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VARIANT SPECIFIC SURFACE ANTIGENS FROM TRYPANOSOMA EQUIPERDUM : CHEMICAL AND PHYSICAL STUDIES

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SUMMARY - Nine variant specific surface antigens were purified from clones of Trypanosoma equiperdum and characterized by amino acid analysis, isoelectric focusing and circular dichroism. The molecules showed extensive differences in their isoelectric points, and by comparison with the corresponding amino acid compositions, this variation seemed to be due to different amide contents. Circular dichroism data allowed one to divide the molecules into 4 groups according to their respective percentages in α-helical and β-sheet structure.

Salivarian trypanosomes evade the immune defence of their mammalian host by sequentially expressing a series of different variable antigen types. The immunological specificity of each living trypanosome is determined by the structure of the single predominant glycoprotein in the parasite surface coat (for review 1-2). In Trypanosoma brucei, this variant specific surface glycoprotein accounts for approximately 95 % of the total coat protein (2).

In chronic infections, successive replacements of characteristic glycoproteins involve subsequent appearances of waves of parasitemia. More than 100 distinct antigens can be identified in experimentally infected rabbits with a single trypanosome clone of T. equiperdum (3). In T. brucei, the expression of the genes specifying the surface antigens has been shown to depend on synthesis and transposition of an "expression-linked" gene copy (4-6).

Variant surface glycoproteins from T. brucei have been purified, characterized (7-11) and also analysed for the presence of cross-reacting antigenic sites (12-14). We have reported the isolation by affinity chromatography on Concanavalin-A Sepharose of

Abbreviations : Botat : Bordeaux Trypanozoon-antigenic type
VSSA : Variant Specific Surface Antigen

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several specific antigens from T. equiperdum (15), as well as some of their chemical and immunological properties (16) and the existence of common antigenic determinants between two variable antigens (17). We give here the results obtained in amino acid composition, isoelectric focusing and circular dichroism of nine variants.

MATERIALS AND METHODS

2.1 - Isolation of specific glycoproteins - Cloned variants of T. equiperdum were isolated at various stages of the infection : BoTat-1, the basic antigenic type and BoTat-2 -3 and -4 within the first 2 weeks of the disease. BoTat-78 and -100 between 3-4 weeks ; BoTat-20, -28 and -51 in the last stages of the infection (3). The specific glycoproteins were purified by means of affinity chromatography on Concanavalin-A Sepharose as described previously (15).

2.2 - Chemical analyses -

* Amino acid analyses

Samples were hydrolysed in sealed tubes for 24 hours, in 5.6 M HCl at 105°C under vacuum. Analysis was performed on a Beckman Multichrom B apparatus. Amino acids and hexosamines were separated with a single column system and a stepwise gradient elution (18). Tryptophan was estimated according to the method of Penke et al. (19).

* Isoelectric focusing

Isoelectric focusing was performed in a thin layer 3 % polyacrylamide gel containing 7 % ampholyte (LKB), between pH 4.5 and 9.5, using a Multiphor apparatus (LKB 2117). The electrode solutions were 1.0 M NaOH at the cathode and 1.0 M phosphoric acid at the anode. Samples were dissolved in distilled water, applied on the gel and focusing was performed with a constant power of 6 W for 5 hours. The pH gradient was determined with a surface glass pH electrode (Orion). The bands were stained with Coomassie brillant blue R-250.

2.3 - Physical analyses -

* Circular dichroism measurements

CD Spectra were recorded with a dichrograph Jobin Yvon RJ mark III. The samples were dissolved in distilled water. Protein content of the solutions was determined according to the method of Lowry, and always amounted to about 1 mg per ml. Cells of 0.01 cm pathlength were used. Spectra were drawn using a constant time of 10 s. and a scanning speed of 0.1 mm/s. Results were given as ellipticity $[\theta]$, expressed in deg. $d\,mole^{-1}.cm^{2}$ taking 111 as mean residue weight. All the ellipticity curves were constructed using at least 3 spectra.

Analysis at variable temperature was performed with a jacketed cell of 0.1 cm pathlength. A platinum probe was used to measure the temperature of the solution.

* Circular dichroism calculations

CD spectra were converted into gaussian curves, with negative maxima at 198, 207, 214 and 222 nm. α-helical and β-sheet structure contents were estimated using respectively the band at 222 and 214 nm, and taking the value of -30.000 for the α standard and -9.200 for the β standard.

RESULTS

The results of the amino acid analysis are given in table 1. Significant amino acids in the selected VSSAs appeared to be aspartic acid and/or asparagine, glutamic

TABLE 1

Amino acid composition of the purified specific surface antigens from variants BoTat-1, -2, -3, -4, -78, -100, -20, -28, -51.

	Early				Semi-Late		Late		
Amino acid	BoTat-1	BoTat-2	BoTat-3	BoTat-4	BoTat-78	BoTat-100	BoTat-20	BoTat-28	BoTat-51
Asp	13.37	8.41	10.32	10.32	9.16	10.99	10.49	11.05	10.99
Thr	8.90	10.74	11.07	8.67	10.48	10.53	10.57	11.20	9.05
Ser	5.42	6.93	5.91	5.69	5.17	4.44	3.33	6.23	5.25
Glu	11.10	12.41	13.62	13.48	14.22	13.49	16.88	11.46	14.29
Pro	3.60	3.64	2.54	5.16	3.19	5.88	3.81	3.68	4.05
Gly	7.96	6.29	6.99	6.30	6.58	7.14	7.32	8.85	6.76
Ala	15.13	15.06	13.02	14.33	14.10	13.22	11.72	15.57	12.18
Val	2.59	3.26	3.73	1.64	3.21	2.78	4.08	3.37	2.81
½ Cys	2.19	1.21	2.15	1.20	1.25	1.32	1.17	1.90	1.45
Met	1.13	0.59	Traces	1.26	0.89	0.49	Traces	0.35	0.45
Ile	2.36	4.11	3.18	4.68	3.51	2.83	3.01	3.45	2.85
Leu	9.16	7.64	6.98	6.93	6.34	5.36	7.07	4.40	8.17
Tyr	2.20	2.61	2.49	2.93	2.04	2.69	2.17	1.87	2.12
Phe	2.14	1.60	1.95	2.59	3.03	1.92	1.87	2.45	3.31
Lys	8.07	9.59	11.86	10.77	11.61	11.32	12.34	9.93	11.65
His	0.57	2.04	2.03	1.59	1.40	2.74	1.29	0.51	1.55
Trp	0.46	0.46	0.40	0.41	1.06	0.58	0.34	0.98	N.D
Arg	3.65	3.41	1.76	2.05	2.76	2.28	2.54	2.75	3.07

Values expressed as residues/100 amino acid residues

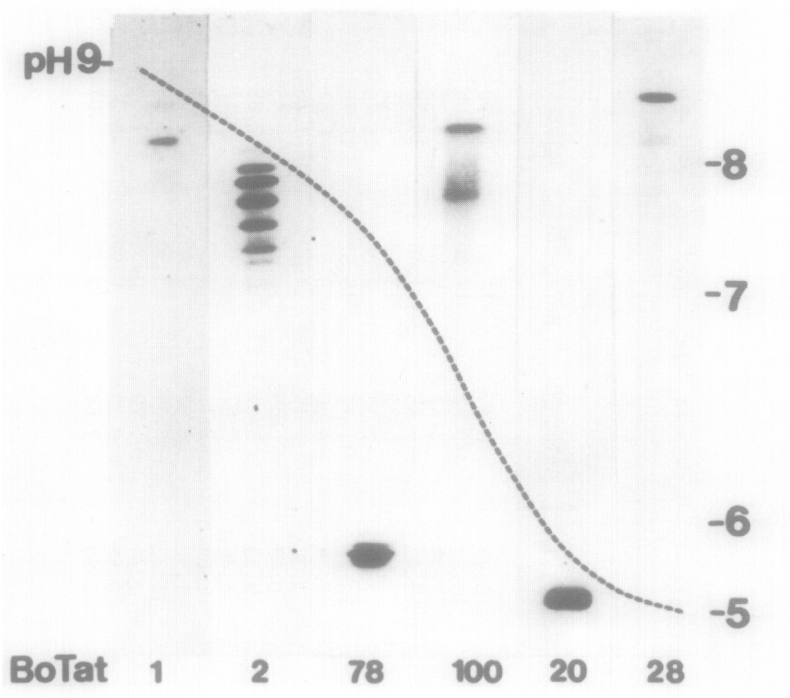

Fig. 1. Isoelectric focusing pattern of the purified specific surface antigens from variants BoTat-1,-2,-78,-100,-20,-28.

acid and/or glutamine, alanine and lysine. Data did not show a strong difference between the glycoproteins. However the specific surface antigens from the successive variants BoTat -1, -2, -3, -4, -78, -100, -28 were characterized by a decrease of the leucine content ; but this feature did not apply to the late variants BoTat -20 and -51.

In contrast, the VSSAs were found to be widely different in isoelectric focusing (Fig. 1). Each glycoprotein was represented by a pattern of several close bands which were stained by Coomassie Blue to different degrees. BoTat-3, -4 and -51 VSSAs, were only slightly soluble, and could not be focused in the conditions employed.

Contents in α-helical and β-sheet structure were estimated from the CD spectra, and the results (Table 2) show that the 9 VSSAs could be divided into 4 groups. The secondary structure of 2 groups was characterized by nearly 50 % of one type of organized structure : α-helical for BoTat-1 VSSA, β-sheet for BoTat-20 and -51 VSSAs. The CD Spectra of BoTat-1, -78, -28 and -51 are represented in fig. 2.

For all the glycoproteins, a variation of $[\theta]_{222}$ according to temperature was drawn, and Fig. 3 gives the typical curves of each defined group. For BoTat-20 and -51 VSSAs the temperature of half-denaturation appeared to be the highest one (nearly 60°C),

Table 2

	Variants	α-helical	β-sheet	Random coil and β-turn
	BoTat-1	49	25	26
EARLY	BoTat-2	38	32	30
EARLY	BoTat-3	35	30	35
EARLY	BoTat-4	36	29	35
SEMI	BoTat-78	37	28	35
LATE	BoTat-100	38	31	31
LATE	BoTat-20	33	49	18
LATE	BoTat-51	37	46	17
	BoTat-28	28	29	43

and for the 3 other groups decreased from 50°C to 45°C respectively from BoTat (-1), BoTat (-2,-3,-4,-78,-100) to BoTat (-28).

Fig. 3 also shows the effect of the decrease in the temperature. BoTat-1 VSSA almost completely recovered its secondary structure. Only partial renaturation was observed for BoTat-20 and -51 VSSAs. For the other glycoproteins, thermal denaturation was irreversible.

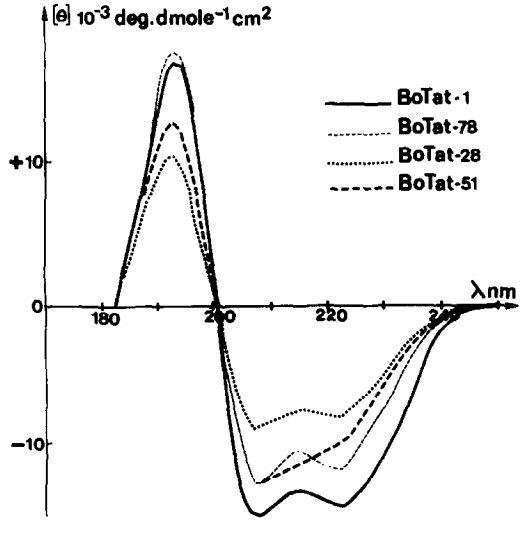

Fig. 2. Circular dichroism spectra of the purified specific surface antigens from the variants BoTat-1,-78,-51 and -28.

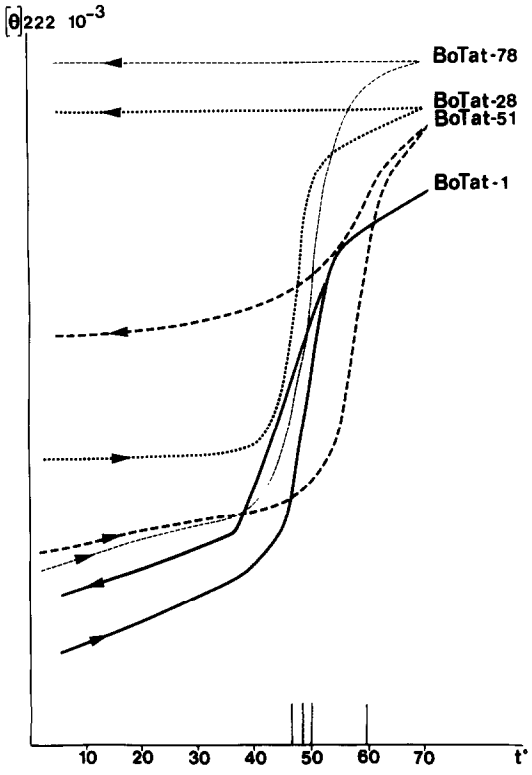

Fig. 3. Thermal denaturation and renaturation of the purified specific surface antigens from the variants BoTat-1,-78,-51 and -28.

DISCUSSION

The amino acid composition of the 9 purified glycoproteins appeared to be very similar. However patterns of close bands obtained in isoelectric focusing were found to lie in different pH areas (Fig. 1). According to previously obtained sugar analyses (16) it is very unlikely that variations observed in average pIs are the consequence of different structure in the oligosaccharide moiety ; the three VSSAs analysed (BoTat-1, -78, -28) had the same qualitative monosaccharide composition. Each of them contained mannose, galactose, glucose and N-acetyl glucosamine, present in very similar amount. No sialic acid could be detected in any of these 3 glycoproteins. Specific surface glycoproteins from T. brucei were also found to have the same qualitative sugar composition, and particularly to lack sialic acid (10) ; however variation in their quantitative sugar composition was reported (10). Large differences in average pIs of VSSAs could be the result of a change in Asn/Asp and/or Gln/Glu ratios.

The purified preparations of VSSAs although homogeneous in SDS-Page, exhibited a pattern of several bands in isoelectric focusing. According to our isolation procedure

(15), the release of the specific surface antigens was obtained by keeping the trypanosomes in phosphate buffer pH 5.5 for 12 hours at 4°C ; that step perhaps may induce some microheterogeneity in the glycoproteins. Nevertheless, VSSAs from T. congolense, isolated by freeze thawing and shearing of the parasites and purified on concanavalin-A, Sephadex G-25, and gradient eluted DEAE-cellulose colonnes, were also found to give a pattern of several bands (20) ; in particular, the two surface glycoproteins that were studied, gave both a pattern of 3 bands.

A detailed study of the different components separated in isoelectric focusing is necessary to determine the exact nature and the origin of the observed microheterogeneity.

According to their secondary structure, the 9 specific glycoproteins were divided into 4 groups (Table 2). Thermal denaturation and renaturation investigations confirmed these 4 groups ; as expected, the temperature of half-denaturation was linked to the $\alpha+\beta$ content, with a prevailing influence of the β structure. In this preliminary classification, it must be emphasized that all the early and semi-late variants studied, except the basic antigenic type, were characterized by the same global secondary structure. On the contrary, the 3 late variants appeared under two radically different structures : BoTat-20 and -51 were particularly organized (49%β-sheet + 33% α-helical) while BoTat-28 was especially disorganized (43% random coil and β-turn). Lastly, the basic antigenic type (BoTat-1) exhibited the highest percentage of α-helical structure (49%).

Using monoclonal antibodies and both primary and secondary structure data, we hope to define the conformational antigenic determinants of the surface of the parasite.

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