GLYCOPROTEINS FROM *TRYPANOSOMA CRUZI*: PARTIAL PURIFICATION BY GEL CHROMATOGRAPHY

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1. Introduction

Glycoproteins from cell membranes have been related to several functions like antigenicity, cellular adhesion and hormone recognition, among others [1]. Glycoproteins of unicellular eukaryotes have been much less studied when compared with higher organisms. In the cell surface of African trypanosomatids these macromolecules were related to antigenicity and host-parasite relationship [2–7]. Recently, we reported a differential agglutination in the presence of concanavalin A between epimastigote and trypomastigote forms of *Trypanosoma cruzi* [8]. The agglutinability of the epimastigote forms suggested the existence of reacting glycoproteins toward con A in their cell surface. This report describes the partial purification and some properties of a glycoprotein complex of whole-cell epimastigote forms of *T. cruzi*.

2. Methods

Polyacrilamide gel electrophoresis was performed either in the absence [9] or in the presence [10] of 0.1% sodium dodecyl sulfate (SDS). Total neutral sugars were determined by the phenol–sulfuric acid method [11] and sialic acid was measured by the thiobarbituric acid assay [12]. Ribose was determined by the orcinol method [13]. The agglutination experiments were as described by Alves and Colli [8]. Epimastigote forms of *T. cruzi* (Y strain) were cultivated in medium LIT [14]. The cells were collected in the late log phase by centrifugation and washed three times with 0.9% NaCl (w/v). The cell suspension was then sonicated (Branson, 4 pulses of 15 sec, medium power) and extracted with 88% phenol (v/v) for 30 min at room temperature. Centrifugation of this mixture at 14 000 *g* for 15 min yielded an aqueous phase which, after extraction (three times), was precipitated with 4 vol of ethanol. This precipitate after standing overnight at −20°C was collected by centrifugation dried under vacuum and dissolved in water (aqueous fraction).

3. Results and discussion

The aqueous fraction contained, respectively, 5.2% and 0.23% of the original carbohydrate and protein present in the cell extract.

The glycoprotein complex behaved as a single band with low penetration in 5% polyacrilamide gels. In the presence of 0.1% SDS four discrete Schiff positive bands were observed (fig.1a, bands A, B, C, and D). The glycoprotein nature of these fractions was confirmed by: 1) staining of independent gels with the Schiff reagent and Coomassie Blue gave four bands with identical pattern; 2) restaining of Schiff prestained gels with amidoblack showed coincident bands [15], a control gel containing only amilose displayed a strong Schiff positive band and did not stain with amidoblack; 3) treatment of the complex with pronase (4 mg/ml, 36 hr, 37°C), produced a modification in the band pattern (fig.2). Except for those described, no other sugar or protein positive material could be detected in the gels.

Partial resolution of this glycoprotein complex could be achieved by Bio-Gel P-150 column chromato-

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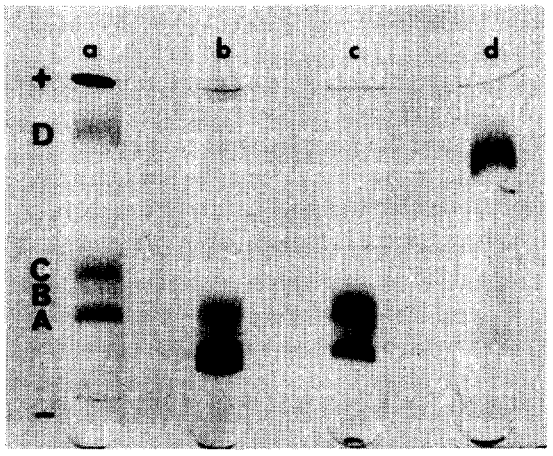

Fig.1. Polyacrilamide gel electrophoresis of the glycoprotein fractions isolated from *T. cruzi*. Gels (10%) were subjected to electrophoresis in 0.1 M sodium phosphate, pH 7.2 containing 0.1% SDS. Staining was made with the Schiff reagent: a) aqueous fraction; b), c) and d) correspond, respectively, to peaks I, II and III of fig. 3. Bromophenol Blue was used as reference. Migration was from – to +.

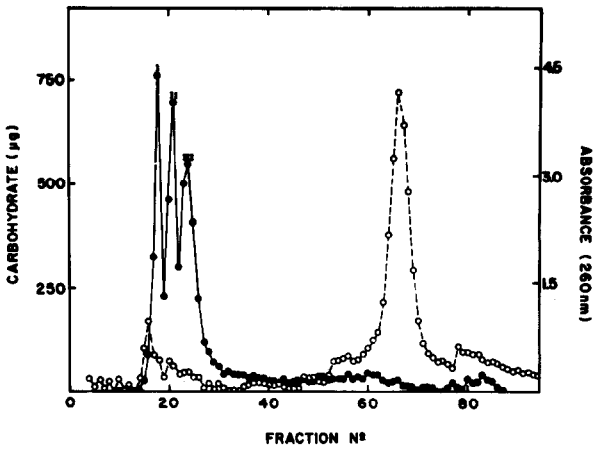

Fig.3. Gel chromatography of the aqueous fraction. The aqueous fraction (4 mg carbohydrate) was dissolved in a buffer containing 0.1 M sodium phosphate, pH 7.2 and 0.1% SDS and applied to a column of Bio-Gel P-150 (2.6 × 80 cm). Pre-equilibration of the column and elution was made with the same buffer; (●——●) carbohydrate was measured by the phenol–sulfuric acid method; (○——○) absorbance at 260 nm.

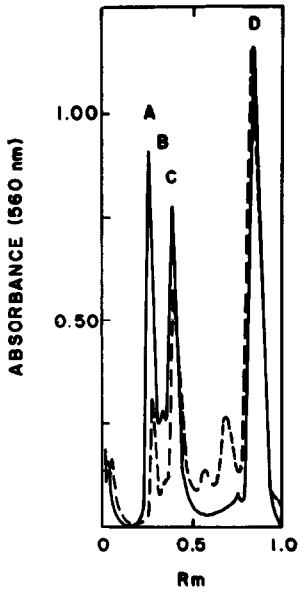

Fig.2. Espectrophotometric profiles of polyacrilamide gels of the aqueous fraction treated (– – – – –) and not treated (———) with pronase. The enzyme was incubated at 80°C for 15 min before use. Scanning was made at 560 nm after staining with the Schiff reagent. A control gel (not shown) containing only pronase did not stain under the same conditions.

graphy in the presence of 0.1% SDS (fig.3). Three sugar positive peaks (I, II and III) were obtained. Polyacrilamide gel electrophoresis of these fractions in the presence of 0.1% SDS (fig.1b,c,d) shows that peak III is almost pure since 88% of its composition is represented only by band D. This result was obtained by calculating the areas under the curves after densitometry of the Schiff stained gels (table 1).

Table 1
Relative proportion of glycoprotein components during purification

Bands	Aqueous	I	II	III
A	22.0	48.8	35.9	5.1
B	12.0	–	–	–
C	28.0	44.1	59.8	7.0
D	3.8	7.1	4.3	87.9

Aqueous fraction and peaks I, II and III obtained by gel chromatography (fig.3) were subjected to polyacrilamide gel electrophoresis in the presence of 0.1% SDS. The gels were stained with the Schiff reagents as described in Methods. The areas under the curves were determined by densitometry at 560 nm. The values are given in percentage of the total area.

The apparent disappearance of band B is probably due to an overlap of bands A and C since all three migrate very close to each other. Column chromatography also eliminated a 260 nm absorbing material which was tentatively identified as composed of ribonucleotides.

It is very difficult to predict the real number of isolated glycoproteins if the aggregation properties of these molecules in aqueous solutions (even in the presence of SDS) are considered [16–19]. This aspect will be further clarified after a complete purification of the glycoprotein components in the presence of various dissociating agents followed by a quantitative characterization of the carbohydrate and aminoacid composition belonging to these components.

A preliminary study on the composition of the aqueous fraction after acid hydrolisis has shown the presence of glucosamine, galactose, glucose and mannose. Xylose, present in diminute quantities could not be detected in some preparations. Sialic acid seems to be present in amounts of 1–2 μg/100 μg total carbohydrate. The aminoacids present in higher relative amounts were Lys, Asx, Glx, Ala, Thr, Ser, Pro and Gly, in that order.

The cellular location of these glycoproteins is not known. However, it could be demonstrated that they sediment with the cell particulate fraction since all bands appeared in a polyacrilamide-SDS gel electrophoresis of the 100 000 *g* sediment of the cell homogenate.

The glycoprotein complex is able to inhibit the agglutination of epimastigotes by con A in concentrations above 10 μg/ml. Taking into consideration the specificity of the lectin and the carbohydrate composition of the isolated glycoprotein complex it is quite possible that these substances belong to the cell surface of *T. cruzi*. These aspects will be further explored by the isolation and characterization of the membrane of *T. cruzi*.

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