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Molecular parameters and physical state of neutral glycosphingolipids and gangliosides in monolayers at different temperatures *

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The effect of temperature on the behaviour of four different gangliosides (G_{M3}, G_{M1}, G_{D1a} and G_{T1b}), sulphatide, ceramide (Cer) and three neutral glycosphingolipids (GalCer, Gg_3Cer, Gg_4Cer) was investigated in monolayers at the air-NaCl (145 mM) interface. G_{M1}, G_{D1a} and G_{T1b} are liquid-expanded in the range of temperatures studied (5–65°C). G_{M3}, sulphatide, Cer and neutral glycosphingolipids show isothermal liquid-expanded → liquid-condensed transitions. The collapse pressure of ganglioside monolayers decreases with temperature, whereas neutral glycosphingolipids may show some maximum values at particular temperatures. The reduction of the molecular area of liquid-expanded glycosphingolipids under compression occurs with a favorable positive entropy change and an unfavorable negative enthalpy. By contrast, the compression of interfaces with a two-dimensional phase transition occurs with an unfavorable entropy but a favorable enthalpy change. From the temperature dependence of the surface pressure at which the two-dimensional phase transition takes place, a minimal temperature above which the isotherm becomes totally liquid-expanded can be obtained. For the different glycosphingolipids this temperature decreases in the order Cer > GalCer > sulphatide > Gg_3Cer > Gg_4Cer > G_{M3} > G_{M1} > G_{D1a} > G_{T1b}. This sequence is similar to that found for the calorimetrically determined transition temperatures (cf. Maggio, B., Ariga, T., Sturtevant, J.M. and Yu, R.K. (1985) Biochemistry 24, 1084–1092).

Introduction

For phospholipids, it has long been demonstrated that both the phase behaviour of bilayer vesicles and the molecular properties in interfacial monolayers are well correlated; a knowledge of the transition temperature for the bulk dispersion permits inferences on the state of the lipid monolayer at a particular temperature and vice versa [1]. For glycosphingolipids, one of the major factors that influence the thermotropic behaviour of their bulk dispersions in dilute aqueous solutions is the type and conformation of the oligosaccharide chain

* Dedicated to Dr. Luis F. Leloir on the occasion of his 80th birthday.

Abbreviations: Cer, ceramide (*N*-acylsphingosine); NeuAc, *N*-acetylneuraminate; GalCer, Galβ1–1Cer; sulphatide, sulphate-I^3-GalCer; LacCer, Galβ1–4Glcβ1–1Cer; Gg_3Cer, GalNAcβ1–4Galβ1–Glc1–1Cer; Gg_4Cer, Galβ1–3GalNAcβ1–4Galβ1–4Glcβ1–1Cer; G_{M3}, NeuGcα2–3Galβ1–4Glcβ1–1Cer; G_{M1}, Galβ1–3GalNAcβ1–4Gal(3–2αNeuAc)β1–4Glc1–1Cer; G_{D1a}, NeuAcα2–3Galβ1–3GalNAcβ1–4Gal(3–2αNeuAc)β1–4Glc1–Cer; G_{T1b}, NeuAcα2–3Galβ1–3GalNAcβ1–4Gal(3–2αNeuAc8–2αNeuAc)-β1–4Glcβ1–1Cer; DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine. Abbreviations are those recommended by IUPAC-IUB (cf. Ref. 5) for neutral glycosphingolipids and by Svennerholm [30] for gangliosides.

present in their polar head group [2]. However, compared to phospholipids, the optimization of energetic, entropic and geometric constraints introduces the possibility of different states of aggregation for glycosphingolipids with oligosaccharide chains of different complexities [3]. Therefore, both the oligosaccharide chain effect and the influence of a different structural organization cannot be easily separated when performing calorimetric studies in aqueous lipid dispersions [2].

In a monomolecular film at the air-water interface the molecules are constrained to the surface and are not free to self-assemble into different three-dimensional structures. In spite of this more similar long-range organization, the physical state of the monolayer arrangement and the possibility for two-dimensional phase transitions vary with the individual properties and interactions of the molecules at the interface [4].

In this work, we have studied the behaviour at different temperatures of monolayers of glycosphingolipids that differ in the complexity of their polar head group. This provides information on the thermodynamic parameters for the two-dimensional packing and phase transitions of these molecules when organized in an oriented array at the aqueous interface, a situation that resembles their state in biological membranes. In addition, a comparison is made of the bulk thermotropic behaviour of glycosphingolipids [2] with the physical state of monolayers at different temperatures. The results indicate that the properties of glycosphingolipids in monolayers at different temperatures and their thermotropic behaviour in bulk dispersions are reasonably well correlated. Both techniques show that the properties of the oligosaccharide chain is one of the primary factors that influence the physical state of glycosphingolipids.

Materials and Methods

As representative lipids we employed Cer, three neutral glycosphingolipids (GalCer, Gg_3Cer, Gg_4Cer) and five anionic glycosphingolipids (sulphatide, G_{M3}, G_{M1}, G_{D1a} and G_{T1b}). The purification and sources of glycosphingolipids and the technique, equipment, calculations and reproducibility for the measurement of monolayer properties at the air-145 mM NaCl interface, at pH 5.6, were described in detail previously [5–7]. DMPC and DPPC were from Sigma Chem. Co. (St. Louis, MO, U.S.A.). The temperature was maintained between 5 and 65°C with a Haake F3C thermocirculating bath with a precision better than 0.1 Cdeg with a thermometer probe immersed directly into the subphase and provided with a digital output redout.

The natural glycosphingolipids employed have a relatively homogeneous hydrocarbon portion. Bovine brain gangliosides and Gg_3Cer and Gg_4Cer, which are derived from them by a desialylation procedure [8], contain mostly C_{18}- or C_{20}-sphingosine bases and the fatty acyl residue is predominantly stearic acid [9]. Ceramide was prepared as the *N*-stearoyl derivative from galactosylsphingosine [5]. GalCer and sulphatide were obtained from bovine brain [10]; these lipids contain longer-chain and hydroxylated fatty acyl residues [11]. It was previously shown that the influence of the hydrocarbon portion heterogeneity on the monolayer properties and on the thermotropic behaviour of bulk dispersions is relatively small compared to the effect of the oligosaccharide chain [2,5].

Results and Discussion

Surface pressure-area isotherms at different temperatures

It was previously reported [4,5] that at 20°C the isotherm for Cer, GalCer, GlcCer and LacCer are liquid-condensed (LC) while those for G_{M2}, G_{M1}, G_{D3}, G_{D1a} and G_{T1b} are liquid- expanded (LE). Gg_3Cer, Gg_4Cer and G_{M3} exhibit a two-dimensional LE-LC phase transition (see inset in Fig. 1).

Similar to phospholipids, the glycosphingolipids can exhibit different types of monolayer states and the value of surface pressure at which the two-dimensional condensation takes place (π_t) is a function of temperature. This is illustrated in Fig. 1 for the surface pressure-area isotherms at different temperatures for GalCer and G_{M3}. As the temperature increases the two-dimensional phase transition occurs at higher values of π_t until, above a certain temperature, the monolayer becomes totally LE. This depends on the glycosphingolipids; the more complex gangliosides (G_{M1}, G_{D1a} and G_{T1b}) still exhibit isotherms fully LE even at the

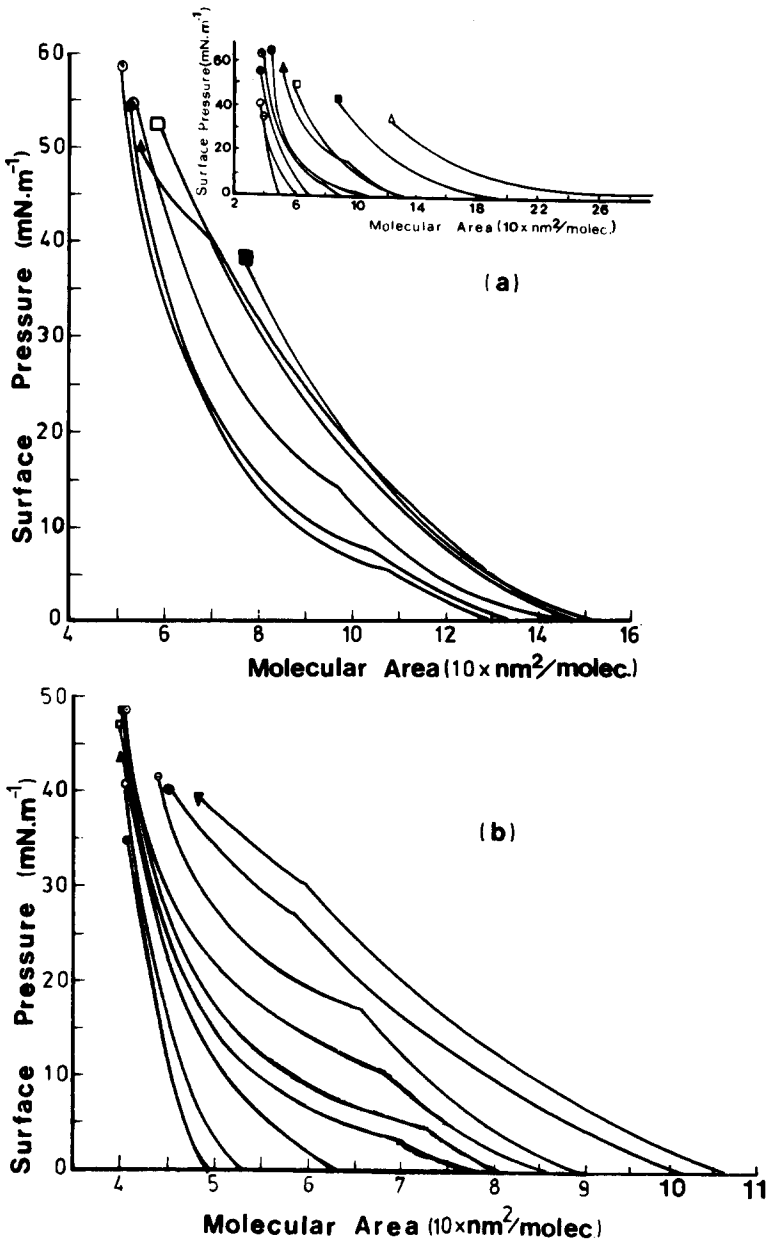

Fig. 1. Surface pressure-area isotherms of glycosphingolipids at different temperatures. The isotherms for G_{M3} (a) are shown at 5°C (⊙); 10°C (●); 20°C (○); 30°C (▲); 35°C (□) and 40°C (■). The isotherms for GalCer (b) are shown at 10°C (●); 20°C (○); 30°C (▲); 35°C (□); 40°C (■); 47.5°C (⊙) 52°C (⊖); 60°C (⊕) and 66°C (▼). The isotherms in the inset correspond to the temperature of 20°C for Cer (○); GalCer (⊖); LacCer (⊕); Gg_3Cer (⊙); Gg_4Cer (●); G_{M3} (▲); G_{M1} (□); G_{D1a} (■) and G_{T1b} (△).

lowest temperature studied (5°C). On the other hand, the glycosphingolipids with shorter polar head-groups (GalCer, sulphatide) and Cer do not reach a totally LE state at the highest temperature studied. The isotherms at different temperatures were reproducible under successive compression and expansion cycles, indicating the absence of monolayer solubility. Also, the films were stable at the different temperatures; the surface pressure remained constant with time if the compression process was stopped at different values of surface pressure along the isotherm. At the collapse pressure, the decay after stopping compression was not more than 1 $mN \cdot m^{-1}$ in the first 3 min and stayed constant thereafter.

Fig. 2 shows the molecular area vs. temperature isobars for representative neutral and anionic glycosphingolipids with different oligosaccharide

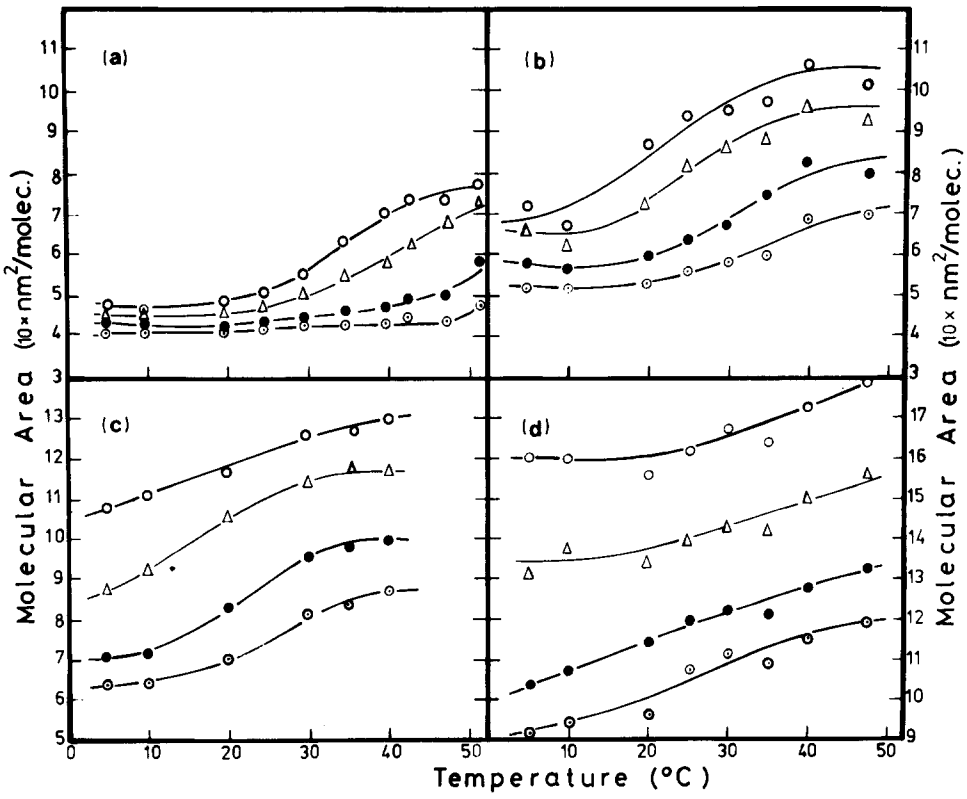

Fig. 2. Molecular area-temperature isobars for representative neutral and anionic glycosphingolipids at different surface pressures. The isobars are shown for GalCer (a); Gg_4Cer (b); G_{M3} (c) and G_{D1a} (d) at a surface pressure of 5 $mN \cdot m^{-1}$ (○); 10 $mN \cdot m^{-1}$ (△); 20 $mN \cdot m^{-1}$ (●) and 30 $mN \cdot m^{-1}$ (⊙).

chains. A steeper increase of the slope of the isobar, indicating a phase transition, occurs at particular temperatures between surface pressures of 0 and 10 $mN \cdot m^{-1}$ for GalCer; for Gg_4Cer this process is detectable at pressures between 5 and 20 $mN \cdot m^{-1}$. At pressures below 5 $mN \cdot m^{-1}$ ganglioside G_{M3} remains totally LE at all temperatures, while isobaric transitions are present between 10 and 30 $mN \cdot m^{-1}$. The isobaric transitions for all these lipids are displaced towards higher temperatures and lower areas per molecule as the lateral surface pressure is higher. The more complex gangliosides (see isobars for G_{D1a} in Fig. 2d) do not exhibit isobaric transitions in the range of temperature studied; only a gradual expansion as a function of temperature is present, probably due to a progressive increase of the molecular kinetic energy.

The above results indicate that, in the range of surface pressures relevant to biological membranes (i.e. about 20–30 $mN \cdot m^{-1}$ [12,13,14]), the more simple neutral glycosphingolipids and the more complex gangliosides may remain in a totally LC or totally LE states, respectively. On the other hand, the neutral glycosphingolipids and gangliosides of intermediate complexity may show isothermal phase transitions depending on the surface pressure. However, it must be kept in mind that in a complex multimolecular system the individual molecular properties are lost [4,6,7,15] and the intermolecular arrangement can be modified considerably by interactions between glycosphingolipids and other membrane lipids [4,6] or proteins [7,15]. In addition, the local molecular density can fluctuate considerably, specially in domains of enhanced compressibility such as the phase-transition region [16], and this may induce isothermal phase transitions in localized domains of the interface.

The values for the collapse pressure (π_c) of a

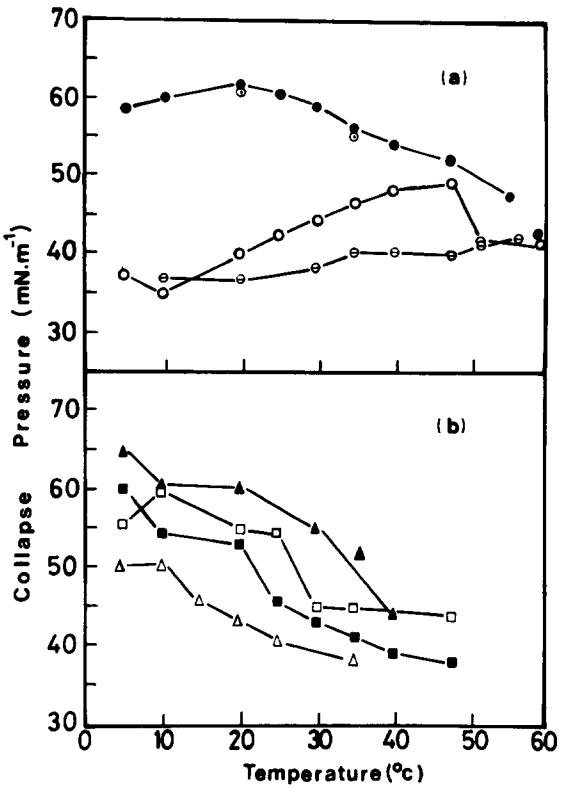

Fig. 3. Collapse pressure of monolayers of neutral and anionic glycosphingolipids at different temperatures. The collapse pressure for neutral glycosphingolipids (a) is shown as a function of temperature for Cer (⊖); GalCer (○); Gg_3Cer (⊙) and Gg_4Cer (●). The collapse pressure for anionic glycosphingolipids (b) is shown as a function of temperature for G_{M3} (▲); G_{M1} (□); G_{D1a} (■) and G_{T1b} (△).

monomolecular film depend on the physical state of the interface and, hence, on the temperature. It results from a balance between intermolecular forces in the film and several factors related to the affinity of the polar head-group for the aqueous subphase [5,17]. At low temperatures the type of oligosaccharide chain appears to influence the hydrophobic-hydrophilic balance for the overall stability of the interface, and different values of π_c for the different glycosphingolipids are observed (Fig. 3). At higher temperatures, on the other hand, the values of π_c for all glycosphingolipids tend to distribute in a very narrow range at about 40 $mN \cdot m^{-1}$ (within ± 5 $mN \cdot m^{-1}$) and the influence of a different polar head-group, if any, has apparently diminished.

The decrease of π_c (and monolayer stability) as the films convert to a more LE character at higher temperature probably reflects an increased kinetic energy and molecular delocalization at the interface [18]. A better and increased hydration of the polar head-group region of glycosphingolipids at higher temperatures [2] may also contribute to this effect. It is interesting that an optimum of π_c can be found in particular ranges of temperature for some of the glycosphingolipids. This suggests that an increase of the molecular kinetic energy does not always, or necessarily, lead to a decreased stability of the monolayer. Also, increases of π_c could result not only from an enhanced stability of the monomolecular arrangement but from a decreased stability of the collapsed phase leading to an increase of its spreading pressure.

Thermodynamic parameters associated with the LE-LC transition and compression process

In principle, the free energy of compression (ΔG_c) may be evaluated directly from the integrated area under the surface pressure-area isotherm. From the temperature dependence of ΔG_c, the enthalpy (ΔH_c) and entropy (ΔS_c) associated with the packing of the molecules during the two-dimensional compression process may be evaluated [19]. Operationally, ΔG_c represents the two-dimensional work involved in bringing together the film-forming molecules to a stable oriented arrangement from a gaseous state ($\pi \rightarrow 0$) up to a certain intermolecular packing [19].

The magnitude of the errors involved in the calculation of ΔG_c can be serious if the gaseous region of the isotherm is neglected. This is usually the case for standard measurements, since the obtention of meaningful values for the very low surface pressure existing in the extremely expanded gaseous region requires sensitivities down to 0.01 $mN \cdot m^{-1}$ or below, which is normally beyond the capabilities of current equipments. However, these limitations only apply if the calculation of ΔG_c attempts to encompass the expanded gaseous region but does not affect the validity of comparisons regarding the work of compression calculated between defined limits of molecular areas and surface pressures. We have therefore performed the calculation of the integrated area under the isotherm between the molecular areas at 0.2 mN ·

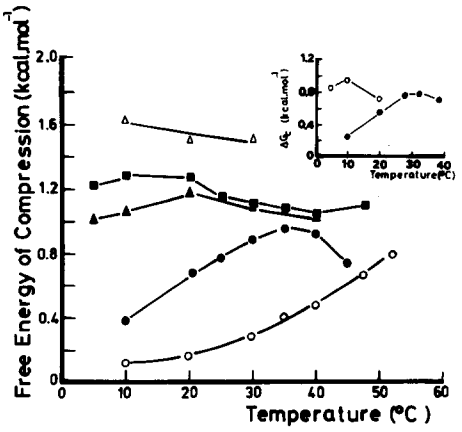

Fig. 4. Apparent free energy of compression of monolayers of glycosphingolipids at different temperatures. The apparent free energy of compression vs. temperature curves are shown for GalCer (○); Gg_4Cer (●); G_{M3} (▲); G_{D1a} (■) and G_{T1b} (△). The inset shows the corresponding curves for DMPC (○) and DPPC (●).

m^{-1} and 30 $mN \cdot m^{-1}$ for each glycosphingolipid. This calculation contains the implicit assumption that the two-dimensional vapor pressure be essentially the same for the different substances employed; this was not possible to estimate due to the sensitivity requirements described above.

In the cases where an isothermal two-dimensional phase transition takes place, the value for the thermodynamic parameters of compression contain energy contributions associated with the phase change besides the work involved in the compression of the molecules. An absolute requirement for these experiments is to ensure that the shape and properties of the surface pressure-area isotherm are completely independent of the rate of compression at the different temperatures. This is necessary for ascertaining that kinetic limitations to the compression process are not present. In our studies the rate of compression was slow enough, since further decreases of it did not introduce modifications of the surface pressure-area isotherms.

Fig. 4 shows that the apparent ΔG_c for the more complex anionic glycosphingolipids is higher than for neutral glycosphingolipids or phospholipids (Fig. 4, inset). This probably reflects the lower intermolecular cohesion for the anionic glycosphingolipids [4,6]. For all glycosphingolipids the values tend to converge as the temperature is increased and the isotherms become more LE. Obviously, the apparent ΔG_c is always positive; however, since this value results from enthalpy-entropy balances, ΔH_c and ΔS_c may acquire different signs depending on the slope of the ΔG_c versus temperature curves. For G_{M3}, Gg_4Cer, DMPC and DPPC, the variation of ΔG_c with temperature shows two defined slopes. The slope is negative at temperatures where the isotherms are totally LE, causing corresponding positive values for ΔH_c and ΔS_c. At temperatures where the lipids show an LE → LC transition the slope is positive and the calculated values of both ΔH_c and ΔS_c are negative. For GalCer (Fig. 4) and Cer (not shown) both the calculated enthalpy and entropy of compression are negative at all temperatures while the values of ΔH_c and ΔS_c calculated for the more complex gangliosides are positive.

No attempt can be made to ascribe an absolute meaning to these apparent thermodynamic quantities in view of the limitations involved in their calculation. However, their comparative values, trends and sign might be due to some of the following effects. For isotherms totally LE, the unfavorable decrease in entropy caused by an increased interfacial ordering of the lipid molecules as these are packed together could be overcome by a favorable increase of entropy if water molecules were released from the polar head-group region. This could occur from the coalescence of the hydration shells of the polar head groups at the interface as the surface molecular density increases [21]. Thus, for interfaces of the LE type an entropically favored (positive ΔS_c) but energetically unfavored (positive ΔH_c) intermolecular organization occurs at high compared to low surface pressures. At temperatures where the isotherms exhibit an LE → LC transition both the calculated ΔS_c and ΔH_c are negative. This may be the result of an increase of order and intermolecular cohesion induced by the two-dimensional condensation; in this case the system becomes energetically favored but entropically unfavored as the pressure increases and the molecules become more closely packed.

The two-dimensional phase change reflected by the LE → LC transition is still a subject under discussion. It has been considered to represent a

TABLE I

LATENT HEAT AND ENTROPY CHANGE ASSOCIATED WITH THE LE → LC TRANSITION OF GLYCOSPHINGOLIPIDS

For definitions of π_t, Q_c and ΔS_t see text. ΔH_{cal} and ΔS^0 are the calorimetrically determined enthalpy and entropy for the phase transitions of bulk aqueous dispersions (taken from Ref. 2).

Glycosphingolipids	π_t (mN/m)	T (°C)	Q_c (kcal/mol)	ΔS_t (cal/K per mol)	ΔH_{cal} (kcal/mol)	ΔS^0 (cal/K per mol)
Cer	5.7	52.0	5.5	16.8	–	–
GalCer	4.3	40.0	9.3	29.8	6.5	19.1
Gg_3Cer	5.0	20.0	9.4	32.2	5.6	16.9
Gg_4Cer	7.7	25.0	14.9	50.0	4.2	12.8
G_{M3}	5.5	5.0	22.9	82.6	2.8	7.8
Sulphatide	6.8	30.0	15.6	51.4	2.8	8.5

kinetic phenomena or a second-order process [22,23]; more recently, a suggestion that compromises both views and considers it as a two-state 'diffuse' first-order process has been proposed [24]. According to this view the occurrence of long-lived 'packing defects' in the transition region can account for a finite slope of the surface pressure-area isotherm. The presence of impurities or mixtures of components (as occurs for purified natural materials) also leads to a diffuse type of transition. Assuming that the transition is first-order, it is possible to use a two-dimensional Clapeyron equation to describe the process [25]:

$$\frac{d\pi_t}{dT} = \frac{Q_c}{T(A_e - A_c)} \quad (1)$$

In our case, it is not necessary to apply a $d\gamma_o/dT$ correction to this equation because the surface pressure of the lipid-free subphase was set equal to zero at each temperature before spreading the film. In Eqn. 1 π_t is the surface pressure at which the LE → LC transition begins, T is the temperature, A_e is the molecular area at π_t and A_c corresponds to the molecular area at the beginning of the LC portion of the isotherm and is obtained by extrapolation of the LC portion down to π_t. While most LC isotherms are steep enough to allow for an acceptable estimate of A_c (within ±2%), for isotherms with a greater slope the uncertainty in A_c can amount to ±5%. This, in turn, introduces maximal uncertainties in the calculated values in Table I of ±0.4 kcal · mol^{-1} for Q_c and ±1.3 cal · K^{-1} · mol^{-1} for ΔS_t.

Q_c, the latent heat associated with the LE → LC transition, cannot be considered simply equal to the enthalpy of the transition. If this is 'diffuse' first-order and does not occur at constant surface pressure Q_c contains additional energetic contributions involved in the change of state that can generally account for about 1 kcal · mol^{-1} [25]. The derivative $d\pi_t/dT$ is obtained from the slope of the π_t vs. temperature plot. The value of $d\pi_t/dT$ is a constant independent of temperature for the glycosphingolipids studied; however, it has been shown that $d\pi_t/dT$ for other systems may not be constant at low pressures [26] and that it may be subphase-sensitive [27]. $d\pi_t/dT$ is an indirect representation of the entropy associated with the condensation process; this can also be concluded from Eqn. 1 written as $d\pi_t/dT = \Delta S_t/\Delta A_t$.

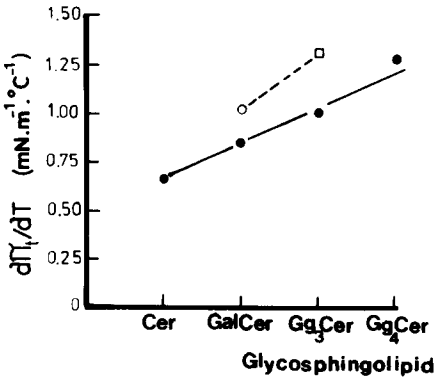

Fig. 5. Changes of the transition surface pressure (π_t) per unit temperature increase. $d\pi_t/dT$ as defined in the text is shown for the glycosphingolipids indicated on the abscissa and for sulphatide (○) and G_{M3} (□).

Fig. 5 shows that $d\pi_t/dT$ increases as the glycosphingolipids become more complex. The values for the different glycosphingolipids are all below those found for DMPC (1.5 $mN \cdot m^{-1} \cdot Cdeg^{-1}$) and DPPC (1.7 $mN \cdot m^{-1} \cdot Cdeg^{-1}$); these values are similar to those reported elsewhere [25]). The smaller value of $d\pi_t/dT$ for glycosphingolipids compared to phospholipids indicates that the entropy change accompanying the expansion of one unit of molecular area for LC → LE transition is less for glycosphingolipids than for phospholipids. Possible factors involved in these effects may be related to the amount of ordered water in the hydration shell of the polar head-group of glycosphingolipids. It is known that more water molecules are associated with the more complex liquid-expanded glycosphingolipids than with the more simple liquid-condensed ones [28]. The gangliosides are also more hydrated than phospholipids and their presence in a lipid interface may lead to an increased micropolarity [29].

A standardized comparison of the values for Q_c and $\Delta S_t = Q_c/T$ for the different glycosphingolipids is complicated because these quantities depend simultaneously on several parameters (π_t, T, changes of molecular area) at which the transition takes place and these vary for each glycosphingolipid. However, the general trend is that the values for Q_c (and ΔS_t) within a range of comparable temperatures are higher for glycosphingolipids with a more complex polar head-group (not shown). A similar trend is found if the values are taken at comparable π_t values (Table I). These results also suggest the participation of enthalpically unfavorable but entropically favored polar head-group hydration-dehydration effects associated with the two-dimensional LE → LC phase transition of glycosphingolipids.

For phospholipids, a satisfactory correlation exists between the physical state of both monolayers and bulk lipid dispersions in aqueous solutions. Above the bulk gel-to-liquid-crystalline phase transition temperature (T_c), the phospholipid monolayers are completely liquid-expanded and π_t, the surface pressure at which the two-dimensional transition takes place, coincides with or is above π_c [1]. From the temperature dependence of π_t it is possible to estimate a minimum temperature at which the isotherms for glycosphingolipids should be fully LE (or, at least, with a π_t above 40 $mN \cdot m^{-1}$, see Fig. 3). For neutral glycosphingolipids the temperature for obtaining a fully LE monolayer should be at least 104°C for Cer, 81°C for GalCer, 54°C for Gg_3Cer and 51°C for Gg_4Cer. For anionic glycosphingolipids, these minimal values are 58°C for sulphatide and 34°C for G_{M3}; the isotherms for gangliosides G_{M1}, G_{D1a} and G_{T1b} did not show LE → LC transitions at the lowest temperature studied (5°C) and from the monolayer behaviour it is clear that bulk aqueous dispersions of these lipids all have T_c values below 20°C. The minimal temperature for reaching a fully LE state decreases as the polar head group of the glycosphingolipids becomes more complex; the sequence is similar to what was previously found for the T_c of glycosphingolipids in dilute aqueous dispersions by high-sensitivity differential scanning calorimetry [1].

For Cer, GalCer and sulphatide the minimal temperatures for obtaining a fully LE monolayer are higher compared with the bulk T_c values observed in direct calorimetric measurements; the latent heat and entropy of the two-dimensional phase transitions of all the glycosphingolipids studied (Table I) are also higher than the calorimetrically determined enthalpies and entropies [1]. The comparisons between the thermotropic behaviour in monolayers and in bulk dispersions assume implicitly that the intermolecular interactions, molecular packing and thermodynamic constraints are the same for both systems. While this may be approximately sound for bilayers or vesicles with a large radius of curvature, it is obviously a gross and inaccurate oversimplification for bilayer vesicles with small radius or for micellar structures [3,14]. When dispersed in aqueous solutions the glycosphingolipids self-assemble into quite different kinds of aggregates according to geometric and thermodynamic restrictions imposed by various concomitant factors [3]. It was previously demonstrated that the properties of the polar head-group region is a major factor mediating the thermotropic behavior of glycosphingolipids, and the T_c of bulk dispersions is linearly dependent on the intermolecular spacings [2]. Our results show that the thermotropic behaviour in monolayers, where the long-range organization for the different glycosphingolipids is more similar than in bulk disper-

sions, is influenced in a comparable manner by the type of oligosaccharide chain and, concomitantly, the molecular area. For the monolayer system, the complex additional factors imposed by the different state of aggregation spontaneously adopted in bulk by the various glycosphingolipids [3] are not present and the two-dimensional thermotropic behaviour may probably be a representation more relevant to the effects occurring in biological membranes.

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