reverse-phase HPLC, eluting with 25% water in acetonitrile. The yield of 1-naphthylamine is less than 4%, and the yield of 1,1'-azonaphthalene is 50% at 10% conversion of 1-NaN_3.

Photolysis of 1-Pyrene Azide in Diethylamine. A solution of 1-PyN_3 (4×10^{-3} M) in neat DEA was purged with nitrogen and irradiated at 350 nm. The product, 1-pyreneamine, was isolated in 82% yield by thin-layer chromatography on silica gel, eluting with benzene, and identified by comparison of its spectral data with those of an authentic sample. A similar solution of 1-PyN_3 (2×10^{-4} M) was irradiated with the nitrogen laser at 337 nm and 2 Hz. After photolysis, diethyl phthalate was added as internal standard. The solvent was removed and the sample analyzed by HPLC, eluting with 1% 2-propanol in hexane. The yield of 1-pyreneamine in this case was 4%.

Triplet Sensitization of 1-Pyrenyl Azide in 1 M Diethylamine. A solution of 1-PyN_3 (4×10^{-4} M), 2-acetonaphthone (2.1×10^{-2} M), and DEA (1.0 M) in benzene was purged with nitrogen and irradiated with a 200-W Hg lamp filtered through a Corning 0-51 glass plate. After photolysis diethyl phthalate was added as an internal standard, and the solvent was removed. The residue was diluted with benzene and analyzed by HPLC, eluting with 1% 2-propanol in hexane. The yield of 1-pyrenamine is 55%. To insure that the 1-pyreneamine was stable to the reaction conditions, a solution of 1-pyreneamine (3×10^{-4} M), 2-acetonaphthone (2.1×10^{-2} M), and 1 M DEA was treated exactly as above. HPLC analysis shows that on prolonged irradiation only a small amount of the 1-pyreneamine is consumed.

Triplet Sensitization of the 2-Aryl Azides. A solution of 2-NaN_3 (1×10^{-3} M), 2-acetonaphthone (1.5×10^{-2} M), and DEA (1.0 M) in benzene was purged with nitrogen and irradiated with the frequency-tripled Nd-YAG at 355 nm and 2 Hz or in the Rayonet photoreactor at 350 nm. The sensitizer absorbs more than 95% of the light under these conditions. After photolysis, diethyl phthalate was added as an internal standard. The sample was analyzed by analytical reverse-phase HPLC for the yield of 2,2'-azonaphthalene and by gas chromatography on an OV-101 column for the yields of 2-(diethylamino)-1-aminonaphthalene and 2-naphthylamine. A similar solution of 2-pyrene azide (3.4×10^{-4} M), 2-acetonaphthone (2×10^{-2} M), and diethylamine (1 M) was treated as above and analyzed by HPLC for 2-pyrenamine and 2-(diethylamino)-1-aminopyrene. Also, a similar sample of 2-PyN_3 without DEA was treated as above. The sample was monitored by UV-vis for 2,2'-azopyrene at 600 nm.

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Registry No. DEA, 109-89-7; 1-$NaNH_2$, 134-32-7; 2-$NaNH_2$, 91-59-8; 1-$PyNO_2$, 5522-43-0; 1-$PyNH_2$, 1606-67-3; 2-$PyNH_2$, 1732-23-6; 1-$NaN_2^+Cl^-$, 3177-49-9; 2-$NaN_2^+Cl^-$, 20893-80-5; 1-PyN_2^+, 41070-21-7; 2-PyN_2^+, 91110-56-4; 1-PyBr, 1714-29-0; 1-NaN_3, 6921-40-0; 2-NaN_3, 20937-86-4; 1-PyN_3, 36171-39-8; 2-PyN_3, 91110-61-1; 1-NaN, 3315-52-4; 2-NaN, 3315-51-3; 1-PyN, 36171-40-1; 1-NaN=NNa-1, 487-10-5; 2-NaN=NNa-2, 582-08-1; 1-NaN=NPh, 2653-70-5; 2-NaN=NPh, 2653-77-2; 2-PyN=NPy-2, 91110-57-5; $K^+NH_2^-$, 17242-52-3; $Na^+N_3^-$, 26628-22-8; $PhNO_2$, 98-95-3; PhN_3, 622-37-7; 2-(drethylamino)-1-aminonaphthalene, 54922-17-7; 1a*H*-naphthaleno[1,2-*b*]azirine, 91110-58-6; 7b*H*-naphthaleno[1,2-*b*]azirine, 91110-60-0; 1a*H*-pyreno[1,2-*b*]azirine, 91110-59-7; 2-acetonaphthone, 93-08-3.

Carbon-13 Spin–Lattice Relaxation Studies of G_{D1a} Micelles. Limited Segmental Motion of Head Group Saccharide Units[1]

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Abstract: Measurements of ^{13}C spin–lattice relaxation times (T_1) for G_{D1a} micelles have been made. The results indicate that the head group is relatively immobile with no overall anisotropic axis of rotation. There is evidence, however, for a small gradient of internal rotation for the individual saccharide units. Significant differences in relaxation were observed between the two sialic acid residues of G_{D1a}. The relaxation data show that carbons in the periphery of the saccharide rings (i.e., saccharide C-6 carbons and the sialic acid glycerol chains) exhibit relaxation behavior similar to the related system, neuraminyllactose (Czarniecki, M. F.; Thornton, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 8279–8282). These phenomena are explained in terms of models derived for neuraminyllactose when possible. The relatively low mobility observed for Cer C-2 and C-3 lent support to the existence of a hydrogen bond in the region of these two carbons. Interestingly, Cer C-1 was found to be significantly more mobile than surrounding carbons.

We wish to report the first complete carbon-13 NMR spin–lattice relaxation time (T_1) measurements for ganglioside micelles. These measurements provide important information regarding conformational mobilities and interactions of gangliosides, which are very significant lipid constituents of neuronal membranes.

Gangliosides, such as G_{D1a} (**1**), are membrane glycosphingolipids in which a sphingosine, usually *trans*-D-*erythro*-2-amino-1,3-dihydroxyoctadec-4-ene, is N-acylated with a fatty acid and linked glycosidically at C-1 to an oligosaccharide containing at least one sialic (NeuAc) acid residue. Variations among gangliosides mainly involve the oligosaccharide region, in which the number of residues and types of linkages can differ.[3]

Gangliosides are found in the outer leaflet of most mammalian cell membranes.[4] The oligosaccharide chains extend into the intercellular space where they can act as receptors for a variety of proteins and cations such as Ca^{2+}.[5,6] Of particular interest

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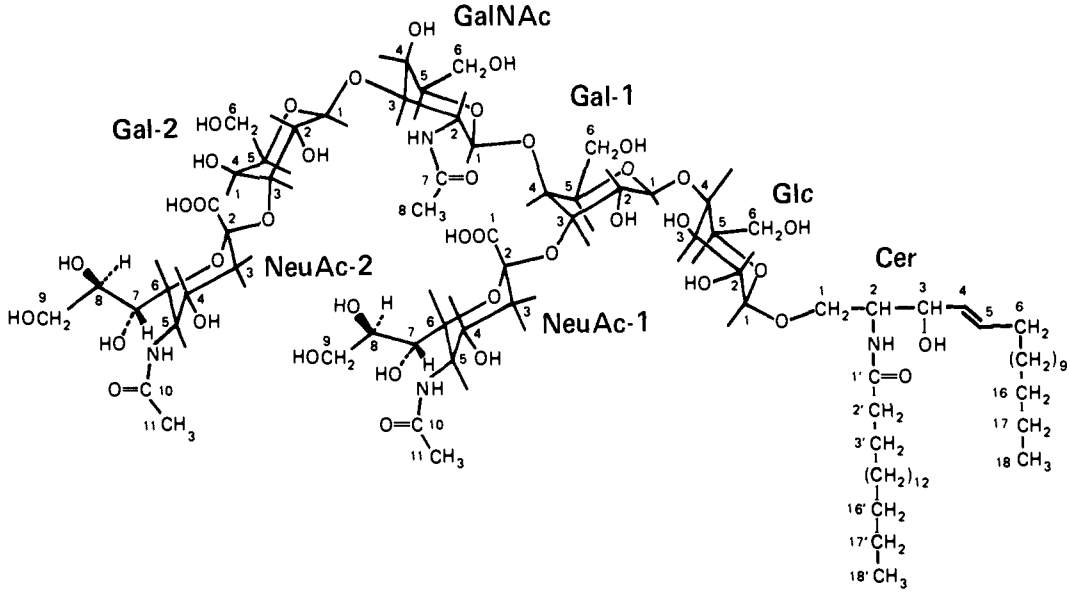

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is the fivefold enrichment of gangliosides in the synaptosomal membranes of the central nervous system.[7] This specific localization, taken in conjunction with properties observed for neuronal gangliosides, implies important structural and dynamic roles in neurofunction.[8]

A possible function for gangliosides, which does not seem to have been suggested previously, is suggested by the fact that very low amounts of gangliosides are detected in synaptic vesicles,[4a] which fuse with the neuronal membrane to release neutrotransmitters exocytotically and are then regenerated by endocytosis.[9] Gangliosides might control endocytosis by exhibiting an optimum concentration in neuronal membranes while being less stable in the highly curved membrane of the tiny synaptic vesicles. Exocytosis of synaptic vesicles, containing no gangliosides, would dilute the gangliosides in the neuronal membrane, and this could trigger or aid in the endocytosis of membrane-bound vesicles without gangliosides until the optimum ganglioside concentration could be re-established in the neuronal membrane.

In this laboratory, we have undertaken experiments designed to probe systematically the conformations, mobilities, and cation binding sites of ganglioside and ganglioside components as monomers and in model membrane systems. We first studied the ^{13}C NMR spectra and T_1 properties of sialic acid (NeuAc) derivatives, including the entire oligosaccharide of G_{M3}.[2,10,11] We have also investigated the complexation of NeuAc and some derivatives with Ca^{2+} via ^{13}C NMR.[12] Other investigators have since contributed closely related experiments.[13-16]

More recently, we used ^{13}C NMR to study three brain gangliosides, as monomers and while incorporated in two model membrane systems.[17] Work by other researchers has contributed the systematic assignment of the ^{13}C NMR spectra of seven brain gangliosides at high magnetic field,[18,19] as well as attempts at elucidating the metal ion and cholera toxin binding sites for G_{M1}.[18,20] The most recent ^{13}C studies of gangliosides involve a comparison of the ^{13}C spectra of G_{M1}, asialo G_{M1}, and the G_{M1} head group.[19b] Studies with ganglioside spin labels have given results in general agreement[21] with our previous ^{13}C work.[17]

Many T_1 studies on phospholipids in model membranes have appeared, leading to an understanding of the motions occurring in the hydrophobic portions of membrane lipids.[22] There are reports of T_1 for phosphatidylcholine methyl groups in the presence of gangliosides,[23,24] and studies have been reported for simple nonganglioside glycolipids.[25-28] We previously reported T_1 data for the main methylene peak of G_{D1a} in model systems.[17]

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Table I. ^{13}C NMR Chemical Shift Data for G_{D1a} Micelle Potassium Salts in D_2O Buffer (0.1 M KNO_3, 0.002 M K_2HPO_4, pH 8.6) at 46 °C

δ_C (Me_4Si) (external)	assignment	δ_C (Me_4Si) (external)	assignment
175.4	Cer C-1′	69.4	Gal-2 C-2
175.1	NeuAc-1 C-10		Cer C-1
	NeuAc-2 C-10	68.6	Gal-2 C-4
174.7	GalNAc C-7		GalNAc C-4
174.1	NeuAc-1 C-1		NeuAc-1 C-4
	NeuAc-2 C-1		NeuAc-2 C-4
134.8	Cer C-4		NeuAc-1 C-7
129.5	Cer C-5		NeuAc-2 C-7
104.6	Gal-2 C-1	63.0	NeuAc-1 C-9
102.9	Gal-1 C-1		NeuAc-2 C-9
	Glc C-1	61.2	Gal-1 C-6
	GalNAc C-1		Gal-2 C-6
101.8	NeuAc-1 C-2		GalNAc C-6
100.1	NeuAc-2 C-2	60.9	Glc C-6
80.9	GalNAc C-3	53.7	Cer C-2
79.3	Glc C-4	52.1	NeuAc-1 C-5
77.7	Gal-1 C-4		NeuAc-2 C-5
75.8	Gal-2 C-3	51.1	GalNAc C-2
74.8	Gal-1 C-5	39.9	NeuAc-2 C-3
	Gal-2 C-5	37.5	NeuAc-1 C-3
	Glc C-3	36.5	Cer C-2′
	Glc C-5	32.6	Cer C-6
	Gal-1 C-3	32.1	Cer C-16,16′
	GalNAc C-5	30.2	Cer C-4′-14′
73.1	NeuAc-1 C-6		Cer C-7-14
	NeuAc-2 C-6	29.7	Cer C-15,15′
	Glc C-2	26.2	Cer C-3′
72.4	NeuAc-1 C-8	23.0	GalNAc C-8
72.0	NeuAc-2 C-8	22.7	Cer C-17,17′
71.7	Cer C-3	22.5	NeuAc-1 C-11
70.1	Gal-1 C-2		NeuAc-2 C-11
		13.8	Cer C-18,18′

No T_1 measurements on the other carbons of gangliosides have been reported, however. Therefore, we report herein T_1 data for all 40 ^{13}C NMR resonances resolved in aqueous micelles of the major brain ganglioside G_{D1a} at 37.73 MHz. The ability to resolve a large number of resonances has enabled us to gain a qualitative understanding of the relative mobility and types of motions occurring for the entire carbon skeleton of G_{D1a} in the micellar environment. From this information, we postulate some organizational properties and interactions for gangliosides in membranes.

Experimental Section

Materials and Methods. Bovine brains were obtained from a local meat packer and transported to the laboratory on ice, whereupon they were used immediately or stored at −20 °C until needed. All solvents and reagents were of reagent grade. Spectral grade tetramethylsilane was used as the reference for all ^{13}C NMR spectra. All solvent systems described via ratios are volume:volume ratios, before mixing. All solutions described as percents are weight/volume percents, before mixing. E. M. Silica gel 60 (70–230 mesh) was used for gravity chromatographic columns. Baker silica gel for flash chromatography (40-μm average particle size) was used for flash chromatography columns. Baker glass-backed TLC plates (without fluorescence indicator) were used in examining the purity of ganglioside samples.

pH measurements were made with a Radiometer pH meter. All ^{13}C NMR spectra for spin–lattice relaxation studies were recorded on a Nicolet NT-150 wide-bore spectrometer (20-mm tubes).

Isolation of Gangliosides. A mixture of the various brain gangliosides was extracted from dried bovine brain according to the method of Gammack,[29] as modified in this laboratory.[30] Pure G_{D1a} was obtained from the crude gangliosides by a system of column chromatographic procedures. Crude separation of gangliosides was achieved by DEAE–cellulose ion exchange chromatography.[31,32] Pure G_{D1a} was obtained by either gravity or flash chromatography on silica gel. At each stage of isolation, the ganglioside content and purity were analyzed with two TLC systems with silica plates. Gangliosides were separated from neutral and polar lipids by development with 65:25:4 chloroform:methanol:water,[33] followed by visualization by application of a 7.40% solution of phosphomolybdic acid in ethanol, and heat.[34] Individual gangliosides were separated by development with 5:1 THF:0.08 M KCl.[35] These plates were visualized with 7.40% ethanolic phosphomolybdic acid as described above.

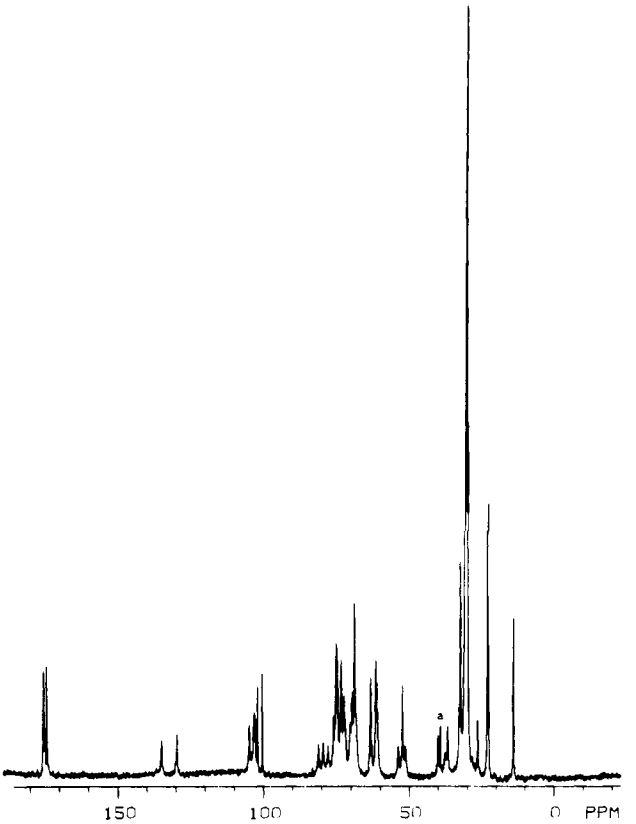

Figure 1. Fully relaxed ^{13}C spectrum for G_{D1a} micelle potassium salt in D_2O buffer (0.1 M KNO_3, 0.002 M K_2HPO_4, pH 8.6) at 46 °C. Peak a is internal Me_2SO (chemical shift standard).

^{13}C NMR Studies. Because of the deleterious effect of paramagnetic ions on T_1 values, special precautions were taken.[2,10,11,17,28,36] All equipment coming in contact with gangliosides to be studied by NMR or used in the preparation of the D_2O buffer was soaked in an alkaline 0.05 M Na_2EDTA solution for 12 h, followed by thorough rinsing with distilled, deionized water and drying in a 100 °C oven. The D_2O (Cambridge Isotopes, 99.8%) used in the buffer for the NMR experiments was distilled. The buffer, once prepared, was extracted three times by centrifugation with an equal quantity of 0.05% diphenylthiocarbazone in CCl_4.

G_{D1a} micelles were studied as the potassium salt in D_2O buffer (0.1 M KNO_3, 0.002 M K_2HPO_4, pH 8.6) at 46 ± 1 °C. The ^{13}C NMR T_1 experiments were performed at 37.73 MHz with complete proton noise decoupling. T_1 values were measured by using the fast-inversion–recovery method over a 4000-Hz band width with 8K time-domain points.[37] Prior to each experiment, the 90° and 180° pulses were determined. The 90° pulse was found to be in the range 20–24 μs. Relaxation times were calculated from sets of spectra representing 10–11 τ values with use of a least-squares fit to the three-parameter equation suggested by Levy and

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Table II. ^{13}C NMR Spin-Lattice Relaxation Times in Seconds[a-c] for G_{D1a} Micelle Potassium Salts in D_2O Buffer (0.1 M KNO_3, 0.002 M K_2HPO_4, pH 8.6) at 46 °C

NeuAc-1	NeuAc-2	Gal-2	GalNAc	Gal-1	Glc	Cer	Cer′
(1) 1.628 ± 0.059	(1) 1.628 ± 0.059	(1) 0.121 ± 0.005	(1) 0.108 ± 0.002	(1) 0.108 ± 0.002	(1) 0.108 ± 0.002	(1) 0.228 ± 0.006	(1′) 1.586 ± 0.099
(2) 1.310 ± 0.019	(2) 1.754 ± 0.093	(2) 0.114 ± 0.003	(2) 0.0904 ± 0.003	(2) 0.102 ± 0.002	(2) 0.120 ± 0.001	(2) 0.0855 ± 0.004	(2′) 0.208 ± 0.019
(3) 0.0918 ± 0.006	(3) 0.115 ± 0.002	(3) 0.120 ± 0.006	(3) 0.100 ± 0.002	(3) 0.120 ± 0.002	(3) 0.120 ± 0.002	(3) 0.121 ± 0.006	(3′) 0.255 ± 0.013
(4) 0.122 ± 0.003	(4) 0.122 ± 0.003	(4) 0.122 ± 0.003	(4) 0.122 ± 0.003	(4) 0.0935 ± 0.011	(4) 0.113 ± 0.007	(4) 0.204 ± 0.004	(4′)-(14′) 0.676 ± 0.006
(5) 0.118 ± 0.002	(5) 0.118 ± 0.002	(5) 0.120 ± 0.002	(5) 0.120 ± 0.002	(5) 0.120 ± 0.002	(5) 0.120 ± 0.002	(5) 0.212 ± 0.011	(15′) 0.892 ± 0.008
(6) 0.120 ± 0.001	(6) 0.120 ± 0.001	(6) 0.224 ± 0.002	(6) 0.224 ± 0.002	(6) 0.224 ± 0.002	(6) 0.166 ± 0.006	(6) 0.350 ± 0.052	(16′) 1.41 ± 0.045
(7) 0.122 ± 0.003	(7) 0.122 ± 0.003		(7) 1.201 ± 0.056			(7)-(14) 0.676 ± 0.006	(17′) 2.25 ± 0.063
(8) 0.120 ± 0.003	(8) 0.132 ± 0.005		(8) 1.929 ± 0.159			(15) 0.892 ± 0.008	(18′) 6.36 ± 0.738
(9) 0.192 ± 0.002	(9) 0.192 ± 0.002					(16) 1.41 ± 0.045	
(10) 1.464 ± 0.136	(10) 1.464 ± 0.136					(17) 2.25 ± 0.063	
(11) 1.446 ± 0.149	(11) 1.446 ± 0.149					(18) 6.36 ± 0.738	

[a] The numbers (1), (2), etc. refer to carbon numbers of G_{D1a} (cf. **1**). [b] Values are given in seconds as NT_1 values, where N is the number of hydrogens directly attached to the carbon. However, T_1 values are given for nonprotonated carbons. It should be noted that equal NT_1 values are given in several cases for carbons that are not resolved from each other (see Table I). In these cases, no evidence could be seen in T_1 plots to indicate significantly different T_1 values; therefore, the individual T_1 values must not be far from equal. [c] The ± figures are standard deviations based on three separate T_1 experiments.

Peat[38] for the calculation of T_1 while correcting for inhomogeneous H_1 fields.

Before and after each spin-lattice relaxation study, the purity of each sample was tested in the two solvent systems previously described.

Results

^{13}C NMR Chemical Shifts. The ^{13}C chemical shifts and corresponding assignments are presented in Table I as averages from three spectra. A representative fully relaxed spectrum of G_{D1a} at 37.73 MHz is shown in Figure 1. Carbon assignments were made by analogy to the chemical shifts of the related compounds β-D-glucose, methyl-β-D-glucopyranoside, β-D-galactose, methyl-β-D-galactopyranoside, β-D-lactose, methyl-β-D-lactopyranoside, *N*-acetylneuraminic acid, *N*-acetylgalactosamine, and neuraminyllactose.[2,39-41] Assignments were confirmed by using previously published ^{13}C data on gangliosides as well as the T_1 data.[17-20] The T_1 data were used when certain trends could be predicted. For example, Cer C-17,17′ should have a T_1 that is intermediate in magnitude between that obtained for Cer C-16,16′ and Cer C-18,18′ on the basis of its relative position in the hydrocarbon chain. Cer C-17,17′ is in a region of the ^{13}C spectrum where three peaks arise, having T_1 values of 0.643, 1.125, and 0.482 s, respectively. Since the T_1 values for Cer C-16,16′ and Cer C-18,18′ are 0.705 and 2.12 s, respectively, Cer C-17,17′ must be assigned to the peak having the T_1 of 1.125 s.

Certain peaks required special experiments for assignment. According to comparison with model compounds, Cer C-1 should have a chemical shift of ca. 70 ppm. Cer C-1 is the only CH_2 group falling in a region of the spectrum containing CH groups. On this basis, a *J*-modulated spin-echo experiment was carried out, which showed only CH_2 in the region to resonate at 69.4 ppm.[42] Another assignment problem involved Cer C-1′ and GalNAc C-7. Considering the different environments of each of the carbons, it was believed that lanthanide shift experiment results could differentiate them.[42] The peak at 174.7 ppm was most subject to lanthanide-induced shifts whereas the peak at 175.4 ppm was not significantly changed. As a result, the upfield peak was assigned to GalNAc C-7, since this carbon is more likely to interact with the lanthanide-shift reagent. GalNAc C-7 is located in the head group where exposure to the reagent is more probable. Since Cer C-1′ is located in a less accessible region of the micelle, it is assigned to the unshifted peak at 175.4 ppm.

^{13}C Relaxation Times. ^{13}C spin-lattice relaxation times (T_1) were measured for the 40 resonances resolved in the G_{D1a} spectrum. These values are presented in Table II as NT_1, where N is the number of hydrogens directly attached to a given carbon. It has been shown previously that for carbons bearing N protons, NT_1 is approximately proportional to $(\tau_c)^{-1}$, the effective rotational correlation time for the C–H bonds (ref 22f, p 217; ref 43). Longer NT_1 values are therefore indicative of greater conformational mobility. This approximate model has been used to interpret the NT_1 values for several relatively complex systems analogous to aqueous G_{D1a} micelles. T_1 data for phospholipids, sections of ganglioside head groups, the G_{M1} head group, and glycolipids including cerebrosides have all been interpreted in this way.[2,10,11,13,22,23,25-28] Although this relationship is not valid outside the region of motional narrowing, it has been shown to hold true for G_{D1a} micelles in D_2O buffer.[17]

Immediately apparent upon study of the spin-lattice relaxation times are the continuous increases in NT_1 along the ceramide hydrocarbon chains. This type of gradient is well documented for phospholipids and is consistent with rapid kink migrations along the chains, the rotation of C–C bonds becoming more favorable toward the terminal methyl group, where steric hindrance due to neighboring hydrocarbon chains is lessened.[22]

Another striking trend observed is the relative constancy and small magnitude of T_1 for carbons comprising the skeleton of the head group. The majority of these carbons have NT_1 values in the narrow range of 0.09–0.12 s, indicating little segmental motion of the individual saccharide rings within the head group. If sizable segmental motions of individual saccharide units were occurring, larger differences in NT_1 would be expected.[2,44]

Consideration of the magnetically distinct ring carbons, i.e., those ring carbons having a unique resonance in the spectrum, should make apparent any gradations in mobility that exist among

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sugar residues. There is at least one protonated carbon in each ring with a unique resonance; the unique carbons are Gal-2 C-1, Gal-2 C-3, GalNAc C-3, Glc C-4, Gal-1 C-2, Gal-1 C-4, NeuAc-1 C-3, and NeuAc-2 C-3. Any changes in NT_1 among magnetically distinct carbons of different residues may be interpreted as differences in the ability of the entire residue to undergo internal rotation as a unit. Since this is an anisotropic motion, problems could arise in analysis if the only magnetically distinct carbon in a residue has a C–H bond parallel to a preferred axis of rotation. The resulting T_1 could be misinterpreted as decreased internal rotation of the whole ring rather than an anisotropy effect. Fortunately, this does not appear to be a problem for this system, since most magnetically distinct C–H bond vectors are axially disposed in the preferred conformer of each sugar. The axial C–H vectors do not lie parallel to any possible preferred axes. Gal-1 C-4 is equatorial, but there is another magnetically distinct carbon in this residue which has an axial C–H vector and an identical T_1 within experimental error.

Considering the standard deviation associated with each NT_1, NeuAc-1 C-3, GalNAc C-3, Gal-1 C-2, and Gal-1 C-4, all relax at the same rate, indicating that NeuAc-1, GalNAc, and Gal-1 are the least mobile residues, with NT_1 ca. 0.10 s. Gal-2 C-1, Gal-2 C-3, and NeuAc-2 C-3 have very similar NT_1 values, the Gal-2 NT_1 values being slightly greater. Thus, the Gal-2 and NeuAc-2 residues appear to have greater overall conformational mobility, with NT_1 ca. 0.12 s. Glc C-4 is less distinct, overlapping with one carbon in each grouping when errors are considered. The differences in mobility between the NeuAc-1 and NeuAc-2 residues are corroborated by the T_1 data obtained for the magnetically distinct carbons NeuAc-1 C-2 and NeuAc-2 C-2. Though these two carbons are nonprotonated, it was shown that this carbon in an analogous system is almost entirely relaxed by neighboring protons, and therefore the T_1 data could be correlated with mobility of the ring in the same way as for protonated carbons.[11] NeuAc-1 C-2 and NeuAc-2 C-2 have T_1 values of 1.310 and 1.754 s, respectively, paralleling the differences observed at the two C-3 carbons.

The carbons found in the peripheral regions of the head group include the glycerol side chains on each of the NeuAc residues, the C-6 carbons on each of the neutral sugars, and the ceramide backbone. Considering the glycerol side chains, the NT_1 values for C-7 and C-8 are very close to those of the NeuAc ring, all values being in the range from 0.120 to 0.132 s.[45] The NT_1 value for both C-9 carbons is significantly longer at 0.192 s.[45] These results show that C-7 and C-8 of both side chains are relaxing similarly to the ring carbons and are therefore moving isotropically with them, since additional motions would require larger NT_1 values, as discussed in detail for NeuAc derivatives previously.[11] In contrast, the increased NT_1 for the C-9 carbons indicates increased internal rotations relative to the saccharide ring. Only the two C-8 carbons are magnetically distinct, so these are the only means by which any differences between the side chains can be evaluated. Of the two, NeuAc-2 C-8 has a significantly longer NT_1 and, therefore, exhibits greater mobility than NeuAc-1 C-8. This difference is consistent with the greater mobility observed for all magnetically distinct ring carbons of NeuAc-2 as discussed previously.

There are two peaks in the G_{D1a} spectrum corresponding to the neutral saccharide C-6 carbons. The NT_1 corresponding to the galactose and GalNAc C-6 carbons is 0.224 s,[45] whereas the value obtained for glucose C-6 is 0.166 s. This very significant change in NT_1 shows that the galactose-derived C-6 carbons are considerably more mobile than the C-6 of glucose. All the C-6 carbons, however, have larger NT_1 values than the saccharide ring carbons and are therefore exhibiting librational or rotational motion relative to their rings.

Carbons comprising the ceramide backbone are Cer C-1, C-2, and C-3. Cer C-1 is not resolved from the ^{13}C peak of Gal-2 C-2. However, T_1 values for both carbons must be close, ca. 0.114 s, since the T_1 plot for this peak is linear ($\ln |A_\tau - A_\infty|$ vs. τ). For the degree of equivalence to be tested, a series of artificial T_1 plots were generated in which the T_1 plot of a peak with a relaxation time of 0.114 s was added to various T_1 plots having T_1 values less than and greater than 0.114 s.[46] The results show that the T_1 of a second peak has a profound effect on the calculated T_1 from a combined plot. For example, if the T_1 of the second peak is 0.05 s, the calculated T_1 of the combined plot is lowered to approximately 0.086 s. These studies showed that NT_1 for Cer C-1 cannot be in the range of NT_1 for Cer C-2 and C-3 without lowering the observed T_1 of the Cer C-1, Gal-2 C-2 peak well below the normal NT_1 range including associated error for Gal-2 saccharide ring carbons (assuming Gal-2 to have an NT_1 in the observed range: 0.114–0.122 s). Furthermore, all Gal-2 ring carbons other than C-2 have NT_1 in the narrow range 0.120–0.122 s. Considering the general equivalence of NT_1 observed for carbons of a given ring, the reduced value observed for Gal-2 C-2 is very probably due to the effect of overlap by the Cer C-1 peak. If Gal-2 C-2 has an actual NT_1 of ca. 0.120 s, these calculations show that Cer C-1 would need a T_1 of 0.108 s to lower the combined T_1 to 0.114 s. This lower T_1 still corresponds to an NT_1 of 0.216 s, a value significantly larger than those obtained for Cer C-2 and C-3, which have NT_1 similar to saccharide ring carbons. Therefore, Cer C-2 and C-3 in the G_{D1a} micelle experience constraints upon mobility similar to those of the ring carbons. Cer C-1, however, exhibits enhanced conformational mobility similar to other peripheral CH_2 carbons in the head group. It relaxes similarly to NeuAc-1 and NeuAc-2 C-9 and the galactose-derived C-6 carbons, but more slowly than the glucose C-6 carbon and much faster than any other CH_2 in the sphingosine chain.

Discussion

The T_1 data show several very interesting features of G_{D1a} molecular dynamics in micelles. This discussion will focus on the hydrophilic oligosaccharide and upper ceramide regions of the molecule, since these are the proposed areas of protein and calcium interaction in neuronal membranes.

Head group T_1's are generally shorter than those measured for analogous systems. Ring carbon NT_1 values for neuraminyllactose were found to range from 0.10 to 0.27 s, most being closer to 0.2 s[2] (see also ref 15). This difference exists despite a 46 °C experimental temperature in these studies vs. the 35 °C conditions used for neuraminyllactose. For identical systems, longer T_1's would be expected at higher temperatures (ref 22f p 218).

The relatively low magnitudes of NT_1 observed in both systems, as compared for instance to the magnitude of NT_1 measured for the main methylene of G_{D1a}, demonstrate the constraints on mobility imposed by the rings and possible interactions causing ring-like structures. The significant decreases in NT_1 values for gangliosides relative to neuraminyllactose shown in these experiments reflect the added constraints imposed by the secondary micellar structure combined with the effects of the larger, branched head group.

As previously described, slight but significant segmental mobility of sugar residues exists. It is apparent that increased mobility radiates outward from a central, restricted region consisting of Gal-1, NeuAc-1, and GalNAc. This type of gradient is reasonable, considering the extensive branching at Gal-1 and the crowding of GalNAc by NeuAc-1. Space-filling models show the central region to be the most restricted and the two terminal residues to have increased rotational freedom. There is literature precedent for this type of gradient and gradients in general. In Allerhand and Doddrell's[44] PRFT study of stachyose, the terminal galactose was shown to have significantly slower relaxation compared to the inner galactose residue. This mobility gradient, like the one observed in the present experiments, radiates outward from the central residues. Czarniecki and Thornton[2] demonstrated the

(45) Though certain peaks are unresolved, their T_1 values must be nearly equal because T_1 plots ($\ln |A_\tau - A_\infty|$ vs. τ) do not show the curvature expected for two significantly different T_1 values in an unresolved peak.

(46) $(A_\tau - A_\infty)$ of peak 1 (T_1 = 0.114 s) was added to $(A_\tau - A_\infty)$ of peak 2 (T_1 = variable) at each τ value. The natural logarithms of these quantities were taken and plotted vs. τ. The negative (slope)$^{-1}$ of the plot was T_1 for the combined plot.

existence of a motional gradient among the residues of neuraminyllactose, a more analogous system. These results differ, however, since the least mobility was observed for the NeuAc residue, which was thought to be anchored in the solvent. Anchoring effects of the two sialic acids may also be at work in G_{D1a}. The G_{D1a} head group may be viewed as three saccharide chains branching out from the Gal-1 residue. At the end of two of the branches are sialic acids which can interact with the solvent. The third branch is attached to the lipid chains which are embedded in the hydrophobic region. The combined effect of the "3 anchors" tying down the three branches would result in a general decrease in mobility and mobility gradient for the various sugar residues. As previously described, NeuAc-2 is slightly less mobile than Gal-2, indicating that there is some anchoring of NeuAc-2 in the solvent. NeuAc-2, however, is considerably more mobile than NeuAc-1. The difference in mobility observed for the two structurally identical "anchors" suggests that the steric and polar interactions in the crowded "central" region of the head group combined with the constraints imposed by the secondary micellar structure have a greater effect on mobility than any specific anchoring effects of the sialic acids.

These results are also unlike preceding work in terms of the magnitude of the gradient. For G_{D1a} micelles, NT_1 ranges from 0.09 to 0.12 s for the skeletal carbons. According to Czarniecki and Thornton,[2] the range of NT_1 for analogous carbons is 0.10–0.27 s. Thus, G_{D1a} micelles contain a considerably more immobile saccharide network. This relative immobility is corroborated by preliminary binding studies completed in this laboratory. These studies show none of the G_{D1a} carbon T_1's to be altered significantly upon interaction with Ca^{2+}, suggesting that the head group is in the approximate conformation for binding prior to addition of Ca^{2+}. A model illustrating the preceding discussion regarding the gradient and steric crowding among residues is presented in Figure 2.

The low degree of segmental mobility of the rings extends into the peripheral regions of the head group. In the glycerol side chains of NeuAc, C-7 and C-8 move isotropically with the ring system, whereas C-9 exhibits segmental motion. These results are like those obtained for the model saccharide neuraminyllactose by Czarniecki and Thornton,[2] who proposed a hydrogen-bonded network in which the C-8 hydroxyl hydrogen is hydrogen bonded to the pyranose ring oxygen and the C-7 hydroxyl oxygen is hydrogen bonded to the hydrogen of the acetamido nitrogen to explain the phenomena they observed. This network was consistent with previously published ^1H NMR data, but several researchers have since disputed this proposal.[13,40,47] The only other model consistent with both the ^1H and ^{13}C NMR data is a theoretical study which explains the observed isotropy at C-7 and C-8 in terms of the inherent steric and electrical energy barriers experienced by these pseudo-tertiary carbons.[47] This study also included measurement of the ^{15}N spectrum for sialic acid in Me_2SO, which indicated that the nitrogen was unassociated. Though these studies cannot be ignored, their theoretical nature and the different solvent used in the NMR measurement make them conjectural. It is clear that the G_{D1a} glycerol chains exhibit the same motional properties as the glycerol chain of neuraminyllactose;[2] however, further study in this area is needed before a model that explains all results can be defined.

The difference in NT_1 between NeuAc-1 C-8 and NeuAc-2 C-8 needs some elaboration. The NT_1 for NeuAc-2 C-8 is significantly larger but still close to the values seen for carbons subject to constraints like those of a ring, as could be provided by an H-bond network or other phenomena not allowing complete (360°) rotations about the C-8 carbon–carbon bonds. The difference might therefore arise from increased libration of NeuAc-2 C-8 relative to NeuAc-1 C-8. Librational effects have been noted previously in several T_1 studies.[11,48–50] The increased librational motion of NeuAc-2 C-8 would then reflect the decreased steric crowding of the outer sialic acid.

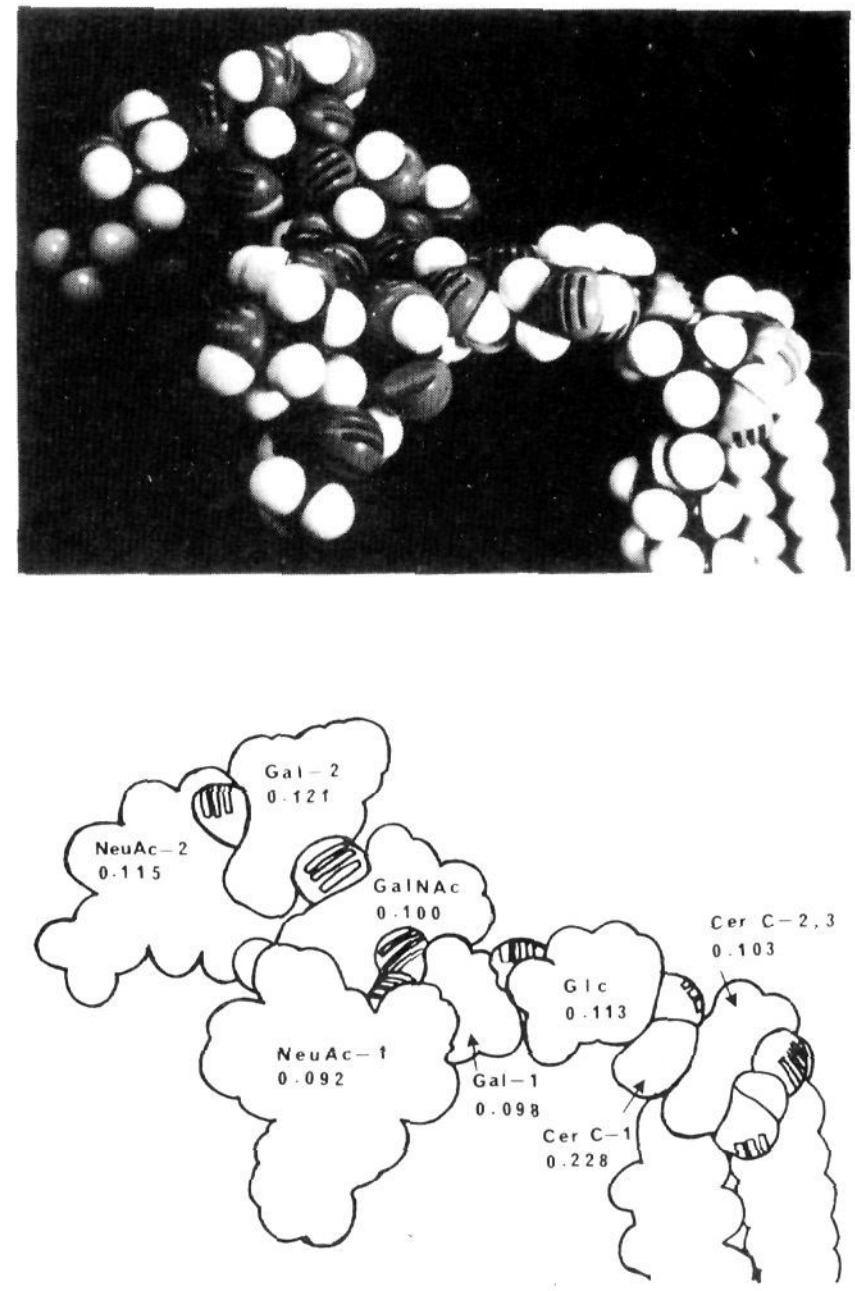

Figure 2. Space-filling molecular model and corresponding outline projection of G_{D1a}, showing the mobility gradient as reflected in NT_1 values and steric interactions among individual saccharide residues and the proposed hydrogen bond in the ceramide backbone. The NT_1 value presented with each saccharide residue is the average NT_1 for the magnetically distinct carbons of that residue. The conformation of G_{D1a} presented is based on several relevant studies. The torsional angles ϕ and ψ between glucose and galactose are based on values obtained for methyl-β-D-lactoside.[52] ϕ and ψ between GalNAc and Gal-2 are similarly based on data obtained for the analogous GlcNAc–Gal linkage in oligosaccharides of blood group determinants.[53] The Gal-1–NeuAc-1 torsional angle, the GalNAc amide linkage, and the NeuAc-1 glycerol side chain are oriented as postulated in previous ^{13}C studies of G_{D1a}.[17] The NeuAc-2 residue is oriented as postulated for G_{T1} in monolayers.[54] The ceramide group conformation is based on X-ray data obtained for cerebroside[51] and sphingosine,[55] incorporating the hydrogen bond proposed in these studies.

The large difference in NT_1 observed for C-6 of glucose vs. that of galactose-derived residues has been demonstrated in analogous systems. It was found that NT_1 for C-6 of glucose was substantially shorter than that for galactose derivatives.[11] This effect was observed in lactose[11] and, according to the present experiments, appears to apply to Glc, Gal-2, and GalNAc in G_{D1a} micelles. Gal-1, however, does not have a free hydroxyl at C-4 to participate in the postulated[11] hydrogen bonding. The explanation of this phenomenon in the case of G_{D1a} is therefore not evident at present.

The motional properties exhibited by the ceramide backbone are particularly interesting. Cer C-1 is capable of enhanced internal rotation relative to the saccharide rings, but there is decreased mobility at Cer C-2 and C-3 on the order of that observed for the saccharide rings of this system. This phenomenon

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could be the result of a postulated[7c] hydrogen bond between the C-3 hydroxyl hydrogen and the Cer C-1′ carbonyl oxygen. This hydrogen bond would spread the two hydrocarbon chains, increasing association between adjacent molecules and thus increasing the stability of the model membrane system. The model presented in Figure 2 also illustrates this proposed interaction. On the basis of these data, Cer C-1 cannot be constrained to a ring as in the hydrogen-bonded network observed for crystalline cerebroside.[51] Rotations about both the Cer C-1—Cer C-2 bond and the Cer C-1—O bond would give Cer C-1 more mobility than the oligosaccharide group or Cer C-2 and C-3 separately and thus explain the larger value of NT_1 for Cer C-1. In this way, Cer C-1 would act as a double hinge between the oligosaccharide and ceramide groups.

There are two types of systems which have been used to study G_{D1a} in a membrane-like environment: micelles and vesicles (small unilamellar vesicles).[17] Micelles have a distinct advantage in this type of study since all resolvable resonances are observed without being obscured by resonances of the colipid necessary for ganglioside vesicles. Though a structurally simpler system than vesicles, there are several important similarities between the chosen experimental system and membrane bilayers. Micelles, like membranes, are spherical structures in which the head group is exposed to the aqueous medium. In both structures, the hydrophobic lipid chains are aggregated together away from the aqueous medium. Though there is no actual bilayer structure in micelles, each pair of micellar lipid chains is directly opposed by another pair of lipid chains as in a bilayer. These analogies show micelles to be a simple experimental model for membranes, and therefore the phenomena observed should represent properties of G_{D1a} in membranes to some extent. This is a particularly reasonable conclusion in the present case, since many of the properties observed for the very simple model systems such as sialic acid and the G_{M3} head group are also observed for G_{D1a} micelles.

Conclusion

With these studies, we have developed a qualitative understanding of the molecular dynamics and interactions of the major brain ganglioside, G_{D1a}, in a simple model membrane system. Significant among findings is the existence of a slight gradient in mobility among the sugar residues of the relatively immobile head group. Of particular interest in this regard are the distinct differences in conformational mobility observed for the structurally identical sialic acid residues. Groups attached to the saccharide rings were found to exhibit phenomena similar to neuraminyllactose,[2] and the first evidence for a previously postulated[7c] hydrogen bond in the ceramide backbone was acquired. These studies provide a comprehensive picture of gangliosides in a resting ground state, which is an essential basis for future experimentation involving these undoubtedly important glycolipids.

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Mass Spectrometry of Phospholipids.[1,2] Some Applications of Desorption Chemical Ionization and Fast Atom Bombardment

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Abstract: Major diagnostic peaks in desorption chemical ionization (D/CI) and fast atom bombardment (FAB) mass spectra of various model synthetic phospholipids and related compounds are reported in conjunction with our ongoing research on marine phospholipids. Similarities and differences with some previous studies are presented. Commercially available or partially synthesized, saturated or unsaturated fatty acid containing phospholipids with different head groups such as choline, ethanolamine, mono- and dimethylethanolamine, serine, and glycerol were investigated. For identification purposes, 1,2-diacetylglycerol, 1,2-diacetyl-*sn*-glycero-3-phosphocholine, and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine-d_9 were also prepared and their mass spectral behavior studied. In terms of diagnostically useful fragmentations ammonia proved to be superior to methane as reagent gas for chemical ionization. The use of deuterated ammonia shed light on the nature of several fragmentations. In most cases, both chemical ionization and fast atom bombardment techniques exhibited molecular ions and/or related peaks. Desorption chemical ionization with ammonia provided more information about fatty acyl moieties, while fast atom bombardment gave diagnostic peaks about various head groups. These two techniques thus offer complementary information about the structures of intact phospholipids.

Phospholipids, together with sterols, are among the main classes of lipids in the cell membranes of living organisms. While electron-impact mass spectrometry has proved to be a very powerful technique in the structure elucidation of sterols, difficulties

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