Thermotropic Characterization of Phosphatidylcholine Vesicles Containing Ganglioside G_{M1} with Homogeneous Ceramide Chain Length†

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ABSTRACT: The thermotropic behavior of dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine large unilamellar vesicles containing ganglioside G_{M1} of homogeneous long chain base composition has been studied by high-sensitivity differential scanning calorimetry and fluorescence spectroscopy. At neutral pH and in the absence of Ca^{2+}, the thermotropic behavior of these systems is independent of the ganglioside chain length composition. The presence of Ca^{2+} at concentrations higher than 5 mM induces ganglioside phase separation in a manner dependent upon the length difference between the ganglioside long chain base and the phosphatidylcholine acyl chains. The analysis of the chain length dependence of the thermotropic behavior suggests that the driving force for ganglioside phase separation is not a Ca^{2+}-induced cross-bridging of the ganglioside head group but a passive ganglioside exclusion from Ca^{2+}-perturbed phosphatidylcholine-rich regions within the bilayer. Experiments with native ganglioside G_{M1}, primarily a mixture of C18:1 and C20:1 long chain bases, indicate that the individual components of the mixture maintain their characteristic behavior within the lipid bilayer matrix. These results, together with the presence of a phase transition in native G_{M1} micellar dispersions, absent in purified C18:1 or C20:1 ganglioside micelles, strengthen the idea of a possible role of chain length composition in the modulation of ganglioside function.

Gangliosides are sialic acid containing glycosphingolipids present in the plasma membrane of vertebrate cells and particularly abundant in neuronal membranes (Ledeen, 1978; Tettamanti et al., 1980). They are asymmetrically located in the outer membrane surface with their hydrophobic portion, the ceramide, inserted in the lipidic core of the membrane and with the oligosaccharide hydrophilic moiety protruding from the membrane and free to interact with external ligands. Gangliosides have been implicated in a variety of cell surface processes such as receptor binding and recognition phenomena and in the functioning of central nervous systems (Mullin et al., 1976; Brady & Fishman, 1978; Ando 1983; Sharom & Grant, 1978). The specificity and the ability of gangliosides to interact with external ligands are generally considered to be related to their oligosaccharide portion; however, the fine modulation of these interactions can also be affected by intrinsic membrane properties modified by the presence of the ganglioside molecules (Masserini et al., 1982; Myers et al., 1984). In this case, the hydrophobic portion of the ganglioside and its interactions with other membrane components may play a significant role in membrane organization and function (Maggio et al., 1981). Previous reports have also noted the potential importance of the lipidic portion of gangliosides in relation to their physicochemical and biochemical properties (Yohe et al., 1976; Kannagi et al., 1982).

The fatty acid portion of the ceramide moiety of most common mammalian brain gangliosides has a rather homogeneous composition (with a prevalence of stearic acid) in contrast with the long chain base. In the G_{M1} ganglioside extracted and purified from beef brain, the most abundant long chain bases are the C18 and C20 compounds in the erythro form, with a double bond in the C4–C5 position (C20:1 and C18:1 long chain bases) (Sonnino et al., 1984). Until today, very little was known regarding the influence of the ceramide portion on the physical and functional properties of gangliosides.

In the present work, we have studied by high-sensitivity differential scanning calorimetry and fluorescence spectroscopy the thermotropic behavior of fused unilamellar vesicles composed of dipalmitoyl- or distearoylphospatidylcholine, containing highly purified G_{M1} molecular species, homogeneous in the ceramide moiety. To the best of our knowledge, this is the first study reporting the behavior of a ganglioside species with homogeneous chain composition. For these experiments, the ganglioside molecules were incorporated into the outer surface of the phospholipid vesicles, thus mimicking the asymmetric distribution found in plasma membranes.

Materials and Methods

Ganglioside G_{M1} was extracted and purified from beef brain according to the procedure described by Tettamanti et al. (1973). The identification, structural analysis, and purity of the samples were assessed as described by Sonnino et al. (1978). In the purification step by silica gel column chromatography, only the central fractions of the eluted peaks were collected. These fractions contained the highest percentage of stearic acid. The final purity of G_{M1} ganglioside was better than 99%. This G_{M1} preparation, homogeneous in the saccharide portion but not in the lipidic moiety, will be referred to as "native G_{M1}".

Separation of G_{M1} into molecular species with homogeneous long chain base composition was accomplished by high-performance liquid chromatography (HPLC) as described by Sonnino et al. (1984). Briefly, the separation was attained on a semipreparative reversed-phase Spherisorb S5ODS2 column (1 × 25 cm; Phase Separations Ltd., Qeensferry, U.K.) with a Gilson HPLC apparatus (Model 303, Paris, France) under the following experimental conditions: temperature 20 °C; solvent system acetonitrile/5 mM sodium phosphate buffer, pH 7.0 (3:2 v/v); flow rate 7.5 mL/min; elution

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Table I: Long Chain Base and Fatty Acid Composition of G_{M1} Ganglioside Species Used in This Investigation

G_{M1} species	long chain base (%)				fatty acid (%)					
	C18:0	C18:1	C20:0	C20:1	C16:0	C16:1	C18:0	C18:1	C20:0	C22:1
G_{M1} purified from beef brain (native)	6.3	59.6	2.7	31.4	0.6	0.2	94.6	0.2	3.2	1.2
C18:1 G_{M1}		99			0.5	0.1	97.1	0.2	1.6	0.5
C20:1 G_{M1}				99	0.3		92.7	0.2	5.1	1.7

monitored with an UV detector at 195 nm. The eluted fractions were dried, dissolved in water, dialyzed, high-speed centrifuged (40 000 rpm/30 min), lyophilized, and further purified by precipitation with cold acetone (Ghidoni et al., 1980). Identification, structural analysis, and purity assays were performed as described by Sonnino et al. (1984). The final purity of the G_{M1} ganglioside species with homogeneous long chain base composition was over 99% regarding both the oligosaccharide portion and the long chain base composition. The fatty acid composition was more than 93% stearic acid. By use of these procedures, homogeneous G_{M1} ganglioside species containing the C20 (C20:1 G_{M1}) and C18 (C18:1 G_{M1}) long chain base in the unsaturated form were prepared. The lipidic composition of ganglioside G_{M1} as extracted and purified from beef brain, native G_{M1}, and the purified molecular species C20 and C18 long chain base in the unsaturated form are reported in Table I.

Dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) were purchased from Avanti Biochemicals (Birmingham, AL) and used without further purification provided they gave a single spot when assayed by thin-layer chromatography (solvent chloroform/methanol/water, 60:35:4 v:v:v; 2-h run at 20 °C; spots revealed with iodine). 1-Palmitoyl-2-(pyrenyldecanoyl)phosphatidylcholine (pyrenyl-PPC) was obtained from KSV-Chemicals (Helsinki, Finland). A stock solution of the probe was prepared with absolute ethanol as the solvent and stored at −20 °C in the dark under argon.

Vesicle Preparation. All the vesicle preparation used for these experiments were fused unilamellar vesicles (FUV's) prepared essentially as described by Schullery et al. (1980). DPPC was first dried from a chloroform solution and lyophilized overnight. The dried lipid was suspended in 50 mM KCl containing 0.02% sodium azide to give a final concentration of 50 mg/mL. The lipid suspensions were sonicated in a bath sonicator (Model G112 SPIG, Laboratory Supplies, Hicksville, NY) and then centrifuged at 15000*g* for 60 min above the lipid phase transition temperature to pellet any residual multilamellar vesicles. The sonicated vesicles were then incubated at 4 °C for 3 weeks before use.

This low-temperature incubation triggers a spontaneous fusion process and produces a homogeneous population of single lamellar vesicles of about 900-Å diameter (Wong et al., 1982). The size and homogeneity of the vesicle preparations were checked by negative-staining electron microscopy. The incorporation of ganglioside G_{M1} into the lipid bilayer was achieved by adding the desired amounts of ganglioside, from an aqueous stock containing 20 mg of ganglioside/mL, to diluted aliquots of the vesicle preparations (5 mol/mL of total lipid) followed by incubation at 45 °C for 120 min. this procedure results in the formation of a stable population of lipid vesicles containing ganglioside molecules asymmetrically located in the outer membrane surface (Felgner et al., 1981; Myers et al., 1984).

Differential Scanning Calorimetry. Calorimetric experiments were performed with a Microcal MC1 differential scanning calorimeter. The sensitivity and precision of the basic calorimetric unit have been improved by the use of two Keithley amplifiers connected to the heat capacity and temperature outputs of the calorimeter and interfaced to an IBM PC microcomputer using a Data Translation (DT-805) A/D conversion board for automated data collection and analysis. With pure lipid dispersions, concentrations lower than 0.5 mg/mL can be used with a total sample volume of 0.7 mL.

For these studies, the concentration of the lipids (total sample volume 0.7 mL) was about 2.5 mM. The calorimetric scans were performed at a scanning rate of 20 °C/h. When required, Ca^{2+} was added to the vesicle preparations from a concentrated $CaCl_2$ stock solution, directly into the calorimeter cell. The addition of Ca^{2+} was always done at a temperature above the phospholipid gel–liquid transition and the calorimeter allowed to cool very slowly (5–6 h) in order to permit a thorough equilibration of the divalent cation across the membrane. Usually this procedure gave calorimetric scans that did not change after subsequent cooling and heating scans, indicating that Ca^{2+} had equilibrated across the membrane. All the calorimetric data reported in this paper are repeated scans of the same sample to assure complete equilibration and reproducibility.

Fluorescence Spectroscopy. All fluorescence spectroscopy experiments were performed by using a Perkin-Elmer LS-5 spectrofluorometer equipped with a thermostated cuvette holder connected to a refrigerated water bath circulator. The temperature of the cuvette was monitored within ±0.1 °C with a Keithley digital thermometer.

The excimer to monomer ratio technique (Galla & Hartmann, 1980) was performed by measuring the fluorescence emission spectrum of pyrenyl-PPC incorporated into DPPC fused unilamellar vesicles at 3–5% molar content. In a typical experiment, 50 μL of pyrenyl-PPC stock solution in ethanol was added to 0.5 mL of vesicle preparation at a 2.5 mM lipid concentration, under vigorous vortexing at 45 °C. The sample was then incubated overnight at the same temperature in order to assure complete equilibration of the fluorescent probe. In the case of G_{M1}–DPPC vesicles, the insertion of the glycolipid was attained after this step. Vesicles were diluted prior to the fluorescence measurements in order to have a total lipid concentration in the cuvette of 0.25 mM. The samples were excited at 340 nm, and the emission of dimer and monomer was taken at their maxima at 395 and 480 nm.

Other Techniques. Gel-sieve column chromatography of fused unilamellar vesicles was performed on Sepharose CL-2B as described by Felgner et al. (1981). Calibration of the column was attained with G_{M1} micelles or pure DPPC fused unilamellar vesicles. The elution was followed by assaying the fraction for phosphorous or sialic acid content. Phospholipid concentrations were estimated from phosphate analysis by a modification of Bartlett's procedure as described by Marinetti (1962). Ganglioside-bound sialic acid (NeuAc) was determined according to Svennerholm (1964).

RESULTS

Differential Scanning Calorimetry (DSC) of G_{M1} Ganglioside in Micellar Form. The DSC scan of micellar native G_{M1} as extracted and purified from beef brain (see Materials and Methods) is shown in Figure 1. In the tested temperature

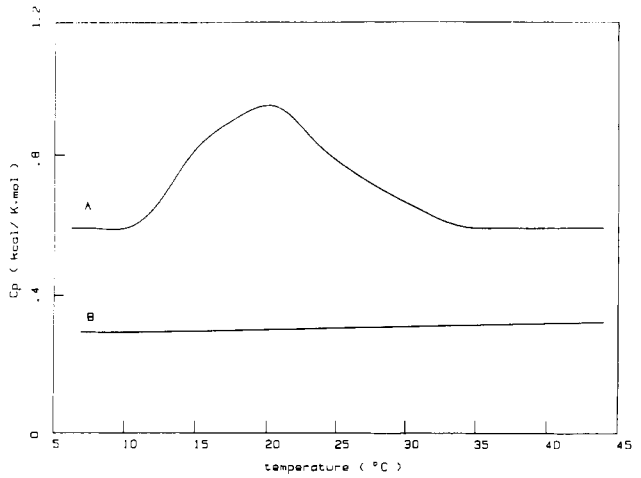

FIGURE 1: Heat capacity function vs. temperature for G_{M1} ganglioside in micellar forms: (A) 2.8 mM native G_{M1}; (B) 10 mM C18:1 or C20:1 long chain base species. See text for details.

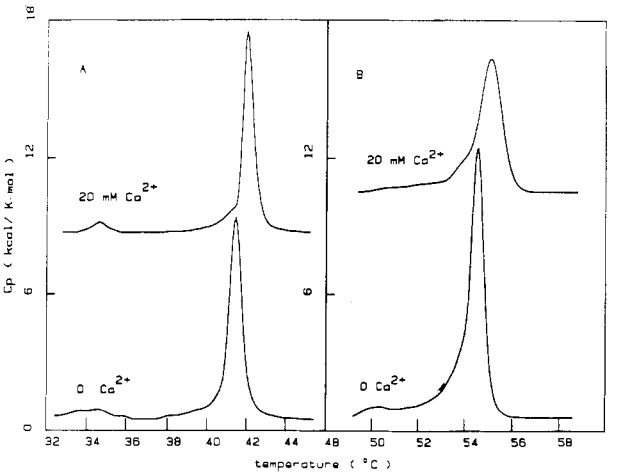

FIGURE 2: Effect of Ca^{2+} on the heat capacity function vs. temperature for DPPC (A) and DSPC (B) fused unilamellar vesicles.

range (5–80 °C) and at a glycolipid concentration of 2.8 mM, the scan shows the presence of a broad peak in the excess heat capacity function centered between 10 and 35 °C (Figure 1). The presence of this peak and its total enthalpy change (ΔH) of 3.9 kcal/mol are in agreement with previous papers (Maggio et al., 1985; Bach et al., 1982) reporting small transitions in the same temperature range with associated enthalpy changes ranging between 0.8 and 4.0 kcal/mol.

Micellar dispersions prepared with highly homogeneous C18:1 or C20:1 ganglioside G_{M1} did not show any appreciable change in the heat capacity function in this temperature range, even at concentrations as high as 10 mM (Figure 1). It should be noted that previously we (unpublished results) and other workers (Sillerud et al., 1979; Hinz et al., 1981) have also been unable to detect a ganglioside micellar transition using ganglioside G_{M1} prepared by neuraminidase treatment of the entire bovine brain ganglioside fraction. The small value of the enthalpy change as well as the apparent dependence of this transition on sample heterogeneity suggests that ganglioside micelles do not undergo a cooperative chain melting transition and that the transition may be associated to structural packing rearrangements of the more heterogeneous samples, like the native G_{M1} preparation. Homogeneous chain length samples like C20:1 and C18:1 ganglioside G_{M1} apparently do not show this behavior.

Differential Scanning Calorimetry of Phospholipid Unilamellar Vesicles. The gel–liquid crystalline transition of DPPC vesicles (shown in Figure 2A) is characterized by an enthalpy change of 8.2 kcal/mol, a transition temperature of 41.5 °C, and a half-height width of 0.6 °C, in close agreement with previous reports (Mabrey & Sturtevant, 1979; Meyers et al., 1984).

The thermodynamic parameters for the transition remain unchanged upon addition of 20 mM $CaCl_2$ except that the position of the calorimetric peak is shifted to a higher temperature value (42.0 °C), probably as the result of the interaction of Ca^{2+} with the phosphatidylcholine bilayer as described by various authors (Akutsu & Seelig, 1981; Borle & Seelig, 1985), which leads to a structural change at the level of the phospholipid head group.

In the case of DSPC (see Figure 2B), the addition of Ca^{2+} at increasing concentrations produces a slightly stronger but qualitatively similar effect to DPPC. The main gel–liquid transition of the phospholipid originally centered at at 54.5 °C moves toward higher temperatures and becomes broader. In the presence of 20 mM Ca^{2+}, the calorimetric peak is

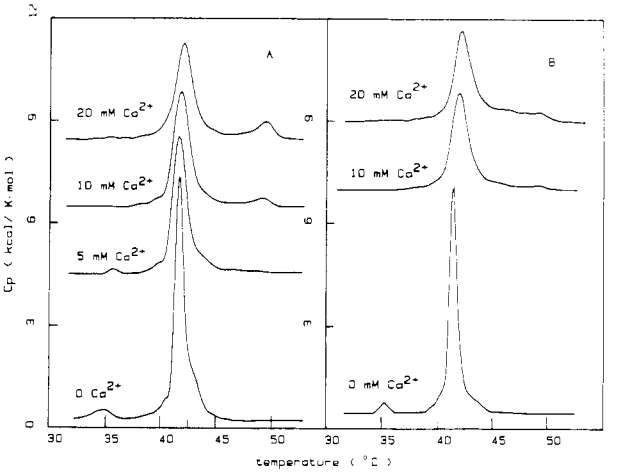

FIGURE 3: Heat capacity function vs. temperature at different Ca^{2+} concentrations for DPPC fused unilamellar vesicles containing 10% (A) or 5% (B) G_{M1} ganglioside with homogeneous (C20:1) long chain base composition.

centered at 55 °C, with a half-height width of 1.1 °C.

Differential Scanning Calorimetry of Phospholipid–G_{M1} Mixed Vesicles. Figures 3 and 4 show the calorimetric scans of DPPC and DSPC fused unilamellar vesicles containing 10 mol % C20:1 G_{M1}. In these vesicle preparations the glycolipid component is asymmetrically located on the outer surface of the vesicle, the ceramide moiety being inserted only in the outer monolayer of the phospholipid bilayer matrix (Felgner et al., 1981). The temperature of the phospholipid gel–liquid crystalline transition remains the same as that in the absence of ganglioside for both phospholipids, but the heat capacity peak becomes broader and skewed toward higher temperatures (in the case of DPPC + G_{M1} vesicles) or lower temperatures (in the case of DSPC + G_{M1} vesicles). The C18:1 long chain base molecular species of G_{M1} and the native ganglioside (as extracted from beef brain) displayed similar calorimetric properties when tested under the same experimental conditions (Figures 5 and 6). Also, similar calorimetric scans were produced with ganglioside G_{M1} obtained by neuraminidase treatment of the entire ganglioside fraction (Goins & Freire, 1985). Under the conditions of these experiments, the enthalpy change for the main lipid transition remains constant within the experimental error.

These results, as well as those obtained by other authors (Goins & Freire, 1985; Sillerud et al., 1979) for DPPC–G_{M1} mixed vesicles using G_{M1}'s of different lipidic composition,

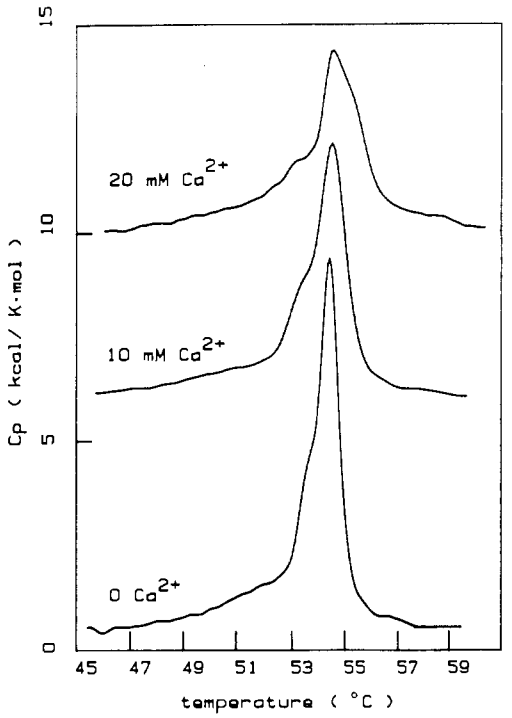

FIGURE 4: Heat capacity function vs. temperature at different Ca^{2+} concentrations for DSPC fused unilamellar vesicles containing 10% G_{M1} ganglioside with homogeneous (C18:1) long chain base composition.

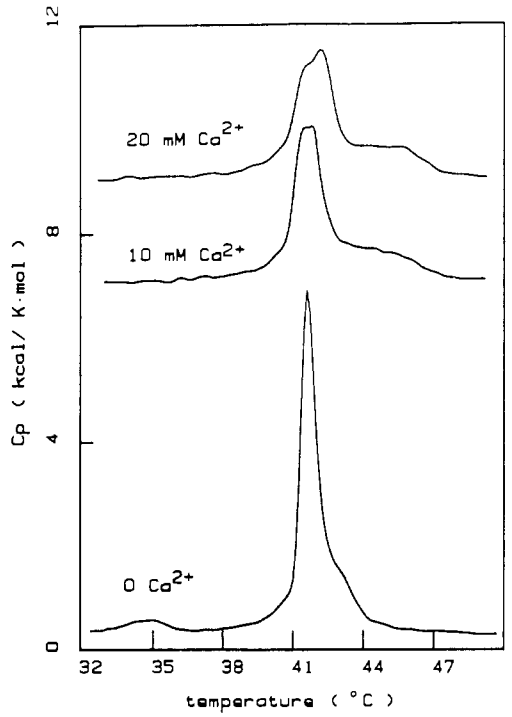

FIGURE 5: Heat capacity function vs. temperature at different Ca^{2+} concentrations for DPPC fused unilamellar vesicles containing 10% G_{M1} ganglioside with homogeneous (C18:1) long chain base composition.

suggest that at neutral pH and in the absence of divalent cations the thermotropic properties of phospholipid–ganglioside vesicles are dominated by the oligosaccharide head group and independent of the exact ganglioside long chain base composition.

Effect of Ca^{2+} on Thermotropic Properties of Phosphatidylcholine–C20:1 G_{M1} Vesicles. The addition of Ca^{2+} produced different and remarkable effects on the different vesicular systems studied. The increase of Ca^{2+} concentration up to 20 mM produced dramatic changes in the heat capacity function of DPPC vesicles containing the homogeneous molecular species C20:1 G_{M1} (Figure 3). First, the position of the main transition peak shifted gradually to slightly higher temperatures, and the amplitude of the peak decreased as the Ca^{2+} concentration was increased. More dramatic, however, was the appearance of a new peak centered at ~49 °C. This high-temperature peak could not be detected calorimetrically at Ca^{2+} concentrations smaller than 5 mM. The position of this high-temperature peak was insensitive to Ca^{2+}, but its associated enthalpy change increased at the expense of the enthalpy of the main transition peak. The total enthalpy change (the sum of the two peaks) remained constant within experimental error. These observations suggest that part of the phospholipid molecules leave, under these conditions, the main transition to assemble in a more rigid, higher melting domain. The area of the phase-separated peak increased with Ca^{2+} concentration for a given G_{M1} molar fraction and with the molar fraction of ganglioside G_{M1} for a given Ca^{2+} concentration. At 20 mM Ca^{2+} concentration and 10 mol % C20:1 G_{M1} (Figure 3A), the area under the high-temperature peak was 18% of the total.

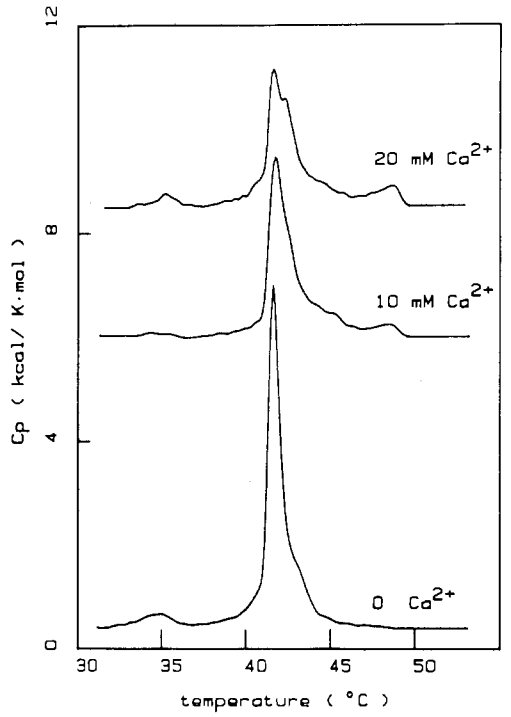

FIGURE 6: Heat capacity function vs. temperature at different Ca^{2+} concentrations for DPPC fused unilamellar vesicles containing native G_{M1} ganglioside.

In order to check the integrity of the vesicle preparations after the calorimetric scans, the samples were analyzed by gel-sieve chromatography on a Sepharose CL-2B column. The ganglioside was always found coeluting with the phospholipid vesicles. On the contrary, when pure G_{M1} micelles or G_{M1}–DPPC mixed micelles (0.75 G_{M1} molar fraction) were mixed with DPPC vesicles in the absence or in the presence of 20 mM Ca^{2+} and immediately chromatographed, the glycolypid was eluted later, in fractions separated from the phospholipid. These data show that under the conditions of these experiments the vesicular structures are devoided of micellar contaminants before [as also shown by Felgner et al. (1984)] and after the addition of Ca^{2+} and repeated temperature scans.

Effect of Ca^{2+} on Thermotropic Properties of Phosphatidylcholine–C18:1 G_{M1} Vesicles. When the homogeneous long

chain base C18:1 G_{M1} was incorporated into the DPPC vesicles, a less dramatic change was produced in the calorimetric scans upon addition of Ca^{2+} (Figure 5). In the presence of excess Ca^{2+} concentration (20 mM), the main DPPC transition was split into two distinguishable but overlapped peaks and a very broad peak centered at 46.0 °C. Under these conditions, the area under the 46 °C peak is 14% of the total even though an exact estimate is difficult due to the strong overlap with the main transition peak.

The apparent splitting of the main transition peak was not observed with the samples containing C20:1 ganglioside G_{M1}. The reason for this effect could be the extent of acyl chain interdigitation as proposed for sphingomyelins of different chain lengths (Estep et al., 1980; Schmidt et al., 1978). The C18:1 G_{M1} is only slightly longer than the DPPC molecules, and therefore, it may not cause a significant perturbation on the opposite monolayer. Under these conditions, the resulting main transition peak will contain a significant fraction of unperturbed component arising primarily from the opposing monolayer and the perturbed component arising from the monolayer where the ganglioside is located. On the contrary, the C20:1 ganglioside G_{M1}, and also the corresponding C20 sphingomyelin (Estep et al., 1980; Schmidt et al., 1978) are apparently able to perturb both monolayers even if these molecules are asymmetrically located. It should be stressed that the vesicles used in these experiments contain 20 mol % ganglioside in the outer monolayer and none in the inner monolayer.

These results seem to imply that for a given ganglioside species the long chain base moiety plays an important role in determining the behavior of the glycosphingolipid in the membrane. More precisely, the difference in lipidic chain length between the DPPC phospholipid matrix (C16:0) and the embedded ganglioside long chain base seems to be responsible for the extent of phase separation induced by the addition of Ca^{2+} ions. In the case of C20:1 G_{M1}, the phase-separated peak is well-defined and centered at 49 °C whereas in the case of C18:1 G_{M1} the phase-separated peak is broader and centered at 46 °C. Experiments with DSPC (C18:0) large unilamellar vesicles containing C20:1 G_{M1} (Figure 4) failed to reveal any evidence of phase separation, suggesting that the Ca^{2+} phase separating effect is mediated by lipid chain length differences and not due to an active clustering or "cross-bridging" of the ganglioside molecules by the divalent cation. In order to evaluate the influence of each of the lipidic components on the thermotropic behavior of G_{M1} embedded in DPPC vesicles, the calorimetric properties of 10% native G_{M1} inserted in DPPC FUV's was also investigated (Figure 6). The calorimetric profile in the presence of 20 mM Ca^{2+} shows characteristic features of the main components in the mixture (C18:1 and C20:1 long chain base) like the appearance of a peak at 49 °C (C20:1 G_{M1}), an overlapped but recognizable broad shoulder at 45 °C (C18:1 G_{M1}), and the presence of a shoulder on the main peak (C18:1 G_{M1}). At 20 mM Ca^{2+}, the area under the two high-temperature peaks is ~18% of the total, and that of the 49 °C peak alone is ~8% of the total, suggesting that their relative contributions are related to the amount of each species in the mixture.

The persistence of an individual behavior of G_{M1} molecules having different lipidic portions even when they are mixed together suggests that ganglioside molecules with different lipidic portions are nct randomly mixed within the bilayer matrix. This phenomenon would give rise to the possibility of selective ganglioside reorganization processes involving only these molecules characterized by certain lipid moiety. In the past, ganglioside properties have only been associated with the oligosaccharide head group. These studies show that the lipidic portion of these molecules is able to modulate ganglioside properties on a given membrane environment.

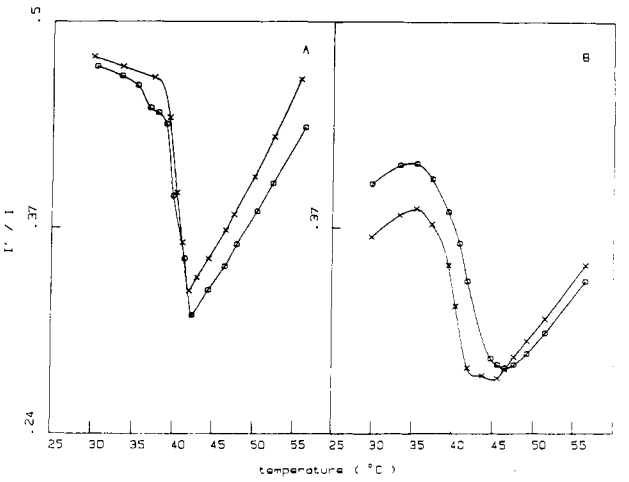

FIGURE 7: Excimer to monomer intensity ratio I'/I vs. temperature for pure DPPC fused unilamellar vesicles (A) and vesicles containing 10 mol % G_{M1} ganglioside with homogeneous (C20:1) long chain base composition (B). The probe was 1-palmitoyl-2-(pyrenyldecanoyl)-phosphatidylcholine. (×) Without Ca^{2+}; (○) with 20 mM Ca^{2+}.

G_{M1} Effect on Phospholipid Lateral Diffusion. The ratio I'/I of the fluorescence intensity of pyrenyl-PC at the maxima of the excimer (I') and monomer (I) bands was measured as a function of temperature (cooling scans) for DPPC vesicles containing up to 10 mol % of C20:1 G_{M1} at different Ca^{2+} concentrations. Figure 7 shows the excimer/monomer ratio scans for DPPC and DPPC + 10% G_{M1} (C20:1 long chain base) vesicles. The sharp increase of the E/M ratio occurring at about 42 °C, for pure DPPC, strongly contrasts with the two-step transition (inflection points at 45.5 and 42 °C) of the DPPC–G_{M1} mixed vesicles. At all temperatures above 42 °C, where the phospholipid matrix is in the liquid-crystalline state, the presence of G_{M1} caused a strong decrease in the rates of excimer formation, indicating that the presence of ganglioside significantly reduces lateral diffusion within the bilayer. These observations are consistent with previous reports suggesting that gangliosides have a rigidifying effect on the bilayer (Uchida et al., 1981; Sharom & Grant, 1978) and with recent fluorescence photobleaching recovery experiments (Goins et al., 1985), which also indicate a reduced lateral diffusion coefficient following ganglioside incorporation into DMPC bilayers above the transition temperature. Figure 7 also shows the effect of Ca^{2+} addition on the fluorometric scans of DPPC and DPPC + G_{M1} vesicles. In both cases, the presence of Ca^{2+} above the phospholipid phase transition temperature caused only a slight decrease in the rate of excimer formation. Also, the mixed DPPC–G_{M1} system shows a distinct inflection point at 47.5 °C, closely resembling the portion of the phase-separated peak observed by differential scanning calorimetry.

Discussion

Gangliosides have been implicated in several cellular recognition processes like receptor binding, virus infection, and the functioning of the central nervous system (Ledeen et al., 1981). As cell surface recognition sites, the most important region of these molecules, from a functional point of view, is their oligosaccharide head group. Gangliosides are characterized by the presence of one or more negatively charged sialic acid residues located at different positions in the head group [for a review, see Wiegandt (1982)]. As of today, more than

35 different ganglioside species have been found in mammalian cells (Wiegandt, 1982). The specific number and location of the sialic acid residues confer various gangliosides their ligated binding characteristics. Cholera toxin, the best known example, binds with high affinity to ganglioside G_{M1} but not to other ganglioside molecules (Van Heyningen, 1983).

While most ganglioside research in recent years has concentrated in the elucidation of the role of the oligosaccharide region in the biological function and properties of gangliosides, these molecules also differ in their acyl chain composition. Native ganglioside G_{M1} for example (see Table I), is actually a mixture containing primarily C18:1 and C20:1 long chain bases. The results of this investigation indicate that these two main ganglioside components respond differently to external stimuli, like Ca^{2+} concentration, suggesting that the ganglioside acyl chain might have a modulating role in ganglioside function.

For several years, conflicting reports have appeared in the literature regarding the existence of a phase transition in pure ganglioside micelles (Maggio et al., 1985; Bach et al., 1982; Sillerud et al., 1979; Hinz et al., 1981). Our results suggest that the existence of this phase transition is related to the chain length composition of the ganglioside sample. Micelles prepared with native ganglioside G_{M1}, which is a mixture containing primarily C18:1 and C20:1 long chain bases, undergo a broad phase transition centered at $\sim$20 °C, in agreement with recent results obtained by Maggio et al. (1985). On the contrary, micelles prepared with the isolated C18:1 or C20:1 ganglioside G_{M1} components do not exhibit this phase transition. These results suggest that this micelle transition might be related to structural rearrangements in ganglioside micelles of certain chain length composition.

The effects of Ca^{2+} upon phospholipid vesicles preparations containing different gangliosides have been studied by several techniques including electron spin resonance and differential scanning calorimetry (Sharom & Grant, 1977, 1978; Bertoli et al., 1981; Myers et al., 1984; Goins & Freire, 1985). These studies have concluded that Ca^{2+} is able to cause ganglioside phase separation in phospholipid vesicles. This effect of Ca^{2+} has been thought to arise from a cross-bridging effect similar to the one reported for phosphatidylserine vesicles (Portis et al., 1979). However, such an effect would imply a strong binding of Ca^{2+} to the oligosaccharide head group of the ganglioside, most likely to the negatively charged sialic acid residues. Recent studies, however, indicate that the binding affinity of gangliosides to Ca^{2+} is indeed relatively poor and similar in magnitude to the affinity of phosphatidylcholines to Ca^{2+} (McDaniel & McLaughlin, 1985a,b).

The calorimetric results in this paper indicate that Ca^{2+} does indeed induce ganglioside phase separation; however, the analysis of this effect as a function of chain length composition suggests that the phase separation effect is not due to an "active" clustering of gangliosides induced by Ca^{2+} cross-bridging but due to the "passive" ganglioside exclusion from the Ca^{2+}-perturbed phosphatidylcholine phase. An active cross-bridging of gangliosides by Ca^{2+} would have induced phase separation independently of the host vesicle system. On the contrary, the calorimetric results indicate that Ca^{2+} only induces ganglioside phase separation under conditions in which there is a chain length difference between the ganglioside and the phospholipid and that the extent of phase separation depends on this chain difference. It is known that Ca^{2+} interacts with phosphatidylcholines (Akutsu & Seelig, 1981; Borle & Seelig, 1985; Aruga et al., 1985; Kataoka et al., 1985) and that this interaction results in a rigidification of the bilayer lattice, as also shown in our calorimetric data. In this more ordered arrangement, gangliosides might be excluded and segregated provided that their chain lengths differ from those of the phosphatidylcholine molecules. In this respect, the driving force for ganglioside phase separation would be similar to that of phosphatidylcholine mixtures of different chain length (Mabrey & Sturtevant, 1976).

One of the most striking observations of this study is the persistent individual behavior of ganglioside molecules having different lipidic portions. The experiments with native ganglioside G_{M1} indicate that the C18:1 and C20:1 components of the mixture maintain their characteristic individual behavior under conditions of phase separation. Thus, in the presence of Ca^{2+}, the lipid bilayer is more likely to consist of pure phosphatidylcholine regions, coexisting with C20:1 G_{M1} rich regions and C18:1 G_{M1} rich regions. This behavior raises the possibility of selective ganglioside redistribution processes involving only those molecules characterized by a specific chain composition. Thus, the lipidic portion of gangliosides may play a role in the modulation of ganglioside function by discriminating among otherwise identical molecules.

Registry No. DPPC, 2644-64-6; DSPC, 4539-70-2; G_{M1}, 37758-47-7; Ca, 7440-70-2.

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Solvent Accessibility of the Adenosine 5′-Triphosphate Catalytic Site of Sarcoplasmic Reticulum CaATPase†

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ABSTRACT: The CaATPase of rabbit skeletal sarcoplasmic reticulum was labeled at or near the ATP catalytic site with fluoresceinyl isothiocyanate (FITC), and the accessibility of the attached probe to the bulk solvent was determined by I^- quenching of its fluorescence. The quenching of free FITC was also measured. In both cases, the quenching was of the Stern–Volmer type and collisional quenching rate constants were obtained over the pH range 5–8 in the presence of ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid and with added Ca^{2+}, vanadate, or phosphate. The fluorescence intensity and susceptibility to quenching by I^- of free FITC were insensitive to the added ligands. In all cases, the intensity decreased with pH, as predicted from the known properties of FITC mono- and dianions. The collisional quenching rate constants increased at lower pH, as expected for I^- quenching of a molecule with decreasing negative charge due to protonation. When FITC was attached to the CaATPase, the FITC fluorescence intensity and I^- collisional quenching rate constants were sensitive to ligand binding as well as pH. The changes in fluorescence intensity with acidity, when compared to the results for free FITC, indicated the pK_a of the FITC was reduced 0.6 unit when it was attached to the CaATPase. Excited-state lifetime measurements indicated that ligand effects at constant pH were not due to protonation-induced changes in FITC quantum yield but to conformational changes of the CaATPase. The ligand-induced changes in the collisional quenching rate constants appeared to be due to changes in steric hindrance to I^- colliding with bound FITC rather than changes in local charge near the probe. At all pH values, the hindrance to I^- quenching of the fluorescence of the FITC at the ATP binding site was vanadoenzyme < enzyme < phosphoenzyme. The effect of Ca^{2+} binding to the CaATPase was to decrease the hindrance. The lower the pH, the greater was the decrease caused by Ca^{2+} binding. At pH 6.0, the transition from E–P to ECa_2 was consistent with an increase of 48% in the accessibility of the ATP binding site to the bulk solvent.

The CaATPase of skeletal muscle sarcoplasmic reticulum (SR)[1] uses the energy of MgATP binding and hydrolysis to move two bound calcium ions from the cytosolic side into the lumen of the membrane (Inesi, 1981; de Meis, 1981; Hasselbach & Oetliker, 1983). This interaction between the ATP binding site and the Ca^{2+} binding sites also is apparent from

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[1] Abbreviations: SR, sarcoplasmic reticulum; CaATPase, (Ca^{2+},-Mg^{2+}) adenosinetriphosphatase from skeletal muscle SR; K_Q, slope of the Stern–Volmer plot; k_Q, Stern–Volmer collisional quenching rate constant; τ, excited-state lifetime; *q*, quantum yield; MOPS, 3-(*N*-morpholino)-propanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; FITC, fluoresceinyl isothiocyanate.

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