Changes in cell-surface carbohydrates of *Trypanosoma cruzi* during metacyclogenesis under chemically defined conditions

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Highly purified lectins with specificities for receptor molecules containing sialic acid, *N*-acetylglucosamine (D-GlcNAc), *N*-acetylgalactosamine (D-GalNAc), galactose (D-Gal), mannose-like residues (D-Man) or L-fucose (L-Fuc), were used to determine changes in cell-surface carbohydrates of the protozoal parasite *Trypanosoma cruzi* during metacyclogenesis under chemically defined conditions. Of the D-GalNAc-binding lectins, BS-I selectively agglutinated metacyclic trypomastigotes, MPL was selective for replicating epimastigotes, whereas SBA strongly agglutinated all developmental stages of *T. cruzi*. WGA (sialic acid and/or D-GlcNAc specific) was also reactive with differentiating epimastigotes and metacyclic trypomastigotes but displayed a higher reactivity with replicating epimastigote forms. A progressive decrease in agglutinating activity was observed for jacaline (specific for D-Gal) during the metacyclogenesis process; conversely, a progressive increase in affinity was observed for RCA-I (D-Gal-specific), although the reactivity of other D-Gal-specific lectins (PNA and AxP) was strong at all developmental stages. All developmental stages of *T. cruzi* were agglutinated by Con A and *Lens culinaris* lectins (specific for D-Man-like residues); however, they were unreactive with the L-fucose-binding lectins from *Lotus tetragonolobos* and *Ulex europaeus*. These agglutination assays were further confirmed by binding studies using ^{125}I-labelled lectins. Neuraminidase activity was detected in supernatants of cell-free differentiation medium using the PNA hemagglutination test with human A erythrocytes. The most pronounced differences in lectin agglutination activity were observed between replicating and differentiating epimastigotes, suggesting that changes in the composition of accessible cell-surface carbohydrates precede the morphological transformation of epimastigotes into metacyclic trypomastigotes.

Introduction

During its life cycle the protozoal parasite *Trypanosoma cruzi* alternates between different morphological and functional types and different hosts, namely mammals and triatomine (cone-nosed) bugs. The infective and non-replicative trypomastigotes result from differentiation of replicative epimastigotes (within the invertebrate host) and of amastigotes (within mammal cells) (Brener, 1973).

Transformation of epimastigotes into metacyclic trypomastigotes (metacyclogenesis) will also occur *in vitro* under chemically defined conditions (Contreras *et al.*, 1985; Bonaldo *et al.*, 1988). In addition to morphological changes, the metacyclogenesis process involves differential gene expression (Contreras *et al.*, 1985; Bonaldo *et al.*, 1988) and changes in fatty acid composition (Esteves *et al.*, 1989). The process also involves changes in surface carbohydrates, since epimastigotes and trypomastigotes display different lectin agglutination profiles (Pereira *et al.*, 1980).

The study of changes in surface components during differentiation is of the utmost importance in view of the interaction of the parasite with its intermediary hosts and its adaptive responses to different environments. In this article we analyse changes in the lectin agglutination profile during metacyclogenesis of *T. cruzi* under chemically defined conditions. The results indicate that important changes occur in the pattern of surface-exposed carbohydrates and that these changes precede

Abbreviations: LIT, liver infusion tryptose medium; TAU(P), artificial triatomine urine medium (+ proline); Ep-TAU(P), epimastigotes from TAU(P); Ep-LIT, epimastigotes from LIT; Mt, metacyclic trypomastigotes.

the morphological transformation of epimastigotes into metacyclic trypomastigotes.

Methods

Parasites. T. cruzi clone Dm28c, isolated from opossum (Contreras *et al.*, 1988) was used in this study. Parasites were maintained by weekly passages in liver infusion tryptose medium (LIT) (Camargo, 1964); epimastigotes were transformed into metacyclic trypomastigotes in artificial triatomine urine medium (TAU) (190 mM-NaCl, 17 mM-KCl, 2 mM-$MgCl_2$, 2 mM-$CaCl_2$, 8 mM-potassium phosphate buffer, pH 6·0) supplemented with 10 mM-L-proline (TAUP) as previously described (Contreras *et al.*, 1985).

Agglutination with lectins. Agglutination tests were done with a Takatsy microtitrator (Cooke Engineering Co.) using suspensions containing 2×10^8 cells ml^{-1}, as described by Saraiva *et al.* (1986). The following lectins, purified as previously described (Pereira *et al.*, 1980; Esteves *et al.*, 1982), were used: *Triticum vulgaris* (WGA), *Bandeiraea simplicifolia* II (BS-II), *Aaptus papillata* (APL), *Wistaria floribunda* (WFH), *Glycine max* (SBA), *Vicia villosa* (VVL), *Maclura pomifera* (MPL), *Bandeiraea simplicifolia* I (BS-I), *Arachis hypogaea* (PNA), *Ricinus communis* I (RCA-I), *Aximella polypoides* (AxP), *Artocarpus integrifolia* (jacaline), *Canavalia ensiformis* (Con A) and *Lens culinaris* (LCL).

Radio-iodination of lectins. The lectins WGA, jacaline, PNA and BS-I were labelled with ^{125}I as described by Saraiva *et al.* (1986). The minimum agglutination dose of each iodinated lectin was the same as that of the unlabelled lectin, as determined with the different development stages of *T. cruzi.*

Neuraminidase activity assay. This was done using the PNA haemagglutination method according to Pereira (1983*a*). Briefly, human A erythrocytes from a healthy donor were incubated at a final concentration of 5% (v/v) with supernatants of cell-free LIT and TAUP for 60 min at 37 °C. The reaction was stopped by washing the erythrocytes three times in phosphate-buffered saline (PBS; 150 mM-NaCl, 20 mM-sodium phosphate buffer, pH 7·5) containing 0·5 mg BSA ml^{-1} (PBS-BSA). Erythrocytes were then resuspended in PBS-BSA and titrated against PNA by agglutination assay.

Results

Metacyclogenesis

The *in vitro* metacyclogenesis of *T. cruzi* under chemically defined conditions consists of incubating replicating (LIT) epimastigotes in TAU for a 2 h period followed by incubating the parasites in TAUP, resulting in the transformation of epimastigotes into metacyclic trypomastigotes after 72 h. Under these conditions, three distinct developmental stages of the parasite can be defined: epimastigotes (Ep-LIT), differentiating epimastigotes (Ep-TAU) and metacyclic trypomastigotes (Mt). Our previous results indicate that TAUP Mt display biological properties identical to those of triatomine Mt (Contreras *et al.*, 1988).

Table 1. *Agglutinating activity of lectins during metacyclogenesis of T. cruzi*

Values given are means of three experiments.

	Minimum concentration for agglutination (μg ml^{-1})		
	Ep-LIT	Ep-TAU	Mt
D-GlcNAc-binding lectins			
WGA	62·5	250·0	250·0
BS-II	63·5	127·0	127·0
APL	30·8	61·6	30·8
D-GalNAc-binding lectins			
WFH	92·4	46·2	23·1
SBA	0·14	0·14	0·14
VVL	>1300·0*	>1300·0*	325·0
MPL	96·2	>770·0*	>770·0*
BS-I	>2000·0*	>1000·0	250·0
D-Gal-binding lectins			
PNA	156·0	39·0	39·0
RCA I	>1000·0*	500·0	125·0
AxP	3·9	3·9	7·8
Jacaline	36·6	146·5	>586·0*
D-Man-binding lectins			
Con A	0·97	0·97	0·97
LCL	500·0	250·0	250·0

* No agglutination seen at the maximum concentration used.

Agglutination tests

Agglutination of the different developmental stages of *T. cruzi* by lectins is shown in Table 1. The binding reaction is considered to be most specific with cells that are agglutinated at the lowest lectin concentration. All types of agglutination were observed with all lectins: somatic–somatic (S–S), somatic–flagellum (S–F) and flagellum–flagellum (F–F). All the developmental stages tested were unreactive with L-fucose-binding lectins, including those of *Lotus tetragonolobus* and *Ulex europaeus.*

D-*GlcNAc-binding lectins.* Ep-LIT, Ep-TAU and Mt were strongly agglutinated by APL. However, a decreased agglutination activity was observed during the metacyclogenesis process for BS-II and WGA. All these agglutinations were inhibited by 0·1 M-D-GlcNAc.

D-*GalNAc-binding lectins.* SBA displayed a strong affinity for all developmental stages. With WFH the transformation of epimastigotes into metacyclic trypomastigotes was accompanied by increased cell interaction. However, the most striking differences were observed for VVL and BS-I, which selectively agglutinated Mt, and MPL, which preferentially agglutinated Ep-LIT. These agglutinations were inhibited by 0·1 M-D-GalNAc.

D-*Gal-binding lectins.* The reactivities of PNA and AxP were strong for all developmental stages. However,

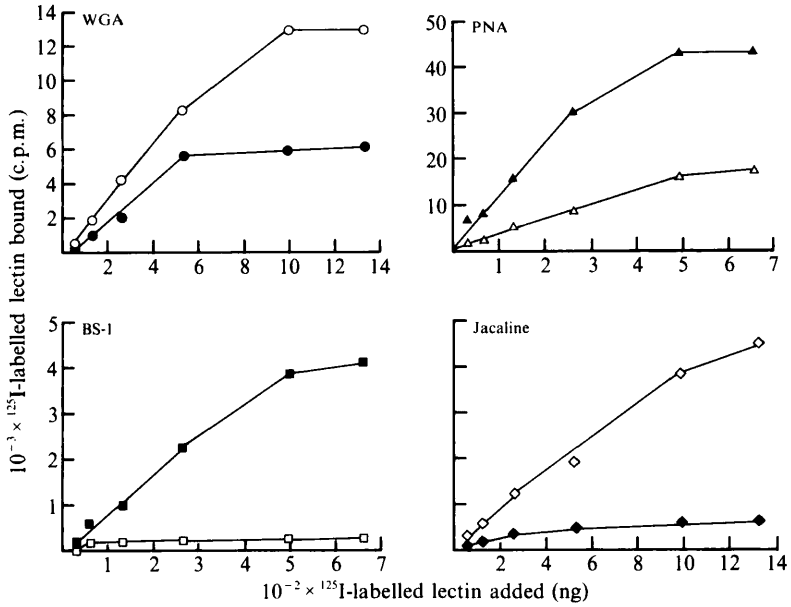

Fig. 1. Binding of ^{125}I-labelled lectins to Ep-LIT (open symbols) and Mt (filled symbols) of *T. cruzi* Dm28c. Each point is the mean of three separate binding experiments, the results from which differed by no more than 10%.

Table 2. *Agglutination of human A erythrocytes in the presence of PNA after incubation with supernatants collected during metacyclogenesis of T. cruzi*

Erythrocytes were in contact with *T. cruzi* culture supernatants for 60 min at 37 °C. Control erythrocytes did not agglutinate with PNA.

Supernatant	Minimum PNA concentration required for agglutination (μg ml^{-1})
LIT epimastigotes	250·0
TAUP metacyclic trypomastigotes	62·5

Table 3. *Binding of ^{125}I-labelled BS-I to Ep-LIT and ^{125}I-labelled jacaline to Mt of T. cruzi*

Values were calculated as in Fig. 2; only the major binding sites for each lectin were considered.

Lectin	$10^{-6} \times k$ (M)	$10^{-5} \times$ No. of lectin sites per cell
Jacaline	5·8	3·3
BS-I	4·5	3·9

differences were observed for jacaline and RCA-I: the former displayed a decreasing affinity and the latter an increasing affinity during the course of the metacyclogenesis process. The specificity of the agglutination reactions was confirmed by their inhibition with 0·1 M-D-Gal.

D-*Man-binding lectins.* Con A and LCL reacted with all developmental stages of *T. cruzi.* However, Con A showed a stronger affinity than LCL. These agglutinations were inhibited by 0·1 M-α-D-methyl mannoside.

Neuraminidase activity

Neuraminidase activity can be assayed by incubating human A erythrocytes with PNA, since these cells do not bind PNA unless they are first treated with neuraminidase (Pereira, 1983*a*; Libby *et al.*, 1986). Incubating erythrocytes with LIT and TAUP cell-free supernatants resulted in their agglutination by PNA, indicating the presence of neuraminidase activities in the supernatants. This activity was more pronounced in TAUP supernatant (Table 2).

Binding of ^{125}I-labelled lectins

In order to verify if the differences detected in the cell agglutination pattern with lectins (Table 1) were compatible with the number of the respective cell-surface-exposed receptors, we tested the binding of ^{125}I-labelled WGA, BS-I, PNA and jacaline to the different developmental forms of *T. cruzi.* This was necessary since the absence of agglutination by lectins does not necessarily mean absence of interaction with a particular cell (Nicolson, 1974). As clearly seen in Fig. 1(*a–d*), all four ^{125}I-labelled lectins tested were able to discriminate Ep-LIT from Mt. The binding was specific since it could be reversed with a 0·1 M concentration of the corresponding

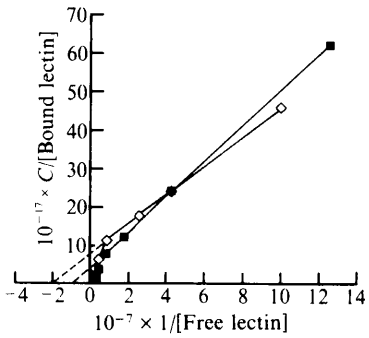

Fig. 2. Binding of ^{125}I-labelled jacaline to Ep-LIT (◇) and ^{125}I-labelled BS-I to Mt (■) of *T. cruzi* Dm28c. The data have been plotted by the method of Steck & Wallach (1965) according to the equation

$$\frac{C}{[\text{Bound lectin}]} = \frac{1}{K \cdot n} \cdot \frac{1}{[\text{Free lectin}]} + \frac{1}{n}$$

where C is the concentration of cells, n is the number of lectin molecules bound per cell and K is the association constant. For each lectin, only the high-affinity receptors were considered. All points represent means of triplicate experiments; the SD is less than 10%.

specific inhibitor saccharide. The differential agglutination of the various developmental forms of *T. cruzi* by these four lectins (Table 1) correlates with the number of exposed ^{125}I-labelled lectin receptor sites on each. The association constant and the mean numbers of lectin receptors per cell (Table 3) were calculated by plotting the binding data for BS-I and jacaline according to Steck & Wallach (1965). BS-I interacted more strongly with Mt (Fig. 1) whereas the binding of jacaline was higher with Ep-LIT (Fig. 1). It should be noted that the interaction of both lectins is heterogeneous, but only their major receptor sites were considered (Fig. 2).

Discussion

The results presented above indicate that important changes in exposed surface carbohydrates occur during metacyclogenesis of *T. cruzi*, although the cell-surface saccharide moieties resembled those detected in other trypanosomatids (Pereira *et al.*, 1980; Esteves *et al.*, 1982, 1987, 1988; Saraiva *et al.*, 1986). However, the pattern of lectin-binding sites obtained for *T. cruzi* Dm28c (isolated from opossum) differed from that obtained for the Y strain (isolated from man) (Pereira *et al.*, 1980). This observation is in agreement with the generally described heterogeneity of the parasite (Morel *et al.*, 1980; Dvorak *et al.*, 1980; Engel *et al.*, 1982; Schottelius & Uhlenbruck, 1983; Tibayrenc *et al.*, 1986; Stevens *et al.*, 1988. Aymerich & Goldenberg, 1989).

The differences observed in agglutination by lectins for the different developmental stages must reflect quite precisely the surface-carbohydrate composition of the parasite since the differentiation medium is chemically defined, containing only proline and glucose as carbon sources. This point is particularly relevant for the surface-sugar composition of trypomastigote forms, which in other studies have been obtained from infected mice and from tissue culture (Pereira *et al.*, 1980). Transformation of epimastigotes into metacyclic trypomastigotes was followed by acquisition of cell-surface-specific receptors for D-GalNAc- and D-Gal-lectins (VVL and RCA-I, respectively). Hence, cells of *T. cruzi* appear to synthesize rather than take up available carbohydrates from the environment.

Significant differences in agglutination by lectins were observed after incubation of Ep-LIT for only 2 h in TAU, for example increased agglutinating activity of PNA and RCA-I and decreased agglutination with WGA and MPL (Table 1). For some lectins, Ep-TAU resembled Mt much more than Ep-LIT, suggesting that changes in the surface sugar composition precede the morphological transformation of epimastigotes into metacyclic trypomastigotes. This is in agreement with previous observations concerning the fatty acid composition of differentiating epimastigotes (Esteves *et al.*, 1989).

Neuraminidase activity could be detected in cell culture supernatants (Table 2). Detection of neuraminidase in TAU suggests that it is secreted by the parasite, which could partially explain the low reactivity of WGA with Ep-TAU, since WGA reacts with *N*-acetylneuraminic acid as well as D-GlcNAc. Furthermore, this finding could also explain the increased binding of PNA (specific for D-Gal) since neuraminidase action leads to the generation of new galactose-receptor sites (Pereira, 1983*a*). Several studies indicate that neuraminidase may be involved in the infection of vertebrate host cells and in the release of trypomastigotes from these cells (Pereira, 1983*b*).

Studies with ^{125}I-labelled WGA, PNA, jacaline and BS-I (Fig. 1) indicate that the agglutination induced by these lectins is clearly dependent on the number and type of cell-surface lectin receptors, and not on other factors such as the mobility and accessibility of the receptors. In addition, these studies allowed the determination of the density of receptor sites during metacyclogenesis of *T. cruzi*. The number of jacaline (on Ep-LIT) and BS-I (on Mt) receptor sites were $3{\cdot}3 \times 10^5$ and $3{\cdot}9 \times 10^5$ per cell, respectively: these values fall well within the range of the number of cell-surface lectin-receptor sites (10^5–10^6) detected for other trypanosomatids (Pereira *et al.*, 1980; Esteves *et al.*, 1982; Saraiva *et al.*, 1986).

The observed changes in surface-exposed carbohydrates during metacyclogenesis of *T. cruzi* under chemically defined conditions, as judged by lectin-binding

studies, might ultimately be related to the differentiation process occurring inside the triatomine intermediary host. It is possible that the interaction of cell-surface glycoproteins with lectins, which have been described in the gut of the insect vector (Pereira *et al.*, 1981), might play a role in the morphogenesis of *T. cruzi*. Clearly, further biochemical characterization of the glycoconjugates on the surface of *T. cruzi* will be needed to help clarify their possible role during differentiation of the parasite.

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