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The Major Acidic Glycolipids from the Kidney of the Pacific Salmon (*Oncorhynchus keta*): Characterization of a Novel Ganglioside, Fucosyl-*N*-acetylgalactosaminyl-GM1[1]

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Two fractions of a major ganglioside from the kidney of the pacific salmon, *Oncorhynchus keta*, were eluted from a DEAE-Sephadex column in the monosialosyl fraction. The faster moving ganglioside (X1) on TLC was separated from the slower moving one (X2) by HPLC using a silica beads column. By methylation analysis, chemical and enzymatic degradation, reaction with monoclonal antibodies, LSIMS, and ^{1}H-NMR spectroscopy, X1 was determined to be a monosialosyl ganglioside belonging to the ganglio-series with a unique Fucα1-3GalNAc linkage at the nonreducing terminal:

Fucα1-3GalNAcβ1-3Galβ1-3GalNAcβ1-4[NeuAcα2-3]Galβ1-4Glcβ1-1Cer

Analysis of the lipophilic moiety indicated predominance of 24:1 fatty acid in combination with sphingenine. X2 was found to have a glycon structure identical to X1. The ceramide of X2 consisted predominantly of saturated fatty acids (18:0 and 16:0). The tissue concentrations of X1 and X2 in kidney were 3.7 and 2.8 nmol/g, respectively.

Key words: fucoganglioside, ^{1}H-NMR spectroscopy, LSIMS, kidney, *Oncorhynchus keta*, salmon.

Glycolipids are the components of the animal plasma membrane (*3, 4*), and those in mammalian kidney have been studied with respect to renal functions (*5-8*). Quantitative estimation of renal sulfoglycolipids in various mammals indicated that the sulfoglycolipid content is a function of metabolic activities (*9, 10*) as well as the environmental osmolality (*11*). Recently, using the system of cultured renal tubular cells, the crucial role of the sulfoglycolipids in adaptation to a change in osmolality was clearly demonstrated (*12, 13*). The osmolality of euryhaline fish is regulated mainly by the kidney and gills (*14*). Of these tissues, glycolipid has been studied only in the gills of eel (*15*), although the compositions of the acidic glycolipid of other fish tissues reported include brain (*16-19*), milt (*20*), liver (*21, 22*), and roe (*23, 24*). Salmon hatched in fresh water undergo parr-smolt transformation on moving into seawater and perform anadromous reproduction. Therefore, we were prompted to study the composition of acidic glycolipids in the salmon kidney. The present paper describes characterization of a novel fucoganglioside of the ganglio-series containing a unique Fucα1-3GalNAc structure as the major sialoglycolipid component of the kidney of the Pacific salmon.

EXPERIMENTAL PROCEDURES

Materials—Kidneys were freshly prepared from salmon captured off the Sanriku Coast of Japan in December and stored at −40°C before extraction. Ganglioside GM1a (*1*) from bovine brain was obtained from Funakoshi Pharmaceutical, Tokyo, and gangliotetraosylceramide (Gg$_4$Cer) was prepared from GM1a by mild acid hydrolysis (*16*). Other reference glycolipids and partially *O*-methylated alditols were prepared in this laboratory (*25*). Rabbit anti-Gg$_4$Cer antibody was purchased from Seikagaku Kogyo,

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Abbreviations: Abbreviations of gangliosides follow the nomenclature system of Svennerholm (*1*) and those of other lipids are according to the recommendation of the Nomenclature Committee, International Union of the Pure and Applied Chemistry (*2*). Cer, ceramide; COSY, chemical-shift-correlated spectroscopy; LSIMS, liquid secondary ion mass spectrometry; d18:1, 4-sphingenine; d18:0, sphinganine; 1-D, one-dimensional; 2-D, two-dimensional; Fuc, fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; HPTLC, high performance TLC; Me$_2$SO, dimethyl sulfoxide; Me$_4$Si, tetramethylsilane; NeuAc, *N*-acetylneuraminic acid; t18:0, 4-hydroxysphinganine.

Tokyo. The affinity-purified peroxidase-goat anti-rabbit IgG (c-chain specific) was purchased from Zymed Laboratories, San Francisco (*26*). β-Galactosidase [EC 3.2.1.23, grade VII] and β-*N*-acetylhexosaminidase [EC 3.2.1.30] from jack bean and neuraminidase [EC 3.2.1.18, type V] from *Clostridium perfringens* were the products of Sigma, St. Louis, USA. Reagents for derivatization and NMR spectroscopy were previously described (*27*).

Thin Layer Chromatography—TLC was performed on Silicagel 60 HPTLC plates, Art. 5641 (E. Merck, Darmstadt, FRG), with the following solvent systems: I, chloroform/methanol/0.2% $CaCl_2$ (55:45:10, v/v); II, chloroform/methanol/water (60:35:8, v/v); and III, chloroform/methanol/3.5 M NH_4OH (55:45:10, v/v). Glycolipids, gangliosides, and sulfoglycolipids were visualized with orcinol, resorcinol (*28*), and Azure A reagent (*27*), respectively. TLC densitometric analysis of resorcinol-stained ganglioside was performed at 580 nm with a Shimadzu Flying-Spot Scanner CS-9000. TLC-immunostaining was performed as described previously (*26*).

Isolation of the Major Monosialosyl Gangliosides—The extraction and purification procedures for acidic glycolipid were similar to the methods of Bligh and Dyer (*29*) and Momoi *et al.* (*30*), respectively. Briefly, 2 kg of kidneys was extracted in two steps with 6 liters of chloroform/methanol (1:2, v/v) and 3.8 liters of chloroform/methanol/water (1:2:0.8, v/v). The combined extracts were partitioned by addition of 1.5 liters each of chloroform and water, and the resulting upper phase was dialyzed and lyophilized. The lower phase lipid was treated with 0.1 M NaOH in methanol (200 ml) at 37°C for 1 h, then neutralized with 1 M acetic acid. The mixture was concentrated, dialyzed and lyophilized.

The upper and the lower phase lipids were combined, redissolved in chloroform/methanol/water (30:60:8, v/v), and applied to a column of DEAE-Sephadex A-25 (3×50 cm) (Amersham Pharmacia Biotech, Tokyo) prepared by equilibration with 2 M sodium acetate in methanol. After washing the column with 4.0 liters of the same solvent to remove neutral lipids, acidic glycolipids were separated with a linear gradient (2.2 liters) of chloroform/methanol/water (30:60:8, v/v) to chloroform/methanol/0.5 M ammonium acetate (30:60:8, v/v). Fractions (15 ml/tube) of eluates were monitored by HPTLC (Fig. 1).

Two major glycolipids in the monosialosyl ganglioside fraction, tentatively designated as X1 and X2 (tube numbers 55-80), were eluted earlier than two monosialosyl gangliosides tentatively assigned to GM1 (tube numbers 94-106) and GM3 (102-114). These bands were identified as gangliosides, because they reacted positively with orcinol and resorcinol but not with Azure A reagent. They were further purified by HPLC with a Shimadzu LC 4A apparatus using a column (1×30 cm) of porous silica gel (Iatrobeads 6RS-8005, 5 μm, Iatron, Tokyo) with chloroform/methanol/water (60:40:2, v/v) at a flow rate of 1 ml/min. The concentration of X1 and X2 determined by TLC-densitometry using GM1a (NeuAc) as the standard was 3.7 and 2.8 nmol sialic acid/g kidney, respectively.

Chemical Analysis—The trimethylsilyl methylglycosides and methyl esters of fatty acids were prepared as described (*7*). GLC of carbohydrates and fatty acid methyl esters was performed on a capillary columns (25 m) of 0.2 μm thick OV-101 and CBP-1 (Shimadzu), respectively, in a Shimadzu GC-7A Gas Chromatograph. *N*-Acetyl-*O*-trimethylsilyl derivatives of sphingoid bases (20 nmol) were prepared by the method of Sweeley and Moscatelli (*31*) after methanolysis (*32*) and analyzed isothermally at 260°C using the CBP-1 capillary column. For methylation study, a portion (20–50 μg) of glycolipid was permethylated (*33*), acetolyzed, reduced with $NaB[^2H]_4$ (*25*), and acetylated according to the published procedures (*34*). The acetates of partially methylated alditols were analyzed by GLC-electron impact mass spectrometry using a Shimadzu Auto GC-MS 6020 apparatus (*25*). GLC separation was achieved using columns of either 3% SP-2340 or 3% OV-17. Peaks were identified by retention times and characteristic fragment ions (*35*). GLC for quantitative analysis of alditol acetates was carried out on a CBP-1 capillary column.

Limited Hydrolysis of Glycolipid—Glycolipid X1 (500 μg) was treated with 1% acetic acid at 100°C for 1 h, and the reaction mixture was lyophilized. The neutral fraction, obtained by passing through a DEAE-Sephadex column (5×10 mm), was applied on a HPLC column (Iatrobeads 6RS-8005, 1×30 cm). Three major products (designated P1, P2, and P3) were separated by elution with chloroform/methanol/water (60:40:1, v/v). For the enzymatic hydrolysis with β-galactosidase, glycolipids were incubated in 50 mM sodium citrate buffer (pH 4.0) containing sodium

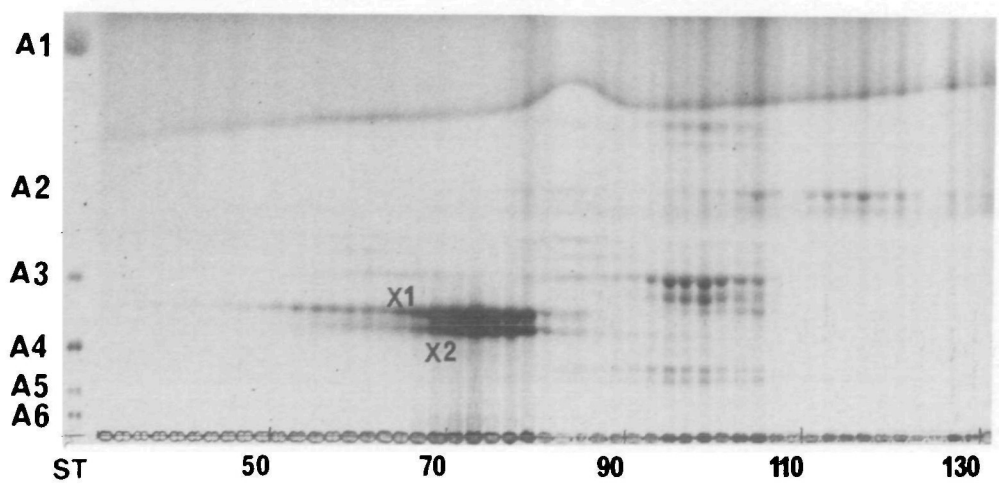

Fig. 1. **Elution of acidic glycolipids of salmon kidneys from a DEAE-Sephadex column.** Elution of acidic glycolipids from the DEAE-Sephadex column was monitored by HPTLC developed in the neutral solvent system I. Glycolipids were located by orcinol reagent. Lane ST, the mixture of rat brain acidic glycolipids for reference (A1, galactosyl sulfatide; A2, GM3; A3, GM1; A4, GD1a; A5, GD1b; A6, GT1b + GQ1b). Details are described in the text.

taurocholate (1 mg/ml) at 37°C for 16 h. For β-*N*-acetylhexosaminidase, the same buffer of pH 5.0 was used. The reaction mixture was adjusted to chloroform/methanol/water (30:60:8, v/v), and sialic acid, taurocholate, and the intact glycolipid were removed by using a DEAE-Sephadex column.

Liquid Secondary Ion Mass Spectrometry (LSIMS)—Negative-ion LSIMS was performed on a Concept IH mass spectrometer (Shimadzu/Kratos, Kyoto) fitted with a cesium ion gun (*36, 37*). About 0.5 nmol of underivatized glycolipid in 1 μl of chloroform/methanol (1:2, v/v) was mixed with 1 μl of triethanolamine as the matrix. Spectra were recorded at an accelerating voltage of 8 kV, with a scan rate of 5 s/decade, and at a resolution of 1000 to 2000.

^1H NMR Spectroscopy—After successive dialysis against phosphate-buffered saline (Ca^{2+} and Mg^{2+} free), pH 7.4, and water, glycolipid X1 (11 mg) and X2 (2 mg) were lyophilized, treated with $CH_3O[^2H]$, and dried thoroughly over P_2O_5 *in vacuo*. ^1H NMR spectra were recorded on a GX-400 spectrometer of Japan Electron Optical Laboratory (JEOL, Tokyo) at 400 MHz (*38*) and 60°C. The protons of the saccharide ring and ceramides were assigned by combined use of spin-decoupling and multiple-relayed chemical-shift-correlated spectroscopy (COSY). Chemical shifts were indicated by ppm from the signal of Me_4Si as an internal standard.

RESULTS

Chemical Analysis of X1 and X2—On HPTLC, gangliosides X1 and X2 migrated between GM1 and GD1a in the neutral solvent system I (Fig. 2).

However, in the basic solvent system III, both gangliosides migrated between GD1a and GD1b. GLC of trimethylsilyl methylglycosides indicated that the component monosaccharides of both X1 and X2 were Glc, Gal, GalNAc, Fuc, and sialic acid in essentially similar proportions of 1.0:2.0:2.1:1.0:0.7. The sialic acid liberated by hydrolysis with 0.05 N HCl/methanol for 1 h (*39*) was identified as NeuAc by GLC using GM1a (NeuAc) as the standard. The partially methylated alditol acetates of X1 and X2 were separated with a SP-2340 column and identified by mass chromatography (*25*) to be 2,3,4-tri-*O*-methyl-fucitol, 2,4,6-tri-*O*-methylgalactitol, 2,3,6-tri-*O*-methylglucitol, and 2,6-di-*O*-methylgalactitol. By using an OV-17 column, 4,6-di-*O*-methyl-*N*-acetylgalactosaminitol was also detected. The ratios of the peak areas of 2,3,4-tri-*O*-methyl-fucitol, 2,4,6-tri-*O*-methylgalactitol, 2,3,6-tri-*O*-methylglucitol, 2,6-di-*O*-methylgalactitol, and 4,6-di-*O*-methyl-*N*-acetylgalactosaminitol in X1 and X2 were essentially similar, approximately 0.4:1.0:1.0:1.2:1.8 (Table I).

Taking into consideration the relative volatility of permethylated fucitol, the molar ratios of partially methylated alditol acetates are in reasonable agreement with the monosaccharide composition, suggesting that the saccharide linkages of X1 and X2 are identical. The major fatty acids were 24:1 (80.0%) for X1 and 18:0 (31.1%) and 16:0 (32.4%) for X2, indicating that the difference in their chromatographic behavior might be ascribed to the fatty acid composition. The preponderant sphingoid of both X1 and X2 was 4-sphingenine (d18: 1) (Table II).

Identification of Limited Hydrolysis Products of X1 by Mild Acid and Glycosidases—Ganglioside X1, being unsusceptible to sialidase from *Clostridium perfringens*, was hydrolyzed with mild acid to give three major products (designated P1, P2, and P3 on TLC upward from the lowest band in lane 3 of Fig. 3).

P1, P2, and P3 were subsequently purified by HPLC to single bands (Fig. 3, lanes 4, 5, and 6). Glycolipid P3 (lane 6) was converted by β-galactosidase into a product shown in lane 7. The product of lane 7 was sequentially converted into a dihexosylceramide (lane 8) by β-*N*-acetylhexosaminidase, and a monohexosylceramide (lane 9) by β-galactosidase. In methylation study, P3 yielded acetates of 2,3,4,6-tetra-*O*-methylgalactitol, 2,3,6-tri-*O*-methylgalactitol, 2,3,6-tri-*O*-methylglucitol, and 4,6-di-*O*-methyl-*N*-acetylgalactosaminitol (Table I). The anomeric region of the ^1H-NMR spectrum of P3 contained a one-proton doublet (4.603 ppm, $J_{1,2}=7.8$ Hz), a two-proton doublet (4.227 ppm (=the internal), $^3J_{1,2}=7.8$ Hz), and a one-proton doublet (4.165 ppm, $J_{1,2}=7.8$ Hz) (Table III) assigned

Fig. 2. **HPTLC of the major gangliosides purified from salmon kidney.** Plates were developed in the neutral solvent system I in panel A, and in a basic solvent system III in panel B. Glycolipids were located by orcinol reagent. Lane 1, rat brain acidic glycolipids (*cf.* Fig. 1); lane 2, X1; lane 3, X2.

TABLE I. **Partially *O*-methylated alditol acetates from the intact ganglioside X1 and the glycolipids obtained by mild acid hydrolysis.** Values were determined by GLC on a CBP-1 capillary column. P1, P2, and P3 are hydrolysis products of X1. +, detected by GC-MS on an OV-17 column.

	2,3,4-Fuc[a]	2,3,4,6-Gal[b]	2,4,6-Gal[c]	2,3,6-Gal[d]	2,3,6-Glc[e]	2,6-Gal[f]	3,4,6-GalNAc[g]	4,6-GalNAc[h]
X1	0.41	–	0.95	–	1.00	1.17	–	1.80
P1	0.97	–	0.98	1.31	1.00	–	–	+
P2	–	–	1.10	0.74	1.00	–	0.62	0.42
P3	–	0.59	–	0.78	1.00	–	–	+
X2	0.39	–	1.02	–	1.00	1.22	–	1.74

[a]2,3,4-tri-*O*-methyl-fucitol, [b]2,3,4,6-tetra-*O*-methylgalactitol, [c]2,4,6-tri-*O*-methylgalactitol, [d]2,3,6-tri-*O*-methylgalactitol, [e]2,3,6-tri-*O*-methylglucitol, [f]2,6-di-*O*-methylgalactitol, [g]3,4,6-tri-*O*-methyl-*N*-acetylgalactosaminitol, [h]4,6-di-*O*-methyl-*N*-acetylgalactosaminitol.

to a β-GalNAc, two β-Gal, and one β-Glc linked to ceramide, respectively. These resonances were similar to those of Gg_4Cer described previously (*33*). Glycolipid P3 was strongly stained with anti-Gg_4Cer anti-body (indicated in "EXPERIMENTAL PROCEDURES") on TLC (data not shown). These results suggested that P3 was Gg_4Cer.

The peak area ratio of 2,4,6- and 2,3,6-tri-*O*-methylgalactitol, 2,3,6-tri-*O*-methylglucitol, 3,4,6-tri-*O*-methyl- and 4,6-di-*O*-methyl-*N*-acetylgalactosaminitol of P2 was approximately equimolar (Table I) suggesting that the structure of P2 is GalNAcβ-3Galβ-3GalNAcβ-4Galβ-4Glcβ-1Cer. In the ^{1}H-NMR spectrum of P2, a two-proton doublet corresponding to anomeric protons of two β-GalNAc was shown at 4.601 ppm in addition to the signals of β-Glc and β-Gal (4.227 and 4.292 ppm) (Table III). The signal (4.292 ppm) was assigned to the anomeric proton of the penultimate Gal, because the glycosylation shift of 0.065 ppm by the terminal GalNAc is reasonable.

Methylation study of glycolipid P1 showed 2,3,4-tri-*O*-methyl-fucitol, 2,4,6- and 2,3,6-tri-*O*-methylgalactitol, and 2,3,6-tri-*O*-methylglucitol in equimolar amounts (Table I). The only amino sugar detected by GC-MS was 4,6-tri-*O*-methyl-*N*-acetylgalactosaminitol. NMR spectrum of P1 showed resonances of H-1, H-5, and H-6 of an α-Fuc in addition to those found with P2 (Table III). From these results, P1 was characterized as Fucα-3GalNAcβ-3Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer, a desialylation product of ganglioside X1. Together with the results of methylation analysis, this suggested the probable structure of X1 to be:

$$\begin{array}{ccccccc} \text{VI} & & \text{V} & & \text{IV} & \text{III} & \text{VII} \\ \text{Fuc}\alpha\text{-3GalNAc}\beta\text{-3Gal}\beta\text{-3GalNAc}\beta\text{-4[NeuAc}\alpha\text{2-3]} \\ \text{II} & & \text{I} \\ \text{Gal}\beta\text{-4Glc}\beta\text{-Cer} \end{array}$$

The Roman numerals correspond to the individual saccharide and were also used for the assignment of NMR spectrum (see below).

Liquid Secondary Ion Mass Spectrometry (LSIMS)—To confirm the structure above, negative LSIMS analysis of X1 was performed (Fig. 4).

TABLE II. **Fatty acids and long chain bases of gangliosides X1 and X2.**

	X1	X2
	(weight % of total)	
Fatty acid		
14:0	2.8	15.9
16:0	4.6	32.4
18:0	10.6	31.1
18:1	2.0	–
24:1	80.0	20.6
Long chain base		
d18:1	79	85
d18:0	13	9
t18:0	8	6

–, not determined.

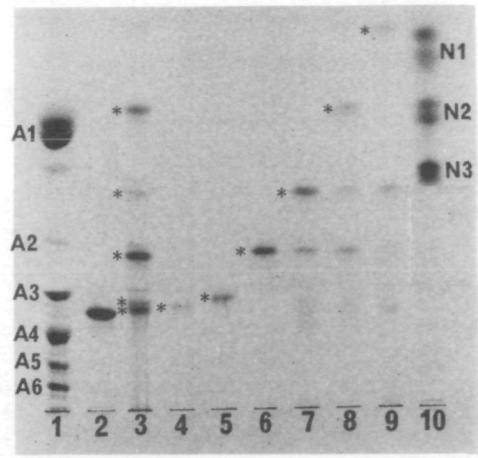

Fig. 3. **Hydrolysis of X1 by mild acid and exoglycosidases.** The HPTLC plate was developed with the neutral solvent system II. Glycolipids were located by orcinol reagent. Lane 1, rat brain acidic glycolipids (*cf.* Fig. 1); lane 2, purified X1; lane 3, the products (*) from X1 by mild acid hydrolysis; lanes 4-6, the purified product (*) P1, P2, and P3, respectively; lane 7, the product (*) from P3 after incubation with β-galactosidase; lane 8, the product (*) from the glycolipids of lane 7 after incubation with β-*N*-acetylhexosaminidase; lane 9, the product (*) from the glycolipid of lane 8 after incubation with β-galactosidase. Lane 10, a neutral glycolipid mixture from horse kidneys as reference: N1, monohexosylceramides; N2, dihexosylceramides; N3, globotriaosylceramide.

TABLE III. **^{1}H chemical shifts δ (ppm from Me_4Si) and vicinal coupling constants *J* (Hz) for intact ganglioside X1 and X2 and the hydrolysis products of X1.** Spectra were acquired in Me_2SO-d_6/$[^2H]_2O$ (98:2, v/v) at 60°C, and the chemical shifts were referenced to internal Me_4Si. The chemical shift value and $^3J_{1,2}$ values are expressed in ppm and Hz, respectively. P1, P2, and P3 are products of limited hydrolysis of X1.

	Fucα1			3GalNAcβ1		3Galβ···	3GalNAcβ			NeuAc			4Galβ··· 3	4Glcβ···Cer
	H-1	H-5	H-6	H-1	N-Ac[a]	H-1	H-1	N-Ac[a]	H-3eq	H-3ax	N-Ac[a]	H-1	H-1
P3 (δ)						4.227	4.603	1.836				4.227	4.165
(*J*)						(7.8)	(7.8)					(7.8)	(7.8)
P2 (δ)				4.601	1.861[b]	4.292	4.601	1.847[b]				4.227	4.165
(*J*)				(8.3)		(6.8)	(8.3)					(7.8)	(7.8)
P1 (δ)	4.758	4.048	1.066	4.679	1.809[b]	4.239	4.599	1.841[b]				4.222	4.166
(*J*)	(2.9)		(6.8)	(8.8)		(6.3)	(8.3)					(6.8)	(7.8)
X1 (δ)	4.765	4.046	1.066	4.657	1.812	4.292	4.871	1.780	2.580	1.634	1.879	4.279	4.158
(*J*)	(3.9)		(6.4)	(8.5)		(6.8)	(8.5)					(7.8)	(7.8)
X2 (δ)	4.763	4.046	1.065	4.657	1.811	4.291	4.871	1.779	2.581	1.632	1.878	4.278	4.158
(*J*)	(4.0)		(6.5)	(8.6)		(6.8)	(8.7)					(7.8)	(7.8)
GM1 (δ)						4.279	4.882	1.769	2.580	1.630	1.878	4.242	4.158
(*J*)						(7.8)	(8.8)					(7.1)	(7.8)

[a]^{1}H chemical shift of *N*-acetylmethyl proton resonance. [b]These resonance assignments are tentative.

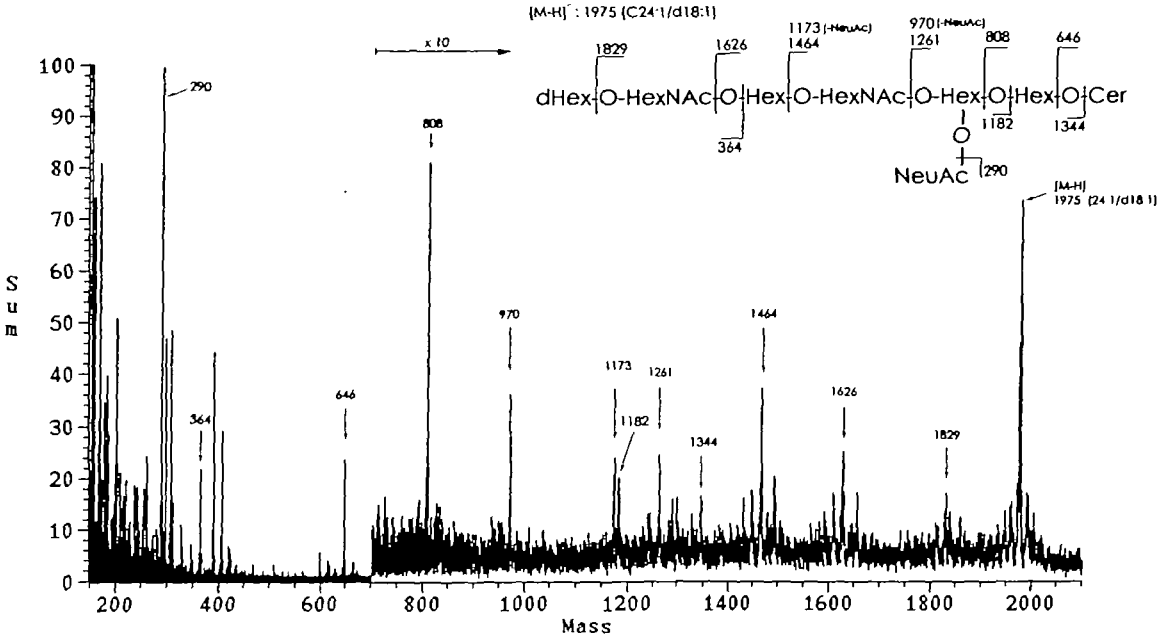

Fig. 4. **Negative-ion LSIMS of X1.** Hex, hexose; HexNAc, *N*-acetylhexosamine; Cer, ceramide; d18:1, 4-sphingenine; $(M-H)^-$, the deprotonated molecule. The peaks in the mass ranges higher than *m/z* 700 were amplified 10-fold.

An intense deprotonated molecule (M minus H)$^-$ was detected at *m/z* 1,975, corresponding to the ceramide species of C24:1 plus d18:1. Furthermore, characteristic fragment ions [*m/z* 1,626 (-O-GM1), 1,464 (-O-GM2), 1,173 (-O-AM2), 1,261 (-O-GM3), 970 (-O-Hex$_2$Cer), 808 (-O-HexCer), 364 (dHex-O-HexNAc-O-), 290 (NeuAc-), and 646 (Cer, C24:1, d18:1)] were obtained. The fragment ion at *m/z* 1,261 suggested that NeuAc is linked to the second hexose from the ceramide (*40*).

^{1}H NMR Spectrum of X1—The ^{1}H-NMR spectrum of X1 is shown in Fig. 5.

A typical H-2 triplet at the highest field of all saccharide protons (3.027 ppm) supported the inference that the β-Glc is next to the ceramide. The connectivity between H-2/H-1, H-2/H-3, H-5/H-6b, H-5/H-6a, and H-6a/H-6b of Glc could be easily distinguished in the COSY spectrum. The connectivity of resonances H-1 to H-4 of Gal (II) and H-1 to H-5 of Gal (IV) allowed the assignment of the sialylated Gal. Cross-peaks of H-2/H-1 (not shown), H-3/H-2, and H-3/H-4 in GalNAc (III and V) were also detected in COSY spectrum. Based on the above results, the resonances of six anomeric protons of the saccharide chain were assigned as listed in Table III.

The anomeric proton of β-GalNAc (III) at 4.871 ppm ($J_{1,2}$=8.5 Hz) adjacent to the sialylated Gal was close to GM1a (4.882). In the product (P1), the anomeric proton of GalNAc resonated at 4.599 ppm, 0.272 ppm higher field than in X1 (see below). This is in agreement with the difference (0.293 ppm) on comparison of GM1 and Gg$_4$Cer (*41*). The doublet of the H-6 methyl proton of Fuc was observed at 1.066 ppm and was coupled with H-5 (4.046 ppm). Since the resonance of H-5 of Fuc is located in the close upfield of the anomeric region, it was suggested that Fuc is linked to the non-reducing terminal of the saccharide chain (*42*) (pyranosides of the L-series mostly occur in the ^{1}C$_4$ conformation). The signals corresponding to double

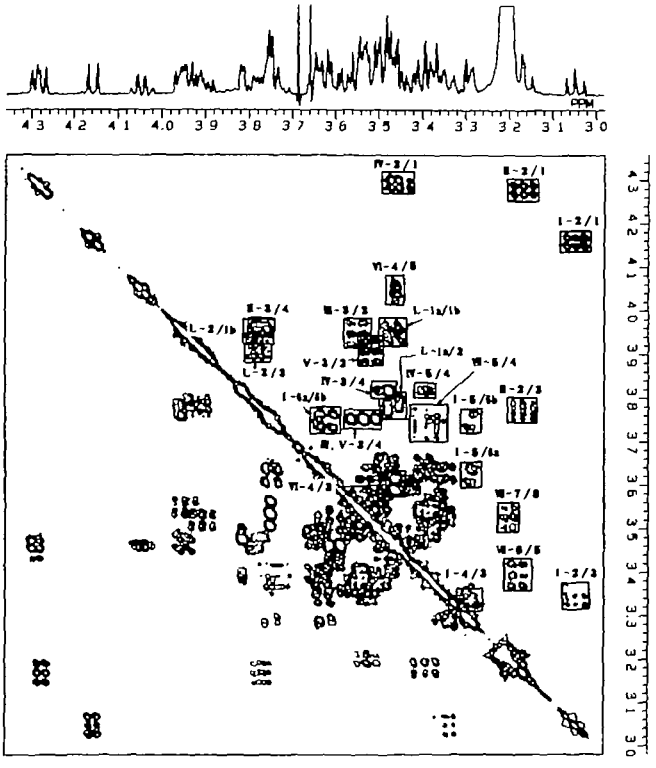

Fig. 5. **^{1}H-^{1}H-COSY spectrum of X1.** NMR spectra of X1 was obtained in 0.5 ml of Me_2SO-d_6 containing 2% $[^2H]_2O$ at 60°C. The spectrum was recorded with 2,048×512 data points and a spectral width of 1,000 Hz (*13, 14*). Free induction decays were multiplied by the Gaussian function (*46*). Arabic numbers refer to the ring protons of sugar residues marked by Roman numerals in the structure drawn in the text. Cross-signals of protons in the Glc residue are indicated in the form I-2/1 (for H-2 and H-1 protons).

doublets of H-3eq and a triplet of H-3ax of NeuAc were found at 2.580 and 1.634 ppm, respectively. In addition to the cross-peaks of H-3ax/H-3eq, H-3ax/H-4 (3.761 ppm), and H-3eq/H-4 (not shown), cross-peaks of H-5 (3.410 ppm, $J_{4,5}=10.5$ Hz, $J_{5,6}=10.5$ Hz)/H-4, H-6 (3.16 ppm, obscured by HDO peak)/H-5 as well as H-7/H-8 could be assigned. Three singlets of *N*-acetyl methyl groups corresponding to NeuAc (VII) and GalNAc (III and V) were observed between 1.8 and 1.9 ppm. The signal at 1.879 ppm may be assigned to *N*-acetyl methyl of NeuAc by comparison with the chemical shift (1.878 ppm) of *N*-acetyl methyl of NeuAc in GM1a (Table III). The resonances at 1.812 and 1.780 ppm were assigned to the *N*-acetyl methyls of GalNAc (V) and (III), respectively, since the latter was very close to the *N*-acetyl methyl (1.769 ppm) of GalNAc in GM1. The signals arising from the long chain base were in agreement with the major component (d18: 1). The NMR spectrum of ganglioside X2 was identical to that of X1 except for the much smaller olefinic signals (5.3 ppm), in agreement with the low content of unsaturated fatty acids (Table III).

Table III lists the chemical shifts and coupling constants for the anomeric protons, H-5 and H-6 of fucose and H-3 of NeuAc, and *N*-acetyl methyl groups of monosaccharides in X1, X2, the hydrolysis products of X1 (P1, P2, and P3), and GM1a. Chemical shifts of the other ring protons of the saccharide residues and the long chain base in X1 are summarized in Table IV. Gal (II): The connectivity from II-1 and II-2=3.17 [much higher than IV-2=3.46, II-2= 3.17 (unknown in P1) is 0.1 ppm higher than GM1a of Koerner (*39*) to II-4 (=3.96 ppm), with II-3 at 3.77 ppm ($J_{2,3}=8.7$; $J_{3,4}=3.7$) (and another Gal (IV-3)=3.49 ppm, IV-4=3.82 ppm] indicated that this is the sialylated internal Gal. Two GalNAc (III and V) could be followed up from H-1 to H-4 (internal or external can be inferred only by comparison with P1-P3; III-3 is at a lower field than V-3).

Based on NMR spectroscopy, methylation studies of the saccharide chain, and the fatty acid analysis, X2 was suggested to have a saccharide identical to X1, with the ceramide containing predominantly more saturated fatty acids.

TABLE IV. **^{1}H chemical shift (ppm) of the saccharide residues and the long chain base in ganglioside X1.** The chemical shift is expressed in ppm referenced to Me_4Si. The Roman numerals correspond to those in the structure described in the text. Sph, sphingosine base; nd, not determined because of obscurity of the cross peak.

	(H-2)	(H-3)	(H-4)	(H-5)	(H-6a)	(H-6b)
Glc (I)	3.05	3.35	3.30	3.28[b,c]	3.63[c]	3.75[b]
Gal (II)	3.17	3.77	3.96	3.48	nd	nd
GalNAc (III)	3.95	3.55	3.76	3.65	nd	nd
Gal (IV)	3.46	3.49	3.82	3.40	nd	nd
GalNAc (V)	3.91	3.52	3.76	3.66	nd	nd
Fuc (VI)[a]	3.44	3.60	3.46			
	(H-4)	(H-5)	(H-6)	(H-7)	(H-8)	(H-9a)	(H-9b)
NeuAc (VII)	3.75	3.40	3.18	3.20	3.53	3.35	3.62
	(H-1a)	(H-1b)	(H-2)	(H-3)	(H-4)	(H-5)	
Sph	3.47	3.95	3.78	3.92	5.35	5.55	

[a]The signals of H-5 and H-6 were described in Table III. [b]Confirmed by spin decoupling of Glc H-6a. [c]Confirmed by spin decoupling of Glc H-6b and H-4 of III and IV GalNAc simultaneously.

VI V IV III VII
Fucα-3GalNAcβ-3Galβ-3GalNAcβ-4[NeuAcα2-3]
II I
Galβ-4Glcβ-Cer

DISCUSSION

One of the euryhaline fishes, Pacific salmon undergoes parr-smolt transformation. The present study, prompted by interest in the adaptation of salmon to osmolality, was also motivated by an interest in the comparative biochemistry of glycolipids of the kidney. A striking feature of the gangliosides in teleost fish kidney is the presence of a major novel ganglioside in which a core structure of GM1a ganglioside is substituted with Fucα1-3GalNAcβ1-3R. A unique ganglioside with oligosaccharide with a ganglioseries core and a Forssmaan antigen determinant, GalNAc-α1-3GalNAcβ1-3R residue, has been identified in the liver of English sole (*21*). In the other non-neuronal tissue of the salmon, we identified GM1b ganglioside (authors' unpublished observation). It appears that gangliosides of the ganglioseries are commonly found in the non-neuronal tissues of teleosts (*29-32*). Unique Fucα1-3GalNAcα- and Fucα1-3GalNAcβ-linkages have been characterized in the glycolipid of abalone (*43*) and English sole liver (*30*), respectively. The disaccharide of Fucα1-3GalNAcβ has already been reported in glycoproteins of salmon eggs (*44, 45*). The teleost brain is rich in polysialosyl gangliosides (*21-26*). However, the majority of gangliosides in the teleost kidney are monosialosyl. A higher degree of unsaturation of the fatty acids was found in the gangliosides of teleost kidney in comparison to mammalian kidney. As already observed in the ganglioside of fish liver (*29*) and brain (*23*), these fatty acids may help maintain membrane fluidity at low temperatures in poikilothermic animals.

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