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Note

Complete ^{1}H and ^{13}C NMR assignment of mono-sulfated galactosylceramides with four types of ceramides from human kidney

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Abstract

The full assignment of ^{1}H and ^{13}C NMR signals of galactosylceramide 3-sulfate (galactosyl sulfatide) and ^{1}H signals of galactosylceramide 6-sulfate was achieved by using $^{1}H-^{1}H$ DQF-COSY and $^{1}H-^{13}C$ heteronuclear COSY. Analyses were performed on a mixture of galactosyl sulfatides with four representative ceramide types consisting of a combination of non-hydroxy or 2-hydroxy fatty acids and sphingenine or 4*D*-hydroxysphinganine (trihydroxysphinganine) as the long-chain bases. The ^{1}H and ^{13}C NMR parameters of galactosyl sulfatide with 4-hydroxysphinganine as well as ^{13}C signals of complex lipids with 4-hydroxysphinganine were elucidated for the first time. Not only sulfation of the galactosyl residue, but also modification of the aglycon, including hydroxylation of fatty acids and hydration of the double bond in sphingoid bases, altered the chemical shifts substantially. In addition, the unique long-range coupling constants, $^{4}J_{H,H}$ and $^{5}J_{H,H}$, in the galactosyl residue of galactosyl sulfatide could be determined.

Keywords: Galactosyl sulfatide; ^{1}H and ^{13}C NMR; Ceramide structure; Long-range proton–proton coupling constant

1. Introduction

Sulfated glycolipids, the unique membrane components of glandular epithelial tissues of vertebrates [1], may function as one of the ion barriers to adapt to the environmental osmolality [2–5]. In recent years, considerable attention has also been directed to the interaction of sulfated glycolipids, especially the major sulfated glycolipid, galactosyl sulfatide (HSO_3-3Galβ1-1Cer, designated as sulfatide in the present paper), with several proteins that possess established biological roles [6,7]. In general, glycolipids have diverse aglycons, including heterogeneities in the hydrocarbon chain length as well as the presence of hydroxyl groups and double bonds, which may in turn affect the conformation of their carbohydrate chain [8–11]. The kidney contains a significant amount of 4-hydroxysphinganine (t18:0) in sphingolipids, whose

Abbreviations: Sulfatide, galactosylceramide 3-sulfate, HSO_3-3Galβ1-1Cer; Sulfatide-6, galactosylceramide 6-sulfate, HSO_3-6Galβ1-1Cer; GalCer, galactosylceramide, Galβ1-1Cer; d18:1, 4-sphingenine; t18:0, 4*D*-hydroxysphinganine; hFA, 2-hydroxy fatty acid; nFA, non-hydroxy fatty acid; COSY, correlated spectroscopy; DQF, double-quantum-filtered.

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4-hydroxyl group is a hydrogen donor to form hydrogen bonds with the polar groups of the membrane lipids [2]. The ^{1}H and ^{13}C NMR parameters of the molecular types of sulfatide have been only partially reported [12–16]. In the present study, we fully assigned the ^{1}H and ^{13}C signals of the four representative molecular types of sulfatides, for the first time including t18:0-containing types.

In the partial DQF-COSY spectrum of sulfatides (Fig. 1) we could observe two extra doublets in the vicinity of two sets of the major doublets in the anomeric region. From the peak area of the four anomeric signals, the molar ratios of four ceramide types, d18:1/hFA, d18:1/nFA, t18:0/hFA and t18:0/nFA, of sulfatides from the whole adult human kidney were determined for the first time in the present report to be 37, 46, 8 and 9%, respectively.

Two spectral features characteristic of t18:0-containing sulfatides were noted. First, H-2′ was found in a lower field than H-1′b due to the shift increment of H-2′ caused by the 4,5-hydration of d18:1 ($\Delta\delta$ are 0.252 and 0.189 ppm for sulfatides containing hFA and nFA, respectively) (Table 1). This proximity of H-2′ particularly in sulfatide(t18:0/hFA) to the anomeric protons, together with its characteristic quasi-quartet line shape due to the $^{3}J_{H-2',H-3'}$ (6.0 Hz) smaller than that of d18:1 (7.7 Hz), is helpful for assignment. Secondly, H-1′b shifted to a higher field and H-1′a to a lower field than the corresponding protons in d18:1, resulting in tighter couplings of H-1′a and b in t18:0. To summarize, the chemical

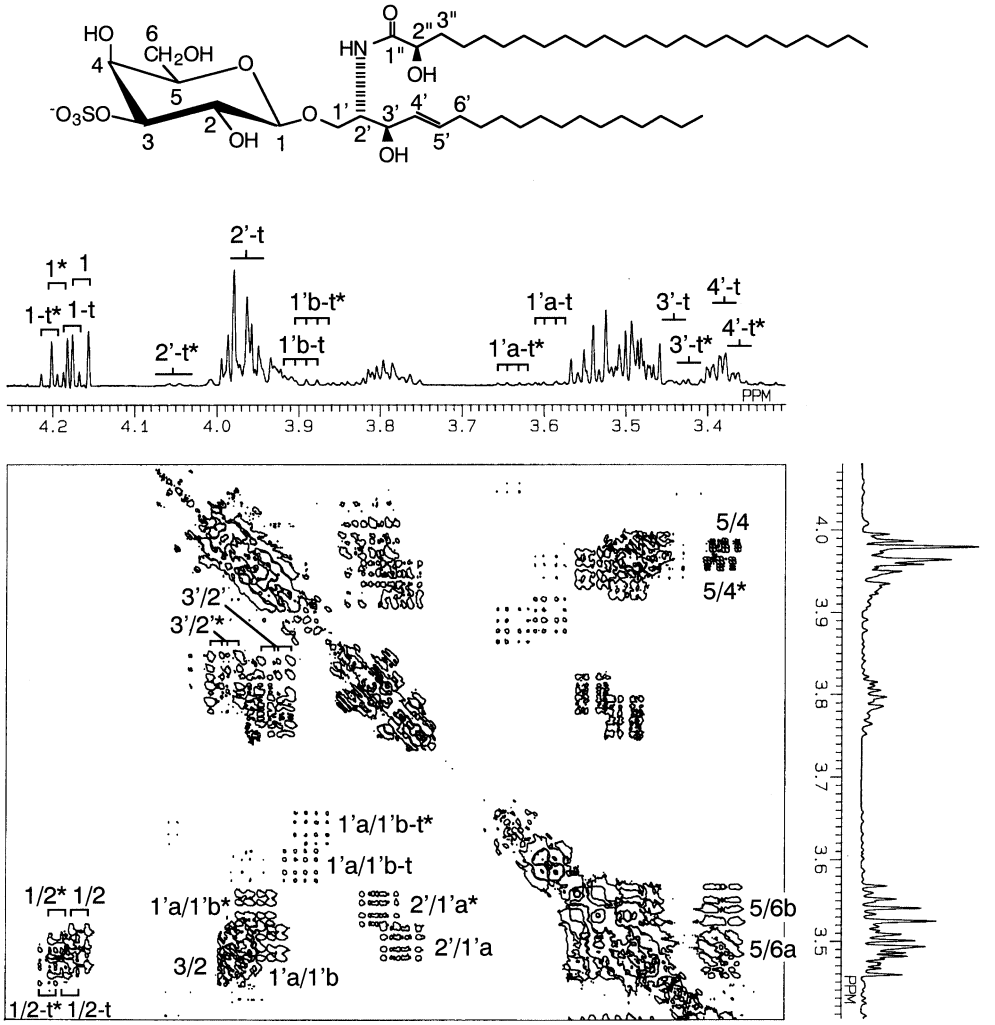

Fig. 1. Partial DQF-COSY spectrum of sulfatide. The figures marked with asterisks (*) refer to the protons in sulfatides containing hFA and the figures accompanied by -t refer to those in sulfatide containing t18:0.

Table 1
^{1}H chemical shifts (ppm) of sulfatide, sulfatide-6 and GalCer

		Galactosyl residue						
Glycolipid	Ceramide type	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
1 sulfatide	d18:1/hFA	4.192	3.480	3.979	3.959	3.385	3.487	3.545
2	d18:1/nFA	4.166	3.492	3.964	3.982	3.379	3.482	3.546
3	t18:0/hFA	4.204	3.469	3.981	3.959	3.385	3.487	3.545
4	t18:0/nFA	4.177	3.485	3.965	3.982	3.379	3.482	3.546
5 sulfatide-6	d18:1/hFA	4.093	3.288	3.285	3.631	3.563	3.794	3.880
6 GalCer	d18:1/hFA	4.088	3.304	3.283	3.644	3.339	3.494	3.546
7	d18:1/nFA	4.052	3.323	3.279	3.649	3.333	3.488	3.549

		Long-chain base							Fatty acid		
Glycolipid	Ceramide type	H-1′a	H-1′b	H-2′	H-3′	H-4′	H-5′	H-6′	H-2″	H-3″(a)	(H-3″b)
1 sulfatide	d18:1/hFA	3.541	3.953	3.800	3.990	5.382	5.576	1.937	3.801	1.423	1.572
2	d18:1/nFA	3.500	3.940	3.773	3.928	5.375	5.551	1.937	2.032	1.451	
3	t18:0/hFA	3.638	3.884	4.052	3.424	3.363	1.272		3.844	1.487	1.606
4	t18:0/nFA	3.593	3.897	3.962	3.445	3.379	1.272		2.073	1.481	
5 sulfatide-6	d18:1/hFA	3.527	3.920	3.817	3.993	5.377	5.584	1.941	3.809	1.427	1.575
6 GalCer	d18:1/hFA	3.540	3.909	3.814	4.004	5.383	5.587	1.945	3.812	1.434	1.569
7	d18:1/nFA	3.449	3.937	3.792	3.919	5.371	5.555	1.943	2.036	1.460	

shift differences between H-1′b and H-1′a in the four types of sulfatide d18:1/hFA, d18:1/nFA, t18:0/hFA and t18:0/nFA were 0.412, 0.440, 0.246 and 0.304 ppm, respectively. The values calculated for t18:0/hFA and t18:0/nFA linked to non-sulfated glucosyl residues [17,18] were similar to our results.

The 2-hydroxylation shifts of H-1′a and H-1′b in sulfatide(d18:1) were different from those of GalCer(d18:1), suggesting that the anisotropic environment of the sphingoid H-1′ protons produced by a fatty acid 2-hydroxyl group was influenced by 3-sulfation of the Gal residue.

^{13}C signals were identified (Table 2) by ^{1}H–^{13}C COSY (Fig. 2). ^{13}C parameters of t18:0 in complex lipids from an animal have never been reported. The C-2′ signal can serve as a marker signal of t18:0 because it resonated at a higher field than the corresponding carbon in d18:1.

The signals of H-2 and H-3 of the galactosyl residue in GalCer were more strongly coupled to each other in 6% D_2O–Me_2SO-d_6 than in 2% D_2O–Me_2SO-d_6, resulting in distorted line shapes of adjacent H-1 and H-4 enabling us to delineate $^4J_{H\text{-}1,H\text{-}3}$ and $^5J_{H\text{-}1,H\text{-}4}$. Spin-simulation studies of GalCer(d18:1/hFA) were performed varying the chemical shifts and coupling constants. Although manipulation of $^3J_{H,H}$ only could not reproduce the observed line shape of the anomeric signal (Fig. 3), substitution of -0.3 and -0.08 Hz for $^4J_{H\text{-}1,H\text{-}3}$ and $^5J_{H\text{-}1,H\text{-}4}$, respectively, could achieve complete reproduction of the spectrum. Actually, the line shape of H-1 was changed by decoupling the H-4 proton, confirming the existence of $^5J_{H\text{-}1,H\text{-}4}$ (data not shown). We could not find any strongly coupled signals suitable for similar studies in the spectrum of sulfatide. But there is a possibility that $^4J_{H\text{-}1,H\text{-}3}$ and $^5J_{H\text{-}1,H\text{-}4}$ have the significant magnitude in sulfatide, because $^3J_{H,H}$ values of galactosyl ring protons in sulfatide were similar to those of GalCer.

2. Experimental

Sulfatide as a mixture of four types of ceramides was isolated in this laboratory from human kidney [6]. Galactosylceramide 6-sulfate (HSO_3-6Galβ1-1Cer, sulfatide-6) containing 2-hydroxy fatty acids and 4-sphingenine (d18:1/hFA) was a kind gift from Dr Y. Sanai

Table 2
^{13}C chemical shifts (ppm) of sulfatide and GalCer

		Galactosyl residue					
Glycolipid	Ceramide type	C-1	C-2	C-3	C-4	C-5	C-6
1 sulfatide	d18:1/hFA	103.94	69.14	79.17	66.52	75.04	60.23
2	d18:1/nFA	104.31	69.24	79.13	66.43	75.00	60.21
3	t18:0/hFA	103.87	69.25	79.17	66.52	75.04	60.23
4	t18:0/nFA	104.18	69.35	79.11	66.43	75.00	60.21
6 GalCer	d18:1/hFA	103.99	70.53	73.28	68.10	75.26	60.43
7	d18:1/nFA	104.37	70.63	73.13	68.06	75.22	60.41

		Long-chain base						Fatty acid			
Glycolipid	Ceramide type	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1″	C-2″	C-3″	C-4″
1 sulfatide	d18:1/hFA	68.26	52.82	70.69	130.81	131.41	31.69	173.58	71.09	34.40	24.59
2	d18:1/nFA	68.79	53.35	70.95	131.13	131.21	31.69	171.86	35.65	25.27	29 [a]
3	t18:0/hFA	68.46	49.87	74.09	70.76	31.80	25.33	173.52	71.01	34.31	24.46
4	t18:0/nFA	68.90	50.47	73.90	70.78	31.80	25.33	171.97	35.58	25.38	29 [a]
6 GalCer	d18:1/hFA	68.46	52.88	70.57	130.76	131.42	31.64	173.69	71.06	34.36	24.50
7	d18:1/nFA	68.85	53.19	70.74	131.23	131.27	31.63	171.91	35.60	25.26	29 [a]

[a] The carbon was assigned to one of the several signals around 29 ppm.

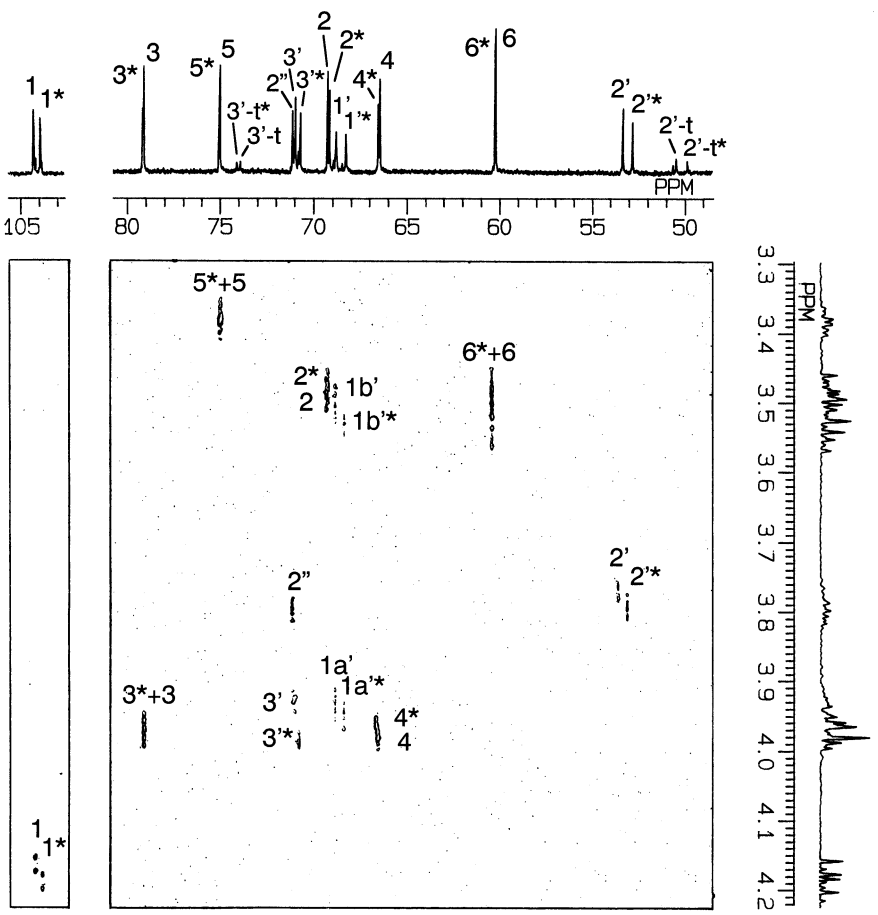

Fig. 2. Partial $^{1}H-^{13}C$ COSY spectrum of sulfatide. The abbreviations of each signal are similar to those in Fig. 1.

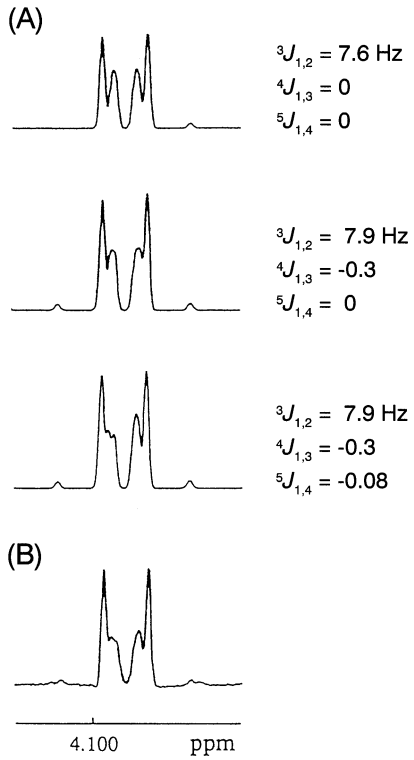

Fig. 3. ^{1}H NMR spin simulation of the H-1 signal in the spectrum of GalCer(d18:1/hFA). (A) The spectra calculated with the parameters $^3J_{1,2}$, $^4J_{1,3}$ and $^5J_{1,4}$ with a line width of 1.0 Hz. (B) The spectrum observed in 6% D_2O–Me_2SO-d_6 at 45 °C.

and Dr Y. Nagai (Department of Biochemistry, Faculty of Medicine, University of Tokyo) [19]. Galactosylceramides from bovine brain consisting of 98% of 2-hydroxy fatty acids (GalCer(d18:1/hFA)) and non-hydroxy fatty acids (GalCer(d18:1/nFA)), respectively, were obtained from Sigma.

NMR spectroscopy.—The purified glycolipids were deuterium-exchanged and lyophilized twice in D_2O, and then dried over P_2O_5 under a high vacuum before dissolution in Me_2SO-d_6–D_2O (98:2, v/v) [20]. The final concentrations of sulfatide, sulfatide-6 and three GalCers were ca. 50, 1 and 20 mM, respectively. ^{1}H and ^{13}C NMR analyses were performed using a JEOL GX-400 spectrometer operating at 400 and 100 MHz, respectively, with a probe temperature of 60 °C. Chemical shifts were referenced to tetramethylsilane. Double-quantum-filtered COSY (DQF-COSY) spectra were recorded with 512 increments in t_1 and 72 scans were collected for each t_1 experiment. Spectral widths were 625 Hz in both dimensions. After zero-filling, the time-domain spectrum was transformed to give 4k × 1k data point matrices with a resolution of 1.2 and 0.3 Hz/point in the ω_1 and ω_2 dimensions, respectively. ^{1}H–^{13}C heteronuclear COSY (^{1}H–^{13}C COSY) spectra were recorded with 256 increments in t_1 and 272 scans were collected for each t_1 experiment. The spectral widths were 20 kHz in ω_2 (for ^{13}C) and 380 Hz in ω_1 (for ^{1}H). After zero-filling, the time-domain spectrum was transformed to give 4096 × 512 data point matrices with a resolution of 9.8 and 1.5 Hz/point, respectively. Spin-simulation studies were performed by using the COMICS program (JEOL).

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