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The Lipid Structure of the Glycosylphosphatidylinositol-anchored Mucin-like Sialic Acid Acceptors of Trypanosoma cruzi Changes during Parasite Differentiation from Epimastigotes to Infective Metacyclic Trypomastigote Forms*

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The major acceptors of sialic acid on the surface of metacyclic trypomastigotes, which are the infective forms of Trypanosoma cruzi found in the insect vector, are mucin-like glycoproteins linked to the parasite membrane via glycosylphosphatidylinositol anchors. Here we have compared the lipid and the carbohydrate structure of the glycosylphosphatidylinositol anchors and the O-linked oligosaccharides of the mucins isolated from metacyclic trypomastigotes and noninfective epimastigote forms obtained in culture. The single difference found was in the lipid structure. While the phosphatidylinositol moiety of the epimastigote mucins contains mainly 1-O-hexadecyl-2-O-hexadecanoylphosphatidylinositol, the phosphatidylinositol moiety of the metacyclic trypomastigote mucins contains mostly (∼70%) inositol phosphoceramides, consisting of a C18:1 sphinganine long chain base and mainly C24:0 and C16:0 fatty acids. The remaining 30% of the metacyclic phosphatidylinositol moieties are the same alkylacylphosphatidylinositol species found in epimastigotes. In contrast, the glycosylphosphatidylinositol glycan cores of both molecules are very similar, mainly Manα1–2Manα1–2Manα1–6Manα1–4GlcN. The glycans are substituted at the GlcN residue and at the third α-Man distal to the GlcN residue by ethanolamine phosphate or 2-aminoethylphosphonate groups. The structures of the desialylated O-linked oligosaccharides of the metacyclic trypomastigote mucin-like molecules, released by β-elimination with concomitant reduction, are identical to the structures reported for the epimastigote mucins (Previato, J. O., Jones, C., Gonçalves, L. P. B., Wait, R., Travassos, L. R., and Mendoça-Prevaito, L. (1994) Biochem. J. 301, 151–159). In addition, a significant amount of nonsubstituted N-acetylgalcosaminitol was released from the mucins of both forms of the parasite. Taken together, these results indicate that when epimastigotes transform into infective metacyclic trypomastigotes, the phosphatidylinositol moiety of the glycosylphosphatidylinositol anchor of the major acceptor of sialic acid is modified, while the glycosylphosphatidylinositol anchor and O-linked sugar chains remain essentially unchanged.

Trypanosoma cruzi, the protozoan parasite that causes Chagas’ disease in humans, has a complex life cycle alternating between the insect vector and the mammalian host. In the vector, it multiplies as noninfective epimastigotes that migrate to the hindgut and differentiate into infective metacyclic trypomastigotes. During the insect blood meal, the metacyclic trypomastigotes are deposited with the feces and urine near a skin wound, initiating the natural infection.

T. cruzi is unable to synthesize sialic acids (SA), but it expresses a unique trans-sialidase (TS), which transfers α2–3-linked SA from host glycoproteins and glycolipids to acceptors containing terminal β-galactosyl residues present on the parasite surface (reviewed in Refs. 1–4). Several studies characterizing the nature and structure of the SA acceptors have been published. These acceptors are abundant on the parasite surface and were first described as major surface glycoproteins of epimastigotes by Alves and Colli (5), who called them bands A, B, and C. Subsequently, a similar cell surface glycoprotein complex, called GP24, GP31, and GP37 was described by Ferguson et al. (6), and Previato et al. (7) first described a 43-kDa SA acceptor. More recently, they have been called 38/43 glycoconjugates (8), and the so called epimastigote lipophosphoglycan-like molecule could belong to the same family of molecules (9). In metacyclic trypomastigote forms, the SA acceptors were reported originally as the 35/50-kDa antigens (10, 11) that were subsequently defined as mucin-like glycoproteins (12). In the trypomastigote forms found in mammals, the SA acceptors were described as a group of molecules that share the stage-specific epitope 3 (Ssp-3)/13), an epitope dependent on parasite

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1 The abbreviations used are: SA, sialic acid(s); TS, trans-sialidase; GPI, glycosylphosphatidylinositol; PI, phosphatidylinositol; ES-MS, electrospray-mass spectrometry; GC-MS gas chromatography-mass spectrometry; HPTLC, high-performance thin layer chromatography; HPLC, high-performance liquid chromatography; AHM*, [1-3H]2,5-anhydromannitol; GlcNAc-ol, N-acetylgalacosaminitol; HexNAc-ol, N-acetylgalactosaminitol; LPPG, lipopetidophosphoglycan; Gu, glucose units; 2-AEP, 2-aminoethylphosphonate; PAGE, polyacrylamide gel electrophoresis.
sialylation (14), and were also identified as mucin-like molecules that appear larger than the epimastigote and metacyclic mucins on SDS-polyacrylamide gel electrophoresis (15, 16). These trypomastigote mucins also contain some terminal α-galactosyl residues (16). In summary, these mucin-like molecules are glycoproteins rich in threonine and serine that are linked to the parasite membrane via a glycosylphosphatidylinositol (GPI) anchor and that contain novel O-linked oligosaccharides. The O-linked oligosaccharides are attached to the protein via GlcNAc residues and act as SA acceptor sites for the parasite TS. The chemical structure of O-linked oligosaccharides of epimastigote mucins of G (8) and Y (17) strains have recently been elucidated. They are quite similar but differ in their average size and in some of the Gal linkages. The glycan structure of the GPI anchor of the epimastigote mucin (Y strain) has also been reported (17).

Several lines of evidence suggest that the 35/50-kDa mucins of metacyclic-trypomastigotes are involved in host cell invasion. Monoclonal antibodies directed against the mucin, and the purified molecule itself, are able to inhibit parasite entry (10, 18), and the 35/50-kDa antigens are capped and locally released during invasion (12). Epimastigotes are unable to enter mammalian cells but express large amounts of mucins with similar size recognized by the same monoclonal antibodies. Therefore, we decided to investigate possible subtle structural differences between the mucins of these two stages. We found that, after differentiation of epimastigotes into metacyclic forms, the lipid portion is modified, while the oligosaccharide chains and the glycan structure of the GPI are conserved. This lipid change might correlate with the increased infectivity of metacyclic forms and the ability of the parasite to shed the mucins upon invasion of the host cell (12).

**MATERIALS AND METHODS**

**Parasites—**Epimastigotes of *T. cruzi* strain G (19) were grown at 28 °C in liver-infusion tryptose medium (20) containing 10% fetal bovine serum. Metacytic trypomastigotes were purified from cultures at stationary phase by passage through a diethylaminoethyl cellulose column, as described in Ref. 19. The purity of the metacytic trypomastigotes preparations was estimated by morphology and/or by complement-mediated lysis assay using normal human sera.

**Purification of Mucins—**Mucins from epimastigotes and metacyclic trypomastigotes were extracted as described for the lipophosphoglycan of *L. donovani* (21). Briefly, parasites (~7 × 10^10 metacyclic mol-ecules and ~8 × 10^10 metacyclic trypomastigotes) were freeze-dried and placed in a sonicating water bath for 10 min with 25 ml of chloroform/methanol/water (1:2:0, by volume). After centrifugation (2,000 × g, 5 min) the insoluble material was re-extracted twice more, as described above, and the final insoluble pellet was used as a source of delipidated parasites. The pooled chloroform/methanol/water soluble fractions were evaporated under nitrogen, and the residue was extracted with 50 ml of butanol-1-ol/water (2:1, by volume). The butanol-1-ol phase, containing the lipid fraction (F1) was collected, and the aqueous phase (F2) was washed twice with 25 ml of water-saturated butanol-1-ol and concent-rated. The delipidated parasites were extracted by sonication (three times) with 25 ml of 9% butanol in water. The soluble material was pooled, concentrated, and freeze-dried to produce a polar fraction (F3). Most of the mucins from epimastigotes were recovered in F2, while mucins of metacyclic trypomastigotes were recovered in the F3 fraction. The mucins were resuspended in 0.5 ml of 0.1 M ammonium acetate in 5% propan-1-ol (v/v) (buffer A) and fractionated on an octyl-Sepharose column (10 × 0.5 cm), previously equilibrated in buffer A. The column was washed with 5 ml of buffer A and NaOH containing 500 mM sodium acetate and 100 mM trifuoroacetic acid (100 °C, 12 h). The samples were dried in a speed-vac and redried twice from 200 μl of water. The hydrolysates were then split into three portions and treated as follows: (i) dephosphorylated with aqueous HF (60 °C, 0 °C), (ii) treated with jack bean α-mannosidase prior to dephosphorylation with aqueous HF, or (iii) passed directly through a 0.2-ml QAE-Sephadex A-25 column. The products were analyzed by HPTLC using solvent A, propan-1-ol/acetone/water (9:6:4, by volume). Radioactive glycans were detected with a radioactivity linear analyzer (Raytest RITA) and visualized by fluorography after spraying with En3Hance (DuPont NEN). A set of standards, terminating in [1-3H]2,5-anhydromannitol (AHM*), was prepared by partial acid hydrolysis (23) of authentic Manol–2Manol–2Manol–6Manol–1AHM* from purified glycoprotein (SPP) (24). This so called ladder contains a mixture of Manol–2Manol–2Manol–6Manol–1AHM* (M4), Manol–2Manol–6Manol–1AHM* (M3), Manol–6Manol–1AHM* (M2), Manol–1AHM* (M1), and AHM*.

**Changes in GPI Lipid Structure during T. cruzi Differentiation**—About 200 nmol of 35/50-kDa mucin of epimastigotes and metacyclic trypomastigotes was isolated and dried twice more with water-saturated butanol and freeze-dried again. Nitrous Acid Deamination and NaBH₄ Reduction of GPI Neutral Glycans and Recovery of the Phosphatidylinositol Moieties—About 20 nmol of 35/50-kDa mucin of epimastigotes and metacyclic trypomastigotes, as judged by the myo-inositol content (see below) were freeze-dried. Mucins were resuspended in 50 μl of 0.1 M ammonium acetate and treated three times with 100 μl of butan-1-ol saturated with water. Then 25 μl of 1 M sodium nitrite was added to the aqueous phase (two times at 1-h intervals), and deamination was performed for 2 h at 37 °C. The released phosphatidylinositol (PI) moieties were recovered by three extractions with 100 μl of butan-1-ol saturated with water and analyzed by high pressure liquid chromatography (ES-MS), as described below. The deaminated molecules remaining in the aqueous phase were reduced with NaBH₄, as described in Ref. 22, and repurified by chromatography on a Sephadex G-10 column, where they eluted in the void volume. The deaminated and NaBH₄-reduced mucins were then dephosphorylated in 48% aqueous HF for 60 h at 0 °C and re-N-acetylated, and the GPI neutral glycans were purified from radiochemical contaminants by downward paper chromatography and high voltage paper electrophoresis (22). The 3H-labeled neutral glycans were analyzed by Bio-Gel P4 chromatography and Dionex high-performance anion-exchange chromatography. The elution positions of the radiolabeled glycans were expressed in glucose units (Gu) and Dionex units, respectively, by linear interpolation of the elution position between adjacent glucose oligomer internal standards (22).

**Microsequencing of the GPI Glycan Cores—**Three aliquots (20,000 cpm) of the neutral glycans fractions recovered from the Bio-Gel P4 column were dried in speed-vac and subjected to digestion with jack bean α-mannosidase (Boehringer Mannheim), Aspergillus saitoi Manol–2Manol–specific α-mannosidase (Oxford Glycosystems) and by partial acidolysis. Digestions were carried out with 0.75 units of jack bean α-mannosidase in 30 μl of 0.1 M sodium acetate buffer, pH 5.0, for 16 h at 37 °C or with 0.01 units of A. saitoi α-mannosidase in 10 μl of 0.1 M sodium acetate buffer, pH 5.0, for 16 h at 37 °C. Acidolysis was performed as described in Ref. 23. The products were analyzed by high performance thin layer chromatography (HPTLC) on silica gel 60 plates (Merck) using solvent A, propan-1-ol/acetone/water (9:6:4, by volume). Radioactive glycans were detected with a radioactivity linear analyzer (Raytest RITA) and visualized by fluorography after spraying with En3Hance (DuPont NEN). A set of standards, terminating in [1-3H]2,5-anhydromannitol (AHM*), was prepared by partial acid hydrolysis (23) of authentic Manol–2Manol–2Manol–6Manol–1AHM* from purified glycoprotein (SPP) (24). This so called ladder contains a mixture of Manol–2Manol–2Manol–6Manol–1AHM* (M4), Manol–2Manol–6Manol–1AHM* (M3), Manol–6Manol–1AHM* (M2), Manol–1AHM* (M1), and AHM*.

**Location of the Ethanolamine Phosphate and α-Aminoethyphospho- nate Groups—**This method was adapted from Ref. 23. Deaminated and NaBH₄-reduced mucins, 500,000 cpm (before dephosphorylation with aqueous HF), were subjected to partial acid hydrolysis (1% nitrous acid, 30 min) and to digestion with jack bean α-mannosidase prior to dephosphorylation with aqueous HF, or (ii) passed directly through a 0.2-ml QAE-Sephadex A-25 column. The products were analyzed by HPTLC using solvent A, as described above. Mild Alkaline Treatment of 35/50-kDa Mucins and Fractionation of Oligosacchari-tols—About 200 nmol of metacyclic 35/50-kDa mucin was freeze-dried twice, resuspended in 250 μl of 0.1 M sodium hydroxide containing 250 mM NaBH₄, and incubated for 24 h at 37 °C. The sample was acidified with 1 M hydrochloric acid and desalted by passage through 0.6 ml of AG 50-X2-H⁺ (10 × 5 mm, Whatman 3MM paper in butan-1-ol/ethanol/water (4:1:0.6, by volume) and digested with 50 μm Arthrobacter ureafaciens sialidase (Oxford Glycosystems) in 25 μl of 50 mM sodium acetate (pH 5.0) for 20 h at 37 °C. After desalting (as described below for the exoglycosidase digestes) the neutral oligosacchari-tols were fractionated by HPLC using a 5-μm hydrophilic interaction Glycoplex™ column (200 × 46 mm,
Cholesterol Esters and Fatty Acids Esters were dissolved in 0.5 ml of 2H2O after repeated exchange in 2H2O. All samples were equipped with a 5-mm triple resonance probe, and the samples were terminated by heating at 100 °C for 5 min. The products were desalted by passage through a column of 0.2 ml of AG50X12 (H+), over 0.2 ml of AG3X4 (OH-) over 0.1 ml of QAEP-Sephadex A-25. Eluates were desalted and the residual acetic acid was removed by co-evaporation with 50 μl of toluene (two times).

Compositional Analysis and Methylation Analysis—Amino acids, ethanolamine, 2-aminoethyolphosphonate, and glucosamine were quantified after strong acid hydrolysis (6 μ HCl, 110 °C, 16 h) and derivatization with phenylisothiocyanate using a Waters Pico-Tag system, as described (22). Gas chromatography-mass spectrometry (GC-MS) analyses were performed with a Hewlett-Packard 5890-5970 system. The myo-inositol content of samples was measured using selected ion monitoring after strong acid hydrolysis and trimethylsilyl derivatization (22). Monosaccharide and lipid contents were measured after methanolysis, re-N-acetylation, and trimethylsilyl derivatization (22). Methylation linkage analysis was performed as described (22).

Electrospray-Mass Spectrometry—ES-MS data were obtained with a VG-Quattro triple-quadrupole mass spectrometer (Fisons Instruments, United Kingdom) coupled to a Micromass microbore HPLC system. Analysis of PI fractions was performed in negative ion mode, and aliquots of PI samples (20 μl of PI dissolved in chloroform/methanol/water (10:10:5, by volume)) were injected into the electrospray source at 5 μl/min. Source and spectrometer parameters were optimized using a standard of soybean PI (26). The masses of the released O-linked oligosaccharidol components were determined in negative ion mode. Samples, dissolved in 20 μl of 50% acetonitrile, were injected into the electrospray source at 5 μl/min. Source and spectrometer conditions were optimized using a standard of maltotetraose (Sigma). The 1H 1D NMR 500-MHz H NMR spectra of the individual oligosaccharidol samples were obtained using a Bruker AM 500 spectrometer equipped with a 5-mm triple resonance probe, and the samples were dissolved in 0.5 ml of 6H2O after repeated exchange in 6H2O. All experiments were performed at 300 K, and chemical shifts were referenced externally to acetone (2.225 ppm). Further assignments for oligosaccharidol c were deduced from two-dimensional 1H-1H experiments. Correlated spectroscopy and triple quantum-filtered correlated spectroscopy experiments were performed using a sweep width of 2,200 Hz, and 4,000 data points, and 512 increments were collected in 1D. The rotating frame Overhauser effect spectroscopy experiment used 64 transients of 4,000 data points, and 512 increments were collected in 1D. The mixing time was 500 ms. In the total correlation spectroscopy experiment, 64 transients of 4,000 datapoints were collected, and 512 experimental increments were collected in 1D. The sweep width was 1,500 Hz, and the mixing time used was 203 ms.

Trans-sialylation of Mucin O-Linked Oligosaccharidols—Purified radiolabeled mucin from metacyclics was hydrolyzed, the oligosaccharidol, obtained after fractionation on a 150-μl Glycoplex™ HPLC column, were dried in a speed-vac and redissolved in 0.02 M HEPES buffer (pH 7.0), 1 ml 3’S-sialylactose (SA23-3Galβ1-4Glc (Boehringer Mannheim), 0.2% bovine serum albumine (Ultrafine, Boehringer Mannheim) and incubated with purified T. cruzi TS (27). After 2 h at 37 °C, the reaction was stopped by the addition of 1 ml of water, and the amount of nonsialylated products was quantified by passage through a 0.5 ml QAE-Sephadex A-25 column equilibrated in water. Sialylated products were recovered after washing the column with 8 ml of water and elution with 1 ml of 1 M ammonium formate.

Alternatively, the products were adjusted to 5 mM sodium acetate buffer, pH 4.0, and chromatographed on a Mono Q column, as described in Ref. 28, to determine the extent of sialylation of each individual oligosaccharidol.

RESULTS

The mucins were purified from epimastigotes and metacyclic trypomastigotes by solvent extraction and octyl-Sepharose chromatography. The material recognized by the monoclonal antibody 10D8, specific for the sialic acid acceptors, eluted at 25% (v/v) propan-1-ol and appeared as two bands with apparent molecular masses of 35 and 50 kDa on SDS-polyacrylamide gel electrophoresis, as shown previously for the metacyclic trypomastigote sialic acid acceptors (12). The significance of the double nature of the antigen is unknown, but it may reflect the presence of at least two different (O-glycosylated/GPI-anchored) gene products. The purified mucins were judged to be free of T. cruzi LPG, which migrates near the front of an SDS-polyacrylamide electrophoresis gel, by silver staining and by Western blot analysis using an LPPG-specific antibody (data not shown). Based on the myo-inositol content of the recovered material (25 nmol/1010 cells), the metacyclic mucin is present at a minimum of 1.5 × 105 copies/parasite. The mucins eluted from the octyl-Sepharose column were subjected to compositional analysis, showing that amino acids (particularly Ser and Thr) and monosaccharides (Man, Gal, GlcNac, and SA) together with myo-inositol, ethanolamine, 1-O-hexadecylglycerol, and fatty acids were present in both preparations in amounts similar to those reported previously (8, 12). In addition, a previously unidentified peak in the amino acid analyses (with a retention time of 3.2 min) was shown to co-elute with an authentic standard of 2-aminoethyolphosphonate (2-AEP). The molar ratio of ethanolamine to 2-AEP was approximately 1:1 for both preparations.

GPI Lipid Structure—the mucins (approximately 20 nmol of each, based on myo-inositol content) were subjected to nitrous acid deamination and extracted with butan-1-ol. The butan-1-ol extracts, containing the released PI fractions, were analyzed by ES-MS. The mucin isolated from epimastigotes produced one major pseudomolecular ion at m/z 795.5 and minor ions at m/z 809.6 and 823.6 (Fig. 1A). No other ions were observed in the mass range m/z 400-1400 (data not shown). The collision-induced dissociation daughter ion spectra of the m/z 823 and 795 parent ions (Fig. 1, C and D), define the parent ions as the [M-1]- pseudomolecular ions of 1-O-(C16:0)alkylkyl-2-(C18:0)acylglycerol-3-HPO4-inositol and 1-O-(C16:0)alkylkyl-2-O-(C24:0)acylglycerol-3-HPO4-, respectively. The common daughter ions at m/z 79, 241, and 377 correspond to [PO4]−, [inositol-1,2-cyclic-phosphate]−, and [HPO4-CH2-CH2-O-(CH2)7-C=CH2]−, respectively. The daughter ions at m/z 283 (panel C) and 255 (panel D) correspond to the carboxylate ions [CH2=(CH2)15-CO2]− and [CH2=(CH2)14-CO2]−, respectively.

In contrast, the mucin from metacyclics showed, in addition to the same alkacil-PI pseudomolecular ion species at m/z 795.5 and 823.6, abundant ions at m/z 892.7 and 780.6 (Fig. 1B). The daughter ion spectra of these species (Fig. 1, E and F) define these ions as the [M-1]- pseudomolecular ions of inositol phosphoceramides (ceramide-PIs). In this case, the daughter ion spectra contain common ions at m/z 79, 97, 241, and 259, corresponding to [PO4]−, [H2PO4]−, [inositol-1,2-cyclic-PO4]−, and [inositol-1-HPO4]−, respectively. The relatively stable amide bond of the ceramide prevents the formation of the carboxylate ions seen previously for the alkacil-PI species. The m/z values of the pseudomolecular ions at m/z 892.7 and 780.6 suggest that the ceramide components are made up of a sphingosine (C18:0) long chain base and C24:0 and C24:0 fatty acids, respectively. The minor pseudomolecular ions at m/z 890.7 and
To determine the structure of the GPI glycan, the aqueous phase obtained after butan-1-ol extraction of the deaminated mucins was reduced with NaB\(_3\)H\(_4\) and dephosphorylated with aqueous HF, and the resulting GPI neutral glycans were purified by paper chromatography and high voltage paper electrophoresis. The neutral glycan fractions were chromatographed by Dionex HP AEC and Bio-Gel P4 gel filtration (data not shown). The neutral glycans derived from the epimastigotes and metacyclic trypomastigotes mucins eluted as one major component on both systems with chromatographic values of 3.0 Dionex units and 5.2 Gu, respectively. These values are identical to those of authentic Man\(_α1\)-2Man\(_α1\)-2Man\(_α1\)-6Man\(_α1\)-4AHM* (Man4-AHM*) (22). This sequence was confirmed by exoglycosidase digestion and partial acetolysis followed by product analysis by HPTLC (Fig. 2A).

For the neutral glycans derived from both mucins, digestion with Man\(_α1\)-2Man-specific A. saitoi \(α\)-mannosidase yielded a major product that comigrated with Man\(_α1\)-6Man\(_α1\)-4AHM* (Man2-AHM*) (Fig. 2A, lanes 2 and 6); partial acetolysis, which selectively hydrolyzes Man\(_α1\)-6Man glycosidic bonds, produced a major product that comigrated with Man\(_α1\)-4AHM* (Man1-AHM*) (Fig. 2A, lanes 3 and 7), and jack bean \(α\)-mannosidase, which removes all unsubstituted nonreducing terminal \(α\)Man residues, produced a major product that comigrated with AHM*. One additional minor neutral glycan structure, with a slower mobility than Man4-AHM*, was also detected in metacyclic and epimastigote preparations (Fig. 2A, lanes 1 and 5). This additional band can be seen more clearly in Fig. 2B, which shows the results for the epimastigote neutral glycan fraction before and after the sequencing digestions. This additional band was sensitive to A. saitoi \(α\)-mannosidase, producing a faint band migrating between Man2-AHM* and Man3-AHM* (Fig. 2B, lane 2), and digestion with jack bean \(α\)-mannosidase produced, in addition to the AHM*, a product that migrated slightly ahead of Man2-AHM* (Fig. 2B, lane 4). Thus, this minor component probably represents a Man4-AHM* structure with an additional, jack bean \(α\)-mannosidase-resistant residue linked to the first \(α\)Man adjacent to the AHM* (i.e. Man\(_α1\)-2Man\(_α1\)-2Man\(_α1\)-6X:2Man\(_α1\)-4AHM*). Due to the low abundance of this glycan, its structure was not studied further.

**Location of the Phosphoryl/Phosphonyl Substituents in the Glycan**—Samples of the deaminated and NaB\(_3\)H\(_4\)-reduced epimastigote and metacyclic mucins were subjected to partial acid hydrolysis, using conditions that retain phosphoryl and phosphonyl substituents but partially cleave glycosidic bonds (23). The hydrolysates were then split into aliquots and (i) dephosphorylated with aqueous HF, (ii) treated with jack bean \(α\)-mannosidase prior to dephosphorylation with aqueous HF, or (iii) passed through a QAE-Sephadex A-25 ion exchange column. The products of these treatments were analyzed by HPTLC (Fig. 3). The dephosphorylated samples produced ladders of the components Man4-AHM*, Man3-AHM*, Man2-AHM*, Man1-AHM*, and AHM* (Fig. 3, lanes 2 and 6). In contrast, all of the bands except for Man3-AHM* and AHM* were diminished in the samples treated with mannosidase prior to dephosphorylation (Fig. 3, lanes 3 and 7). At least part of the less intense bands, migrating below Man3-AHM* and above Man2-AHM*, probably originated from the Man\(_α1\)-2Man\(_α1\)-2Man\(_α1\)-6X:2Man\(_α1\)-4AHM*-containing mucin species. This result indicates that the Man3-AHM* component was fully protected from \(α\)-mannosidase digestion by an aqueous HF-sensitive substituent attached to the third \(α\)Man residue distal to the AHM*. This substituent is most likely an ethanolamine phosphate moiety, attached to the 6-position of the third \(α\)Man residue, that is the conventional GPI anchor bridge to the polypeptide chain (29). Another possibility is that part of this aqueous HF-sensitive substituent is 2-aminoethylphosphonate, as described for the GPI of epimastigote mucin of Y strain (17). Both ethanolamine and 2-aminoethylphosphonate were identified in the compositional analyses of the epimastigote and metacyclic trypomastigote mucins. When the partial acid hydrolysis products were passed through QAE-Sephadex, most of the Man4-AHM*, Man3-AHM*, Man2-AHM*, Man1-AHM*, and AHM* radioactivity was lost, indicating that all of the GPI glycans were negatively charged prior to dephosphorylation. This, in turn, suggests that most of the AHM* residues were originally substituted with phosphoryl (i.e. ethanolamine phosphate) and/or phosphonyl (i.e. 2-AEP) substituents, both of
These assignments are given below. The experimental data supporting the separation in the Glycoplex™ column, except for oligosaccharides separated on a Glycoplex™ hydrophilic interaction HPLC column. The purity of individual neutral oligosaccharides was assessed by HPTLC (Fig. 5). The migration on HPTLC followed the separation in the Glycoplex™ column, except for oligosaccharide b (identified as Galβ1-4GlcNAc-ol; see below), which was found to migrate slightly ahead of fraction a (GlcNAc-ol). The two minor fractions, labeled b’ and d’ were shown to contain unique structures that have not been fully characterized in this study. The structures of the components of peaks a–f are shown in Fig. 6. The experimental data supporting these assignments are given below.

Peak a contained a component that produced an [M-1]⁻ pseudomolecular ion at m/z 223 in negative ion ES-MS and that contained only 1-²H]GlcNAc-ol, as judged by GC-MS composition analysis (Table II). These data define peak a as the alditol GlcNAc-ol.

Peak b contained a component that produced an [M-1]⁻ pseudomolecular ion at m/z 385 in negative ion ES-MS (deuterio-reduced Hex-HexNAc-ol = 386 Da) and that contained only 1-²H]GlcNAc-ol and Gal, as judged by GC-MS composition analysis (Table II). The GC-MS methylation analysis revealed the presence of a terminal Gal residue and 4-O-substituted [1-²H]GlcNAc-ol (Table II). The radiolabeled (NaB³H₄-reduced) form of this component had a size of 3.5 Gu that was reduced to 2.6 Gu (the size of HexNAc-ol) after cleavage of the Gal residue by mild acid hydrolysis (Table IV). The chemical shift (5.154 ppm; Table II) and extremely small J₁,₂ coupling constant (data not shown), of the Gal H-1 proton defined the galactofuranosidic linkage as β (8). Taken together, these data define the peak b component as Galβ1-4GlcNAc-ol.

Peak c contained a component that produced an [M-1]⁻ pseudomolecular ion at m/z 547 in negative ion ES-MS (deuterio-reduced Hex₂-HexNAc-ol = 548 Da) and that contained only 1-²H]GlcNAc-ol and Gal, as judged by GC-MS composition analysis (Table II). The GC-MS methylation analysis revealed the presence of a terminal Gal residue by mild acid hydrolysis (Table IV). The chemical shift (5.154 ppm; Table II) and extremely small J₁,₂ coupling constant (data not shown), of the Gal H-1 proton defined the galactofuranosidic linkage as β (8). Taken together, these data define the peak b component as Galβ1-4GlcNAc-ol.

Peak f contained a component that produced an [M-1]⁻ pseudomolecular ion at m/z 785.5 in negative ion ES-MS (deuterio-reduced Hex₂-HexNAc-ol = 786 Da) and that contained only 1-²H]GlcNAc-ol, as judged by GC-MS composition analysis (Table II). The GC-MS methylation analysis revealed the presence of a terminal Gal residue by mild acid hydrolysis (Table IV). The chemical shift (5.154 ppm; Table II) and extremely small J₁,₂ coupling constant (data not shown), of the Gal H-1 proton defined the galactofuranosidic linkage as β (8). Taken together, these data define the peak b component as Galβ1-4GlcNAc-ol.

which have been found attached to the 6-position of the GlcN residue of kinetoplastid GPI structures (29). The nature of the intense radioactive bands running below the Man4-AHM* band (Fig. 3, lanes 4 and 8) is unknown. However, these samples (unlike those in lanes 2, 3, 6, and 7) had not undergone high voltage paper electrophoresis prior to analysis and may be non-carbohydrate radiochemical contaminants. The deduced structures of the GPI anchors for the epimastigote and metacyclic trypomastigote mucin are shown in Fig. 4.

Structure of the O-Linked Oligosaccharides of the Epimastigote and Metacyclic Trypomastigote Mucins—The O-linked oligosaccharides were released by β-elimination in the presence of NaB³H₄ or NaB⁴H₄, desialylated with neuraminidase, and separated on a Glycoplex™ hydrophilic interaction HPLC column. The column profiles revealed the presence of 6 major peaks (peaks a–f) that were similar in both preparations (Fig. 5, A and B). The purity of individual neutral oligosaccharides was assessed by HPTLC (Fig. 5C). The migration on HPTLC followed the separation in the Glycoplex™ column, except for oligosaccharide b (identified as Galβ1-4GlcNAc-ol; see below), which was found to migrate slightly ahead of fraction a (GlcNAc-ol). The two minor fractions, labeled b’ and d’ were shown to contain unique structures that have not been fully characterized in this study. The structures of the components of peaks a–f are shown in Fig. 6. The experimental data supporting these assignments are given below.

Peak a contained a component that produced an [M-1]⁻ pseudomolecular ion at 272 Da in negative ion ES-MS and that contained only [1-²H]GlcNAc-ol, as judged by GC-MS composition analysis (Table II). The GC-MS methylation analysis revealed the presence of a terminal Gal residue and 4-O-substituted [1-²H]GlcNAc-ol (Table II). The radio- and Metacyclic Trypomastigote Mucins—The O-linked oligosaccharides were released by β-elimination in the presence of NaB³H₄ or NaB⁴H₄, desialylated with neuraminidase, and separated on a Glycoplex™ hydrophilic interaction HPLC column. The column profiles revealed the presence of 6 major peaks (peaks a–f) that were similar in both preparations (Fig. 5, A and B). The purity of individual neutral oligosaccharides was assessed by HPTLC (Fig. 5C). The migration on HPTLC followed the separation in the Glycoplex™ column, except for oligosaccharide b (identified as Galβ1-4GlcNAc-ol; see below), which was found to migrate slightly ahead of fraction a (GlcNAc-ol). The two minor fractions, labeled b’ and d’ were shown to contain unique structures that have not been fully characterized in this study. The structures of the components of peaks a–f are shown in Fig. 6. The experimental data supporting these assignments are given below.

Peak a contained a component that produced an [M-1]⁻ pseudomolecular ion at m/z 223 in negative ion ES-MS and that contained only [1-²H]GlcNAc-ol, as judged by GC-MS composition analysis (Table II). The GC-MS methylation analysis revealed the presence of a terminal Gal residue and 4-O-substituted [1-²H]GlcNAc-ol (Table II). The radio- and Metacyclic Trypomastigote Mucins—The O-linked oligosaccharides were released by β-elimination in the presence of NaB³H₄ or NaB⁴H₄, desialylated with neuraminidase, and separated on a Glycoplex™ hydrophilic interaction HPLC column. The column profiles revealed the presence of 6 major peaks (peaks a–f) that were similar in both preparations (Fig. 5, A and B). The purity of individual neutral oligosaccharides was assessed by HPTLC (Fig. 5C). The migration on HPTLC followed the separation in the Glycoplex™ column, except for oligosaccharide b (identified as Galβ1-4GlcNAc-ol; see below), which was found to migrate slightly ahead of fraction a (GlcNAc-ol)
revealed the presence of a terminal Galf residue, a terminal Galp residue, and 4,6-di-O-substituted \([1\text{-}2H]\)GlcNAc-ol. The radiolabeled (NaB\(_3\)H\(_4\)-reduced) form of this component had a size of 4.5 Gu that was reduced to 3.5 Gu after cleavage of the Galp residue by mild acid hydrolysis or after the cleavage of the Galf residue with jack bean \(\beta\)-galactosidase (Table IV). The sensitivity of the structure to jack bean \(\beta\)-galactosidase, which does not efficiently cleave \(\text{Galp}_{\beta 1-4}\text{GlcNAc-ol}\), and its resistance to bovine testicular \(\beta\)-galactosidase, which does cleave \(\text{Galp}_{\beta 1-4}\text{GlcNAc-ol}\) (16), suggest that the Galp residue is attached to the 6-position of the GlcNAc-ol residue and that the peak c component has the structure \(\text{Galp}_{\beta 1-6}(\text{Galf}_{\beta 1-4})\text{GlcNAc-ol}\). The chemical shifts of the protons of this component (Table III), which are identical to those described in (8) for the same structure, confirm this assignment. The data in Table III add some additional assignments compared with Ref. 8: (i) the H-5 resonance of the Galp residue, which was assigned by chemical shift arguments in Ref. 8, was confirmed from the rotating frame Overhauser effects spectroscopy experiment, and (ii) the H-6 \(^9\) proton of the Galf residue was assigned via the triple quantum-filtered correlated spectroscopy experiment, which is selective for the H-5, H-6, and H-6 \(^9\) protons.

**Fig. 3.** Location of phosphoryl/phosphonyl substituents. HPTLC analysis of the mucins subjected to partial acid hydrolysis followed by sequential exoglycosidase digestion and dephosphorylation. Deaminated and reduced mucins from epimastigotes (EP) and metacyclic trypanomastigote (META) mucins were subjected to partial acid hydrolysis (H\(_1\)) followed by aqueous HF dephosphorylation (HF) (lanes 2 and 6), partial acid hydrolysis, jack bean \(\alpha\)-mannosidase digestion, and aqueous HF dephosphorylation (lanes 3 and 7) or to partial acid hydrolysis and passage through QAE-Sephadex A-25 column (lanes 4 and 8). The products were analyzed by HPTLC in solvent A and fluorography. The migration of standards (DEX), and the glycans of T. cruzi LPPG (as described in Fig. 2) are shown in lanes 1 and 5.

**Fig. 4.** Proposed GPI anchor structures of T. cruzi mucins. See text for details.

**Fig. 5.** Fractionation of mucin oligosaccharitols released by reductive \(\beta\)-elimination. Epimastigote (panel A) and metacyclic trypanomastigote (panel B) mucins were submitted to mild alkaline \(\beta\)-elimination, with concomitant reduction with NaB\(_3\)H\(_4\), and separated by HPLC using a Glycoplex\textsuperscript{TM} column, as described under "Materials and Methods." C, the purity of individual oligosaccharitols from metacyclic sample (lanes a–f) versus the unfractionated oligosaccharitols mixture (lane T) was assessed by HPTLC in solvent system B. Lane S contains a \([1\text{-}3H]\)GlcNAc-ol standard, and lane DEX contains the \(^3\)H-reduced dextran hydrolysate standard.

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tuted [1-2H]GlcNAc-ol. The radiolabeled (NaB3H4-reduced) form of this component had a size of 5.6 Gu that was reduced to 4.5 Gu after cleavage of the Galp residue by mild acid hydrolysis and to 3.5 Gu after the cleavage of 2 βGalp residues with jack bean β-galactosidase (Table IV). Taking into account the methylation data, the accessibility of both βGalp residues to jack bean β-galactosidase suggests that they are linked together in the form Galpβ1–3Galpβ1–6(Galpβ1–4)GlcNAc-ol. The bovine testicular β-galactosidase enzyme was capable of removing the terminal β1–3-linked Gal residue only, to yield a 4.5-Gu product, again suggesting that the βGalp branch was attached to the 6-position of the GlcNAc-ol residue. Taken together, these data suggest that the peak d component has the structure Galpβ1–3Galpβ1–6(Galpβ1–4)GlcNAc-ol. The chemical shifts of the anomeric protons of this component (Table II), which are identical to those described in Ref. 8 for the same structure, confirm this assignment.

Peak e contained a component that produced an [M-1]- pseudomolecular ion at m/z 871 in negative ion ES-MS (deu-
tero-reduced Hex, HexNAc-ol = 872 Da) and which contained only [1-2H]GlcNAc-ol and Gal, as judged by GC-MS composi-
tion analysis (Table II). The GC-MS methylation analysis revealed the presence of a terminal Galp residue, two terminal Galp residues, a 2,3-di-O-substituted Galp residue, and 4,6-di-
O-substituted [1-2H]GlcNAc-ol. The radiolabeled (NaB3H4-re-
duced) form of this component had a size of 6.4 Gu that was reduced to 5.4 Gu after cleavage of the Galp residue by mild acid hydrolysis (Table IV). Interestingly, the structure was resistant to both jack bean β-galactosidase and bovine testicular β-galactosidase enzymes.2 The chemical shifts of the anomeric protons of this component (Table II) are identical to those described in Ref. 8 for the structure Galpβ1–3Galpβ1–2Galpβ1–6(Galpβ1–2Galpβ1–4)GlcNAc-ol, and all of the above data are consistent with this structure.

Peak f contained a component that produced an [M-1]- pseudomolecular ion at m/z 1,034 in negative ion ES-MS (deu-
tero-reduced Hex, HexNAc-ol = 1,034 Da) and that contained only [1-2H]GlcNAc-ol and Gal, as judged by GC-MS composi-
tion analysis (Table II). The radiolabeled (NaB3H4-reduced) form of this component had a size of 7.1 Gu that was reduced to 5.5 Gu after mild acid hydrolysis, suggesting that the structure contained either 2 Galp residues or 1 Galp residue that is sub-
stituted by a Galp residue (Table IV). The structure was resis-
tant to both jack bean β-galactosidase and bovine testicular β-galactosidase enzymes.2 The chemical shifts of the anomeric protons of this component (Table II) are identical to those described in Ref. 8 for the structure Galpβ1–3Galpβ1–2Galpβ1–6(Galpβ1–2Galpβ1–4)GlcNAc-ol, and all of the above data are consistent with this structure.

Trans-Sialidase Acceptor Activity of the O-Linked Oligosac-
charides—Neuraminidase-treated, HPLC-purified, and radi-
labeled oligosaccharitols were incubated with purified TS and
3'-sialyllactose as sialic acid donor, and the reaction products were fractionated by QAE-Sephadex chromatography. As ex-
pected, all of the oligosaccharitols containing terminal βGalp

![Table II](image)

Summary of mass spectrometry and 1H NMR spectroscopy analysis of O-linked carbohydrates of maccins from metacyclic trypanostigotes

| Glycoplex peak | ES-MS analysis | 1H NMR chemical shifts | Methylation analysis |
|---------------|----------------|------------------------|---------------------|
|               | m/z ppm        | Galp                  | Galp(1)  | Galp(2)  | Galp(3)  | Galp(4)  | 4GlucNAc-ol | 4,6-GlucNAc-ol | t-Galp | t-Galp | 3-Galp | 2,3-Galp |
| a             | 223            | (HexNAc-ol)           |          |          |          |          |          |          |        |        |        |        |
| b             | 385            | (Hex-HexNAc-ol)       | 5.154    |          |          |          |          |          |        |        |        |        |
| c             | 547            | (Hex, HexNAc-ol)      | 5.215    | 4.432    |          |          |          |          |        |        |        |        |
| d             | 709            | (Hex, HexNAc-ol)      | 5.217    | 4.493    | 4.613    |          |          |          |        |        |        |        |
| e             | 871            | (Hex, HexNAc-ol)      | 5.273    | 4.582    | 4.662    | 4.862    |          |          |        |        |        |        |
| f             | 1033           | (Hex, HexNAc-ol)      | 5.534    | 4.642    | 4.656    | 4.850    | 4.603    |          |        |        |        | ND |

a Components contained GlcNAc-ol (a-f) and Gal (h-f) by GC-MS compositional analysis.

b [M-1]- pseudomolecular ions detected by negative-ion ES-MS.

c Data obtained from one-dimensional 500-MHz NMR spectroscopy. Chemical shifts were referenced relative to acetone (2.225 ppm.) at 300 K.

d Obtained from analysis of partially methylated alditols acetate (PMAAs).

e Two terminal Galp residues detected.

f Not determined.
were sialylated, while the oligosaccharitol b, which contains only a terminal βGal residue, was not a sialic acid acceptor (not shown). To further evaluate the degree of sialylation of oligosaccharitols c–f, the TS reaction products were fractionated in a Mono-Q column. Oligosaccharitols c and d, which contain single βGal termini, accepted only one sialic acid residue, as expected (Fig. 7, A and B). Oligosaccharitol e, which contains two terminal βGal residues (attached to the 2- and 3-position of the same subterminal residue) accepted only one sialic acid residue (Fig. 7C). This result indicates that either only one of the terminal βGal residues can be sialylated or sialylation of either residue precludes sialylation of the adjacent residue for steric reasons. Oligosaccharitol f, which, compared with oligosaccharitol e, has one additional βGal attached to a different branch, was both monosialylated and bisialylated (Fig. 7D). These sialylation patterns are included in Fig. 6.

**DISCUSSION**

We have compared the structures of the O-linked oligosaccharides and the GPI anchors of the major mucin-like SA acceptors from metacyclic trypanosomes and epimastigotes forms of *T. cruzi*. We found that when epimastigotes transform into metacyclic trypanosomes the O-linked oligosaccharides and the GPI glycan core structures remain unchanged, whereas the lipid portion of the GPI anchor changes substantially from alkylacylglycerol-PI to mostly ceramide-PI. The ES-MS analyses showed that the epimastigote mucins contain alkylacyl-PI species, the principal component being 1-O-hexadecyl-2-O-hexadecanoyl-PI, whereas 70% of the metacyclic mucins contain inositol phosphoceramides (ceramide-Pi), with the principal ceramide components identified as lignoceroyl-sphinganine and palmitoyl-sphinganine. The remaining 30% of the metacyclic mucins contain the same alkylacyl-PI species as the epimastigote mucins. The lipids present in the epimastigote mucins of strain Y, identified as 40/45-kDa glycoconjugates, were also shown to contain 1-O-hexadecyl-2-O-palmitoylglycerol and 1-O-hexadecyl-2-O-stearoylglycerol (17), in similar proportions to those found in G-strain epimastigotes in this study. The lipid portion of a molecule called lipophosphoglycan-like glycoconjugate, isolated from *T. cruzi* epimastigotes (Peru strain) also contains the same alkylacylglycerol-PI species (9). No direct evidence for the presence of phosphosaccharide repeats, characteristic of *Leishmania* lipophosphoglycans, was presented in that study, and, given its similarity in composition and properties to the mucin-like molecules reported here and in Refs. 8, 12, 16, and 17, it should be considered as a member of the *T. cruzi* mucin family.

The GPI anchors of both trypanosome Tc-85 (30) and the metacyclic 90-kDa 1G7-antigen (26, 31) have been shown to contain mostly 1-O-hexadecyl-2-acyl-PIs, which are similar to those reported here and in Refs. 9 and 17 for the epimastigote mucin membrane anchors. In the case of the metacyclic mucins, the GPI anchor ceramide-PI structures are identical to the main type found in LPPG, the major surface glycoconjugate of *T. cruzi* (Y strain) epimastigotes (32, 33); i.e. they contain palmitoyl-sphinganine and lignoceroyl-sphinganine. The LPPG molecule also shares the same Man₆GlcN core glycan structure as the mucin GPI anchor and contains a phosphate (or 2-AEP) bridge to Man₆-GlcN-(ceramide-PI) to form the GPI anchor precursor or the addition of two terminal Gal residues to form LPPG. The relationship between the ceramide-PI type GPI structures and the alkylacyl-PI structures is not clear. In the case of *Saccharomyces cerevisiae*, the GPI precursors are based on diacyl-PIs that on most (but not all) glycoproteins are exchanged to ceramide-PIs after transfer to protein in an as yet undefined lipid-remodelling reaction (34, 35). It is possible that a similar mechanism may operate in *T. cruzi*, as discussed in Ref. 26. The observation that all of the early GPI intermediates in *T. cruzi* epimastigotes are based on alkylacyl-PI is consistent with this notion (36). Interestingly, a glycositophilospholipid (called GIEL A) with the same glycan core structure as LPPG, but containing a lipid

TABLE III

| Proton | βGal | βGalp | GlcNAc-εl |
|--------|------|-------|-----------|
| H-1    | 5.21 | 4.43  | 3.73      |
| H-1'   |     |       | 3.66      |
| H-2    | 4.14 | 3.56  | 4.17      |
| H-3    | 4.08 | 3.66  | 3.93      |
| H-4    | 4.09 | 3.92  | 3.82      |
| H-5    | 3.82 | 3.71  | 4.03      |
| H-6    | 3.63 | 3.76  | 4.14      |
| H-6'   | 3.72 | 3.78  | 3.84      |
| NAc    |     |       | 2.06      |

**TABLE IV**

*Bio-Gel P4 analysis of neutral O-linked carbohydrates of mucins from metacyclic trypanosomes.*

Individual O-linked carbohydrates separated on HPLC, were chromatographed on a Bio-Gel P4 column, and the elution position relative to a series of dextran oligomers was expressed as glucose units (Glu). The oligosaccharides were further treated with bovine testicular β-galactosidase (BTBG), jack bean β-galactosidase (JBBG) and 40 mM trifluoroacetic acid (TFA).

| Oligosaccharide | Size on P4 | Treatment |
|-----------------|------------|-----------|
| a               | 2.6        | NTa       |
| b               | 3.5        | NTb       |
| c               | 4.5        | NTc       |
| d               | 5.6        | NTd       |
| e               | 6.4        | NTe       |
| f               | 7.1        | NTf       |

a NT, not treated.
b TFA treatment produced products correspondent to 7.0 and 5.5 Gu.
c No modification after treatment.

d, e, f Oligosaccharitol c–f, the TS reaction products were fractionated in a Mono-Q column. Oligosaccharitols c and d, which contain single βGal termini, accepted only one sialic acid residue, as expected (Fig. 7, A and B). Oligosaccharitol e, which contains two terminal βGal residues (attached to the 2- and 3-position of the same subterminal residue) accepted only one sialic acid residue (Fig. 7C). This result indicates that either only one of the terminal βGal residues can be sialylated or sialylation of either residue precludes sialylation of the adjacent residue for steric reasons. Oligosaccharitol f, which, compared with oligosaccharitol e, has one additional βGal attached to a different branch, was both monosialylated and bisialylated (Fig. 7D). These sialylation patterns are included in Fig. 6.

**FIG. 7.** Sialylation of O-linked oligosaccharitols of metacyclic trypanosomes mucin accessed by Mono Q chromatography. Purified and labeled neutral oligosaccharitols c–f (panels A, B, C, and D) of metacyclic trypanosomes were incubated with TS and sialylactose, as described under "Materials and Methods." After 3 h of incubation, the samples were diluted with 5 mM sodium acetate, pH 4.6, and loaded onto a Mono Q column. The number above each peak represents the amount of sialic acid residues per oligosaccharitol.

Changes in GPI Lipid Structure during *T. cruzi* Differentiation

| Treatment | Radioactivity (cpm) | NaOAc (mM) |
|-----------|---------------------|------------|
| A         | 3000                | 600        |
| B         | 2000                | 500        |
| C         | 1000                | 400        |
| D         | 500                 | 300        |

**TABLE IV**

*Bio-Gel P4 analysis of neutral O-linked carbohydrates of mucins from metacyclic trypanosomes.*

Individual O-linked carbohydrates separated on HPLC, were chromatographed on a Bio-Gel P4 column, and the elution position relative to a series of dextran oligomers was expressed as glucose units (Glu). The oligosaccharides were further treated with bovine testicular β-galactosidase (BTBG), jack bean β-galactosidase (JBBG) and 40 mM trifluoroacetic acid (TFA).

| Oligosaccharide | Size on P4 | Treatment |
|-----------------|------------|-----------|
| a               | 2.6        | NTa       |
| b               | 3.5        | NTb       |
| c               | 4.5        | NTc       |
| d               | 5.6        | NTd       |
| e               | 6.4        | NTe       |
| f               | 7.1        | NTf       |

a NT, not treated.
b TFA treatment produced products correspondent to 7.0 and 5.5 Gu.
c No modification after treatment.
moiety composed exclusively of 1-O-hexadecyl-2-O-palmitoyl-
glycerol, has been detected in early cultures of Y strain T. cruzi
epimastigotes (37). This glycosylphosphatidylinositol could be the
immediate precursor to LPPG. If so, the shift from alkylacyl-PI
to ceramide-PI seen in the mucins upon transformation of late
epimastigotes to metacyclic trypomastigotes may be similar to
the shift from GIPL A to LPPG seen upon the transformation
from early to late epimastigotes. Thus the change in the PI
lipid structure of the mucin molecules of T. cruzi appears to be
under developmental control. Interestingly, the ES-MS anal-
sis of the metacyclic 1G7-antigen PI moieties also revealed that
a small quantity (>15 mol %) of the GPI anchors contained
linear and branched side-chains to the 6-position of the Glc-
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exhaustively pre-extracted with butan-1-ol to remove glycolipid
blot with an anti-LPPG antibody; (iv) the mucin samples were
epimastigotes (38), suggesting that any LPPG contamination
tose medium was used throughout this mucin study, it is pos-
tible that the nutrient conditions might affect the fatty acid
content of the ceramide components.

The possibility that the ceramide-PI content of the metacy-
cyclic trypomastigote mucin preparation might be due to contam-
nation with LPPG can be ruled out for the following reasons:
(i) the mucin fractions were eluted from the octyl-Sepharose
column at >40% propan-1-ol, while LPPG is known to elute from this column at >40% propan-1-ol (33); (ii) metacy-
cyclic trypomastigotes express about 10 times less LPPG than epimastigotes (38), suggesting that any LPPG contamination
would be greater in epimastigote preparations; (iii) no LPPG
could be detected in the mucin preparations by SDS-polyacryl-
amide gel electrophoresis and silver staining and by Western
blot with an anti-LPPG antibody; (iv) the mucin samples were
exhaustively pre-extracted with butan-1-ol to remove glycolipid
and phospholipid contaminants prior to deamination; (v) the
Man3-AHM* fragment, generated by partial acid hydrolysis of
the deaminated NaBH4-reduced mucin, was completely pro-
tected from jack bean α-mannosidase digestion by an aqueous
HF-sensitive substituent (Fig. 3, lane 7). The corresponding
LPPG Man3-AHM* fragment would be digested to AHM*.

The structure of the major GPI glycan (shown to be as
Manα1–2Manα1–2Manα1–6Manα1–4GlcN-myoo-inositol) was
identical in epimastigote and metacyclic mucins. A small pro-
portion of the GPI glycans contained an additional unidentified
sugar residue attached to the αMan residue adjacent to the
GlcN residue. Similar results have been reported recently for
the epimastigote mucin of Y strain (17). The major Man3 GIPI
glycan structure described above is also found in the 1G7-
noted. As Grace’s medium was used to induce metacyc-
genesis in the 1G7-antigen work, whereas liver infusion trypod-
tose medium was used throughout this mucin study, it is pos-
tible that the nutrient conditions might affect the fatty acid
content of the ceramide components.

The possibility that the ceramide-PI content of the metacy-
cyclic trypomastigote mucin preparation might be due to contam-
nation with LPPG can be ruled out for the following reasons:
(i) the mucin fractions were eluted from the octyl-Sepharose
column at >40% propan-1-ol, while LPPG is known to elute from this column at >40% propan-1-ol (33); (ii) metacy-
cyclic trypomastigotes express about 10 times less LPPG than epimastigotes (38), suggesting that any LPPG contamination
would be greater in epimastigote preparations; (iii) no LPPG
could be detected in the mucin preparations by SDS-polyacryl-
amide gel electrophoresis and silver staining and by Western
blot with an anti-LPPG antibody; (iv) the mucin samples were
exhaustively pre-extracted with butan-1-ol to remove glycolipid
and phospholipid contaminants prior to deamination; (v) the
Man3-AHM* fragment, generated by partial acid hydrolysis of
the deaminated NaBH4-reduced mucin, was completely pro-
tected from jack bean α-mannosidase digestion by an aqueous
HF-sensitive substituent (Fig. 3, lane 7). The corresponding
LPPG Man3-AHM* fragment would be digested to AHM*.

The structure of the major GPI glycan (shown to be as
Manα1–2Manα1–2Manα1–6Manα1–4GlcN-myoo-inositol) was
identical in epimastigote and metacyclic mucins. A small pro-
portion of the GPI glycans contained an additional unidentified
sugar residue attached to the αMan residue adjacent to the
GlcN residue. Similar results have been reported recently for
the epimastigote mucin of Y strain (17). The major Man3 GIPI
glycan structure described above is also found in the 1G7-
noted. As Grace’s medium was used to induce metacyc-
genesis in the 1G7-antigen work, whereas liver infusion trypod-
tose medium was used throughout this mucin study, it is pos-
tible that the nutrient conditions might affect the fatty acid
content of the ceramide components.

The possibility that the ceramide-PI content of the metacy-
cyclic trypomastigote mucin preparation might be due to contam-
nation with LPPG can be ruled out for the following reasons:
(i) the mucin fractions were eluted from the octyl-Sepharose
column at >40% propan-1-ol, while LPPG is known to elute from this column at >40% propan-1-ol (33); (ii) metacy-
cyclic trypomastigotes express about 10 times less LPPG than epimastigotes (38), suggesting that any LPPG contamination
would be greater in epimastigote preparations; (iii) no LPPG
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293–304

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