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Lipids and fatty acids of tachyzoites and purified pellicles of *Toxoplasma gondii*

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Abstract. Various lipids were extracted from tachyzoites and from purfied pellicles of *Toxoplasma gondii*. Extracts from both sources were found to have a low cholesterol/phospholipid ratio. The major phospholipid in these fractions was phosphatidylcholine associated with a low amount of sphingomyelin. Oleic acid represented one-third of whole-cell fatty acids and 44% of pellicular fatty acid content. The lipid composition of the pellicle of *T. gondii* is consistent with the previously reported high fluidity of this membrane.

Toxoplasma gondii (Protozoa: Coccidia) exhibits a particularly high membrane fluidity when studied by fluorescence polarization (Gallois et al. 1988). Because of the probable involvement of the pellicle in host cell invasion, this fluidity may play an important role in host-parasite interactions. High fluidity is particularly linked to the cholesterol/phospholipid molar ratio and to the phospholipid and fatty acid composition, which are the main chemical modulators of fluidity (Shinitsky and Inbar 1976). We therefore analyzed these characteristics in the *Toxoplasma* pellicle. We have previously described a new procedure for isolating pellicles from *T. gondii* tachyzoites (Foussard et al. 1990). In the present report we describe the lipid composition of the pellicles of *T. gondii* as compared with that of the whole cell. Our results show that the membrane lipid composition of *T. gondii* correlates well with its high membrane fluidity.

Materials and methods

Purification of the pellicle fraction

Parasites were harvested and purified as previously described (Gallois et al. 1988). Pellicles were prepared using a discontinuous sucrose gradient (Foussard et al. 1990). The pellet obtained by centrifugation from the 0.25- to 1-M interface was washed with 0.2 M phosphate-buffered saline (PBS, pH 7.2) and resuspended in a small amount of PBS. One part of the suspension was used for control by electron microscopy; another portion, for protein determination; and the remainder, for lipid analysis.

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Analytical procedures

Proteins were estimated according to the method of Lowry et al. (1951) using bovine serum albumin as the standard, except that samples and standards were initially suspended in aqueous solution containing 1% sodium dodecyl sulfate.

From the pellicle preparation, total lipids were assayed according to the method of Folch et al. (1957). Total phospholipids were determined using a commercially available enzymatic kit [Biomerieux phospholipid enzymatic (PAP)] and compared with lipid phosphorus obtained after combustion with $HClO_4$ as assayed by the method of Bottcher et al. (1961). Individual phospholipids were analysed by thin-layer chromatography on silica gel G-coated plates (0.25 mm, Merck) as described elsewhere (Gallois et al. 1988). Cholesterol was assayed using cholesterol esterase and oxidase (Boehringer test-combination cholesterol) and by the Liebermann method (1885).

A semi-quantitative estimate of the relative amount of neutral lipids was obtained by microdensitometry (Clinscan Helena) of high-performance thin-layer chromatography (HPTLC) plates (Merck). The chromatography was performed in hexane/ether/acetic acid (75:23:2, by vol.). The plates were sprayed with $CuSO_4$ (10%) in phosphoric acid (8%) and then heated at 120° C for 20 min (Touchstone et al. 1981).

Glycolipids were obtained after silicic acid chromatography. Silicic acid in *n*-heptane was poured into a 10-mm × 100-mm column and washed as described by Rouser et al. (1967). The total lipid extract in chloroform was introduced into the column and sequentially eluted with chloroform/acetone/methanol (10:40:10, by vol.). The lipid fraction eluted by acetone was chromatographed on silica-gel plates (0.25 mm, Merck) in 28% chloroform/methanol/ammonia (65:25:5, by vol.). Glycolipids were stained by spraying the plates with 20% naphthylamine or H_2SO_4 followed by heating at 100° C for 20 min.

Total fatty acids in the lipid extract were methylated using the $BF_3 + CH_3OH$ procedure (Morrison and Smith 1964). Hydrolysis and methylation were performed over 10 min for phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolyamine (PE) and phosphatidylinositol (PI) and over 90 min for sphingomyelin (SM) and for the total extract. This was followed by hexane extrac-

tion, washing and dehydration with anhydrous Na_2SO_4. The extracts were then evaporated to a few microliters of hexane solution. They were subsequently assayed by gas chromatography using a Carlo Erba gas chromatograph equipped with a fused silica capillary column (50 m × 0.22 mm) coated with a 0.19 μm-thick film of cyanopropylpolysiloxane (CP-SIL 88, Chrompack, The Netherlands). Fatty acids were identified by their retention time as compared with standards (Sigma). All chemical analyses were done in triplicate for each preparation.

Results

The chemical composition of the pellicle fraction is shown in Table 1 and is compared with that of whole cells (Gallois et al. 1988). Proteins represented 75.5% of the dry weight in whole cells and 55%–60% of the dry weight in pellicles. Lipids comprised 17.5% of the dry weight of the cell and 35% of the dry weight in the enriched pellicle fraction. The most notable feature was a 2.4-fold enrichment in phospholipid and a 4.5-fold enrichment in cholesterol in the pellicle fraction. As a consequence, the cholesterol/phospholipid molar ratio increased from 0.21 in homogenate to 0.4 in the pellicle fraction.

As shown in Table 2, the membrane fraction was enriched in phosphatidylcholine (75.4% vs 62% in tachyzoites) and displayed a lower sphingomyelin content (1% vs 8% in tachyzoites). No major change was observed for the other phospholipids. Lysophospholipids and cardiolipin were not detected. Glycolipids eluted from silicic acid by acetone constituted a very small fraction of whole-cell lipids. Only three pale spots were observed, making their identification difficult.

Table 3 lists the major fatty acids identified in tachyzoites and in the pellicle fraction. The various preparations displayed a similar distribution of fatty acids, with

Table 1. Chemical composition of the enriched membrane fraction and of whole-cell preparations of *Toxoplamsa gondii*

	Whole-cell preparations	Enriched membrane fraction
Proteins[a]	75.5 ±3.8	55 −60
Lipids[a]	17.5 ±0.56	32 −38
Phospholipids (μmol mg protein^{-1})	0.24±0.01	0.56± 0.08
Cholesterol (μmol mg protein^{-1})	0.05±0.003	0.23± 0.03
Cholesterol/phospholipid (mol mol^{-1})	0.21±0.01	0.4 ± 0.01

Data represent mean values ±SD for 5 whole-cell preparations made in duplicate and for 4 preparations of enriched membrane fraction

[a] Results are expressed as the mean percentage of the dry weight ±SD for 5 whole-cell preparations made in duplicate (date from Gallois et al. 1988) and 2 preparations of enriched membrane fraction

Table 2. Phospholipid composition of whole-cell preparations and enriched membrane fractions of *Toxoplasma gondii*

Phospholipid	Total phospholipids (%)		
	Whole-cell preparations ($n=6$)	Enriched membrane fractions ($n=4$)	
		Mean	Range
PC	62 ±4.2	75.4	71.1−83.5
PS	8.4±3.5	12	5.5−13
PE	11.2±4.2	6.9	4.3− 9.1
PI	6.6±2.1	5.5	3.2− 7.7
SM	8 ±4.3	1	0.6− 3.9

Lipids were extracted from cellular material by the method of Bligh and Dyer and thin-layer chromatography was carried out using the solvent system of Esko and Raetz. Following chromatography, the phosphorus in each spot was estimated colorimetrically. Results are expressed as the mean percentage of phosphorus ±SD for whole-cell preparations (data from Gallois et al. 1988); the mean value and range is shown for enriched membrane fractions. PC, Phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin

Table 3. Fatty acid composition of *Toxoplasma gondii* whole-cell preparations and enriched membrane fractions and phospholipid species in the membrane fraction

Fatty acid	Total phospholipids (weight/100%)		Phospholipid species (weight/100%)				
	Whole-cell preparations	EM fraction	PC	PS	PI	PE	SM
$C_{14:0}$	9 ±0.8	18.4 ±6.15	24.5±3.5	6.85±1.85	9.6 ±2.9	4.3±3.1	19.4±0.5
$C_{16:0}$	16.6 ±1.5	24.2 ±5.3	28 ±5.4	31.4 ±5.6	36 ±3	20.8±2.7	46 ±4.6
$C_{16:1}$				7.5 ±2.2		4.2±3	
$C_{18:0}$	10.8 ±1.15	8.5 ±2.8	4 ±2.75	16.8 ±2.25	15 ±5	16.4±4.85	22 ±3.8
$C_{18:1}$	30.6 ±2.5	40 ±4.5	40 ±3.1	11.9 ±4.45	24.8 ±5.75	34.5±14.6	9.3±3.1
$C_{18:2}$	19.8 ±1.8	7.5 ±4.25	3 ±3	6.6 ±4	11.65±1.95	10.1± 6.7	7 ±6.3
$C_{18:3}$				6.5			
$C_{20:4}$[a]	5.8 ±0.6	2.6 ±0.85	0.5	11.3	5.4	9.9	
Satd/unsatd	0.78±0.45	0.66±0.07					

Results for major fatty acids represent the mean value ±SD for 3 preparations in dublicate. Fatty acids contributing <1% to the whole fatty acid composition were omitted. Satd/unsatd, Ratio of saturated to unsaturated fatty acids; EM, enriched membrane

[a] Identified in 2 preparations

oleic acid ($C_{18:1}$) accounting for about one-third of the fatty acids identified. Each of the eight other fatty acids accounted for $<1\%$ of the total. Fatty acids of different classes of phospholipids that were extracted from the enriched pellicle fraction are also shown in Table 3. Phosphatidylcholine, which represented 75% of the total phospholipids, was esterified by about 40% of the oleic acid, and phosphatidylethanolamine was esterified by about 30% of the oleic acid. Phosphatidylserine and phosphatidylinositol were esterified by about 33% of the palmitic acid and sphingomyelin was esterified by about 50% of the palmitic acid.

Discussion

The lipid/protein ratio found in the whole-cell pellicle fraction of *Toxoplasma gondii* was in agreement with results published for other cell types (Dawidowicz 1987). However, the lipid composition of the whole-cell material and the enriched pellicle fraction showed some peculiarities:

1. The low cholesterol level associated with the low cholesterol/phospholipid molar ratio in *T. gondii* is comparable with results previously obtained for *Gregarina*, another sporozoan, which possesses a similar pellicle and exhibits cholesterol/phospholipid ratios of 0.2–0.36 (Philippe and Schrevel 1982).
2. The unusual phospholipid composition involving large amounts of phosphatidylcholine (75%) and the very low amount of sphingomyelin (1%) in *T. gondii* have also been observed in *Leishmania collosoma* and *L. donovani donovani* (Chaudhuri and Banerjee 1987).
3. Oleic acid was the major fatty acid found both in whole-cell and in pellicular phospholipids in *T. gondii*, and its content in the latter parasite may be compared with the fatty acid composition of *L. collosoma*, in which oleic acid represents 35.5% of plasma-membrane fatty acids, and with that of *L. d. donovani*, in which it represents 60% of total fatty acids (Chaudhuri and Banerjee 1987). The ratio of saturated vs unsaturated fatty acids found in the pellicle fraction was lower (0.78) than that previously described for the eucaryotic plasma membrane (about 0.9; Spector and Yoreck 1985).

Our results are in good agreement with the high fluidity observed by fluorescence polarization (Gallois et al. 1988). It has previously been suggested that a decrease in the sphingomyelin/phosphatidylcholine ratio and in the cholesterol/phospholipid molar ratio increases membrane heterogeneity and domain formation (Yechiel and Barenholz 1985). It should therefore be worthwhile to investigate the relationships between these peculiar chemical and physical properties, which may play a role in parasite host-cell interactions.

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