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STRUCTURE OF A NOVEL SULFATED SIALOGLYCOSPHINGOLIPID FROM BOVINE GASTRIC MUCOSA

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SUMMARY - A novel sulfated sialoglycosphingolipid was isolated from the lipid extract of bovine gastric mucosa in a yield of 6.15 μM per kg of wet tissue. Based on the results of partial acid hydrolysis, sequential degradation with specific glycosidases, periodate oxidation, and methylation analysis of the native and partially degraded compound, the structure of this glycolipid is proposed to be: SO_3H→8Siaα2→8Siaα2→3Galβ1→4Glc→ceramide. The sialic acid of this glycolipid consisted of N-acetyl and N-glycolylneuraminic acids, with the former being predominant component (65%) of the mixture.

INTRODUCTION - Sialoglycosphingolipids of animal tissues display considerable heterogeneity with respect to sugar composition, length of oligosaccharide chains, degree of sialylation and the type of sialosyl residues (1,2). The sialosyl residues identified in these compounds are N-acetyl and N-glycolyl derivatives of neuraminic acid. The occurrence of N-acetyl and N-glycolylneuraminic acids appears to reflect both organ and species specificity (3). N-Acetylneuraminic acid is the only sialic acid occurring in the glycolipids of human tissues, equine erythrocytes contain N-glycolylneuraminic acid, whereas sialoglycosphingolipids of bovine tissues contain both N-acetyl and N-glycolylneuraminic acids (3-5).

Some of the sialoglycolipids in addition to N-substituted neuraminic acid also contain O-acetylated forms of N-acetyl and N-glycolylneuraminic acids. 4-O-Acetyl-N-glycolylneuraminic acid has been found in the hematoside of equine erythrocytes (6), and 9-O-acetyl-N-acetylneuraminic acid in the sialoglycosphingolipids from the brain of mouse (7). In this report, we describe the isolation and structural characterization of the disialyllactosylceramide containing 8-sulfate-sialosyl residue.

MATERIALS AND METHODS - Frozen bovine stomachs used for mucosa preparation were obtained from Pel-Freez (Rogers, AR). Enzymes, α-galactosidase and β-galacto-

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sidase were kindly donated by Drs S.C.Li and Y.T.Li (Tulane University, LA). The Clostridium perfringens neuraminidase was purchased from Miles (Elkhart, IN). Silicic acid (100-200 mesh) was from Bio-Rad (Richmond, CA), DEAE-Sephadex A-25 from Pharmacia (Piscataway, NJ), and silica gel HR and HL plates from Analtech (Newark, DE). Standard fatty acid methyl esters and long-chain bases were purchased from Applied Science (State College, PA). Methyl ether derivatives of glucose, galactose and N-acetylneuraminic acid were from the same source as reported previously (8,9).

Extraction of lipids from gastric mucosa scrapings was performed by the sodium acetate procedure as described previously (10). The lipid extract was subjected to alkaline methanolysis (10), dialyzed and lyophilized. The lyophilizate was dissolved in a small volume of methanol/chloroform/water (60/30/8) and chromatographed on a DEAE-Sephadex column (10). The crude acidic glycolipid fraction eluted from the column with 0.4 M sodium acetate in methanol/chloroform/water (60/30/8) was dialyzed, lyophilized, dissolved in chloroform and chromatographed on a silicic acid column into three fractions (11). Further purification of the sulfated sialoglycolipid, eluted from the column with chloroform/methanol (3/2), was accomplished by preparative thin-layer chromatography in chloroform/methanol/water (65/35/8), chloroform/methanol/water (65/25/4) and chloroform/methanol/NH_4OH/water (60/35/1/7).

Methyl esters of fatty acids and methyl glycosides were obtained by methanolysis of the glycolipid in 1.2 M methanolic HCl (10). For long-chain bases the glycolipid was hydrolyzed in 1.0 M HCl in aqueous methanol (11). Gas-liquid chromatography analyses of fatty acid methyl esters and trimethylsilyl derivatives of methyl glycosides, and long-chain bases were performed on the columns packed with 3% SE-30 on Gas Chrom Q (10). Identification of N-acetyl and N-glycolylneuraminic acids was performed according to Yu and Ledeen (12). Purified glycolipid was assayed for sulfatide and sulfate by the procedures in (13,14). The thin-layer chromatographic visualization of glycolipid was performed with orcinol, rhodizonate and resorcinol reagents (11).

Solvolysis of the isolated glycolipid was performed with 0.05 M HCl in dry methanol at room temperature for 4 h and the resulting desulfated compound was separated by thin-layer chromatography in chloroform/methanol/water (65/25/4).The neutral derivative of the glycolipid was prepared by hydrolysis in 1 M formic acid at 100°C for 1.5 h (15). Neuraminidase treatment of the native and desulfated glycolipid was performed according to Rauvala (16). The sialic acid-free glycolipid was subjected to enzymatic hydrolysis with α- and β-galactosidases according to the procedure reported previously (10). Periodate oxidation of the native and desulfated glycolipid was performed with 0.015 M sodium metaperiodate at room temperature for 24 h in the dark (10). Following reduction and methanolysis, the products were analyzed by gas-liquid chromatography for carbohydrates (10).

Methylation of the native, desulfated and desialyzed glycolipid was performed with iodomethane in the presence of methylsulfinyl carbanion, in dimethylsulfoxide (17). The permethylated glycolipids were recovered from the methylation mixture by extraction with chloroform and purified by thin-layer chromatography (10). Following hydrolysis, reduction, and acetylation (18) the mixtures of partially methylated sugar alcohols were analyzed by gas-liquid chromatography on a 1% ECNSS-M columns (9,10). For the analysis of methylated neuraminic acid, the permethylated native and desulfated glycolipid was subjected to acid methanolysis in 0.5 M methanolic HCl at 85°C for 24 h, re-N-acetylated, derivatized with silylating reagent and analyzed by gas-liquid chromatography on a 3% SE-30 columns (8).

RESULTS - Extraction of lipids from bovine gastric mucosa with sodium acetate in methanol/chloroform/water, followed by alkaline methanolysis and column fractionation on DEAE-Sephadex and silicic acid afforded 77.1 μM of sulfato-

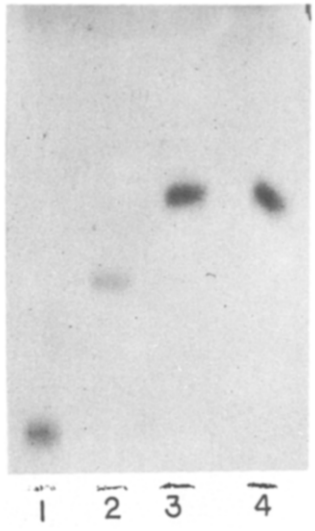

Fig.1. Thin-layer chromatogram of the native (1), desulfated (2), and formic acid desialyzed (3) sulfated sialoglycosphingolipid purified from bovine gastric mucosa. (4) Lactosylceramide standard. Conditions: Silica gel HL, 250 nm, developed in chloroform/methanol/water (65/25/4). Visualization: orcinol reagent.

glycosphingolipids per kg of wet tissue. Of this 69% was represented by monohexose sulfatide, 18.5% by dihexose sulfatide and about 8% by the investigated glycolipid. This compound, unlike mono- and dihexose sulfatides,reacted positively on thin-layer plates with resorcinol reagent and contained 1.01 mol of sulfate/1 mol of glucose. The glycolipid reacted also as sulfolipid in the assay procedure of Kean (13) and was susceptible to desulfation by acid solvolysis. The glycolipid product of solvolysis exhibited enhanced migration on thin-layer chromatography (Fig 1) and did not contain sulfate.

The results of sugar analysis established the presence of glucose, galactose and sialic acid in the molar ratio of 1:1:2 (Table I). Gas liquid chromatography of the sialic acids released from the glycolipid under mild acid methanolysis conditions (12) revealed presence of N-acetylneuraminic acid (65.6%) and N-glycolylneuraminic acid (34.4%). Mild acid hydrolysis in formic acid resulted in the conversion of glycolipid to a sialic acid-free compound (Fig 1) which was identified as a lactosylceramide. These results indicate

Table I. The composition and molar ratios of carbohydrates in the native, desulfated and enzyme-degraded glycolipid.

Glycolipid	Molar ratios			
	Gal	Glc	Sia	SO_4^{2-}
Native	1.03	1.0	1.94	1.01
Desulfated	1.01	1.0	1.89	
Desulfated treated with neuraminidase	0.98	1.0		
Desialyzed treated with β-galactosidase		1.0		

that the native glycolipid contained lactosyl chain to which two residues of sialic acid and one residue of sulfate were attached.

The intact glycolipid was completely resistant to the action of neuraminidase, regardless of the presence or absence of sodium cholate in the incubation mixtures. The desulfated glycolipid, however, was readily converted (24 h of incubation) to a dihexosylceramide even in the absence of cholate. The ceramide dihexoside was further degraded to glucosylceramide by β-galactosidase. The results presented above indicate that the desulfated glycolipid has the structure: Siaα→Sia α→Galβ→Glc→ceramide. Resistance of the native glycolipid to degradation by neuraminidase suggest that the intact compound contained sulfate ester group situated at the terminal sialic acid residue.

The point of attachment of the sulfate ester group to sialic acid as well as the type of linkages between the sugars in the studied glycolipid were elucidated by periodate oxidation and methylation analysis. The sialic acid in the native glycolipid was resistant to periodate, whereas in the desulfated compound 56% of it was destroyed. The permethylated native and desulfated glycolipid after hydrolysis, reduction and acetylation gave, on gas-liquid chromatography peaks corresponding to the acetates of 2,4,6-tri-O-methylgalactitol and 2,3,6-tri-O-methylglucitol. The alditol acetates of 2,3,4,6-tetra-O-methylgalactitol and 2,3,6-tri-O-methylglucitol were found in the hydrolyzates of

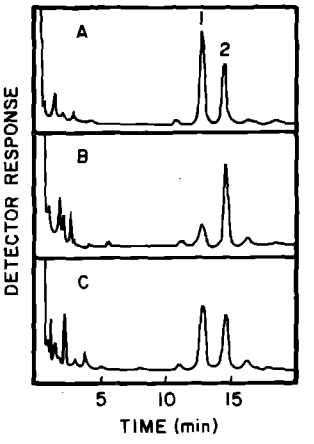

Fig.2. Gas-liquid chromatograms of the trimethylsilyl derivatives of partially methylated methyl ester of N-acetylneuraminic acid found in the acid methanolysates of permethylated GT1b ganglioside (A), and in the permethylated native (B) and desulfated (C) glycolipid. Peak 1, 4,7,8,9-tetra-O-methyl derivative of the methyl glycoside methyl ester of N-acetylneuraminic acid; peak 2, 8-O-trimethylsilyl-4,7,9-tri-O-methyl derivative of the methyl glycoside methyl ester of N-acetylneuraminic acid. Conditions: 3% SE-30 columns, temperature programmed at 1°C/min from 210-230°C.

permethylated desialyzed glycolipid. Gas-liquid chromatograms of the partially methylated sialic acid derivatives obtained from the permethylated native and desulfated glycolipid are shown in Fig 2. Only one peak, coinciding with 8-O-trimethylsilyl-4,7,9-tri-O-methyl derivative of the methyl glycoside methyl ester of N-acetylneuraminic acid was found among the permethylation products of the intact glycolipid. The permethylated desulfated glycolipid gave two neuraminic acid derivatives which corresponded in their retention times to that of 4,7,8,9-tetra-O-methyl and 4,7,9-tri-O-methyl methyl glycoside methyl esters of N-acetylneuraminic acid. These results clearly indicate that terminal residue of sialic acid in the intact glycolipid is substituted at C-8 by sulfate ester group and that subterminal sialic acid unit is attached to C-3 of galactose.

The fatty acid and long-chain base composition of the studied glycolipid is given in Table II. Docosenoate and tetracosenoate were the principal fatty acids, whereas sphingosine accounted for 86% of the total bases present in this compound.

Table II. Fatty acid and long-chain base composition of the isolated sulfated sialoglycosphingolipid.

Fatty acid	Amount	Long-chain base	Amount
	% total		% total
16:0	13.9		
18:0	13.6	Dihydrosphingosine	5.2
18:1	6.7	Sphingosine	85.6
18:0 (^{2}OH)	3.9	Phytosphingosine	7.8
22:1	21.4	Unidentified	1.4
22:0 (^{2}OH)	9.9		
24:0	5.1		
24:1	16.3		
Unidentified	9.2		

In the abbreviations for fatty acids, the number before the colon denotes the number of carbon atoms, the number following the colon is the number of double bonds and ^{2}OH refers to 2-hydroxy fatty acids.

DISCUSSION - A novel sulfated glycosphingolipid containing sialic acid has been isolated from the lipid extract of bovine gastric mucosa. The results of chemical and enzymatic analyzes strongly suggest that this compound is a sulfated disialyllactosylceramide with the following structure: SO_3H→8Siaα2→8Siaα2→3Galβ1→4Glc→ceramide.

This glycolipid differs from the currently known sulfatoglycosphingolipids of gastric mucosa with respect to sugar composition and the site of sulfation (10,11,18). In the sulfatoglycosphingolipids described to date, the sulfate ester group is situated at C-3 of the galactose or C-6 of the N-acetylglucosamine and the carbohydrate portion of these glycolipids consist of glucose, galactose and N-acetylglucosamine. Recently, a sulfated trihexoside containing N-acetylgalactosamine was also described (19). Although the presence of sulfated sialoglycosphingolipid composed of sulfated sialic acid and glucosyl-

ceramide was reported in sea urchin, there has been no reports on the occurrence of sulfated sialoglycosphingolipids in mammalian tissues.

The occurrence of sialoglycosphingolipids containing O-substituted sialic acids in the tissues of higher animals is well documented (3,6,7). The O-acetylated sialic acids in these glycolipids, like sulfated sialic acid in our compound, are considerably less sensitive to the action of neuraminidase. This property may be of great physiological importance for the function of the sialosyl groups in the cell membranes.

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