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CHEMICAL COMPOSITION OF THE PLASMA MEMBRANE FROM EPIMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI*

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The chemical composition of two plasma membrane fractions from epimastigote forms of *Trypanosoma cruzi* is reported. Fraction M, a preparation obtained by conventional methods of cell fractionation is composed of 31% proteins, 34% lipids, 16% carbohydrates and 3% of the lipopeptidophosphoglycan. Phospholipids and sterols account for 7.5 and 9%, respectively, of the total mass. Phosphatidylethanolamine is the major phospholipid in fraction M, representing 45% of the total membrane phospholipids. The other fraction, fraction V (vesicles), was obtained by treatment of the cell with a vesiculating agent. This fraction contains 42% lipids, 20% carbohydrates, 13% proteins and 21% of the lipopeptidophosphoglycan. Phospholipids and sterols make up 17 and 8%, respectively, of the total mass of this fraction. Phosphatidylcholine and phosphatidylethanolamine are the main phospholipids found in fraction V. Phosphonolipids and sialic acid have not been detected in either membrane fraction. Sodium dodecyl sulphate polyacrylamide gel electrophoretic analysis show that the glycoproteins ABC and the lipopeptidophosphoglycan are 50- and 10-times more concentrated, respectively, in fractions V and M than in the whole cell homogenate. The high molar sterol/phospholipid ratio found in fraction M suggests that this fraction is less fluid than fraction V, perhaps reflecting a migration of certain membrane components in the presence of the vesiculating agent. Hence, fraction M is, probably, more representative of the epimastigote plasma membrane as a whole than fraction V.

Introduction

Trypanosoma cruzi, the causative agent of Chagas' disease, is an unicellular organism whose life cycle involves a series of intimate interactions with the host structures. Cell penetration, signal recognition for differentiation, and evasion from immune mechanisms, represent only a few of the phenomena vital for the maintenance of this biological cycle. As in other cell systems, these phenomena may be reasonably presumed to be dependent on plasma membrane madiation. Thus, examination of the properties of the plasma membrane from this protozoan may contribute to a better understanding of the mechanisms which ultimately lead to infection (cf. Ref. 1).

Two methods for isolation of plasma membrane from *T. cruzi* have recently been developed in our laboratory [2,3]. In the first method, cells are ruptured by sonication under controlled conditions, yielding a fraction enriched in plasma membrane fragments upon differential and equilibrium density centrifugation [2]. In the other method, membrane vesicles have been obtained from epimastigote cell upon incubation with acetate pH 4.0, a condition which induces membrane vesiculation in *T. cruzi* [3]. The purity of both preparations has been checked by ultrastructural, biochemical and immunological methods [2,3].

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The latter method, which is rather drastic yields vesicles which are glycoprotein rich relative to the membranes obtained by the former method. The importance of comparing both membrane preparations with respect to their chemical composition stems directly from the fact that the vesicle preparation also displays antigenic properties.

Materials and Methods

Materials. All solvents utilized were analytical grade and have been redistilled before use. Phospholipids, acylglycerols, palmitic acid, cholesterol, sialic acid, 2-aminoethylphosphonic acid, potassium borohydride, 2,6-di-*tert*-butyl-*p*-cresol and dihydrosphingosine were purchased from Sigma. Silica gel H plates (0.25 mm thick) were obtained from Merck, silicic acid from Carlo Erba, and borontrifluoride-methanol from Applied Sciences. Radioactive products were obtained from New England Nuclear.

Cell culture. Epimastigote forms of *Trypanosoma cruzi*, Y strain [4], were cultured in LIT medium [5] in a rotary shaker (New Brunswick) at 120 rev./min, 28°C. Cells were harvested during late exponential growth (80–100 h) by centrifugation at $1000 \times g$, 4°C. The protozoa were washed three times with 0.9% NaCl. Cell numbers were determined in a Coulter counter.

Plasma membrane isolation. Fraction M (formerly called P'_3 [2]) was obtained by cell sonication, followed by differential and equilibrium centrifugation, as previously described [2]. Fraction V (vesicles) was prepared by exposing *T. cruzi* epimastigotes to sodium acetate pH 4.0, for 30 min at 37°C. Several centrifugation steps, which include a discontinuous sucrose gradient, provide pure vesicles, as described in detail in Franco da Silveira et al. [3].

Lipid extraction and fractionation. The ratios indicated below for all solvent systems represent volume ratios. Whole cells or membranes (10–20 mg protein) were extracted twice with 7 ml of chloroform/methanol (2 : 1, v/v) containing 10 mg/l of 2,6-di-*tert*-butyl-*p*-cresol, stirring 1–2 h at room temperature in each extraction. Supernatants were washed with 5 ml of 0.1 M KCl and, after careful removal of the upper phase, the lower phase was washed three times with chloroform/methanol/0.1 M KCl (3 : 48 : 47, v/v). The lower phase was concentrated and purified by chromatography on Sephadex G-25 [6]. This procedure resulted in the recovery of 100% [^{14}C]cholesterol and 90–95% phosphatidyl-1,2-[^{14}C]choline added to the preparation before extraction in the first Sephadex column eluate (chloroform/methanol (19 : 1, v/v), saturated with water). Sialic acid, phospholipids neutral carbohydrate and sterols have not been detected in the second column eluate (chloroform/methanol/acetic acid/water (32 : 2 : 7 : 1, v/v)).

The lipids contained in the first column eluate were separated into individual classes by one-dimensional thin-layer chromatography on Silica gel H. The following solvent systems were employed: *n*-heptane/diethyl ether/acetic acid (70 : 30 : 1, v/v), *n*-heptane/diethyl ether/acetic acid (90 : 10 : 1, v/v) and benzene/diethyl ether/acetic acid (45 : 70 : 1, v/v) [7,8]. After spot visualization with iodine vapors, the lipids were extracted from the silica gel as previously described [7]. The extraction efficiency was shown to be 95–100% using known standards of acylglycerols (monopalmitin, D-1,3-dipalmitin, tripalmitin), palmitic acid and cholesterol.

The classes of phospholipids were separated using the following solvent systems: (a) acetone/petroleum ether (1 : 3, v/v); (b) chloroform/methanol/acetic acid/water (25 : 15 : 4 : 1, v/v) [7]; (c) chloroform/methanol/acetic acid/water (65 : 28 : 8 : 4, v/v); (d) chloroform/methanol/acetic acid/water (38 : 65 : 8 : 4, v/v) [9]; (e) chloroform/methanol/28% aqueous NH_4OH (65 : 25 : 5, v/v); (f) chloroform/acetone/methanol/acetic acid/water (3 : 4 : 1 : 1 : 0.5, v/v) [10]; (g) chloroform/acetic acid/water (65 : 35 : 4, v/v) [11]. For one-dimensional chromatography, solvent systems (a) (1st run) and (b) (2nd run) were employed. For two-dimensional chromatography, the following pairs were used: (c) then (d); (e) then (f); (e) then (g). Phospholipids were detected with iodine and isolated by extraction as described [7]. Under these conditions, recoveries (based on phosphorus analysis) of phospholipid standards were 75–80%. The phosphorus present in each phospholipid class was also determined directly in the silica gel material corresponding to each spot after removal from the plates [12,13]. Specific dyes were used to detect lipid classes: ninhydrin for primary amines; Dragen-

dorf reagent for choline [7]; periodate-Schiff reagent for glycolipids, phosphatidyl glycerol and phosphatidyl inositol; ammoniacal silver nitrate for glycerol and inositol [14]; anthrone (0.4% in benzene) for lipid-containing sugar.

In some experiments, chromatography on silicic acid columns was used to separate neutral from polar lipids [6,15] or to separate each lipid class individually [8].

Analytical procedures. The sterols present in the total lipid extracts, or in samples purified by thin-layer and silicic acid column chromatography were determined as previously described [16], using cholesterol as standard. The concentration of free sterol in total lipid extracts was determined after precipitation with digitonin [17]. Esters were measured as hydroxamates [18,19], using methyl-palmitate, monopalmitin, dipalmitin and tripalmitin as standards. Total neutral carbohydrate was estimated by the phenol-sulfuric acid reaction [20], using glucose as standard. Protein was estimated by the method of Lowry et al. [21], using bovine serum albumin as standard. Nucleic acids were extracted as described [22]. DNA and RNA were measured by the diphenylamine reaction [23] and the orcinol method [24], respectively. Sialic acid was determined by the thiobarbituric acid assay [25]. Quantitative determination of long-chain bases was carried out by the methyl orange method [26] after alkaline hydrolysis with 2 ml of 1 M NaOH for 40 h at 100°C and extraction (twice) with ethyl acetate. Dihydrosphingosine was used as standard. Total phosphorus was determined by the method of Ames and Dubin [27]. The same method was used for the determination of inorganic phosphate, omitting the fusion with magnesium nitrate. In addition, the modified method of Fiske and SubbaRow [12,13] was employed after sample digestion with 70% perchloric acid for 4.5 to 12 h. In this case total phosphorus was estimated after 12 h hydrolysis (175°C) and mono- and diester phosphate after 4.5 h hydrolysis (140–155°C). Phosphonate phosphorus was calculated as the difference between total phosphorus and ester phosphorus as recommended [28]. Standards included disodium phosphate, phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid, sodium β-glycerophosphate and 2-aminoethylphosphonic acid.

The isolated free fatty acids were methylated with 10% boron trifluoride in methanol (2 min at 100°C). Lipopeptidophosphoglycan was isolated from epimastigote forms of *T. cruzi* according to Lederkremer et al. [29,30]. The isolated lipopeptidophosphoglycan was labelled with tritiated potassium borohydride after oxidation with sodium periodate [31,32]. This treatment does not alter the electrophoretic mobility of lipopeptidophosphoglycan in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sugar-containing macromolecules were analysed by electrophoresis in 15% polyacrylamide gels containing 0.1% sodium dodecyl sulfate after staining with the periodic acid-Schiff reagent [29].

Results

Overall chemical composition of isolated plasma membrane and whole cells

Table I shows the general chemical composition of the plasma membrane fractions and whole cell trypanosomes. The plasma membrane fractions are enriched in carbohydrates (2.6- to 3.2-fold) and lipids (1.8- to 2.2-fold) as compared to the total cell homogenates. Nucleic acids are practically absent from the membrane preparations. The small percentage of RNA in the vesicle preparation (fraction V) is probably due to the entrapping of cytoplasmic RNA during the vesiculation procedure.

Quantitative differences between the two membrane preparations are apparent in Table I. Fraction V is relatively protein poor and lipid rich as compared to fraction M. Moreover, the lipopeptidophosphoglycan, a characteristic glycophosphoceramide of *T. cruzi* epimastigotes [29,30,32,33], contributed significantly to the total mass in the vesicle preparation (21%, as opposed to 3.2% of the mass of fraction M). This calculation was made from determinations of long-chain amino alcohols in delipidized membranes.

Lipid-depleted plasma membranes

Since lipopeptidophosphoglycan is not extracted by chloroform/methanol (2 : 1, v/v), the relative amounts of this compound in the membrane fractions were measured following extraction with this solvent to eliminate interference from other lipid-bound long-chain amino alcohols. Table II

TABLE I

CHEMICAL ANALYSIS OF PLASMA MEMBRANE FRACTIONS FROM EPIMASTIGOTE CELLS

Data are expressed in mg per 100 mg of total dry weight. The results express the average of four to five experiments. Extreme values for each determination are given between parentheses.

Component	Plasma membrane fractions		Whole cells
	Fraction V	Fraction M	
Protein	12.8 (10.7–13.9)	31.3 (30.0–32.5)	47.3 (45.0–50.0)
Carbohydrate	20.0 (17.2–22.2)	16.4 (13.6–19.4)	6.2 (5.0– 7.0)
Lipid	42.0 (41.0–43.0)	34.2 (33.0–35.0)	19.0 (18.0–21.0)
DNA	0	0	2.3 (2.3)
RNA	0.9 (0.9)	0	5.5 (5.0– 6.0)
Total phosphorus	2.2 (2.2– 2.4)	1.0 (0.9– 1.0)	n.d. *
Lipopeptidophosphoglycan **	20.7 (17.0–28.0)	3.2 (3.1– 3.3)	n.d. *
Phospholipid	17.4 (16.9–18.3)	7.5 (7.0– 8.0)	5.8 (5.4– 6.4)
Sterol	8.4 (7.6–10.0)	9.2 (7.9– 9.9)	5.0 (4.8– 5.1)
Lipid-bound carbohydrate	2.6	2.1	0.9
Lipid-bound phosphorus	0.8	0.3	0.3

* n.d., not determined.

** Based on analyses of lipid-free membrane fractions (Table II).

summarizes the general composition of these delipidized plasma membrane fractions.

Lederkremer et al. [35] demonstrated that sphinganine and 17-methyl sphinganine account for 6.3% of the composition of the lipopeptidophosphoglycan. The recovery of lipopeptidophosphoglycan in the residue of extracted membranes is approx. 80%, as measured by the addition of the tritiated compound to the preparation before extraction. If the reasonable assumption is made that all long chain amino alcohols which remain in the membrane preparations after total lipid extraction derive from the lipopeptidophosphoglycan, determination of these amino alcohols provides the amount of lipopeptidophosphoglycan in the membrane. Following this rationale, the proportion of lipopeptidophosphoglycan in lipid-free residues from V and M is estimated to be 33 and 5%, respectively. Thus, taking into account the relative proportions of sugar and phosphorus in the lipopeptidophosphoglycan [29, 30], one can estimate that 50% of the total sugar and 26% of the total phosphorus present in the vesicles are associated with this substance. Similarly, 22% of the carbohydrates and 8% of the total phosphorus present in lipid-free residues from M are estimated to derive from the lipopeptidophosphoglycan.

Densitometric determinations on periodic acid-Schiff stained sodium dodecyl sulphate polyacrylamide gels (cf. Ref. 33) also indicate that the lipo-

TABLE II

CHEMICAL ANALYSIS OF LIPID-FREE *T. cruzi* PLASMA MEMBRANE FRACTIONS

Data are expressed in mg per 100 mg of the total dry weight of the membrane fractions after extraction with chloroform/methanol (2 : 1, v/v). The results express the average of four to six preparations. Extreme values for each determination are given between parentheses.

Component	Fraction V	Fraction M
Protein	24.0 (24.0–24.1)	47.5 (47.4 –47.6)
Carbohydrate	37.7 (34.4–42.1)	13.5 (11.2 –19.3)
Phosphoryl phosphorus	1.9 (1.8– 2.0)	1.06 (1.03– 1.06)
Phosphonyl phosphorus	0.6 (0.5– 0.7)	0.15 (0.14– 0.17)
Long-chain amino alcohols	2.0 (1.8– 2.2)	0.23 (0.22– 0.23)
Lipopeptido-phosphoglycan	32.5 (29.2–34.7)	4.9 (4.8 – 5.1)
Sialic acid	0 *	0 *

* Not detected by the thiobarbiturate assay method [25].

peptidophosphoglycan, as well as the carbohydrate-rich substances A, B, C are 30- to 50-fold more concentrated in fraction V (solid line) and 5- to 10-fold more concentrated in fraction M (dotted line) when compared to the same amount of protein from the total homogenate (in Fig. 1 coincident with the abcissa and thus not shown).

Of the membrane-bound total phosphorus present in the lipid-depleted membranes only 76–87% can be liberated by strong acid hydrolysis (6 M HCl, 48 h, 105°C); hence, the remaining 12–24% must be involved in phosphonyl bonds. These results suggest that phosphorus-containing macromolecules other than lipopeptidophosphoglycan exist in the membrane fractions.

Sialic acid could not be detected in these membranes using the thiobarbiturate reaction after hydrolysis of up to 1.4 mg of membrane dry weight in 0.01 M H_2SO_4 at 80°C for 2 h.

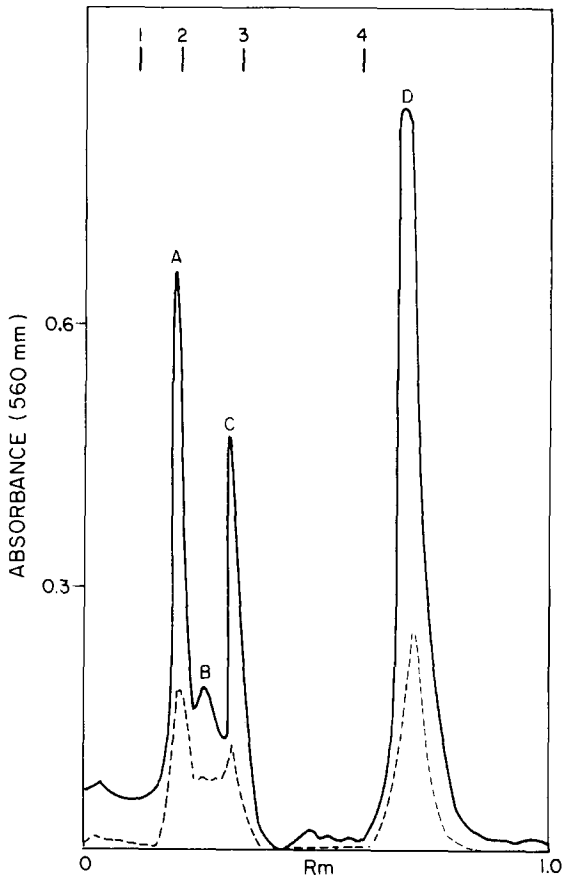

Fig. 1. Densitometer profiles of polyacrylamide gels. Fractions V (———) and M (- - - - -) were electrophoresed in 0.1% sodium dodecyl sulfate polyacrylamide gels (15%). Each gel contained 4.2 μg protein. After staining with periodic acid-Schiff reagent, gels were scanned at 560 nm. A, B and C are the carbohydrate-rich macromolecules (cf. Ref. 33) and D is the lipopeptidophosphoglycan. Molecular weight markers are shown at the top of the figure: 1, bovine serum albumin (68 000); 2, ovalbumin (43 000); 3, chymotrypsinogen (25 700); 4, cytochrome *c* (12 400).

Neutral lipids

Neutral lipids and phospholipids account for 71% of the total lipids in the whole cell homogenates (Table III). Among the neutral lipids, the most prominent are sterols (69% of the neutral lipids), along with acylglycerols (21%) and free fatty acids (10%). In the plasma membrane fractions, phospholipids and neutral lipids account for 58–73% of the total lipids. In fraction M, the neutral lipids are basically sterols (74%) and free fatty acids (26%). Sterols (63%), free fatty acids (2.2%) and acylglycerols (6.4%) are the main neutral lipids found in fraction V.

Although compounds having the same chromatographic behavior as acylglycerols could be detected in thin-layer plates, their concentrations in the lipid fraction from whole cells and membranes were relatively low or undetectable by the ferric hydroxamate method [17,18]. Since this method does not measure alkyl and alkenyl glyceryl compounds, it is likely that the total amounts of acylglycerols have been underestimated.

The molar ratio between total sterols and phospholipids differs significantly when fractions M and V are compared (Table IV). Another feature worthy of note is the appreciable concentration of sterol esters (30–40% of the total sterols) found in the membrane preparations.

Phospholipids and other polar lipids

Table V presents the phospholipid composition of whole cells and membrane fractions after separation by two-dimensional thin-layer chromatography (solvent systems (b) and (c), Materials and Methods). Other solvent systems used to separate phospholipids and other polar lipids revealed the same phospholipid classes.

The phospholipid composition of whole cell epi-

TABLE III

NEUTRAL AND POLAR LIPID COMPOSITION OF THE PLASMA MEMBRANE FRACTIONS FROM EPIMASTIGOTE CELLS

Data are expressed in mg of each lipid class per 100 mg of a total lipid extract dry weight. (I) Results obtained by separation of the neutral lipids on thin-layer chromatography on silica gel. The values express the average of three to five experiments. (II) Results obtained directly from the non-fractionated total lipid extract. The values express the average of four to eight preparations. (III) Results obtained by fractionation of the total lipid extract on silicic acid columns [8]. The values express the average of two preparations. Extreme values for each determination are given between parentheses. tr, trace.

Component	Plasma membrane fractions				Whole cell		
	Fraction V		Fraction M		I	II	III
	I	II	I	II			
Neutral lipids							
Free sterol	13.2 (12.2–15.4)	15.4 (13.4–18.3)	15.0 (13.0–16.0)	16.0 (14.0–17.0)	10.9 (8.5–13.1)	8.5 (5.7–12.6)	8.6 –
Sterol ester	6.7 (6.0– 8.3)	4.0 (3.7– 4.9)	12.0 (11.0–13.0)	14.0 (11.7–16.6)	17.2 (14.5–22.4)	14.6 (12.0–20.0)	19.0 (17.7–20.3)
Monoacylglycerol	1.0 (0.6– 1.3)	–	tr.	–	1.8 (1.3– 2.2)	–	1.0 –
Diacylglycerol	3.0 (1.9– 4.2)	–	tr.	–	3.3 (2.0– 4.1)	–	1.6 (1.4– 1.8)
Triacylglycerol	2.4 (1.5– 3.2)	–	tr.	–	3.6 (1.7– 6.9)	–	2.6 –
Free fatty acids	5.2 (2.8– 6.7)	–	9.4 (9.3– 9.7)	–	4.1 (1.8– 5.3)	4.7 (4.3– 5.0)	4.0 –
Phospholipids	–	41.5 (40.2–43.9)	–	22.0 (20.5–23.4)	–	30.4 (27.5–31.8)	–
Carbohydrates	–	6.3 (6.0– 6.7)	–	6.0 (5.9– 6.0)	–	4.9 (3.3– 6.2)	–
Long-chain amino alcohols	–	4.9 (3.8– 6.0)	–	2.1 (1.8– 3.5)	–	4.5 (3.7– 5.3)	–

* The phospholipid content was obtained by multiplying the total phosphorus found in the lipid extract by 22.58.

TABLE IV

RELATIVE CONTENT OF STEROLS AND LONG-CHAIN AMINO ALCOHOLS IN THE LIPID FRACTIONS FROM PLASMA MEMBRANE FRAGMENTS OF EPIMASTIGOTE CELLS

Component	Plasma membrane fractions		Whole cell
	Fraction V	Fraction M	
Total sterol (mol/mol of phospholipid)	0.87	2.22	1.68
Sterol (mol of free sterol/mol of sterol ester)	1.97	1.25	0.63
Long-chain amino alcohols (mol/mol of total phosphorus)	0.27	0.22	0.24
Long-chain amino alcohols (mol/mol of carbohydrate)	0.46	0.21	0.34

TABLE V

PHOSPHOLIPID COMPOSITION OF THE PLASMA MEMBRANE FROM *T. cruzi*

Data are expressed as the percentage of total phospholipid content. The results represent the average of four to five different preparations. Extreme values for each determination are given between parentheses. tr, trace. The figures in the columns 4–6 refer to, respectively: (a) this work; (b) Oliveira et al. [9]; (c) phospholipid isolated from cells previously subjected to the vesiculation procedure.

Component	Plasma membrane fractions		Whole cell		
	Fraction V	Fraction M	a	b	c
Lysophosphatidylcholine	9.0 (6.9–11.3)	13.4 (11.6–15.2)	1.2 (1.0– 1.7)	0.7 –	24.6 (22.3–26.8)
Sphingomyelin	tr.	tr.	3.0 (2.9– 3.0)	3.8 –	2.5 (1.0– 3.9)
Phosphatidylcholine	32.6 (28.5–34.5)	11.4 (9.1–16.1)	45.7 (42.5–50.9)	44.2 –	22.7 (20.0–24.0)
Phosphatidylethanolamine	30.8 (29.0–32.0)	44.4 (41.5–47.1)	22.4 (20.1–23.2)	27.8 –	25.8 (23.1–28.5)
Lysophosphatidylethanolamine	5.8 (5.5– 6.0)	6.0 (5.8– 6.2)	2.5 (2.0– 3.0)	–	2.5 (2.0– 3.0)
Phosphatidylinositol	9.7 (9.1–11.4)	3.9 (3.5– 4.5)	11.7 (10.5–12.8)	12.3 –	10.7 (10.5–10.9)
Phosphatidic acid	tr.	tr.	4.0 (4.0– 5.0)	4.2 –	4.6 (4.2– 4.8)
Cardiolipin	tr.	10.9 (10.4–11.4)	5.8 (5.0– 6.2)	2.1 –	1.0
Unidentified *	12.5 (12.0–13.0)	10.9 (10.7–11.0)	5.3 (5.0– 5.5)	2.9 –	5.3 (5.0– 5.5)

* Unidentified, R_f = 0.28 in solvent (c) and R_f = 0.68 in solvent (d).

mastigotes is similar to that described previously by Oliveira et al. [9]. The majority of the phospholipids present in the cell are also present in the membrane fractions, with the exceptions of phosphatidic acid and sphingomyelin. The high concentration of lysophosphatides in the membrane preparations is due to hydrolysis of the corresponding phosphatides during membrane isolation. Thus, the concentration of lysophosphatidylcholine in the whole cell extract increases from 1 to 25% when the cells are subjected to the same treatment employed to obtain membrane vesicles (Fraction V) (Table V). Consequently, assuming that the majority of the lysophosphatides found in the membrane fractions are derived from hydrolysis of phosphatidylcholine and, to a lesser extent, phosphatidylethanolamine, the phospholipids which contain choline and free aminogroups constitute 75–80% of the total phosphorus-containing membrane lipids. No phosphatidylserine was detected in either the whole cell extracts or the membrane preparations. Likewise, no phosphonyl phosphorus was detected in total lipid extract or in the isolated phospholipid fractions.

Long-chain bases were determined in total lipid extracts and isolated phospholipids from whole cells and membranes (Table III and IV). Sphingomyelin was the only compound in the total cell lipid extracts which contained long-chain bases. Since only 3% of the lipid-bound phosphorus is associated with sphingomyelin (Table V) it can be estimated that approx. 10% of the long-chain lipid-associated bases in the total cell derive from sphingomyelin. Since this complex lipid is almost absent in the membrane fractions, practically all of the long-chain bases present in our membrane preparations must be associated with compounds that do not

belong to the phospholipid fraction.

Neutral carbohydrates were present in the lipids from whole cells and membranes (Tables III and IV). The presence of lipid-bound carbohydrates could be detected by anthrone staining after separation by thin-layer chromatography. An anthrone positive spot migrating similar to a diacyl-threalose standard, i.e. with a R_f higher than that of cardiolipin, has been detected by using solvent pair (a), (b). The use of another solvent system (chloroform/methanol/water; 90 : 10 : 1, v/v) [34] revealed anthrone positive spots in the lipids from whole cell trypanosomes (R_f = 0.53; 0.36), fraction M (R_f = 0.53; 0.42; 0.22; 0.17) and fraction V (R_f = 0.53; 0.42; 0.36; 0.31; 0.22; 0.17). Although no attempts have been made to identify these glycolipids, the possibility that some of them are associated with the above mentioned long-chain bases to form sphingoglycolipids cannot be ruled out. Sialic acid was not detected in the lipid fractions from whole cells and membranes.

Discussion

The plasma membrane fractions from *T. cruzi* show a net enrichment in lipids and carbohydrates when compared to the whole cell homogenate, in general agreement with data for plasma membrane fractions from other protozoa and mammalian cells [36,41]. As observed in other systems [42–45], the method of cell surface vesiculation yields plasma membrane fragments characterized by a lower protein content and a higher total lipid content than the fraction M membrane preparation.

Sterols account for 70%, or more, of the neutral lipids isolated from the *T. cruzi* plasma membrane fragments. In fraction V (vesicles), the sterol/phospholipid molar ratio is very similar to that found in plasma membranes isolated from mammalian cells and certain protozoa [37–40]. On the other hand, this molar ratio is much higher in fraction M, being comparable to that in the plasma membranes from *Tryponosoma brucei* [46].

A peculiar aspect of the *T. cruzi* plasma membrane is the high proportion of sterol esters in relation to total sterols (30–40%). Sterol esters are not very common in plasma membranes of mammalian cells and protozoa; ever, when present, they rarely exceed 10% of the total sterol fraction [37–39]. A possible explanation for the elevated proportion of sterols and sterol esters in the plasma membrane of *T. cruzi* might be the existence of a preferential interaction with proteins or other components; indeed, specific lipid-protein interactions have been described in other systems [47]. The high concentration of sterols in the membrane of *T. cruzi* might also play an important role in the control of membrane fluidization, as has been proposed for mammalian cells and *Tetrahymena pyriformis* [48–50].

Phosphatidylcholine and phosphatidylethanolamine are the main phospholipids in vesicles, while phosphatidylethanolamine is the major phospholipid in fraction M. Although the proportion of lysophosphatides in the *T. cruzi* membrane preparations is similar to that found in membranes from mammalian cells and other protozoa [37,39, 50], the high concentration of lysophosphatidylcholine in our preparations is certainly due to hydrolysis of phosphatidylcholine during membrane isolation. This conclusion is evident from a comparison of the phospholipid compositions of cells before and after treatment with acetate (Table V, columns a, b and c). Both we and Oliveira et al. [9] have found that cell manipulation, i.e. freezing and thawing, incubation with acid buffers, sonication or lyophilization, leads to the appearance of lysophosphatides, principally, lysophosphatidylcholine. One possibility is that *T. cruzi* contains a phospholipase which preferentially hydrolyzes phosphatidylcholine when the cell is subjected to mistreatment. Phospholipases of this type have been described in the plasma membranes of erythrocytes and *Acanthamoeba castellanii* [51–52].

The membrane fractions of *T. cruzi* are enriched in the macromolecular complex composed of carbohydrate-rich electrophoretically defined bands A, B and C and in the lipopeptidophosphoglycan [33]. While the enrichment of these substances in fraction M is only 5- to 10-fold, the enrichment in the case of vesicles is approx. 50 times over the amounts observed in the total homogenates (Fig. 1). This fact, together with the mode of preparation [2] and overall chemical composition of fraction M, suggests that the fraction M plasma membrane fragments are more representative of the membrane

as a whole than fraction V. Indeed, in other systems [42,44,45] membrane fractions obtained using vesiculating agents exhibit a chemical composition significantly different from that of plasma membranes isolated from the same source by more conventional techniques of the type employed to obtain fraction M.

In addition to the net enrichment in bands A–C, lipopeptidophosphoglycan and phosphatidylcholine (40% of the total phospholipids after taking into account conversion of phosphatidylcholine to lysophosphatidylcholine), our fraction V is protein poor when compared to fraction M. This is consistent with either of two limiting situations: either the *T. cruzi* membrane is inherently microheterogeneous, favoring vesiculation in pre-existent 'weaker' domains, or the membrane is relatively homogeneous and becomes microheterogeneous due to vesiculating-agent-induced lateral migration of these components along the plane of the membrane. Several lines of evidence favor the latter hypothesis: (a) the distribution of antigenic determinants, concanavalin A receptors, colloidal iron binding sites, cationized ferritin and ruthenium red is homogeneous on the whole *T. cruzi* cell surface [53–57]; (b) except in the cytostome and flagellar regions, the intramembranous particles exhibit a homogeneous distribution along the plane of the membrane of the epimastigote cell [54,55]. As has been hypothesised for erythrocytes [58–62]. the aggregation of intramembranous particles induced by the acid pH leads to the liberation of vesicles rich in lipids, glycoproteins and glycolipids. Moreover, an acid pH might modify the lipid-lipid and lipid-protein interaction at the cell surface in such a manner as to facilitate the concentration of lipopeptidophosphoglycan (a glycophosphoceramide) and phosphatidylcholine in the vesiculating region.

Finally, it is important to note that although the vesicles possess a relatively low protein content, the protein banding pattern in polyacrylamide gel electrophoresis under denaturing conditions is not very different from that of the membranes prepared by conventional methods, mainly in the higher molecular weight regions (not shown). Both the chemical and antigenic properties of fraction V are currently under study in our laboratory. Preliminary observations suggest that fraction V can induce partial protection against experimental infection in mice (Martin, N.F. and Colli, W., in preparation).

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