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PARTIAL CHARACTERIZATION OF THE HIGHLY COMPLEX FUCOLIPIDS FROM GASTRIC MUCOSA

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SUMMARY - Six highly complex fucolipids containing 18, 20-21, 24, 28, 32, and 35-36 sugar residues, respectively, have been isolated from hog gastric mucosa. All six compounds exhibited blood-group (A+H) activities, and were different from each other with respect to the number of fucose, galactose, N-acetylglucosamine and N-acetylgalactosamine residues. Based on the results of chemical, immunological and enzymatic analyses, we suggest that the carbohydrate chains of these glycolipids are highly branched. The branches, number of which is proportional to the degree of molecular complexity, are terminated by GalNAcα1→3(Fucα1→2)Gal, Fucα1→2Gal, Galβ→GlcNAc and βGlcNAc.

INTRODUCTION - Blood-group active glycosphingolipids of erythrocyte membrane and glandular epithelial tissue of different animal species show a considerable degree of heterogeneity with respect to sugar composition and the length of carbohydrate chains (1-5). The simple glycosphingolipids are readily extractable with chloroform/methanol, whereas those containing longer oligosaccharide chains exhibit considerable solubility in the aqueous systems (6-8). This property was considered in the development of new procedures for the isolation of complex glycosphingolipids from erythrocyte membrane and gastric mucosa (6,7,9,10). Among the procedures successfully applied for the isolation of these compounds are extractions with aqueous butanol (6,7,9), and with sodium acetate in methanol/chloroform/water (10). By employing butanol extraction, Koscielak et al (9) isolated from erythrocyte membrane a series of complex glycosphingolipids containing 22-60 sugar residues. Two glycosphingolipids with 12 and 18 sugar residues were obtained by this technique from gastric mucosa (7).

Application of the sodium acetate extraction procedure (10) to hog gastric mucosa resulted in the isolation of several complex glycosphingolipids (11,12). The structures of the four simplest compounds of this series have

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been elucidated recently (12). Here, we present structural and immunological studies on six more complex glycosphingolipids, the carbohydrate portions of which consist of 18-36 sugar residues.

MATERIALS AND METHODS - Frozen hog stomachs used for mucosa preparation were purchased from Pel-Freez (Rogers, AR). Human red cells ABO types, human blood grouping anti-A and anti-B serum, and anti-H lectin (Ulex europeus agglutin I) were obtained from Biol. Corp. Am. (Port Reading, NJ). Enzymes, α-galactosidase, β-galactosidase and β-N-acetylhexosaminidase, were donated by Drs. Y.T. Li and S.C. Li (Tulane Univ., LA). Silicic acid (100-200 mesh) was from Bio-Rad (Richmond, CA), DEAE-Sephadex A-25 from Pharmacia (Piscataway, NJ) and silica gel HR plates from Analtech (Newark, DE).

The acetone-dried mucosal scrapings were homogenized with 10 volumes of chloroform/methanol (2/1), filtered through a sintered glass funnel and the filtrate was discarded. The residue was then extracted with 0.4 sodium acetate in methanol/chloroform/water (60/30/8), as detailed in (10). The filtrate was dried, treated with methanolic NaOH, dialyzed extensively against ice-cold water and lyophilized (11). The lyophilizate was dissolved in a small volume of methanol/chloroform/water (60/30/8) and chromatographed on a DEAE-Sephadex column (12). The glycolipids eluted from the column in neutral lipid fraction were dried, acetylated with acetic anydride/pyridine (2/3), and fractionated on a silicic acid column into four fractions (12). The final purification of the acetylated fucolipids, eluted from the column with acetone/methanol (1/1) and methanol/chloroform/water (90/10/2) was accomplished by preparative thin-layer chromatography in chloroform/methanol/water (70/25/2), chloroform/methanol/water (60/40/10) and chloroform/methanol/2N NH_4OH (40/15/1.5).

Hemagglutination and hemagglutination-inhibition assays were performed with the Takatsy microtitractor as described in (12). Prior to assay, the fucolipids were deacylated with sodium methoxide (13) and dialyzed. The anti-A and anti-B sera were diluted to 4 units; the anti-H was commercially available at a potency of 2 units.

Removal of fucose from the isolated fucolipids was accomplished by hydrolysis in 0.1 M trichloroacetic acid at 100°C for 2 h (14). Enzymatic hydrolysis of saccharide chains in the native and defucosylated glycolipids was performed by incubating the substrates at 37°C for 24-36 h with α-galactosidase, β-galactosidase and β-N-acetylhexosaminidase in 0.05 M sodium citrate buffer pH 4.0 (15). Following each enzymatic treatment, 3 volumes of chloroform was added and the reaction mixtures were dialyzed. Dialyzates were dried and analyzed for the released monosaccharides (12).

Methyl esters of fatty acids and methyl glycosides were obtained by methanolysis of the glycolipids in 1.2 M methanolic HCl (12), and the alditol acetate derivatives of monosaccharides by the procedure in (16). Gas-liquid chromatography analyses of fatty acid methyl esters and trimethylsilyl derivatives of methyl glycosides were performed on the columns packed with 3% SE-30 on chromosorb, W, AW, DMCS (12). Alditol acetates were analyzed on 1% ECNSS-M columns (14). The long-chain bases were determined colorimetrically (17). The orcinol reagent and iodine vapors were used for thin-layer chromatographic visualization of glycolipids.

RESULTS - Column chromatography of the acetylated glycolipids, contained in the neutral lipid fraction from DEAE-Sephadex, on silicic acid gave four

Table I Molar ratios of sphingosine and carbohydrates in the isolated fucolipids.

Fucolipid	Molar ratios[a]					
	Fuc	Gal	Glc	GlcNAc	GalNAc	Sphingosine
I	2.01	7.86	1.0	5.92	1.05	1.0
II	2.84	7.50	1.0	6.77	1.78	0.9
III	2.90	9.81	1.0	7.85	2.02	0.8
IV	3.81	9.77	1.0	9.68	3.12	0.8
V	3.85	11.78	1.0	11.89	2.79	0.9
VI	4.63	12.40	1.0	13.60	3.04	0.7

[a]Relative to Glc=1

fractions. The fractions eluted with 1,2-dichloroethane/acetone (1/1) and acetone contained fucolipids with 12-14 sugar residues. These were characterized previously (11,12). The carbohydrate and sphingosine composition of the major fucolipids purified from the acetone/methanol and methanol/chloroform/water fractions are given in Table I. The fucolipids I-III were present in the acetone/methanol fraction, whereas methanol/chloroform/water eluate contained fucolipids IV-VI. Thin-layer chromatograms of the purified fucolipids are shown in Fig. 1. The yield of isolated fucolipids I,II,III,IV,V and VI per 100 g of dry mucosa was 0.70, 0.91, 0.62, 0.54, 0.71 and 0.6 mg, respectively.

In hemagglutination-inhibition assays all six fucolipids were potent inhibitors of agglutination of human group A-cells by anti-A serum (1.5-3.1 μg/0.1 ml) and human O-cells by anti-H lectin (2.1-4.5 μg/0.1 ml). None of the fucolipids inhibited agglutination of human group-B red cells by anti-B serum. These results indicate that carbohydrate chain of each fucolipid bears two types of blood-group determinants, A and H.

Analyses of carbohydrate components revealed in each fucolipid the presence of fucose, galactose, glucose, N-acetylglucosamine and N-acetylgalacto-

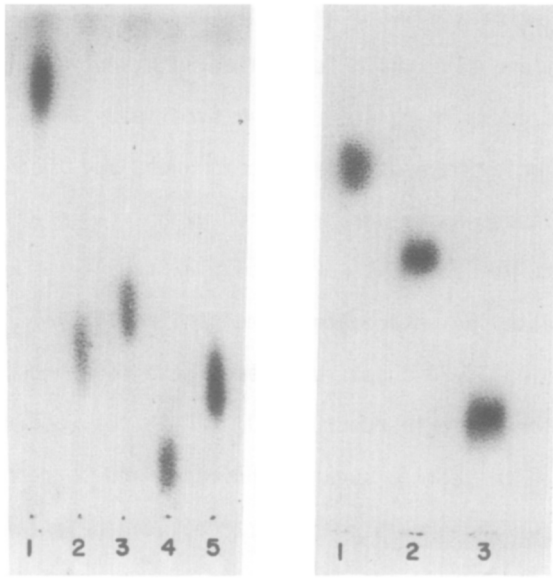

Fig. 1. Thin-layer chromatograms of the acetylated highly complex fucolipids from hog gastric mucosa.
Left plate, developed in chloroform/methanol/2 M NH_4OH (40/15/1.5), 1, ceramide dodekahexoside (refr. 12); 2, fucolipid II; 3, fucolipid I; 4, fucolipid IV; 5, fucolipid III. Right plate, developed in chloroform/methanol/water (60/40/10). 1, fucolipid IV; 2, fucolipid V; 3, fucolipid VI. Visualization: orcinol reagent.

samine. The results presented in Table I indicate that the oligosaccharide chains of fucolipids I-VI contained 18, 20-21, 24, 28, 32 and 35-36 sugar residues, respectively. The native and defucosylated fucolipids I-V were resistant to the action of α-galactosidase and β-N-acetylhexosaminidase. The intact fucolipid VI was resistant to the action of α-galactosidase, but one N-acetylglucosamine residue was lost when treated with β-N-acetylhexosaminidase. Treatment of the native fucolipids I-VI with β-galactosidase resulted in the loss of two galactose residues in fucolipids I,III,IV and VI, three in fucolipid V and one in fucolipid II. Incubation of the defucosylated glycolipids with β-galactosidase resulted in the release of two galactose residues from glycolipid II, three from glycolipids I,III and IV, and four from glycolipids V and VI. These data indicate that the saccharide chains of the isolated fucolipids are highly branched. The branches are terminated by

blood-group A and H determinants, and galactose. In addition, the fucolipid VI contains at least one side chain terminated by N-acetylglucosamine.

Analyses of the ceramide portion of the fucolipids revealed that sphingenine was the major base of all six compounds, and that hexadecanoate, octadecanoate and octadecenoate were the predominant fatty acids.

DISCUSSION - The data presented show that complex fucolipids extractable from hog gastric mucosa with sodium acetate in methanol/chloroform/water consist of compounds containing 12-36 sugar residues. These compounds, like complex fucolipids of human erythrocyte membrane (9), exhibit a considerable degree of heterogeneity with respect to sugar composition and degree of branching. The number of branches appears to be proportional to the extent of molecular complexity of the fucolipid. The simpler compounds of this series, structures of which were elucidated recently (11,12), contain side chains terminated by one blood-group A and one blood-group H determinants. Two of the more complex fucolipids, in addition to the blood-group A and H determinants, also contained a single side chain terminated by galactose (12). The results of structural analyses of the fucolipids described here indicate that the saccharide chains of these compounds are even more complex.

Based on the results of carbohydrate analysis, immunological assays and the susceptibility of the native and defucosylated glycosphingolipids to the action of specific exoglycosidases, we suggest that the carbohydrate chain of fucolipid I contains four branches, two terminated by β-galactose, one by blood-group A (GalNAcα1→3[Fucα1→2]Gal-) antigenic determinant and one by blood-group H (Fucα1→2Gal-) determinant; fucolipid II contains two branches terminated by blood-group A determinant, one by H determinant and one by β-galactose; fucolipid III contains two branches terminated by A antigenic determinant, one by H determinant and two by β-galactose; fucolipid IV contains three branches terminated by A determinant, one by H and two by β-galactose; fucolipid V contains three branches terminated by A determinant, one by H determinant and three by β-galactose; and that the fucolipid VI contains three

branches terminated by A determinant, two by H determinant, two by β-galactose and one by β-N-acetylglucosamine.

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