CELL SURFACE ANALYSIS OF TRYPANOSOMES OF THE SUBGENUS *SCHIZOTRYPANUM* ISOLATED FROM BATS

PINTO A.S.*, TEIXEIRA L.F.M.*, SOUTO-PADRÓN T.** & ANDRADE A.F.B.***

Summary :

Two stocks (M5, M29) of trypanosomes of the subgenus *Schizotrypanum* were isolated from the bat *Phyllostomus hastatus* and analyzed for cell electrophoretic mobility (EPM) and lectin binding surface sites. Epimastigotes from the M5 and M29 stocks presented a mean EPM of around -0.57 and $-0.56 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$, respectively. Differences in the agglutination profiles were detected between epimastigotes or trypomastigotes from the two parasite populations using lectins with specificity for D-GlcNAc, D-GalNAc, D-Gal and D-Man as probe. Major variation was observed between epimastigote forms. Additionally, the D-GlcNAc binding lectins WGA and BS II strongly interacted with the trypomastigote from both M5 and M29 stocks; this fact is evidence that these trypanosomes are distinct from *Trypanosoma (Schizotrypanum) cruzi*.

KEY WORDS : *Schizotrypanum* from bats, electrophoretic mobility, lectin agglutination.

Résumé : ANALYSE DE LA SURFACE CELLULAIRE DES TRYPANOSOMES APPARTENANT AU SOUS-GENRE *SCHIZOTRYPANUM* ISOLÉS DE LA CHAUVE-SOURIS *PHYLLOSTOMUS HASTATUS*

Deux stocks de trypanosomes appartenant au sous-genre *Schizotrypanum*, isolés de la chauve-souris *Phyllostomus hastatus*, ont été analysés pour leur mobilité électrophorétique (MEP) et leurs profils de liaison de sites de superficie lectiniques. Les épimastigotes des stocks M5 et M29 ont présenté des moyennes MEP approximatives de -0.57 et $-0.56 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$ respectivement. Des différences des profils d'agglutination ont été détectés entre les épimastigotes et les trypomastigotes dans les deux populations de parasites, en utilisant des lectines de spécificités D-GlcNAc, D-GalAc, D-Gal et D-Man. Les variations les plus fortes ont été observées dans les formes épimastigotes. De plus, les lectines WGA et BS II, de spécificité D-GlcNAc, ont présenté une forte interaction avec les trypomastigotes des deux stocks. Ce résultat montre que ces trypanosomes ne se rattachent pas à l'espèce *Trypanosoma (Schizotrypanum) cruzi*.

MOTS CLÉS : *Schizotrypanum* de la chauve-souris, mobilité électrophorétique, interaction avec lectines.

INTRODUCTION

Trypanosomes of the subgenus *Schizotrypanum* are morphologically almost indistinguishable from each other and those from bats have cosmopolitan distribution (Hoare, 1972). In Latin America, *Trypanosoma (Schizotrypanum) cruzi*, the causative agent of Chagas' disease, can be found in several mammalian orders, including bats (Pinto and Costa Bento, 1986). Thus, in this region, it is of considerable importance to public health to determine whether isolates from bats are distinct from *T. (S.) cruzi*. Cell electrophoretic mobility (EPM) and lectin agglutination patterns have successfully been applied to characterize trypanosomes of the subgenus *Schizotrypanum*, as well as to discriminate *T. (S.) cruzi*

developmental stages (Pereira *et al.*, 1980; Shottelius *et al.*, 1983; Souto-Padron *et al.*, 1984; Souto-Padron *et al.*, 1990).

To determine whether bats isolates are distinct from *T. cruzi*, we analyzed the cell surface charge and the surface lectins binding sites of two stocks of parasites, of the subgenus *Schizotrypanum*, isolated from the bat *P. hastatus* in Minas Gerais, Brazil.

MATERIALS AND METHODS

PARASITES

The M5 and M29 stocks of the subgenus *Schizotrypanum* were isolated from *P. hastatus* bats collected in Pedro Leopoldo and Serrania, Minas Gerais, Brazil, respectively. Isolation was performed by hemoculture in Brain-Heart-Infusion (BHI) medium supplemented with 10 % (v/v) heat-inactivated fetal calf serum (FCS) and 2.5 (v/v) of 10 % rabbit hemoglobin solution, 100 IU penicillin/ml, and 50 g streptomycin/ml. Flagellates were maintained by serial passages every 10 days. They were cryopre-

* Departamento de Microbiologia, Instituto de Ciências Biológicas, CP 486, Universidade Federal de Minas Gerais 31270-010, Belo Horizonte, MG, Brasil.

** Instituto de Biofísica Carlos Chagas Filho,

*** Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, 21949-900, Rio de Janeiro, RJ, Brasil.

served in liquid nitrogen after adding 10 % glycerol to the cultures.

PARASITE MASS

Parasite cells were obtained in 18 × 180 mm screw-capped tubes containing 5.0 ml of BHI medium supplemented with FCS and hemoglobin as described above but without antibiotics. Inocula always consisted of about 4 % (v/v) of a mid-log phase growth culture kept at 28 °C. Epimastigote and trypomastigotes forms were harvested from 8-days or 27-days-old cultures, respectively.

ELECTROPHORETIC MOBILITY ASSAY

Epimastigotes were collected by centrifugation (1.500 g, 10 min, 4 °C), twice washed with 0.1 M phosphate buffer, pH 7.2 and fixed for 1 hr in 2.5 % glutaraldehyde in 0.1 M phosphate buffer. After fixation cells were washed twice in a 0.85 % sodium chloride solution with an ionic strength of 0.145 mol. dm⁻³, pH 7.2. The electrophoretic mobility (EPM) of the cells was determined in a Zeiss cytopherometer with a current of 4-6 mA and a final voltage of 100 V. The cell suspension was placed into a chamber and then allowed to equilibrate for 10 minutes. Measurements were made at 25 °C. When the current was switched on, the time necessary for one cell to travel across two vertical lines, separated by a distance of 16 mm was measured. Then the polarity was reversed and time was measured again for the cell traveling in the opposite direction. About 35 cells were measured for each sample analyzed. Calibration of the equipment was made by measuring the electrophoretic mobility of fresh human erythrocytes. Statistical analysis was performed using the T-test.

LECTIN AGGLUTINATION ASSAY

Epimastigotes or trypomastigotes were collected and washed as described above. Agglutination assays were performed with a Takasys microtritor plate (Cooke Engineering, VA). Equal volume (25 ml) of the parasite suspension containing $1-5 \times 10^8$ cells/ml and the lectin dilution were mixed and placed at room temperature for 1 h. The agglutination of cells was always scored visually with a hand lens after gently resuspending the settled cells and by microscopic observations. Agglutination inhibition assays were performed with four agglutinating units of lectin and 0.1 M concentration of each specific carbohydrate. The lectins used and their concentrations are identified in Table I. All lectins were purchased from Sigma Chemical Co. (St.-Louis).

RESULTS

ELECTROPHORETIC MOBILITY

Epimastigote forms of the two stocks of trypanosomes studied under standard conditions had negative surface charge. Population analysis indicated that the cells of both M5 and M29 stocks were homogeneous in their EPM (Fig. 1). The orientation of the parasites was random during the migration towards the positive electrode.

Fig. 1. — Electrophoretic mobility (EPM) distribution among epimastigote forms of trypanosomes of the subgenus *Schizotrypanum* isolated from the bat *Phyllostomus hastatus* in Minas Gerais, Brazil. A, M5 Stock; B, M29 stock.

LECTIN AGGLUTINATION

The agglutination patterns of epimastigote and trypomastigote stages of the two stocks are shown in Table I. Results are presented as minimum concentration required to agglutinate the flagellates. The binding reaction is considered to be most specific with cells that are agglutinated at the lowest lectin concentration. When epimastigotes and trypomastigotes were agglutinated, mixed types of clumps were observed, *i.e.*, body-body, body-flagellum and flagellum-flagellum. Quantitative and qualitative differences in the agglutination patterns were observed. Major variations were observed between epimastigotes, where WGA, SBA, HP, dB, NP and RCA I lectins clearly discriminated the two parasite populations. On the other hand, WGA, WFH, SBA and Con A strongly interacted with epimastigote and trypomastigote stages from the two stocks of parasites. Evidence for the interaction of WGA with sialic residues on carbohydrate determinants was observed. The digestion of epimastigotes of the M5 or M29 stocks with *Clostridium perfringens* sialidase completely abolished the ability of WGA to agglutinate the cells. Such treatment exposed receptor sites for the interaction with PNA lectin (data not shown).

DISCUSSION

Epimastigotes of the two parasite populations analyzed presented very similar values of surface negative charge. These values are closely related to those observed for *T. (S.) cruzi* or *Trypanosoma (Schizotrypanum) vespertilionis* and distinct from *Trypanosoma (Schizotrypanum) dionisii* or *Trypanosoma (Schizotrypanum) myoti* (Souto-Pradón *et al.*, 1990).

On the contrary, the lectins used clearly discriminated the two stocks of trypanosomes from each other. Indeed, these two populations are also different in their *Eco*R1 k-DNA digestion products and glucose phosphate isomerase (GPI) isoenzymatic patterns (Teixeira *et al.*, 1993).

Probably the most interesting finding in our study was the agglutination of trypomastigotes from both parasite populations with the *D-GlcNAc*-binding lectins, WGA and BSII. These lectins did not react with *T. (S.) cruzi* trypomastigotes obtained in similar conditions (Pereira *et al.*, 1980). Additionally, the isoenzymatic patterns of these two stocks studied are different from those of *T. (S.) cruzi* Z1, Z2, Z3 and ZB *T. (S.) cruzi* reference zymodemes (Teixeira *et al.*, 1993).

Carbohydrate specificity and lectins	Minimum concentration required to agglutinate (µg/ml)			
	Epimastigotes		Trypomastigotes	
	M5 stock	M29 stock	M5 stock	M29 stock
<i>D-GlcNAc</i>				
WGA (Weat germ agglutinin)	7.8	62.5	31.2	31.2
BS II (<i>Bandeiraea simplicifolia</i> II)	ND	31.2	125.9	250.0
<i>D-GalNAc</i>				
WFH (<i>Wistaria floribunda</i>)	0.12	0.24	0.12	0.12
SBA (Soybean agglutinin)	7.8	31.2	15.6	31.2
HP (<i>Helix pomatia</i>)	250.0	500.0	250.0	500.0
DBL (<i>Dolichos biflorus</i>)	500.0	1,000.0	1,000.0	1,000.0
<i>D-Gal</i>				
PNA (Peanut agglutinin)	500.0	250.0	1,000.0	1,000.0
RCAI (<i>Ricinus communis</i>)	250.0	7.8	31.2	15.6
<i>D-Man and similar</i>				
Con A (<i>Canavalia ensiformis</i>)	0.49	0.98	0.98	0.49
LCL (<i>Lens culinaris</i>)	250.0	250.0	500.0	500.0

ND = not determined.

Table I. — Activity of various lectins for parasites of the subgenus *Schizotrypanum* isolated from the bat *Phyllostomus hastatus* in Minas Gerais, Brazil.

Chagas' disease remains a serious public health problem in Latin America where bats were frequently found infected by trypanosomes of the subgenus *Schizotrypanum* (Pinto and Costa Bento, 1986). More extensive studies are required to characterize surface components of flagellates of this subgenus. In particular, isolates from bats collected over a wider geographic area need to be examined. Perhaps, improved agglutination tests with trypomastigotes using the *D-GlcNAc*-binding lectins, mainly WGA, may be useful tool to distinguish *T. (S.) cruzi* from others *Schizotrypanum* isolate from bats in endemic areas.

analysis of trypanosomes of the subgenus *Schizotrypanum* from the bat. *Parasitology Research*, 1983, 79, 497-500.

Reçu le 27 octobre 1995
 Accepté le 10 janvier 1996

ACKNOWLEDGEMENTS

This work was supported by grants from CNPq, FAPEMIG and Pro-Reitoria de Pesquisa da Universidade Federal de Minas Gerais, Brazil. We thank Dr. Michel Tibayrenc for the "résumé" revision.

REFERENCES

- HOARE C.A. The trypanosomes of mammals. A zoological monograph. Blackwell, Oxford, Edinburgh, 1972.
- PEREIRA M.E.A., LOURDES M.A., VILLALTA F. & ANDRADE A.F.B. Lectin receptors as markers for *Trypanosoma cruzi*. *Journal of Experimental Medicine*, 1980, 152, 1375-1392.
- PINTO A.S., RENAULT C.P., TAFURI W.L. & CHIARI E. *Trypanosoma cruzi*-like trypanosomes isolated from the bat *Phyllostomus hastatus*. *Memorias do Instituto Oswaldo Cruz*, 1987, 82, BI-10.
- PINTO A.S. & COSTA BENTO D.N. *Trypanosoma cruzi*-like bloodstream trypomastigotes in bats from the State of Piauí, Northeastern Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 1986, 19, 31-34.
- SCHOTTELIUS J., KOCH O. & UHLENBRUCK G. Differentiation of *Trypanosoma cruzi* Chagas, 1909 and *Trypanosoma vespertilionis*, Battaglia, 1884 by various lectins. *Tropenmedizin und Parasitenkunde*, 1983, 34, 89-92.
- SOUTO-PADRÓN T., CARVALHO T.U., CHIARI E. & DE SOUSA W. Further studies on the cell surface charge of *Trypanosoma cruzi*. *Acta Tropica*, 1984, 41, 215-225.
- SOUTO-PADRÓN T., CHIARI E. & DE SOUSA W. The surface charge of trypanosomatids of the genus *Trypanosoma*. *Memorias do Instituto Oswaldo Cruz*, 1990, 85, 215-219.
- TEIXEIRA L.F.M., GONÇALVES A.M., ROMANHA A.J., STEINDEL M. & PINTO A.S. Schizodeme and zymodeme