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VARIANT SPECIFIC GLYCOPROTEINS OF TRYPANOSOMA EQUIPERDUM : CROSS REACTING DETERMINANTS AND CHEMICAL STUDIES

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SUMMARY - Two variant specific surface antigens (VSSA) (BoTat-1 and BoTat-28) were purified from clones of Trypanosoma equiperdum and tested for immunological cross reactivity. Competitive radioimmunoassay revealed the presence of cross reacting determinants. Limited tryptic cleavage of BoTat-28 resulted in the purification of two fragments F_1 and F_2 with respectively 38,000 and 28,000 apparent molecular weight. F_1 represents a large N-terminal fragment containing 30 % of the total sugar content. F_2 is characterized by an N-terminal amino acid sequence different from that of the native glycoprotein and contains all the carbohydrate. Inhibition of the heterologous precipitation reaction was only achieved with F_2 and reaches 100 % as with the native glycoproteins. These results demonstrate the presence of cross reacting determinants located in the C-terminal part of the molecule.

INTRODUCTION

Antigenic variation appears to be the main mechanism enabling salivarian trypanosomes to evade the immune system of their hosts (for review 1 to 3). During the course of a chronic infection, trypanosomes express a series of different variant specific surface antigens (VSSA). Within a clone of Trypanosoma equiperdum, up to 100 variants (BoTat-1, -100) : Bordeaux Trypanozoon-antigenic-type) appearing in a particular sequence have been isolated in experimentaly infected rabbits (4). The VSSA were purified and characterized (5) from several variants which were isolated at different stages of the infection. The different VSSAs studied have a molecular weight (M.W.) ranging from 52,000 to 56,000 daltons and show extensive differences within their amino acid compositions. No homology of N-terminal amino acid sequence could be found in the VSSAs of BoTat- and BoTat-28 (unpublished results). In contrast, sugar contents seem to be constant in total amount (7 to 8 %) and in composition (mainly : mannose, galactose, N-acetylglucosamine and traces of glycose).

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The existence of cross immunological reactions between different VSSAs of T. brucei and T. congolense (6, 7) suggesting the presence of common antigenic determinants led us to undertake a structural study of two T. equiperdum VSSAs : BoTat-1 and BoTat-28. Variant BoTat-1 is the basic antigenic type to which all variants revert, whereas variant BoTat-28 appears always late during the infection of rabbits (4). Our aim was to study by competitive radioimmunoassay the presence of cross reacting determinants and to localize them within the glycoprotein with the aid of tryptic fragments purified from BoTat-28 VSSA.

We report here the tryptic cleavage results of BoTat-28 VSSA and the chemical and immunological properties of the resulting fragments. A fraction of molecular weight 28,000, showing common antigenic determinants with BoTat-1 VSSA, was obtained. This fragment contains the major part of the polysaccharides and shows an N-terminal amino acid sequence different from the native glycoprotein.

MATERIAL AND METHODS

Purification of the glycoproteins. The VSSAs of BoTat-1 and BoTat-28 were purified as described previously (8).

Trypsin digestion and purification of the products. Trypsin digestion was performed by incubating BoTat-28 VSSA at 4 mg/ml in 0.01 M ammonium bicarbonate, pH = 8.5 at 37°C for 1 hour with trypsin in a molar ratio of 1/200.

Reactions were terminated by addition of acetic acid and the cleavage products were purified by gel filtration on Biogel P 100 (100 - 200 mesh, column dimensions : 2 x 170 cm). The column was eluted with 0.1 N acetic acid, the effluent was monitored at 230 nm and tested by the ninhydrin reaction.

Analytical methods. Trypsin digest products were analysed on polyacrylamide gradient gel electrophoresis (5 - 30 %) using the Laemmli method (9).

Amino acid and sugar analyses were performed as previously described (5). N-terminal amino acid sequence analysis of BoTat-28 VSSA and fragment F_2, were performed by the Edman degradation method (10). Fragment F_1 amino acid was determined with the aid of a Beckman 890 C sequencer. In all cases, the phenyl thiohydantoin derivatives were identified by HPLC (11).

Immunological methods. Antisera were raised in rabbits by 5 injections at 2 weeks intervals of 1 mg glycoprotein emulsified with complete Freund adjuvant.

Radioimmunoassay (RIA). 5 µg of BoTat-1 VSSA was labeled by the chloramine T method (12) with 1 mCi ^{125}I. Antisera dilutions were incubated (3 hours at 37° and then overnight at 4°C) in a total volume of 0.2 ml RIA buffer (0.01 M Tris, 0.05 M NaCl, 0.001 M EDTA, 0.1 % Triton x 100, pH = 7.9), containing 1 % bovine serum albumin (BSA) with 1-3 ng of labeled antigen (approximatively 2 x 10^4 cpm).

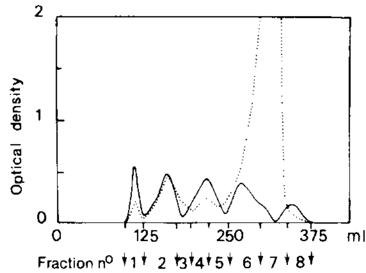


Fig. 1 - Biogel P 100 chromatography of tryptic digest of BoTat-28 VSSA. Fractions of 2.5 ml were collected. (——— optical density at 230 nm, ········ ninhydrin reaction)

The antigen-antibody complexes were precipited with an optimal amount (100 - 150 µl) of a 10 % w/v St. aureus Cowan 1 suspension (in 0.01 M Tris, 0.15 M NaCl, 0.25 % NP 40, pH = 7.9) 30 mm at 4°C. They were diluted with 0.5 ml buffer (0.01 Tris, 0.1 M NaCl, 0.001 M EDTA, 0.1 % Triton x 100, pH = 7.9) centrifuged, washed with the same buffer and the pellets were counted in a gamma counter.

Competitive RIA. Unlabeled competitors diluted in RIA buffer were incubated with an antiserum dilution giving 60 % precipitation, 1 hour at 37°C. All other steps were performed as described above.

RESULTS

In order to localize the common antigenic determinants, tryptic fragments of BoTat-28 VSSA were purified and checked by antigen competition radioimmunoassay. To prevent complete proteolysis of the glycoprotein, mild trypsinisation conditions were used. After gel filtration chromatography of the tryptic digest on Biogel P 100, 8 fractions were obtained (Fig. 1).

Polyacrylamide gel electrophoresis in the presence of SDS revealed a major component in fraction F_1 (M.W. 38,000) and in fraction F_2 (M.W. 28,000). In the same conditions, BoTat-28 VSSA had a M.W. of 54,000. Fraction 4 and 5 were heterogeneous in size and showed M.W. ranging from 14,000 to 28,000. Fractions 6, 7 and 8 seemed to correspond to a mixture of small peptides or/and glycopeptides. The amino acid compositions of the 8 fractions are summarized in Table 1. All of them exhibit a high content of Asp, Thr, Glu, Ala and Lys residues.

Fractions F_1 and F_2 were further studied for their N-terminal amino acid sequence (Table II) and their sugar content (Table III). As in the native glycoprotein the major sugars remained mannose and galactose.

TABLE I - Amino acid analysis of BoTat-28 VSSA and corresponding tryptic fragments (results are expressed as residues per cent amino acid residues).

Amino acid	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	BoTat-28 VSSA
Asp	9.74	17.30	15.04	15.51	10.84	6.98	8.26	8.57	9.11
Thr	10.38	8.80	9.91	11.30	11.89	9.72	7.42	10.68	11.11
Ser	6.44	4.12	5.72	4.90	6.84	6.92	8.35	8.09	5.54
Glu	11.68	12.92	12.13	10.22	9.95	12.55	18.25	8.97	14.20
Pro	2.82	5.33	5.02	6.17	5.29	1.03	1.79	2.29	4.50
Gly	9.23	7.98	9.31	9.25	9.26	12.36	7.95	8.69	7.54
Ala	16.14	11.23	11.50	13.33	15.61	15.24	13.01	23.93	15.50
Val	4.23	1.42	2.88	3.03	3.97	5.16	2.10	0.53	3.82
½Cys	1.85	7.58	4.93	5.66	4.14	1.66	-	-	1.50
Met	0.23	-	-	-	-	-	-	-	0.61
Ile	4.00	0.90	2.26	1.75	3.33	3.97	5.31	0.55	2.42
Leu	6.15	0.64	3.23	3.13	5.99	8.05	3.98	0.67	3.77
Tyr	2.11	2.73	2.61	1.83	2.33	3.07	1.00	8.61	2.30
Phe	3.07	0.59	2.23	2.20	2.48	2.80	3.12	8.25	3.15
Lys	8.16	17.20	11.18	10.55	6.94	7.22	10.74	7.54	10.33
His	0.72	0.39	0.59	0.30	0.67	0.82	0.58	0.52	0.73
Arg	2.96	0.88	1.44	0.86	0.48	2.44	8.14	2.10	3.01

TABLE II - N-terminal amino acid sequence of BoTat-28 VSSA and tryptic fragments (F_1 and F_2).

BoTat-28 VSSA	GLY-ASP-ILE-GLY-ALA-GLY-ALA ....
F_1	GLY-ASP-ILE-GLY-ALA-GLY-ALA-ASN-ARG-ASP.......
F_2	ASN-LYS-GLN-ARG-PRO-LYS..........

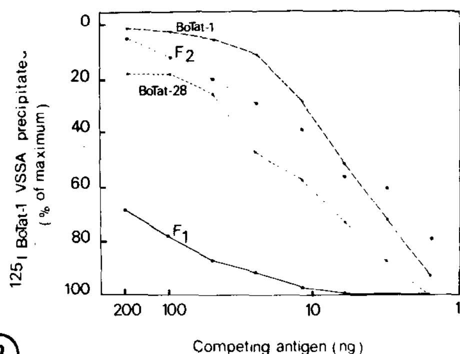
Glucose was mainly detected in fraction F_1. It is of interest to note the higher content of galactose in F_2 compared to BoTat-28 VSSA and F_1 fragment.

Immunological results. The results of Figure 2 show that the antiserum prepared against BoTät-28 VSSA cross reacts with the antigen of BoTat-1. However the concentration of heterologous serum required to precipitate 50 % of radioactive label is 50 fold higher than the effective concentration of homologous antisera. In order to eliminate the possiblity of BoTat-28 VSSA contamination by BoTat-1 VSSA (to which variant BoTat-28 tends to revert), competitive RIA was performed with antisera dilutions precitating approximatively 60 % of the labeled antigen.

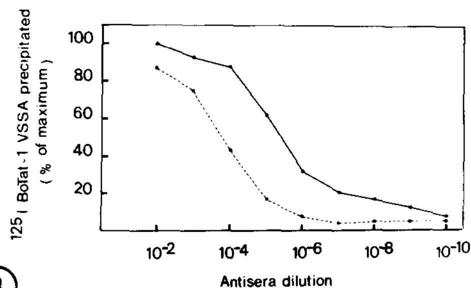
The homologous reaction (BoTat-1 VSSA^{125}I, anti-BoTat-1 VSSA) was inhibited (50 % inhibition) by 3 ng of homologous antigen BoTat-1, whereas 200 ng of BoTat-28 VSSA were ineffective.

TABLE III - Sugar analysis of BoTat-28 VSSA and tryptic fragments F_1 and F_2 (results are expressed as mol/mol of glycoprotein or glycopeptide, apparent SDS polyacrylamide M.W. were used for calculation).

Sugars	BoTat-28 VSSA	F_1	F_2
Mannose	12	3.3	14.9
Galactose	7.2	0.7	13.6
Glucose	1.9	1.4	0.5
N-acetyl glucosamine	2.4	1.5	2.6



(2)



(3)

Fig. 2 - Radioimmunoprecipitation of ^{125}I BoTat-1 VSSA with rabbit anti-BoTat-1 (———) and anti-BoTat-28 (- - - - -) VSSA sera.

Fig. 3 - Competitive RIA for the BoTat-1 VSSA with anti-BoTat-28 VSSA serum. The competing proteins BoTat-1, BoTat-28 VSSA and fragments F_1, F_2 are indicated in the figure.

In contrast the heterogolous reaction (BoTat-1 VSSA^{125}I-anti-BoTat-28 VSSA) was completely inhibited by the variable antigens of both variants and by the fraction F_2 of BoTat-28 VSSA (Fig. 3). 6 to 15 ng of competitor caused 50 % inhibition.

These results clearly demonstrate the presence of cross reacting determinants located within the fragment F_2 of BoTat-28 VSSA.

DISCUSSION

Several conclusions can be drawn from the chemical compositions and N-terminal amino acid sequences :

- Fraction F_1 (M.W. 38,000) contains 30 % of the total sugar content of BoTat-28 VSSA. Its N-terminal sequence is identical to that of the native glycoprotein. This clearly demonstrates that F_1 is a large N-terminal fragment (70 % of the native glycoprotein).
- Fraction F_2 (M.W. 28,000) contains roughly the total sugar amount of BoTat-28 VSSA and represents half of the peptide backbone. The N-terminal amino acid sequence differs totaly from that of the native glycoprotein. Comparing F_1 and F_2 amino acid compositions differences appeared : fraction F_2 is characterized by a high content of lysine and cysteine probably involved in disulfide bridges and by a low percentage of hydrophobic amino acid residues Val, Ile, Leu and Phe.

This clearly demonstrates that F_2 is overlaping in its N-terminal portion with F_1. The difference of sugar composition found in both fractions indicates the presence of at least two glycosylation sites in the peptide axis of BoTat-28 VSSA. The low sugar content of F_1 (2/3 of the entire glycoprotein) shows that the majority (70 %) of the sugars are located in the 30 % C-terminal region. These results agree with those obtained by Johnson and Cross (13) on VSSA of T. brucei where most of the glucosamine was found in the C-terminal portion.

The results of competitive RIA show the presence of cross reacting determinants between BoTat-1 and BoTat-28. As was previously reported (5), no cross reactivity could be demonstrated by immunodiffusion.

Cross reacting determinants have also been reported in T. Brucei and T. congolense variable antigens (6). In our case determinants are mainly located in the F_2 tryptic fragment. F_1 inhibits slightly the heterologous reaction and only at high concentration, suggesting the presence of most of the antigenic site, within 30 % of the C-terminal region of BoTat-28 VSSA. Similar results were reported with the VSSA of T. brucei where the cross reacting determinants were located within the 25 % C-terminal part of the molecule (7). Recently, Barbet et al. (14) reported the localization of these determinants in the glycopeptide fraction isolated after pronase digestion of T. brucei VSSA.

Further work is in progress to have more information on these cross reacting determinants and the role of sugars.

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