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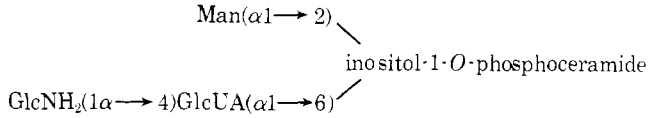
Structure of a Major Glycophosphoceramide from Tobacco Leaves, PSL-I: 2-Deoxy-2-acetamido-D-glucopyranosyl(α1→4)-D-glucuronopyranosyl(α1→2)myoinositol-1-*O*-phosphoceramide†

Thomas C.-Y. Hsieh, Karan Kaul,‡ Roger A. Laine, and Robert L. Lester*

ABSTRACT: The chemical structure of a major glycophosphoceramide from tobacco leaves, called PSL-I [K. Kaul and R. L. Lester (1975), *Plant Physiol. 55,* 120], has now been characterized as 2-deoxy-2-acetamido-D-glucopyranosyl-(α1→4)D-glucuronopyranosyl(α1→2)myoinositol-1-*O*-phosphoceramide. Sites of glycosidic linkage were determined by (1) methylation analysis on a trisaccharide isolated by degradation of carboxyl-reduced PSL-I and (2) periodate oxidation experiments on PSL-I. The resulting products were identified with gas chromatography/mass spectrometry. Anomeric configurations were determined by resistance of the sugars in the peracetylated trisaccharide to chromium trioxide treatment.

A class of complex glycophosphoceramides containing phosphoinositol occurs in higher plants and fungi (Carter et al., 1965; Lester et al., 1974). These lipids may be analogous to the glycolipids found in animal cell plasma membrane, which in the animal cells carry ABO, Lewis and Forssmann antigen activities, among many others (Hakomori, 1970; Hakomori et al., 1972; Siddiqui and Hakomori, 1971), as well as receptors for cholera toxin (Cuatrecasas, 1973; Holmgren et al., 1975), clostridial toxins (Van Heyningen and Miller, 1961; Berheimer and Van Heyningen, 1961; Van Heyningen, 1974), hormones (Mullin et al., 1978), and perhaps viruses (McCrea, 1953; Klenk and Uhlenbrook, 1958; Kathan et al., 1959; Kathan and Winzler, 1963). Finding the true function of glycolipids in either plants or animals is a challenging research goal.

Carter and his co-workers (Carter et al., 1958, 1962; Carter and Koob, 1969) were the first to study phosphoinositol-containing glycophosphoceramides prepared from a variety of plant materials such as commercial vegetable oil, corn and flax seeds, and leaves of bush beans. Apparently, homogeneous glycolipids were not obtained, since alkaline hydrolysis of these preparations yielded a mixture of oligosaccharides, suggesting the occurrence of a diverse series of glycolipids in plants (Carter and Kisic, 1969). The major oligosaccharide from corn seed was isolated in large quantity and was chemically characterized leading to the following structural proposal for corn "phytoglycolipid" (Carter et al., 1969):



The isolation procedures employed by Carter and his coworkers involved treatment with alkali to remove acyl esters (Carter et al., 1958) or refluxing with 70% ethanol containing 0.1 N HCl (Carter and Koob, 1969).

To preclude possible breakdown of labile bonds in the glycolipids, a relatively mild extraction procedure was devised for the preparation of a glycophosphoceramide concentrate from fresh tobacco leaves, yielding about 100 μmol of P/kg fresh weight (Kaul and Lester, 1975). Eight components have been

† From the Department of Biochemistry, University of Kentucky College of Medicine, Lexington, Kentucky 40506. *Received February 8, 1978.* This investigation was supported by Research Grant PCM76-09314 from the National Science Foundation and Project KTRB053 from the Tobacco and Health Research Institute, University of Kentucky.

‡ Present address: Department of Chemistry, University of Colorado, Boulder, Colo. 80302.

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purified comprising about 50–60% of this concentrate (Kaul and Lester, 1975, 1978). The two most abundant components are PSL-I and PSL-II, each constituting about 20% of the glycophosphoceramide concentrate. Partial chemical structures reported earlier (Kaul and Lester, 1975) are PSL-I, GlcNAc[GlcUA, myoinositol]-1-*O*-phosphoceramide, and PSL-II, $GlcNH_2$[GlcUA, myoinositol]-1-*O*-phosphoceramide. This report describes the complete chemical characterization of the carbohydrate moiety in PSL-I, including the anomeric configuration and position of glycosidic linkages.

Materials and Methods

PSL-I was isolated from the glycophosphoceramide concentrate of tobacco leaves directly by column chromatography on porous silica as previously described (Kaul and Lester, 1975) or by DEAE-cellulose (acetate form) column chromatography followed by porous silica bead column chromatography (Kaul and Lester, 1978).

Preparation and Purification of a Trisaccharide from Carboxyl-Reduced PSL-I. Na_2PSL-I was converted to the acid form by passing a 6 mM aqueous solution (10 mL) through a 30-mL Dowex-50 (H^+) column. The acid-form PSL-I was reduced by the following procedure adapted from Taylor et al. (1973). To an 80-mL 0.75 mM aqueous solution of acid-form PSL-I, 1.528 g of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide was added and the solution was stirred for 2 h at room temperature. The pH was maintained at 4.75–4.80 during this period by the addition of 1 N HCl. The pH of the solution was then adjusted to 8.0 with 14 N NH_4OH; 0.5 mL of 1-octanol (to prevent foaming) followed by 6.0 g of $NaBH_4$ was added, and the reduction was allowed to occur at 55 °C for 18 h. The reaction mixture was neutralized with 120 mL of packed Dowex-50 (H^+) resin. The resin slurry was filtered and washed with 300 mL of H_2O; the pH of the filtrate was adjusted to 6 with 14 N NH_4OH and applied to a 15-mL column of DEAE-cellulose (acetate form) in H_2O. The column was washed with 2 column volumes each of H_2O–CH_3OH (1:1), H_2O–CH_3OH (1:3), and $CHCl_3$–CH_3OH–H_2O (16:16:5) to remove the components of the reaction mixture other than carboxyl-reduced PSL-I. Carboxyl-reduced PSL-I was eluted with $CHCl_3$–CH_3OH–H_2O (16:16:5) containing 0.5 M ammonium acetate; 88% of the starting total P was recovered. As judged by the hexuronic acid to total P ratio, 15% of the PSL-I remained unreduced. The products were precipitated from the column eluate by adding 0.5 volume of acetone. The precipitate was washed with acetone, air dried, and stored in a desiccator at 5 °C.

To cleave the ceramide from the glycolipid, a sample containing 20 μmol of the above carboxyl-reduced PSL-I preparation was hydrolyzed in 4 mL of aqueous 0.5 N KOH at 100 °C for 9 h, acidified with 0.5 mL of 5 N CH_3COOH, and extracted after adding 1.5 mL of H_2O, 6 mL of $CHCl_3$, 4.5 mL of CH_3OH, and 1.5 mL of toluene. The aqueous phase, which contained trisaccharides and phosphotrisaccharides, was evaporated to dryness with a stream of N_2 and redissolved in 2 mL of water, and the pH was adjusted to 8 with 14 N NH_4OH. Four units of *E. coli* alkaline phosphatase (Sigma Chemical Co.) was added, and the mixture was incubated at room temperature overnight, after which all of the organic phosphate in the sample was released from the trisaccharide phosphate. Since the GlcNAc residue was de-N-acetylated during the alkaline hydrolysis, the trisaccharide was N-acetylated by three 20-min incubations at room temperature with the acetic anhydride reagent of Roseman and Daffner (1956) which rendered the sample ninhydrin negative. After evaporation of the acetic anhydride at 100 °C for 5 min, the sample was passed through a 20-mL column of mixed-bed resins [Dowex-50 (H^+) and AG-1X1 (OH^-), Bio-Rad Laboratories] to remove the salts and any remaining glucuronic acid-containing trisaccharide. The eluate of 3 column volumes of water was lyophilized, redissolved in 0.5 mL of water, and purified by gel filtration on a 1 × 100 cm column of Bio-Gel P-2 (Bio-Rad Laboratories) eluted with water. Column fractions were assayed for carbohydrate by the anthrone method (Seifter et al., 1950), and the fractions were pooled and lyophilized. Reduced trisaccharide was obtained in 46% molar yield based on hexosamine analysis.

Preparation and Purification of Acetylated Trisaccharide. The trisaccharide (9.5 μmol) from carboxyl-reduced PSL-I was dissolved in 1.0 mL of pyridine–acetic anhydride (1:1) using a sonication bath followed by reaction at 100 °C for 2 h. The reagents were removed under a stream of N_2 in the presence of toluene. The acetylated trisaccharide was dissolved in a small volume of chloroform, chromatographed on a 0.32 × 100 cm column of Porasil A-60 (37–75 μm, Waters Associates, Inc.), eluted with chloroform–*n*-hexane–methanol (49:49:2), at a flow rate of 1.0 mL/min, and monitored with a moving-wire flame-ionization detector (Model Pye LCM2, Pye Unicam). The peak fractions were combined, evaporated to dryness, and redissolved in 1 mL of chloroform. An aliquot of this solution, chromatographed on silica gel thin-layer (Quanta/Gram LQ, Kontes Glass Co.) with $CHCl_3$–CH_3OH (75:1) revealed a single orcinol-positive spot.

Methylation Linkage Analysis by GC/MS.[1] Permethylation of the carboxyl-reduced trisaccharide (Hakomori, 1964) and subsequent preparation of the partially methylated alditol and myoinositol acetates were performed as described by Björndal et al. (1967, 1970) and Stellner et al. (1973) with deuterium labeling at the C-1 position of alditols. These acetates were chromatographed on a 2 mm × 1.6 m glass column of OV-17 (3% on Supelcoport) at 180 °C in a Finnigan automated gas chromatograph/mass spectrometer system, Model 3300-6110, using both EI and CI. Ion source temperature was 60 °C, and the emission current was set at 0.6 mA. The electron energy was 70 eV in the EI mode and 150 eV in the CI mode using methane as carrier and reagent gas at 1 Torr ion source pressure (Hancock et al., 1976; Laine et al., 1977).

Nitrous Acid Deamination of Carboxyl-Reduced De-N-acetylated Trisaccharide. A modified procedure of Bayard and Roux (1975) was used to prepare glucosylmyoinositol in order to determine the ring form of the glucosyl residue in the trisaccharide. To 0.5 μmol of the carboxyl-reduced PSL-I trisaccharide (de-N-acetylated) was added 1.0 mL of the following reagent: 6 mL of H_2O, 166.8 mg of sodium nitrite, and 0.90 mL of glacial acetic acid. After standing at room temperature for 16 h with occasional stirring, the mixture was passed through a 10-mL column of AG 50W-X4 resin (H^+ form, 50–100 mesh, Bio-Rad Laboratories) and eluted with water. The product was passed through a 10-mL column of AG 1-X2 resin (acetate form, 200–400 mesh, Bio-Rad Laboratories), eluted with water, and evaporated to dryness. Partially methylated glucitol and myoinositol acetates were prepared from this sample and analyzed by GC/MS as described above.

Determination of Linkage between Glucuronic Acid and Myoinositol by Periodate Oxidation and GC/MS. To a 0.98-mL aqueous solution containing 12.3 μmol of PSL-I was

[1] Abbreviations used are: GlcNAcp, 2-deoxy-2-acetamido-D-glucopyranoside; GlcUAp, D-glucuronopyranoside; GC/MS, combined gas–liquid chromatography/mass spectrometry; EI, electron impact mode of MS; CI, chemical ionization mode of MS; TLC, thin-layer chromatography.

added 3.0 mL of 50 mM $NaIO_4$ in 0.2 M sodium acetate buffer at pH 5.0. The oxidation reaction was allowed to take place in the dark at room temperature. At various time points, 25-μL aliquots were taken from the reaction mixture and mixed with 10 mL of 1 mM sodium acetate buffer at pH 5.0. The absorbance at 250 nm was measured, and the amount of periodate present at each time point was calculated. At 61 h, the periodate consumption reached 6 mol/mol of PSL-I (theoretical maximum consumption of periodate for oxidation of all possible vicinal hydroxyls). The reaction was stopped by the addition of 50 μL of ethylene glycol to destroy the excess periodate. Ten milligrams of sodium borodeuteride in 0.5 mL of 1 N NH_4OH was added (at this stage pH was 8), and the mixture was allowed to react at room temperature overnight. To destroy excess $NaBD_4$, glacial acetic acid was added until D_2 evolution ceased. Evaporation in vacuo was carried out with six additions of 1.8 mL of redistilled methanol to remove borate. The residue was dissolved in 2 mL of H_2O and passed through a 2-mL column of AG 50W-X4 resin (50–100 mesh, H^+ form, Bio-Rad Laboratories) to remove Na^+ ions. The volume was reduced to 2.9 mL by evaporation in vacuo and after addition of 2.9 mL of 2 N HCl hydrolysis was carried out at 100 °C for 1 h.

The phospho alcohol in the hydrolysate was separated from other periodate oxidation products by anion-exchange chromatography on AG-1X2 resin (200–400 mesh, HCO_3^- form, Bio-Rad LABORATORIES(/ The column (60 mm × 80 cm) was eluted with 0.3 M NH_4HCO_3. The fractions containing phosphorus were pooled and evaporated in vacuo, and the phosphate group was removed from the product by incubation with 2 units of *E. coli* alkaline phosphatase (Sigma Chemical Co.) in 0.2 M ammonium acetate buffer at pH 8.5 at room temperature overnight. The incubation mixture was dried under N_2 and then acetylated at 120 °C for 30 min with 200 μL of pyridine–acetic anhydride (1:1). The acetylated, deuterated alcohol was analyzed by methane CI GC/MS on an OV-275 (3% on Supelcoport) column (2 mm × 1.6 m) at 170 °C (Hancock et al., 1976; Laine et al., 1977).

Mass Chromatography. Partially methylated alditol and myoinositol acetates as well as periodate oxidation products were also examined by mass chromatography (Hites and Biemann, 1970). The gas chromatographic effluents of these samples were analyzed in the EI and the methane CI mode (Hancock et al., 1976; Laine et al., 1977; McNiel and Albersheim, 1977), and the mass spectra were recorded each 3–5 s and stored on a magnetic disk prior to reconstructing the mass chromatograms of specific ions.

CrO_3/CH_3COOH Oxidation of the Acetylated Trisaccharide. A sample of acetylated carboxyl-reduced PSL-I trisaccharide was subjected to CrO_3 oxidation (Hoffman et al., 1972) and partitioned between water and chloroform under the conditions specified by Laine and Renkonen (1975). The chloroform phase was evaporated to dryness and hydrolyzed with 2 N trifluoroacetic acid at 120 °C for 90 min. This hydrolysate was evaporated to dryness and redissolved in 0.5 mL of H_2O, and the pH was adjusted to 8 with 1 N NH_4OH. After the addition of 10–15 mg of $NaBH_4$ and treatment in a sonic bath for 3 min, the reduction was continued overnight at room temperature. Excess $NaBH_4$ was destroyed with glacial acetic acid, and the samples were evaporated to dryness four times in the presence of methanol to remove borate. The samples were treated with 0.4 mL of pyridine–acetic anhydride (1:1) in a sonic bath for 1 h and heated at 120 °C for 30 min, followed by evaporation to dryness in the presence of toluene. The dried sample was partitioned between $CHCl_3$ and H_2O, and the $CHCl_3$ phase was evaporated to dryness, redissolved in 50 μL of acetone, and analyzed by GC on a 2 mm × 1 m column of 3% OV-275 on 100/120 mesh Chromosorb W AW (Supelco, Inc.) at 180 °C. Nitrogen was the carrier gas at 54 mL/min. The organic phase after CrO_3 oxidation was also assayed for hexosamine colorimetrically by the method of Gatt and Berman (1966).

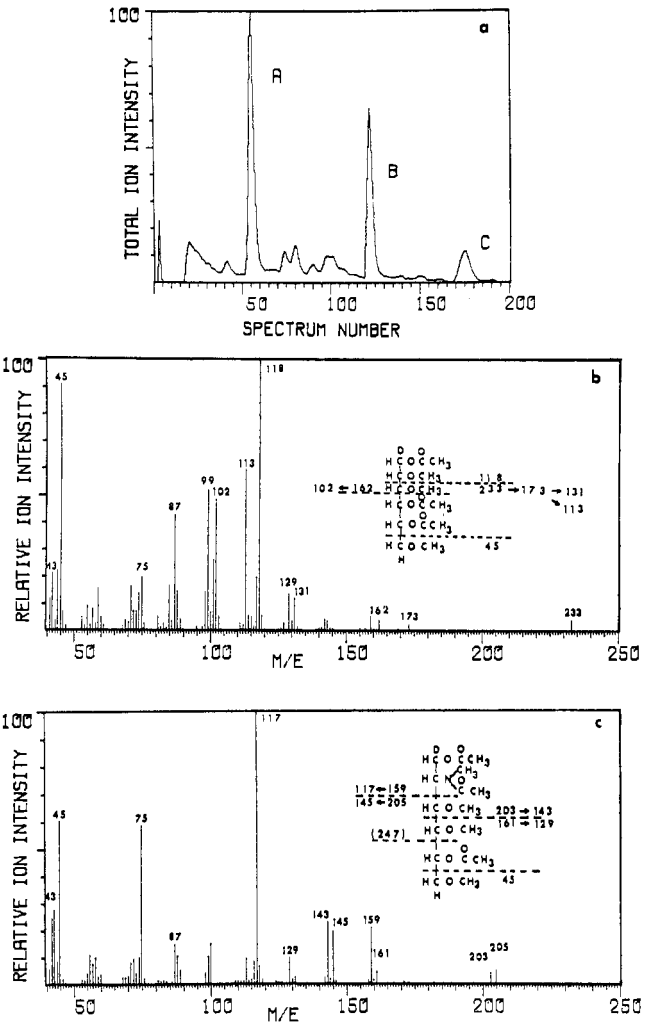

FIGURE 1: EI GC/MS of the partially methylated alditol and myoinositol acetates derived from the carboxyl-reduced trisaccharide of PSL-I. For details of experimental conditions, see Materials and Methods: (a) total ion current recording; (b) EI mass spectrum of peak B in a; (c) EI mass spectrum of peak C in a.

Results

Methylation Linkage Analysis by GC/MC. The composition of the carboxyl-reduced trisaccharide was 1.0 inositol:0.82 glucose:0.84 *N*-acetylglucosamine based on GC of alditol acetates after 2 N trifluoroacetic acid hydrolysis. Figure 1a shows the total ion current recording by EI GC/MS of the partially methylated alditol and myoinositol acetates from carboxyl-reduced PSL-I trisaccharide. Peak B can be assigned the structure 2,3,6-tri-*O*-methyl-1,4,5-tri-*O*-acetylglucitol, which most likely arises from 4-linked glucose (reduced glucuronic acid) in the trisaccharide. The methylation data does not distinguish between a 5-linked furanose and a 4-linked pyranose. Therefore, glucosylmyoinositol was prepared by nitrous acid treatment (Bayard and Roux, 1975) of the carboxyl-reduced trisaccharide and subjected to a separate methylation analysis. The only partially methylated glucitol acetate derived from the glucosylmyoinositol was found to be 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetylglucitol, indicating that

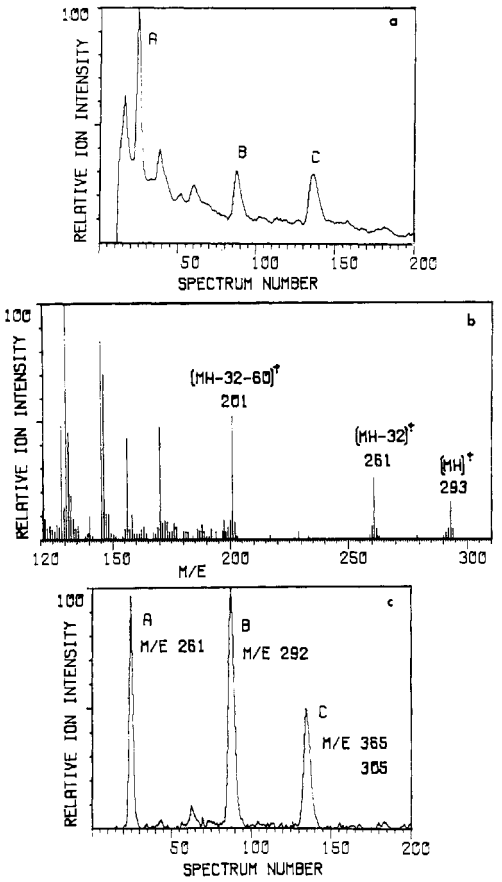

FIGURE 2: CI GC/MS of the partially methylated alditol and myoinositol acetates derived from the carboxyl-reduced trisaccharide of PSL-I. For details of experiments, see Materials and Methods: (a) total ion current recording; (b) CI mass spectra of peak A in a; (c) CI mass chromatogram for specific ions at *m/e* 261, 292, and 365, plus 305 from a.

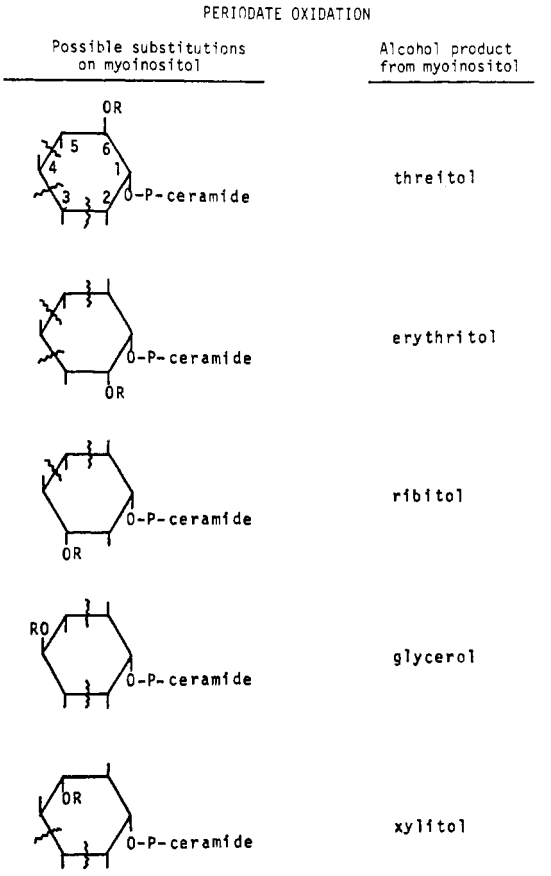

FIGURE 3: Possible substitutions on myoinositol by GlcUA. Shown are the bonds susceptible to periodate oxidation (wavy lines) and the predicted corresponding final myoinositol-derived alcohol products after periodate oxidation (followed by $NaBD_4$ reduction, hydrolysis, anion-exchange chromatography, and dephosphorylation).

the glucose residue of the carboxyl-reduced trisaccharide is in the pyranosyl form. Figure 1b shows the mass spectrum of peak B in Figure 1a. The inset illustrates the fragmentation scheme. Peak C in Figure 1a can be assigned the structure 3,4,6-tri-*O*-methyl-1,5-di-*O*-acetyl-2-deoxy-2-*N*-methylacetamidoglucitol from terminal *N*-acetylglucosamine. Figure 1c shows the mass spectrum of peak C in Figure 1a. The inset diagram depicts the EI-induced cleavage scheme. A mass chromatographic search (Hites and Biemann, 1970) for derivatives from all possible linkages of glucose and *N*-acetylglucosamine was carried out. Only the derivatives from 4-linked glucose and terminal *N*-acetylglucosamine were found. The information in the EI mass spectrum of peak A in Figure 1a was considered inadequate for the assignment of peak A to monosubstituted myoinositol, since ions closely related to the expected molecular ion were not apparent. Chemical ionization GC/MS was therefore used to analyze the same mixture of partially methylated alditol and myoinositol acetates prepared from the carboxyl-reduced PSL-I trisaccharide. Figure 2a shows the total ion current recording. The CI mass spectra of peaks B and C (not shown) were wholly consistent with the assignment of 4-linked glucose and terminal N-acetylglucosamine, giving $[MH - 60]^{+\cdot}$ ions in high abundance. Figure 2b shows the CI mass spectrum of peak A in Figure 2a. Peak A was assigned the structure penta-*O*-methylmono-*O*-acetylmyoinositol derived from monosubstituted myoinositol in the PSL-I trisaccharide. Three ions support this assignment: *m/e* 293 $[MH]^{+\cdot}$, *m/e* 261 $[MH - \text{methanol}]^{+\cdot}$, and *m/e* 201 $[MH - \text{methanol} - \text{acetic acid}]^{+\cdot}$ (Figure 2b). Figure 2c shows the CI mass chromatogram of specific ions at *m/e* 261, $[MH - \text{methanol}]^{+\cdot}$ (for penta-*O*-methylmono-*O*-acetylmyoinositol), *m/e* 292, $[MH - 60]^{+\cdot}$ (for 2,3,6-tri-*O*-methyl-1,4,5-tri-*O*-acetylhexitol), and *m/e* 365 $[MH]^{+\cdot}$ and *m/e* 305 $[MH - 60]^{+\cdot}$ (for 3,4,6-tri-*O*-methyl-1,5-di-*O*-acetyl-2-deoxy-2-*N*-methylacetamidohexitol). In summary, both EI and CI GC/MS methylation linkage analyses were consistent with the structure of the carboxyl-reduced PSL-I trisaccharide as being GlcNAcp(1→4)Glcp(1→?)myoinositol.

Linkage of Glucuronic Acid to Myoinositol. Since the phosphate group is linked to the myoinositol at the C-1 position of the ring (Kaul and Lester, 1975), PSL-I was formulated as GlcNAcp(1→4)GlcUAp(1→?)myoinositol-1-*O*-phosphoceramide with the GlcUAp–myoinositol bond remaining unknown. CI mass spectrometry allowed us to decipher the number of methyl substitution sites on the myoinositol ring in the trisaccharide by molecular weight (Figure 2b, *m/e* 293 = $[MH]^{+\cdot}$). The exact substitution site on the myoinositol ring by glucuronic acid in the intact PSL-I was determined to be the 2 position by periodate oxidation, and CI GC/MS identification of the final periodate oxidation product from myoinositol in PSL-I was determined as acetylated, dideuterated erythritol. Figure 3 shows all the possible glycosidic substitutions on 1-phosphomyoinositol of PSL-I by glucuronic acid, the bonds on the resulting disubstituted myoinositol ring which are susceptible to periodate oxidation, and the corresponding final alcohol product derived from disubstituted myoinositol in PSL-I. Figure 4 summarizes the proposed major reaction scheme of the periodate oxidation of PSL-I and the subsequent processing leading to the final product—the acetylated di-

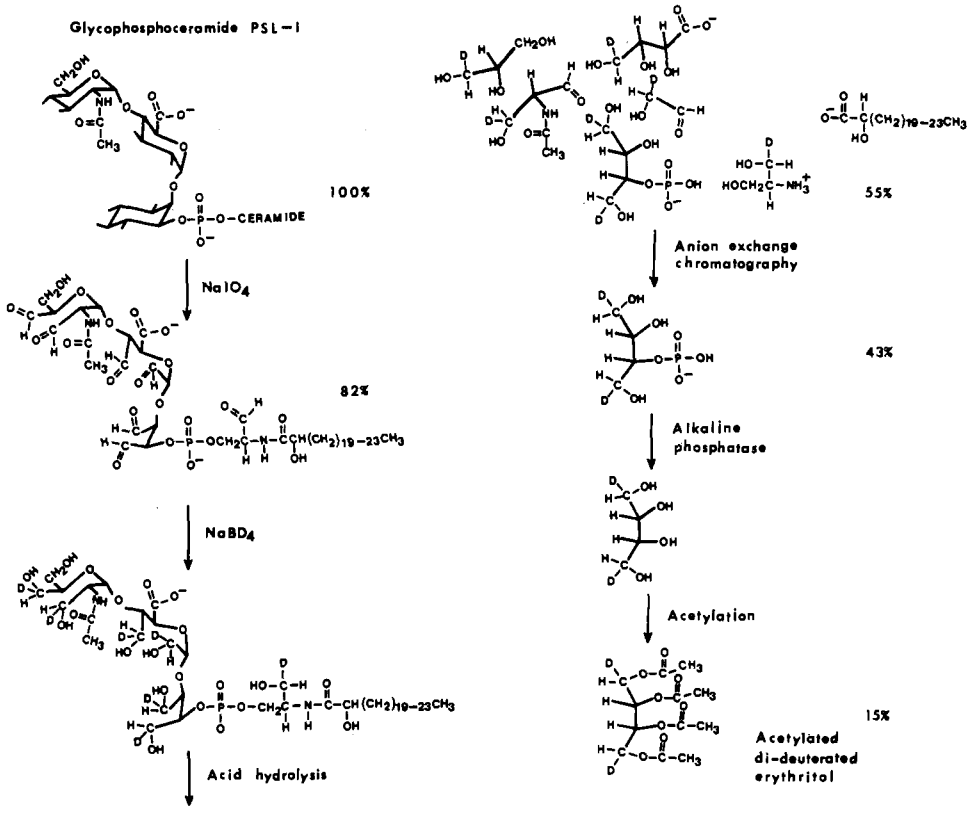

FIGURE 4: Proposed major reaction scheme of the periodate oxidation of PSL-I and subsequent processing leading to the acetylated dideuterated erythritol. Overall yields of the phosphate-containing products are based on total phosphorus determination. The final overall yield at the last step is based on the amount of acetylated dideuterated erythritol determined by CI GC/MS.

deuterated erythritol. Overall yields of the major products at the degradation steps are shown in the figure.

Since periodate oxidation of myoinositol, glucuronic acid, and *N*-acetylglucosamine could give similar products after hydrolysis, separation of the 1-phosphomyoinositol oxidation product from those of glucuronic acid and *N*-acetylglucosamine was important. Acid hydrolysis was carried out under controlled conditions which allowed the hydrolysis of (1) all glycosidic bonds and (2) the phosphodiester bond (to form the phosphomonoester products). Subsequent anion-exchange chromatography and monitoring of phosphorus in the fractions allowed purification of the phospho alcohol derived from the myoinositol ring. After removal of the phosphorus and acetylation of the reduced product, CI GC/MS gave unequivocal identification of the resulting dideuterated erythritol based on both the GC retention time and the exact molecular weight information.

Figure 5a shows the CI total ion current recording of the acetylated products (after phosphatase incubation) which contained the final dideuterated alcohol product derived from the myoinositol ring. A mass chromatographic search for diagnostic ions ($[MH]^+$·, $[MH - 60]^+$·) derived from acetylated myoinositol (*m/e* 433, 373) as well as acetylated dideuterated glycerol (*m/e* 221, 161), tetritols (*m/e* 293, 233), and pentitols (*m/e* 365, 305) was carried out. Only acetylated dideuterated erythritol was found, as shown (*m/e* 233) in Figure 5b. Authentic threitol and erythritol were acetylated and analyzed by CI GC/MS under identical conditions. The acetylated dideuterated tetritol derived from the myoinositol ring was found to have an identical GC retention time with that of the acetylated authentic erythritol (well separated from the peak of acetylated authentic threitol) as shown in Figure 5. Figures 5c and 5d show the total ion current recording and mass chromatogram for *m/e* 231 of the acetylated authentic erythritol. Figures 5e and 5f show the CI mass spectra of the acetylated dideuterated tetritol derived from PSL-I and authentic erythritol, respectively. Inset diagrams explain the formation of the major diagnostic ions (*m/e* 233 and 231). Upon coinjection of the acetylated dideuterated tetritol with the acetylated authentic erythritol, these two compounds were found to cochromatograph. Mass chromatograms for *m/e* 233 and 231 gave reconstructed peaks with identical retention times, and the composite CI mass spectrum gave both ions at *m/e* 233 and 231 (not shown).

The establishment of dideuterated erythritol as the final product derived from the periodate oxidation of the myoinositol ring led us to conclude that glucuronic acid is linked to the C-2 position of the myoinositol ring. Hence, PSL-I is GlcNAcp(1→4)GlcUAp(1→2)myoinositol-1-*O*-phosphoceramide.

Anomeric Configuration of the Carboxyl-Reduced Trisaccharide. Hoffman et al. (1972) described a procedure whereby the anomeric configuration of carbohydrate residues in an acetylated oligo- or polysaccharide can be determined by their resistance to CrO_3 oxidation in the presence of acetic acid. Residues in the β configuration are converted to 5-hexulosonates, while residues in the α configuration remain unchanged.

Based on the colorimetric assay, the *N*-acetylhexosamine residue in the acetylated trisaccharide derived from carboxyl-reduced PSL-I was found to be resistant to the CrO_3 oxidation (Table I). Carbohydrate assay by GC also indicated that both *N*-acetylglucosamine and glucose residues were resistant to CrO_3 oxidation (Table I). These results indicate that both *N*-acetylglucosamine and glucuronic acid (from which the glucose residue was derived) are in the α anomeric con-

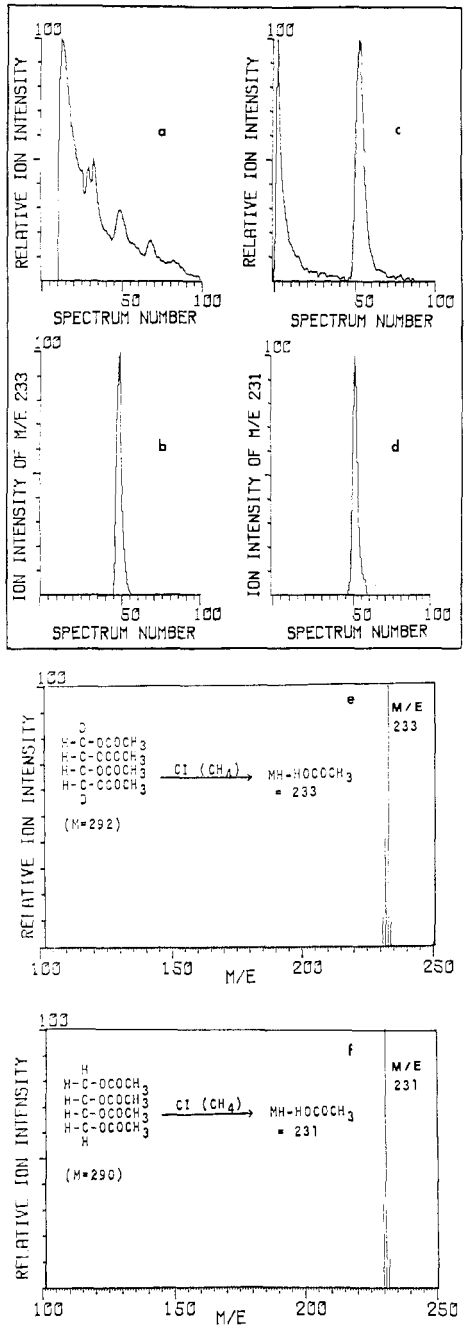

FIGURE 5: CI GC/MS of the acetylated final product derived from periodate oxidation of the myoinositol ring in PSL-I (a, b, e) and acetylated authentic erythritol (c, d, f). For details of the experiment, see Materials and Methods: (a) total ion current recording of the oxidation product from myoinositol in PSL-I; (b) mass chromatogram for *m/e* 233 $[MH - 60]^+$ from a; (c) total ion current recording of acetylated authentic erythritol; (d) mass chromatogram for *m/e* 231 $[MH - 60]^+$ from c; (e) and (f), CI mass spectrum of the peak in b and d, respectively.

figuration in PSL-I, giving the complete structure as GlcNAcp(α1→4)GlcUAp(α1→2)myoinositol-1-*O*-phosphoceramide as shown in Figure 6.

Discussion

It is evident from the present work that the structure of PSL-I is closely related to that of the "phytoglycolipid" as described by Carter et al. (1969). However, there are two clear differences: (1) there is no mannose in PSL-I and (2) the glucuronic acid is linked to C-2 of myoinositol instead of C-6 as reported by Carter et al. (1969) for the phytoglycolipid from corn seed. Mannose-containing phosphosphingolipids surprisingly constitute only a very minor part (less than 0.5%) of the total glycophosphoceramide concentrate prepared from tobacco leaves (Kaul and Lester, 1978).

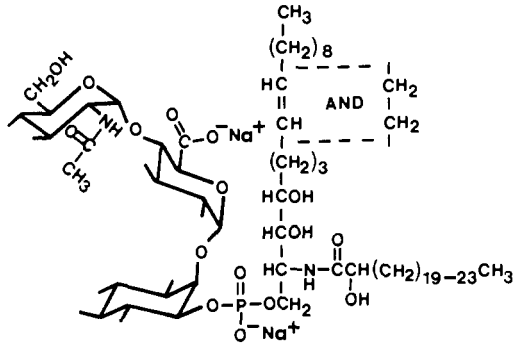

FIGURE 6: Proposed structure of PSL-I: GlcNAcp(α1→4)-GlcUAp(α1→2)myoinositol-1-*O*-phosphoceramide.

TABLE I: Sensitivity of Carbohydrate Residues in Acetylated Trisaccharide from Carboxyl-Reduced PSL-I to CrO_3 Oxidation.

assay	% hexosamine recov after CrO_3 ox.	Glc before	Glc after	GlcNAc before	GlcNAc after
colorimetry	96.3				
GLC[a]		0.82	0.83	0.84	0.78

[a] Based on mol of glucose of *N*-acetylglucosamine per mol of myoinositol detected.

It is interesting that in both PSL-I and the reported structure for phytoglycolipid the *N*-acetylglucosamine residue is in the α anomeric configuration linked to the C-4 position of the glucuronic acid. This situation is unlike that found in glycosphingolipids from animal cells where *N*-acetylglucosamine is almost always in the β configuration. However, heparin has a similar structure in its repeating oligosaccharide units of sulfated [GlcNH$_2$(α1→4)GlcUA(α1→4)].

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Use of Fluorescence Polarization to Monitor Intracellular Membrane Changes during Temperature Acclimation. Correlation with Lipid Compositional and Ultrastructural Changes†

Charles E. Martin‡,* and Guy A. Thompson, Jr.

ABSTRACT: Fluorescence polarization of 1,6-diphenylhexatriene (DPH) was used to study the effects of temperature acclimation on *Tetrahymena* membranes. The physical properties of membrane lipids were found to be highly dependent on cellular growth temperature. DPH polarization in lipids from three different membrane fractions correlated well with earlier freeze-fracture and electron spin resonance observations showing that membrane fluidity progressively decreases in the order microsomes > pellicles > cilia throughout a wide range of growth temperatures. Changes in membrane lipid fluidity following a shift from high to low growth temperatures proceed rapidly in the microsomes, whereas there is a pronounced lag in the changes of peripheral cell membrane lipids. These data support previous observations that adaptive changes in membrane fluidity proceed via lipid modifications in the endoplasmic reticulum, followed by dissemination of lipid components to other cell membranes. The rapid changes in polarization observed in the microsomal lipids following a temperature shift correspond closely with the time-dependent alterations in both lipid fatty acid composition and freeze-fracture patterns of membrane particle distribution, suggesting that, in the endoplasmic reticulum, lipid phase separation is the primary cause of membrane particle rearrangements.

Biological membranes are formed through complex interactions between lipids and proteins. Such associations allow for the existence of stable, highly ordered structures while permitting remarkable mobility of their individual molecular components (Singer & Nicolson, 1972; Edidin, 1974). Because the unique physical characteristics of membrane lipids account in large part for the observed fluid properties of cellular membranes, a great deal of effort has recently been applied to the study of lipid contributions to cell structure and function. A persuasive body of evidence now indicates that the function of many membrane-bound enzymes is dependent upon specific associations with lipids (Finean, 1973) and is strongly dependent upon the physical state of the surrounding lipid environment (Singer, 1974; Vik & Capaldi, 1977).

Where highly purified membranes have been studied, the lipids have been found to consist of heterogeneous mixtures of many molecular species. In fact, each functionally distinct membrane in eukaryotic cells appears to be maintained with its own specific lipid composition (for reviews, see Ansell et al., 1973). The physical properties of artificial membranes composed of single phospholipid molecular species have been determined by a number of corroborating physical techniques

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