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Effect of calcium ions on the thermotropic behaviour of neutral and anionic glycosphingolipids

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In the concentration range of 10^{-5} to 10^{-1} M Ca^{2+} modulates the thermotropic properties of several neutral and anionic glycosphingolipids (galactosylceramide, asialo-G_{M1}, sulfatide, G_{M1}, G_{D1a}, G_{T1b}) and of their mixtures with dipalmitoylphosphatidylcholine. The transition temperature of gangliosides is not appreciably changed while the transition enthalpy increases by 20% in the presence of Ca^{2+}. The more marked effect of Ca^{2+} is on the thermotropic behavior of systems containing sulfatide. Increasing concentrations of Ca^{2+} between 10^{-5} and 10^{-3} M (up to a molar ratio of Ca^{2+}/sulfatide 1:2) induce a progressive increase of both the transition temperature and enthalpy. Further increases up to 10^{-1} M Ca^{2+} induce a new phase transition at a lower temperature. No evidence is found for induction of phase separation of pure glycosphingolipid-Ca^{2+} domains in mixtures of any of the glycosphingolipids with dipalmitoylphosphatidylcholine. The modification of the phase behavior of anionic glycosphingolipids by Ca^{2+} does not involve detectable variations of the intermolecular packing but is accompanied by marked modifications of the dipolar properties of the polar head group region.

Introduction

Glycosphingolipids modify their interfacial behaviour depending on the interactions established with phospholipids and proteins [1–3]. As a result, the intermolecular organization, phase state and surface electrical potential of lipid interfaces containing glycosphingolipids behave in a non-ideal manner and are influenced by the charge, size, and overall conformation of the oligosaccharide chain present in their polar head group [1,3]. Similarly, the thermotropic behaviour of glycosphingolipids and of their mixtures with DPPC depends markedly on the type and properties of carbohydrate residues present in their oligosaccharide chain [4,5].

The presence of Ca^{2+} affects considerably the molecular parameters and phase behaviour of anionic phospholipids [6,7] and an increase of the intermolecular packing is observed for monolayers of PS or PA in the presence of Ca^{2+} [6]. On the other hand, zwitterionic phospholipids such as PC

Abbreviations: Cer, ceramide (*N*-acylsphingoid); GalCer, Galβ1 → 1Cer; Gg$_4$Cer, Galβ1 → 3GalNAcβ1 → 4Galβ1 → 4Glcβ1 → 1Cer; NeuAc, *N*-acetylneuraminate; sulfatide, Gal(3-sulfate)β1 → 1Cer; G_{M1}, Galβ1 → 3GalNAcβ1 → 4Gal(3 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer; G_{D1a}, NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4Gal(3 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer; G_{T1b}, NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4Gal(3 ← 2αNeuAc8 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer; DPPC, dipalmitoylphosphatidylcholine; PS, phosphatidylserine; PA, phosphatidic acid.

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and PE do not exhibit noticeable changes of their molecular area in the presence of Ca^{2+} but their surface electrical potential is increased due to polar head group interactions with the cation [8]. A similar effect is found with gangliosides which show, in addition, changes of surface potential in the presence of Ca^{2+}. This depends on the dipolar properties of the sialosyl residues and is related to the different orientations of the resultant vertical dipole moment vectors that vary with the location and linkage of the sialosyl residues along the oligosaccharide chain [9].

In this work we study the effect of Ca^{2+} on the thermotropic behaviour of some neutral (GalCer, Gg_4Cer) and anionic (sulfatide, G_{M1}, G_{D1a}, G_{T1b}) glycosphingolipids and some of their mixtures with DPPC by high sensitivity differential scanning calorimetry.

Materials and Methods

The source and purification of glycosphingolipids have been described elsewhere [4]. Calorimetric studies were performed with a DASM-1M Privalov calorimeter at a nominal scan rate of 0.5 K/min with lipid dispersions prepared as described previously [4,5], in water containing 145 mM NaCl and the required concentration of $CaCl_2$. Some experiments were performed in which $CaCl_2$ was added to lipid dispersions pre-formed in 145 mM NaCl. However, this method led to rather high noise levels and considerable oscillations in the base line as compared with the method of co-dispersion in the presence of $CaCl_2$. This may be due to the random formation of unstable aggregates and clumps (visually observable in some samples after the addition of $CaCl_2$) that tend to move with convection currents or settle within the calorimetric cell. For these reasons, the method of co-dispersion with $CaCl_2$ was routinely employed. Under these conditions a stable and reproducible thermotropic behaviour was found for successive cooling and reheating cycles. A systematic study of detailed morphological changes induced by Ca^{2+} was outside the scope of the present work but routine observations by negative staining electron microscopy (2% sodium phosphotungstate) were made of some representative systems (sulfatide, DPPC-sulfatide, DPPC-G_{D1a}, DPPC-GalCer). Multilamellar structures were seen for the pure neutral glycosphingolipids and for the mixtures of neutral or anionic glycosphingolipids with DPPC (at ganglioside molar ratios between 0.08 and 0.09); pure gangliosides showed spherical or elipsoidal micelles [4,5,10]. The presence of Ca^{2+} in concentration of 0.01 M did not induce significant changes except that the mixtures containing anionic glycosphingolipids showed a tendency to become aggregated.

Results

The presence of Ca^{2+} induces minor changes of the t_m of GalCer (Fig. 1) and Gg_4Cer (Table I) and of gangliosides G_{M1}, G_{D1a} (Fig. 1) and G_{T1b}

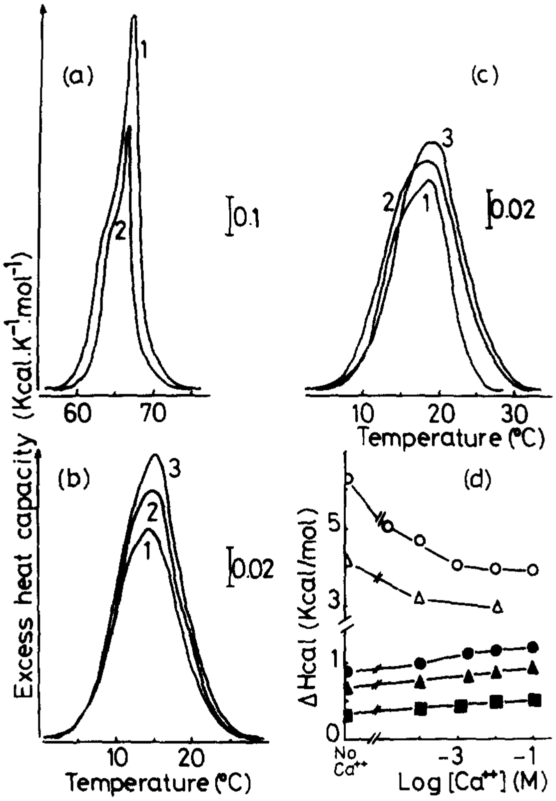

Fig. 1. Excess heat capacity-temperature curves for: (a) GalCer with no added Ca^{2+} (1), 10^{-1} M Ca^{2+} (2); (b) G_{D1a} with no added Ca^{2+} (1), $3.2 \cdot 10^{-3}$ M Ca^{2+} (2), 10^{-1} M Ca^{2+} (3); (c) G_{M1} with no added Ca^{2+} (1), $1.6 \cdot 10^{-3}$ M Ca^{2+} (2), 10^{-1} M Ca^{2+} (3). The dependence of ΔH_{cal} with the Ca^{2+} concentration (d) is shown for GalCer (○), Gg_4Cer (△), G_{M1} (●), G_{D1a} (▲) and G_{T1b} (■).

(Table I). The effect of Ca^{2+} on the transition enthalpy of these lipids is more marked. An approximately 20% increase in ΔH_{cal} is found for gangliosides in the presence of 0.1 M Ca^{2+}, while a 40% decrease occurs with the neutral glycosphingolipids (Table I and Fig. 1).

In spite of the changes induced by Ca^{2+} on the thermotropic properties of gangliosides and neu-

TABLE I

t_{max} and C_{pmax} correspond, respectively, to the temperature and magnitude of the point of maximal excess heat capacity of the phase transition. ΔH_{cal} is the transition enthalpy and CU is the cooperative unit defined as the ratio between the van't Hoff enthalpy and ΔH_{cal}. (T), (C1), (C2) and (C3) refer to the total transition, and the component transitions obtained by deconvolution analysis of the excess heat capacity-temperature curves assuming independent transitions.

Lipid	Parameter	Calcium concentration (M)							
		0.0	10^{-5}	10^{-4}	10^{-3}	0.005	0.010	0.03	0.10
GalCer	t_m	67.10	66.80	66.85	67.02		66.75		67.00
	C_{pmax}	0.98	0.84	0.76	0.72		0.70		0.68
	ΔH_{cal}	6.30	5.10	4.60	4.02		3.91		3.85
	CU	23	30	33	41		42		42
Gg_4Cer	t_m	54.20		54.50			53.90		
	C_{pmax}	0.70		0.53			0.40		
	ΔH_{cal}	4.10		3.10			2.54		
	CU	35		47			53		
Sulfatide									
(T)	t_m	49.95	50.10	54.54	55.50	54.35	42.52	42.80	43.50
	C_{pmax}	0.31	0.33	0.43	0.61	0.46	0.33	0.28	0.32
	ΔH_{cal}	2.62	3.00	4.49	7.48	7.80	6.40	3.98	2.92
	CU	37	30	18	9	6	6	14	29
(C1)	t_m			50.60	50.34	50.20			
	C_{pmax}			0.16	0.13	0.08			
	ΔH_{cal}			1.08	0.82	0.43			
	CU			114	160	359			
(C2)	t_m			55.80	56.10	55.95	55.88	55.90	
	C_{pmax}			0.36	0.56	0.41	0.25	0.05	
	ΔH_{cal}			3.31	6.46	5.25	2.83	0.70	
	CU			28	12	13	27	88	
(C3)	t_m				43.21	43.20	43.3	43.15	43.15
	C_{pmax}				0.04	0.19	0.28	0.20	0.32
	ΔH_{cal}				0.25	1.92	3.41	3.30	2.92
	CU				509	41	19	15	30
G_{M1}	t_m	19.00		18.75	19.00		18.95		19.10
	C_{pmax}	0.11		0.12	0.13		0.14		0.15
	ΔH_{cal}	0.93		1.02	1.12		1.15		1.17
	CU	86		78	70		72		74
G_{D1a}	t_m	15.05		15.09	15.19		15.03		15.10
	C_{pmax}	0.09		0.11	0.12		0.13		0.14
	ΔH_{cal}	0.68		0.78	0.82		0.85		0.87
	CU	128		119	118		119		122
G_{T1b}	t_m	7.42		7.54	7.38		7.50		7.45
	C_{pmax}	0.07		0.08	0.09		0.11		0.12
	ΔH_{cal}	0.34		0.36	0.38		0.41		0.43
	CU	379		387	390		410		406

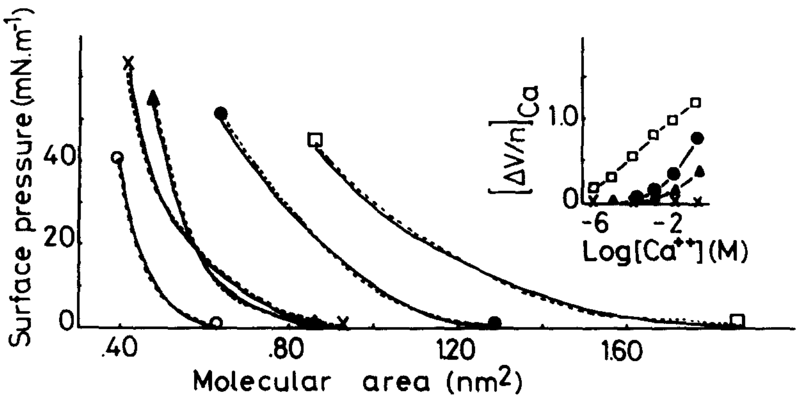

Fig. 2. Surface pressure-area curves at 20±1°C for GalCer (○), Gg_4Cer (×), Sulf (▲), G_{M1} (●) and G_{D1a} (□) on subphases containing 145 mM NaCl with no added Ca^{2+} (———) or 10^{-1} M Ca^{2+} (- - - - - -). The inset shows for each lipid the surface potential per molecule ($fV \cdot cm^2 \cdot mol^{-1}$) at 30 $mN \cdot m^{-1}$ obtained on subphases containing the indicated concentration of Ca^{2+} after discounting the surface potential per molecule on 145 mM NaCl. As described in Ref. 1, the surface pressure-area isotherm for G_{T1b} at 20 °C is fully liquid-expanded and extends from about 15.0 to 30.0 nm^2. This is not modified on subphases containing 10^{-1} M Ca^{2+}; the surface potential per molecule of G_{T1b} shows values slightly higher than those of G_{D1a} and a similar variation with the Ca^{2+} concentration (see Ref. 9)

tral glycosphingolipids, no modification of the molecular area at different surface pressures is observed in lipid monolayers at 20 °C over Ca^{2+}-containing subphases (Fig. 2, see also Ref. 9). However, Ca^{2+} induces a considerable modification of the surface electrical potential of ganglioside monolayers (Fig. 2). The range of Ca^{2+} concentration in which this change becomes noticeable is different for the various ganglioside species; it depends on the dipolar properties and type of linkage of a particular sialosyl residue in the oligosaccharide chain [9].

A more marked effect of Ca^{2+} is on the thermotropic behaviour of sulfatide (Fig. 3 and Table I). The excess heat capacity-temperature curves can be resolved into two or three components

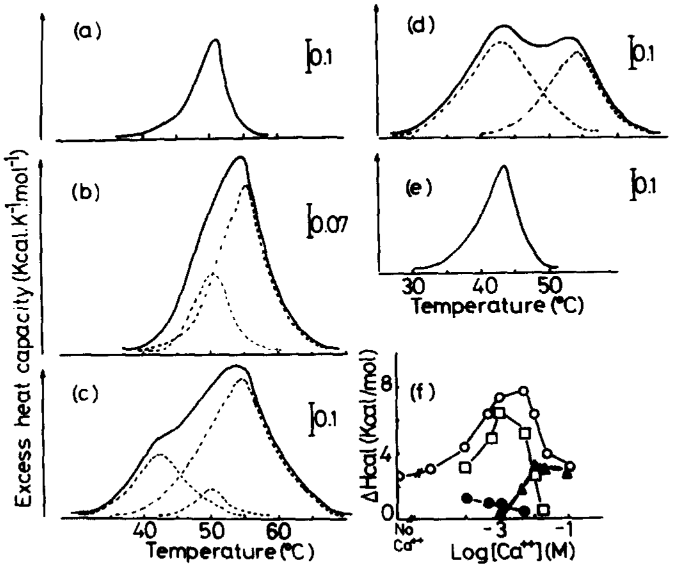

Fig. 3. Excess heat capacity-temperature curves for sulfatide in the presence of: no added Ca^{2+} (a); 10^{-4} M Ca^{2+} (b); $5 \cdot 10^{-3}$ M Ca^{2+} (c); 10^{-2} M Ca^{2+} (d); 10^{-1} M Ca^{2+} (e). The dependence of ΔH_{cal} with the Ca^{2+} concentration (f) is shown for the total transition (○), and for the component transitions with the t_m values at about 50 °C (●), 56 °C (□), and 43° C (▲).

depending on the concentration of Ca^{2+} and assuming either independent or sequential transitions. Ca^{2+} induces at first the progressive appearance of a component with a higher t_m at about 56°C and higher transition enthalpy up to a concentration of $6.7 \cdot 10^{-4}$ M to $5 \cdot 10^{-3}$ M. In this range of Ca^{2+} concentration the point of maximal excess heat capacity of the whole curve is shifted by 4–5 K and the total ΔH_{cal} is increased considerably (Fig. 3 and Table I). Further increases of the concentration of Ca^{2+} have a contrasting effect and bring about a progressive decrease of the transition enthalpy of the total transition, and a component with a lower t_m centered at about 43.2°C gradually shows up. At 0.1 M Ca^{2+} the t_m is decreased by about 6 K and the transition enthalpy has decreased to a value only slightly higher than that of sulfatide in the absence of Ca^{2+}. The t_m values of both the component with a higher and a lower transition temperature and that corresponding to the t_m of pure sulfatide remain approximately constant (within 0.5 K) in the presence of the different concentrations of Ca^{2+}. Their relative contributions to the total enthalpy, on the other hand, vary according to the cation concentration (Fig. 3 and Table I). The dispersion of sulfatide becomes flocculent and some precipitation occurs above a concentration of Ca^{2+} of 10 mM. Similar to gangliosides, no modification of the intermolecular packing of sulfatide is found in the presence of 10^{-1} m Ca^{2+} in the subphase (Fig. 2).

Below a concentration of 10 mM Ca^{2+} only minor changes of the thermotropic behaviour of DPPC are observed. The behaviour of equimolar mixtures of GalCer-DPPC (Fig. 4) or Gg_4Cer-DPPC (Table II) in the presence of Ca^{2+} shows a slight but consistent decrease of the t_m and a broadening of the excess heat capacity-temperature curve while the total ΔH_{cal} is moderately increased. Resolution of the curves by fitting of

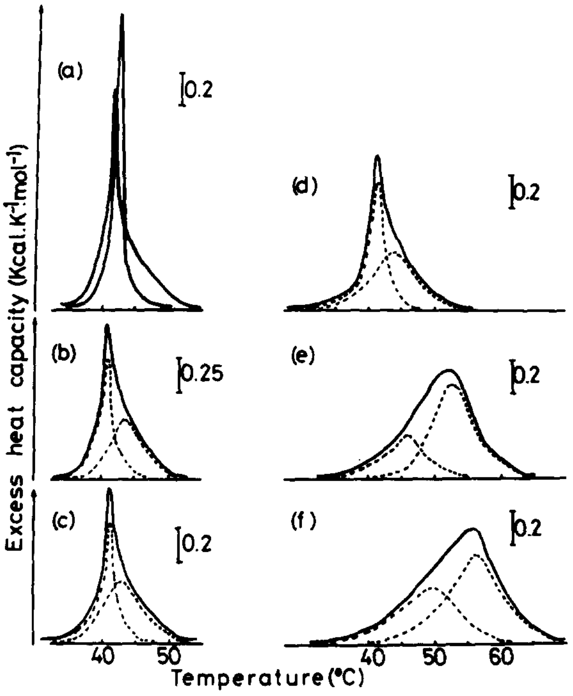

Fig. 4. Excess heat capacity-temperature curves for: (a) mixtures of GalCer-DPPC (molar ratio 1:1) with no added Ca^{2+} (1) and $6.3 \cdot 10^{-4}$ M Ca^{2+} (2); (b) $1.3 \cdot 10^{-2}$ M Ca^{2+}; (c) 10^{-1} M Ca^{2+}; (d) mixture of Sulf-DPPC (molar ratio 1:1) with no added Ca^{2+}; (e) $2.5 \cdot 10^{-3}$ M Ca^{2+}; (f) 10^{-1} M Ca^{2+}.

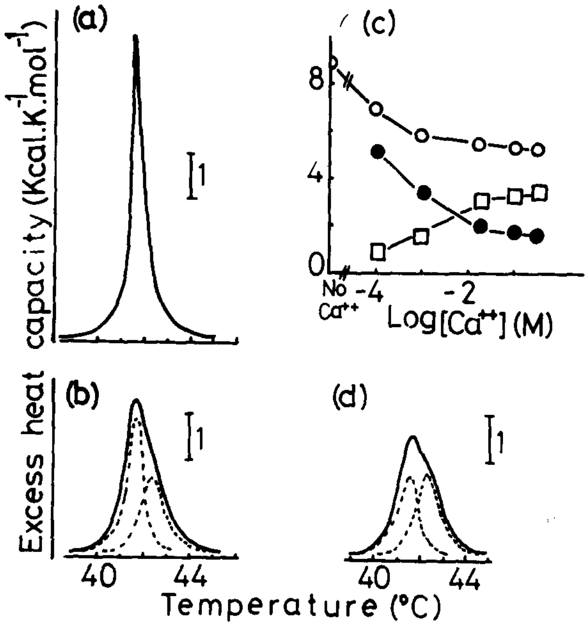

Fig. 5. Excess heat capacity-temperature curves for mixtures of G_{M1}-DPPC (molar fraction of $G_{M1} = 0.09$) with no added Ca^{2+} (a), 10^{-3} M Ca^{2+} (b), $2 \cdot 10^{-2}$ M Ca^{2+} (d). The dependence of ΔH_{cal} with the Ca^{2+} concentration (c) is shown for the total transition (○) and the component transitions with the t_m values at about 41.4°C (●) and 42.3°C (□). Effects similar to these in the presence of Ca^{2+} are found for mixtures of G_{D1a}-DPPC and G_{T1b}-DPPC (molar fraction of ganglioside = 0.08) (see also Table III).

two sequential or independent transitions suggest that Ca^{2+} induces the appearance of two new components in these mixtures. Depending on the concentration of the bivalent cation, one component remains centered between about 41 and 41.5 °C and another between 43.9 and 44.1 °C for DPPC-GalCer; for DPPC-Gg_4Cer the two component transitions occur between 44.4 and 45.6 °C and between 48.6 and 48.8 °C (Fig. 4 and Table II). With mixtures of sulfatide-DPPC (mole ratio 1 : 1) the whole curve shifts to higher temperatur eand exhibits a greater ΔH_{cal} in presence of increasing concentrations of Ca^{2+} (Fig. 4 and Table II). The curves are rather broad; the deconvolu-

TABLE II

For denomination of the thermodynamic parameters of the total and components transitions see the legend under Table I.

Lipid	Parameter	Calcium concentration (M)				
		0.0	10^{-4}	10^{-3}	0.010	0.10
DPPC-GalCer	t_m	42.20	42.10	41.50	41.00	40.90
(1:1)	C_{pmax}	1.79	1.68	1.19	1.13	0.92
	ΔH_{cal}	3.80	3.70	4.30	4.74	4.94
	CU	98	97	51	39	30
(C1)	t_m			41.30	41.30	41.40
	C_{pmax}			0.95	0.85	0.70
	ΔH_{cal}			2.60	2.35	2.30
	CU			110	121	104
(C2)	t_m			43.90	44.10	43.90
	C_{pmax}			0.29	0.42	0.36
	ΔH_{cal}			1.58	2.34	2.39
	CU			93	61	50
DPPC-Gg_4Cer	t_m	46.50		46.00	45.70	45.30
(1:1)	C_{pmax}	1.15		0.76	0.73	0.60
	ΔH_{cal}	3.50		3.90	4.40	4.50
	CU	76		40	30	24
(C1)	t_m			45.50	45.40	45.55
	C_{pmax}			0.60	0.56	0.48
	ΔH_{cal}			2.16	2.21	2.10
	CU			104	92	88
(C2)	t_m			48.70	48.80	48.65
	C_{pmax}			0.20	0.30	0.22
	ΔH_{cal}			1.65	2.10	2.20
	CU			60	56	37
DPPC-sulfatide	t_m	41.60	51.55	52.40		56.29
(1:1)	C_{pmax}	0.93	0.58	0.62		0.67
	ΔH_{cal}	4.40	4.82	5.59		6.15
	CU	38	21	17		15
(C1)	t_m	41.10	44.89	45.55		49.55
	C_{pmax}	0.64	0.30	0.26		0.34
	ΔH_{cal}	1.59	1.42	1.85		2.30
	CU	199	120	61		53
(C2)	t_m	43.80	50.95	52.69		56.60
	C_{pmax}	0.36	0.55	0.56		0.51
	ΔH_{cal}	2.55	3.32	3.44		3.76
	CU	44	42	40		31

tion analysis suggests the presence of two components both showing progressively higher t_m values and ΔH_{cal} values as the concentration of Ca^{2+} is increased.

For mixtures of G_{M1}, G_{D1a} or G_{T1b} with DPPC in mole ratios at which the favored stable structure corresponds to bilayer vesicles and a single type of phase behaviour occurs [5,10] (i.e., below 10 mole% of ganglioside in the mixture) a behaviour that reproduces, in general, that described previously by Myers et al. [11] is found in the presence of Ca^{2+} (Fig. 5 and Table III). Concentrations of Ca^{2+} higher than 10^{-4} M induce a broadening of the excess heat capacity-tempera-

TABLE III

For denomination of the thermodynamic parameters of the total and components transitions see the legend under Table I. The molar fraction of ganglioside in the mixture with DPPC is 0.09 for G_{M1} and 0.08 for G_{D1a} and G_{T1b}.

Lipid	Parameter	Calcium concentration (M)					
		0.0	10^{-4}	10^{-3}	0.010	0.10	0.50
DPPC-G_{M1}	t_m	41.49	41.40	41.44	41.55	41.43	42.43
	C_{pmax}	6.79	4.80	3.29	2.61	2.50	2.48
	ΔH_{cal}	8.27	6.70	5.85	5.49	5.35	5.33
	CU	78	84	76	68	69	69
(C1)	t_m		41.45	41.40	41.35	41.30	41.35
	C_{pmax}		4.10	2.90	1.60	0.85	0.90
	ΔH_{cal}		5.20	3.40	2.25	1.65	1.68
	CU		119	197	248	245	251
(C2)	t_m		42.40	42.50	42.30	42.40	42.45
	C_{pmax}		0.90	1.60	1.80	1.76	1.80
	ΔH_{cal}		1.05	2.30	3.26	3.30	3.44
	CU		646	240	134	128	120
DPPC-G_{D1a}	t_m	41.22	42.20	42.31	42.67	42.65	42.70
	C_{pmax}	4.90	4.33	2.41	2.21	2.15	2.09
	ΔH_{cal}	6.90	6.53	5.55	5.29	5.15	5.01
	CU	81	80	62	63	64	66
(C1)	t_m		41.70	41.80	41.75	41.80	41.70
	C_{pmax}		3.06	1.45	1.05	0.50	0.48
	ΔH_{cal}		4.40	2.50	1.74	1.18	1.14
	CU		125	183	273	283	291
(C2)	t_m		42.60	42.90	42.80	42.85	42.90
	C_{pmax}		1.25	1.20	1.70	1.52	1.70
	ΔH_{cal}		2.10	2.80	3.75	3.94	4.15
	CU		225	122	96	78	78
DPPC-G_{T1b}	t_m	41.23		42.35	42.46	42.60	42.55
	C_{pmax}	6.46		4.55	3.48	2.80	2.70
	ΔH_{cal}	7.11		6.40	5.60	5.30	5.20
	CU	100		88	88	79	79
(C1)	t_m			41.90	41.85	41.90	41.80
	C_{pmax}			3.12	1.20	0.60	0.55
	ΔH_{cal}			3.70	1.90	1.32	1.26
	CU			180	262	272	273
(C2)	t_m			42.60	42.80	42.95	42.90
	C_{pmax}			1.32	1.90	1.85	1.90
	ΔH_{cal}			2.50	3.90	3.80	3.75
	CU			167	99	101	107

ture curve exhibiting a t_m centered at 41.5 °C and the presence of shoulders on the high temperature side of the transition. The theoretical deconvolution of the excess heat capacity function indicates the possible presence of two component transitions. For the various mixtures these remain centered between 41.3 and 41.9 °C and between 42.3 and 43.0 °C at the different concentrations of Ca^{2+}. However, the shoulders or other less defined deformations, occurring between 42 and 43 °C never resolve into distinct peaks even at a concentrations of 0.5 M Ca^{2+}. The two component transitions reach a relative contribution to the total transition change that remains practically invariant between 0.01 M and 0.5 M Ca^{2+} (Fig. 5 and Table III).

Discussion

Calcium is directly or indirectly involved in a variety of biological processes related to enzymatic activations, protein conformation or phosphorylation, hormone action, nerve stimulation and conduction, and membrane processes related to endo and exocytosis [12]. At least two important membrane processes elicited by glycosphingolipids such as cell fusion and neurotransmitter movements in nerve endings also require Ca^{2+} [13,14]. These effects are usually interpreted as resulting mainly from an increased membrane permeability to the bivalent cation although it is generally recognized that the intermolecular organization in the membrane itself is modified to different degrees by interaction with Ca^{2+}. The apparent association constant of Ca^{2+} with gangliosides has been studied by different methods and reported to be in the range of 10^4 to 10^6 M^{-1} [15,16]. However, it has recently been proposed that this high value for the constant results from a concomitant double layer potential effect in solutions of low ionic strength [17]. When this effect is taken into account the apparent association constant of Ca^{2+} to gangliosides is similar to that occurring with zwitterionic phospholipids and is in the order of 100 M^{-1} [17]. Also, very little binding of Ca^{2+} to ganglioside micelles was found [18].

A screening or reduction of the effect of negatively charged sialosyl residues by interaction with Ca^{2+} may presumably affect intermolecular cohesion. However, this is not reflected by measurable changes of the cross-sectional molecular area of gangliosides in the presence of Ca^{2+} at different surface pressures [9]; certain mixed effects on the molecular packing of some gangliosides are apparently induced by Ca^{2+} at different temperatures [19]. On the other hand, in lipid monolayers the interaction of Ca^{2+} with gangliosides can be revealed by changes of the surface potential that are of a different magnitude and occur at different ocncentrations of Ca^{2+} depending on the type of sialosyl residue present in the ganglioside [9]. This effect is related to the orientation of the resultant dipole moment vector of the sialosyl residue according to its position along the oligosaccharide chain and the type of linkage in which it is involved so as to allow for a stable complexing of the cation [9,20]. Sela and Bach [21] reported no effect of Ca^{2+} on the transition enthalpy and t_m of G_{M1} whereas we observed only a modification of the transition enthalpy at concentrations above 1 mM Ca^{2+} (see Fig. 1). According to the monolayer data, these effects do not appear to result simply from increased hydrophobic chain interactions leading to strengthened intermolecular cohesion. More complex phenomena involving the properties of the polar head group are occurring and hydration-dehydration effects in the presence of different ions or solutes probably participate [22,23,25].

The effect of Ca^{2+} on aqueous dispersions of sulfatide are more marked and complex. Two types of behaviour are present that appear to correspond to immiscible components able to interconvert as a function of the concentration of Ca^{2+}. A component with higher ΔH_{cal} and t_m of about 56 °C is formed at lower concentrations of Ca^{2+}. This increases proportionally and reaches a maximum around 10^{-3} M Ca^{2+} (Fig. 3), this range of concentration corresponds to a mole ratio of Ca^{2+} to sulfatide of 1 : 3 to 1 : 2. This higher melting component may represent some kind of sulfatide-Ca^{2+} association due to stoichiometric binding or negative charge screening thus leading to greater intermolecular cohesion revealed by the increased ΔH_{cal}. Concomitantly to this, a component probably representing sulfatide not interacting with Ca^{2+} gradually decreases its contribution to the total enthalpy change (Fig. 3 and Table I). However, even if the t_m of this component remains centered at the value corresponding to pure sulfatide, some of its phase transition properties are modified by the presence of the rest of the sulfatide-Ca^{2+} phase and shows up to 10-fold increase of its cooperativity at a concentration of Ca^{2+} of $5 \cdot 10^{-3}$ M (Table I).

At concentrations of Ca^{2+} higher than 10^{-3} M, a component with lower t_m becomes predominant until, between about 10 and 100 mM Ca^{2+} (i.e. molar ratios of Ca^{2+} to sulfatide of 6 : 1 to 50 : 1) the component with the t_m at 56 °C and high ΔH_{cal} is no longer detectable. The new component becomes noticeable from a mole ratio of sulfatide to Ca^{2+} of about 1 : 1 and shows a lower t_m at about 43 °C (Table I). This is the only component present above $1.6 \cdot 10^{-2}$ M Ca^{2+} and showing a lower enthalpy of about 2.9 kcal/mol. Also, this component exhibits a degree of intermolecular cooperativity for the phase transition similar to that of pure sulfatide and may be revealing a decrease of the intermolecular cohesion by an excess Ca^{2+} in the lipid interface. In spite of these changes, no modification of the intermolecular packing in monolayers is found in the presence of Ca^{2+}. The monolayer technique is extremely sensitive to small changes of interactions or conformation of the polar head group region that may affect the intermolecular spacings and cohesion energies [1,9]. Due to this and in view of the correlation existing between the behavior of glycosphingolipids in monolayers and bulk dispersions [22] it can be concluded that the changes of thermotropic behavior can not be due to a modification of the intermolecular organization of sulfatide. Interactions taking place in the polar head group region probably participate in these effects since changes of the surface potential are present. These may be related to reorientations or modification of the magnitude of molecular dipoles affecting hydration of the interface. Similarly, the effect of Ca^{2+} on the thermotropic behavior of anionic phospholipids can not be explained solely by charge neutralization and increased intermolecular cohesion effects [24]. Recently we have described changes of the dielectric character that may be related to hydration of surfaces containing anionic glycosphingolipids in the presence of Ca^{2+} [25]. Modifications of the hydrophilic character and hydration of the surface of anionic phospholipid dispersions induced by Ca^{2+} were described before [26,27].

As discussed elsewhere [5], mixtures containing more than about 80 mole% DPPC gel and liquid phase immiscibility is present with the formation of phase-separated DPPC. The asymmetric excess heat capacity-temperature curve of the equimolar mixture of DPPC with sulfatide in absence of Ca^{2+} is contributed by two components (Table II), one corresponds to a mixed sulfatide-DPPC phase and the other represents the phase separated DPPC [5]. In mixtures of sulfatide-DPPC in presence of Ca^{2+} the excess heat capacity-temperature curve is broadened and the t_m and ΔH_{cal} increase. In the presence of increasing concentrations of Ca^{2+} the broadened curve can still be resolved into two components but these acquire a progressively different thermotropic behavior depending on the concentration of Ca^{2+} (Table II). Even at concentration of Ca^{2+} of 0.5 M no component with the characteristics of low t_m (at about 43 °C) corresponding to the apparently complete titration of sulfatide with Ca^{2+} is found. No clear evidence exists for assuming the presence of phase separation of immiscible domains of pure sulfatide interacting with Ca^{2+}. Rather, the results suggest that Ca^{2+} is able to modulate the thermotropic properties of the whole mixture of sulfatide-DPPC. A similar conclusion is to be reached for the mixtures of ganglioside-DPPC, in agreement with Myers et al. [11]. For the latter mixtures the presence of deformations and shoulders in the excess heat capacity-temperature curves may be indicative of the coexistence of phases in which the proportion of ganglioside deviates from the ideal average. The curves can be resolved into two component transitions. However, these are strongly overlapping and the best fit for the deconvolution analysis could not be improved beyond a standard deviation of 5–8%. This makes it very difficult to perform an analysis of their relative contribution and ascertain their individuality.

An effect that should be emphasized is that the transition enthalpy of the neutral glycosphingolipids, GalCer and Gg_4Cer, is higher and the excess heat capacity-temperature curve of mixtures of these lipids with DPPC is considerably broadened in the presence of Ca^{2+}. However, in lipid monolayers this cation does not bind to these neutral glycosphingolipids or induce any change of their dipolar properties or intermolecular packing [9]. Similar to the case of gangliosides mixed with DPPC, the mixtures containing the neutral glycosphingolipids can be deconvoluted into two strongly overlapping independent transitions that could not be resolved to a statistical certainty better than about 6%. It is also possible that Ca^{2+} may induce only an asymmetry of the excess heat capacity-temperature curve due to a preferential interaction or partition of the cation into the liquid compared to the gel state of the lipid phase [28]. The modification of their thermotropic behaviour may also result from longer range effects derived from a surface adsorption of Ca^{2+} resulting in diminished intermolecular association through positive charge repulsion or modification of the lipid hydration shell.

In conclusion, Ca^{2+} in the concentration range of 10^{-5} to 10^{-1} M can modulate the thermotropic properties of lipid interfaces containing either neutral or anionic glycosphingolipids of different complexities and their mixtures with DPPC. These phase changes apparently do not involve detectable variations of the intermolecular packing but are accompanied by considerable modifications of the dipolar properties of the lipid interface or its state of hydration that may be modified by Ca^{2+}.

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