A Surface Antigen of Giardia lamblia with a Glycosylphosphatidylinositol Anchor*

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Since Giardia lamblia trophozoites are exposed to high concentrations of fatty acids in their human small intestinal milieu, we determined the pattern of incorporation of [3H]palmitic acid and myristic acid into G. lamblia proteins. The pattern of fatty acylation was unusually simple since >90% of the Giardia protein biosynthetically labeled with either [3H]palmitate or myristate migrated at ~49 kDa (GP49) in reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis. These observations suggest that GP49, the first surface antigen, which does not appear to be GPI-anchored, may be a common antigen with properties suggesting that it has a glycosylphosphatidylinositol (GPI) anchor. Moreover, phospholipase A2 (PLA2) or mild alkaline treatment released free fatty acids, indicating that it has a glycosylphosphatidylinositol (GPI) anchor.

In the present study, we show that the pattern of fatty acylation of G. lamblia proteins is very simple and does not change during differentiation. We found that the major fatty-acylated species is a protein of ~49 kDa which is linked to the outer face of the plasma membrane by a GPI anchor. In contrast, we show that the predominant 66/85-kDa cysteine-rich surface antigen described previously, GP49, was identified in Western blots of every isolate tested, as well as in subclones of a single isolate which differ in expression of a major cysteine-rich 85/66-kDa surface antigen, which does not appear to be GPI-anchored. These observations suggest that GP49, the first common surface antigen to be described in G. lamblia, may play an important role in the interaction of this parasite with its environment.

The parasitic protozoan Giardia lamblia is a major cause of waterborne enteric disease worldwide (Craun, 1984). This flagellate is of great biologic interest as well as medical importance because it belongs to the earliest identified lineage to diverge from the eukaryotic line of descent (Sogin et al., 1989). Giardia has two life cycle stages which are well adapted to survival in very different and hostile environments. The dormant cyst form, which is responsible for transmission, survives well in cold water (Bingham et al., 1979). In contrast, the flagellated trophozoite form, which causes disease, colonizes the human upper small intestine where it is exposed to fatty acids and bile, as well as digestive enzymes. Therefore, it is important to understand how the outer surface of this unique parasite enables it to survive in such a degradative milieu. Key surface antigens of many parasites have glycolipid anchors. However, little is known of how Giardia proteins are anchored in the plasma membrane.

Previously, we have shown that a mixture of biliary lipids (Gillin et al., 1984) or fatty acids complexed to BSA (Wieder et al., 1983) could replace serum in supporting growth of G. lamblia in vitro. Moreover, exposure of cultured G. lamblia trophozoites to bile salts and fatty acids at the slightly alkaline pH (pH 7.8) of the human small intestine triggered encystation (Gillin et al., 1988). The importance of fatty acids for growth and differentiation stimulated us to assay the proteins of both life cycle stages of G. lamblia for fatty acylation.

The abbreviations used are: BSA, bovine serum albumin; PBS, phosphate-buffered saline; TLCK, N-p-tosyl-L-lysine chloromethyl ketone; PCMPS, p-chloromercuribenzenesulfonate; VSG, variant surface glycoprotein; CRD, cross-reactive determinant of VSG; E-64, trans-epoxysuccinyl-L-1-leucylamido(4-guanidino)butane; PMSF, phenylmethyl-sulfonfluoride; PIP2, phosphatidylinositol bisphosphate; DTT, dithiothreitol; GPI, glycosyl-phosphatidylinositol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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G. lamblia Surface Antigen

SDS-PAGE. Fig. 2 shows that GP49 (labeled with either palmitate or myristate) is an amphiphilic protein which partitions into the Triton X-114 detergent phase. After Triton X-114 phase separation, GP49 consistently migrates slightly faster, at around 46 kDa. We do not yet know the relationship between these two species.

In order to determine the native molecular weight of this protein, the phase-separated [3H]palmitate-labeled GP49 was analyzed on nonreducing SDS-PAGE. Fig. 3 shows major fatty acylated species of \( M_r = 90,000 \) and 49,000 and a minor band at 46,000. We do not yet know whether the 90-kDa species represents a dimer of GP49 or GP49 complexed to another protein.

**Bioisotopic Labeling of GP49 with GPI Components and Nitrous Acid Deamination**—Next we asked whether the fatty acid associated with GP49 is part of a phospholipid anchor. Fig. 4 shows the metabolic incorporation of GPI components into GP49, as analyzed by SDS-PAGE. [3H]myoinositol and [14C]ethanolamine, as well as [3H]glucosamine (not shown), were each incorporated into GP49, but [3H]choline was not (data not shown). Fig. 5A shows immunoprecipitation of [125I]-surface-labeled GP49 using monospecific rabbit antiserum against GP49, showing that this protein is exposed on the cell surface and supporting the specificity of this antibody. Fig. 5B shows that GP49 biosynthetically labeled with [14C]palmitate is also immunoprecipitated by this antibody. In another study (data not shown), double-labeled GP49 ([14C] palmitate and [3H]myoinositol) was immunoprecipitated with anti-GP49 antibody, and the [3H]palmitate and [3H]myoinositol were incorporated into the same protein.

The biosynthetic labeling pattern (Fig. 4, lane 4) shows that [14C]ethanolamine is incorporated into numerous proteins in G. lamblia including GP49, suggesting that other proteins may be modified by ethanolamine. Previously, Wiest

**RESULTS**

**Fatty Acylation Is Not Stage-specific**—We have observed (Gillin et al., 1987, 1988) that exposure of cultured trophozoites to free fatty acids in the presence of a primary bile salt stimulates encystation. Therefore, we performed biosynthetic labeling experiments to determine if [3H]palmitate or myristate is incorporated into stage-specific proteins of growing or encysting trophozoites. The major protein labeled with [3H] palmitate or myristate migrated at \( \sim 49 \) kDa (GP49) with a fainter band at 46 kDa in SDS-PAGE (Fig. 1). Since the 49-kDa and minor labeled proteins were observed equally in both encysting and nonencysting trophozoites, fatty acylation did not appear to be altered during encystation. Surface iodination (see below) revealed that GP49 is exposed on the cell surface of nonencysting trophozoites. In contrast, the major 66- and 85-kDa surface-iodinated species of strain WB clone C6 (Gillin et al., 1990) did not appear to be fatty-acylated (Fig. 1).

**Differential Extraction of GP49 by Triton X-114**—Since most fatty acylated proteins are membrane-associated, radiolabeled proteins were solubilized in Triton X-114, and the detergent-rich and detergent-poor phases were analyzed on

**MATERIALS AND METHODS**

**FIG. 1. Metabolic labeling of proteins from growing and encysting G. lamblia with [3H]palmitate and -myristate.** Attached trophozoites were harvested by chilling after 24 h of incubation in growth or encystation medium. Cell pellets were washed, labeled, and harvested as described under "Materials and Methods," then lysed by freezing and thawing, and analyzed on 10% SDS-PAGE and fluorography. The exposure time was 6 weeks. Lanes 1 and 2, nonencysting trophozoites (T), lanes 3 and 4, encysting cells (E). Lanes 1 and 3 show labeling with [3H]palmitate and lanes 2 and 4 with [3H] myristate. 100 \( \mu \)g of sample protein was loaded in each lane. GP49 is the major protein labeled with [3H]palmitate or -myristate in both nonencysting and encysting trophozoites.

**FIG. 4. Incorporation of glycosylphosphoinositol precursors into GP49.** Replicate samples of G. lamblia trophozoites (1.2 \( \times \) 10^6 cells each) were metabolically labeled with [3H]palmitate (10 \( \mu \)Ci/ml), [3H]myristate (20 \( \mu \)Ci/ml), [3H]myoinositol (50 \( \mu \)Ci/ml), or [14C] ethanolamine (20 \( \mu \)Ci/ml) for 2 h at 37°C. Cells were washed three times with cold PBS and analyzed by reducing 10% SDS-PAGE and fluorography. Lane 1, [3H]palmitate; lane 2, [3H]myristate; lane 3, [3H]myoinositol; lane 4, [14C] ethanolamine. Fatty acid and other GPI precursors are incorporated into GP49. The minor fatty-acylated bands are consistently observed with longer exposures of autoradiograms.
et al. (1988) have shown that ethanolamine is incorporated into several proteins of the parasitic trematode *Schistosoma mansoni*, which are not GPI-anchored. However, GP49 is the major protein which is labeled with fatty acids, myoinositol, and ethanolamine, suggesting that it is GPI-anchored.

The free amino group of the glucosamine residue is susceptible to nitrous acid deamination which cleaves its glycolic linkage (Shively and Conrad, 1976). Fig. 6 shows that the $^1$H- and $^{14}$C-labeled radioactive product released by nitrous acid treatment of immunopurified GP49 migrated faster ($R_f = 0.7$–0.8) than the PI marker ($R_f = 0.5$–0.6), suggesting that it is more hydrophobic. In comparison, the deaminated product of VSG, migrated with authentic PI (Fig. 6). Recently, Walter et al. (1990) have demonstrated that human erythrocyte de-cay-accelerating factor contains an ester-linked fatty acid substitution on the inositol ring of its GPI anchor and that the product released by nitrous acid deamination migrates faster than the authentic PI in TLC.

The nitrous acid deamination result, along with the observations that GP49 is not cleaved by *S. aureus* PI-PLC and is not recognized by anti-CRD antibody (see below), suggests that it may contain a substituted inositol ring (Roberts et al., 1988a, 1988b; Walter et al., 1990). The observation that treatment of the nitrous acid-deaminated anchor with methylene released all $^{14}$C radioactivity supports this hypothesis. This procedure releases ester-linked fatty acids, but not ether-linked fatty alcohols. Moreover, the water-soluble product of deacylation contained only $^3$H label and co-migrated with glycerophosphoinositol in HPLC (Fig. 7).

**Hydrolysis of GP49 by Phospholipases**—Bacterial PI-specific PLCs, which release 1,2-diacylglycerol from phosphoglycerides, are widely used to identify GPI-anchored molecules. These enzymes have little or no specificity for the acyl or alkyl group of the PI substrate, but differ in specificity toward the inositol head group (see Ferguson and Williams, 1988, for review). Therefore, gel-purified $^3$H palmitate-labeled GP49 and $^3$H myristate-labeled mVSG were treated with PI-PLC from various sources. The results in Table I

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### Table I

| Phospholipase               | Percent of VSG hydrolyzed | Percent of GP49 hydrolyzed |
|-----------------------------|---------------------------|----------------------------|
| None                        | 0                         | <0.1                       |
| *S. aureus* PI-PLC (0.25 μg)| 68.8                      | <0.1                       |
| *T. brucei* PI-PLC (0.2 unit)* | 85.7                      | 65                         |
| PLD, rabbit plasma (25 μl)  | 65                        | 10.0                       |
| PLD, human plasma (25 μl)   | 28.7                      | <0.1                       |

*One unit of PI-PLC is defined as the amount of enzyme that hydrolyzes 1 μmol of phosphatidylinositol per min and for GPI-PLC as 2 μg of VSG hydrolyzed under standard conditions.*
Release of $^3$H-Fatty Acid from GP49 by Mild Alkali and Phospholipase A. Treatment—The release of radioactive fatty acid from metabolically labeled acyl proteins by treatment with nucleophilic hydroxylamine has been widely used to identify amide or ester-linked fatty acids. In GPI-anchored proteins, fatty acids are attached to the 1,2-sn-diaclylglycerol backbone via ester linkages. When GP49 was digested with mild alkali, more than 90% of its radioactivity was released as free and esterified palmitic acid (Fig. 9A), showing ester linkages (Ferguson and Cross, 1984).

PLA$_2$ hydrolyzes the fatty acid ester bond in position 2 of phospholipids. As reported previously (Ferguson et al., 1985a), phospholipase A$_2$ removed 50% of the myristate from $^3$H myristic acid-labeled VSG. Similarly, when $^3$H-palmitate-labeled GP49 was treated with snake venom PLA$_2$, approximately 40% of the fatty acid was released. This observation was confirmed by identifying the released product on TLC (Fig. 9B). Taken together, the results of alkali and PLA$_2$ treatments indicate a 1,2-sn-diaclyglycerol moiety in GP49 which is typical of GPI anchors.

GP49 Is a Common Surface Antigen—The G. lamblia surface antigens which have been well characterized to date are a group of extremely cysteine-rich proteins which vary both among isolates and between subclones of a single isolate (Adam et al., 1988; Aggarwal et al., 1989). Therefore, we asked whether GP49 is also a variable antigen. Recently, we (Gillin et al., 1990) cloned and sequenced the major cysteine-rich 66/46-kD protein which permits them to survive in this hostile environment.

FIG. 11. Presence of GP49 in different isolates of G. lamblia. A, immunoblot; B, autoradiogram of the immunoblot. Four different isolates of G. lamblia labeled with $^3$H-palmitate were tested on an immunoblot with anti-GP49 polyclonal rabbit serum (1:100 dilution) prepared against strain WB, clone C6. Lane 1, isolate from Turkey; lane 2, Portland, OR; lane 3, Alaska; lane 4, Peru.

migration of the major fatty acid-labeled species. These results show that GP49 is present in all isolates tested and does not vary with the major cysteine-rich species.

DISCUSSION

The two life cycle stages of G. lamblia are remarkably well adapted to survival in very different and inhospitable environments. The dormant, egg-shaped cyst form which is responsible for transmission, survives for months in fresh water at 4 to 8 °C. After ingestion and passage through the stomach and into the small intestine, flagellated trophozoites emerge from the cysts to colonize the human upper small intestine, a complex environment containing everchanging concentrations of fatty acids, bile salts, hydrogen ions, and food and digestive enzymes as well as their products. The structure and function of cell surface components of G. lamblia trophozoites which permit them to survive in this hostile environment are

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*S. Das and F. D. Gillin, unpublished observations.*
largely unknown. Therefore, it is important to identify the surface components of *G. lamblia* which play a key role in its adaptation and survival.

Farrington et al. (1985) proposed that in the small intestine, *G. lamblia* might obtain its lipids from bile. Similarly, we reported that defined mixtures of biliary lipids containing bile salts, phospholipids, and cholesterol support the growth of *G. lamblia in vitro* (Gillin et al., 1986). Moreover, Jarroll et al. (1981) showed that these parasites have a limited capacity for *de novo* lipid biosynthesis. Blair and Weller (1987) reported the preferential incorporation of polyunsaturated and saturated fatty acids into different phospholipids. Interestingly, palmitic acid was mainly incorporated into phosphatidylinositol. We have also shown that exposure of cultured *G. lamblia* trophozoites to bile salt and free fatty acid triggers encystation (Gillin et al., 1988). Since fatty acids are important for both growth and differentiation, we asked whether crucial proteins or antigens of *G. lamblia* are fatty acylated.

GPI anchors have been found in many proteins with diverse biological activities, including cell surface antigens, membrane-bound enzymes, and adherence proteins. In *Trypanosoma brucei*, VSG anchored through its GPI molecule is susceptible to cleavage by an endogenous phospholipase C and is released quickly during host-parasite interactions. This may also be true of the lipophosphoglycan antigen of Leishmania (Turco et al., 1984) or the membrane form of the P90 antigen of Toxoplasma (Nagel and Boothroyd, 1989), which are also GPI-anchored.

In the present study, the biosynthetic labeling experiments with [3H]palmitic and [3H]myristic acid show that the 49-kDa *G. lamblia*, under nonreducing conditions, a substantial portion of GP49 migrates as a dimer, at ~90 kDa. However, the major species is still 49 kDa and the 46-kDa minor species is apparent. We do not at present understand the relationship between these three protein species.

Since many surface antigens of protozoan and mammalian cells are anchored on the cell surface by a GPI anchor, it was of interest to determine whether this is true of GP49. Presence of a GPI anchor was supported by the metabolic incorporation of [14C]ethanolamine, [3H]myoinositol, and fatty acids into GP49 (Fig. 4). This was confirmed by enzymatic and chemical cleavage experiments (Figs. 6–9). However, the GPI anchor of GP49 differed from that of the well characterized VSG in three respects. While GP49 is susceptible to cleavage by *B. cereus* PI-PLC, it was not hydrolyzed by *S. aureus* PI-PLC, *T. brucei* GPI-PLC, or rabbit or human plasma PLD (Table 1), suggesting a major difference between the GPI moiety of GP49 and VSG. Moreover, antibody to the VSG (CRD) which cross-reacts with many other GPI-anchored molecules did not recognize GP49 (not shown). Finally, GP49 reacts with wheat germ agglutinin, a lectin specific for N-acetylglucosamine or sialic acid residues (data not shown), which are usually not part of the GPI glycan. Further work is needed to determine whether N-acetylglucosamine and/or sialic acid are present in the GP49 GPI glycan or elsewhere on the molecule.

Recently, Roberts et al. (1988a, 1988b) proposed that the presence of palmitic acid in the 2-position of the inositol ring in human erythrocyte acetylcholinesterase is responsible for its resistance to cleavage by *S. aureus* PI-PLC. In the present study, we have shown that the purified GP49 of *G. lamblia* is resistant to PI-PLC from *S. aureus*, but not from *B. cereus*. However, *B. cereus* PI-PLC did not release GP49 in soluble form from the intact membrane (data not shown). When palmitoylated GP49 was subjected to nitrous acid deamination, no radioactivity was detected either in the glycan moiety or in the protein part of the molecule (not shown). Sequential degradation (nitrous acid deamination followed by methylamine treatment) of purified GP49 double-labeled with [14C]palmitate and [3H]myoinositol supported the hypothesis that, like human erythrocyte acetylcholinesterase, the GP49 of *G. lamblia* may contain a fatty-acylated inositol ring. Although the chemical degradation studies of GP49 are consistent with an acylated GPI similar to that of human erythrocyte acetylcholinesterase, the results with *B. cereus* PI-PLC and plasma PLD are not, for reasons which are not yet understood. A detailed structural analysis of *Giardia* GP49 is necessary to locate the exact site of acylation in the inositol ring as well as to elucidate the total structure of the anchor molecule.

The *G. lamblia* surface antigens which have been characterized to date are a group of extremely cysteine-rich proteins which vary both among isolates and between subclones of a single isolate (Aggarwal et al., 1980). Adam et al. (1988) have demonstrated that a cloned isolate of *G. lamblia* expresses a cysteine-rich 170-kDa surface antigen (CRP 170) which undergoes antigenic variation. In their study (Adam et al., 1988), a monoclonal antibody against CRP 170 was used to select subclones in which the 170-kDa protein was replaced by other cysteine-rich proteins ranging from ~50 to 170 kDa. Recently, we have cloned and sequenced the entire gene (TSA 417) which encodes the major 66- and 85-kDa cysteine-rich trophozoite surface antigen species of *G. lamblia* strain WB, clone C6 (Gillin et al., 1990). Although sequence analysis showed a hydrophilic protein with a hydrophilic C-terminal membrane-spanning region, the present studies show that this is probably not replaced by a GPI anchor since the 66/85-kDa protein does not appear to be metabolically labeled by GPI precursors. TSA 417 negative subclones were isolated with or without antibody selection. In contrast, GP49 did not appear to vary among TSA 417 positive and negative subclones (Fig. 10). Moreover, despite repeated selection with GP49 antisera and complement, surviving parasites still expressed GP49 as detected by Western blots (not shown). Furthermore, *G. lamblia* isolates from Turkey, Portland, Alaska, Peru, and Afghanistan, whose expression of TSA 417 varied from ~0.01 to >80% of the population, showed identical patterns of fatty acylation and reactivity with anti-GP49 in Western blots (Fig. 11A). Thus, GP49 is a common, possibly invariant, antigen which could play a crucial role in the survival of *G. lamblia* and may be a target for vaccine development.

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Infectious Material: 10^6

A Common Surface Antigen of Giardia lamblia with a GlycosphingolipidContaining Holohexosamine.

by

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The Chinese Flag and Francis 2 Gillin
**G. lamblia Surface Antigen**

Immunoprecipitation and purification of GCP by immunoaffinity column:

Rabbit polyclonal antiserum directed against GCP was precipitated with 10% ammonium sulphate and centrifuged at 2000 g for 10 min in the cold. The pellet was resuspended in water and dialyzed against 0.9% NaCl/0.05 M Tris/0.1 M EDTA pH 7.4. The sedimentation supernatant was further purified by immunoaffinity column chromatography by using a column packed with 2 ml of immobilized antiserum, collected by centrifugation, washed with PBS and divided into two parts. One part was used for immunoprecipitation following the method of Fraser et al. (1986). The second part, antibody-bound to Affigel, was packed in a 3 ml plastic syringe. Lysed radiolabelled cells were centrifuged at 500 g for 10 min in the cold and the supernatant loaded on the Affigel column. After extensive washing with immunoprecipitation buffer (Fergeron et al., 1986), the radiolabelled GCP was eluted from the column by 10% glycerol, pH 7.4 containing 0.18 Triton X-100 and immediately neutralized with 1 M Tris-HCl, pH 9.5. The eluted fractions were pooled, dialyzed extensively against 10 mM ammonium acetate, pH 4.5, and concentrated. The concentrated (5 ml) fractions were subjected to sodium dodecylsulphate electrophoresis as described by Harel et al. (1985) for the isolation of 16 VSG from 7. brevis.

**Penetration assay digestion**

Approximately 2000 rpm of gel-purified [3H] palate labelled GCP was resuspended in 0.5 ml of 0.9% NaCl/0.05 M Tris/0.05 M EDTA pH 7.3, 10% glycerol and 1% Triton X-100 and was subjected to 3 h of digestion. The reaction product was then precipitated as described by Fergeron et al. (1986).

**Results**

**Histocompatibility**

Gel-purified GCP (approximately 2000 rpm) was treated with 40% ethanol containing 30 mM NaCl for 30 min at room temperature and the gel-purified product was analyzed on TLC (Fergeron, 1986).

Nitrogen acid methanolation:

Immunoprecipitated GCP labelled with [14C] palmitate and [3H] myristate was incubated at room temperature for 3 h with 150 ml of 150 mM sodium acetate (pH 5.5) and 150 ml of 0.5 M acetyl propionate in a final volume of 0.5 ml. 0.5 M NaCl was added in control tubes (Fergeron et al., 1986). The reaction product was labeled with an acetylated CH3/NaCl mixture (5:1), resuspended, and the CH3 phase was separated by centrifugation. Evaporated, mixed with phospholipid standards and analyzed by TLC in a CH3-acetate/acetic acid/H2O (88:20:24:16) solvent system.

**Myelin treatment**

Affinity-purified GCP double-labeled with [14C] palmitic acid and [3H] myristic acid was first subjected to nitric acid methanolation (see above). After extensive methylation of the aminophenyl carrier, the reaction product was isolated from the lower CH3 phase. The CH3 phase was extracted with dioxane nitrogen, and treated with methylene chloride in MeOH/chloroform (1:1) for 1 h at 50°C, and processed as described previously and separated by HPLC using glycofer-PL, glycofer-PL and glycofer-PL as standards (Kanegae-Kajitani et al., 1995).

**[14C]GCP and immunoblot analysis**

Reducing SDS-polyacrylamide gels were run as described by Laemmli (1970). For non-reducing gels, sample buffer was prepared without SDS or 2-mercaptoethanol. Gels were subjected to fluorography by treatment with Enhance for 1 h. Radiolabeled proteins were visualized by fluorography using En3HANCE (Bio-Rad) on XAR-3 X-ray film (Kodak). Western blot analysis was carried out by transferring gels according to the method of Towbin et al. (1979). The filter was first blocked with 5% milk and then reacted with affinity-purified rabbit polyclonal antibody to the cross-reacting determinant (CRD) of the ankle form of T. brucei [14C] GCP and [3H] CRP antibody (1:2000) for 1 h at 4°C, washed, and subjected to electrophoresis (10000 volts) for 1 h and developed in a chloroform/methanol substrate (Raham et al., 1984).

**Immunoprecipitation of GCP**

A 10% Triton X-100 soluble [3H] palate and myristate labelled GCP. Radiolabelled trophozoites were solubilized at 4°C in 1% Triton X-100 detergent, subjected to phase separation, and analyzed by reducing SDS-PAGE and fluorography. Lane 1 and 2, labelled protein before phase separation. Lane 3 and 4, detergent-insoluble fractions. Lane 5 and 6, detergent-soluble fractions. Lane 7, anti-CRD antibody; lane 8, GCP labelled with [14C] palmitic acid and [3H] myristate labelled cells. Lane 1, total precipitated extract; lane 2, preimmune serum; lane 3, unrelated antibody; lane 4, antiGCP antibody.

**Relative Mobility, cm**

Fig. 3: Non-reducing SDS-PAGE of [3H] palate labelled GCP.

[14C] palate labelled cells were phase-separated in Triton X-100 and the detergent-insoluble fraction was analyzed on non-reducing SDS-PAGE. After fluorography, the X-ray film was exposed to film and the radioactive bands were visualized by autoradiography. In parenthesis, the position of proteins bands above 90 KDa and 40 KDa, suggesting that GCP may exist. In part, as a dimer held together by disulfide bonds.
G. lamblia Surface Antigen

Fig. 6: Analysis of phospholipase-C cleavage products.

A. [125I]myristate labelled of VSG and B. [14C]palmitate labelled GNS were treated with G. lamblia ST-PS. Released products were extracted with toluene and analyzed on TLC. NF, monoarachidonate; SF, dipalmitate; PA, palmitic acid; TT, triglyceride and HD, methylylycerides. The radioactive product migrates with the 5-kD diacylglycerol standard.

Fig. 9: TLC analysis of the products of mild alkali and phospholipase A2 treatment.

A. Gel purified [125I]palmitate labelled GNS was treated separately with (A) mild alkali for 10 min at room temperature (RT) with phospholipase A2 (1 mg/mL) for 1 h at 37°C. Released products were extracted with toluene and small aliquots (approx. 600-800 cpm) were spotted and analyzed on TLC using petroleum ether/diethyl ether/acetic acid (80:20:1). The lipids standards are, NF, monopalmitate; SF, dipalmitate; PA, palmitic acid; TT, triglyceride and HD, methylylycerides (which co-migrates with ethyl palmitate). The released product migrates with the free and esterified palmitic acid standards showing cleavage of [125I]palmitate from a diacylglycerol backbone.