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Plasmodium falciparum biosynthesizes sulfoglycosphingolipids

Malena Landoni [a], Vilma G. Duschak [b], Valnice J. Peres [c], Hiroshi Nonami [d], Rosa Erra-Balsells [a], Alejandro M. Katzin [c], Alicia S. Couto [a,*]

[a] *CIHIDECAR, Departamento de Química Orgánica, Pabellón II, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, 1428, Argentina*
[b] *Departamento de Investigación, Instituto Nacional de Parasitología 'Dr Mario Fatala Chaben', ANLIS-Malbrán, Ministerio de Salud, Buenos Aires, Argentina*
[c] *Departamento de Parasitología, Instituto de Ciencias Biomédicas, Universidade de São Paulo, São Paulo, Brazil*
[d] *College of Agriculture, Ehime University, Matsuyama CP 790-8566, Japan*

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Abstract

Sulfated glycosphingolipids are present on the surface of a variety of cells. They are active participants in adhesion processes in many systems and appear to be involved in the regulation of cell proliferation, differentiation and other developmental cellular events. However, the body of knowledge about synthesis, structure, and function of glycolipids in parasitic protozoa is very limited so far. In this work, we show by metabolic incorporation of [^{14}C]palmitic acid, [^{14}C]glucose and $Na_2{}^{35}SO_4$ that sulfoglycosphingolipids are biosynthesized in the three intraerythrocytic stages of *Plasmodium falciparum*. After saponification, purification of the labelled acidic components was achieved and two components named SPf1 and SPf2 were characterized. Chemical degradations and TLC analysis pointed out to sulfolipidic structures. Analysis by UV-MALDI-TOF mass spectrometry in the negative ion mode using *nor*-harmane as matrix showed for SPf2 a structure consisting in a disulfated hexose linked to a 20:1 sphingosine acylated with C18:0 fatty acid. Interestingly, parasite treatment with low concentrations of D,L-*threo*-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP) caused an arrest on parasite development associated to the inhibition of sulfoglycolipid biosynthesis. Taking into account that sulfoglycolipidic structures are currently involved in adhesion processes, our findings open the possibility to study the participation of this type of structures in the described aggregation phenomena in severe malaria and may contribute to clarify the pathogenesis of the disease. This report shows for the first time the synthesis of sulfoglycolipids in Apicomplexa.

Keywords: *Plasmodium falciparum*; Sulfoglycosphingolipids; Malaria; Glycoconjugates

1. Introduction

Plasmodium falciparum is one of the world's most pathogenic microbes. It kills millions annually and is especially lethal to young children and pregnant women in areas with high transmission [1]. The resistance of this parasite to conventional antimalarial drugs, such as chloroquine is growing at an alarming rate indicating that new efficient drugs are urgently needed [2]. Therefore, the biochemistry of the parasite needs to be understood for achieving rational approaches to chemotherapy.

Lipid metabolism has been attracting a lot of attention in the last years with respect to basic biology and applications for malaria chemotherapeutic purposes [3]. The results of biochemical and genomic studies show that apicomplexa parasites are capable of scavenging lipids and lipid precursors, including long-chain fatty acids, from their hosts. These resources provide lipids for protein membrane anchors and signal transduction cascades [4–7]. However, latest studies also showed that these parasites are competent to synthesize lipids *de novo* from simple metabolites. In labelling experiments, *P. falciparum* incorporated acetate into fatty acids [8]. Metabolic incorporation of [^{3}H]serine and [^{3}H]glucosamine was used to evidence the presence of glycosphingolipids [9], chloroplast galactolipids in apicomplexa parasites were also reported [10] and incorporation of [^{14}C]palmitic acid and [^{14}C]glucose into

* Corresponding author. Tel.: +54 11 4576 3346; fax: +54 11 4576 3346.
E-mail address: acouto@qo.fcen.uba.ar (A.S. Couto).

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neutral glycosphingolipids was performed and their structural determination by MALDI-TOF MS was achieved [11]. In addition, the potential of new antimalarial chemotherapeutics based on ceramide cytotoxic activity has been recently proposed [12].

Among acidic glycolipids, gangliosides (containing sialic acid) and sulfoglycolipids which contain sulfate monoesters are usually found as membrane components. Previous reports showed that *P. falciparum* does not biosynthesize sialic acid [13]. Cell surface sulfoglycolipids appear to be involved in the regulation of cell proliferation, differentiation and other developmental events [14–20]. Recently, the interaction of the major cardiotoxin from Taiwan cobra with sulfatides in the cell membrane has been shown to induce pore formation and cell internalization [21]. However, the body of knowledge about synthesis structure and function of glycolipids in parasitic protozoa is very limited so far [9].

It is known that the pathogenesis of *P. falciparum* infection involves intercellular adhesive interactions as critical events; the infected erythrocytes adhere both to endothelial cells [22,23] and, in a strain-specific manner, to uninfected red blood cells, thereby leading to the formation of rosettes [24]. Adhesion to endothelium is thought to be a critical factor in the pathogenesis of the disease [25,26] and rosetting, which may contribute to vascular plugging, has been associated with severe malaria [27,28]. The biosynthesis of sulfoglycolipids, active participants in adhesion processes in many cellular systems, might be related to a crucial role during plasmodial life cycle.

In this work, we show by metabolic incorporation of [^{14}C]palmitic acid, [^{14}C]glucose and $Na_2{}^{35}SO_4$ that sulfoglycosphingolipids are biosynthesized in the three intraerythrocytic stages of *P. falciparum*. After purification of acidic lipids and alkaline hydrolysis, the labelled components were analyzed by TLC. Chemical degradations pointed out to a sulfoglycolipidic structure for two labelled components SPf1 and SPf2. SPf2 structure was confirmed by UV-MALDI-TOF mass spectrometry. In addition, experiments with low concentrations of D,L-*threo*-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP) in the different stages of *Plasmodium falciparum* were performed showing that the drug caused an arrest on parasite development associated to the inhibition of the biosynthesis of sulfoglycolipids. In conclusion, in this report, we show for the first time the synthesis of sulfoglycolipids in Apicomplexa.

2. Material and methods

2.1. Materials

Lipid standards and BSA were purchased from Sigma. AlbuMax I® was obtained of Gibco BRL Life Technologies (NY, USA). D,L-*threo*-Phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP) was from Matreya (Pleasant Gap, PA, USA). Percoll® was purchased from Pharmacia Chemicals (Uppsala, Sweden). TLC was performed on silica gel 60 pre-coated plates (Merck) using the following solvent systems: (A) chloroform/methanol/water (65:25:3, v/v/v), (B) propanol/NH_3/H_2O (75:5:5, v/v/v) and (C) chloroform/methanol/water (65:25:4, v/v/v); reverse-phase TLC (RP-TLC) was performed on RP-18 f254 (Merck) plates using acetonitrile: acetic acid (1:1, v/v) (solvent D).

In all cases, radioactive samples were located by fluorography at −70 °C using EN^{3}HANCE (NEN) and Kodak X-OMAT AR films.

The β-carboline (9H-pyrido[3,4-*b*]indole) *nor*-harmane and 2,5-dihydroxybenzoic acid (DHB, gentisic acid, GA, USA) used as matrices for UV-MALDI-TOF MS were obtained from Aldrich Chemical Co. Calibrating chemicals for UV-MALDI-TOF analysis: α-Cyclodextrin (cyclohexaamylose, mw 972.9), β-cyclodextrin (cycloheptaamylose, mw 1135.0), γ-cyclodextrin (cyclooctaamylose, mw 1297.1), angiotensin I (mw 1296.49), neurotensin (mw 1672.96) and bovine insulin (mw 5733.5) were purchased from Sigma–Aldrich.

Solvents: methanol, ethanol and acetonitrile (Sigma–Aldrich HPLC grade), trifluoroacetic acid (TFA, Merck) were used as purchased without further purification. Water of very low conductivity (Milli Q grade; 56–59 nS/cm with PURIC-S, ORUGANO Co., Ltd., Tokyo, Japan) was used.

2.2. Parasite culture

The experiments were performed with an isolate NF54, clone 3D7 of *P. falciparum*. Parasites were cultured according to the method of Trager and Jensen [27] as modified by Kimura et al. [28]. The gas mixture of the tissue culture flasks (75 cm^2) contained 5.05% CO_2, 4.93% O_2 and 90.2% N_2. Parasite development and multiplication was monitored by microscopic evaluation of Giemsa stained thin smears.

[U-^{14}C]Palmitic acid (Amersham 822 mCi/mmol, 2.91 mCi/mg) originally supplied in toluene was dried under nitrogen, re-dissolved in ethanol and coupled with defatted BSA at a 1:1 molar ratio. Parasites (5.7% ring stages, 5.7% trophozoites stages, 4% schizont stages) were labelled for 18 h with 6.25 μCi/ml of the radioactive precursor in medium RPMI 1640 containing 0.5% Albumax and 0.05% BSA.

D-[U-^{14}C]Glucose (Amersham, 291 mCi/mmol, 1.54 mCi/mg) was incorporated at a concentration of 6.25 μCi/ml in RPMI 1640 medium without addition of 11 mM of glucose [30]. Parasites (5.9% ring stages, 5.4% trophozoites stages, 3.7% schizont stages) were labelled for 18 h.

$Na_2{}^{35}SO_4$ (Amersham, 1 mCi/mmol, 2.2 mCi/ml) was incorporated at a concentration of 12.5 mCi/ml in RPMI 1640 medium. Parasites (6.3% ring stages, 7.3% trophozoite stages, 4.6% schizont stages) were labelled for 18 h.

The viability of the parasites was verified by microscopic evaluation of Giemsa stained smears. Each stage was purified on a 40/70/80% discontinuous Percoll gradient (15,000 × *g*, 30 min, 25 °C). This procedure yielded an upper band (40%) containing schizont stages, another band with trophozoite stages (70–80% interface) and a pellet of ring stages [29]. A control containing a similar number of uninfected erythrocytes was incubated with the different radioactive precursors and further processed under the same conditions.

In another experiment, NBD-ceramide (NBD-Cer) purchased from Sigma was coupled to BSA and incorporated in parallel experiments to the parasite cultures at a concentration of 5 μM in RPMI 1640 medium. Parasites (6% ring stages, 4% trophozoite stages, 4% schizont stages) were labelled for 18 h.

2.3. Isolation and purification of glycosphingolipids

After lyophilization, each intraerythrocytic stage of *P. falciparum* was extracted with chloroform/methanol 1:1 (3 × 1 ml). Each extract was fractionated by anionic exchange chromatography on DEAE-Sephadex A-25 (acetate form) column, which was eluted with chloroform/methanol/water (30:60:8, v/v/v) to recover neutral GSLs and zwitterionic lipids. Anionic lipids were bulk eluted with chloroform/methanol/0.8 M NaAcO (30:60:8, v/v/v) evaporated to dryness and treated with 0.1 M NaOH in methanol (500 μl), at 37 °C for 3 h. The mixture was neutralized with HCl 1 M in the presence of 1 M phosphate buffer pH 7 (50 μl) to avoid over-acidification. After evaporation, salts were removed by reverse-phase chromatography, using a Sep-Pack C18 cartridge (Worldwire monitoring, PA, USA).

In another experiment, total lipids from schizont stages were extracted and purified as described above. The purified acidic fraction was analyzed in parallel with an analogous fraction obtained from [U-^{14}C]palmitic acid labelled parasites by TLC in solvent B. Spots corresponding to the sulfoglycolipids were extracted from the plate and analyzed by UV-MALDI-TOF MS.

2.4. Treatment of parasites with D,L-*threo*-phenyl-2-palmitoylamino-3-morpholino-1-propanol (D,L-*threo*-PPMP)

Parasite cultures (6.4% ring forms, 2.4% trophozoites, 1.2% schizonts) were incubated with 5 μM D,L-*threo*-PPMP. After 24 h of treatment, parasites were labelled with [^{14}C]palmitic acid or [^{14}C]glucose for 18 h in the presence of the drug. After the labelling period, each stage was purified on a Percoll® gradient as described above and freeze-dried prior to lipid extraction. The effect of the drug on parasite development was monitored by microscopy of Giemsa-stained blood smears. In all cases, control cultures without the inhibitor and a similar amount of uninfected erythrocytes were labelled under the same conditions.

2.5. Acid methanolysis and methylation

The sample was hydrolyzed for 18 h at 80 °C with 12 M HCl/MeOH/water (3:29:4, v/v/v). The hydrolysate was dried and the acid eliminated by several evaporations with addition of water. Methylation of fatty acids was carried out with BF_3/MeOH in dry toluene under nitrogen at 80 °C during 90 min [30].

2.6. Solvolysis

Desulfation was performed by treatment with 9 mM H_2SO_4 in 9:1 Me_2SO–MeOH at 80 °C for 3 h, after neutralization with dilute ammonia, the mixture was concentrated to dryness and the residue was dissolved in 1:1 $CHCl_3$–MeOH [31].

2.7. UV-MALDI-TOF MS analysis

Measurements were performed using a Shimadzu Kratos, Kompact MALDI 4 (pulsed extraction) laser-desorption time-of-flight mass spectrometer (Shimadzu, Kyoto, Japan) equipped with a pulsed nitrogen laser (λ_{em} = 337 nm; pulse width = 3 ns), tunable PDE, PSD (MS/MS device) and a secondary electron multiplier (SEM).

Experiments were performed using firstly the full range setting for laser firing position in order to select the optimal position for data collection, and secondly fixing the laser firing position in the sample sweet spots. The samples were irradiated just above the threshold laser power for obtaining molecular ions and with higher laser power for studying cluster formation. Thus, the irradiation used for producing a mass spectrum was analyte-dependent with an acceleration voltage of 20 kV. Usually 50 spectra were accumulated. All samples were measured in the linear and the reflectron modes, in both the positive- and the negative-ion mode. Best spectra were obtained with *nor*-harmane in negative ion mode and with GA in positive ion mode.

Sample preparation [32,33]: matrix stock solutions were made by dissolving 1 mg of the selected compound in 0.5 ml of 1:1 (v/v) methanol/water. Analyte solutions were freshly prepared by dissolving the samples (0.05 mg) in chloroform/methanol, 1:1 (v/v) (0.025 μl). To prepare the analyte/matrix deposit the sandwich method was used.

3. Results and discussion

Little is known about glycosphingolipid biosynthesis in *P. falciparum* [9]. We have previously reported the presence of an active glucosylceramide synthase in the intraerythrocytic stages of the parasite, a key enzyme for neutral glycosphingolipid biosynthesis. In addition, the structure of the ceramide, monohexosylceramide, trihexosylceramide and tetrahexosylceramide fractions was analysed by UV-MALDI-TOF mass spectrometry [11] increasing the body of knowledge about apicomplexa parasites lipidic metabolism. In this study, we show for the first time that acidic sphingolipids are biosynthesized in the three intraerythrocytic stages of *P. falciparum*. To achieve this goal, parasites metabolically labelled with [^{14}C]palmitic acid were extracted with Chloroform:methanol and the extracts were fractionated by DEAE-Sephadex column chromatography. The bound fraction eluted with Clo:Me:NaAcO 0.8 M corresponding to the acidic lipids was further subjected to alkaline hydrolysis and analyzed by TLC (Fig. 1A). The three intraerythrocytic stages showed the presence of labelled components. Apart from the fast migrating products that correspond to labelled fatty acids released by saponification, two double bands migrating lower than galactosylsulfatide (SM4s), named SPf1 and SPf2, resulted of special interest. When a similar number of parasites of each stage was analyzed, the schizont stages showed to incorporate a higher amount of the precursor than the others, as it has been

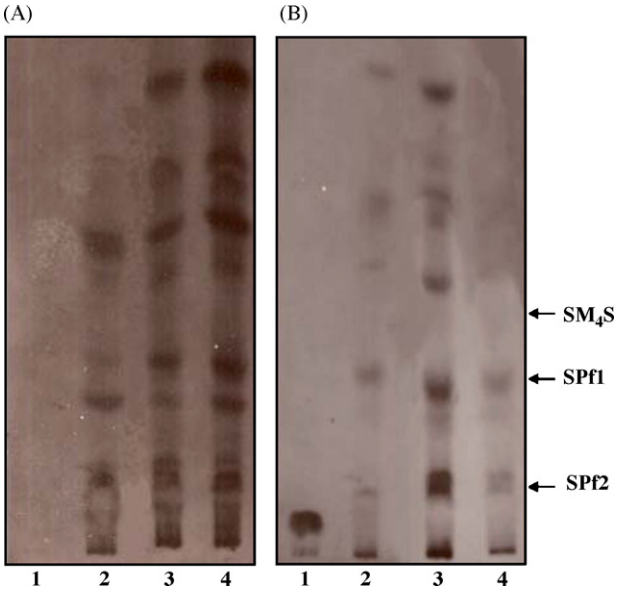

Fig. 1. TLC analysis of the labelled acidic lipids of *P. falciparum* in solvent system A. (A) [^{14}C]Palmitic acid incorporation: lane 1, control uninfected red blood cells; lane 2, ring stages; lane 3, trophozoite stages; lane 4, schizont stages. (B) [^{14}C]Glucose incorporation: lane 1, control uninfected red blood cells; lane 2, ring stages; lane 3, trophozoite stages; lane 4, schizont stages.

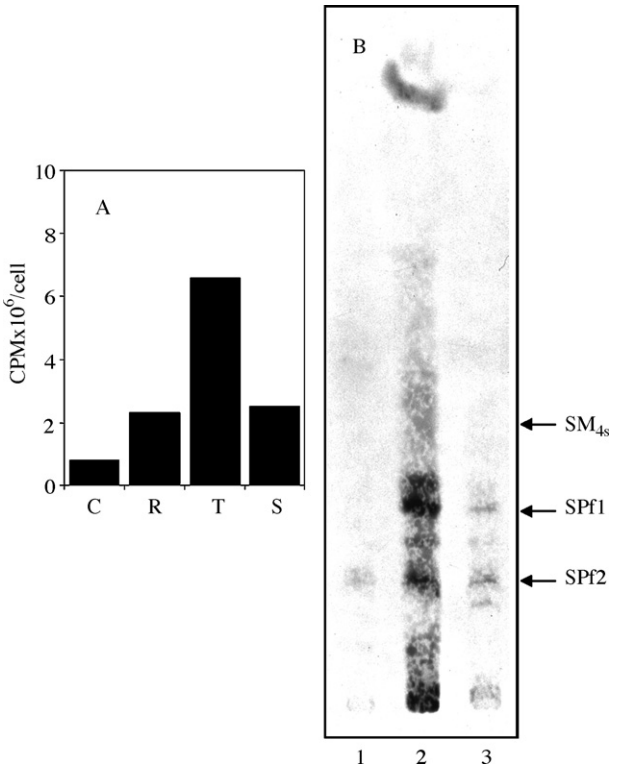

Fig. 2. Incorporation of $Na_2{}^{35}SO_4$ into acidic lipids of *P. falciparum*. (A) Comparison of the radioactivity per cell recovered in the fraction bound to the DEAE-Sephadex column when extracts corresponding to each stage were chromatographed. C, uninfected erythrocytes; R, ring stages; T, trophozoite stages; S, schizont stages. (B) TLC analysis in solvent system A of the acidic lipids: lane 1, ring stages (10×10^8 cells); lane 2, trophozoite stages (12×10^8 cells); lane 3, schizont stages (8×10^8 cells).

previously shown for incorporation of palmitic acid into neutral glycosphingolipids [11]. The same analysis was performed with parasites metabolically labelled with [^{14}C]glucose and also acidic GSLs from the three intraerythrocytic stages resulted labelled (Fig. 1B). It is worth noting that using the sugar precursor, the trophozoite stage showed to be the best incorporated.

It has been earlier described that *P. falciparum* does not synthesize sialic acids [13]. Thus, considering that inositol phospholipids [34–37] had been eliminated by the saponification step, the labelled compounds obtained pointed out to the presence of sulfoglycolipids in *P. falciparum*. To investigate the sulfolipidic nature of these components, a $Na_2{}^{35}SO_4$ metabolic incorporation was tried. The three-labelled stages were extracted as above and each extract was further purified by DEAE-Sephadex column chromatography. After saponification, the corresponding acidic fractions were desalted. As well as the sugar precursor, $Na_2{}^{35}SO_4$ was best incorporated in the trophozoite stage (Fig. 2A). When the acidic components from the three-labelled stages were analyzed by TLC (Fig. 2B) at least two major labelled components named SPf1 and SPf2, mainly present in the trophozoite stage, were detected. The fact that both bands, SPf1 and SPf2, were also identified with $Na_2{}^{35}SO_4$ as precursor, suggests the sulfoglycolipidic nature of these structures. Under the conditions used, uninfected erythrocytes showed no labelled components and the migration of both SPf1 and SPf2, was different from those corresponding to major sulfatides in mammalian cells [38] indicating that the biosynthesis of these compounds in the three parasite stages tested is genuine.

In order to confirm the sulfoglycosphingolipid nature of the labelled components, SPf1 was subjected to solvolysis with 9 mM H_2SO_4 in DMSO and the product was analyzed by TLC (Fig. 3A). As expected, solvolysis of SPf1 produced a fast migrating component as consequence of the release of the sulfate group (Fig. 3A, lane 2). In addition, when both sufatides were separately subjected to acid methanolysis and the resultant fatty acid components were extracted, methylated and analyzed by RP-TLC (Fig. 3B), spots coincident with the methyl esters of C18:0 and C16:0 fatty acids were detected for SPf1 and C18:0 and C14:0 fatty acids for SPf2. As a second approach, a fluorescent ceramide (NBD-Cer) was incorporated to the parasite culture. After purification, the corresponding acidic glycolipidic fraction was analyzed by TLC (Fig. 3C). Despite free fluorescent-acid due to partial decomposition was observed, the spots corresponding to the fluorescent sulfatides were evidenced, confirming the biosynthesis of acidic sphingolipids in *P. falciparum*. Taking into account that plasmodial GCS does not use NBD-ceramide as substrate [11], the fact that NBD-ceramide was incorporated into these acidic components suggests they are involved in a different biosynthetic pathway. Ongoing studies are being performed to address this issue.

In order to make a better characterization of these sulfoglycosphingolipids, an extract obtained from a mixture of the three forms of the parasite was prepared as above described and fractionated by DEAE-Sephadex column chromatography; the acidic fraction was further saponified and subjected to TLC in parallel with an analogous [^{14}C]palmitic acid labelled fraction.

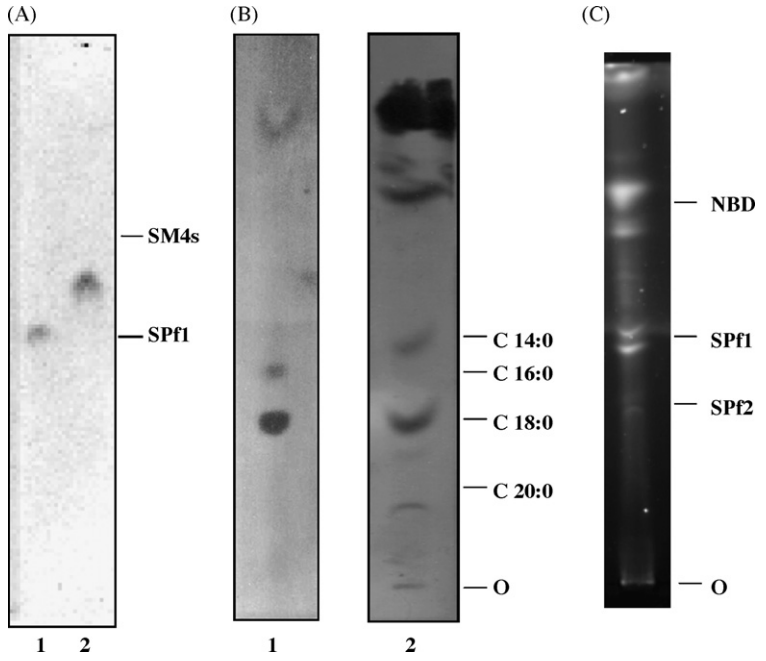

Fig. 3. (A) TLC developed in solvent system B of SPf1 (lane 1) and SPf1 after desulfation (lane 2). (B) RP-TLC analysis in solvent system D of the methyl esters of the fatty acids obtained after methanolysis of (1) SPf1 and (2) SPf2. C20:0, arachidic acid methyl ester; C18:0, stearic acid methyl ester; C16:0, palmitic acid methyl ester; C14:0, myristic acid methyl ester. (C) TLC analysis of the incorporation of NBD-ceramide into the purified lipid acidic fraction of *P. falciparum*.

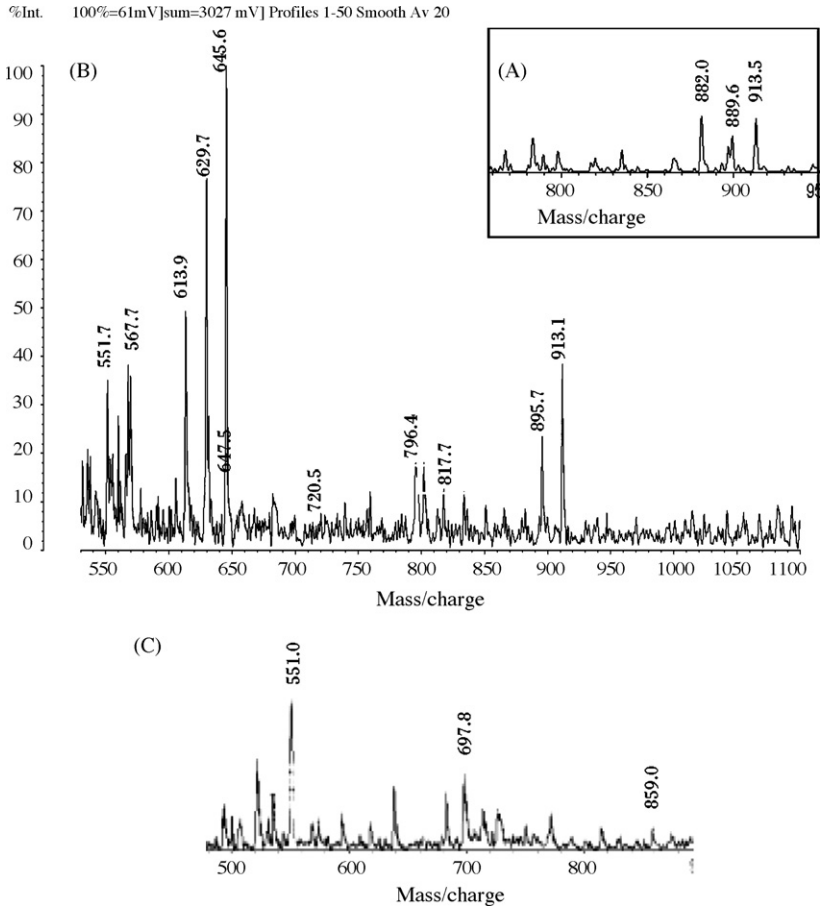

Fig. 4. (A) Linear UV-MALDI-TOF mass spectrum of SPf2 in the negative ion mode using *nor*-harmane as matrix. (B) reflectron UV-MALDI-TOF mass spectrum of SPf2 in negative ion mode using *nor*-harmane as matrix. (C) Linear UV-MALDI-TOF mass spectrum of SPf1 in the positive ion mode using gentisic acid as matrix.

The spot corresponding to SPf2 was extracted from the plate and analyzed by UV-MALDI-TOF mass spectrometry (Fig. 4). The spectra of SPf2 performed in the negative linear ion mode using *nor*-harmane as matrix showed a disulfated monohexosyl structure composed by a d20:1 sphingosine acylated mainly with a C18:0 fatty acid (as $[M–H]^-$: calculated *m/z* 914.5, observed *m/z* 913.5) (Fig. 4A). The spectrum performed in the reflectron mode (Fig. 4B) helped to get deeper in the structure as it also showed some laser-induced fragmentations. The loss of a sulfate group $[M–H–HOSO_3]^-$ may account for *m/z* 817.6 (observed *m/z* 817.7). The loss of another sulfate group is compatible with *m/z* 720.6 (observed *m/z* 720.5), the loss of water, $[M–H–H_2O]^-$ would account for *m/z* 896.5 (observed 895.7). From the latter, the loss of a C18:0 fatty acid moiety is compatible with *m/z* 630.3 (observed *m/z* 629.7) and the ion $[M–H–(C18:0)]^-$ is compatible with *m/z* 648.3 (observed *m/z* 647.5) [39], both of them are in accordance with the data obtained from the metabolic incorporation of [^{14}C]-fatty acid (Fig. 3B). In addition, the fragment derived from the cleavage of the sphingoid base between C2 and C3 left a fragment corresponding to the disulfated hexose with C1 to C2 of the sphingoid base acylated with C18:0 and would account for the main ion *m/z* 645.3 (observed *m/z* 645.6). By contrast, the UV-MALDI-TOF analysis of SPF_1 showed no signals when performed in the negative ion mode, whereas those obtained in the positive ion mode using GA as matrix, would account for a monosulfated hexose structure linked to a d20:1 sphingosine acylated with a C18:0 fatty acid ($[M + Na]^+$ calculated *m/z* 858.6 (observed *m/z* 859.0), confirming also a sulfated nature (Fig. 4C).

In *P. falciparum*, PPMP has been described as a potent inhibitor of the intraerythrocytic maturation leading to an arrest of the parasites at ring stage. Rings formed in the presence of the drug contain no tubular structures. On the contrary, mature trophozoites and schizonts that contain a fully extended tubular network are not affected by the drug [40–44]. In order to determine the effect of this inhibitor in sulfoglycolipid synthesis, treatment of parasite cultures with *threo*-PPMP for 24 h was performed followed by incorporation of [^{14}C]glucose or [^{14}C]palmitic acid in the presence of the drug. Extracts from treated and non-treated parasites were purified and further fractionated as above to achieve the corresponding acidic glycolipidic fractions.

As expected, the experiments showed that PPMP caused alterations in the parasite growth. As previously reported [11], when the inhibitor was added in parasite cultures, an arrest on parasite development was observed. Parasites collected at the ring stage had been treated with PPMP at the trophozoite stage (aproximately 40 h before) and resulted affected in a low degree. On the other hand, parasites collected at the schizont stage had received the inhibitor at the ring stage, consequently they were not able to evolve and died. As the fatty acid precursor is actively incorporated, a comparison of samples containing the same number of parasites corresponding to each of the three intraerythrocytic stages by TLC, was performed. Noticeably, the analysis showed that sulfoglycolipid biosynthesis was seriously affected mainly in schizont and ring stages (Fig. 5A). Similar differences among different stages were observed when NBD-ceramide was incorporated into these acidic components (data not shown). On the contrary, the low amount of [^{14}C]glucose incorporated in the early stages precluded the comparative analysis of these stages with the sugar precursor. Nevertheless, while the fraction obtained from non-treated schizont forms showed

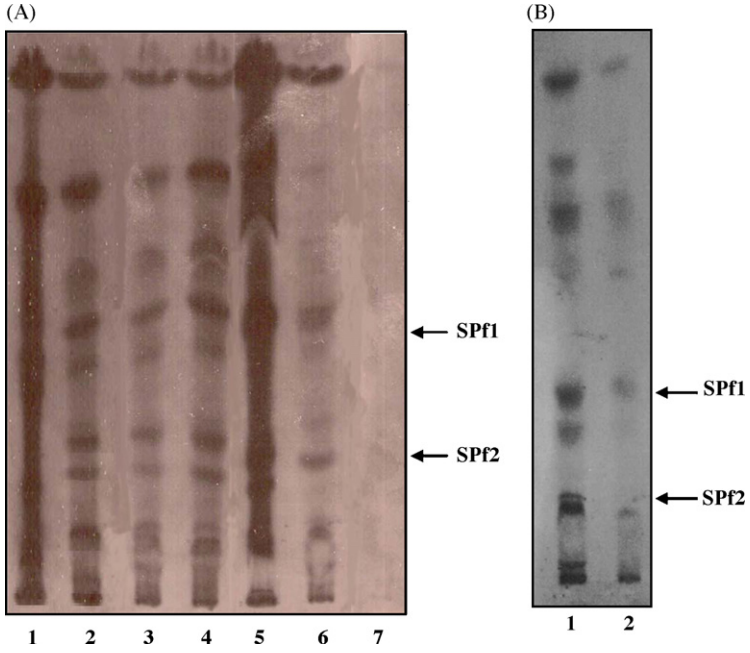

Fig. 5. TLC of the purified acidic lipid fraction obtained from parasites treated or non-treated with PPMP. (A) [^{14}C]Palmitic acid labelled acidic lipids. Lanes 1, 3 and 5 non-treated ring stage, trophozoites and schizonts, respectively; lanes 2, 4 and 6, PPMP-treated ring stages, trophozoites and schizonts, respectively; lane 7 uninfected erythrocytes. (B) [^{14}C]Glucose labelled acidic lipids obtained from (1) non-treated schizonts and (2) PPMP-treated schizonts.

spots corresponding to SPF1 and SPF2, the analogous fraction obtained from parasites treated with PPMP, showed a significant decrease in the acidic lipid biosynthesis (Fig. 5B).

Sulfated glycosphingolipids are present on the surface of a variety of cells including oligodendrocytes, renal tubular cells, and certain tumor cells. They seem to be involved in nerve conduction and cell adhesion [40–44], however, their exact physiological function is still unknown. In addition, diversities of sulfatide molecular species, not only with respect to sugar but also to ceramide moieties, probably important in lipid membrane microdomains were recently analyzed [17]. The parasitic sulfated lipids herein described, although minor structures in the three stages, might play an important role in physical and structural membrane stabilization, might be involved in the adhesion phenomena of intraerythrocytic forms, as well as in the sequestration phenomena [45]. In addition, our findings show a differential behaviour in the sulfoglycolipidic biosynthesis in the different intraerytrocitic stages of the parasite, suggesting some regulation on sulfatide levels along the parasite life cycle. Further studies will clarify the biological role of the sulfatides in *P. falciparum* development and/or in the pathogenesis of the malarial disease. As far as we know this constitutes the first report of the biosynthesis of sulfoglycolipids in Apicomplexa.

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