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Ultracytochemistry of the Surface Coat/Pellicle Complex in *Trypanosoma brucei*

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Abstract

Ultracytochemistry of polysaccharides and specific sugar residues reveals differences in the surface staining pattern between developmental forms of *Trypanosoma brucei*. The techniques used were the PA (periodic acid) – TCH (thiocarbohydrazide) – silver albumose reaction for the polysaccharides, and the Concanavalin A (Con A) – peroxidase – DAB coupling method for specific sugar residues.

Blood and metacyclic forms, both possessing a surface coat, stain distinctly for carbohydrates at the level of the pellicular membrane. The external portion of the bloodform coat lacks any positive staining.

Pellicles of non-coated culture and vector forms react only faintly for polysaccharides, whereas heavy staining of oxidized peroxidase/DAB reaction product, indicative of sugar bound Con A, occurs.

It is suggested that the sugar moieties of the coat glycoproteins are located close to the membrane-coat junction.

Introduction

The surface coat of *Trypanosoma brucei* bloodforms is composed of glycoproteins (NJOGU & HUMPHRIES 1972), and contains the variant antigens (VICKERMAN & LUCKINS 1969). It is lost during transformation to the culture/midgut stage (VICKERMAN 1969; STEIGER 1973) with the concomitant disappearance of the variant antigens (SEED 1963). The surface coat is built up anew as the infective metatrypomastigotes develop in the salivary glands of the vector (VICKERMAN 1966; STEIGER 1971).

Cytochemical staining has revealed the presence of polysaccharides in the trypanosome pellicle (WRIGHT & HALES 1970; STEIGER 1973). Biochemical investigations on surface coat material have demonstrated protein-carbohydrate complexes containing neutral sugars, such as D-mannose (ALLSOPP, NJOGU & HUMPHRIES 1971). Preliminary experiments have indicated differences in amino acid composition between coat proteins isolated from closely related cloned populations, these alterations being suggestive of a major rôle in antigenic variation (CROSS 1973).

In a related organism, *T. lewisi*, which possesses a morphologically somewhat different coat (VICKERMAN 1969), cytochemical staining of trypsinized cells gives positive reactions for polyanionic saccharides in the pellicular membrane (DWYER 1974a).

Furthermore, the same author suggests that glycoproteins and glycopeptides are major components of the coat/pellicle complex of *T. lewisi*; α -D-mannose-like sugar residues could be demonstrated by Con A binding.

The present cytochemical study describes the distribution of coat/pellicle saccharides in different developmental stages of *T. brucei*. Preliminary findings have recently been reported (STEIGER & JENNI 1974).

Material and Methods

Three strains (STIB 33, EATRO 1093, EATRO 1961) of *Trypanosoma brucei* bloodforms and their respective fly stages were used. Culture forms (strain S 42) were grown in a modification (S. Böhringer, pers. communication) of the defined medium HX 25 (CROSS & MANNING 1973). Harvesting, processing and micrographic recording of the parasite material were essentially as described earlier (STEIGER 1973), however, with the omission of agar encapsulation of the blood specimens.

The two cytochemical experiments were carried out as follows:

a) For the cytochemical demonstration of polysaccharides 60 nm thick sections were mounted on uncoated 300 mesh gold grids and stained by means of the PA-TCH-silver albumose technique (THIÉRY 1969), with the controls recommended. Incubation in TCH was performed for varying time periods (3, 6, 8, 10, 12 hours).

b) For the specific demonstration of terminal α -D-mannopyranosyl and α -D-glucopyranosyl residues (e.g. PORETZ & GOLDSTEIN 1970) the Concanavalin A-peroxidase coupling method (BERNHARD & AVRAMEAS 1970) was used with similarly fixed blood and culture forms (2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3). Agglutinin incubations were performed for 30 min. at 20 °C at Con A concentrations of 100, 150 and 200 μ g/ml. Control incubation mixtures contained 0.2 M α -methyl-D-mannoside (Schwarz/Mann) as an inhibitor. For positive controls rat blood cells were simultaneously included in some of the samples, processed and treated the same way as the parasites.

All experiments were done in triplicates.

Results

Staining for polysaccharides: incubations of the sections in TCH for 8–10 hours proved to be optimal. They yield silver deposits of high intensity and precise localization with reduced overstaining effects, and with all controls being negative.

No differences in the staining pattern are found between strains of *T. brucei* (blood and vector stages).

In bloodforms, which contain a surface coat, the pellicular and flagellar membranes stain very distinctly showing dark granulations of reduced silver deposits (Fig. 1). The outer leaflet of the membrane is somewhat more opaque (Fig. 1, inset). The central lamina of the unit membrane structure remains more or less translucent. The external portion of the surface coat is virtually unstained (Fig. 1 + inset), whereas cytoplasmic membranes (e.g. rer) react very strongly.

Upon transformation to the culture/vector stage (midgutforms, epimastigotes), when the coat is absent, a marked reduction in staining intensity occurs. The culture, midgut and epimastigote forms exhibit the same picture: only a reduced, less conspicuous granulation is left, whereas the mitochondrial membranes remain intensively blackened (Figs. 2, 3).

In metatrypomastigotes of the salivary gland (= infective, metacyclic forms), where the coat is reacquired, the bloodform-like staining pattern reappears. Here, the stronger contrast of the membrane's outer leaflet is even more evident. The external coat appears unstained (Fig. 4 + inset).

Staining for D-mannose and D-glucose residues: again, the external part of the surface coat of bloodforms is generally devoid of dark reaction products

(oxidized DAB), whereas the surface of rat blood cells, serving as positive controls, is heavily stained indicating the presence of Con A binding sites (Fig. 5). In a few cases, however, a slight staining of the trypanosome surface coat occurs, though irrespective of the strain used. In control incubations with the haptenic inhibitor no staining is found whatsoever.

In the coatless culture forms, on the other hand, a thick continuous layer of reaction product covers the trypanosome pellicle uniformly (Fig. 6). Again, all controls are negative. The results are the same with all concentrations of the phytohaemagglutinin used.

Discussion

The results demonstrate cytochemical changes in the pellicle/coat complex of *Trypanosoma brucei* in the course of the life cycle. The presence of a strong positive reaction for carbohydrates in coat-bearing stages and its diminution in culture and vector stages without coat seem to indicate that at least some of the pellicle-associated sugar moieties are an intrinsic part of the coat.

Apparently specific Con A binding sites, e. g. branched terminal α -D-mannopyranosyl and α -D-glucopyranosyl residues (PORETZ & GOLDSTEIN 1970), are

Fig. 1. Bloodform of *T. brucei*: localization of polysaccharides (PA-TCH-silver albumose technique). Distinct coarse granulation in the pellicular membrane (\rightarrow). External surface coat is negative (\rightarrow), cytoplasmic membranes (c) stain positive, nucleus (n). 86,000 \times .

Inset: Details from a bloodform: trilaminar pellicular membrane with the outer leaflet being more opaque and the central lamina translucent (\rightarrow). External portion of the coat is not stained (\rightarrow); flagellar profile (f). 130,400 \times .

Fig. 2. Culture form of *T. brucei*: staining of the pellicle is less conspicuous (\rightarrow); mitochondrial profile (m). 86,000 \times .

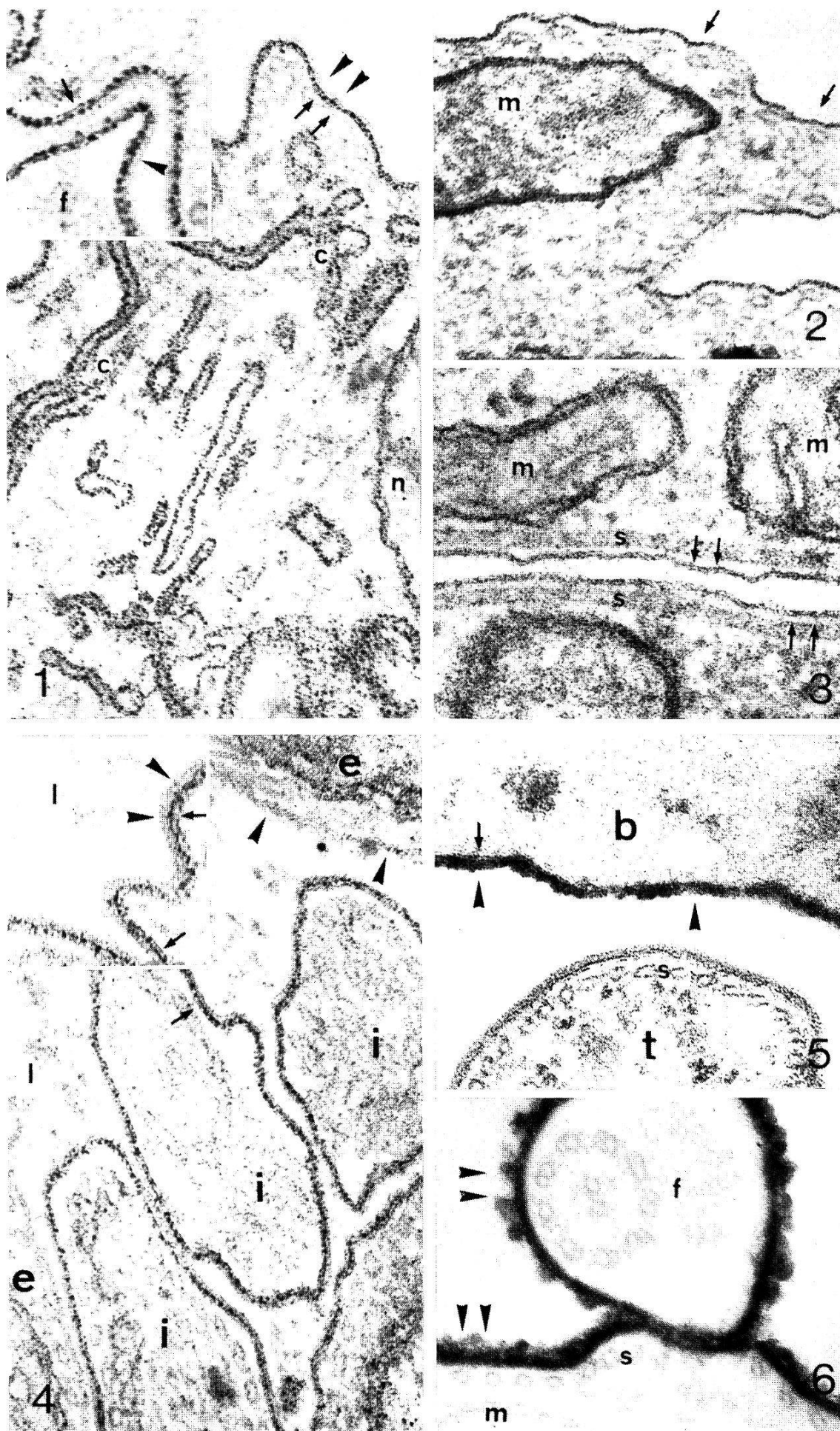
Fig. 3. Vector (= midgut) forms of *T. brucei*: weak staining of the pellicle (\rightarrow); membranes of the mitochondrion (m) are contrasted; subpellicular microtubules (s). 86,000 \times .

Fig. 4. Vector (= salivary gland) forms of *T. brucei*: pellicles of infective metacyclic forms (i) are heavily stained (\rightarrow). Those of epimastigotes (e) show reduced staining (\rightarrow); lumen of the salivary gland (l). 86,000 \times .

Inset: Details from a metacyclic form: surface coat is essentially unstained (\rightarrow), whereas the membrane shows a positive reaction (\rightarrow). Again, the outer leaflet is more electron-dense; lumen of the gland (l). 130,400 \times .

Fig. 5. Bloodform of *T. brucei* (t) and rat blood cell (b) – Concanavalin A – peroxidase staining method: the outer leaflet of the blood cell membrane (\rightarrow) is covered with a continuous layer of DAB reaction product (\rightarrow); the trypanosome surface remains negative; subpellicular microtubules (s). 86,000 \times .

Fig. 6. Culture form of *T. brucei* – Concanavalin A – peroxidase staining: note thick continuous layer of DAB reaction product on the trypanosome surface (\rightarrow), profile of apposed flagellum (f); subpellicular microtubules (s), mitochondrion (m). 86,000 \times .



located on the outer leaflet of the limiting membrane, but not in the external portion of the surface coat. It is therefore suggested that the carbohydrate moiety of the coat glycoproteins is restricted to the coat/pellicle junction site. Differences in end product density of the PA-silver reaction between coated and non-coated pellicles could as well delineate changes in membrane glycoproteins and/or glycolipids. Alterations in the overall lipid composition of *T. rhodesiense* blood and culture forms have been described (DIXON & WILLIAMSON 1970), and glycoproteins are thought to be major constituents of the *T. lewisi* pellicular membrane (DWYER 1974a). The same author has suggested from results obtained by enzyme digestion experiments that related organisms, *Leishmania donovani* promastigotes, contain polysaccharides, such as glucans randomly dispersed on the pellicular membrane – trypsinization of the cells does not alter the agglutination pattern (DWYER 1974b). Polysaccharides can be demonstrated, however, in the surface coat of *T. lewisi* by means of cationic dyes (DWYER 1974a), which signifies that the coat of the archaic species *T. lewisi* differs not only morphologically from that of *T. brucei*. Absence of ruthenium red staining in the latter species (VICKERMAN 1969) supports this. Other workers, using a similar PA-silver method, have found an “outer reaction line” in the surface coat of *T. brucei* (WRIGHT & HALES 1970). Such a feature has never been observed with our material.

The question arises as to whether the two cytochemical methods applied are specific enough.

The specificity of cytochemical techniques for demonstrating polysaccharides on thin sections is far from clear (RAMBOURG 1971). However, it has been claimed that by using double fixation, as in the present experiments, unspecific staining, e. g. by absorbed glutaraldehyde, is minimized (THIÉRY 1967). Moreover, qualitative specificity in these experiments seems warranted by thoroughly negative controls.

From the quantitative point of view this seems not to be the case: recent work has shown that prefixation of cells in glutaraldehyde, also carried out with our material, results in a greater amount of peroxidase-DAB reaction product possibly due to the stabilization of the membrane so that no redistribution of the Con A binding sites occurs (COLLARD & TEMMINK 1974).

Besides it has recently been shown that the sugar-binding specificity of Con A is to be understood in a broader sense (GOLDSTEIN et al. 1973), since also internal sugar residues of glycoproteins and polysaccharides can be reactive. The very slight staining of the coat of bloodforms sometimes encountered may reflect binding of horseradish peroxidase to the coat as suggested before in pinocytosis experiments (GEIGY, STEIGER & HECKER 1970).

Biochemical studies are needed to elucidate the nature, composition and function of the sugar moieties of the coat/pellicle complex, especially concerning the question if the sugar composition of the coat glycoproteins is constant, and whether saccharides play a rôle in coat attachment.

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