Biochimica et Biophysica Acta, 727 (1983) 403–411
Elsevier Biomedical Press

BBA 71505

LECTIN RECEPTORS IN *TRYPANOSOMA CRUZI* AN *N*-ACETYL-D-GLUCOSAMINE-CONTAINING SURFACE GLYCOPROTEIN SPECIFIC FOR THE TRYPOMASTIGOTE STAGE

ALEJANDRO M. KATZIN and WALTER COLLI

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, C.P. 20780, São Paulo (Brazil)

(Received July 20th, 1982)

Key words: Glycoprotein; Acetylglucosamine; Lectin receptor; Chagas' disease; (Trypanosoma cruzi)

We have investigated the interaction of three lectins, differing in their sugar specificities, with the surface of the three differentiation stages of *Trypanosoma cruzi*. The Scatchard constants for each lectin and parasite stage imply that differentiation of *T. cruzi* is accompanied by changes in the cell surface saccharides. Trypomastigotes obtained from two different sources do not differ appreciably as to the number and affinity of binding sites for the three lectins employed, suggesting a similar cell-surface saccharide composition. These conclusions are reinforced by sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of the ^{131}I-labeled surface glycoproteins, following isolation by affinity chromatography. The surface membrane of trypomastigotes, the infective stage to *T. cruzi* for mammalian cells, possesses a specific glycoprotein of apparent M_r 85000 (Tc-85) which is absent from the other two stages and can be isolated by affinity chromatography on wheat germ agglutinin-Sepharose columns. This glycoprotein also binds to concanavalin A, but not to *Lens culinaris* lectin. The binding of Tc-85 to wheat germ agglutinin is unnafected by treatment of either the isolated glycoprotein or intact living trypomastigotes with neuraminidase. Since *N*-acetyl-D-glucosamine inhibits internalization of trypomastigotes by cultured mammalian cells, it is suggested that Tc-85 might be involved in adhesion and/or interiorization of *T. cruzi* into mammalian cells, possibly via recognition of an ubiquitous host-cell surface *N*-acetyl-D-glucosamine-specific receptor activity.

Introduction

Trypanosoma cruzi, the causative agent of Chagas' disease, is a parasite with at least three well-defined differentiation stages: the amastigote, which lives and reproduces inside vertebrate cells; the epimastigote, a dividing form that inhabits the midgut of a reduviid insect; and the trypomastigote, a non-dividing cell which, in nature, is the link between the vertebrate and the invertebrate phases of the protozoan biological cycle. These forms are distinct not only in terms of their host environments, but also with respect to their morphologies and biological behaviour [1–3].

Some of the differences are expressed at the level of the cell surface, as indicated by variations in the reactivity of the three forms towards lectins. However, the published results, based largely on direct agglutination or fluorescence techniques, are sometimes at variance with each other [4–8].

Attempts to characterize *T. cruzi* surface glycoproteins have also led to equally contradictory results. Snary and Hudson [9] reported finding a major glycoprotein of apparent M_r 90000 common to the three stages of the parasite. On the other

Abbreviations: PMSF, phenylmethylsulfonyl fluoride; TLCK, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone; TPCK, L-1-tosylamide-2-phenylethyl chloromethyl ketone.

hand, Nogueira et al. [10,11] reported that the 90 kDa glycoprotein is specific to the trypomastigote form and that epimastigotes and metacyclic forms from acellular cultures possess a major glycoprotein of apparent M_r 75 000. Finally, Zingales et al. [12] encountered a somewhat more complex surface protein pattern for each differentiation stage of the parasite. Thus, in trypomastigotes, several proteins, all nonexistent in epimastigotes, were found at apparent M_r higher that 95 000 and in the range between 80 000 and 95 000. Of particular interest is the trypomastigote-specific protein of apparent M_r 85 000, uncovered by sequential immunoprecipitation studies.

In an attempt to elucidate the origin of these divergent results, we have carried out a systematic study of the number and affinity of the *T. cruzi* surface receptors for concanavalin A, wheat germ agglutinin and soy bean agglutinin. In addition, we have employed affinity chromatography to characterize several of the glycoproteins presumably involved in the binding of these lectins to the parasite surface.

Materials and Methods

Parasites. Epimastigotes of the Y strain were grown in liver infusion-tryptose (LIT) medium [13,14] under constant agitation at 28°C. The parasites were collected on the third day after inoculation by centrifugation at $800 \times g$ for 10 min and washed three times with a solution of 140 mM NaCl containing 10 mM sodium phosphate, pH 7.2 (phosphate-buffered saline). Trypomastigotes of the Y strain were maintained by weekly transfers in A/Snell mice. Blood was collected on the seventh day after infection in a 3.8% solution of sodium citrate. Trypomastigotes were freed from other cells by centrifugation in a discontinuous gradient (10–15%) of metrizamide at $1000 \times g$ for 1 h [8] and subsequently washed with 1% bovine serum albumin [15]. Cell culture trypomastigotes were obtained from infected LLC-MK_2 epithelial cell monolayers maintained in Dulbecco's modified Eagle medium containing 10% fetal calf serum [16]. The trypomastigotes bursting into the medium were purified by differential centrifugation and washed three times in medium 199 at $800 \times g$ for 10 min. Amastigotes were also obtained from mice. Liver and spleen from each animal were sliced in small pieces, washed with cold phosphate-buffered saline, and homogenized in a Potter disrupter (20 μm thickness) at 150 rpm with 5 strokes. The homogenate was centrifuged at $500 \times g$ for 10 min at 4°C and the supernatant recentrifuged at 5000 $\times g$ for 10 min at 4°C. The pellet was suspended in a minimum volume of phosphate-buffered saline, layered on a discontinuous gradient of metrizamide (10–15%), and centrifuged at $1000 \times g$ for 1 h at 4°C. Parasites, collected from the interphase, were washed three times with phosphate-buffered saline and layered in a discontinuous gradient (30–60%) of Percoll [17], at $800 \times g$ for 15 min. Parasites, essentially free of cell debris, were collected from the interphase and washed three times with phosphate-buffered saline by centrifugation at $6000 \times g$ for 10 min at 4°C.

Lectins. Concanavalin A, wheat germ agglutinin and soy bean agglutinin were obtained from Pharmacia. Lectins were labeled with $Na^{131}I$ (IPEN, São Paulo) by the Iodogen (1,3,4,6, tetrachloro-3α,6α-diphenylglycoluril, Pierce) method [18]. A solution containing 500 μg of each lectin and 10 mM specific protective carbohydrate (vide infra) was incubated for 10 min at 4°C in glass tubes containing 20 μg of Iodo-gen and 300 μCi of $Na^{131}I$, in a final volume of 300 μl. The labeled lectin was dialysed for 24 h against 4 liters of 10 mM Tris-HCl (pH 7.2), containing 150 mM NaCl, 0.5 mM $CaCl_2$ and 0.5 mM $MnCl_2$. The specific activities obtained were: concanavalin A, $5 \cdot 10^5$ cpm/μg; wheat germ agglutinin, $3 \cdot 10^5$ cpm/μg; and soy bean agglutinin, $2.5 \cdot 10^5$ cpm/μg. Specific competitive carbohydrates employed for each lectin were methyl α-D-mannoside and methyl α-D-glucoside for concanavalin A, *N*-acetyl-D-glucosamine for wheat germ agglutinin and D-galactose and *N*-acetyl-D-galactosamine for soy bean agglutinin.

Surface labeling of parasites. A suspension of 10^8 parasites/ml in phosphate-buffered saline was incubated for 10 min at 4°C with 500 μCi of $Na^{131}I$ n glass tubes containing 20 μg of Iodo-gen [12]. The labeled cells were washed twice with phosphate-buffered saline and then lysed with 2% Nonidet-P40 in phosphate-buffered saline containing 1 mM TLCK and 1 mM PMSF. The lysate was centrifuged at $10\,000 \times g$ for 30 min at 4°C and the supernatant subjected to affinity chromatography.

Affinity column chromatography. Con A-Sepharose and Sepharose-WGA (wheat germ agglutinin-Sepharose) were obtained from Pharmacia. Sepharose-SBA (soy bean agglutinin-Sepharose) was prepared from CNBr-activated Sepharose 4B as described [19]. All columns were tested for their retention capacity with known glycoproteins, viz. perox idase (type IV, Sigma) for Con A-Sepharose, ovomucoid (Sigma) for Wheat germ agglutinin-Sepharose, and thyroglobulin (Sigma) for Soy bean agglutinin-Sepharose. The column volume was 0.6 ml with a height of 5 cm and a flux of 0.9 ml/5 min. The column was equilibrated with phosphate-buffered saline/2% Nonidet-P40 prior to adsorption of the proteic material. The amount of protein adsorbed was 1 mg (1 mg/ml) and elution was performed with 0.2 M specific competitive carbohydrate.

Quantification of the lectin receptors. Suspensions of $2 \cdot 10^6$ cells were incubated with increasing concentrations of non-radioactive lectin (0.2–100 μg) at a constant concentration of iodinated lectin (0.5 μg) in a final volume of 1 ml. In each experiment, controls containing specific competitive carbohydrates were run in order to measure non-specific binding. Samples were incubated at 4°C and 30°C for different times and at different lectin concentrations. After incubation, the parasites were filtered through separate 0.2 μm cellulose triacetate (Metricell) filters and washed with 20 ml of cold phosphate-buffered saline. The radioactivity retained on each filter was determined in a Beckman LS-250 spectrometer, the response for the β emission of ^{131}I being linear up to 200000 cpm. The amount of bound lectin per $2 \cdot 10^6$ parasites was calculated from the specific activities and the data plotted in accord with the Scatchard equation [20] to obtain the number of receptors (n) and the affinity constant (K_a). The molecular weights assigned to each lectin were: concanavalin A, 110000; wheat germ agglutinin, 36000; and soy bean agglutinin, 120000 [21].

Affinity chromatography of the cell surface labeled proteins. Labeled parasites were adsorbed [22] onto the detergent equilibrated Lectin-Sepharose columns after lysis with Nonidet-P40. Fractions (0.9 ml) were collected and the radioactivity was measured directly. After all the non-adsorbed material had eluted, specific carbohydrate (0.2 M final) was added to the elution buffer. The carbohydrate-eluted material was precipitated by addition of three volumes of cold ethanol and maintaining for 48 h at −20°C. The precipitated material was analysed by sodium dodecyl-polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis. Electrophoresis in the presence of 0.1% sodium dodecyl sulfate was performed on a linear gradient of 8–15% polyacrylamide according to Laemmli [23]. Phosphorylase *a* (95000), bovine serum albumin (68000), IgG heavy chain (55000) and IgG light chain (25000) previously labeled with Na^{131}I were employed as molecular weight markers. Sharp bands were always obtained with IgG peptides as long as the quantities utilized were less than 1 μg per slot. After drying, gels were subjected to autoradiography [12].

Results

The saturation kinetics were examined at two lectin concentrations (25 and 50 μg). At 30°C, 80% of the saturation value was reached after 15 min, while at 4°C binding was somewhat slower, the same level of binding being attained after 25 min. At the higher temperature, however, 30 min of incubation was sufficient to achieve saturation. Saturation times were similar for all three lectins with the three differentiation stages of the parasite. Thus, all binding experiments were performed by incubating cells at 30°C for 30 min with variable lectin concentrations (0.2–100 μg) of known specific activities. Controls in the presence of 0.1 M specific competitive carbohydrate were run, in order to measure non-specific binding. All lectin binding data were corrected for non-specific binding observed in the presence of the specific carbohydrate.

The binding results are presented in the form of Scatchard plots in Fig. 1. The capacity (n) and the affinity constant (K_a) for each lectin and differentiation stage derived from these plots are collected in Table I. Epimastogotes appear to have at least two types of concanavalin A receptors, i.e., low capacity-high affinity receptors and high capacity-lower affinity receptors. A similar bimodal distribution was found for wheat germ ag-

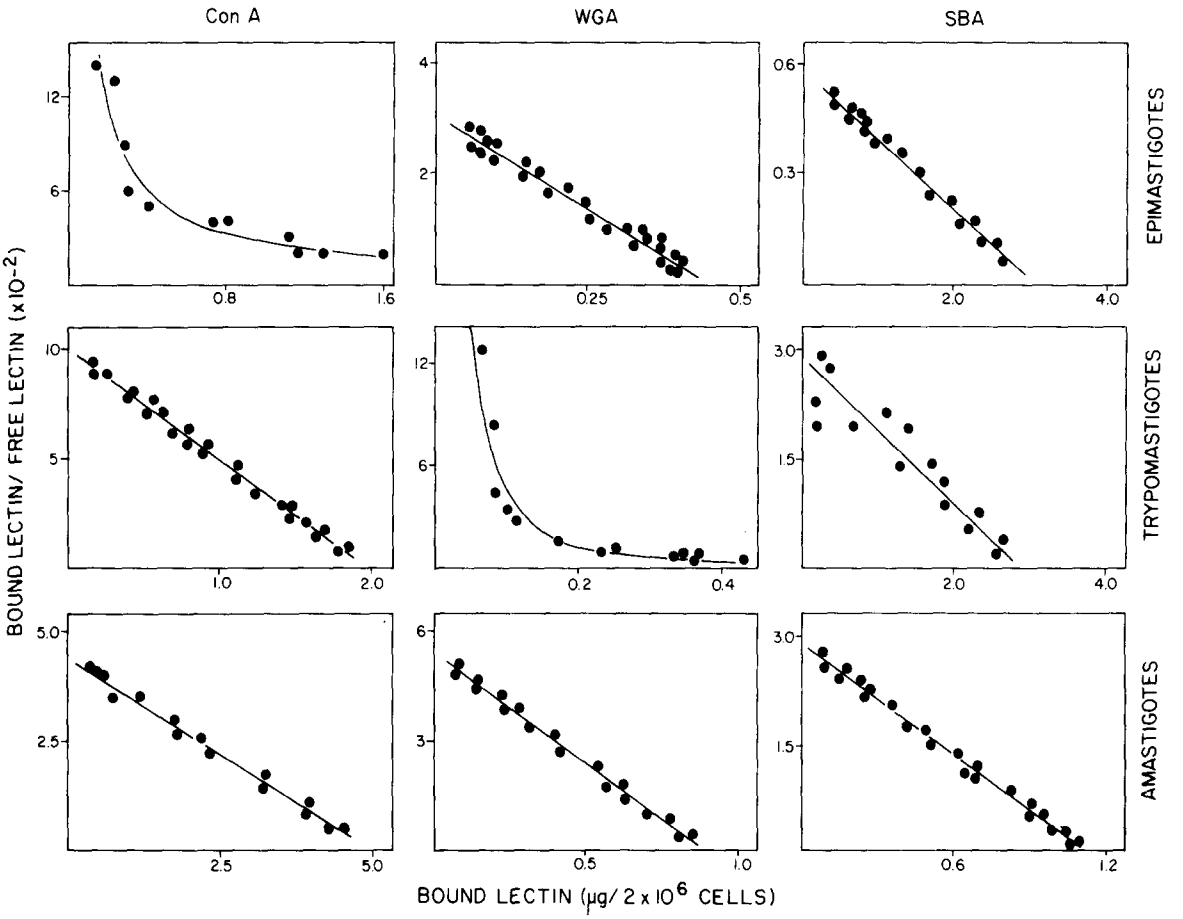

Fig. 1. Scatchard plots of lectin binding to *T. cruzi*. Experimental data were analyzed by computer (HP-85) using a standard multiple regression program. The values of n and K_a are presented in Table I.

glutinin receptors of trypomastigotes, irrespective to their origin (blood or tissue culture).

When surface-labeled and detergent-lysed parasites were chromatographed on the lectin-Sepharose columns, two peaks of radioactivity were obtained: peak 1, which contained all material

TABLE I

BINDING OF LECTINS TO THE THREE DIFFERENTIATION STAGES OF *T. CRUZI*

The values of K_a (in M^{-1}) and n (number of receptors per cell) are multiplied by 10^{-6}. The numbers of parenthesis correspond to the parameters for the lower affinity binding site.

Lectin tested	Epimastigotes		Trypomastigotes				Amastigotes	
			Tissue culture		Blood			
	n	K_a	n	K_a	n	K_a	n	K_a
Concanavalin A	2.1(4.8)	30.0(6.0)	6.0	5.0	22.0	4.6	13.0	8.3
Wheat germ agglutinin	3.0	3.0	1.2(5.9)	33.0(1.5)	2.3(8.2)	11.2(1.6)	4.6	2.4
Soy bean agglutinin	7.4	0.3	8.0	1.0	25.0	0.8	1.9	3.0

that did not adsorb to the column and peak 2, which was eluted from the column by the specific competitive carbohydrate. In all cases, the net recovery of radioactivity was 95–98%. Peak 2 usually corresponded to 1–2% of the total radioactivity. Peak 2 was absent from epimastigote preparations chromatographed on Wheat germ agglutinin-Sepharose and Soy bean agglutinin-Sepharose and from amastigote preparations chromatographed on Soy bean agglutinin-Sepharose.

Following precipitation with ethanol, column eluates were analysed by gradient gel polyacrylamide electrophoresis in the presence of sodium dodecyl sulfate. Con A-Sepharose retained several glycoproteins with apparent M_r ranging from 55 000 to 140 000 present in the three differentiation stages. Although several bands coincide, at least with respect to their molecular weights, the overall patterns are clearly stage-specific (Fig. 2, a–c). In particular, the column retains two highly radioactive epimastigote-derived bands of apparent M_r 80 000 and 95 000, confirming previous findings [12] that the major surface proteins in the epimastigote stage are glycoproteins. These glycoproteins are also found in trypomastigotes and amastigotes. In general, the proteins that bind strongly to concanavalin A appear to have a much lower affinity for wheat germ agglutinin or soy bean agglutinin since most of them appear in the flowthrough (Peak 1) of Wheat germ agglutinin-Sepharose and Soy bean agglutinin-Sepharose columns (Fig. 2d, e). Fig. 2 (f,i) indicates that trypomastigotes and amastigotes possess glycoproteins of apparent M_r higher than 95 000 which are absent in epimastigotes. As a rule, most of the proteins present in the total surface patterns of trypomastigotes and amastigotes bind to concanavalin A (Fig. 2h, k). Significantly, the same patterns are obtained even when the three protease inhibitors TLCK, PMSF and TPCK are present throughout all experimental steps.

The number of bands encountered in the concanavalin A experiments exceeds that reported for epimastigotes and trypomastigotes in previous studies [9], probably as a result of the use of *Lens culinaris* lectin bound to sepharose to identify surface glycoproteins. Thus, although both lectins have approximately the same carbohydrate specificity, the affinity constant of concanavalin A

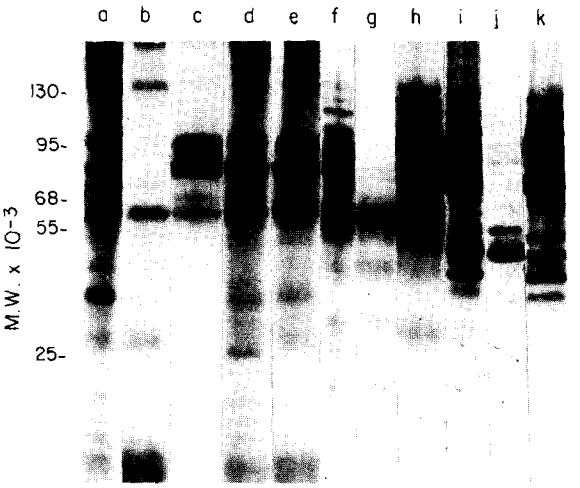

Fig. 2. Gel patterns of labeled surface proteins from *T. cruzi* stages after affinity chromatography. Epimastigotes: (a) total protein pattern, (b) peak 1 from Con A-Sepharose, (c) peak 2 from Con A-Sepharose, (d) peak 1 from Wheat germ agglutinin-Sepharose, (e) peak 1 from Soy bean agglutinin-Sepharose. Trypomastigotes: (f) total protein pattern, (g) peak 1 from Con A-Sepharose, (h) peak 2 from Con A-Sepharose. Amastigotes: (i) total protein pattern, (j) peak 1 from Con A-Sepharose, (k) peak 2 from Con A-Sepharose.

are several order of magnitude higher than those of *L. culinaris* lectin [21]. This is confirmed by the data of Fig. 3. Fig. 3c shows the banding pattern of the trypomastigote surface glycoproteins that elute in peak 2 from a *L. culinaris* Lectin-Sepharose column. The proteins that elute in the peak 1 (flowthrough) of this column (Fig. 3a) are retained on a Con A-Sepharose column and can be eluted only with the specific competitive carbohydrates (Fig. 3b). Indeed, the binding to concanavalin A is so strong that a mixture of 0.1 M methyl α-D-mannoside and 0.1 M methyl α-D-glucoside is required to elute all of the bound glycoproteins.

Only one intensely radioactive band from surface labeled trypomastigotes was retained on Wheat germ agglutinin-Sepharose (Fig. 4b). This band of apparent M_r 85 000, absent in epimastigotes and denominated Tc-85 (cf. Ref. 24), also binds to concanavalin A, but not to *L. culinaris* lectin (Table II). When Tc-85, isolated as peak 2 on Wheat germ agglutinin-Sepharose, is chromatographed on Con A-Sepharose it can be eluted only with the mixture of methyl α-D-glucoside and methyl α-D-mannoside (Table II). This material migrated as authentic Tc-85 on gel electrophoresis

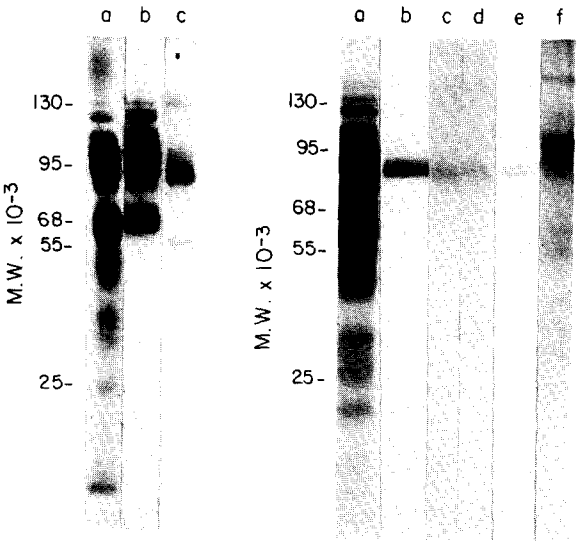

Fig. 3. Protein profiles of surface labeled trypomastigotes after affinity chromatography on Con A-Sepharose and *L. culinaris* Lectin-Sepharose. (a) Peak 1 from *L. culinaris* Lectin-Sepharose; (b) peak 1 from *L. culinaris* Lectin-Sepharose column rechromatographed on Con A-Sepharose and eluted in peak 2; (c) peak 2 from *L. culinaris* Lectin-Sepharose.

Fig. 4. Isolation and properties of trypomastigote-specific Tc-85. Trypomastigotes: (a) Peak 1 from Wheat germ agglutinin-Sepharose. (b) peak 2 from Wheat germ agglutinin-Sepharose (Tc-85), (c) Tc-85 after rechromatography on Con A-Sepharose and eluted in peak 2, (d) peak 2 from Con A-Sepharose rechromatographed on Wheat germ agglutinin-Sepharose and eluted in peak 2, (e) rechromatography of Tc-85 on Wheat germ agglutinin-Sepharose (peak 2) after neuraminidase treatment. Amastigotes: (f) peak 2 from Wheat germ agglutinin-Sepharose.

TABLE II

AFFINITY FOR *LENS CULINARIS* LECTIN AND CONCANAVALIN A AND RESISTANCE OF ISOLATED Tc-85 TO NEURAMINIDASE TREATMENT

Trypomastigotes were surface-labeled with ^{131}I, lysed with detergent, and chromatographed on a Wheat germ agglutinin-Sepharose column. Tc-85, eluted with 0.1 M *N*-acetyl-D-glucosamine, was divided in two equal aliquots. One aliquot was dialysed against an excess of phosphate-buffered saline containing 2% Nonidet-P40 and the other against distilled water. After dialysis, the first aliquot was divided into two equal parts that were chromatographed directly on either *L. culinaris* Lectin-Sepharose or Con A-Sepharose, respectively. Elution of bound material (peak 2) was performed with a mixture of 0.1 M methyl α-D-mannoside and 0.1 M methyl α-D-glucosamide. The other aliquot was precipitated with ethanol, resuspended in 0.5 ml of 50 mM sodium acetate, pH 5.0, and divided in two equal parts. One was treated with neuraminidase (0.3 units, 14 h, 37°C) and chromatographed on a Wheat germ agglutinin-Sepharose column; the other was treated identically except that neuraminidase was omitted (control). Elution of bound material (peak 2) from Wheat germ agglutinin-Sepharose was performed with 0.1 M *N*-acetyl-D-glucosamine. WGA, wheat germ agglutinin.

Fraction	Counts/min			
	L. culinaris	Con A	WGA [a]	WGA [b]
Input	51910	51910	49000	50000
peak 1 [c]	50310	3800	3360	4760
peak 2	800	48380	41600	42000

[a] Tc-85 treated with neuraminidase.
[b] Tc-85 without neuraminidase treatment (control).
[c] Flowthrough.

(Fig. 4c). Similarly, when peak 2 from Con A-Sepharose was rechromatographed on a Wheat germ agglutinin-Sepharose column, the bound material which could be specifically eluted with *N*-acetyl-D-glucosamine, also migrated as Tc-85 on gel electrophoresis (Fig. 4d).

Treatment of isolated Tc-85 with neuraminidase did not significantly alter the wheat germ agglutinin binding properties of the glycoprotein (Table II, Fig. 4e). Tc-85 is also unaffected by neuraminidase treatment of living trypomastigotes, but appears to be modified upon treatment of trypomastigotes with trypsin (150 μg/ml per 10^8 parasites, 37°C for 20 min) under conditions in which parasites do not lose viability [24].

Finally, at least three bands are apparent in the specific eluate when a lysate of surface-labeled amastigotes is chromatographed on the Wheat germ agglutinin-Sepharose column. However, since none of these has an apparent M_r of 85000 (Fig. 4f), the binding of these amastigote glycoproteins to concanavalin A was not investigated further.

Discussion

The degree of binding of the three ^{131}I-labelled lectins employed in our studies to the three differentiation stages of the parasite was substantially

lower in the presence of the specific competitive carbohydrates than in their absence, suggesting that only a small fraction of the lectin binding is due to non-specific interaction with the parasites. Binding was complete within the first 30 min at 30°C, incubation for an additional 30 min resulting in no further increase in the amount of ^{131}I-labeled lectin associated with the parasites, suggesting that endocytosis is unimportant on the time scale of experimental observation. Binding at 4°C was found to be identical to that at 30°C, reinforcing the lack of significant endocytosis. The rate of binding was, however, temperature depend ent, as has been described before for other cell systems [25], being somewhat lower at 4°C than at 30°C.

The Scatchard binding parameters n and K_a for each lectin indicate both qualitative and quantitative differences, reflecting the heterogeneity of the three main differentiation forms of *T. cruzi* with respect to carbohydrate composition of the plasma membrane. Other authors have reported differences of this type, though with less quantitative methods [26,27]. The values of n and K_a found by Pereira et al. [8] for binding of wheat germ agglutinin to epimastigotes are very similar to ours. Using the Scatchard equation [20], which is very sensitive to deviations from linearity, it was possible to demonstrate that epimastigotes and trypomastigotes have at least two receptors with different affinities and capacities for concanavalin A and wheat germ agglutinin, respectively. Except in these two cases, linearity was observed with the three lectins and parasite stages, indicating that each lectin is interacting with relatively homogenous receptors on the *T. cruzi* surface. No marked quantitative differences in the binding constants for the three lectins were found between blood and tissue culture-derived trypomastigotes. This suggests that the similarities in the carbohydrate composition of the surface membrane of trypomastigotes obtained from these two sources outweigh any eventual differences. In the case of epimastigotes one of the two types of concanavalin A receptors has a 5-fold larger affinity constant than that of trypomastigotes. This may explain the contrast between ready agglutination of epimastigotes by low amounts of concanvalin A [4] and the contradictory results [4–8] for agglutination of trypomastigotes with this same lectin. Thus, the relatively lower affinity for concanavalin A may make agglutination of trypomastigotes more sensitive to variations in the incubation conditions.

Affinity chromatography of surface-labeled parasites from the three differentiations stages on Con A-Sepharose demonstrated the common existence of two glycoproteins of M_r 95 000 and 80 000 coincident with two common antigens that undergo immunoprecipitation [12]. Snary and Hudson [9] have described a common antigen of M_r 90 000 isolated by *L. culinaris* Lectin-Sepharose affinity chromatography. The identity between their 90 000 and our 95 000 antigen has been established elsewhere [12]. In view of its much larger affinity constant (several orders of magnitude) and similar carbohydrate specificity, concanavalin A is far superior to *L. culinaris* lectin for identification and isolation of glycoproteins. This is clear in Fig. 3, which shows that trypomastigote-specific surface glucoproteins of M_r between 95 000 and 140 000 do not bind to *L. culinaris* lectin but are retained on Con A-Sepharose. The latter has been avoided in affinity chromatography of trypanosomes because recoveries are not quantitative upon elution with methyl α-D-mannoside. In this respect we have found that a mixture of 0.1 M methyl α-D-mannoside and 0.1 M methyl α-D-glucoside very efficiently elutes all concanavalin A-bound material.

Our results suggest that most of the antigens of M_r above 95 000, found in trypomastigotes but absent from epimastigotes [12], are glycoproteins with affinity for concanavalin A.

Although receptors for WGA and soy bean agglutinin should be demonstrated on the epimastigote plasma membrane (Table I), no glycoprotein from this differentiation stage exhibited binding to Wheat germ agglutinin-Sepharose or Soy bean agglutinin-Sepharose, suggesting that the cell-surface receptors for these lectins may differ in architecture from the isolated glycoproteins or may belong to a class of chemical compounds other than glycoproteins. These receptors might well be components of the epimastigote-specific glycoconjugate complex ABCD described previously [28,29]. In fact, the components of this complex do not bind to lectin columns. Alternatively, the lability of the epimastigote wheat germ ag-

glutinin receptors towards neuraminidase treatment, described by Pereira et al. [8], might indicate the existence of ganglioside or ganglioside-like compounds on the epimastigote plasma membrane.

Affinity chromatography with Wheat germ agglutinin-Sepharose yielded a glycoprotein of M_r 85 000 (Tc-85) specific to the trypomastigote stage. Although three faint bands are present in autoradiographs of the Wheat germ agglutinin-Sepharose peak 2 from surface-labeled amastigotes, none has an M_r of 85 000. The wheat germ agglutinin-binding properties of Tc-85 are unchanged upon treatment of either intact trypomastigotes or the isolated glycoproteins with neuraminidase, strongly suggesting that *N*-acetyl-D-glucosamine, and not sialic acid, is the residue responsible for the affinity for the lectin. The fact that Tc-85 is retained by Con A-Sepharose, suggests that mannosyl or sterically related residues are also exposed in the molecule.

Significantly, sera from human chagasic patients immunoprecipitate an antigen of M_r 85 000 from surface labeled trypomastigote [12]. Immunoprecipitation of Tc-85 also occurs with these sera, suggesting that this glycoproteins and the 85 000 antigens are identical [24]. In this context, we have found that *N*-acetyl-D-glucosamine specifically inhibits the interiorization of trypomastigotes into LLC-MK_2 and HeLa cells [24,30]. Similar results have been reported with BESM cells [31]. It is possible that Tc-85 is somehow involved in the interaction between parasite and host-cells, perhaps via recognition by Tc-85 of an ubiquitous (cf. Ref. 16) *N*-acetyl-D-glucosamine specific receptor activity present on the surface of mammalian cells. Since the existence of penetration-blocking antibodies has been already demonstrated [12,24], this hypothesis would be confirmed if antibodies developed against pure Tc-85 were found capable of inhibiting internalization of *T. cruzi* into mammalian cells.

Acknowledgements

This investigation was supported by grants from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Projeto CNPq/FINEP), the Financiadora de Estudos e Projetos (FINEP), the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), and PNUD/UNESCO RLA 78/024 to W.C. The authors are indebted to N.W. Andrews and Dr. B. Zingales for stimulating discussions throughout the experimental work and Dr. F.H. Quina for helpful suggestions during the preparation of the manuscript. A.M.K. is a recipient of a fellowship from CONICET, Argentina.

References

1 Hoare, C.A. (1972) in the Trypanosomes of Mammals, pp. 327–400, Blackwell Scientific Publications, Oxford and Edinburgh
2 Colli, W. (1979) in Tropical Diseases Research Series: 2 (Wallach, D.F.H., ed.), pp. 131–153, Schwabe and Co., Basel
3 Brener, Z. (1980) Adv. Parasitol. 18, 247–292
4 Alves, M.J.M. and Colli, W. (1974) J. Protozool. 21, 575–578
5 Chiari, E., De Souza, W., Romanha, A.J., Chiari, C.A. and Brener, Z. (1978) Acta Trop. 35, 113–121
6 Araujo, F.G., Handman, E. and Remington, J.S. (1980) J. Protozool. 27, 397–400
7 Katzin, A.M., Lajmanovich, S. and Gonzalez Cappa, S.M. (1980) Medicina (Beunos Aires) 40, 85–90
8 Pereira, M.E.A., Loures, M.A., Villalta, F. and Andrade, A.F.B. (1980) J. Exp. Med. 152, 1375–1392
9 Snary, D. and Hudson, L. (1979) FEBS Lett. 100, 166–170
10 Nogueira, N., Chaplan, S., Tydings, J.D., Unkeless, J. and Cohn, Z. (1981) J. Exp. Med. 153, 629–639
11 Nogueira, N., Unkeless, J. and Cohn, Z. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 1259–1263
12 Zingales, B., Andrews, N.W., Kuwajima, V.Y. and Colli, W. (1982) Mol. Biochem. Parasitol. 6, 111–124
13 Camargo, E.P. (1964) Rev. Inst. Med. Trop. (São Paulo) 6, 93–100
14 Castellani, O., Ribeiro, L.V. and Fernandes, J.F. (1967) J. Protozool. 14, 447–451
15 Katzin, A.M., Lajmanovich, S. and Gonzalez Cappa, S.M. (1977) J. Parasitol. 63, 925–927
16 Andrews, N.W. and Colli, W. (1982) J. Protozool. 29, 264–269
17 Abrahamsohn, I.A., Katzin, A.M. and Milder, R.V. (1983) J. Parasitol., in the press
18 Fraker, F.J. and Speck, J.C. (1982) Biochem. Biophys. Res. Commun. 80, 849–857
19 Cuatrecasas, P. (1970) J. Biol. Chem. 245, 3059–3065
20 Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 54, 660–672
21 Goldstein, I.J. and Hayes, C. (1978) Adv. Carbohydr. Chem. Biochem. 35, 127–340
22 Lotan, R. and Nicolson, G.L. (1979) Biochim. Biophys. Acta 559, 329–376
23 Laemmli, U.K. (1970) Nature 227, 680–685

24 Colli, W., Andrews, N.W., Katzin, A.M., Kuwajima, V.Y. and Zingales, B. (1983) in Genetic Engineering for Biotechnology (Crocomo, O.J., Tavares, F.C.A., Evans, D. and Sharp, W.R., eds.), MacMillan Publishers, New York, in the press
25 Nicolson, G.L. (1974) Int. Rev. Cytol. 39, 89–190
26 Gachelin, G., Buc-Caron, M.H., Lis, H. and Sharon, N. (1976) Biochim. Biophys. Acta 436, 825–832
27 Maher, P. and Molday, R.S. (1981) Biochim. Biophys. Acta 647, 259–269
28 Alves, M.J.M., Da Silveira, J.F., De Paiva, C.H.R., Tanaka, C.T. and Colli, W. (1979) FEBS Lett. 99, 81–85
29 Lederkremer, R.M., Alves, M.J.M., Fonseca, G.C. and Colli, W. (1976) Biochim. Biophys. Acta 444, 85–96
30 Andrews, N.W. and Colli, W. (1981) 8th Annual Meeting on Basic Research on Chagas' Disease, Caxambu, Brazil, Abstr. 104
31 Crane, M.J. and Dvorak, J.A. (1982) Mol. Biochem. Parasitol. 5, 333–341