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STRUCTURE OF THE CERAMIDE OCTADEKAHEXOSIDE ISOLATED FROM GASTRIC MUCOSA

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SUMMARY - A fucose-containing ceramide octadekahexoside exhibiting blood-group (A+H) activity has been isolated from hog gastric mucosa. Based on the results of partial acid hydrolysis, sequential degradation with specific glycosidases, oxidation with periodate and chromium trioxide, and permethylation analysis, we propose that the carbohydrate chain of this fucolipid contains four branches. Two of the branches are terminated by βGal1→4βGlcNAc, one by αFuc1→2βGal1→3/4β GlcNAc and one by αGalNAc1→3(αFuc1→2)βGal1→3/4βGlcNAc.

INTRODUCTION - Systematic investigations into the nature of ABH antigens of human erythrocyte membrane and hog gastric mucosa have led in recent years to isolation of blood-group specific fucolipids containing highly complex carbohydrate chains (1-5). In erythrocyte membrane, these compounds contain up to 60 sugar residues and exhibit high degree of branching (1,3).

In the previous report (6), we have described some of the chemical and immunological characteristics of the six complex fucolipids isolated from gastric mucosa. The carbohydrate portion of these glycosphingolipids consisted of 18-36 sugar residues. Here, we present the structural characterization of the simplest compound of this series, the carbohydrate portion of which consists of eighteen sugar residues.

MATERIALS AND METHODS - Complex fucolipid I (ceramide octadekahexoside), used in this study, was prepared previously (6). Enzymes, α-galactosidase, β-galactosidase and β-N-acetylhexosaminidase, were kindly donated by Drs. Y.T. and S.C. Li (Tulane Univ.,LA). Human red cells ABO types, human blood grouping anti-A and anti-B serum, and anti-H lectin (Ulex europeus agglutinin I) were obtained from Biol. Corp. Am. (Port Reading, NJ). Standard fatty acid methyl esters, sphingosine and sphingenine were purchased from Applied Science (State College, PA). Methyl ethers of neutral and amino sugars were from the same source as reported previously (7,8). All other reagents were of commercial origin.

Methyl esters of fatty acids and methyl glycosides were obtained by methanolysis of the glycolipids in 1.2 M methanolic HCl at 85°C for 24h (7), and the alditol acetate derivatives of monosaccharides according to the procedure in (9). Gas-liquid chromatography analyses of the trimethylsilyl derivatives of methyl glycosides and long chain bases, and fatty acid methyl esters were performed on the columns packed with 3% SE-30, and the alditol acetates

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on 1% ECNSS-M columns (7). Hemagglutination and hemagglutination inhibition assays were performed as described in (10). Chromium trioxide oxidation of the acetylated fucolipid was performed as detailed in (7). The oxidized samples were subjected to acid methanolysis and analyzed for methyl glycosides. For the analysis of carbohydrate composition of the original acetylation mixture, CrO_3 Oxidation step was omitted.

Removal of fucose from the native and enzyme degraded fucolipid was accomplished by hydrolysis in 0.1 M trichloroacetic acid as in (6). The native and enzyme degraded fucolipid was also partially degraded by acid methanolysis (10). Enzymatic hydrolysis of saccharide chain in the native and defucosylated glycolipid was performed by incubating the substrates (0.4-0.6 mg) at 37°C for 36h with 0.5-1 unit of α-galactosidase, β-galactosidase and β-N-acetylhexosaminidase in 0.05 M sodium citrate buffer pH 4.0 (11). Following each enzymatic treatment, 3 vol. of chloroform was added and the reaction mixtures were dialyzed. Dialyzates were dried and analyzed for the released monosaccharides (7). The glycolipid contained in the dialysis bag was dried, an aliquot taken for carbohydrate analysis and the remaining portion was used for further enzymatic treatments or permethylation analysis.

Periodate oxidation of the fucolipid was performed with 0.015 M sodium metaperiodate in methanol/water (4). Following reduction and destruction of excess borohydride with diluted acetic acid, the reaction mixture was dialyzed and subjected to completion of Smith degradation (4).

Methylation of the native and enzyme degraded fucolipid was performed with iodomethane in the presence of methylsulfinyl carbanion, in dimethylsulfoxide (12). The permethylated glycolipids recovered from the methylation mixture by extraction with chloroform were dried and purified by thin layer chromatography (7). Following hydrolysis, reduction and acetylation the mixture of partially methylated sugar alcohols were analyzed by gas-liquid chromatography (7,8).

RESULTS - The results of sugar analysis (Table I) and immunological assays established that the studied fucolipid is a ceramide octadekahexoside, carbohydrate portion of which bears two blood-group determinants, A and H. The extent of A activity of fucolipid against 4 unit of anti-A serum was 1.8 μg/0.1 ml and H activity, against 2 unit of anti-H lectin, 4.2 μg/0.1 ml. Analyses of the ceramide portion of the fucolipid revealed that sphingenine constitute over 95% of total bases present in this compound, whereas octadecanoate, hexadecanoate, octadecenoate and eicosanoate account for 85% of its total fatty acids.

Partial acid methanolysis of the native fucolipid resulted in the formation of Gal→Gal→Glc→ceramide, Gal→Glc→ceramide and Glc→ceramide. However, when fucolipid was first digested with the mixture of β-galactosidase, β-N-acetylhexosaminidase and then subjected to partial acid degradation, the larger fragments were identified. These were GlcNAc→Gal→Gal→Glc→ceramide and Gal→GlcNAc →Gal→Gal→Glc→ceramide. These results suggest that the sequential arrangement

Table I. Effect of serial periodate oxidation on the monosaccharide composition of fucolipid 1.

Fucolipid	Molar ratios				
	Fuc	Gal	Glc	GlcNAc	GalNAc
Native	2.01	7.86	1.0	5.92	1.05
Initial oxidation	-	4.07	1.0	5.86	-
First Smith degradation	-	-	1.0	-	-

of the sugar units in the saccharide chain adjacent to the ceramide core in enzyme degraded fucolipid is Gal→GlcNAc→Gal→Gal→Glc→ceramide. Isolation of Gal →Gal→Glc→ceramide (largest fragment) from the native fucolipid indicates that intact fucolipid contained side chain (composed of Gal and GlcNAc) attached to the galactose adjacent to lactosylceramide.

Oxidation of fucolipid with periodate (Table I) resulted in the loss of fucose, N-acetylgalactosamine and four out of eight galactose residues. Analysis of glycolipid fragment recovered after one complete step of Smith degradation revealed only the presence of Glc→ceramide. This indicates that the galactose residue adjacent to glucosylceramide was susceptible to periodate.

The results of partial enzymatic degradation of saccharide portion of the native and defucosylated glycolipid are given in Table II. Treatment with β-galactosidase resulted in the loss of two galactose from native compound and three galactose residues from defucosylated glycolipid. Two N-acetylglucosamine residues were lost from native compound when the treatment with β-galactosidase was followed by β-N-acetylhexosaminidase. Incubation of the intact and defucosylated glycolipid with the mixture of β-galactosidase, β-N-acetylhexosaminidase resulted in the removal of three galactose and three N-acetylglucosamine residues from intact fucolipid, and four galactose and four N-acetylglucosamine residues from defucosylated compound. The above results suggest that studied fucolipid contained two βGal→βGlcNAc branches attached to βGal→βGlcNAc at the glycolipid core and one side chain, βGal→βGlcNAc, which was substituted at the galactose by fucose.

Table II. Partial degradation of the native and defucosylated glycolipid with specific glycosidases.

Reaction number	Substrate	Enzyme	Carbohydrate in the isolated product				
			Fuc	Gal	Glc	GlcNAc	GalNAc
			mol/mol				
1.	Native	none	2.01	7.86	1.0	5.92	1.05
2.	Defuco-sylated	none	-	7.90	1.0	5.88	1.02
3.	Native or de-fucosylated	α-galactosidase, β-N-acetylhexos-aminidase	no reaction				
4.	Native	β-galactosidase	1.91	6.05	1.0	5.90	1.02
5.	Defuco-sylated	β-galactosidase	-	5.10	1.0	5.87	1.04
6.	Glycolipid product of reaction 4	β-N-acetylhexos-aminidase	1.94	6.08	1.0	4.03	1.00
7.	Native	β-galactosidase, β-N-acetylhexos-aminidase	1.96	5.06	1.0	3.12	1.02
8.	Defuco-sylated	β-galactosidase, β-N-acetylhexos-aminidase	-	3.91	1.0	2.10	0.98

The molar ratios of partially methylated alditol acetates found in the hydrolysates of permethylated native and enzyme degraded fucolipid are given in Table III. Obtained data indicate that native fucolipid contained two residues of fucose, two galactose and one N-acetylgalactosamine at non-reducing termini. Analysis of permethylated enzyme degraded fucolipid revealed presence of one terminal residue of N-acetylgalactosamine and two residues of fucose. Since studied fucolipid exhibited blood-group (A+H) activity, the above data suggest that one fucose residue is linked to C-2 of galactose in the chain bearing H determinant, and one to penultimate galactose in the chain bearing A determinant. The presence of three residues of 2,4-di-O-methylgalactitol in the hydrolyzates of permethylated native fucolipid and only one in the hydrolyzates of permethylated enzyme degraded fucolipid indicate that one 1→3/6 Gal structure is involved in branching point of chains bearing A and H determinants and two in branching points of side chains bearing βGal→βGlcNAc termini. Decrease in the ratio of (C-4)/(C-3)-substituted N-acetylglucosamine in enzyme degraded fucolipid as compared to that of native compound indicate that (C-3)-

Table III. Molar ratios of the alditol acetates found in the hydrolyzates of permethylated native and enzyme degraded fucolipid.

Methylated sugar	Molar ratios[a]	
	Native	Enzyme degraded[b]
2,3,4-Tri-O-methylfucositol	1.8	1.7
2,3,4,6-Tetra-O-methylgalactitol	2.3	-
2,4,6-Tri-O-methylgalactitol	-	0.9
2,3,6-Tri-O-methylgalactitol	0.8	0.8
3,4,6-Tri-O-methylgalactitol	0.9	0.7
2,3,6-Tri-O-methylglucitol	1.0	1.0
4,6-Di-O-methylgalactitol	0.7	0.7
2,4-Di-O-methylgalactitol	2.6	0.8
3,4,6-Tri-O-methyl-N-methylacetamidogalactitol	0.9	1.1
3,6-Di-O-methyl-N-methylacetamidoglucitol	5.1	2.0
4,6-Di-O-methyl-N-methylacetamidoglucitol	0.5	0.6

[a]The ratios are expressed relative to 2,3,6-Tri-O-methylglucitol.

[b]Fucolipid incubated with the mixture of β-galactosidase, β-N-acetylhexosaminidase.

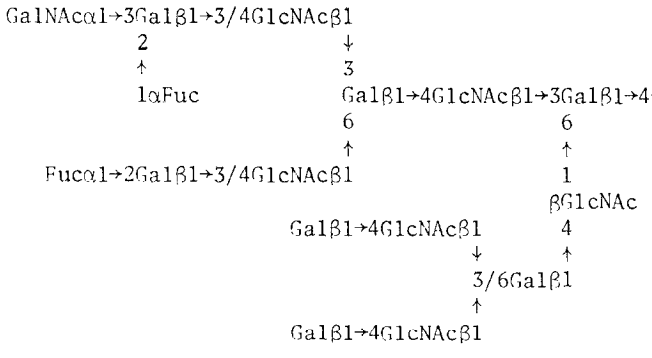
substituted N-acetylglucosamine resides in the branches bearing blood-group determinants (type 1 and type 2 chains).

The anomeric nature of the glycosidic linkages between the sugar residues in intact fucolipid was elucidated with the aid of oxidation with CrO_3 . Over 94% of fucose and 97% of N-acetylgalactosamine were resistant to oxidation, whereas glucose, N-acetylglucosamine and most of the galactose (15% recovery) were destroyed. These data are indicative of α-pyranosidic configuration of fucose and N-acetylgalactosamine residues, and β-pyranosidic configuration of glucose, galactose and N-acetylglucosamine.

DISCUSSION - Although the existence of highly complex fucolipids in erythrocyte membrane and gastric mucosa is well established (1-7), less is known about the structure of carbohydrate chains of this group of compounds. According to some investigators (13, 14), the complex fucolipids contain a long oligosaccharide chain composed of alternating residues of galactose and N-acetylglucosamine with a single symetrical branching at the non-reducing end. Others (1,3-7) suggest that carbohydrate chains of complex fucolipids are highly branched and contain, in addition to alternating residues of galactose and N-acetylglucosamine, di-(N-acetyl)chitobiose sequences. This sequence,

originally found in fucolipids of hog gastric mucosa (4), has also been recently detected in complex glycolipids of human erythrocyte membrane (3).

The data presented here together with previous studies (4,7) suggest the presence of two types of complex fucolipids in gastric mucosa. One type containing di-(N-acetyl)chitobiose and the other in which this sequence is absent. The glycolipid described here belongs to the latter type. The carbohydrate chain of this compound contains four branches. Two of the branches, terminated by βGal1→4βGlcNAc, are linked through 1→3/6βGal1→4βGlcNAc1→6 to the galactose adjacent to glucosylceramide, whereas the galactose adjacent to tetraglycosylceramide core serves as branching point for the chains bearing blood-group A and H determinants. The proposed structure for this fucolipid is shown below.



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REFERENCES

1. Koscielak, J., Miller-Podraza, H., Krauze, R. and Piasek, A. (1976) Eur. J. Biochem. 71, 9-18.
2. Dejter-Juszynski, M., Harpaz, N,, Flowers, H.M. and Sharon, N. (1978) Eur. J. Biochem. 83, 363-373.
3. Zdebska, E. and Koscielak, J. (1978) Eur. J. Biochem. 91, 517-525.
4. Slomiany, B.L. and Slomiany, A. (1977) FEBS Lett. 73, 175-180.
5. Slomiany, A. and Slomiany, B.L. (1978) FEBS Lett. 90, 293-296.
6. Slomiany, B.L., Slomiany, A. and Murty, V.L.N. (1979) Biochem. Biophys. Res. Commun. 88, 1092-1097.
7. Slomiany, B.L. and Slomiany, A. (1978) Eur. J. Biochem. 90, 39-49.
8. Slomiany, B.L. and Slomiany, A. (1978) J. Biol. Chem. 253, 3517-3520.
9. Yang, H. and Hakomori, S.I. (1971) J. Biol. Chem. 246, 1192-1200.
10. Slomiany, A. and Slomiany, B.L. (1977) Chem. Phys. Lipids 20, 57-69.
11. Li, S.C., Mazzota, M.Y., Chien, S.F. and Li, Y.T. (1975) J. Biol. Chem. 250, 6786-6791.
12. Hakomori, S.I. (1964) J. Biochem.(Tokyo) 55, 205-208.
13. Gardas, A. (1976) Eur. J. Biochem. 68, 177-183.
14. Gardas, A. (1976) Eur. J. Biochem.68, 185-191.