Structural Characterization of a Novel Series of Fucolipids from the Marine Annelid, *Pseudopotamilla ocellata**

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Edited by S. Wada, Tokyo Univ. Fisheries, and accepted January 18, 2001 (received for review December 21, 2000)

Abstract : A novel series of fucolipids, provisionally named CPS and CHS in the previous study, was obtained from whole tissues of the marine annelid, *Pseudopotamilla ocellata*. The structures of these fucolipids were determined by compositional analysis, methylation analysis, gas-liquid chromatography, gas chromatograph-mass spectrometry, proton nuclear magnetic resonance spectroscopy, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The structures were shown to be Xyl β 1-4Fuca1-3GlcNAc β 1-3Gal β 1-4Glc β 1-1Cer (CPS₁) and Gal2Me α 1-3Fuca1-3GlcNAc β 1-3Gal β 1-4Glc β 1-1Cer (CPS₂) for CPS, and Xyl β 1-4(Gal2Me α 1-3)Fuca1-3GlcNAc β 1-3Gal β 1-4Glc β 1-1Cer for CHS, respectively. They were structurally related to the previously described ceramide trisaccharide (GlcNAc β 1-3Gal β 1-4Glc β 1-1Cer, Amino-CTH) except that a fucose-containing di- and/or trisaccharides were linked to the *N*-acetylglucosamine residue of the latter lipid. The predominant fatty acids were monoenoic acids, C20:1- and C22:1 acids amounting to about 80% of the total acids, and octadeca-4-sphingene was the sole sphingoid base. The fatty acid and sphingoid base compositions showed nearly the same distribution in these fucolipids.

J. Oleo Sci. **50**, 537-544 (2001).

Key words : ceramide, glycosphingolipid, fucolipid, marine annelida glycolipid (*Pseudopotamilla ocellata*)

1 Introduction

In the previous work (1), the structural investigation of neutral glycolipids of the marine annelid, *Pseudopotamilla ocellata* was performed on ceramide mono-, di- and trisaccharides, which were characterized as Glc β 1-1Cer, Gal β 1-1Cer, Gal α 1-4Gal β 1-1Cer, Gal β 1-4Glc β 1-1Cer, and GlcNAc β 1-3Gal β 1-4Glc β 1-1Cer. In addition, the preliminary results indicated the presence of fucolipids, ceramide penta- (CPS) and

hexasaccharides (CHS) with 2-(*O*-methyl)galactose as a sugar component, in a more complex glycolipid fraction. Their sugar sequences were tentatively assigned as a mixture of Xyl(1-4 or 3)Fuc(1-3)Amino-CTH and Gal2Me(1-3 or 4)Fuc(1-3)Amino-CTH for CPS, and as Gal2Me(Xyl)(1-3 or 1-4)Fuc(1-3)Amino-CTH for CHS, respectively.

In this paper, we describe the clear separation of the mixture corresponding to CPS, and the complete structural determination of CPS and CHS.

2 Experimental

2.1 Materials

The materials used were essentially the same as

* The previous paper : *J. Jpn Oil Chem. Soc. (J. Oleo Sci.)*, **48**, 671-679 (1999)

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described in the previous work (1). Organic solvents were of reagent grade and were freshly distilled before use. Other organic and inorganic reagents of analytical grade or of the highest quality commercially available were used without further purification. 2-(*O*-Methyl)galactose was prepared in our laboratory from 4-*O*-(2-*O*-methyl- β -D-galactopyranosyl)-D-glucopyranose (Gal2Me β 1-4Glc, Sigma Chemical Co.).

2.2 Isolation and Purification of Fucolipids

The basic procedures for the isolation of neutral glycolipids from *P. ocellata* (10 kg, commercial origin) were reported in our previous publication (1). Briefly, after pretreatment by acetone extraction to remove neutral fatty substances, the tissues were extracted at room temperature with chloroform-methanol mixtures. The total lipid extract was treated in 0.5 M methanolic KOH to eliminate glycerolipids and was then acidified, dialyzed and concentrated to near dryness. The residual alkali-stable product was precipitated by addition of acetone (yield : 18.2 g). Precipitates (10 g) were applied to a QAE-Sephadex A-25, OH⁻ form (Pharmacia LKB Biotechnology Inc.) to obtain neutral glycolipids (yield : 6.2 g). The neutral glycolipid fraction was acetylated and then fractionated by Florisil column chromatography (US Silica Co.). The acetylated glycolipid fraction eluted by dichloroethane-acetone was deacetylated with 0.5 M methanolic KOH, dialyzed and lyophilized (yield : 762 mg). The deacetylated product was applied to Iatrobeds 6RS-8060 column chromatography (Iatron Chemical Co.), and was eluted with two consecutive linear gradient systems of chloroform-methanol-water, 80:20:1 ~ 50:50:5 and 55:45:4 ~ 50:60:10 (v/v). Fractions of 5 mL each were collected and an aliquot from each tube was analyzed by thin-layer chromatography (TLC). Based on the TLC chromatographic evidence, the appreciable neutral glycolipids were divided into six fractions (Fr1~Fr.6).

Abbreviations : CMS, ceramide monosaccharide ; CDS, ceramide disaccharide ; CTS, ceramide trisaccharide ; CQS, ceramide tetrasaccharide ; CPS, ceramide pentasaccharide ; CHS, ceramide hexasaccharide ; Cer, ceramide ; Glc, glucose ; Gal, galactose ; GlcNAc, *N*-acetylglucosamine ; Fuc, fucose ; Xyl, xylose ; Gal2Me, 2-(*O*-methyl)galactose ; Amino-CTH, GlcNAc β 1-3 Gal β 1-4Glc β 1-1Cer ; TLC, thin-layer chromatography ; GC, gas-liquid chromatography ; GC-MS, gas chromatograph-mass spectrometry ; ¹H-NMR, proton nuclear magnetic resonance spectroscopy ; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

2.3 Thin-Layer Chromatography (TLC)

Silica gel 60 precoated plates (E. Merck) were developed with chloroform-methanol-water, 60:40:10 and 50:50:15 (v/v), and glycolipids were visualized by spraying with orcinol-H₂SO₄ reagent followed by heating at 110°C. For separation into subgroups, the glycolipid fraction was subjected to preparative TLC on 5% borate-impregnated plates (about 2.5 mg materials/10×5 cm plate) prepared according to the method of Hirabayashi *et al.* (2).

2.4 Preparation of Defucosylated Glycolipids

The purified fucolipids were hydrolyzed with 0.2 M HCl at 100°C for 90 min and dialyzed against water. The partially hydrolyzed glycolipids, defucosylated glycolipids, were recovered from the dialysates and purified by Iatrobeds column chromatography using chloroform-methanol-water, 65:35:8 (v/v) as an eluting solvent. The purified compounds were checked by TLC, and subjected to sugar analysis and methylation analysis.

2.5 Gas-Liquid Chromatography (GC) and Gas Chromatograph-Mass Spectrometry (GC-MS)

For determination of the composition of fatty acids and sugars, 50~200 μ g of glycolipids were methanolized in thick glass test tubes (16×125 mm with Teflon-lined screw caps : Pyrex, Iwaki Glass Co.) with 200 μ L of freshly prepared 1 M anhydrous methanolic HCl using a microwave oven (Sharp RE-Z3W6, 100 V, 60 Hz : Sharp Electric Co.) (3~5). The samples were exposed to the maximum power (500 W) of the microwave oven for 1 min (4,5). After methanolysis, the samples were cooled to room temperature inside the microwave oven. The fatty acid methyl esters produced were extracted three times with 400 μ L each of *n*-hexane and analyzed by using a Shimadzu GC-18A gas chromatograph and a capillary column of Shimadzu HiCap-CBP 5 (0.22 mm×25 m) programmed at 2°C/min from 170°C to 240°C. The methanolic phase that remained was evaporated to dryness for deacidification under N₂ gas. The residue containing methylglycosides was trimethylsilylated with a mixture of pyridine, hexamethyldisilazane and trimethylchlorosilane, 9:3:1 (v/v). The reaction mixture was injected into the same capillary column programmed at 2°C/min from 140°C to 230°C. Sphingoids prepared from each glycolipid by methanolysis with 1 M aqueous methanolic HCl at 70°C for 18 h were converted to their *O*-trimethylsilyl (*N*-free) derivatives and analyzed with the same capillary column at 240°C

(6). For the methylation study, permethylation of the samples was performed, using 300 μ g each of the purified glycolipids, with NaOH and CH₃I in dimethylsulfoxide according to the method of Ciucanu and Kerek (7). The partially methylated glycolipids were acetylated and hydrolyzed with 300 μ L of a mixture of HCl-water- acetic acid, 0.5:1.5:8 (v/v) by exposing to the maximum power of the microwave oven for 1 min (5,8), reduced with NaBH₄, and then acetylated with a mixture of acetic anhydride-pyridine, 1:1 (v/v) at 100°C for 15 min. The partially methylated alditol acetates thus obtained were analyzed by GC and GC-MS on the same HiCap-CBP 5 capillary column as described above. Electron impact mass spectra were taken by using a Shimadzu GCMS-QP 5050 gas chromatograph-mass spectrometer under the following conditions: ionizing voltage, 70 eV; ionizing current, 60 μ A; oven temperature, 80°C (2 min)→180°C (20°C/min)→240°C (4°C/min); interface temperature, 250°C; injection port temperature, 240°C; helium gas pressure, 100 kPa.

2·6 Proton Nuclear Magnetic Resonance Spectroscopy (¹H-NMR)

NMR spectra were obtained with a JEOL α -500 500 MHz ¹H-NMR spectrometer at an operation temperature of 50°C. Samples (2 mg) were dissolved in 0.65 mL of dimethylsulfoxide-d₆ containing 2% D₂O.

2·7 Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS analysis of purified glycolipids was performed using a Shimadzu/KRATOS KOM-PACT MALDI I mass spectrometer equipped with a Workstation SPARC station, operating in positive-ion linear mode. Ions were formed by a pulsed ultraviolet laser beam (N₂ laser, 337 nm; 3-nanosecond-wide pulses/sec). The matrix used was 7-amino-4-methylcoumarin (coumarin 120; Sigma). External mass calibration was provided by the [M + H]⁺ ions of ceramide mono-~nonasaccharides (M.W. 699~2201) prepared from the green bottle fly, *Lucilia caesar* (1,9).

3 Results and Discussion

3·1 Isolation of Three Fucolipids (CPS₁, CPS₂ and CHS)

Extraction with chloroform-methanol, 2:1 and 1:1 (v/v), followed by alkaline hydrolysis, dialysis and acetone precipitation yielded 18.2 g of a crude

sphingolipid fraction starting from 10 kg of *P. occelata* (the wet tissues with tubes). The crude sphingolipid fraction was subjected to three modes of column chromatography, ion exchange Sephadex (QAE-Sephadex), magnesium silicate (Florisil) and silicic acid (Iatrobeds), and was finally separated into six fractions, Fr.1 to Fr.6 based on the TLC migration properties. The yields were as follows: Fr.1, 33.3 mg; Fr.2, 20.8 mg; Fr.3, 26.2 mg; Fr.4, 13.3 mg; Fr.5, 35.6 mg; and Fr.6, 258.1 mg (recovered from 762 mg). Lane 1 in **Fig. 1A** shows the TLC pattern of the neutral glycolipid fraction containing CMS (Fr.1), CDS (Fr.2), CTS (Fr.3), CPS (Fr.4), CHS (Fr.5), and more complex glycolipids (Fr.6). Our previous work dealt with five neutral glycolipids, Glc β 1-1Cer, Gal β 1-1Cer, Gal α 1-4Gal β 1-1Cer, Gal β 1-4Glc β 1-1Cer and GlcNAc β 1-3Gal β 1-4Glc β 1-1Cer, which were obtained from these fractions, namely ceramide mono- (CMS), di- (CDS) and trisaccharides (CTS) (1). In addition, the limited structural studies that were possible of ceramide penta- (CPS) and hexasaccharides (CHS) obtained earlier suggested the possible occurrence of the novel fucolipids, tentatively assigned as a mixture of 2-(*O*-methyl) galactosyl-fucosyl-*N*-acetylglucosaminylgalactosylglucosyl-ceramide and xylosylfucosyl-*N*-acetylglucosaminylgalactosylglucosylceramide for CPS and 2-(*O*-methyl) galactosyl(xylosyl) fucosyl-*N*-acetylglucosaminylgalactosylglucosylceramide for CHS, based on their sugar constituent molar ratios (1).

At this point in the purification process, substances CPS in Fr.4 and CHS in Fr.5 each showed a single band, which migrated to positions corresponding to ceramide penta- and hexasaccharides on TLC (**Fig. 1A**, lanes 2 and 3). However, substance CPS was shown to consist of two sub-fractions CPS₁ and CPS₂ by TLC employing a 5% borate-impregnated plate (**Fig. 1B**, lane 1). Subsequently, a preparative TLC of the substance CPS satisfactorily resolved the two CPS sub-fractions as shown in **Fig. 1B**, lanes 2 and 3. The yields from the two sub-fractions were as follows: CPS₁, 1.9 mg; and CPS₂, 7.7 mg (recovered from 13.3 mg of CPS fraction).

3·2 Aliphatic Components of CPS₁, CPS₂ and CHS

The aliphatic components of CPS₁, CPS₂ and CHS were determined by GC and GC-MS. Their main fatty acids were monoenoic acids, C20:1- and C22:1 acids, accounting for about 80% of the total acids, and the sphingoid base was entirely d18:1-sphingoid (**Table**

Fig. 1 Thin-Layer Chromatograms of Neutral Glycolipids obtained from *P. ocellata*. Panel A: lane 1, neutral glycolipid fraction eluted with dichloroethane-acetone (1:1, v/v) using Florisil column chromatography (Frs.1~3, positions corresponding to ceramide mono-, di- and trisaccharide fractions reported previously (1); Frs. 4 and 5, ceramide penta- and hexasaccharide fractions described in this paper; Fr.6, a much more complex glycolipid fraction); lanes 2 and 3, CPS and CHS fractions obtained from Iatrobeds column chromatography using a chloroform-methanol-water gradient elution system. Panel B: lane 1, CPS fraction (the same as Panel A, lane 2) after Iatrobeds column chromatography; lanes 2 and 3, purified CPS₁ and CPS₂ after preparative thin-layer chromatography of CPS fraction. The separations were performed on a precoated silica gel 60 plate developed in chloroform-methanol-water (60:40:10, v/v) for 15 min for Panel A and on a 5% borate-impregnated silica gel 60 plate developed in chloroform-methanol-water (50:50:15, v/v) for 30 min for Panel B. The spots were visualized using a orcinol-H₂SO₄ reagent.

1). It is also noteworthy that in the fatty acid compositions of CPS₂ and CHS, branched C20:1 acid comprises more than 10% of the total fatty acids. Based on the structural similarity of the aliphatic moieties it appears likely that a relationship joins the five neutral glycolipids, CDS, CTS, CPS₁, CPS₂ and CHS in one metabolic pathway, although no glycolipid component corresponding to CQS, Fuc(α 1-3)Amino-CTH, could be detected during the analyses.

3.3 Sugar Constituents, Sequences and Linkages in CPS₁, CPS₂ and CHS

Sugar constituents were analyzed quantitatively by GC and GC-MS analyses as methylglycoside-TMS and alditol acetate derivatives. Their appropriate molar ratios were determined after correction for their relative molar responses as shown in Table 2. Both of

Table 1 Chemical Compositions of the Ceramide Portions of *P. ocellata* Neutral Glycolipids.

	CDS ^a	CTS ^a	CPS ₁	CPS ₂	CHS
Fatty acid ^b (%)					
16:0	10.9	6.8	5.4	5.3	3.0
18:0	7.5	1.7	12.2	4.6	7.0
br20:1	—	—	1.2	10.2	13.7
20:1	20.3	32.3	9.3	10.0	9.7
22:1	61.3	59.2	71.9	69.9	66.6
Sphingoid ^c (%)					
d18:1	100	100	100	100	100

^a CDS, Gal β 1-4Glc β 1-1Cer; CTS, GlcNAc β 1-3Gal β 1-4Glc β 1-1Cer (1).

^b br, branched.

^c d, dihydroxy sphingoid.

CPS₁ and CPS₂ consisted of one mole each of five sugar components : glucose, galactose, *N*-acetylglucosamine, fucose and xylose for CPS₁; and glucose, galactose, *N*-acetylglucosamine, fucose and 2-(*O*-methyl)galactose for CPS₂; and CHS consisted of one mole each of six sugar components : glucose, galactose, *N*-acetylglucosamine, fucose, xylose and 2-(*O*-methyl)galactose. 2-(*O*-Methyl)galactose was identified by comparison of the retention time with that of the authentic sample (Sigma Chemical Co.) as their methylglycoside-TMS derivatives, and also detected as an alditol acetate, which was assigned as a 2-(*O*-methyl) hexitol acetate appearing to have the same mass spectrum as that of 1,2,4,5-tetra-(*O*-acetyl)-2-(*O*-methyl)hexitol reported by Jansson *et al.* (10). In this study we have isolated two neutral glycolipids, CPS₂ and CHS, containing 2-(*O*-methyl)galactose in their sugar components. This is the first case of glycolipids having a 2-(*O*-methyl)galactose residue, although 3-(*O*-methyl)galactose and/or 4-(*O*-methyl)galactose have been found in the aquatic molluscan glycolipids (11-14).

By partial acid hydrolysis (0.2 M HCl for 90 min at 100°C), three lipidic fragments were obtained from each glycolipid. They were characterized as ceramide mono- (Glc-Cer), di- (Gal-Glc-Cer) and trisaccharide (GlcNAc-Gal-Glc-Cer). Furthermore, their methylation experiments established the presence of a ceramide trisaccharide with the sugar sequence and linkages, GlcNAc(1-3)Gal(1-4)Glc. This is identical with that of the CTS-oligosaccharide previously reported in the same organism (1).

Subsequently, methylation analysis of the intact CPS₁, CPS₂ and CHS demonstrated the presence of terminal xylose (1,5-di-(*O*-acetyl)-2,3,4-tri-(*O*-methyl)xylitol, 1Xyl), 4-substituted fucose (1,4,5-tri-(*O*-acetyl)-2,3-di-(*O*-methyl)fucitol, 1,4Fuc) (Fig. 2A), 3-substituted *N*-acetylglucosamine (1,3,5-tri-(*O*-acetyl)-

4,6-di-(*O*-methyl)-*N*-acetylglucosaminitol, 1,3GlcNAc), 3-substituted galactose (1,3,5-tri-(*O*-acetyl)-2,4,6-tri-(*O*-methyl)galactitol, 1,3Gal), and 4-substituted glucose (1,4,5-tri-(*O*-acetyl)-2,3,6-tri-(*O*-methyl)glucitol, 1,4Glc) for CPS₁, respectively; terminal galactose (1,5-di-(*O*-acetyl)-2,3,4,6-tetra-(*O*-methyl)galactitol, 1Gal) derived from 2-(*O*-methyl) galactose (Gal2Me),

Fig. 2 EI-Mode Mass Spectra of Partially Methylated Fucitol Acetate Derivatives. A, 1,4,5-tri-(*O*-acetyl)-2,3-di-(*O*-methyl)fucitol (1,4Fuc) derived from permethylated CPS₁; B, 1,3,5-tri-(*O*-acetyl)-2,4-di-(*O*-methyl)fucitol (1,3Fuc) from permethylated CPS₂; and C, 1,3,4,5-tetra-(*O*-acetyl)-2-(*O*-methyl)fucitol (1,3,4Fuc) from permethylated CHS.

Table 2 Sugar Compositions and Their Molar Ratios of *P. ocellata* Neutral Glycolipids.

	Glc	Gal	GlcNAc	Fuc	Xyl	Gal2Me
CDS ^a	1.0	0.9	—	—	—	—
CTS ^a	1.0	1.0	0.9	—	—	—
CPS ₁	1.0	1.1	0.8	0.9	0.8	—
CPS ₂	1.0	1.1	0.9	1.2	—	0.9
CHS	1.0	1.0	0.9	0.9	1.0	1.0

^a CDS, Galβ1-4Glcβ1-1Cer; CTS, GlcNAcβ1-3Galβ1-4Glcβ1-1Cer (1).

3-substituted fucose (1,3,5-tri-(*O*-acetyl)-2,4-di-(*O*-methyl)fucitol, 1,3Fuc) (**Fig. 2B**), 1,3GlcNAc, 1,3Gal and 1,4Glc for CPS₂, respectively; and two terminal sugars, galactose (1Gal) derived from Gal2Me and xylose (1Xyl), 3,4-substituted fucose (1,3,4,5-tetra-(*O*-acetyl)-2-(*O*-methyl)fucitol, 1,3,4Fuc) (**Fig. 2C**), 1,3GlcNAc, 1,3Gal and 1,4Glc for CHS, respectively. The identification of these partially methylated alditol acetate derivatives was accomplished by GC-MS analysis according to the data of Björndal *et al.* (15), Stellner *et al.* (16) and Tai *et al.* (17). From these results, the structures of CPS₁ and CPS₂ would be Xyl(1-4)Fuc(1-3)GlcNAc(1-3)Gal(1-4)Glc(1-1)Cer and Gal2Me(1-3)Fuc(1-3)GlcNAc(1-3)Gal(1-4)Glc(1-1)Cer, respectively. On the other hand, the presence of 2-(*O*-methyl)fucitol acetate in partially methylated alditol acetates derived from permethylated CHS suggested the presence of a branching structure at the fucose residue, that is, either Gal2Me(1-3)[Xyl(1-4)]Fuc or Xyl(1-3)[Gal2Me(1-4)]Fuc. In order to examine the linkage at this fucose moiety, we tried to liberate the fucose-containing disaccharides from CHS by the mild acidic hydrolysis. However, such hydrolysis did not result in any fucose-containing disaccharides. Therefore, in consideration of the sugar linkages of two fucolipids, CPS₁ and CPS₂ whose structures have been established, and in view of their likely role as biosynthetic intermediates, the structure for CHS should be supported as Gal2Me(1-3)[Xyl(1-4)]Fuc(1-3)GlcNAc(1-3)Gal(1-4)Glc(1-1)Cer.

For determination of anomeric configurations of the

sugar residues of CHS, proton NMR spectroscopy was employed. As shown in **Fig. 3**, in the anomeric region of the spectrum, six anomeric proton resonances were observed at 4.16 ppm ($J_{1,2}=7.8$ Hz) indicative of β -glucose; at 4.18 ppm ($J_{1,2}=7.1$ Hz), β -galactose; at 4.27 ppm ($J_{1,2}=6.6$ Hz), β -xylose; at 4.71 ppm ($J_{1,2}=8.0$ Hz), β -*N*-acetylglucosamine; at 4.84 ppm ($J_{1,2}=3.9$ Hz), α -fucose; and at 5.17 ppm ($J_{1,2}=1.8$ Hz), α -2-(*O*-methyl)galactose; finally, one fucose H-5 resonance was noted at 4.21 ppm.

3.4 MALDI-TOF MS Analysis of CPS₁, CPS₂ and CHS

The putative chemical analysis of CPS₁, CPS₂ and CHS was confirmed by positive-ion MALDI-TOF MS analysis as shown in **Fig. 4**. The mass spectra revealed pseudomolecular ions $[M+H]^+$ at m/z 1396 (**Fig. 4A-a**, predicted molecular species of ceramide: fatty acid-sphingoid, br20:1, 20:1-d18:1) and 1424 (**Fig. 4A-b**, 22:1-d18:1) for CPS₁, at m/z 1440 (**Fig. 4B-a**, br20:1, 20:1-d18:1) and 1468 (**Fig. 4B-b**, 22:1-d18:1) for CPS₂, and at m/z 1572 (**Fig. 4C-a**, br20:1, 20:1-d18:1) and 1600 (**Fig. 4C-b**, 22:1-d18:1) for CHS, which corresponded to Xyl-Fuc-GlcNAc-Gal-Glc-Cer, Gal2Me-Fuc-GlcNAc-Gal-Glc-Cer and Gal2Me-(Xyl)-Fuc-GlcNAc-Gal-Glc-Cer, respectively.

From all the results described above, we propose the structures of CPS₁, CPS₂ and CHS to be as follows: CPS₁, Xyl β 1-4Fuc α 1-3GlcNAc β 1-3Gal β 1-4Glc β 1-1Cer; CPS₂, Gal2Me α 1-3Fuc α 1-3GlcNAc β 1-3Gal β 1-4Glc β 1-1Cer; and CHS, Xyl β 1-4(Gal2Me α 1-3)Fuc α 1-3GlcNAc β 1-3Gal β 1-4Glc β 1-1Cer. These gly-

Fig. 3 Anomeric Proton Regions of ¹H-NMR Spectrum of CHS.

Fig. 4 Positive-Ion Linear Mode MALDI-TOF Mass Spectra of Three Fucolipids. A, CPS₁: a and b, [M+H]⁺ at m/z 1396.3 and 1424.0; B, CPS₂: a and b, [M+H]⁺ at m/z 1439.9 and 1468.0; and C, CHS: a and b, [M+H]⁺ at m/z 1572.4 and 1600.1.

colipids contain some remarkable structural features. Their oligosaccharide moieties contain an internally located fucose and a 2-(*O*-methyl)galactose. The internal fucosyl residue has been found also in the glycolipids of the fresh water bivalves, *Corbicula sandai* (11,13) and *Hyriopsis schlegelii* (18,19), the parasitic trematode, *Schistosoma mansoni* (20), and the oldest "living fossil", *Lingula unguis* (21,22). To our knowledge, this is the first instance of the occurrence of 2-(*O*-methyl)galactose as a glycolipid

constituent. It is of interest that 2-(*O*-methyl)galactose occupies the nonreducing side of the oligosaccharide moiety. The *O*-methyl substitution may impart a specific surface property or antigenicity for the cell membranes of the particular aquatic animals. *O*-Methylation may also regulate the physical properties of the glycolipid by changing its hydrophobicity, though the ceramide portion contributes, of course, to the overall hydrophobicity. Additionally, when visualized on a TLC plate, the neutral glycolipid fraction of the marine annelid showed the existence of much more complex glycolipids (Fr. 6 in Fig. 1A). In a preliminary analysis, we have found a highly novel extension of CHS consisting of Xyl(Gal2Me)Fuc repeating units (the complete structural elucidation of these glycolipids is in progress).

References

1. Sugita, M., Yamake, N., Hamana, H., Sasaki, K. & Dulaney, J.T. (1999) *J. Jpn Oil Chem.Soc. (J. Oleo Sci.)*, **48**, 671-679.
2. Hirabayashi, Y., Hamaoka, A., Matsumoto, M. & Nishimura, K. (1986) *Lipids*, **21**, 710-714.
3. Khan, M.U. & Williams, J.P. (1993) *Lipids*, **28**, 953-955.
4. Takahashi, M., Aoki, K. & Sugita, M. (1999) *Mem. of the Faculty of Ed. Shiga Univ.*, **49**, 1-7.
5. Itonori, S., Takahashi, M. & Sugita, M. (2000) *Proceedings of JOCS/AOCS World Congress*, Kyoto, p.214.
6. Gaver, R.C. & Sweeley, C.C. (1965) *J. Am. Oil Chem. Soc.*, **42**, 294-298.
7. Ciucanu, I. & Kerek, F. (1984) *Carbohydr. Res.*, **131**, 209-217.
8. Itonori, S., Takahashi, M., Aoki, K. & Sugita, M. (2000) *Mem. of the Faculty of Ed. Shiga Univ.*, **50**, in press.
9. Aoki, K., Hamana, H., Yamaoka, H. & Sugita, M. (1998) *Mem. of the Faculty of Ed. Shiga Univ.*, **48**, 11-17.
10. Jansson, P-E., Kenne, L., Liedgren, H., Lindberg, B. & Lönngrén, J. (1976) *Chem. Commun. of University of Stockholm*, **8**, 1-75.
11. Itasaka, O. & Hori, T. (1979) *J. Biochem.*, **85**, 1469-1481.
12. Hayashi, A. & Matsubara, T. (1982) *New Vistas in Glycolipid Research* (Makita, A., Handa, S., Taketomi, T. & Nagai, Y., eds.) Plenum Publishing Corp., New York, pp.103-114.
13. Itasaka, O., Kosuge, M., Okayama, M. & Hori, T. (1983) *Biochim. Biophys. Acta*, **750**, 440-446.

14. Araki, S., Satake, M., Ando, S., Hayashi, A. & Fujii, N. (1986) *J. Biol. Chem.*, **261**, 5138-5144.
 15. Björndal, H., Hellerqvist, C.C., Lindberg, B. & Svensson, S. (1970) *Angew. Chem.*, **82**, 643-652.
 16. Stellner, K., Saito, H. & Hakomori, S. (1973) *Arch. Biochem. Biophys.*, **155**, 464-472.
 17. Tai, T., Yamashita, K. & Kobata, A. (1975) *J. Biochem.*, **78**, 679-686.
 18. Hori, T., Sugita, M., Ando, S., Kuwahara, M., Kumauchi, K., Sugie, E. & Itasaka, O. (1981) *J. Biol. Chem.*, **256**, 10979-10985.
 19. Hori, T., Sugita, M., Ando, S., Tsukada, K., Shiota, K., Tsuzuki, M. & Itasaka, O. (1983) *J. Biol. Chem.*, **258**, 2239-2245.
 20. Levery, S.B., Weiss, J.B., Salyan, M.E.K., Roberts, C.E., Hakomori, S., Magnani, J.L. & Strand, M. (1992) *J. Biol. Chem.*, **267**, 5542-5551.
 21. Aoki, K. & Sugita, M. (1999) *Proceedings of J.C.B.L.*, Kamakura, **41**, 113-116.
 22. Aoki, K., Hada, N., Itonori, S., Takeda, T. & Sugita, M. (2000) *Abstracts of XXIst Japanese Carbohydrate Symposium*, Nagoya, p.95.
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