# CHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION OF SPECIFIC GLYCOPROTEINS FROM *TRYPANOSOMA EQUIPERDUM* VARIANTS

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## 1. Introduction

Antigenic variation has been described in different species of trypanosomes: *Trypanosoma brucei* [1–3], *Trypanosoma congolense* [4], *Trypanosoma vivax* [5–6] and *Trypanosoma equiperdum* [7–8]. The capacity of variation within a clone of *Try. equiperdum* seems to be very extensive; up to 100 variants appearing in a particular sequence have been isolated in experimentaly infected rabbits [8]. It seems now established, that *Try. brucei* [9] and *Try. equiperdum* [10] undergo antigenic variation by changing their cell coat composed of antigenically different glycoproteins.

Several clone specific glycoproteins have been purified by isoelectric focusing in the species *Try. brucei* [9] and shown to have no sequence homology in the N-terminal region [11].

Recently, we reported the isolation by affinity chromatography on concanavalin A–Sepharose of several clone specific antigens from *Try. equiperdum* [10]. The glycoproteins of two species of trypanosomes (*Try. bucrei* and *Try. equiperdum*) seem to have the same mol. wt: 65 000 and 66 000, respectively, as determined by polyacrylamide gel electrophoresis.

We describe here the results of amino acid and carbohydrate analysis of several clone specific glycoproteins of *Try. equiperdum* and report some of their immunological properties.

## 2. Methods

### 2.1. *Isolation of specific glycoproteins*

The clone specific glycoproteins were purified by affinity chromatography on concanavalin A–Sepharose (Con A–Sepharose) as described previously [10].

Trypanosomes were isolated from rats infected for 2 days on DEAE cellulose columns as described by Lanham [12]. After gentle agitation overnight at 4°C, the suspension of trypanosomes (1 vol. pellet + 6 vol. phosphate buffer 0.12 M, pH 5.5 containing 1% glucose) was centrifuged at 4000 × *g* for 30 min. The supernatant was dialysed against 0.5 M NaCl and centrifuged at 90 000 × *g* for 1 h. 30 ml of the soluble extract containing 10–20 mg clone-specific glycoprotein was applied to a 10 ml Con A–Sepharose column previously equilibrated with 0.5 M NaCl. After washing the column with 0.5 M NaCl, the glycoprotein was eluted with 0.5 M NaCl containing 10% (w/v) α-methyl-D-mannoside. The glycoprotein fraction was extensively dialysed against distilled water at 4°C and lyophilized.

The glycoproteins were purified from several *Try. equiperdum* variants appearing at different stages of the infection. BoTat-1, the basic antigenic type and BoTat-2 appear during the first 10 days of the disease. BoTat-78 and BoTat-100 always develop in 3–4 weeks; BoTat-28 appears later during the infection of the rabbits.

The purity of the different preparations was checked by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate [13] and by immunoelectrophoresis.

### 2.2. *Immunological studies*

Antisera were raised in rabbits by giving five injections at 2 week intervals of 1 mg purified glycoprotein emulsified with complete Freund's adjuvant. The immunological purity of the clone specific antigens was confirmed by immunoelectrophoresis.

The immunogenicity and ability of the basic strain (BoTat-1)-specific antigen to induce immunity in mice were tested. Groups of 10 mice were injected intraperitoneally on days 0, 14 and 21 with different concentrations of glycoprotein dissolved in physiological saline. Mice were challenged by injection of 1000 trypanosomes 14 days after the last injection of antigen.

### 2.3. *Chemical analyses*

Amino acid analysis were carried out on a Multichrom B Beckman analyser after hydrolysis of the samples in 5.6 N HCl for 24 h at 110°C in evacuated sealed tubes in the presence of nitrogen.

Quantitative gas–liquid chromatography of carbohydrates was performed by the slightly modified method of Reinhold [14,15].

## 3. Results and discussion

The results of the amino acid compositions are expressed in table 1. The different glycoproteins are rich in aspartate, glutamate, threonine, alanine and lysine. The antigens differ greatly in their leucine content. The diversity of amino acid compositions confirms the mutation hypothesis of the origin of variants which are probably the result of genotypic selection.

The glycosylated nature of the variant specific antigens was indicated by the periodic acid Schiff method and by their affinity for Con A [10]. Table 2 summarizes the carbohydrate compositions of three glycoproteins as found by gas chromatography. Apart from traces of glucose, mannose, galactose and

Table 2
Carbohydrate content of purified glycoproteins from variant BoTat-1, -78, -28

| Sugars | BoTat-1 | BoTat-78 | BoTat-28 |
|---|---|---|---|
| Mannose | 222 | 230 | 222 |
| Galactose | 128 | 119 | 133 |
| Glucose | 11 | 33 | 36 |
| *N*-Acetylglucosamine | 45 | 52 | 44 |

Values expressed as μmol/g dry wt preparations

Table 1
Amino acid composition of purified glycoproteins from variant BoTat-1, -2, -78, -100, -28

| Amino acid | | BoTat-1 | BoTat-2 | BoTat-78 | BoTat-100 | BoTat-28 |
|---|---|---|---|---|---|---|
| Asp | → | 13.81 | 10.21 | 11.12 | 12.45 | 10.57 |
| Thr | → | 8.91 | 10.82 | 10.96 | 10.22 | 10.72 |
| Ser | | 7.15 | 8.35 | 6.35 | 6.54 | 6.66 |
| Glu | → | 10.08 | 11.21 | 12.82 | 11.26 | 12.45 |
| Pro | | 3.08 | 3.58 | 2.99 | 5.48 | 3.37 |
| Gly | | 8.40 | 7.81 | 7.78 | 8.95 | 9.32 |
| Ala | → | 15.36 | 14.15 | 13.53 | 12.32 | 14.83 |
| Val | | 3.01 | 3.32 | 3.94 | 3.53 | 4.30 |
| 1/2 Cys | | 1.02 | 1.77 | 2.35 | 2.21 | 2.46 |
| Met | | – | 0.46 | 0.18 | 0.34 | – |
| Ile | | 2.25 | 3.50 | 2.87 | 2.81 | 3.43 |
| Leu | → | 10.46 | 7.34 | 6.23 | 5.66 | 4.21 |
| Tyr | | 2.09 | 2.38 | 1.94 | 2.25 | 2.08 |
| Phe | | 2.01 | 1.43 | 2.68 | 1.58 | 2.66 |
| Lys | → | 8.57 | 8.61 | 10.49 | 9.84 | 9.38 |
| His | | 0.85 | 2.05 | 1.46 | 2.51 | 0.91 |
| Arg | | 2.94 | 3.00 | 2.32 | 2.07 | 2.67 |

Values expressed as residues/100 amino acid residues

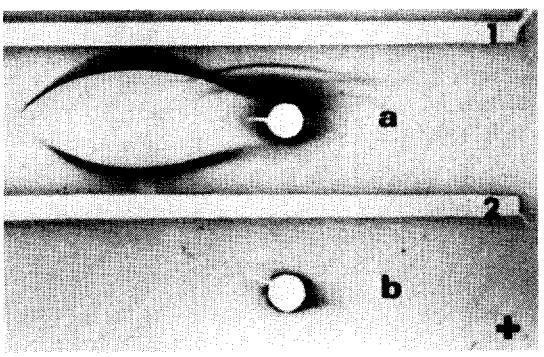


Fig.1. Immunoelectrophoretic patterns of total soluble extracts from variant BoTat-28 (a) and Botat-1 (b) after reaction with antisera raised in rabbits with a total soluble extract (1) and the purified glycoprotein (2) from BoTat-28.

*N*-acetyl glucosamine were the only sugars found. No sialic acid could be detected. The three sugars are present in constant amounts (7–8%, w/w) and proportions. Identical sugar compositions were found when the glycoproteins were eluted with α-methyl-D-glucoside instead of α-methyl-D-mannoside.

Preliminary results obtained in our laboratory have shown that some variants (BoTat-78, -100) are agglutinated specifically by *Ricinus communis* 120 agglutinin and Con A (to be published).

Taking into consideration the affinity for the lectins [16,17] and the carbohydrate composition of the isolated glycoproteins, it is quite possible that the carbohydrate structures are exposed at the surface of the parasites and may play an important role in the host–parasite relationship.

Hyperimmune sera raised in rabbits gave only a single precipitin line by immunoelectrophoresis when tested against a homologous total extract of trypanosomes (fig.1). No interaction between heterologous antisera and glycoprotein extracts could be detected.

These results show the immunological purity of the glycoproteins and the lack of common antigenic determinants of the glycoproteins. Clone-specific protection against homologous infection was obtained by immunizing mice with as little as 3 μg soluble purified antigen (table 3). Trypanosomes were never detected in the blood of mice which survived a challenge showing the high immunogenicity of the glycoprotein component. The protection confirms the specificity of the variant glycoproteins responsible for the antigenic variation of *Try. equiperdum*. Preliminary results have indicated the absence of sequence homology in the N-terminal region of several clone-specific glycoproteins (unpublished results); the differences in their amino acid compositions and the absence of immunological cross reactions offer an explanation for the antigenic variation. However, in contrast to the amino acid compositions, sugar analyses seem to indicate the presence of a constant carbohydrate structure.

Further work is in progress to localize the lectin receptors on the parasite surface and to investigate the glycoprotein structure.

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Table 3
Variant BoTat-1 specific immunization of mice by purified glycoprotein

| Mice challenged with: | Total amount of soluble BoTat-1 glycoprotein injected | Survival at day 30 |
|---|---|---|
| BoTat-1 | 0.3 μg | 0/10 |
| BoTat-1 | 3.0 μg | 7/10 |
| BoTat-1 | 30.0 μg | 6/10 |
| BoTat-28 | 30.0 μg | 0/10 |

(No protection could be obtained by challenging mice with an heterologous variant : ex. BoTat-28)

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