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PARTIAL RESOLUTION OF THE SUGAR CONTENT OF TOXOPLASMA GONDII MEMBRANE

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SUMMARY

The glycoconjugates on Toxoplasma gondii membrane have been investigated in several ways, usually used for the study of cell surfaces : binding of $|^{14}C|$ lectins, Concanavalin A (Con A) and Wheat germ agglutinin (WGA) to parasites ; parasites membrane labelling, using galactose oxidase/tritiated sodium borohydride (NaB$|^{3}H_4|$) ; determination of sialic acid amount, sensitive to the neuraminidase. Toxoplasma gondii binds Con A but did not bind significantly WGA. The affinity constant for Con A binding was on the same order of magnitude as for other cell systems, but the number of sites per parasite was low ($3x10^4$ sites per parasite). In contrast, with other cell systems, no surface incorporation of tritium was obtained using galactose oxidase/NaB$|^{3}H_4|$. Moreover the determination of sialic acid, sensitive to the neuraminidase gave a negative result. These results shown that Toxoplasma gondii surface do not contain all sugars, usually found in oligosaccharidic chains of membrane glycoconjugates and suggest the presence of low amount of surface glycoconjugates.

INTRODUCTION

Current biochemical studies on the parasite Toxoplasma gondii are focused into two major area ; interaction of the parasite with the host cell and improvement of the diagnosis tests used for the detection of human infection. In both cases, analysis of the membranes components of Toxoplasma gondii seems to be a useful tool for the resolution of these problems. Cellular life cycle of the parasite began by adsorption and penetration of Toxoplasma gondii to the host cell (1). Then some events occur which protect the parasites from digestion by lysosomial enzymes of the host cell. One hypothesis for explain this protection could be, at least in part, a peculiar structure of Toxoplasma gondii membrane constituants.

Studies on the diagnosis test of the human disease are also dealing with analysis of Toxoplasma gondii membrane. Most of the available

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technics used for the diagnosis require the determination of plasmatic level of anti-parasite antibodies (2,3,4). This means that an antigen preparation is used for such a determination. One can then speculates that characterisation and purification of membrane surface antigen from Toxoplasma gondii may lead to an earlier and more sensitive diagnosis of the human infection. In previous reports from this laboratory, we have reported results obtained in the course of Toxoplasma gondii membrane purification (5,6). An other approach of surface antigen analysis consists in direct studies of structural membrane constituants of the whole parasite cell. Among these constituants, characterisation of the sugars may be easily carried out since several technics have already been designed for other cell systems (7,8). We reported, here, the partial resolution of the sugar content of Toxoplasma gondii membrane, using lectin binding, parasite surface labelling by galactose oxidase/ tritiated sodium borohydride and sialic acid determination.

MATERIAL AND METHODS

Preparation of parasites. Trophozoïtes from the virulent Rh strain of Toxoplasma gondii were allowed to multiply in TG 180 transformed cells (9). The parasites and the host cells were maintained separatly by intraperitoneal passages in adult female mice. The production of a large amount of Toxoplasma gondii was obtained by intraperitoneal injection of a mixed suspension of parasite and TG 180 cells ($1,2x10^7$ Toxoplasma gondii and 10^7 TG 180 in 0,2ml). In order to prevent hemorrage, mice were beheaded 72 hours after injection. The ascite suspension was collected separatly from each mouse. Each suspension was washed three times in PBS buffer (phosphate, 0,01M ; NaCl, 0,14M ; pH 7,2) by centrifugation at 400g for 10mn. As estimated by red color, the pellets containing erythrocytes were discarded. The other pellets were pooled and the few cells contamination (TG 180 cells and macrophages), were removed from the suspension by one of the following filtration methods (submitted manuscript):
- Filtration on Sephadex G25 (Pharmacia) ; toxoplasmas prepared on this way were used for WGA binding experiments and for labelling with galactose oxidase/tritiated sodium borohydride system.
- Filtration on Ultrogel AcA 202 (IBF), for the purification of parasites used on Con A binding experiments.

In both cases, the filtrates were centrifuged at 400g for 10mn, and the pellets were washed three times, in PBS buffer. Parasite suspension was counted in hemocytometer and adjusted to 10^9 cells per ml in PBS buffer. Each set of experiments was carried out with a freshly preparation of Toxoplasma gondii. These preparations contained less than 0,1% of erythrocytes, as estimated by cell counting of four separated samples in hemocytometer.

Lectins. Concanavalin A and Wheat germ Agglutinin were purchased from Pharmacia (Uppsala-Sweden).
Labelling of lectins : Radiolabelling of the lectins was carried out according to the method of Miller and Great (10), using $|^{14}C|$ acetic anhydride (30mCi/mM, Radiochemical Center, Amersham-France). Labelled lectin was purified by gel filtration on Sephadex G25 (Pharmacia) for WGA and on Biogel P6 (Biograd-France) for Con A, the eluting buffer being PBS buffer. The specific activity of lectins was 3,6µCi/mg for Con A and 1,7µCi/mg for WGA. The labelled lectins behaved exactly like

native lectin in the erythroagglutination test and polyacrylamide gel electrophoresis. Labelled lectin solution was prepared at a final concentration of 0,5mg/ml in PBS buffer.

Lectin binding study : For the binding experiments, 10^8 parasites were incubated in a final volume of 0,3ml PBS buffer with different concentrations of labelled lectins. After incubation for 15mn at 4°C with shaking, the parasites were harvested and washed three times in PBS buffer by centrifugation at 400g. The parasites were then resuspended in 10ml of scintillation fluid (Phase combining system, Amersham-France) and counted in a liquid scintillation spectrometer (SL 300, Intertechnique-France). The specific binding was determined as the difference between the quantity of lectin bound in the absence and in the presence of 0,2M methyl-D-mannopyranoside for Con A and 0,2M N acetyl-glucosamine for WGA. Experimental data were plotted by the method of Scatchard (11) taking into account the molecular weight of the lectin (110.000 for Con A and 36.000 for WGA). The results were averages from four separate experiments and a new preparation of parasites was used for each experiment.

Labelling of parasite surface using galactose oxidase/NaB$|^3H_4|$.

The labelling was carried out according to the method of Gahmberg and Hakomori (8). In a typical experiment, $5x10^8$ parasites were suspended in 5ml of PBS buffer and were incubated 90mn at 37°C with 5 to 100 units per 10^8 parasites of galactose oxidase (KABI-KB, Stockholm-Sweden). The parasites were then washed and resuspended in PBS buffer. The reduction was performed by addition of tritiated sodium borohydride (420mCi/mmole, Radiochemical Center, Amersham-France), at a final concentration of 15nM per 10^8 parasites. The reaction was allowed to proceed 5mn at room temperature and was stopped by dilution in a large excess of PBS buffer. Parasites were then washed four times by centrifugation at 400g for 10mn, and the radioactivity was determined as described above. Galactose oxidase was devoid of protease activity as estimated by the azocoll test (12). The results were averages from four separate experiments and a new preparation of parasites was used for each experiment.

Sialic acid determination. The cell surface sialic acid were released by Vibrio cholerae neuraminidase (Behringwerke, 500 units/ml). The parasites, at different concentrations (10^8 to $2,5x10^9$ parasites/ml of PBS buffer) were incubated 90mn at 37°C with 100 units per 10^8 parasites of neuraminidase. The parasites were then centrifugated at 1000g for 10mn and sialic acid content of supernatant was estimated by Warren's method (13). Neuraminidase was devoid of protease activity as estimated by azocoll test (12). The total sialic acid of parasites was determined after acid hydrolysis (H_2SO_4, 0,1M) of different concentrations of parasites (10^8 to $2,5x10^9$ parasites/ml of PBS buffer) during 60mn at 80°C.

Parasites viability. The viability of parasites was tested as described by Desmonts (4), after purification, binding and labelling experiments and after treatment by enzymes.

RESULTS AND DISCUSSION

Toxoplasma gondii parasites were tested for their ability to bind $|^{14}C|$ Con A and $|^{14}C|$ WGA. The $|^{14}C|$ Con A binding reached a plateau within 15mn of incubation at 4°C (Fig.1A). The parasite bound at saturation 0,5±0,04µg of Con A per 10^8 parasites. This binding reflected the specific one because $|^{14}C|$ Con A binding to parasites were inhibited (80%) by αmethyl-mannoside. When the competitive sugar inhibitor was ad-

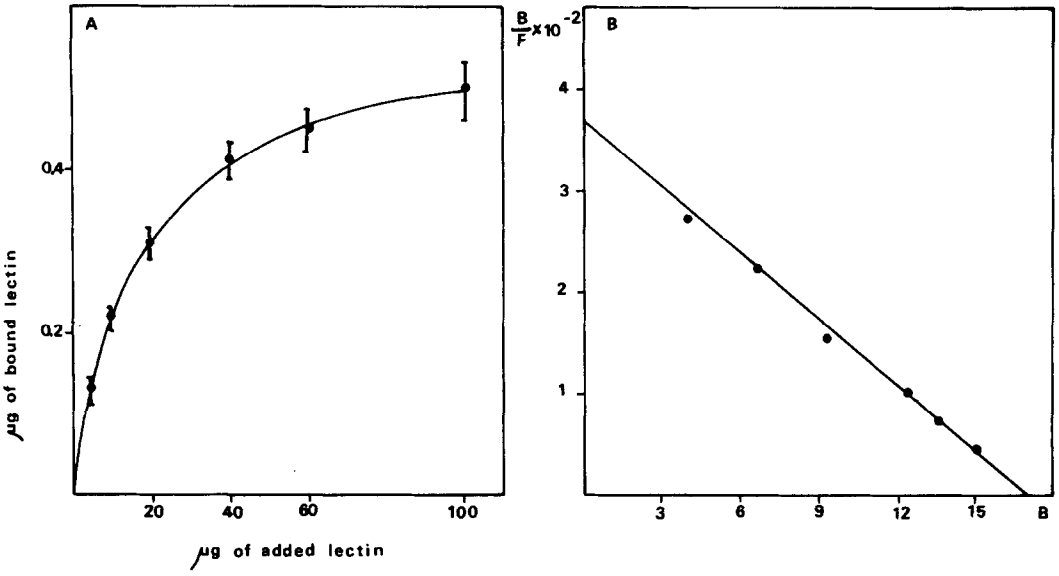

Figure 1A : Saturation curve for Con A binding to Toxoplasma gondii. Each point gives the range of values obtained from four separate experiments.

Figure 1B : Binding to Con A with data plotted according to the method of Scatchard.
B represents the concentration (nM) of Con A bound and F represents the concentration (nM) of free Con A.

ded to the parasites after the Con A was allowed to bind, the specific binding was deplaced (95%). Scatchard analysis of saturation curve (Fig.1B) gave monophasic curve suggesting that only a particular class of receptor sites was available for Con A on the surface of parasites. Toxoplasma gondii possesses $3x10^4$ sites for Con A per parasite and the affinity constant was estimated at $2,2x10^6 M^{-1}$. Compared to other cell systems (14,15), the number of binding sites per parasite was low, but the affinity constant was on the same order of magnitude as that determined for other cells.

In contrast to Con A binding, the specific binding of WGA, estimated either by the difference between total bound amount and non specific bound amount or by the amount of bound WGA released by the sugar inhibitor (N acetyl glucosamine) was very low. The ratio of WGA specific bound/WGA free was estimated at $3x10^{-3}$ to $1x10^{-3}$, using 10µg to 100µg of WGA/10^8 parasites. These results cannot thus be used for the quantitative determination and suggested an absence or a low number of WGA receptors on the surface of Toxoplasma gondii.

Using fluorescent lectins (Con A and WGA), Sethi and al (16) failed to observe a binding of both lectins to Toxoplasma gondii. Our

Table I : Labelling of Toxoplasma gondii surface using galactose oxidase/tritiated sodium borohydride.
The labelling was carried out according to the method of Gahmberg and Hakomori, and was described in "Material and methods".
The results were averages from four separate experiments, using a new preparation of parasites for each experiment.

	cpm, $\lvert^3H\rvert$ incorporated/10^8 parasites
Parasite + NaB $\lvert^3H_4\rvert$	2300 + 200
Parasite treated by galactose oxidase + NaB $\lvert^3H_4\rvert$	2200 + 150
Parasite treated by neuramidase + galactose oxidase + NaB $\lvert^3H_4\rvert$	3500 ± 300

results were in agreement with these authors for WGA binding but not for Con A binding. The difference between preparation procedures of Toxoplasma gondii and/or the use of specific and quantitative method for the binding experiments could account for this discrepancy.
The size of parasite did not explain the low number of Con A binding sites. For example, the sizes of erythrocytes and Toxoplasma gondii (17) are similar, but the Con A binding sites number for mice erythrocytes as determined in parallel experiments was 100 times higher than for the parasites (18). Then the lectin data show a low but significant amount of sugar per parasite cell.

Terminal galactosyl and N-acetyl galactosaminyl residues of membrane glycoconjugates can be estimated by incorporation of tritium, using their oxidation by galactose oxidase followed by their reduction by NaB $\lvert^3H_4\rvert$ (8,19). The results were reported in Table I. No difference was noted between the tritium incorporation in the parasites treated with galactose oxidase (5 to 100 units per 10^8 parasites) and parasites untreated (control). The sialic acid are often linked to subterminal galactosyl or N-acetyl galactosaminyl residues, preventing their oxidation by galactose oxidase (8,19). So the parasites were treated by neuraminidase before incubation with galactose oxidase. The difference of tritium incorporation between parasites treated with galactose oxidase

without and after neuraminidase treatment was not significant (Table I) compared to other cell systems (8,19,20), suggesting that Toxoplasma gondii surface glycoconjugates contain a little or no galactosyl and N acetyl galactosaminyl residues. Moreover, no amount of sialic acid, sensitive to the neuraminidase, can be detected by Warren's method, although high concentrations of parasites have been used ($2,5x10^9$ parasites). This result cannot be explained by the lack of specificity of neuraminidase, since the total sialic acid determination, released by acidic hydrolysis of Toxoplasma gondii ($2,5x10^9$ parasites) was negative. Furthermore, these last results were consistent with the low specific binding of WGA. It has been shown, indeed, that WGA recognize N acetyl glucosaminyl, N acetyl galactosaminyl and N acetyl neuraminic acid residues (21).

Cell surface sugars have been investigated in other protozoan parasite systems ; no sialic acid were found in Trypanosoma equiperdum (22), but their surface glycoconjugates contain N acetyl glucosaminyl, N acetyl galactosaminyl and mannosyl residues. It have also been reported that Trypanosoma congolense contains a single Con A receptor glycoprotein with only mannosyl and (or) glucosyl residues (23). Moreover, in the plasmodium, either no sialic acid (24), or a low amount of sialic acid (25,26), have been found. These results are probably of interest for the analysis of host-parasite interactions. At any rate, we have shown that Toxoplasma gondii is able to synthetise some sugar. This can be used in the controversy about the origin of the wall of Toxoplasma cyst (27,28). It may be thought from our data that polysaccharide found at the surface of cyst wall (29,30) are, in part, synthetised by the parasite. However, a more detailled analysis of sugar content from isolated Toxplasma gondii membrane (manuscript in preparation) would bring additional evidence to this point.

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