Structure of the Major Carbohydrate Fragment of the *Leishmania donovani* Lipophosphoglycan†

Salvatore J. Turco,* Steven R. Hull, Palmer A. Orlandi, Jr., and Steven D. Shepherd
Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40536
Steven W. Homans, Raymond A. Dwek, and Thomas W. Rademacher
Oxford Oligosaccharide Group, Department of Biochemistry, University of Oxford, Oxford, England OX1-3CU
Received April 1, 1987; Revised Manuscript Received May 18, 1987

ABSTRACT: The major carbohydrate fragment from the lipophosphoglycan of *Leishmania donovani* was generated by mild acid hydrolysis (0.02 N HCl, 5 min, 100 °C) and purified by chromatography on DE-52 cellulose and thin layer. By a combination of analyses including gas–liquid chromatography–mass spectrometry and ^{1}H NMR, the structure of the fragment was elucidated as PO_4→6Gal(β1→4)Man. Approximately 16 of these phosphorylated disaccharide units occur in the overall glycoconjugate structure. NMR analysis of an alkaline phosphatase treated phosphorylated tetrasaccharide generated from lipophosphoglycan showed that the phosphorylated disaccharide units are linked together via α-glycosidic linkages. Complete characterization of the phosphorylated disaccharide units of lipophosphoglycan provides the first example of a defined carbohydrate anchored in membranes by a derivative of phosphatidylinositol.

The protozoan parasite *Leishmania donovani* is able to live successfully in two harsh hydrolytic environments in its digenetic life cycle: as an extracellular promastigote in the alimentary tract of its sandfly vector and as an intracellular amastigote in lysosomes of cells of the reticuloendothelial system of its mammalian host. That *L. donovani* has adapted to survive in such hostile environments is most likely due to protection conferred by specialized molecules on the parasite's cell surface. In addition to glycoproteins and glycolipids, *L. donovani* promastigotes synthesize and express on their cell surface a novel glycoconjugate called lipophosphoglycan (Turco et al., 1984; King et al., 1987). This unusual macromolecule of *L. donovani* may be similar to a glycoconjugate in *Leishmania tropica major*, which has been implicated in host–parasite interactions (Handman et al., 1984; Handman & Goding, 1985). LPG[1] is a major glycoconjugate since it contains over half of the total carbohydrate bound to macromolecules in *L. donovani*. Structurally, LPG is a lipid-containing polydisperse species that is heterogeneous in its carbohydrate portion and is extremely labile to hydrolysis by mild acid. In previous observations (Turco et al., 1984), the major carbohydrate fragment generated by mild acid hydrolysis of LPG was partially characterized as a phosphorylated disaccharide unit of phosphorylgalactosyl-β-mannose, and it appeared that these units comprised a repeating sequence in LPG.

In this paper, the complete structure of the phosphorylated disaccharide is determined. The average number of these units in LPG is estimated to be approximately 16, and they are linked together by α-glycosidic linkages.

Experimental Procedures

Materials. All materials were obtained as follows: Dulbecco's modified Eagle medium (DME No. 430-2100) from Gibco; brain–heart infusion from Difco; silica gel G thin-layer plates from E. Merck; DE-52 cellulose from Pharmacia; $NaBD_4$ from Stohler Isotope Chemicals; D_2O from Aldrich. Partially methylated sugar derivatives used as standards in GC–MS analysis were prepared as described elsewhere (Hull & Turco, 1985). All other reagents were of the highest purity commercially available.

Cells and Starter Culture. Promastigotes of *Leishmania donovani* were obtained and passaged as described elsewhere (Turco et al., 1984). Starter cultures were grown at 25 °C to an average density of 3×10^7 cells/mL in Dulbecco's modified Eagle medium supplemented with 0.3% bovine serum albumin, adenosine (0.05 mM), xanthine (0.05 mM), biotin (1 mg/L), Tween 80 (40 mg/L), hemin (5 mg/L), and triethanolamine (0.5 mL/L). For isolation and purification of large amounts of LPG, 1-L cultures of brain–heart infusion supplemented with ethanolamine (1 mL/L), hemin (5 mg/L), and xanthine (0.01 mM) were seeded with 50–100 mL of starter cultures. Cells were grown at 25 °C in a controlled environment incubator shaker to a density of $(4–5) \times 10^7$ cells/mL.

Extraction and Purification of LPG. Ten liters of exponentially growing *L. donovani* promastigotes (approximately 10^{11} cells) was extracted as described previously (Turco et al., 1984). Briefly, the cells were separated from the culture medium by centrifugation and washed with 250 mL of phosphate-buffered saline (Turco et al., 1984). The cells were then extracted sequentially (with 15 mL of each solvent at 4 °C) as follows: 2 times with chloroform/methanol/water (3:2:1), 4 times with 4 mM $MgCl_2$, and 3 times with chloroform/methanol/water (1:1:0.3). LPG was then extracted from the resulting delipidated residue fraction by four extractions at 4 °C with 15 mL of water/ethanol/diethyl ether/pyridine/NH_4OH (15:15:5:1:0.017). The extract was dried by evaporation under reduced pressure, resuspended in 5 mL of

† This investigation received financial support from UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, NIH Grant AI20941, and NIH Research Career Development Award AM01087 to S.J.T. The Oxford Oligosaccharide Group was supported by Monsanto Co.
* Author to whom correspondence should be addressed.

[1] Abbreviations: LPG, lipophosphoglycan; GC–MS, gas–liquid chromatography–mass spectrometry; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride; EI, electron impact; CI, chemical ionization.

0006-2960/87/0426-6233$01.50/0

40 mM NH_4OH and 1 mM EDTA, and applied to a column (4.6 cm × 30 cm) of Sephadex G-150 equilibrated in 40 mM NH_4OH and 1 mM EDTA. The fractions containing LPG were pooled, dried by lyophilization, and desalted by elution through a column (1 cm × 5 cm) of Sephadex G-25 equilibrated in 40 mM NH_4OH. The dried LPG was resuspended in 4 mL of water/ethanol/diethyl ether/pyridine/NH_4OH (15:15:5:1:0.017) and was precipitated from the pooled fractions by adding an equal volume of methanol and chilling the sample at −20 °C for 16 h. LPG purified in this manner was judged to be homogeneous by analysis of SDS–polyacrylamide gels and thin-layer chromatography as reported elsewhere (Orlandi & Turco, 1987).

Alkaline Phosphatase Digestion. Alkaline phosphatase (0.1–0.3 unit) digestion was done in 1 mM Tris-HCl, pH 8, at 37 °C for 16 h.

Phosphate Determination. Phosphate was quantitated by the procedure by Barlett (1959).

Methylation. Methylation linkage analysis of carbohydrate samples converted to their partially methylated alditol acetate derivatives was performed as described earlier (Hull & Turco, 1985) with modifications. Samples were dried under nitrogen and kept under a constant nitrogen atmosphere throughout methylation. The sample residue was resuspended in 2.0 mL of redistilled dimethyl sulfoxide and sonicated for 30–45 min. Sodium methylsulfinyl carbanion (1.0 mL) was then added, and the sample was sonicated for 4–5 h. Subsequently, two additions of methyl iodide (1.5 mL) were made during 1-h intervals with continual sonication. After sonication, the sample was left a room temperature for 18 h, at which time 5 mL of water was added to terminate methylation. The long reaction times were used to ensure complete methylation of the sample. Chloroform (5 mL) was added to the sample to extract the fully methylated species. The chloroform fraction was washed 4 times with an equal volume of water and then dried under nitrogen. The sample was resuspended in 2 mL of 2 N trifluoroacetic acid and the reaction vessel sealed under vacuum and hydrolyzed at 120 °C for 4 h. The hydrolysate was cooled, dried under nitrogen, evaporated in the presence of methanol 3–4 times to remove traces of acid, resuspended in 1 mL of water, and reduced with 10 mg of $NaBD_4$. Reduction was stopped by addition of 1–2 drops of glacial acetic acid. The sample was dried under nitrogen, and boric acid was removed by repeated evaporations with 10% acetic acid in methanol. The dried sample was then acetylated and analyzed by GC–MS as described below.

GC–MS Analysis of Partially Methylated Alditol Acetates. Samples after methylation, hydrolysis, and reduction were acetylated by resuspending the sample residue in pyridine/acetic anhydride (20:80) and heating at 100 °C for 2 h. After the mixture was cooled, the sample was dried under nitrogen and repeatedly dried with toluene. The partially methylated alditol acetates were resuspended in 1 mL of chloroform and extracted 3 times with 3 mL of water. The chloroform phase was dried under nitrogen and resuspended in 50–200 μL of $CHCl_3$, of which 1–2 μL was analyzed by EI and/or CI capillary GC–MS. Capillary gas chromatography was on a DB-5 (J&J) (30 m × 0.25 mm), with helium carrier gas at 35 mL/min and a programmed temperature of 160 °C held isocratically for the first 3.0 min and then elevated to 240 °C at 8 deg/min with injectors and transfer lines at 250 °C. Chemical ionization mass fragmentography was performed essentially as described by Laine (1980, 1981). CI spectra were obtained with a Finnigan 3300-6110 mass spectrometer coupled to a Technivent interactive GC–MS data system.

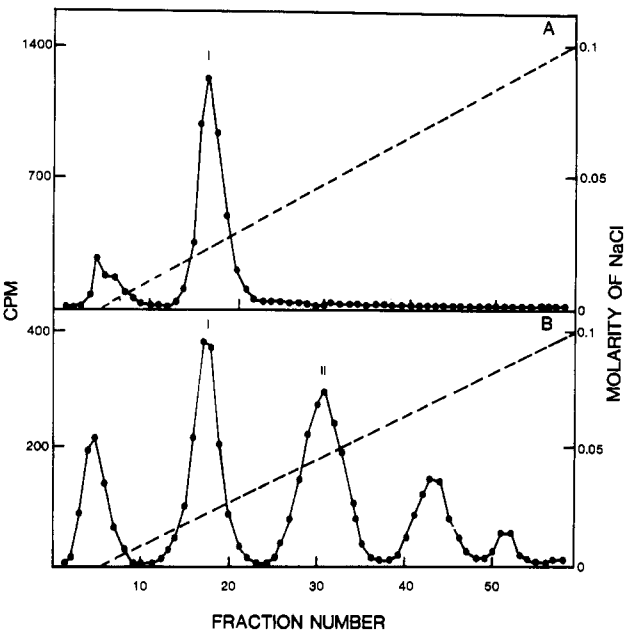

FIGURE 1: Chromatography on DE-52 cellulose of the mild acid hydrolysis products of [^{3}H]Man-labeled LPG. The labeled glycoconjugate was treated with 0.02 N HCl at 100 °C for either 5 min or 50 s, neutralized with 40 mM NH_4OH, dried by evaporation under N_2, resuspended in 1 mM Tris-HCl, pH 8, and applied to a column of DE-52 cellulose (0.5 cm × 1 cm) equilibrated in 1 mM Tris-HCl, pH 8. Fractions of 0.6 mL were collected and measured for radioactivity. After the fifth fraction was collected, a gradient of NaCl (0–0.1 M) in 1 mM Tris-HCl, pH 8, was applied to the column. (Panel A) [^{3}H]Man-labeled LPG pretreated with mild acid for 5 min; (panel B) [^{3}H]Man-labeled LPG pretreated with mild acid for 50 s.

Methane in the ion source was 1 Torr. Ionizing electron energy was 150 eV. Electron-impact mass fragmentography was performed as described by Bjorndal et al. (1970) and Stellner et al. (1970). Gas chromatographic conditions were identical with those for CI mass fragmentography. Ionizing electron energy was 70 eV.

NMR Analysis. One-dimensional ^{1}H NMR spectra were recorded at 500 MHz on a custom-built spectrometer at a probe temperature of 298 K, a sweep width of ±600 Hz, and 8K real data points. Two-dimensional ^{1}H–^{1}H phase-sensitive correlated spectroscopy (COSY) was performed at 500 MHz as described previously (Homans et al., 1986). The final data matrix is composed of 2K × 2K real data points with a sweep width of ±600 Hz and 64 transients per t_1 increment. Both positive and negative contour levels were plotted, and the time domain data were weighted in each dimension with phase-shifted sine-bell functions.

Samples were prepared for NMR studies by repeated dissolution in 99.96% D_2O with intermediate flash evaporation. Finally, the sample was dissolved in 400 μL of 99.96% D_2O to a concentration of approximately 0.4 mM. Chemical shifts are given relative to acetone, 2.225 ppm at 298 K.

RESULTS

Purification of the Phosphorylated Disaccharide. As reported earlier (Turco et al., 1984), mild acid hydrolysis (0.02 N HCl, 5 min, 100 °C) of [^{3}H]mannose-labeled lipophosphoglycan yields several carbohydrate fragments that can be separated by ion-exchange chromatography on DE-52 cellulose (Figure 1A). The predominant carbohydrate fragment (peak I, fractions 14–21) was previously partially characterized as $PO_4 \rightarrow$GalβMan (Turco et al., 1984). To obtain sufficient amounts of this fragment for chemical characterization of the galactosylmannose glycosidic linkage,

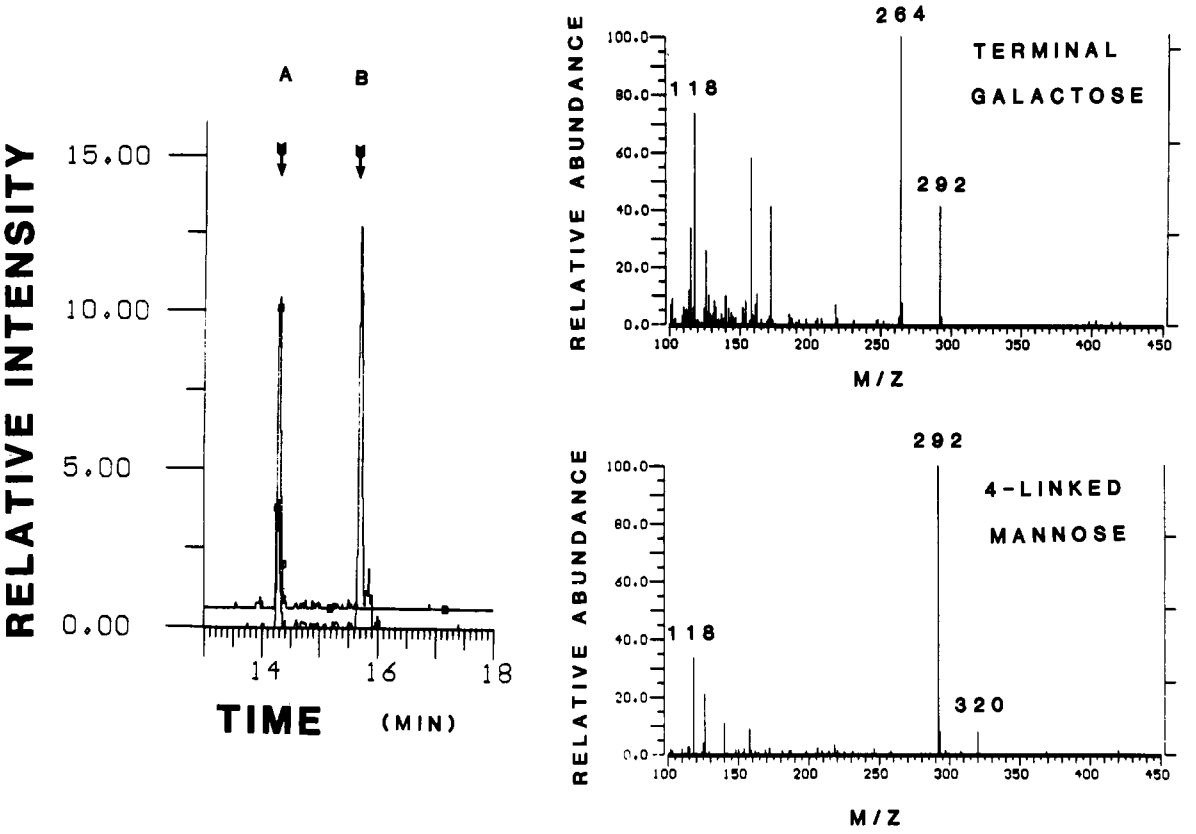

FIGURE 2: GC–MS of the partially methylated alditol acetates derived from the dephosphorylated disaccharide. Preparation of the sample and chromatographic analysis were carried out as described under Experimental Procedures. (Left panel) Total ion current; (upper right panel) mass spectrum of peak A in the total ion current; (lower right panel) mass spectrum of peak B in the total ion current.

1 mg of LPG containing [^{3}H]Man-labeled LPG was subjected to mild acid hydrolysis. Following elution from the ion-exchange support, fractions containing peak I were pooled, dried by evaporation under reduced pressure, resuspended in water, and desalted on a column of Bio-Gel P-2 equilibrated in water. The sample was chromatographed on a thin-layer plate of silica gel and developed in 1-propanol/pyridine/water (1:1:1). The phosphorylated disaccharide was localized by spraying a corresponding aliquot with orcinol–H_2SO_4 (Skipski & Barclay, 1969) and was scraped and eluted from the silica with water. Upon thin-layer chromatography in 1-propanol/pyridine/water (4:7:4) and high-voltage paper electrophoresis in 0.1 M sodium phosphate, pH 8, the phosphorylated disaccharide sample yielded a single spot resulting from spraying with orcinol–H_2SO_4 and, therefore, was judged to be pure (data not shown).

Determination of the Galactosylmannose Glycosidic Linkage. To determine the glycosidic linkage of disaccharide, the phosphorylated disaccharide was treated with alkaline phosphatase to remove the phosphate group and desalted on a column of Bio-Gel P-2 equilibrated in water. The dephosphorylated sample was permethylated, acid hydrolyzed, and analyzed by GC–MS. As shown in Figure 2, GC–MS analysis of the partially methylated alditol acetates revealed equivalent amounts of terminal galactose and 4-substituted mannose. Identification of the sugar derivatives was determined from the chemical ionization mass fragmentation patterns as well as their coelution on DB-5 fused silica with authentic standards. The characteristic MH – 60 fragments for a terminal hexose (264) and a monosubstituted hexose (292) were used for quantitative purposes. With lactose as a standard to determine response factors, the ratio of terminal hexose to 4-substituted hexose was found to be 1:1. Thus, it was concluded that the galactose residue was linked to the reducing mannose residue by a 1,4-glycosidic linkage. Moreover, this glycosidic linkage was confirmed by ^{1}H NMR analysis of the phosphorylated disaccharide (data not shown).

Isolation of a Phosphorylated Tetrasaccharide. The position of the phosphate group on the galactose residue and the anomeric configuration of the mannosyl phosphate groups linking the disaccharides together were elucidated with a larger carbohydrate fragment isolated from LPG. [^{3}H]Man-labeled LPG was subjected to partial acid hydrolysis in 0.02 N HCl at 100 °C for 50 s, and then the sample was neutralized with the addition of NH_4OH. Radioactive fragments from the sample were resolved by chromatography on DE-52 cellulose (Figure 1B). Compared to the profile obtained by total mild acid hydrolysis of LPG (Figure 1A), the proportion of PO_4→Gal(β1→4)Man (peak I) decreased, and a series of other anionic species increased. One of these (peak II, fractions 26–36) was pooled. An aliquot of peak II was treated with 0.02 N HCl at 100 °C for 5 min and reapplied to a column of DE-52 cellulose. The results indicated that peak II was quantitatively converted to peak I under this stronger acidic condition (data not shown).

Chromatographic Analysis of the Phosphorylated Tetrasaccharide. The identity of [^{3}H]mannose-labeled peak II was determined by a combination of mild acid hydrolysis and alkaline phosphatase digestion followed by paper chromatographic analysis of the products. The untreated fragment remained near the origin after paper chromatography (Figure 3A) as did samples treated with mild acid or alkaline phosphatase alone (data not shown). However, the fragment treated with mild acid and then with alkaline phosphatase did migrate with a mobility slightly faster than that of the disaccharide lactose and with an identical mobility with Gal(β1→4)Man (Figure 3B). Furthermore, digestion of peak II with alkaline phosphatase followed by mild acid hydrolysis revealed two products (Figure 3C); one remained near the

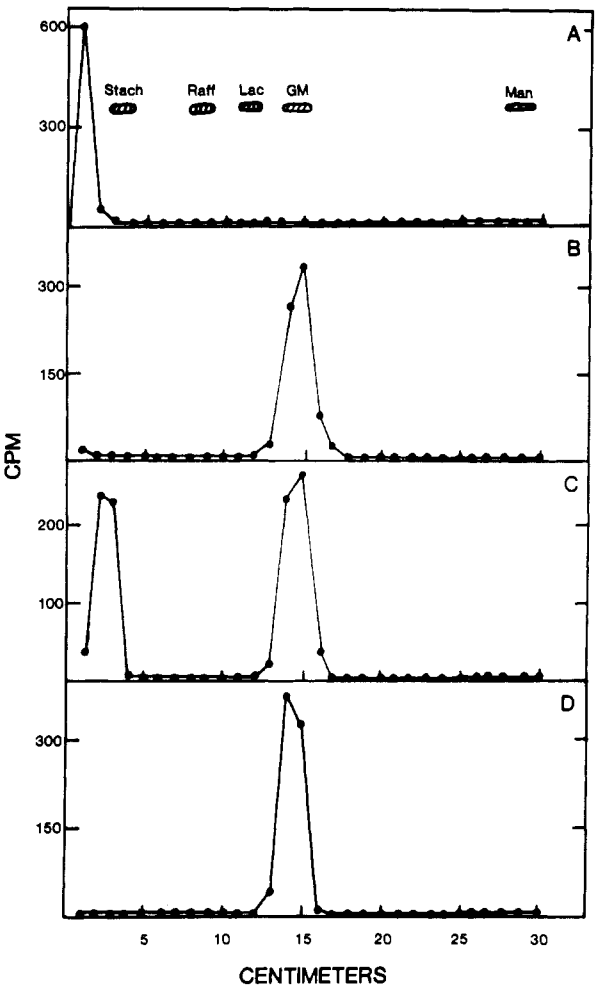

FIGURE 3: Paper chromatography of the phosphorylated tetrasaccharide fragment. Aliquots of [^{3}H]Man-labeled peak II (Figure 1B, fractions 26–36) were treated with 0.02 N HCl at 100 °C for 5 min and with alkaline phosphatase for 16 h. Paper chromatography was carried out in n-butyl alcohol/pyridine/water (6:4:3) for 16 h. Quantitation of radioactivity was accomplished by cutting the paper strips into 1-cm segments and counting in a vial containing scintillation fluid. Standard sugars were detected by staining with alkaline silver nitrate (Anet & Reynolds, 1954). (Panel A) Untreated fragment; (panel B) fragment treated with mild acid and then with alkaline phosphatase; (panel C) fragment treated with alkaline phosphatase and then with mild acid; (panel D) fragment as in panel C followed by treatment with alkaline phosphatase. Standards: Stach, stachyose; Raff, raffinose; Lac, lactose; GM, Gal(β1→4)Man; Man, mannose.

origin, and the other migrated as in Figure 3B. As shown in Figure 3D, sequential treatment of [^{3}H]mannose-labeled peak II with alkaline phosphatase, mild acid, and alkaline phosphatase again yielded a single radioactive peak that comigrated with Gal(β1→4)Man. Similar information was obtained by analyzing the treated fragment with high-voltage paper electrophoresis in 0.1 M sodium phosphate buffer, pH 8 (data not shown). Taken together, peak II obtained by partial acid hydrolysis of LPG was believed to be the phosphorylated tetrasaccharide PO_4→Gal(β1→4)Man–PO_4→Gal(β1→4)-Man.

NMR Analysis of the Phosphorylated Tetrasaccharide. High-resolution ^{1}H NMR spectroscopy was used to confirm the anomeric configuration of the Gal(β1→4)Man linkage, to determine the nature of linkage of the disaccharide units, and to elucidate the position of the phosphate group on the galactose residue. To facilitate the interpretation of the resulting NMR spectrum, the phosphorylated tetrasaccharide PO_4→Gal(β1→4)Man–PO_4→Gal(β1→4)Man was treated with alkaline phosphatase to remove the terminal phosphate

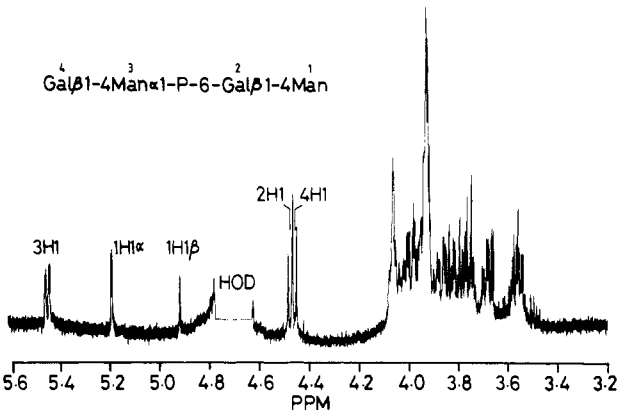

FIGURE 4: Conventional ^{1}H 500-MHz NMR spectrum of the tetrasaccharide. The assignments for the resolved anomeric (H1) protons are noted.

group. The ^{1}H 500-MHz NMR spectrum of the resulting tetrasaccharide is shown in Figure 4. In view of their positions and J couplings, the resonances at 4.92 ppm ($J \sim 1$ Hz) and 5.18 ppm ($J \sim 1.8$ Hz) can immediately be assigned to the C1 protons of β- and α-anomers, respectively, of the terminal mannose (De Bruyn et al., 1975). Similarly, the doublets at 4.45 ppm ($J \sim 7.8$ Hz) and 4.47 ppm ($J \sim 7.8$ Hz) are characteristic of β-galactose (De Bruyn et al., 1975), thus confirming the anomeric nature of both Gal(β1→4)Man linkages. The remaining resonance at 5.44 ppm is a doublet of doublets with a small J coupling of 1.8 Hz and a larger coupling of ~8 Hz. Since this resonance can only arise from the mannose residue that is linked to PO_4–Gal, these data strongly suggest that the anomeric configuration of the mannosyl phosphate bond is α, in view of the characteristic J coupling ($J_{12} \sim 1.8$ Hz). The larger J splitting thus arises from the coupling of the mannosyl C1 proton to the heteronucleus.

The position of phosphorylation of PO_4–Gal in the tetrasaccharide could not be determined from the one-dimensional spectrum. Thus, two-dimensional ^{1}H–^{1}H correlated spectroscopy (COSY) was employed in order to determine the position of this substitution. Inspection of the COSY spectrum (Figure 5) shows the characteristic through-bond connectivities between the Gal H1's and H2's. The H2's in turn show characteristic connectivities to the H3's. Working stepwise in this manner around the rings of each galactosyl residue, the H1's, H2's, and H3's were found to have essentially degenerate chemical shifts. In addition, the H4, H5, and H6 connectivities for the terminal galactose residue were found to be essentially identical with those observed in free β-D-galactose, whereas the H4, H5, and H6 resonances of the remaining Gal residue had significantly different chemical shifts, suggesting that the nonterminal galactose residue is either PO_4→4Gal or PO_4→-6Gal. However, the former can be excluded since the cross-peak multiplicities of the H3–H4 couplings in each galactose residue (Figure 5) are identical with those observed in free galactose (data not shown), indicating that there is no substitution at the C4 position by the heteronucleus. Furthermore, NMR analysis of the phosphorylated disaccharide PO_4→-Gal(β1→4)Man confirmed the phosphate substitution at C6 of galactose (data not shown). It can therefore be concluded that the primary structure of the tetrasaccharide is Gal(β1→-4)Manα1–PO_4→6Gal(β1→4)Man.

Estimation of the Number of PO_4→6Gal(β1→4)Man Units in LPG. To determine the number of repeating phosphorylated disaccharide units in LPG, advantage was taken of the mild acid lability of LPG. LPG (1.8 μmol of phosphate) containing

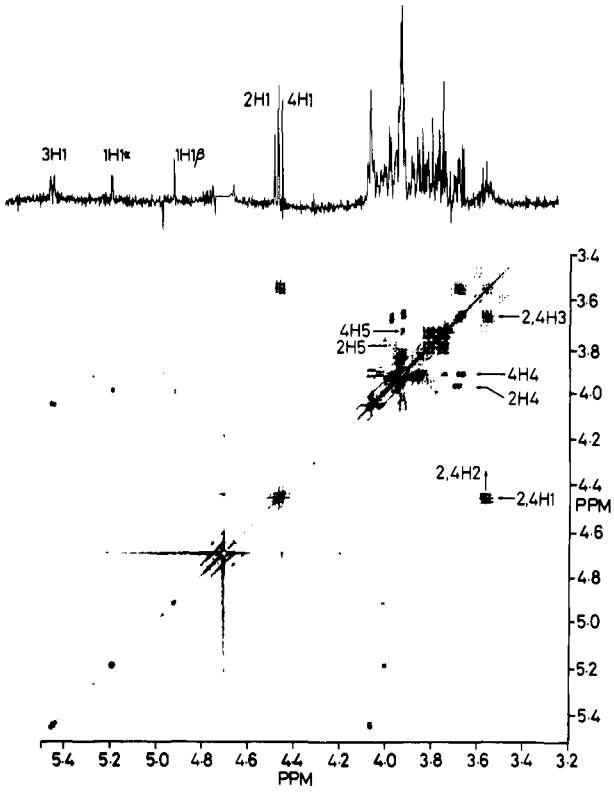

FIGURE 5: Two-dimensional ^1H–^1H 500-MHz COSY spectrum of the tetrasaccharide. Stepwise assignments around the ring are shown as far as H5 for each galactose residue (for notation, see Figure 4). The strong resonance at 4.7 ppm is residual HOD. Shown above the two-dimensional plot is the spectrum derived from the first t_1 increment.

50 000 cpm of [^{3}H]Man-labeled LPG was treated with 0.02 *N* HCl at 100 °C for 5 min, dried under a stream of nitrogen, resuspended in 1 mM Tris-HCl, pH 8, and applied to a column of DE-52 cellulose (0.5 cm × 1 cm). The major peak of radioactivity, PO_4→6Gal(β1→4)[^{3}H]Man, was pooled. Recovery following the ion-exchange chromatographic step was monitored by the amount of radioactivity in each fraction; total recovery was 95%. Phosphate analysis yielded 1.5 μmol of phosphate in the phosphorylated disaccharide pool, representing 84% of the total phosphate in LPG. Quantitative analysis had previously revealed the presence of 18.5 mol of phosphate/mol of LPG (Orlandi & Turco, 1987). Thus, it is concluded that there are an average of 16 phosphorylated disaccharide units per mole of LPG.

Discussion

In this study, the major carbohydrate fragment obtained by mild acid hydrolysis of the *L. donovani* lipophosphoglycan has been elucidated as PO_4→6Gal(β1→4)Man. The average number of these phosphorylated disaccharide units in the glycoconjugate is approximately 16, and they are linked together by α-glycosidic linkages. Most, or possibly all, of the units are believed to constitute a linear, repeating sequence in LPG since partial acid hydrolysis of LPG yielded an array of larger fragments whose profile upon ion-exchange chromatography (Figure 1B) and gel filtration (Turco et al., 1984) is suggestive of an oligomeric series. Furthermore, in the structural characterization of peaks I and II (Figure 1), only monosubstituted sugars were observed, demonstrating the absence of branching. In other recent data, the hydrophobic moiety of LPG has been elucidated as a novel lysoalkylphosphatidylinositol lipid containing a saturated, unbranched C_{24} or C_{26} aliphatic chain (Orlandi & Turco, 1987). The

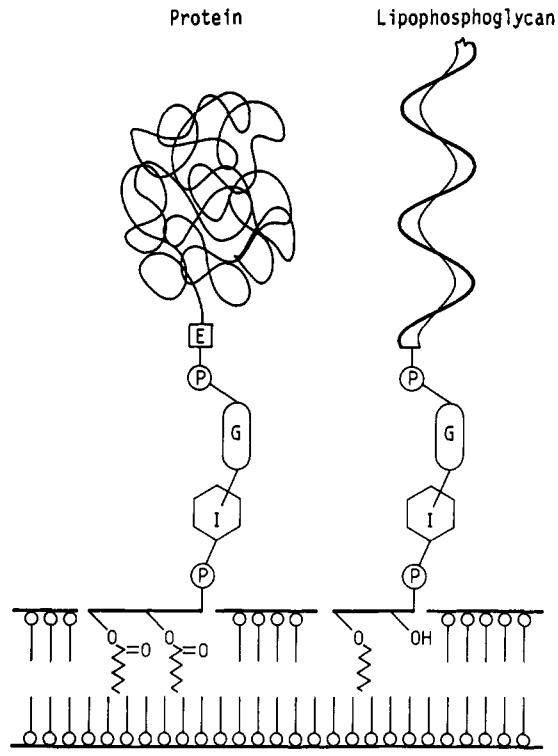

FIGURE 6: Schematic representation of proteins, such as the trypanosomal VSGs (Ferguson et al., 1985), and lipophosphoglycan anchored in membranes. Abbreviations: I, inositol; G, glycan core; P, phosphate; E, ethanolamine. In this schematic representation, all 16 phosphorylated disaccharide units are illustrated as a ribbon-like, consecutive sequence. However, it is possible that one or more of the units are attached to a second site in the heptasaccharide core (G).

repeating phosphorylated disaccharide units are linked to the lipid via an undefined phosphate-containing heptasaccharide core (unpublished observations). On the basis of these results, a tentative structure of lipophosphoglycan is proposed as

$$[PO_4\rightarrow 6Gal(\beta 1\rightarrow 4)Man\alpha 1]_{16}\rightarrow PO_4\rightarrow \text{heptasaccharide core}\rightarrow \text{lysoalkyl-PI}$$

Interestingly, the lipid domain of LPG is similar to the carbohydrate-containing phosphatidylinositol anchor that has been reported for a growing number of eukaryotic membrane proteins [see Cross (1987) for a review]. LPG is the first reported occurrence of an analogous lipid anchoring a polysaccharide. The main structural difference in the two lipid anchors is a conventional diacylated PI for proteins and a lysoalkyl-PI for LPG (Figure 6). The biological significance of this distinction is unknown.

The presence of 4-substituted mannose in lipophosphoglycan is extremely unusual. The only other reported occurrence of 4-substituted mannose in a glycoconjugate in eukaryotic cells is in the mannan of ivory nuts (Aspinall et al., 1958). Thus, LPG may likely be shown to be antigenic due to an epitope possessing this unusual carbohydrate linkage.

Handman and co-workers (Handman & Goding; 1985; Handman et al., 1986) have recently reported that a similar, but antigenically distinct, lipid-containing glycoconjugate in *Leishmania tropica major* is necessary for survival of the parasite in macrophages and also serves as a receptor for binding to host macrophages. It is intriguing to speculate how LPG may function in a protective role for the parasite in lysosomes of phagocytic cells. Lysosomes possibly may not possess the necessary glycosidases or lipases that are capable of degrading the cell surface LPG and, consequently, may preclude *L. donovani* from being attacked by other hydrolytic enzymes. Alternatively, the highly anionic nature of LPG may

afford protection for the parasite against enzymatic attack in lysosomes.

The biological effect of LPG in a defensive role may also be much more complex. An alkyl lysophospholipid has been shown to inhibit the phospholipid, Ca^+, and *sn*-diacylglycerol-dependent protein kinase (protein kinase C) in cultured leukemic cells (Helfman et al., 1983). Phagocytic cells can be stimulated to undergo an oxidative burst with phorbol myristic acetate (Johnston et al., 1978; Nathan et al., 1979; Murray et al., 1979), an activator of protein kinase C (Castagna et al., 1982; Niedel et al., 1983). It is therefore conceivable that LPG or a lysoalkyl-PI-containing fragment of LPG may inhibit the protein kinase C responsible for oxidative killing events in phagocytic cells, enabling intracellular *L. donovani* to avoid destruction.

ACKNOWLEDGMENTS

We thank Dr. Michael A. J. Ferguson for critical reading of the manuscript and confirmatory GC–MS analysis and Marie A. Wilkerson for technical assistance.

Registry No. $PO_4 \rightarrow 6Gal(\beta 1 \rightarrow 4)Man$, 109786-72-3.

REFERENCES

Anet, E. F. L. J., & Reynolds, T. M. (1954) *Nature (London) 174*, 930.

Aspinall, G. O., Rashbrook, R. B., & Kessler, G. (1958) *J. Chem. Soc.*, 215–220.

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466–468.

Bjorndal, H., Lindberg, B., Pilotti, A., & Svennsson, S. (1970) *Carbohydr. Res. 15*, 339–349.

Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., & Nishizuka, Y. (1982) *J. Biol. Chem. 257*, 7847–7851.

Cross, G. A. M. (1987) *Cell (Cambridge, Mass.) 48*, 179–181.

De Bruyn, A., Anteunis, M., & Verhegge, G. (1975) *Acta Cienc. Indica 2*, 83–88.

Ferguson, M. J. A., Low, M. G., & Cross, G. A. M. (1985) *J. Biol. Chem. 260*, 14547–14555.

Handman, E., & Goding, J. W. (1985) *EMBO J. 4*, 329–336.

Handman, E., Greenblatt, C. L., & Goding, J. W. (1984) *EMBO J. 3*, 2301–2306.

Handman, E., Schnur, L. F., Spithill, T. W., & Mitchell, G. F. (1986) *J. Immunol. 137*, 3608–3613.

Helfman, D. M., Barnes, K. C., Kinkade, J. M., Jr., Vogler, W. R., Shoji, M., & Kuo, J. F. (1983) *Cancer Res. 43*, 2955–2961.

Homans, S. W., Dwek, R. A., Boyd, J., Mahmoudian, M., Richards, W. G., & Rademacher, T. W. (1986) *Biochemistry 25*, 6342–6350.

Hull, S. R., & Turco, S. J. (1985) *Anal. Biochem. 151*, 554–560.

Johnston, R. B., Godzik, C. A., & Cohn, Z. A. (1978) *J. Exp. Med. 148*, 115–127.

King, D. L., Chang, Y. D., & Turco, S. J. (1987) *Mol. Biochem. Parasitol. 24*, 47–54.

Laine, R. (1980) in *27th International Congress of Pure and Applied Chemistry* (Varmavuori, A., Ed.) pp 193–198, Pergamon, Elmsford, NY.

Laine, R. (1981) *Anal. Biochem. 116*, 383–356.

Murray, H. W., Juangbhanich, C. W., Nathan, C. F., & Cohn, Z. A. (1979) *J. Exp. Med. 150*, 950–964.

Nathan, C. F., Nogueira, N., Juangbhanich, C., Ellis, J., & Cohn, Z. A. (1979) *J. Exp. Med. 149*, 1056–1068.

Niedel, J. E., Kuhn, L., & Vandenbark, G. R. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 36–40.

Orlandi, P. A., Jr., & Turco, S. J. (1987) *J. Biol. Chem. 262*, 10384–10391.

Stellner, K., Saito, H., & Hakomori, S. I. (1973) *Arch. Biochem. Biophys. 155*, 464–472.

Turco, S. J., Wilkerson, M. A., & Clawson, D. R. (1984) *J. Biol. Chem. 259*, 3883–3889.