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MONOLAYER CHARACTERISTICS OF SOME GLYCOLIPIDS AT THE AIR-WATER INTERFACE

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SUMMARY

Surface pressure and surface potential versus molecular area data have been obtained for some galactosyldiglycerides and some galactosphingolipids at the air-water interface. The physical states of galactolipid monolayers (and bilayers) parallel those of the phospholipids. The molecular packing of the monolayers is determined primarily by the interactions between the hydrocarbon chains and chain melting causes the transition from condensed to expanded monolayer. Thus the long saturated chain cerebrosides from myelin have high chain-melting temperatures and form condensed monolayers with the chains in a quasi-crystalline array. The galactosyldiglycerides from chloroplast membranes contain polyunsaturated chains and form liquid-expanded monolayers. The surface potentials of monolayers of neutral galactosyldiglycerides are similar to those of equivalent lecithins; the contributions of the hydrated galactose and phosphorylcholine moieties to the surface potential are approximately equal. The various galactosphingolipid monolayers studied have quite different surface potentials; this indicates that relatively small variations in molecular structure which do not lead to appreciable changes in the average packing density can cause large changes in surface potential.

INTRODUCTION

Now that the structures formed by phospholipids at the air-water interface and in aqueous dispersion have been well characterised (for a review, see ref. 1) attention is being directed to glycolipid systems. The limited work [2–9] reported so far suggests that the structural behaviour of galactolipids parallels that of phospholipids. In particular the bilayer seems to predominate and therefore membranes rich in galactolipid will contain this structure. However, there is no information available on the effects that variations in the galactolipid structure have on the molecular packing in bilayers of these lipids. Monolayer studies [1] provide a convenient way of obtaining some of this information. Apart from some measurements on cerebroside monolayers [2, 5, 6]

there has been no systematic study of galactolipid monolayers. The aim of this work is to compare the surface pressure and surface potential characteristics of a series of related galactosphingolipids and of a series of galactosyldiglycerides; the effects of changes in the chemical nature of both the apolar and polar parts of the molecules are considered. The galactosphingolipids are constituents of brain myelin whereas the galactosyldiglycerides occur mainly in the photosynthetic tissues of plants, although small proportions of this latter class of glycolipid have been observed in brain.

EXPERIMENTAL

Materials

The structural formulae of some of the lipids used in this work are shown in Fig. 1. The monogalactosyl and digalactosyldiglycerides were isolated from pelargonium leaves. The sulphoquinovosyldiglyceride (sulpholipid) was obtained from lipid extracts of a mixed algal colony and used as the sodium salt. Full details of the preparation and fatty acid composition have been given previously [7]. The galactosphingolipid, phrenosine, occurs in brain myelin as does cerasine which has the same structure except that it contains a simple saturated fatty acid rather than a 2 hydroxy-saturated fatty acid. We used synthetic and natural (from bovine brain) samples of both cerasine and phrenosine; these were kindly provided by Dr M. Martin-Lomas and have been described elsewhere [10]. Cerasine and phrenosine have been identified as organ-specific lipid haptens [11]. Hydrogenation of the *trans* double bond and shortening of the fatty acid chain substituted on the nitrogen of cerasine gives *N*-palmitoyl dihydrogalactocerebroside (1-0(*β*-D-galactopyranosyl)-*N*-hexadecanoyl-DL-dihydrosphingosine). Removal of the galactose moiety yields *N*-palmitoyl-DL-dihy-

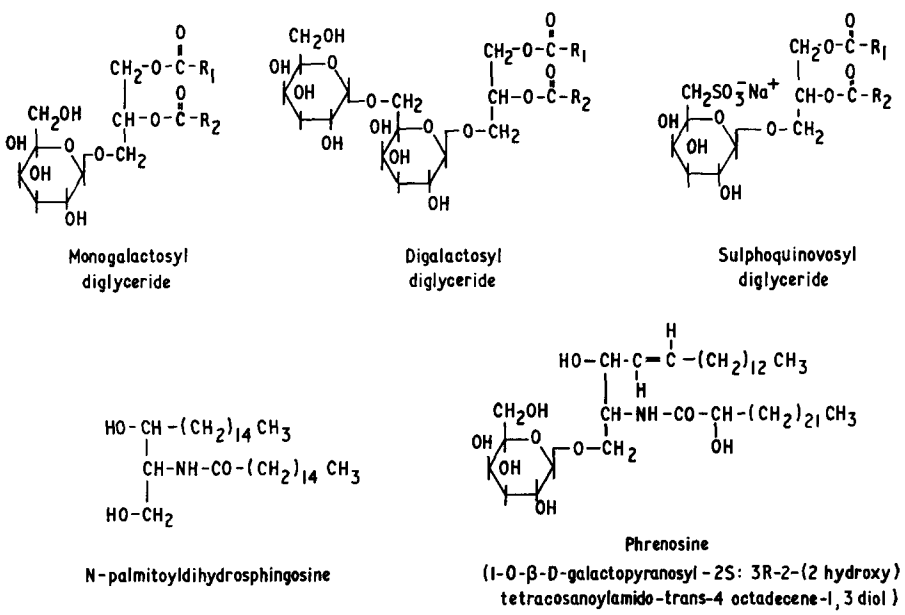


Fig. 1. Structural formulae of some of the lipids used in this investigation.

drosphingosine (see Fig. 1). The latter two sphingolipids were chromatographically pure, synthetic, samples purchased from Miles-Yeda Research Division (Miles Laboratories Inc., Maidenhead, U.K.).

Methods

The apparatus and procedures used to obtain continuous, simultaneous, surface pressure (π) and surface potential (ΔV) measurements as a function of surface area/molecule (A) have been described before [12]. The sphingolipids were spread from 4:1 (v/v) *n*-hexane/ethanol and the galactosyldiglycerides from 4:1 (v/v) chloroform/methanol onto 0.1 M NaCl solutions (pH 5) at 22.5±0.5 °C unless otherwise stated.

RESULTS

The original data were obtained as continuous recorder tracings and the symbols in Fig. 2 and 3 are simply used as labels. The π vs A curve in Fig. 2 is that of digalactosyldiglyceride containing polyunsaturated fatty acid chains (saturated/unsaturated fatty acid ratio 1:4). The equivalent curves for monogalactosyldiglyceride and sulphoquinovosyldiglyceride are not shown but do not differ from that of the digalactosyldiglyceride by more than 0.04 $nm^2 \cdot molecule^{-1}$ at any pressure. Apart from the sulpholipid at pressures above 10 $mN \cdot m^{-1}$, the monolayers were stable. The π vs A curve for the monolayers at room temperature is characteristic of liquid-expanded films. The curve was not significantly affected by cooling to 6 °C indicating that no change in physical state occurred. The π vs A curves

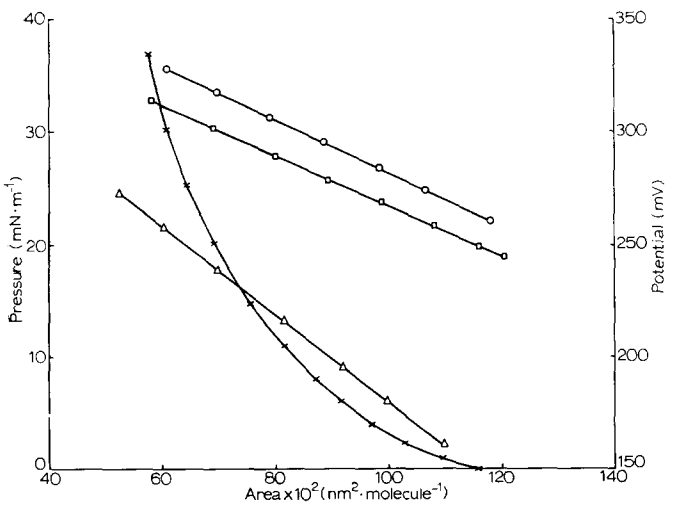


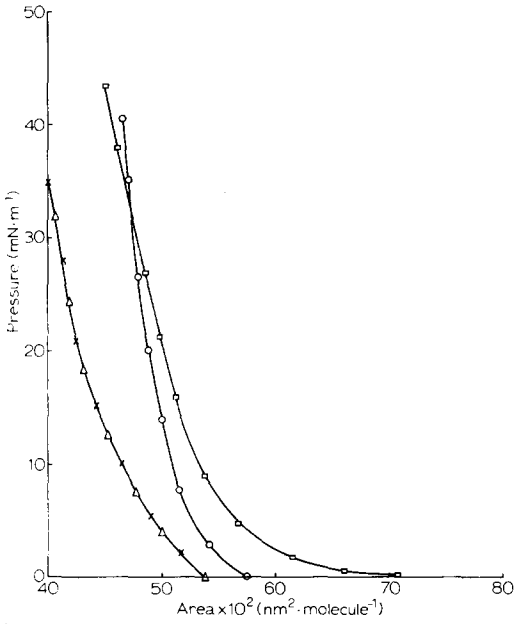
Fig. 2. Surface pressure (π) and surface potential (ΔV) versus molecular area (A) curves for monogalactosyl- and digalactosyldiglycerides and sulphoquinovosyldiglyceride (sulpholipid) on 0.1 M NaCl at room temperature. ×, π for digalactosyldiglyceride (see text for details of other π vs A curves); ○, ΔV for monogalactosyldiglyceride; □, ΔV for digalactosyldiglyceride; △, ΔV for sulphoquinovosyldiglyceride.

of the galactosyldiglycerides are similar to those of lecithins containing polyunsaturated chains [13]. This shows that replacement of the galactose ring by the phosphorylcholine zwitterion does not give rise to large changes in the average molecular packing density in the plane of a monolayer. Because the packing in monolayers correlates with that in bilayers [1, 15] this condition should also apply to bilayers.

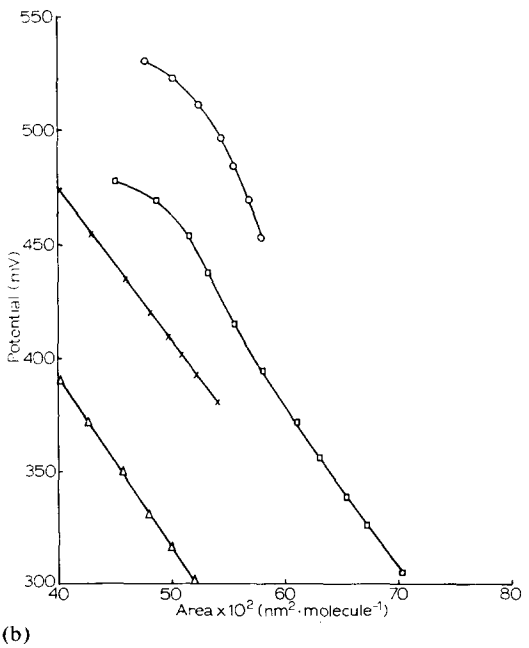
With all of the ΔV vs A curves presented, it was found that at areas greater than "lift-off" ΔV showed random variations of the order of 100 mV which indicates that the films were liquid-expanded and not vapour-expanded (for a definition of terms see ref. 24). The ΔV vs A curves (Fig. 2) for the galactosyldiglycerides have been smoothed to give the straight lines shown. In fact the recorder tracings showed small deviations from linearity during compression but these small fluctuations were not reproducible. Because the surface potentials were only reproducible to ± 10 mV the data indicate that the presence of one or two galactose ring gives similar ΔV values. As expected, the anionic sulphonate group in the sulphoquinovosyldiglyceride causes a reduction in ΔV (cf. ΔV for cerebroside and cerebroside sulphate monolayer [5]). The ΔV vs A curves for the neutral galactosyldiglycerides are similar to those of the equivalent lecithins [12]; for the galactolipids ΔV increases from about 250 to 320 mV as the liquid-expanded film is compressed whereas the equivalent change for lecithin monolayers is from about 250 to 380 mV. Thus the contributions of the hydrated galactose and phosphorylcholine moieties to the surface potential are approximately equal.

The π vs A and ΔV vs A isotherms at room temperature for the series of sphingolipids studied are shown in Figs 3a and 3b, respectively. Phrenosine and cerasine form identical, condensed, monolayers (cf. refs 2 and 6) and the π vs A curve shown is in good agreement with that of Quinn and Sherman [5] for a mixture of cerasine and phrenosine. Monolayer data for *N*-palmitoyldihydrosphingosine and dihydrogalactocerebroside have not been reported before; they also form condensed monolayers and the π vs A curves are presumably slightly more expanded than those of phrenosine and cerasine because of the shorter fatty acid chains present. Warming the monolayers to 35 °C did not lead to any significant expansion. All of these condensed films were unstable [14, 15] in that, even at low compressions, π decreased more than 1 $mN \cdot m^{-1}$ in the first minute after cessation of compression when the films were held at constant area. This decay was the same when the experiments were repeated on a 2 M NaCl substrate indicating that the monolayers were not dissolving and that the films were collapsing because because of three-dimensional aggregation. This behaviour has also been observed with condensed monolayers of saturated, long-chain, phosphatidylethanolamines but not with condensed monolayers of lecithin [15].

At molecular areas below "lift-off" ΔV increases more-or-less linearly (Fig. 3b) until the collapse point is approached. The four ΔV vs A curves are approximately parallel indicating that the variation in ΔV with molecular packing density is the same for each of the sphingolipids. Comparisons with other work are not possible because there are no ΔV data in the literature for pure single component glycolipid monolayers. However, ΔV values for the condensed monolayers of sphingolipid are lower than those of condensed monolayers of lecithin [12], diglyceride and phosphatidylethanolamine [14]. Substitution of the galactose ring on cerasine with phosphorylcholine gives sphingomyelin and, as described above, this change does not have a dramatic



(a)



(b)

Fig. 3. (a) Surface pressure (π) versus molecular area (A) curves for phrenosine, cerasine, *N*-palmitoyldihydrogalactocerebroside and *N*-palmitoyldihydrosphingosine on 0.1 M NaCl at room temperature. × and △, π for phrenosine and cerasine; ○, π for *N*-palmitoyldihydrogalactocerebroside, □, π for *N*-palmitoyldihydrosphingosine. (b) Surface potential (ΔV) versus molecular area curves for the four sphingolipids. The symbols are the same as in (a).

effect on ΔV; at 0.5 $nm^2 \cdot molecule^{-1}$ ΔV for a monolayer of beef heart sphingomyelin is about 250 mV [23] whereas at the same area/molecule ΔV for cerasine is 315 mV (Fig. 3b). Removal of the galactose ring from *N*-palmitoyldihydrogalactocerebroside to form *N*-palmitoyldihydrosphingosine causes a reduction in ΔV of approx. 60 mV. Besides these alterations in ΔV due to changes in the structure of the polar groups, the data in Fig. 3b also show that changes in the polar regions of the fatty acid moieties (i. e. removal of the 2- hydroxy group and hydrogenation of the *trans* double bond in phrenosine) can cause changes of 80–200 mV in ΔV. It is clear from the above data that variations in the molecular structure of the galactolipids can cause large changes in ΔV without any appreciable changes in the average molecular packing, as reflected by π.

DISCUSSION

Comparison of π vs A curves

The physical states of lipid monolayers are equivalent to those of bilayers of the same lipids dispersed in excess water [1, 15]. As depicted in Fig. 4, on warming a phospholipid monolayer the hydrocarbon chains can melt and cause a sudden increase in the molecular area at constant π. The temperature at which the transition from condensed to expanded monolayer occurs is a function of π and only equals the bilayer gel to liquid-crystal transition temperature (T_c) when $\pi = \pi_e$ where π_e is the equilibrium spreading pressure. π_e is the point at which the spread monolayer is in

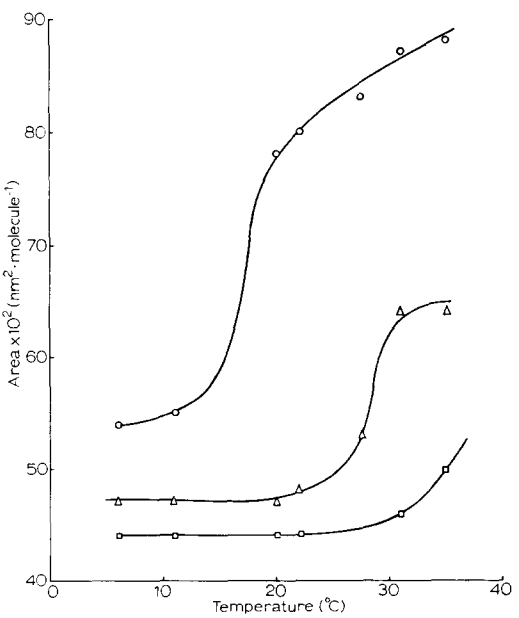


Fig. 4. The effect of chain melting on the molecular packing of lipid monolayers. The variations with temperature of the molecular area of dipalmitoyllecithin monolayers at different, constant, film pressures are shown. ○, $\pi = 5$ mN · m^{-1}; △, $\pi = 20$ mN · m^{-1}; □, $\pi = 40$ mN · m^{-1}. The data are taken from ref. 15.

equilibrium with the bulk lipid phase [16]. For the example of dipalmitoyllecithin $\pi_e \approx 50$ mN · m^{-1} [17] and $T_c = 41$ °C [18]. From the above analysis it follows that it is possible to estimate, for any temperature (T), the film pressure (π_m) at which the monolayer transition occurs from a knowledge of T_c for the lipid in the lamellar phase in excess water because

$$\pi_{m(T)} = \pi_{e(T_c)} - (T_c - T)\frac{d\pi_m}{dT}. \quad (1)$$

$d\pi_m/dT$ is a measure of the entropy of the chain-melting process and is constant over readily accessible temperatures for a given monolayer [15]; for various lipids such as fatty acids, diglycerides, phosphatidylethanolamines and lecithins it lies in the range 1.2–1.6 mN · m^{-1} per degree. From Eqn 1 it is possible to calculate the temperature at which the force vs area isotherm should first show the chain-melting transition (i.e. $\pi_{m(T)} > 0$). When this calculation is performed for the dipalmitoyllecithin monolayer system, using previously published data [15, 17] Eqn 1 indicates that only when $T >$ approx. 10 °C should the π vs A isotherms start to show the chain-melting transition (i.e. have an intermediate region); this is in accord with experiment [15].

Since the phase behaviour of glycosphingolipids in water parallels that of phospholipids where the bilayer is the predominant structure [6, 7, 19] it is possible to use Eqn 1 to make rough estimates of the temperature at which the condensed π vs A curves of cerasine or phrenosine should show the phase transition. $d\pi_m/dT$ and $\pi_{e(T_c)}$ are assumed to have the same values as for lecithin monolayers (i.e. 1.6 mN · m^{-1} per degree and 50 mN · m^{-1}, respectively) while the T_c for phrenosine/cerasine is taken as 70 °C [6]. Substitution into (1) gives $T >$ approx. 40 °C which is consistent with the observation of no expansion at 35 °C; it was not possible to obtain π vs A isotherms at above 40 °C with our apparatus. Eqn 1 also shows that when $T > T_c$, $\pi_{m(T)} > \pi_e$ which means that stable monolayers will always be fully expanded and not undergo the transition (cf. the π vs A curve of digalactosyldiglyceride in Fig. 2, $T_c = -50$ °C [7]).

Because the cerasine and phrenosine molecules in the condensed monolayers aggregate to give a three-dimensional lipid structure (crystals) it would be expected from the monolayer-bilayer correlation that in excess water when $T < T_c$ the coagel (crystals+water) rather than the hydrated gel state would be stable; X-ray studies have shown that bovine brain cerebroside forms the coagel below T_c [6]. It is clear that the close-packed, condensed state adopted by phrenosine and cerasine in monolayers and bilayers is consistent with their occurrence in the relatively impermeable, metabolically inert, myelin membrane. In contrast and consistent with their occurrence in more permeable membranes, the highly unsaturated galactosyldiglyceride molecules are in a very fluid state at normal temperatures because they are so far above T_c. The molecular area in bilayers of the galactosyldiglyceride is approx. 0.75 nm^2 · molecule^{-1} [7] which is equivalent to a film pressure of approx. 15 mN · m^{-1} for the monolayer (Fig. 2). In conclusion, as for the phospholipids the physical state of galactolipid monolayers under given conditions is determined primarily by the nature and packing requirements of the fatty acid chains.

Comparison of ΔV vs A curves

The surface potential data shown in Figs. 2 and 3b contain information about the structure of the polar regions of the monolayers. ΔV for neutral monolayers can be expressed in terms of a surface dipole monent (μ) where

$$\Delta V = 4\mu\pi/A. \tag{2}$$

The significance of the overall perpendicular moment μ and the various contributions to it have been dicussed before [12, 20].

Since the ΔV vs A curves for monogalactosyl- and digalactosyldiglycerides are similar to those of lecithins which form expanded films, μ is approximately the same for the three compounds. The principal contribution to μ in these cases comes from the dipole of the carbonyl groups in the fatty acid ester linkages [12]. Because the presence of galactose rings or phosphorylcholine zwitterions gives rise to similar μ values, the average dipole interaction in the interface is the same in both cases. This means that the long-range influence of the two interfaces through dipole interactions will be similar; at long range an interacting species only experiences the effects of a smeared array of dipoles. Since glycosphingolipids, unlike phospholipids, have immunological properties and may be of importance in cellular adhesion, it is clear that these specific properties are dependent on short-range interactions of the sugar residues.

Specific variations in the structures of the glycosphingolipid molecules can lead to large changes in ΔV. Generally ΔV for these compounds is less than that for diacylglycerides because of the absence of ester linkages. This is consistent with the observation that ΔV for dialkylphospholipids is less than that of diacylphospholipids [12]. Replacement of an hydroxyl group by a proton in phrenosine to give cerasine leads to a reduction in ΔV of about 90 mV. It is not possible to readily account for this decrease in μ in terms of bond moments because the effects of the associated water molecules cannot be allowed for. ΔV is particularly sensitive to the presence of the *trans* double bond in phrenosine or cerasine (Fig. 1). Thus, reduction of this group in cerasine to give *N*-palmitoyldihydrogalactosylcerebroside (shortening of the fatty acid chain does not affect ΔV) increases ΔV by some 200 mV at 0.5 $nm^2 \cdot$ molecule^{-1}. This is equivalent to an increase in μ of $9 \cdot 10^{-31}$ Cm (1 Debye $= 3.336 \cdot 10^{-30}$ Coulomb metre). With sphingomyelin the equivalent structural change decreases ΔV by about 100 mV [21]. The large effects of double bonds is due to the induction of dipoles in them by nearby polar groups [12, 21]. Clearly the induced dipoles in sphingomyelin and cerasine are of opposite polarity. The various ΔV vs A data presented here yield by Eqn 2 values of μ in the range $1.7–2.7 \cdot 10^{-30}$ Cm.

The dipole interaction energies for two-dimensional arrays of point dipoles in various lattice arrangements have been estimated [22]. For a hexagonal array of dipoles of moment μ arranged with their axes perpendicular to the lattice plane, the interaction energy U is

$$U = (+)5.515\ \mu^2/Dr^3 \tag{3}$$

where r is the nearest neighbour distance and D the permittivity. Since surface potential measurements give the overall moment perpendicular to the plane of the interface, substitution of the μ values quoted above into (3) allows an estimate of the repulsive,

dipolar interaction energy arising from the overall perpendicular component. When this is carried out assuming a molecular area of 0.5 $nm^2 \cdot molecule^{-1}$, unit permittivity and $\mu = 1.7 \cdot 10^{-30}$ Cm, U is calculated as about 190 $J \cdot mol^{-1}$. Since this energy is much less than the thermal energy of the molecules, it cannot be significant in determining the molecular packing in the monolayer. It should be stressed that other interactions in the polar group region (e.g. hydrogen bonding) are important in determining the molecular packing.

In summary, the monolayer (and bilayer) behaviour of the galactolipids is analogous to that of the phospholipids and, in this sense, the glycolipid systems do not hold any surprises. The net interactions of the hydrated galactose ring and hydrated phosphorylcholine zwitterion seem to be similar and the packing density in the plane of the monolayer is determined primarily by the interactions between the hydrocarbon chains.

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