearing conditions: tsetse control, diagnosis and chemotherapy using nuclear techniques. IAEA-TECDOC-634. International Atomic Energy Agency, Vienna, pp 91–103