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A novel mono-sulfated pentaglycosylceramide with the isoglobo-series core structure in rat kidney

Keiko Tadano-Aritomi [a,*], Masaru Okuda [a], Ineo Ishizuka [a], Harumi Kubo [b], Philip Ireland [c]

[a] *Department of Biochemistry, Teikyo University School of Medicine, Kaga 2-11-1, Itabashi-ku, Tokyo 173, Japan*

[b] *Shimadzu Corporation, Nakagyo-ku, Kyoto 604, Japan*

[c] *Kratos Analytical, Urmston, Manchester M31 2LD, United Kingdom*

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Abstract

A five-sugar sulfated glycosphingolipid containing an isoglobo-series carbohydrate core was isolated from rat kidney and its structure characterized by compositional analysis, FTIR spectroscopy, methylation analysis and ^{1}H NMR spectroscopy of the intact glycolipid and its limited degradation products, and negative liquid secondary ion mass spectrometry (LSIMS). The two dimensional chemical shift correlated spectroscopy and NOE spectroscopy provided information on the sugar sequence and linkage as well as anomeric configurations, so as to establish the presence of a 3-*O*-sulfated galactose and a Galα1-3Gal structure within the molecule. Negative LSIMS with collision-induced dissociation defined the sugar sequence and ceramide composition, allowing to confirm the presence, and indicating the position, of the sulfate group. The glycosphingolipid was found to be a mono-sulfated derivative of the isoglobo-series core, with the following structure:

HSO_3^--3Galβ1-3GalNAcβ1-3Galα1-3Galβ1-4Glcβ1-1Cer (iGb$_5$Cer V^3-sulfate).

Keywords: Isoglobopentaosylceramide sulfate; Rat kidney; Isoglobo-series core; Sulfated glycolipids; Tumour antigens

* Corresponding author.

[1] Abbreviations for sulfated glycolipids [1] follow the modifications of the nomenclature system of Svennerholm for gangliosides [2], and those of other lipids follow the recommendations of the IUPAC-IUB Commission of Biochemical Nomenclature [3].

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1. Introduction

Rat kidney is rich in sulfated glycolipids containing core structures of the gala-series [GalCer I^3-sulfate (SM4s[1])], and the ganglio-series such as GlcCer I^3-sulfate [4], LacCer II^3-sulfate (SM3), Gg_3Cer II^3-sulfate (SM2a) [5], Gg_3Cer II^3, III^3-bis-sulfate (SB2) [6], and Gg_4Cer II^3, IV^3-bis-sulfate (SB1a) [7]. Recently, the presence of a novel sulfated glycosphingolipid, isoglobotetraosylceramide sulfate (iGb_4Cer IV^3-sulfate), based on the isoglobo-series core, has been demonstrated in rat kidney [8]. In the present study, a more complex sulfated glycolipid (designated as Kc) was isolated and its structure elucidated, providing an additional example of the isoglobo-series sulfated glycolipid in rat kidney [9].

2. Experimental

Materials.—Normal rat kidneys (3.9 kg) were obtained from Sprague Dawley rats. iGb_4Cer IV^3-sulfate, isoglobotetraosylceramide (iGb_4Cer), and globotetraosylceramide (Gb_4Cer) were isolated from rat kidney [8]. Gangliotriaosylceramide (Gg_3Cer) from guinea pig red blood cells was purchased from Seikagaku Kogyo, Tokyo. Gangliotetraosylceramide (Gg_4Cer) was prepared from GM1 ganglioside ($II^3\alpha$NeuAc-Gg_4Cer) by mild acid hydrolysis [10]. DEAE-Toyopearl 650M was supplied by Tosoh, Tokyo. All other materials including glycolipids, reference compounds, reagents, and chemicals were from the same sources as described in Ref. [8].

Isolation of Kc.—The extraction and isolation procedure of the acidic glycolipid fraction from rat kidney by column chromatography on DEAE-Sephadex A-25 was essentially as previously described [8]. The mono-sulfated glycolipid fraction was also isolated by chromatography on DEAE-Toyopearl [11] using the FPLC system (Pharmacia LKB Biotechnology, Uppsala, Sweden), consisting of an LCC-500 PLUS control unit and two P-500 pumps. A batch of total lipids, from 500 g of rat kidneys, was dissolved in 500 mL of 5:10:1 $CHCl_3$–MeOH–H_2O and applied to a column (2.5 × 45 cm) of DEAE-Toyopearl 650M (acetate form). After neutral lipids were washed out, the acidic lipids were separated by the combination of two linear gradients using 1.0 L each of 5:10:1 $CHCl_3$–MeOH–ammonium acetate in water, 0 to 0.5 M, followed by 0.5 to 2 M, with a flow-rate of 5 mL/min. These procedures were repeated six times to extract a total of 3.9 kg rat kidneys. The fractions containing Kc together with other mono-sulfated glycolipids were combined and desalted. In order to remove other mono-sulfated glycolipids, particularly a large amount of SM4s, the combined fraction containing Kc was first separated by column chromatography using Iatrobeads 6RS-8060 (1.8 × 30 cm, 80 mL) and a linear gradient with a total of 2.2 L of 9:1 $CHCl_3$–MeOH to 50:50:5 $CHCl_3$–MeOH–H_2O [8]. The fractions containing Kc, with a slight overlapping of iGb_4Cer IV^3-sulfate, were combined and finally purified by HPLC using an Iatrobeads (6RS-8010) column (1 × 30 cm, 23.5 mL) with a linear 1.5-L gradient of 85:15:1 to 60:40:5 $CHCl_3$–MeOH–3 M NH_4OH.

Analysis.—TLC was performed on silica gel 60 high-performance TLC plates (Merck, Darmstadt, Germany); solvent and detection were as given in the legend of Fig. 1. Solvolysis of Kc (140 nmol) was performed [8] with 0.3 mL of 5 mM HCl in Me_2SO containing 0.5% MeOH at 80°C for 2 h and the products (designated as KcN-1 and KcN-2) purified

by HPLC on Iatrobeads 6RS-8005 (0.46×2 cm, 3.3 mL), using a linear gradient with a total of 250 mL of 85:15:1.5 to 40:60:5 $CHCl_3$–MeOH–3 M NH_4OH. The compositions of sugar, fatty acid, and sphingoid base were determined by analysis of the trimethylsilyl derivatives by GLC (GC-14A, Shimadzu, Kyoto) and GLC–MS (QP-1000, Shimadzu) [5,8,14]. The sulfate group was estimated after peracetylation using a cationic dye, azure A [1]. A modified procedure [8] of Ciucanu and Kerek [12] was used to permethylate the intact molecule and the products of solvolysis, and the acetates of partially methylated alditols were analyzed by both GLC and GLC–MS [5,8]. FTIR spectroscopy was performed on an FTIR-4200 (Shimadzu) [8]. ^1H NMR spectra were acquired at 400 MHz on a GX-400 spectrometer (Japan Electron Optical Laboratory, Tokyo). Double quantum filtered-chemical shift correlated spectroscopy (DQCOSY) and NOE spectra were obtained with spectral widths of 800 Hz either at 30 and 60°C in 98:2 $(CD_3)_2SO$–D_2O. The mixing time used for two-dimensional NOE experiments was 300–500 ms. The solvent resonance was suppressed by selective irradiation during the preparation period of 0.1 s. Spectral interpretation followed essentially the same procedure as previously outlined [8]. Negative liquid secondary ion mass spectrometry (LSIMS) was performed on a Concept IH mass spectrometer (Shimadzu/Kratos, Kyoto) fitted with a cesium ion gun. Spectra of native glycolipid (ca. 0.5 nmol) were recorded at an accelerating voltage of 8 kV, with a scan rate of 10 s/decade, and at a resolution of 2000. Triethanolamine was used as the liquid matrix. Linked scan spectra were obtained at constant B/E ratio after collision-induced dissociation (CID) had occurred in the first field-free region with air as the collision gas [13].

3. Results

Isolation of Kc.—Although similar elution profiles were obtained on both anion exchangers, DEAE-Sephadex and DEAE-Toyopearl, elution positions considerably differed. Kc was eluted simultaneously with iGb_4Cer IV^3-sulfate and a large amount of SM4s from the DEAE-Sephadex column with ca. 30 mM ammonium acetate in 5:10:1 $CHCl_3$–MeOH–H_2O. SM4s eluted from the DEAE-Toyopearl column at ca. 15 mM ammonium acetate, followed by both Kc and iGb_4Cer IV^3-sulfate, which eluted at ca. 20 mM ammonium acetate in 5:10:1 $CHCl_3$–MeOH–H_2O. Complete separation of Kc, which eluted later, from iGb_4Cer IV^3-sulfate was achieved by column chromatography on Iatrobeads. The isolated Kc was examined for purity by TLC in both neutral and basic solvent systems, and found to be a homogeneous band which stained with orcinol, but not with Dittmer and Lester reagent or resorcinol (Fig. 1). These staining characteristics indicated the presence of hexose and the absence of phosphate or sialic acid in the molecule. Kc migrated slower than iGb_4Cer IV^3-sulfate and similarly to globopentaosylceramide sulfate (Gb_5Cer V^3-sulfate) from human kidney [14] (data not shown). The yield of Kc was determined to be 0.11 nmol/g wet tissue by colorimetric analysis of the sulfate [1].

Solvolysis, compositional analysis, and FTIR spectroscopy.—After solvolysis at 80°C for 2 h, visual examination of a TLC separation indicated that ca. 50% of Kc was converted to a compound (KcN-1) with a similar R_f value to Gg_4Cer. Because neither iGb_5Cer or Gb_5Cer was available, Gg_4Cer, which has a similar R_f value to Gb_5Cer, was run as the standard glycolipid (Fig. 1). Additionally, ca. 20% of Kc was converted to a compound

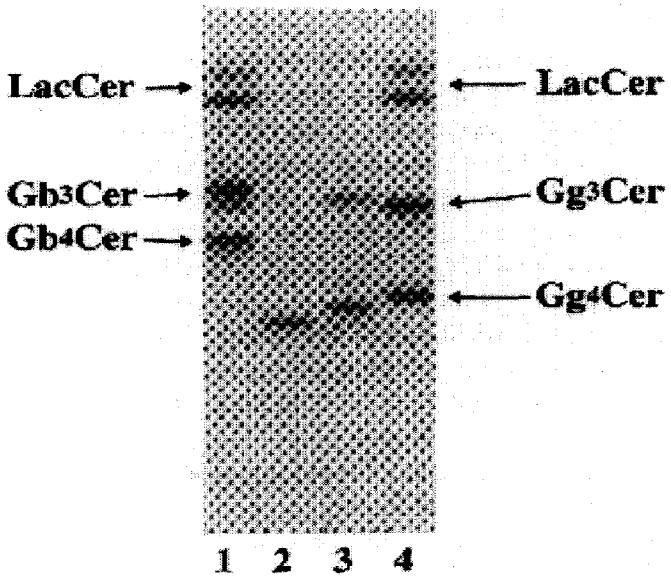

Fig. 1. TLC of isolated Kc and the products of solvolysis. Lane 1, LacCer, Gb_3Cer, and Gb_4Cer, from top to bottom; lane 2, Kc; Lane 3, products after solvolysis; lane 4, LacCer, Gg_3Cer, and Gg_4Cer, from top to bottom. The position of each standard glycosphingolipid is marked with arrows. The plate was developed with 60:40:9 $CHCl_3$–MeOH–0.2% $CaCl_2$. Glycolipid spots were visualized with orcinol reagent.

(KcN-2) with a similar R_f value to globotriaosylceramide (Gb_3Cer). Thus, ca. 30% of Kc appeared to remain unchanged under these conditions (Fig. 1). Complete desulfation does not appear to be possible without cleavage of the sugar components. This has already been observed for the solvolysis of several sulfated glycolipids with longer carbohydrate chains than SM3 [5–8,14].

Analysis of the sugar constituents of Kc established the presence of 3 mol of galactose and 1 mol each of glucose and *N*-acetylgalactosamine. KcN-1 contained galactose, glucose, and *N*-acetylgalactosamine with ratios of 3:1:1, and KcN-2 contained 2 mol of galactose and 1 mol of glucose (Table 1). These data indicated that the loss of a sulfate from Kc produced KcN-1, and that a Gal-GalNAc (or GalNAc-Gal) structure is situated at the non-reducing terminal of Kc. The colorimetric analysis of Kc demonstrated the presence of a sulfate ester group (Table 1). The FTIR spectrum (data not shown) showed the presence of absorptions at 1240 cm^{-1} (S=O stretching) and 810 cm^{-1} (C–O–S vibration), char-

Table 1
Carbohydrate composition of native and partially degraded Kc

Compound	Glucose [a]	Galactose	*N*-acetylgalactosamine	Sulfate [b]
	mol/mol [c]			
Kc	1.0	2.7	0.9	1.0
KcN-1	1.0	2.8	0.9	
KcN-2	1.0	1.8		

[a] The trimethylsilyl derivatives of methyl glycosides were analyzed by GLC. [b] The sulfate ester was estimated by the peracetylation method using azure A [1]. [c] The molar ratio was calculated with glucose as 1.0.

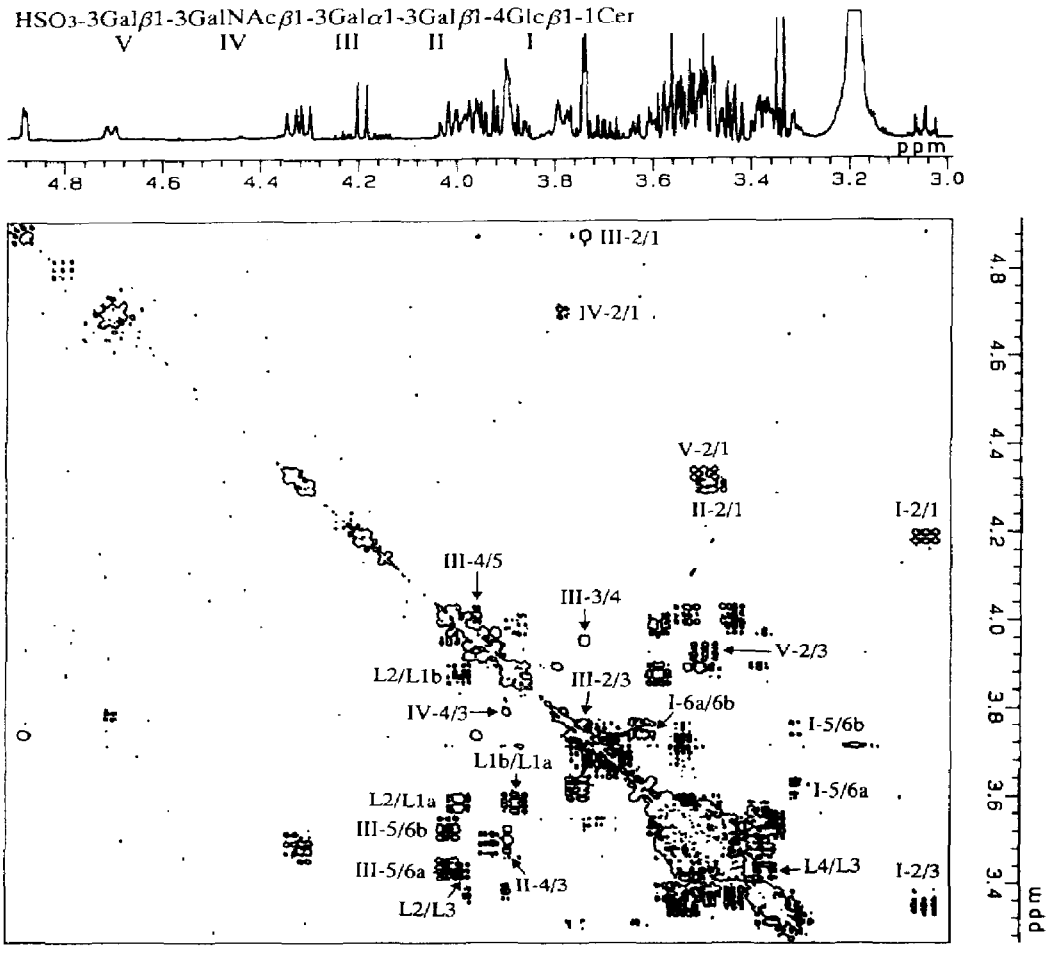

Fig. 2. Contour plot of the two-dimensional DQCOSY spectrum of Kc (300 nmol) at 60°C. The spectrum was recorded using 512 increments in t_1 and 96 scans for each t_1 experiment. Prior to the Fourier transformation, the time domain matrix was zero-filled to 2048 points in t_1 and the final matrix of 2K×2K data points yielded a resolution of 1.0 Hz/point. Spectral widths were 1050 Hz. The spectrum was weighted with Lorenzian–Gaussian functions before processing. The abbreviations labeling each cross-peak correspond to the numbering of sugar residues (Roman numerals), followed by the proton assignment (Arabic numeral). The figure immediately after the Roman numeral denotes the proton whose chemical shift defines the F_2 coordinate (horizontal axis), and the figure next to a slash (/) refers to the proton whose chemical shift is to be read on the F_1 (vertical axis).

acteristic of an equatorial sulfate ester of hexose [15,16]. The absorption arising from C–OH (1060 cm^{-1}) was slightly more intense than that of iGb_4Cer IV^3-sulfate, supporting the hypothesis that Kc contains one more sugar moiety in the molecule. The ceramide part mainly contained 4-hydroxysphinganine (t18:0) (89.2%) and saturated fatty acids, 24:0 (22.5%) and 22:0 (22.1%). These results were consistent with Kc being a mono-sulfated pentaglycosylceramide. The coelution of Kc with iGb_4Cer IV^3-sulfate as well as other mono-sulfated glycolipids from the anion exchange columns also supported the fact that Kc has one sulfate ester group in the molecule.

^1H NMR spectroscopy.—The one-dimensional spectra of the anomeric and ring proton region indicated that Kc (Fig. 2) and KcN-1 contained an α-hexose, three β-hexoses, and

Table 2
Chemical shifts (ppm from tetramethylsilane) and coupling constants (Hz, in parentheses) for Kc, KcN-1, KcN-2, and iGb_3Cer at 60°C

H atom	Chemical shifts (J)				
	V Galβ1-3	IV GalNAcβ1-3	III Galα1-3(1-4) [a]	II Galβ1-4	I Glcβ1-
Gb_5Cer V^3-sulfate [a,b]					
H-1($^3J_{1,2}$)	4.325(7.8)	4.615(8.3)	4.828(3.9)	4.27(7.3)	4.183(7.8)
Kc					
H-1($^3J_{1,2}$)	4.336(7.5)	4.705(7.3)	4.881(2.7)	4.307(7.3)	4.192(7.8)
H-2($^3J_{2,3}$)	3.496(8.7)	3.783(7.8)	3.739(9.8)	3.476(8.1)	3.045(8.2)
H-3($^3J_{3,4}$)	3.931(3.3)	3.801(2.9)	3.727(2.6)	3.511(2.9)	3.352 [c]
H-4	3.977 [c]	3.896 [c]	3.956 [c]	3.893 [c]	
KcN-1					
H-1($^3J_{1,2}$)	4.229(7.3)	4.719(6.8)	4.887(3.4)	4.308(7.3)	4.191(7.8)
KcN-2					
H-1($^3J_{1,2}$)			4.851(3.4)	4.300(7.3)	4.190(7.3)
iGb_3Cer from iGb_4Cer IV^3-sulfate [d]					
H-1($^3J_{1,2}$)			4.849(3.7)	4.302(7.3)	4.189(7.8)
Sphingoid					
	L1a ($^2J_{a,b}/^3J_{a,2}/^3J_{b,2}$)	L1b	L2 ($^3J_{2,3}$)	L3 ($^3J_{3,4}$)	L4
Kc	3.586 (−10.3/4.8/6.5)	3.878	3.995 (6.4)	3.433 (5.1)	3.37 [d]

[a] Gb_5Cer V^3-sulfate has a Galα1-4 structure instead of a Galα1-3 [14]. [b] Data from Ref. [14]. [c] Coupling constants could not be obtained due to overlapping signals, the secondary line shape, and/or the limit of resolution. [d] Data from Ref. [8].

a β-*N*-acetylhexosamine. KcN-2 was found to be formed from Kc by the loss of a sulfate, a β-hexose, and a β-*N*-acetylhexosamine according to the chemical shifts and coupling constants similar to those of iGb_3Cer [8,17]. The proton–proton shift correlations of Kc (Fig. 2), iGb_3Cer, and iGb_4Cer helped to follow all of the connectivities needed for the assignment of H-1 to H-4 of each component monosaccharide and the protons at C-1 to C-4 of the sphingoid (Table 2).

The NOE spectrum of Kc obtained at 30°C (data not shown) showed interresidue NOEs between the proton pairs, β-GalH-1/β-GalNAcH-3, β-GalNAcH-1/α-GalH-3, α-GalH-1/β-GalH-3, and β-GalH-1/β-GlcH-4. The relative magnitudes of interresidue NOEs of Gal(III)H-1/Gal(II)H-3 and Gal(III)H-1/Gal(II)H-4 were approximately equal, implying that the preferred solution conformation of the α-(1→3)-linkage results in close proximity (<3 nm) of Gal(III)H-1 to both H-3 and H-4 of Gal(II) [8]. The similar interresidual NOE contours had been observed across the glycosidic linkage of β-Gal(V)H-1/β-GalNAc(IV)H-3 [18]. These interresidual NOE assignments suggested the saccharide linkages of Kc are Galβ(V)1-3GalNAcβ(IV)1-3Galα(III)1-3Galβ(II)1-4Glcβ.

H-1, H-2, H-3, and H-4 of the terminal β-galactose had a larger downfield shift by 0.09–0.11, 0.16–0.17, 0.64–0.67, and 0.34–0.35 ppm, respectively, as compared to those of the

Table 3
Partially methylated alditol acetates obtained by degradation of the permethylated Kc before and after solvolysis

Compound	Gal- [a]	-3Gal1-	-3GalNAc1-	-4Glc1-
	mol/mol [b]			
Kc		2.9	0.9	1.0
KcN-1	0.9	1.8	0.8	1.0
KcN-2	0.7	1.0		1.0

[a] Peaks were identified by GLC and GLC–MS, as described in the text. The sugar residues are shown as for -3Gal1- which is an abbreviation of 1,3,5-acetyl-2,4,6-methylgalactitol. [b] The molar ratio of alditol acetates was calculated with 2,3,6-tri-*O*-methylglucitol acetate (-4Glc1-) as 1.0 and corrected using the values obtained from standard glycolipids.

terminal galactose in Gg_4Cer, GM1, or Gg_4Cer II^3-sulfate (SM1a) (unpublished results). The difference in the chemical shifts was the largest (0.64–0.67 ppm) with H-3, suggesting [8] that the sulfate group was located on C-3 hydroxyl of the β-galactose (V). The downfield shift (0.34–0.35 ppm) of H-4 was similar to that observed for H-4 of β-galactose of other sulfoglycolipids [14], and relatively smaller than that of the vicinal H-4 of 3-*O*-sulfated *N*-acetylgalactosamine [8] in iGb_4Cer IV^3-sulfate (0.450 ppm) and SB2 (0.471 ppm).

A pair of methylene double doublets observed at 3.586 and 3.878 ppm, coupled with a quadruple doublet centered at 3.995 ppm was assigned to L1a, L1b, and L2 of 4-hydroxysphinganine (t18:0), respectively (Table 2). L2 was further connected to a double doublet, L3, which was further coupled with a single proton at C-4 (L4). The line shape of L4 was a quadruple doublet, indicating coupling with two protons at C-5. The above results were at variance with the signals from 4-sphingenine (d18:1) structure [19], but completely compatible with the t18:0 structure of sphingoid. The chemical shifts are very close to those reported by Inagaki et al. [20].

Methylation analysis.—Permethylation analysis of Kc gave three major peaks on GLC, which co-chromatographed with the acetates of 2,3,6-tri-*O*-methylglucitol (-4Glc1-), 2,4,6-tri-*O*-methylgalactitol (-3Gal1-), and 4,6-di-*O*-methyl-2-*N*-methylacetamidogalactitol (-3GalNAc1-), in the molar ratio of 1:3:1, respectively (Table 3). These peaks were also analyzed by GLC–MS [8]. Desulfation before permethylation and degradation resulted in the disappearance of -3Gal1-; instead a terminal galactose (Gal1-) appeared, showing that the sulfate group is linked to the third position of the terminal galactose. In KcN-2, 1 mol each of -3Gal1- and -3GalNAc1- disappeared; instead, 1 mol of Gal1- appeared, indicating that a -3Gal-3GalNAc (not a -3GalNAc-3Gal) structure is situated at the non-reducing terminal of Kc, and that *N*-acetylgalactosamine was attached to the C-3 hydroxyl of the internal galactose. These results supported the linkage assignments given by NOE.

Negative liquid secondary ion mass spectrometry.—In the mass spectrum (Fig. 3A), an intense deprotonated molecular anion $[M-H]^-$ was detected at m/z 1597. This is consistent with a structure derived from a glycosphingolipid containing a sulfate ester, an *N*-acetylhexosamine, four hexoses, and a ceramide which corresponds to the combination of the major fatty acid (24:0) plus 4-hydroxysphinganine (t18:0). Less-abundant molecular anions, corresponding to other ceramide compositions, were also detected at m/z 1569

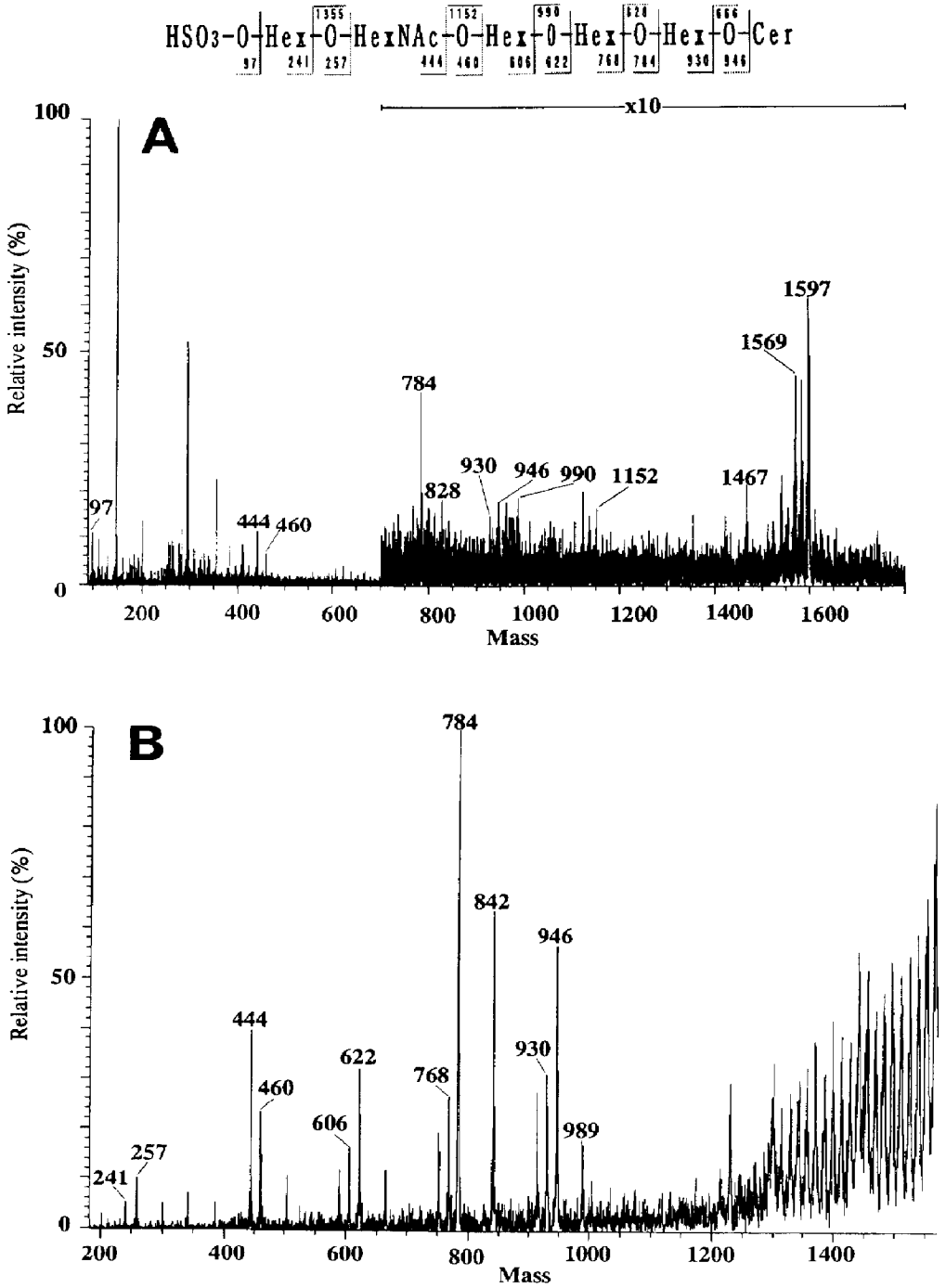

Fig. 3. Mass spectra of Kc: A, negative ion liquid secondary ion mass spectrum in triethanolamine matrix; B, collision-induced dissociation linked scan spectrum at constant B/E ratio of the molecular anion $[M-H]^-$ at m/z 1597.

(22:0 plus t18:0), 1541 (20:0 plus t18:0), 1523 (20:0 plus d18:1), and 1467 (16:0 plus d18:1). The ion at m/z 97 corresponds [21–23] to the hydrogen sulfate anion $[OSO_3H]^-$. The CID linked scan spectrum of the $[M-H]^-$ ion (m/z 1597) (Fig. 3B) showed the series of product ions that arose by the sequential cleavage of the glycosidic bonds on either side of the oxygen atom [24–26]. The peaks at m/z 241 and 257 correspond to the terminal sulfated hexose. The ions arising from the terminal sulfated disaccharide $[HSO_3$-HexNAc-O-Hex-(O) $-2H]^-$ were observed at m/z 444 and 460. By analogy, the peaks at m/z 606, 622, 768, 784, 930, and 946 were derived from the sulfated tri-, tetra-, and penta-saccharides, respectively. The peak at m/z 842 could originate from the cleavage of the glucose ring linked to the ceramide [26]. In addition, the normal spectrum (Fig. 3A) showed a group of ceramide-containing fragments, -O-Hex-O-Hex-O-Hex-O-Cer (m/z 1152), -O-Hex-O-Hex-O-Cer (m/z 990), and -O-Hex-O-Cer (m/z 828), for the C24:0/t18:0. The CID spectrum (Fig. 3B) also contained a series of ions at the higher mass region ($m/z > 1200$) as a result of C–C bond cleavages by charge-remote fragmentation [23]. These results further confirmed the structure for Kc, which is a mono-sulfated isoglobopentaosylceramide, HSO_3^--3Galβ1-3GalNAcβ1-3Galα1-3Galβ1-4Glcβ1-1Cer (iGb_5Cer V^3-sulfate).

4. Discussion

The present results show that the structure of the new sulfoglycolipid (Kc) is HSO_3^--3Galβ1-3GalNAcβ1-3Galα1-3Galβ1-4Glcβ1-1Cer (iGb_5Cer V^3-sulfate). The rat is, so far, the only known source of the GalNAcβ1-3Galα1-3Gal sequence, but the novelty is that this structure is extended by HSO_3^--Galβ1-3. A similar extension by Galβ1-3 of iGb_4Cer was found in rat gastric mucosa [27] to allow for further addition of Fucα1-2 and Galα1-3. The yield of iGb_5Cer V^3-sulfate was 0.11 nmol/g wet tissue, which was about one third of that of iGb_4Cer IV^3-sulfate from rat kidney [8]. This value was ca. 0.6- and 3-fold of Gb_5Cer IV^3-sulfate and Gb_4Cer V^3-sulfate, respectively, from human kidney [14,28].

The intact substance was analyzed by negative ion LSIMS and high-energy CID of the deprotonated molecular anion. From the LSIMS spectrum it is possible to deduce the combination of fatty acids and sphingoid bases present in the various molecular species. In the CID experiment, lower mass matrix background peaks are eliminated and a series of ions corresponding to sulfated mono- to penta-saccharides clearly showed the linear sugar sequence. Several earlier reports on FABMS of glycolipids and saccharides showed that permethylated samples gave more intense spectra and more easily interpretable fragment ions than native samples [29,30]. Leffler et al. [22] analyzed Gg_4Cer IV^3-sulfate (SM1b) by FABMS in various ways and concluded that analysis of permethylated derivative (for ca. 2 μg of sample) in the negative ion mode was preferred. In the present case the emphasis was to obtain molecular and fragment ions, including the sulfate group plus 1–5 residues of the sugar chain, and these were easily observed from 0.6 nmol (ca. 1 μg) of native sample by negative LSIMS and CID.

Expression of sulfated glycolipids has recently been shown to be associated with human cancers [31–37]. The normal human liver contains essentially no detectable amount of sulfated glycolipids, however, the remarkable accumulation of SB1a was observed in human hepatocellular carcinoma cells and tissues [35]. Sulfated glycolipids increased markedly

in human renal cell carcinoma and this increment was due to the elevation of the sulfotransferase activities of the cancer cells themselves [36]. As shown in these cases, glycolipids in a human tumor often reflect quantitative and qualitative changes of the particular lipids present in an organ from which the tumor is derived. iGb_4Cer, the species specific glycolipid of rat, is not characteristic of rat tumor but found to be associated with some malignant neoplasms [27]. Therefore, it seems relevant to clarify how the expression of the sulfate esters of iGb_4Cer and iGb_5Cer relates to the malignant alteration of rat kidney. Further investigations are necessary to clarify the biological significance of these novel sulfated amphiphiles in rat kidney.

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