A Comparative Monomolecular Film Study of 1,2-Di-*O*-palmitoyl-3-*O*-(α- and β-D-glucopyranosyl)-*sn*-glycerols†

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ABSTRACT: The polar headgroup contribution to monolayer behavior of dipalmitoylglucosylglycerol has been examined through studies of 1,2-di-*O*-palmitoyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol (di-16:0-αGlcDG) and 1,2-di-*O*-palmitoyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (di-16:0-βGlcDG) in which the sugar headgroup is linked via an α or β linkage to the diacylglycerol moiety. The results indicate that the limiting areas per molecule of the resultant condensed states are smaller than those of the corresponding phosphatidylcholine (DPPC) but larger than those of dipalmitoylphosphatidylethanolmine (DPPE). In the expanded state, while the areas per molecule are similar to those of DPPC at low pressures, both glycolipids occupy smaller areas at higher pressures. The expanded-state areas of the glucolipids are also slightly greater than those of DPPE. The initial compressional phase transition pressure of the glucolipid liquid-expanded/liquid-condensed transition (π_t) is, however, less sensitive to temperature than are the π_t values of phospholipids. Both of these effects must relate to strong headgroup/water interactions, which, in turn, result in a stabilization of the liquid-expanded states. In the expanded states the α anomers are slightly less tightly packed than the β anomers, as is indicated by the somewhat higher areas per molecule of the expanded states and the lower transition temperatures. These differences in chain-melting temperatures are slightly smaller than those observed in bilayers. While the areas per molecule of the dipalmitoyl glucolipids are greater than those of dipalmitoylphosphatidylethanolamine, they nevertheless exhibit a greater tendency to form nonbilayer structures. Such observations indicate that other factors besides geometric shape play a role in bilayer/nonbilayer transitions.

Studies of the role of lipid polar headgroups in the structure and function of biological membranes have been frequently carried out with model membrane systems. In the past these studies have concentrated mainly on phospholipids (Cadenhead et al., 1967; Hauser & Phillips, 1979). Recently, more attention has been given to the systematic study of glycolipids, due to their frequent occurrence in plants (Quinn & Williams, 1978, 1983; Gigg, 1980), in bacteria (Langworthy et al., 1976; Gigg, 1980; Boggs, 1980), and in mycoplasmas (de Kruijff et al., 1972). A number of these recent studies of glycolipids having a single sugar residue suggest that they bear some resemblance to phosphatidylethanolamines (PEs),[1] at least as far as their calorimetric behavior, hydration capabilities, and molecular shapes are concerned (Wieslander et al., 1978; Iwamoto et al., 1982; Endo et al., 1982; Hinz et al., 1985; Sen et al., 1983; Mannock et al., 1985, 1988a). More than one functional role is proposed for glycolipids in various membranes (Curatolo, 1987). The observed stability of glycolipid membranes has been interpreted in terms of strong polar headgroup interactions involving hydrogen bonding among the sugar residues (Pasher, 1976; Iwamato et al., 1982; Weislander et al., 1978; Hinz et al., 1985; D. A. Mannock, R. N. A. H. Lewis, A. Sen, and R. N. McElhaney, unpublished results).

Monomolecular film studies at the air/water interface should be able to provide useful information about the overall packing and monolayer phase behavior of such glycolipids. Here we report our monolayer film studies of glucosyldiacylglycerols in which a single glucopyranose residue is attached via an α or β linkage to a 1,2-di-*O*-acyl-*sn*-glycerol containing palmitoyl chains. The results are systematically compared with those obtained for two corresponding phospholipids. The monomolecular film behavior of dipalmitoyl phosphatidylcholine (DPPC) has already been established (Albrecht et al., 1978; Phillips & Chapman, 1968; Rice et al., 1987). Since a complete film study of dipalmitoylphosphatidylethanolamine (DPPE) over the entire LE/LC coexistence temperature range on a pure water substrate has not previously been reported, it was felt necessary to include such a study to facilitate a detailed comparison.

Experimental Procedures

The synthesis and purification of the α and β glucolipids of high anomeric and chiral purity used in this study have already been described elsewhere (Mannock et al., 1987,

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[1] Abbreviations: PE, phosphatidylethanolamine; DPPE, dipalmitoylphosphatidylethanolamine; PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; di-16:0-αGlcDG, 1,2-di-*O*-palmitoyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol; di-16:0-βGlcDG, 1,2-di-*O*-palmitoyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol; T_0, the lowest temperture at which an expanded state can exist; T_r, the reduced temperature defined as $(T - T_0)/T_0$, where T is the absolute temperature; T_c, the critical temperature of the LE/LC phase change; T_m, the temperature of the main gel/liquid-crystalline bilayer transition; T_n, the temperature of the bilayer/nonbilayer transition; π, the monolayer surface pressure at the air/water interface; π_{esp}, the equilibrium spreading pressure from a pure crystalline state; π_t, the surface pressure at the compressional onset of the liquid-expanded (LE)/liquid-condensed (LC) transition; A, the area/molecule of the monolayer; A_t, the area/molecule at π_t.

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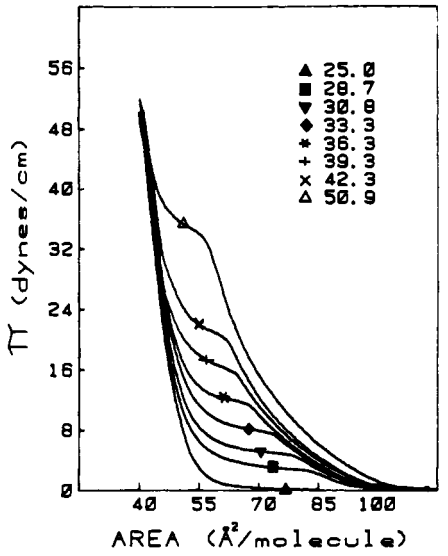

FIGURE 1: Surface pressure (π) vs area/molecule isotherms for stated temperatures for 1,2-di-*O*-palmitoyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol (di-16:0-αGlcDG) on water. Temperatures: (▲) 25.0; (■) 28.7; (▼) 30.8; (◆) 33.3; (*) 36.3; (+) 39.3; (×) 42.3; and (△) 50.9 °C.

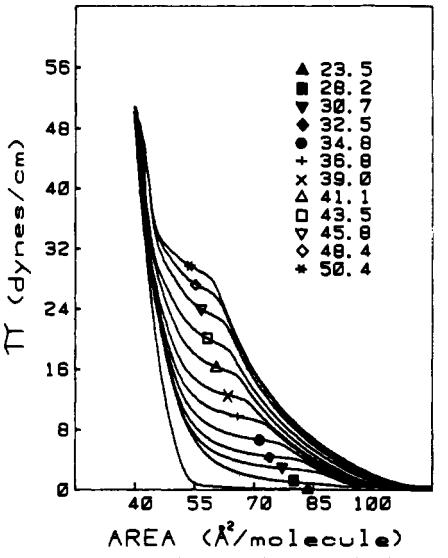

FIGURE 2: Surface pressure (π) vs area/molecule isotherms for stated temperatures for 1,2-di-*O*-palmitoyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (di-16:0-βGclDG) on water. Temperatures: (▲) 23.5; (■) 28.2; (▼) 30.7; (◆) 32.5; (●) 34.8; (+) 36.8; (×) 39.0; (△) 41.1; (□) 43.5; (▽) 45.8; (◇) 48.4; and (*) 50.4 °C.

1988b). DPPE was originally obtained from Sigma. However, since it was found to have trace impurities, presumably due to aging, it was recrystallized twice from ethanol before use. The spreading solvent used to spread the glucolipid films was a 9:1 volume ratio of *n*-hexane/ethanol. Chloroform was used to spread DPPE. Both chloroform and *n*-hexane were further purified by passing them through a column of alumina and subsequently subjecting them to a final distillation. All film compressions were carried out over a quadruply distilled water substrate pH ~5.6 or, where specified, on a pH 2, 0.1 M HCl substrate. The film balance system, as well as the general techniques and procedures, has already been described elsewhere (Cadenhead, 1969). All compressional isotherms were obtained on a computer-interfaced film balance system previously described in detail (Rice, 1986). Equilibrium spreading pressures were measured by depositing a few small crystals of the materials to be studied at the air/water interface and monitoring the pressure until no further increase in pressure was noted.

RESULTS

Film Behavior of Straight-Chain Glucolipids. Pressure (π) vs area (A) plots for 1,2-di-*O*-palmitoyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol (di-16:0-αGlcDG) and 1,2-di-*O*-palmitoyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (di-16:0-βGlcDG) over the wide temperature range 24–51 °C are shown in Figures 1 and 2, respectively. Although the limiting areas could be taken directly from these isotherms, this would probably result in an underestimation of the true areas occupied by these molecules in the condensed state. This is because during a typical compression of most diacyl, saturated, straight-chain compounds, a great deal of overcompression can occur. This, in turn, is due to the slow kinetics of film collapse, especially at higher compressional rates, and can result in apparent film pressures as high as 70 dyn/cm. We have observed that these glucolipids are especially capable of forming highly condensed, rigid structures at high pressures, as was indicated by Wilhelmy plate shifts. All of the above factors make a limiting area evaluation through continuous compressional isotherms less than accurate.

A more reliable method of evaluating limiting areas in such cases is to derive them from isotherms obtained through stepwise compression. In this technique the film is allowed to relax before initiating the next compressional step. The highest pressure obtained in this way for both the above listed glucolipids was 47 dyn/cm at 36 °C. Compression above this point resulted in a relaxation back to the same pressure. By this method, though the pressure increases with temperature at the limiting area, the limiting areas themselves are more or less independent of temperature. The molecular areas measured by this method were about 42 Å² for di-16:0-αGlcDG and 41 Å² for di-16:0-βGlcDG. Figure 3 shows the π_t shift with temperature. Both anomers follow the same temperature dependence, though the α anomer shows a lower T_0 [the lowest temperature at which at liquid-expanded phase can be observed (Kellner et al., 1978)] of 26.0 °C, whereas the β anomer had a T_0 of 28.5 °C.

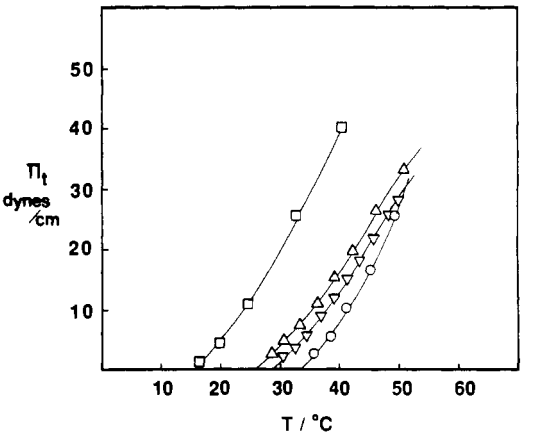

FIGURE 3: Temperature dependence of the compressional onset of the expanded/condensed transition (π_t) for DPPC (□) (Rice et al., 1987), di-16:0-αGlcDG (△), di-16:0-βGlcDG (▽), and DPPE (○).

The equilibrium spreading pressures (π_{esp}) of the two glucolipid anomers were measured at 24 and 36.5 °C. They both showed the same π_{esp} of 21 ± 1 and 47 ± 1 dyn/cm, respectively, at these temperatures, showing that the two films have equal stability.

The effect of substrate pH on film behavior was measured at 36.5 °C. The isotherms showed very little expansion of the expanded state and the intermediate region with no shift in π_t when the pH was shifted from 5.6 to 2. Mixed films of the

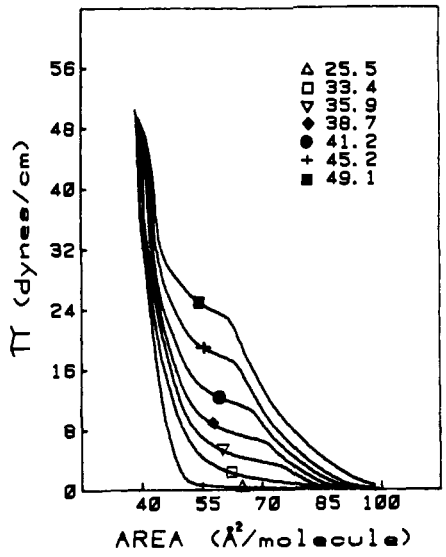

FIGURE 4: Surface pressure (π) vs area/molecule isotherms of DPPE at stated temperatures. Temperatures: (△) 23.5; (□) 33.4; (▽) 35.9; (◆) 38.7; (○) 41.2; (+) 45.2; and (■) 49.1 °C.

two anomers were also examined at the air/water interface for selected compositions (0.25, 0.50, and 0.75 mole fraction) at 36.5 °C and π_t was observed to shift linearly with concentration.

Dipalmitoylphosphatidylethanolamine (DPPE). Surface pressure (π) vs area/molecule (A) isotherms of DPPE are illustrated in Figure 4 and indicate the thermal extent of the LE/LC transition. On the basis of these data, we estimate the close-packed area of DPPE to be 40 Å^2/molecule, precisely twice the close-packed area of an acyl chain. The low-temperature isotherms show a break or change in slope in the high-pressure region (~36 dyn/cm), which corresponds to the liquid-condensed (LC)/solid-condensed (SC) phase transition exhibited by single-chain fatty acids, alcohols, and other related compounds (Gaines, 1966). This constitutes further evidence that the acyl chains are the limiting factor in the close packing of PEs. The extrapolation of π_t to zero pressure gave a T_0 value of 32.9 °C (Figure 3).

In terms of absolute temperatures, when both are in a liquid-expanded state, the glucolipids occupy areas/molecule approximately equal to those of DPPC at low pressures but occupy smaller areas/molecule at higher pressures. Their areas/molecule are also slightly greater than those of DPPE. To compare the liquid-expanded areas of DPPE, DPPC, and the two glucolipids (di-16:0-α- and β-GlcDG), it is best to carry out the comparison at a reduced temperature $[(T - T_0)/T_0]$. This comparative basis is justifiable insofar as the transition regions between the T_0 and T_c values are similar in all cases (29.3 ± 3.4 °C). The data are illustrated in Figure 5 with the values for DPPC being taken from Rice et al. (1987). As expected (Cadenhead et al., 1967), it is clear that DPPE always occupies smaller areas than DPPC at the same reduced temperature. At low reduced temperatures, the glucolipids occupy about the same areas/molecule as DPPC, but at higher reduced temperatures they approach the values for DPPE. Thus, any comparison of the packing of the glucolipids with either DPPC or DPPE must specify the reduced temperature at which the comparison is made.

Discussion

Effects of Anomeric Linkage. If we compare our data for the two glucolipids, di-16:0-α- and -βGlcDG, we can see that in the condensed state the former has a slightly larger close-packed area/molecule (by about 1 Å^2). Previous studies of 1,2-*O*-dialkyl-3-*O*-(β-D-glucosyl)-*sn*-glycerols (Hinz et al., 1985) have indicated similar close-packed areas/molecule (40–41 Å^2) for glucolipids having alkyl chains. Other studies on semisynthetic saturated monogalactosyldiacylglycerols (Bishop et al., 1980; Sen et al., 1981) also indicate similar condensed-state areas/molecule. It would seem, therefore, that the condensed-state packing of these glucolipids is not greatly influenced by either the nature of the sugar–glycerol linkage, the glycerol–hydrocarbon chain linkage, or even the precise nature of the sugar residue. Thus, in the condensed state the glycolipid headgroup packing would appear to be similar with that of the hydrocarbon chains to be the determining factor with little or no chain tilting.

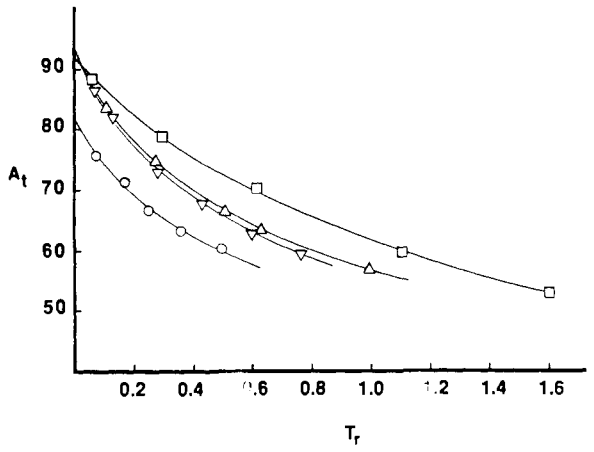

FIGURE 5: Transition area (A_t) vs reduced temperature plots of DPPC (□) (Rice et al., 1987), di-16:0-αGlcDG (△), di-16:0-βGlcDG (▽), and DPPE (○).

In the expanded states it would seem more likely that the areas/molecule of di-16:0-α- and -βGlcDG would differ. Figure 5, however, shows that, up to a reduced temperature (T_r) of about 0.3 °C, the areas/molecule at the compressional onset of the transition (A_t) are about the same. But at higher T_r values, where the corresponding areas are lower, di-16:0-αGlcDG has a slightly greater area/molecule. Jarrell et al. (1986, 1987a) carried out a ^{2}H NMR study of ditetradecylglucosylglycerols having α or β linkages in both lamellar and hexagonal phases and concluded that, whereas the sugar group of the β-linked glucolipid is fully extended perpendicular to the bilayer surface, the α anomer is essentially parallel to the bilayer surface. While these results are qualitatively in agreement with our findings, we see a significantly smaller difference between the two anomers. That the α and β anomers do not differ greatly in their packing is further emphasized by the additivity of the areas/molecule in mixed films. Since the components are clearly not immiscible, the only reasonable interpretation of this observation is that the α and β anomers form a nearly ideal, two-dimensional solution.

Our clearest indication that the α and β glucolipids do indeed differ in their headgroup orientation is seen in the temperature dependence of the phase transitions of these compounds. The α anomer shows a T_0 about 2.5 °C below that of the β anomer, while $d\pi_t/dT$ values show essentially the same trend (see Figure 3). This clearly shows that the α anomer shifts to an expanded state at a lower absolute temperature and remains in an expanded state until a higher surface pressure. The similar $d\pi_t/dT$ values suggest that the temperature dependence of the polar headgroup interactions is similar in magnitude, suggesting the T_0 difference may well be due to a differing headgroup orientation. Such a conformational difference could influence the absolute head-

Table I: T_0 Values for Selected Phospho- and Glucolipids

lipid	T_0 (°C)	close-packed limiting areas/molecule (Å^2)
DPPC	15.5[a]	44–45
DPPE	32.9	40
di-16:0-α-GlcDG	26.0	42
di-16:0-β-GlcDG	28.5	41

[a] Rice et al. (1987).

Table II: Bilayer Calorimetric Data

lipid sample	T_m (°C)	ΔH_{cal} (kcal/mol)	T_h (°C)
DPPC	41.4	7.7[a]	
DPPE	64.4	7.9[b]	118.0
di-16:0-α-GlcDG	61.0	9.5[c]	75.0
di-16:0-β-GlcDG	57.2	9.0[d]	79.1[e]

[a] Lewis et al. (1987). [b] Seddon et al. (1983). [c] D. A. Mannock, R. N. A. H. Lewis, A. Sen, and R. N. McElhaney, unpublished results. [d] Mannock et al. (1988a). [e] Nonbilayer cubic phase.

group/headgroup interactions and hydration and dehydration properties. A conformational difference could also explain the more stable monolayer expanded state of the α anomer.

Comparison of the Glucolipids with DPPC and DPPE. In the condensed state, the molecular areas of the dipalmitoyl glucolipids are smaller than those of DPPC but slightly larger than those of DPPE (see Table I). The close-packed areas listed in Table I are in good agreement with the data for PEs and PCs of Phillips and Chapman (1968) and also those of Suzuki and Cadenhead (1985). Table I also shows a decrease in T_0 values with an increase in size of the headgroups. Therefore, it would seem that the glucolipids have properties intermediate between those of PCs and PEs. Table II provides related bilayer calorimetric data on the same glucolipids (Mannock et al., 1988a, unpublished results) with DPPC (Lewis et al., 1987) and DPPE (Seddon et al., 1983). As is expected, the bilayer gel/liquid-crystalline phase transition (T_m) shifts in parallel with the monolayer T_0 values in Table I. Also, the fact that the molecular areas of the glucolipids are smaller than those of DPPC and tend toward those of DPPE is not unexpected since, unlike DPPC, which is a typical bilayer-forming lipid, both the glucolipids and DPPE spontaneously form inverted nonbilayer structures at elevated temperatures (Seddon et al., 1983; Mannock et al., 1988a, unpublished results). The stronger preference of molecules, such as the glucolipids and DPPE, for inverted nonbilayer structures can be rationalized by a pure geometric argument (Israelachvilli et al., 1977, 1980), based on the fact that their headgroups are smaller than that of DPPC. However, using the same geometric arguments, one would not expect glucolipids to form nonbilayer assemblies more readily than does DPPE, and the lamellar/hexagonal transition temperature (T_h) values in Table II clearly show that this is indeed the case. Clearly other factors need to be considered here.

A recent study (Jarrell et al., 1987a,b) of dialkyl glucolipids in a liquid-crystalline phase have shown that, at least for the β anomer, the glycerol backbone has an orientation relative to the bilayer normal which is similar to that found for phospholipids. Therefore, differences in the properties of glucolipids and phospholipids most probably reflect the glucopyranosyl and phospholipid functional group environment and the ways in which headgroup/headgroup or headgroup/aqueous substrate interactions take place.

In the expanded state the areas of the glucolipids relative to those of DPPC and DPPE are more strongly temperature dependent. When examined on a reduced temperature scale, glucolipids were found to occupy areas comparable to those of DPPC at low temperatures, whereas at elevated temperatures their molecular areas are reduced and progressively approach those of DPPE (Figure 5). The variation in molecular area requirements must reflect some difference in hydration properties. It is noteworthy that our π_{esp} measurements indicate that the glucolipids are capable of spreading at the air/water interface at considerably lower temperatures than phospholipids. Phillips and Hauser (1974) have pointed out that PCs spread at the air/water interface from the monohydrate crystal at lower temperatures than the equivalent PEs do. This was explained in terms of a lower crystal lattice energy of PCs. The fact that glucolipids are capable of spreading at even lower temperatures indicates that the glucosyl/water interactions are stronger than the glucosyl/glucosyl headgroup interactions. This explanation is supported by solution studies of monosaccharides (Barone et al., 1981a,b, 1983a,b, 1984), which indicate sugar/water interactions are favored over those of sugar/sugar in dilute aqueous solutions. In addition, the large space requirements of the glucolipids in the expanded state at lower temperatures would suggest a bulkier hydration shell, more stable than that of phospholipids. It is known that with increasing temperature the extent of carbohydrate hydration decreases in solutions of simple carbohydrates (Suggett, 1975; Mannock et al., unpublished results). This could explain the reduced molecular areas found at elevated temperatures for glucolipids. In addition, this unusual temperature-dependent space requirement of these glucolipids may also explain the observed stability of the expanded state that decreases with temperature. This concept may also explain why glucolipids are capable of forming expanded states at lower temperatures than can PEs (lower T_0 values). The bulk of the data so far suggest that glucolipid/water interactions are stronger than those of PE headgroup/water, at least at lower temperatures.

The reduced flexibility of the glucosyl group of glucolipids coupled with the decrease in hydration at elevated temperatures appears the most likely reason why the glucolipids are capable of forming nonbilayer structures at lower temperatures than PEs (see Table II). Such a mechanism might also play an important part in the regulation of the bilayer–nonbilayer phase transitions and, indeed, determine the "nonbilayer-forming ability" of lipids as a whole. This may provide an explanation for the lower L_α/nonbilayer phase transition temperatures of the glucolipids studied here vs PEs. However, it is also interesting to speculate that such differences may originate from a change in headgroup conformation that results in a more compact headgroup and consequently a smaller surface area per molecule. Such a conformational change has been observed by ^{2}H NMR at the L_α/NBL phase transition of the ditetradecyl-β-D-glucosyl-*sn*-glycerol but not of the corresponding α anomer (Jarrell et al., 1987a). The absence of such an event at the l_α/NBL phase transition of the α anomer may, however, reflect a difference in the structure of the nonbilayer phase formed at this transition, as has recently been demonstrated for the corresponding diacyl compounds, where conversion from an intermediate nonbilayer phase to a H_{II} phase was not observed until considerably higher temperatures, or longer chain lengths (Mannock et al., unpublished results; Sen et al., 1988; M. Akiyama, personal communication). Thus, a conformational change in the α-glucosyl headgroup of the type seen for the β anomer might not be observed until significantly higher temperatures.

At present it is not clear whether such changes in headgroup hydration and conformation are connected. Unfortunately, there is insufficient evidence from the studies of lipids, gly-

cosides, or phosphoryl amino alcohols to answer this question at the present time. However, if changes in hydration and conformation of this type are a common feature of glycolipid phase behavior, then the surface area per molecule in the liquid-condensed state cannot be used as a reliable index of the ability of these molecules to form nonbilayer structures.

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