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Interactions of Proteins with Ganglioside-Enriched Microdomains on the Membrane: The Lateral Phase Separation of Molecular Species of GD1a Ganglioside, Having Homogeneous Long-Chain Base Composition, Is Recognized by *Vibrio cholerae* Sialidase†

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ABSTRACT: The thermotropic behavior (studied by high-sensitivity differential scanning calorimetry) and susceptibility to *Vibrio cholerae* sialidase hydrolysis of large unilamellar vesicles of dipalmitoylphosphatidylcholine, containing native GD1a ganglioside or the molecular species of GD1a containing C18:1 or C20:1 long-chain base (C18:1 GD1a; C20:1 GD1a), were studied. Vesicles containing ganglioside (10% in molar terms) showed the presence in the heat capacity function of a second minor peak besides the phospholipid main transition peak. The presence of a second peak is much more evident with C20:1 GD1a than with C18:1 GD1a, the difference being potentiated by Ca^{2+} and indicating a different tendency of the GD1a molecular species to undergo lateral phase separation. The scans of vesicles containing native GD1a showed the features of those obtained with C18:1 GD1a and C20:1 GD1a, indicating that the main components of native GD1a, C18:1 GD1a and C20:1 GD1a, maintain their individual aggregative properties. *V. cholerae* sialidase affects vesicle-bound GD1a at a much higher rate (17–25-fold) than it does micellar GD1a, the activation by Ca^{2+} being 3- and 2-fold, respectively. The V_{max} values were identical on C18:1 GD1a and C20:1 GD1a in micellar dispersions, whereas they were markedly higher (from 20 to 50%) on C18:1 GD1a than on C20:1 GD1a in vesicular dispersions. Exhaustive sialidase hydrolysis of vesicles carrying native GD1a produced C18:1 GM1 and C20:1 GM1 in the same proportion as the C18:1 and C20:1 species present in native GD1a (53.9% and 46.1%). Conversely, sialidase treatment producing about 10% of GD1a hydrolysis gave origin to C18:1 GM1 and C20:1 GM1 in the proportions of 65–69% and 31–35%, indicating the preference by the enzyme to affect C18:1 GD1a. These data show that *V. cholerae* sialidase is able to recognize GD1a molecules with different long-chain base moieties on the basis of their tendency to undergo lateral phase separation on the membrane, realizing higher V_{max} values of the substrate molecules more dispersed on the surface.

Gangliosides are amphiphilic components of the plasma membrane of vertebrate cells and are asymmetrically located in the outer face of the membrane with the oligosaccharide portions that protrude from the cell surface and interact with a variety of external ligands. Gangliosides are known to participate in a number of cell surface events where the oligosaccharide chains seem to be instrumental to functional performance (Sharom & Grant, 1978; Brady & Fishman, 1979; Ando, 1983). The actual availability of the ganglioside oligosaccharide chains to interactions has been shown (Masserini et al., 1982; Myers et al., 1984) to depend on the physicochemical properties of the embedding membrane, as well as on the aggregative properties of the ganglioside molecules. In turn, these latter properties are largely determined by the chemical characteristics of the ganglioside lipidic portion (ceramide) which is inserted into the lipid bilayer, intercalating with the other hydrophobic components of the membrane

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Table I: Lipid Composition of the Gangliosides Used in the Present Investigation

	long-chain base (%)				fatty acid (%)			
ganglioside	C18:1	C18:0	C20:1	C20:0	C16:0	C18:0	C20:0	others
native GD1a	51.2	2.6	43.9	1.3	3.2	91	2.5	3.3
C18:1 GD1a	99				1.8	95	2.1	1.1
C20:1 GD1a			99		1.3	95.6	1.9	1.2

(Yohe et al., 1976; Kannagi et al., 1982; Hitzemann, 1987; Maggio et al., 1987).

It was demonstrated (Masserini & Freire, 1986) in large unilamellar vesicles (LUVs)[1] containing molecular species of ganglioside, highly homogeneous in both the long-chain base and oligosaccharide moieties, that the lipidic portion is crucial in governing the lateral distribution of the gangliosidic component within the membrane and leads to the formation of ganglioside-enriched (and hence oligosaccharide chain enriched) microdomains.

An important question is whether gangliosides can segregate in enriched microdomains in natural membranes too, these microdomains being dependent on the compositional differences between the ganglioside lipidic portion and that of the surrounding partners in the membrane.

The present investigation approached this question starting from the observation (Venerando et al., 1982) that some enzymes affecting gangliosides, like *Vibrio cholerae* sialidase, exhibit a much lower apparent V_{max} on micellar dispersions than on vesicular dispersions; should the enzyme distinguish molecular dispersions from segregated microdomains of gangliosides on the membrane, a lower V_{max} would be expected in the latter case.

The study model adopted was constituted by *V. cholerae* sialidase and ganglioside GD1a[2] inserted into the outer layer of large unilamellar vesicles of dipalmitoylphosphatidylcholine so as to mimic the ganglioside distribution in the cell membrane (Felgner et al., 1981). Different molecular species of the ganglioside were used, each containing a single long-chain base. Segregation of the ganglioside into microdomains on the membrane was monitored by high-sensitivity differential scanning calorimetry (Masserini & Freire, 1986).

Materials and Methods

The reagents used (analytical grade) and high-performance thin-layer chromatography (HPTLC) silica gel plates were purchased from Merck GmbH (Darmstadt, FGR); dipalmitoylphosphatidylcholine (DPPC) was from Fluka AG (Buchs, Switzerland). *V. cholerae* sialidase (1 unit/mL, 40 μg as protein/mL; 1 unit defined as the amount releasing 1 μmol/min NeuAc from human acid α1-glycoprotein at 37 °C) was from Calbiochem-Behring Corp. (La Jolla, CA); crystalline bovine serum albumin and *N*-acetylneuraminic acid were from Sigma Chemical Co. (St. Louis, MO).

Preparation and Radiolabeling of Gangliosides. GD1a ganglioside was extracted and purified from beef brain according to the procedure described by Tettamanti et al. (1973). This preparation of GD1a, homogeneous in the saccharide portion but not in the lipidic moiety (see the compositional analysis in Table I), will be referred to as "native" GD1a.

Preparation of native GD1a, tritium labeled at the 3-position of the long-chain base, was accomplished by the method described by Ghidoni et al. (1982). Separation of native (radiolabeled or not) GD1a ganglioside into molecular species with homogeneous long-chain base composition in the erythro configuration (in particular, the species containing C18 and C20 unsaturated long-chain bases, the most abundant in native GD1a) was performed by high-performance liquid chromatography (HPLC) as described by Sonnino et al. (1984).

Identification, structural analysis, and purity assays of the native GD1a and its molecular species containing C18:1 (C18:1 GD1a) and C20:1 (C20:1 GD1a) long-chain bases were performed as described by Sonnino et al. (1978, 1984). The final purity of all prepared gangliosides was over 99%. The lipidic compositions of native GD1a, C18:1 GD1a, and C20:1 GD1a are reported in Table I.

Preparation of Vesicles. All the vesicle preparations used for these experiments were LUVs, prepared according to Schullery et al. (1980) with the modifications introduced by Wong et al. (1982). This procedure gives a homogeneous population of large unilamellar vesicles (LUVs) of about 800-Å diameter.

Preparation of Ganglioside Samples. (*a*) *Micellar Dispersions.* In order to prepare micellar dispersions of native GD1a, C18:1 GD1a, or C20:1 GD1a, a known amount of ganglioside dissolved in chloroform/methanol (2:1 by volume) was dried by a gentle flow of nitrogen, resuspended in water, and vortexed. The mixtures were allowed to stand overnight at room temperature.

(*b*) *Ganglioside-Containing Vesicles.* The insertion of native GD1a, C18:1 GD1a, or C20:1 GD1a into LUVs was done following the method of Felgner et al. (1981, 1983), which allows the preparation of vesicles bearing gangliosides asymmetrically embedded into the outer leaflet only. That the ganglioside molecules were really inserted and intercalated with the phospholipid components of the vesicles and not simply associated (as micelles) to the vesicle surface was always checked, according to Felgner et al. (1981). Vesicles containing 10% (molar) GD1a were prepared and used within 1 day of preparation.

Differential Scanning Calorimetry. Calorimetric experiments were performed with a Microcal MC2-D (Microcal, Amherst, MA) differential scanning calorimeter interfaced to an IBM PC microcomputer for automatic data collection and analysis. The total lipid concentration was about 1 mM. Calorimetric scans were performed at a scan rate of 20 °C/h. When required, Ca^{2+} was added to the vesicle preparation from a concentrated 1 M $CaCl_2$ solution, following the suggestions of Masserini and Freire (1986).

Sialidase Activity Assay. The incubation mixtures contained, in a final volume of 100 μL, 0.3–3.0 μL of sialidase solution, 0.05–0.6 mM GD1a in micellar or vesicular form, radiolabeled native GD1a, C18:1 GD1a, or C20:1 GD1a (250 000 dpm, corresponding to less than 0.15 nmol), if requested, and 25 mM Tris-HCl buffer, pH 6.8. The incubation mixtures, started by the addition of the enzyme, were incubated under shaking at 37 °C for 5–10 min. Incubation was stopped by dipping the tubes in an acetone/solid CO_2 mixture. The

[1] Abbreviations: LUVs, large unilamellar vesicles; DPPC, dipalmitoylphosphatidylcholine; HPLC, high-performance liquid chromatography; DSC, differential scanning calorimetry; HPTLC, high-performance thin-layer chromatography.

[2] This paper follows the ganglioside nomenclature of Svennerholm (1970) and the IUPAC–IUB Commission on Biochemical Nomenclature (1972, 1982): GM1, II^{3}NeuAc-GgOse4; GD1a, IV^{3}NeuAcII^{3}NeuAc-GgOse4.

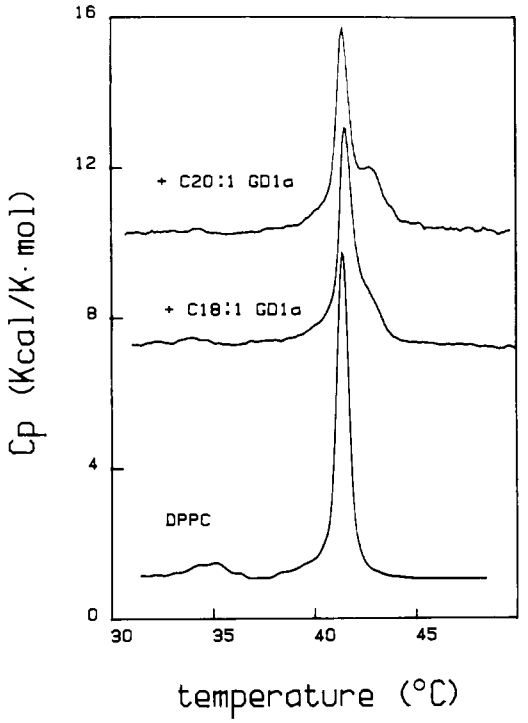

FIGURE 1: Heat capacity function vs temperature for DPPC LUVs and the same vesicles containing 10% (molar) C18:1 GD1a or C20:1 GD1a.

sample was then submitted to lipid extraction by the addition of 0.4 mL of tetrahydrofuran (Tettamanti et al., 1973) and vortexed for 5 min; the mixture was cleared by centrifugation at 12000 rpm for 5 min in a bench-top centrifuge (Eppendorf Gerateban, Hamburg, FRG). Sialidase activity was measured by quantification of the released GM1 ganglioside with the radiochemical method described by Chigorno et al. (1986). In the control incubation mixtures (blanks), the enzyme was omitted. K_m and V_{max} were determined by the double-reciprocal plot method of Lineweaver–Burk (Lineweaver & Burk, 1934).

Determination of the Long-Chain Base Composition of Residual GD1a and GM1 Released after Treatment of Native GD1a with Sialidase. Native GD1a was treated with *V. cholerae* sialidase under the assay conditions specified above, different periods of time giving a different extent of hydrolysis (from 10% to 99%). The C18:1 and C20:1 molecular species of both residual GD1a and GM1 released were then separated and quantitated with a single injection following the HPLC technique described by Gazzotti et al. (1984).

Other Methods. Ganglioside-bound sialic acid (NeuAc) was determined by the method of Svennerholm (1957) using NeuAc as standard; protein concentration was determined according to Lowry et al. (1951) with crystalline bovine serum albumin as standard.

RESULTS

Differential Scanning Calorimetry of DPPC Large Unilamellar Vesicles Containing Molecular Species of GD1a Having Homogeneous Long-Chain Base Composition. The calorimetric scans of DPPC LUVs, with or without 10% molar C18:1 GD1a or C20:1 GD1a, are reported in Figure 1. The temperature of the phospholipid gel–liquid crystalline transition (41.5 °C) remains unchanged after incorporation of the ganglioside, but the heat capacity peak becomes broader. The total enthalpy change (8.2 kcal/mol) remains constant within the experimental error, with no apparent contribution deriving from gangliosides, in agreement with previous data (Maggio et al., 1985; Myers et al., 1984). The scan of the C20:1 GD1a containing vesicles shows, in addition to the main transition peak, a partially overlapping but distinguishable peak at a higher temperature, while the scan of the C18:1 GD1a containing vesicles displays a slight shoulder in the same zone. This indicates, particularly in the presence of C20:1 GD1a, that part of the phospholipids are assembled in a higher temperature melting domain.

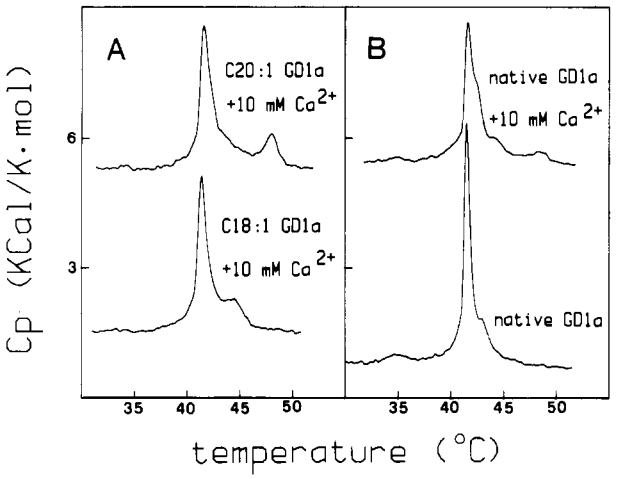

FIGURE 2: Heat capacity function vs temperature for DPPC LUVs containing 10% (molar) C18:1 GD1a or C20:1 GD1a in the presence of 10 mM Ca^{2+} ions (panel A) and for DPPC LUVs containing 10% (molar) native GD1a in the presence or absence of 10 mM Ca^{2+} ions (panel B).

Addition of 10 mM Ca^{2+} to the above ganglioside-containing vesicles (Figure 2, panel A) causes a shift of the main peak at a slightly higher temperature (+0.3 °C). In the case of the C20:1 GD1a containing vesicles a new minor peak appears in the scan. This peak is centered at 48 °C and covers about 19% of the total area. The scan referring to the C18:1 GD1a containing vesicles shows, besides the major peak at 41.8 °C, the presence of a broad minor peak centered at 44.5 °C.

Both in the absence and in the presence of Ca^{2+}, the area of the peaks indicating phase separation increased with the molar fraction of ganglioside in the vesicles, while the position of the peaks in the scan remained unchanged (data not shown).

Differential Scanning Calorimetry of DPPC Large Unilamellar Vesicles Containing Native GD1a. The calorimetric scan relative to that of vesicles containing 10% molar native GD1a (Figure 2, panel B) is characterized by the presence of the main transition peak followed by a second, partially overlapping but evident peak centered at a higher temperature. After the addition of 10 mM Ca^{2+} (Figure 2, panel B) the features of the vesicles containing C18:1 or C20:1 GD1a are exhibited, that is, the presence of an overlapping but recognizable second peak at 44.5 °C (C18:1 GD1a) and a third peak at 48 °C (C20:1 GD1a).

These data, when compared to those obtained with pure C20:1 GD1a and C18:1 GD1a, show that when the native ganglioside is embedded in the phospholipid matrix, the component molecular species retain their individual behavior, forming separate microdomains within the membrane.

Activity of V. cholerae Sialidase upon DPPC LUVs Containing Native GD1a. V. cholerae sialidase was allowed to act on GD1a embedded in LUVs; residual GD1a, as well as formed GM1, was extracted and submitted to HPLC for separation into the molecular species containing C18:1 or C20:1 long-chain bases.

Typical retention times of separated gangliosides were C18:1 GD1a = 8 min, C20:1 GD1a = 13 min, C18:1 GM1 = 21.5

Table II: Kinetic Parameters Relative to the Activity of *V. cholerae* Sialidase Acting upon C18:1 GD1a and C20:1 GD1a in Different Physicochemical Dispersions

type of dispersion	V_{max}[a]	K_m (mM)
(a) micelles		
C18:1 GD1a	2.45	0.075
C20:1 GD1a	2.37	0.075
C18:1 GD1a + 2 mM Ca^{2+}	6.74	0.078
C20:1 GD1a + 2 mM Ca^{2+}	6.42	0.078
(b) DPPC + 10% GD1a (LUVs)		
C18:1 GD1a	61.25	0.1
C20:1 GD1a	40.75	0.08
C18:1 GD1a + 2 mM Ca^{2+}	112.25	0.09
C20:1 GD1a + 2 mM Ca^{2+}	96.25	0.092
C18:1 GD1a + 10 mM Ca^{2+}	91.25	0.109
C20:1 GD1a + 10 mM Ca^{2+}	72.0	0.083
C18:1 GD1a (50%) + C20:1 GD1a (50%)	62.5 (C18:1 GD1a)	0.09 (C18:1 GD1a)
	44.0 (C20:1 GD1a)	0.1 (C20:1 GD1a)

[a] V_{max} expressed as nanomoles of hydrolyzed substrate per minute per milligram of protein.

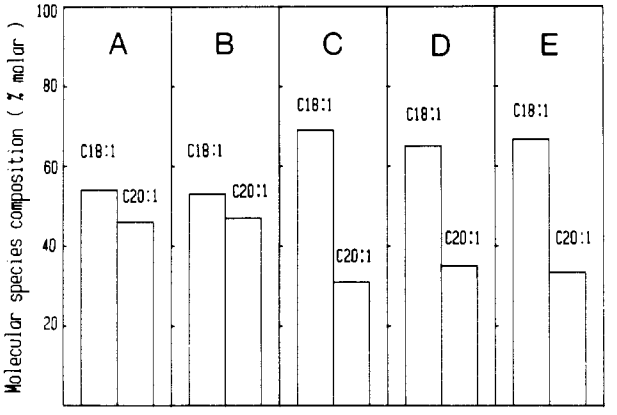

FIGURE 3: Molecular species composition (C18:1 and C20:1 long-chain bases), determined by reverse-phase HPLC of native GD1a embedded in DPPC LUVs before and after partial (10%) or exhaustive (99%) hydrolysis by *V. cholerae* sialidase: (A) native GD1a ganglioside (0 time of incubation with sialidase); (B) GM1 ganglioside released by exhaustive hydrolysis (99%) of native GD1a by *V. cholerae* sialidase; (C) GM1 ganglioside released by partial hydrolysis (10%) of native GD1a by *V. cholerae* sialidase; (D) the same as (C), obtained in the presence of 10 mM Ca^{2+}; (E) the same as (C), obtained at 46 °C.

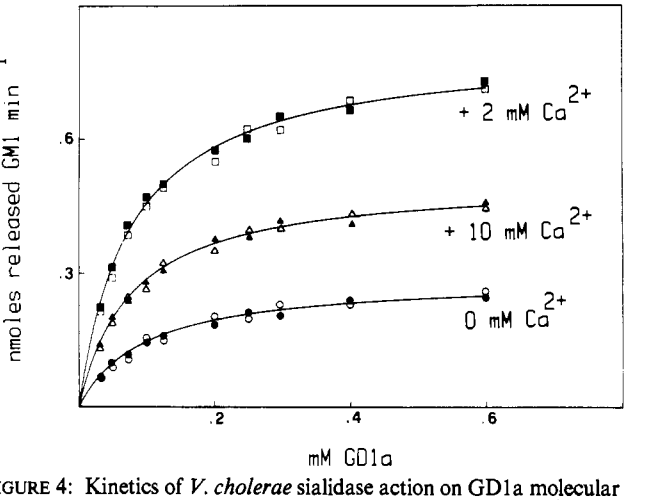

FIGURE 4: Kinetics of *V. cholerae* sialidase action on GD1a molecular species in micellar dispersion, in the presence or absence of Ca^{2+} ions. Experimental conditions: enzyme, 3 milliunits; incubation time, 10 min. Full symbols = C18:1 GD1a; open symbols = C20:1 GD1a.

min, and C20:1 GM1 = 25.5 min. As shown in Figure 3C, after a sialidase treatment that produced only 10% hydrolysis of GD1a, released GM1 was composed of the C18:1 and the C20:1 species in the proportion 68.7% to 31.3%.

Similar proportions (65% and 35%) of C18:1 and C20:1 were observed (Figure 3D) when sialidase treatment was carried out in the presence of 10 mM Ca^{2+} and with about 10% hydrolysis of the starting GD1a. Instead, exhaustive enzymatic hydrolysis (99%) of the native GD1a ganglioside (3-h incubation) produced C18:1 and C20:1 GM1 in the proportion of 53.9% to 46.1% (Figure 3B), very close to that of the starting native GD1a (see Figure 3A and Table I).

In a parallel set of experiments carried out at 46 °C (i.e., above the gel–liquid transition temperature of the bilayer) with about 10% hydrolysis of the native GD1a, the composition of released GM1 was found to be as follows: C18:1 GM1, 66.5%; C20:1 GM1, 33.5% (Figure 3E).

The different ratios between formed C18:1 GM1 and C20:1 GM1 at the short or prolonged time of incubation indicate that *V. cholerae* sialidase, at the initial stage of action upon native GD1a, has a preferential action on the ganglioside molecules carrying the shorter long-chain base.

Kinetics of V. cholerae Sialidase Action on Micellar and Vesicular Dispersions of C18:1 GD1a and C20:1 GD1a. The *V/S* plots referring to *V. cholerae* sialidase acting on micellar dispersions of C18:1 GD1a or C20:1 GD1a in the presence or absence of Ca^{2+} are reported in Figure 4. The corresponding values of apparent V_{max} and K_m are given in Table II. Neither in the presence nor in the absence of Ca^{2+} were significant differences in the V_{max} and K_m values between C18:1 GD1a and C20:1 GD1a observed. Calcium ions appear to provide a marked activation which is maximal (about 3-fold) at 2 mM. The K_m values are not affected by calcium. These data show that, upon micellar systems, the enzyme has no intrinsic capacity to discriminate between molecules of GD1a having different lipidic composition. On the contrary, striking differences were noticed in the enzyme kinetics when vesicular dispersions of C18:1 GD1a or C20:1 GD1a were used. In general, the rate of GD1a hydrolysis increased considerably (17–25-fold) from micellar to vesicular dispersions while the K_m value remained substantially unmodified (Table II), confirming previous evidence (Venerando et al., 1982). Moreover, a substantial difference in the kinetic parameters was observed for the two molecular species of gangliosides (Figure 5). The V_{max} values were higher for the C18:1 species than for the C20:1 species [61.25 and 40.75 nmol of hydrolyzed substrate min^{-1} (mg of protein)$^{-1}$, respectively] (Table II).

In the presence of Ca^{2+} the capacity of the enzyme to process C18:1 GD1a more rapidly than C20:1 GD1a was maintained. In the presence of 10 mM Ca^{2+} the rate of hydrolysis increased to 91.25 nmol of hydrolyzed substrate min^{-1} (mg of protein)$^{-1}$ for C18:1 GD1a (72.0 for C20:1 GD1a) and in the presence of 2 mM Ca^{2+} to 112.25 for C18:1 GD1a (96.25 for C20:1 GD1a).

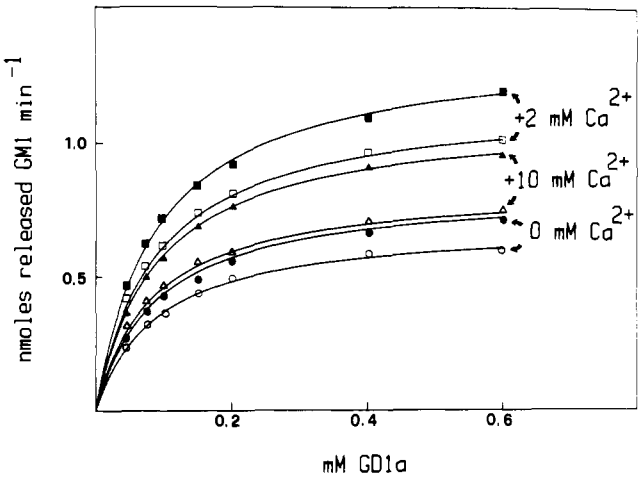

FIGURE 5: Kinetics of *V. cholerae* sialidase action on GD1a molecular species inserted into DPPC LUVs, in the presence or absence of Ca^{2+} ions. Experimental conditions: enzyme, 0.3 milliunit; incubation time, 5 min. Full symbols = C18:1 GD1a; open symbols = C20:1 GD1a.

The enzyme kinetics on vesicles containing a mixture (1:1) of C18:1 GD1a and C20:1 GD1a (total GD1a, 10% molar) were also studied. In these experiments radiolabeled C18:1 GD1a or C20:1 GD1a were added in turn to the mixture of the cold gangliosides. The V_{max} values obtained and reported in Table II [62.5 and 44.0 nmol of hydrolyzed substrate min^{-1} (mg of protein)$^{-1}$ for C18:1 GD1a and C20:1 GD1a, respectively] show that the susceptibility of the molecular species to sialidase action remains the same as when the same species was separately loaded in the vesicles.

Discussion

The most striking piece of evidence emerging from the present investigation is the ability of the sialidase from *V. cholerae* to distinguish between GD1a ganglioside molecules containing different long-chain bases (C18:1 or C20:1). This peculiar ability is linked to the type of ganglioside dispersion. In fact, when the ganglioside molecules are embedded in the vesicles (LUVs), the enzyme displays a much lower activity on C20:1 GD1a than on C18:1 GD1a, while no difference in the enzyme activity is expressed when the same gangliosides are in micellar dispersions.

Since *V. cholerae* sialidase has no recognition site for the ganglioside aglycon (Corfield et al., 1981), the capacity to distinguish ganglioside molecules on the basis of their long-chain base composition is not an intrinsic attitude of the enzyme but should reside in the particular features of the membrane-mimicking system that contains the ganglioside.

The model system adopted in our studies (DPPC LUVs with the gangliosides inserted only in the outer layer) was extensively characterized in a series of papers (Felgner et al., 1981, 1983; Thompson et al., 1985; Acquotti et al., 1987) and utilized in a number of investigations, as an artificial membrane model mimicking the ganglioside asymmetry in natural membranes. The calorimetric data obtained show that in this vesicular system C20:1 GD1a molecules segregate in ganglioside-enriched ganglioside–phospholipid microdomains on the membrane, while C18:1 GD1a molecules do so a much lesser extent. Presumably sialidase recognizes molecular dispersions of GD1a molecules from molecules packed in ganglioside-enriched domains (a situation more similar to that occurring in micelles) providing higher activity on C18:1 GD1a (more dispersed) than on C20:1 GD1a (more packed).

The calorimetric data obtained in this investigation bring to mind data obtained by Masserini and Freire (1986) using GM1 ganglioside and phosphatidylcholines of different chain length and suggest that the main driving force toward ganglioside phase separation is the chain-length difference between the ganglioside long-chain bases and the phospholipid acyl chains, in analogy with mixtures of phosphatidylcholines of different chain length (Mabrey & Sturtevant, 1976). Therefore, it is conceivable that with phospholipids other than DPPC different results can be expected regarding the capacity of ganglioside molecular species to undergo lateral phase separation.

It is worthy of note that the calorimetric data presented in this work were obtained with the instrument operating in the heating direction and the enzymatic determinations were carried out at 37 °C. The lateral phase separation of gangliosides, recognized by sialidase, is therefore present when the bilayer is in the gel state. However, the enzymatic data obtained at 46 °C showed the same difference in recognizing the ganglioside molecular species as data at 37 °C suggesting that lateral phase separation is also present when the bilayer is in the fluid state. Preliminary calorimetric data obtained on cooling are consistent with this interpretation (paper in preparation).

It should be emphasized that the kinetic parameter affected by the different distribution of gangliosides on the surface is the V_{max} and not the K_m. Therefore, it is conceivable that it is not the formation of the enzyme–substrate complex that is influenced by the even or packed distribution of ganglioside molecules but the subsequent steps of catalysis such as the formation and/or liberation of the reaction product.

In the presence of millimolar concentrations of Ca^{2+} ions, the activity of sialidase undergoes a 1.5–3-fold increase in both micellar and vesicular dispersions of GD1a. In the case of micellar dispersions the enzyme does not differentiate C18:1 GD1a from C20:1 GD1a; in the case of vesicular dispersions a higher V_{max} is obtained on C18:1 GD1a than on C20:1 GD1a. These data are in line with the above interpretation, considering that Ca^{2+} ions have an activating effect on *V. cholerae* sialidase (Rosenberg et al., 1960) on the one hand and facilitate the formation of ganglioside-enriched microdomains in the bilayer (Sharom & Grant, 1978; Bertoli et al., 1981) on the other. In theory in the presence of calcium ions one would have expected, as a consequence of the higher degree of lateral phase separation of gangliosides, a greater differentiation between vesicular systems containing C18:1 GD1a or C20:1 GD1a; however, the concomitant strong activation of the enzyme makes it more difficult to establish conditions emphasizing this effect.

In conclusion, the results presented here show that *V. cholerae* sialidase is able to recognize and affect in different ways the same substrate in even molecular dispersion or in laterally segregated domains on artificial membranes. Since the enzyme activity dramatically changes in these two situations, a transition from freely dispersed to segregated ganglioside molecules on the membrane may be an efficient device for regulating the activity of proteins interacting with gangliosides. On the other hand, *V. cholerae* sialidase appears to be a potential biological tool to monitor the lateral phase separation and segregation of individual molecular species of ganglioside within natural membranes.

Registry No. GD1a, 12707-58-3; DPPC, 2644-64-6; Ca, 7440-70-2; sialidase, 9001-67-6.

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