Head-Group Contributions to Bilayer Stability: Monolayer and Calorimetric Studies on Synthetic, Stereochemically Uniform Glucolipids

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ABSTRACT: Monolayer and differential scanning calorimetry studies have been performed on synthetic, stereochemically uniform glyceroglucolipids having saturated, ether-linked alkyl chains. The limiting area, A_0 = 40 Å^2·molecule^{-1}, resulting from the monolayer measurements of the glucolipids is comparable to the A_0 value found for phosphatidylethanolamine lipids. The area corresponds to twice the value observed with saturated straight chain fatty acids, which indicates that at high surface pressure the space requirement of the glucose head group does not exceed that of the two alkyl chains. The apparent specific heat capacities of the glucolipid dispersions have been found to be higher than those of corresponding phospholipids. They can be approximated from group parameters with the additional assumption that the experimental partial molar heat capacity of glucose is valid for the glucose head groups of the lipids. The transition enthalpies of the C_{16} and C_{18} glyceroglucolipids are clearly larger than the ΔH values of corresponding phospholipids, while the C_{14} glyceroglucolipid has the same transition enthalpy as dimyristoylphosphatidylethanolamine or ditetradecylphosphatidylethanolamine. Glucolipids exhibit gel to liquid-crystalline phase transition temperatures which are only slightly lower than those of their phosphatidylethanolamine analogues, although they are uncharged molecules. Like phosphatidylethanolamine the glucolipids do not show a pretransition; however, with the C_{14} glucolipid a highly cooperative posttransition, approximately 5 deg above the main transition, has been found. Calorimetric experiments with a C_{14} glucolipid, in which the hydroxyl protons of the glucose moiety have been exchanged by deuterium, suggest that the posttransition might reflect structural changes of the head group. It has been demonstrated that no unique correlations between transition parameters and the nature of the head group or the alkyl chain linkage can be evaluated on the basis of presently available data. This is probably due to the complexity introduced by the variety of opposing interactions which stabilize the structures of charged phospholipids. Agreement between transition temperatures estimated from monolayer studies at high surface pressures and those obtained from scanning calorimetry on bilayers has been taken as supporting evidence for the hypothesis that monolayers behave similar to bilayers when subject to surface pressures around 50 mN·m^{-1} (Hui et al., 1975; Nagle, 1980).

It has been emphasized repeatedly (Chapman, 1975; White, 1973; Boggs, 1980) that control of membrane fluidity is not the only purpose of the large variety of lipids occurring in biological membranes. The finding of unique fatty acid and lipid compositions that are characteristic of the type of the membrane rather than of the species (Boggs, 1980) suggests specific roles of the lipids in membrane function. These functional roles are determined by the molecular forces governing the lipid–lipid and lipid–protein interactions. Parameters instrumental in understanding these interactions are molecular areas, expansion coefficients, and heat capacities as well as temperatures, enthalpies, and entropies of the gel to liquid-crystalline transition (Phillips et al., 1970; Albrecht et al., 1978, 1981; Nagle, 1980; Wilkinson & Nagle, 1982; Blume, 1983; Ladbrook & Chapman, 1969; Melchior & Steim, 1976, 1979; Mabrey & Sturtevant, 1978; McElhaney, 1982). The majority of studies has been devoted to substantiating the influence of length and degree of saturation of the hydrocarbon chains on the thermodynamic transition properties of bilayer systems. A smaller number of studies has been addressed to systematic elucidation of the importance of the nature of the head group. Calorimetric and other studies of a number of lipids having phosphatidylethanolamine (Mabrey & Sturtevant, 1976, 1977; Simon et al., 1975; Nagle, 1976; van Dijck et al., 1976; Jackson & Sturtevant, 1977; Yang et al., 1979; Wilkinson & Nagle, 1981), phosphatidylserine (Jacobson & Papahadjopoulos, 1975; van Dijck et al., 1978; McDonald et al., 1976), and diphosphatidylglycerol head groups (Rainier et al., 1979) have demonstrated the significance of surface charge densities and hydrogen bonding for the magnitude of the thermodynamic transition quantities. A comparison of the transition properties of these compounds is, however, complicated by the fact that it is difficult to separate charge effects from size effects. For instance, electrostatic effects cannot entirely account for the consistently higher transition temperatures observed for phosphatidylethanolamine and phosphatidylserine lipids as compared to those of phosphatidylcholines even at pH values where all have one positive and one negative charge (Boggs, 1980).

To render a proper evaluation of head-group contributions to the magnitude of lipid interactions possible, which does not suffer from the intrinsic ambiguities involved in separating charge and size effects, a series of dialkylglycerol ether glycosides was synthesized (Six et al., 1983), which will permit a clear separation of the two effects. Besides their promising use as model systems for unraveling the question of head-group contributions to bilayer stability, they have an interesting biological aspect. The synthetic glycolipids are representative of the large number of glyceroglycolipids occurring in plant

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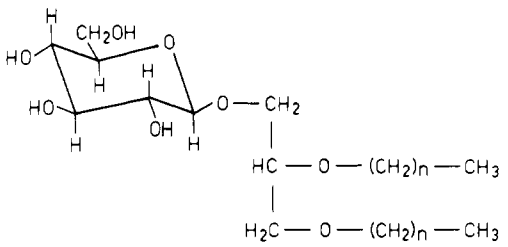

FIGURE 1: Structure of synthetic glucolipids; n = 13, 15, and 17.

tissues (Quinn & Williams, 1978), bacteria (Boggs, 1980, Oshima & Yamakowa, 1974), and mycoplasma membranes (de Kruijff et al., 1972). Instead of the usual diester linkage, the synthetic glucolipids contain ether bonds, which make them extremely pH stable. Ether linkage apparently occurs frequently in nature in bacteria which live under extreme environmental conditions, like some subgroups of archaebacteria, including extreme halophiles and certain thermoacidophiles (Langworthy et al., 1982).

In the present work we report monolayer and high sensitivity scanning microcalorimetry studies on saturated, stereochemically uniform 1,2-*O*-dialkyl-3-*O*-β-D-glucosyl-*sn*-glycerols having 14-, 16-, or 18-carbon atoms in the alkyl chains, to characterize the charge independent packing and transition properties of these lipids.

Materials and Methods

Detailed procedures for the stereospecific synthesis and the purification of the glycolipids have been described (Six et al., 1983). Figure 1 shows the structure of the glucolipids employed in the present study. The alkyl chain length varied between 14- carbon and 18-carbon atoms. The extreme sensitivity of DSC measurements to impurities required rigorous tests of the purity and stereospecific uniformity of the lipids used. The samples were found to be pure on the basis of elemental analysis, infrared spectroscopy, mass spectroscopy, and thin-layer chromatography (Six et al., 1983). Stereospecific uniformity of 1,2-*O*-ditetradecyl-3-*O*-β-D-glucosyl-*sn*-glycerol was checked by 400-MHz ^{1}H NMR spectra of the lipid dissolved in $CDCl_3$–CD_3OD, 1:1.

The doublet observed at δ 4.25 ($J_{1,2}$ = 7.69 Hz) unambiguously demonstrates that the aglycon is linked via a β-glycosidic bond to the glucose. There is no signal at shifts $\delta > 5$ which proves the absence of α anomers.

This result is particularly important for the interpretation of the high-temperature transition of 1,2-*O*-ditetradecyl-3-*O*-β-D-glucosyl-*sn*-glycerol (14-1,2-G).[1] To facilitate reference to the lipids the following abbreviations will be used: 16-1,2-G, 1,2-*O*-dihexadecyl-3-*O*-β-D-glucosyl-*sn*-glycerol; 18-1,2-G, 1,2-*O*-dioctadecyl-3-*O*-β-D-glucosyl-*sn*-glycerol.

Preparation of Lipid Suspensions. A characteristic feature of the glucolipids studied is their extreme tendency to aggregate in aqueous solution. When treated by ultrasound above their transition temperatures, it was not possible to form stable suspensions even at concentrations as low as 0.1 mg/mL. Within 2 or 3 min after the ultrasonic treatment the lipids aggregated into fast sedimenting lumps, which prevented proper filling of the calorimeter. The only practicable procedure to obtain homogeneous aqueous suspensions of the lipids, which permitted reproducible fillings of the calorimeter, was the following low-temperature treatment. Exactly weighed quantities of the dry lipids (2–8 mg) were mixed with the respective amounts of water or buffer in a vial to give concentrations between 0.1 and 4 mg/mL. The 10-mL vials were placed into a 50-mL, water-filled beaker and cooled by ice. A Branson sonifier (Model B-12) equipped with a microtip was used to agitate the water in the beaker (position 6, 70-W output power, 20-min duration). This treatment yielded homogeneous suspensions, which remained stable for the duration of the filling procedure.

Calorimetric Measurements. The heat capacity measurements were made on an electronically modified, high sensivity Privalov type DASM 1 microcalorimeter, which allows heating rates from 0.005 to 2 deg/min. Runs were performed in the temperature range of 5–90 °C, employing various heating rates. The heat capacity and temperature data were routinely registered every tenth or hundreth of a degree by a computer. Three to four repetitions of the measurements, employing the same sample, were usually made, as well as at least two buffer runs to establish the base line and to perform the electrical calibration. The concentrations of the lipid suspensions employed in the calorimetric measurements were varied between 0.1 and 4 mg/mL, though most frequently a concentration of approximately 0.2 mg/mL was used. The molecular weights used for calculation of the molar thermodynamic quantities were 647.0, 703.1, and 759.2, respectively, for the glucolipids containing 14-, 16-, and 18-carbon atoms in their alkyl chains.

The transition temperatures, T_m, reported in this study are the temperatures of half-conversion. Due to small asymmetries of the transition peaks, they are usually a few tenths of a degree lower than the temperatures corresponding to the maxima of the peaks. Cooperative units, CU, were derived from the heat capacity measurements as ratios of the van't Hoff enthalpy, ΔH_{vH}, and the calorimetric enthalpies, ΔH_{cal}. van't Hoff enthalpies were calculated from the calorimetric measurements by using the formula

$$\Delta H_{vH} = 4RT_m^2C_m/\Delta H_{cal} \quad (1)$$

where T_m and C_m refer to absolute temperature and excess heat capacity, respectively, of half-conversion, and R is the gas constant (Hinz & Sturtevant, 1972).

Determination of Apparent Specific Heat Capacity. The specific heat capacity of the lipids, c_l, has been obtained by using the formula (Privalov & Khechinashvili, 1974)

$$c_l = c_w\left(\frac{v_l}{v_w}\right) - \frac{\Delta}{m_l}$$

where c_l and c_w refer to the specific heat capacities of lipid and water, respectively. v_l and v_w are the corresponding specific volumes (0.95 and 1 mL·g^{-1}, respectively, were used), m_l is the mass of lipid in grams, and Δ the measured difference between the heat capacity of the sample and that of the water or buffer base line (in J·K^{-1}). For a discussion of the accuracy of the method see, e.g., Blume (1983). The specific volume of the lipid has been measured by using a digital density meter,

[1] Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; 14-1,2-G, 1,2-*O*-ditetradecyl-3-*O*-β-D-glucosyl-*sn*-glycerol; 16-1,2-G, 1,2-*O*-dihexadecyl-3-*O*-β-D-glucosyl-*sn*-glycerol; 18-1,2-G, 1,2-*O*-dioctadecyl-3-*O*-β-D-glucosyl-*sn*-glycerol; 16-1,2-α,β-G, 1,2-*O*-ditetradecyl-3-*O*-α,β-D-glucosyl-*sn*-glycerol; 14-1,2-PC, 1,2-*O*-ditetradecyl-*sn*-glycero-3-phosphocholine; 16-1,2-PC, 1,2-*O*-dihexadecyl-*sn*-glycero-3-phosphocholine; 18-1,2-PC, 1,2-*O*-dioctadecyl-*sn*-glycero-3-phosphocholine; 14-1,2-PE, 1,2-*O*-ditetradecyl-*sn*-glycero-3-phosphoethanolamine; 16-1,2-PE, 1,2-*O*-dihexadecyl-*sn*-glycero-3-phosphoethanolamine; 18-1,2-PE, 1,2-*O*-dioctadecyl-*sn*-glycero-3-phosphoethanolamine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DSPE, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; DSC, differential scanning calorimetry.

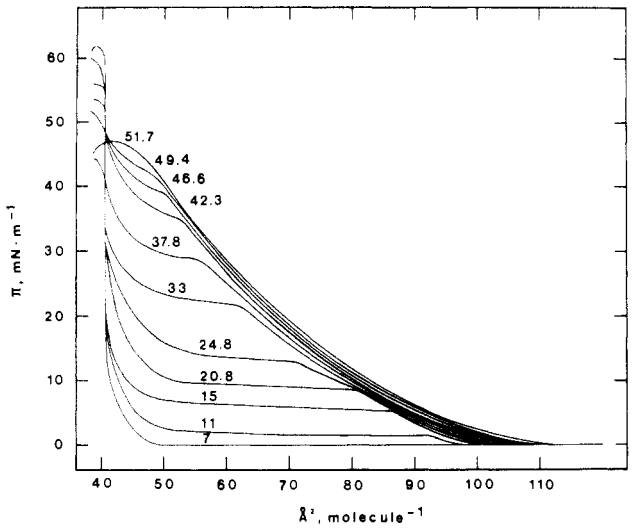

FIGURE 2: Surface pressure (Π)–molecular area (A) isotherms of 14-1,2-G on pure water. The numbers indicate the temperature to which the isotherm refers. Experimental procedures are described under Materials and Methods.

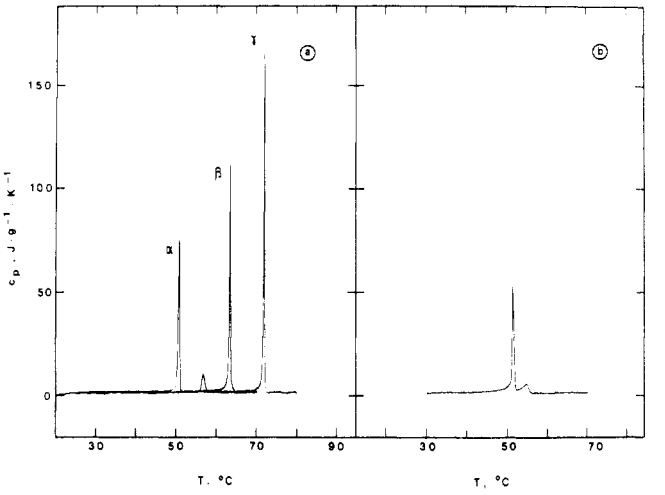

FIGURE 3: (a) Variation with temperature of the apparent specific heat capacity of glucolipid suspensions. (α) 14-1,2-G, 1.03 mg/mL; heating rate, 0.197 °C/min. (β) 16-1,2-G, 1.08 mg/mL; heating rate, 0.094 °C/min. (γ) 18-1,2-G, 1.13 mg/mL; heating rate, 0.047 °C/min. (b) Variation with temperature of the apparent specific heat capacity, c_p, of partially deuterated 14-1,2-G dispersed in D_2O (heating rate, 0.476 °C/min; concentration, 0.42 mg/mL). This curve does not show absolute values of c_p; c_p has been arbitrarily adjusted to $c_p = 0$ at $t = 25$ °C.

DMA 02 (Anton Paar KG, Graz, Austria).

Monolayer Film Studies. Monolayers of the glucolipids were spread from solutions of the lipids in $CHCl_3$–CH_3OH (1:1 v/v) on a subphase of doubly distilled water; 50-μL portions of the stock solution [(1–2) × 10^{-3} M] were applied by using a Hamilton syringe. Surface areas were varied by moving the barrier in a thermostated Langmuir trough (Lauda, Model FW-1/S). Surface pressure and area were registered by an X–Y recorder.

Measurements were routinely started 3–5 min after application of the lipid monolayer. The film was compressed at a rate of approximately (3–5) × 10^{-3} nm²·molecule^{-1}·s^{-1}.

Results

Monolayer Studies. Figure 2 shows for various temperatures the dependence of surface pressure Π on molecular area A of 14-1,2-G. At 7 °C, 14-1,2-G does not form a liquid-expanded film [for nomenclature, see Gaines (1966)] under the conditions of the experiment. At approximately 50 Å²·molecule^{-1}, the monolayer goes directly from the gaseous state into the liquid-condensed film. Below a surface pressure of 1.5 mN·m^{-1}, the 11-deg isotherm exhibits a liquid-expanded film, which turns rather sharply into an intermediate state at approximately 92 Å²·molecule^{-1}, when the pressure is increased.

Increase of temperature results in an increase of the liquid-expanded state region at the expense of the intermediate state. Higher surface pressures are required to drive the transition to the intermediate state, and the transition occurs at correspondingly smaller molecular areas. At 51.7 °C, the film collapses before reaching the fully condensed region.

A parameter characteristic of the molecular area, A_0, required by the film-forming molecule at high packing density, can be obtained by extrapolating the steep, high pressure, linear part of the Π–A diagram back to Π = 0. The extrapolation is rather safe in Figure 2 due to the low degree of curvature in the high pressure parts of the Π–A curves.

The molecular area, A_0, obtained is approximately 40–41 Å²·molecule^{-1}. This value is identical with twice the value determined for the homologous series of fatty acids, which all occupy areas near 20 Å²·molecule^{-1} (Alexander, 1941,1942a,b). To elucidate the influence of the hydrocarbon chain length on the Π–A curves, measurements were also made on 16-1,2-G and 18-1,2-G. These studies showed an analogous chain length dependence of the isotherms as previously found with racemic mixtures (Six et et., 1983).

DSC Measurements. Figure 3a shows the temperature dependence of the apparent specific heat capacity of the homologous lipids, 14-1,2-G, 16-1,2-G, and 18-1,2-G, dispersed in H_2O.

The curves give absolute values of apparent specific heat capacity. The measurements shown have been made by using up to 4 mg/mL lipid and small heating rates (0.05 and 0.1 K·min^{-1}). However, equilibrium was also established at higher heating rates (0.5 and 1 K·min^{-1}) as was demonstrated by the identity of T_m and enthalpy values. A noteworthy feature of the transition curves is the absence of pretransitions, which are generally observed with saturated phosphatidylcholines (Hinz & Sturtevant, 1972). Surprisingly, 14-1,2-G shows instead a posttransition approximately 5–6 deg above the main phase transition. Its enthalpy is only about 20% of that of the main transition; however, its cooperativity is higher than that of the main transition. While the main phase transition of 14-1,2-G can be safely identified with the gel to liquid-crystalline transition, it is unknown, at present, which structural change is reflected by the apparent increase in heat capacity above the main transition temperature. To obtain some preliminary information about the origin of this posttransition, the OH protons of glucose in 14-1,2-G were exchanged with deuterium by repeated freeze-drying. DSC measurements on these samples (Figure 3b) revealed that partially deuterated 14-1,2-G exhibits the posttransition at a lower transition temperature than the undeuterated compound. This result suggests that the posttransition may be associated with some structural change of the head group.

Inspection of the thermodynamic transition parameters of the glucolipids obtained from the calorimeric studies shows that the thermodynamic quantities are probably not a strictly linear function of the number of carbon atoms in the alkyl chains. However, deviation from linearity is not strong.

Numerical values of the thermodynamic transition quantities, obtained with the glucolipids in the present study, have been summarized in Table I together with published param-

Table I: Comparison of Thermodynamic Transition Properties of Phospholipids Having Various Head Groups[a]

lipid	linkage	T_{m1} (°C)	T_{m2} (°C)	ΔH_1 (kJ/mol)	ΔS_1 [J/(mol·K)]	ΔH_2 (kJ/mol)	ΔS_2 [J/(mol·K)]	CU_1	CU_2	ref[b]
14-1,2-G[c]	ether		50.8 ± 0.4			24.9 ± 2.9	76.9		133 ± 67	A
14-1,2-PC	ether		27.4			31.4	105.0		66	B
14-1,2-PE	ether		55.5			24.0	24.0			C
DMPC	ester	14.2	23.9	4.2	14.6	22.8	76.8	280	330	D
DMPC	ester	15.3	24.0	5.4	18.7	27.2	91.5			E
DMPE	ester		49.9			27.6	85.4			E
DMPE	ester		49.5			24.3	75.3		140	D
DMPE	ester		49.5			24.2	75.0			C
16-1,2-G	ether		63.6 ± 0.4			40.4 ± 5.2	120.0		115 ± 48	A
16-1,2-α,β-G	ether		59.0			20.9	62.9			F
16-1,2-PC	ether	33.0	43.5	5.9	19.2	35.6	112.0			E
16-1,2-PC	ether		43.4			36.8	116		72	B
16-1,2-PE	ether		68.5		31.8	31.8	93			E
16-1,2--PE	ether		68.5			33.1	91			C
16-1,2-PE	ether		69.1			31.3 ± 1.5	92			G
DPPC	ester	35.3	41.4	7.7	24.9	36.6	116	290	260	D
DPPC	ester	35.5	41.5	6.7		36.4	116			E
DPPE	ester		64			33.1	98			C
DPPE	ester		63.8			40.3	120		140	D
DPPE	ester		63.9			36.0	107			E
18-1,2-G	ether		72.0 ± 0.3			46.6 ± 4.3	135			A
18-1,2-PC	ether		55.5			40.6	124		52	B
18-1,2-PE	ether		77.0			39.1	112			C
DSPC	ester	51.5	54.9	7.7	23.7	44.4	135	160	130	D
DSPC	ester	51.0	54.3	7.5		45.6				E
DSPE	ester		74.0			43.9	126			C
DSPE	ester		70.4			43.9	128			E

[a] Subscript 1 refers to pretransition, subscript 2, to gel to liquid-crystalline transition, and subscript 3, to posttransition. T, ΔH, ΔS, and CU are transition temperature, enthalpy, entropy, and cooperative unit, respectively. [b] References: A, this work; B, Bittman et al. (1981); C, Seddon et al. (1983); D, Mabrey & Sturtevant (1978); E, Blume et al. (1983); F, Endo et al. (1982); G, Boggs et al. (1981). [c] With 14-1,2-G a posttransition has been observed. The thermodynamic parameters of this transition are T_{m3} = 56.9 ± 0.5 °C, ΔH_3 = 5.0 ± 1.7 kJ/mol, ΔS_3 = 15.1 J/mol·K), and CU_3 = 512 ± 155.

Table II: Apparent Specific, c_p (J·g^{-1}·K^{-1}), and Molar, C_p (J·mol^{-1}·K^{-1}), Heat Capacities of Ether-Linked Glucolipids Having Saturated Alkyl Chains[a]

	25 °C		T_{m2} − 10 °C		T_{m2} + 5 °C		
	c_p	C_p	c_p	C_p	c_p	C_p	$\Delta c_p(T_2)$
14-1,2-G	2.39 ± 0.32	1546 ± 207	2.69 ± 0.36	1740 ± 231	2.26 ± 0.48	1462 ± 314	−0.66 ± 0.2
16-1,2-G	2.34 ± 0.24	1645 ± 168	2.78 ± 0.40	1955 ± 278	2.16 ± 0.33	1519 ± 232	−1.2 ± 0.5
18-1,2-G	2.37 ± 0.36	1799 ± 274	2.91 ± 0.14	2209 ± 111	2.28 ± 0.18	1731 ± 138	−0.73 ± 0.2

[a] $\Delta c_p(T_2)$ is the change in apparent specific heat capacity associated with the gel to liquid-crystalline transition at the transition temperature T_2. The average increase in apparent molar heat capacity per methylene group, calculated on the basis of the data for 14-1,2-G and 18-1,2-G at 25 °C, is 63 J·mol^{-1}·K^{-1}.

eters of analogous glycerolipids having saturated hydrocarbon chains but different head groups.

A comparison of the data shows that the uncharged ether glucolipids exhibit transition temperatures slightly lower than the temperatures of the corresponding ether phosphatidylethanolamines, however, considerably higher than the T_m values of both the ester or ether phosphatidylcholines.

The situation is not as clear with regard to the transition enthalpies. 16-1,2-G and 18-1,2-G obviously show the highest transition enthalpies; the lowest ΔH values in this series are associated with the transition of the corresponding diether ethanolamines. For the C_{14} diether lipids the situation is again different: the highest transition enthalpy has been obtained with the C_{14} diether phosphatidylcholine 14-1,2-PC (Bittman et al., 1981), while the ΔH values measured with 14-1,2-G and with C_{14} diether ethanolamine, 14-1,2-PE, can be assumed to be identical within the limits of experimental error.

Thus, only the finding that ether-bond-linked lipids show slightly higher transition temperatures than the corresponding ester-bond-linked lipids appears to be generally verifiable (Vaughan & Keough, 1974; Bittman et al., 1981; Abramson, 1970; Chen & Barton, 1971; Chupin et al., 1979; Lee & Fitzgerald, 1980; Eibl & Blume, 1979; Harlos et al., 1979; Blume & Eibl, 1979; Boggs et al., 1981). However, no unique correlation seems to exist for the enthalpies. The variation between results from different groups is too large as to allow any systematic trend to be recognized.

Apparent Specific Heat Capacities. At glucolipid concentrations between 1 and 4 mg·mL^{-1} reliable values of the apparent specific heat capacity can be determined on the DASM microcalorimeter. Scans have been performed at heating rates between 0.0089 and 1 K·min^{-1} with identical results. The heat capacities have been calculated employing formula 2.

Some typical heat capacity data have been summarized in Table II. Since lipids appear to exist in roughly corresponding states at temperatures equally below or above the characteristic transition temperatures, T_m, c_p data have been listed at 298.15 K and two reduced temperatures T_{m2} − 10 K and T_{m2} + 5 K.

Discussion

Monolayer Studies. Monolayer investigations reveal important information relevant and complementary to the data obtained from the study of the bilayer phase transition. Molecular areas at high and low compression are a characteristic measure of the packing properties of lipids (Philips & Chapman, 1968; Hui et al., 1975; Albrecht et al., 1980). They

are also significant thermodynamic variables from a theoretical point of view (Nagle, 1980).

It is apparent from Figure 2 that the limiting area, A_0, of 14-1,2-G is about 40 Å^2·molecule^{-1}. This value is practically identical with that obtained by Phillips & Chapman (1968) for phosphatidylethanolamine, whereas the corresponding molecular area of the phosphatidylcholines was 44 Å^2·molecule^{-1}. Recalling that 40 Å^2·molecule^{-1} is twice the value found for saturated straight chain fatty acids, one can conclude that the packing properties of the glucolipids at high surface pressure are determined by the properties of the alkyl chains. It should be mentioned, however, that the difference in limiting area between DPPE and DPPC has been challenged by the results of monolayer studies by Hui et al. (1975) and Albrecht et al. (1981). Both groups observed also for DPPC only a limiting area A_0 of approximately 40 Å^2·molecule^{-1}.

The similarities between the glucolipids and the phosphatidylethanolamine-containing lipids become again apparent when one compares the areas corresponding to the gaseous to condensed state transition (Gaines, 1966). From the 7 °C isotherm of 14-1,2-G in Figure 2 an area of approximately 50 Å^2·molecule^{-1} can be estimated, which again is comparable to the value of about 53 Å^2·molecule^{-1} for DSPE at 22 °C [as judged from the paper of Phillips & Chapman (1968)] but clearly smaller than the 58–60 Å^2·molecule^{-1}, observed at 22 °C for DSPC by Phillips & Chapman (1968). Also the corresponding areas measured for DPPC by Hui et al. (1975) (57–58 Å^2·molecule^{-1} at 9 °C), and for 16-1,2-PC (57–58 Å^2·molecule^{-1}) by Albrecht et al. (1981), decidedly exceed the values found for the glucolipid and for DSPE. This conclusion is also supported by results of Paltauf et al. (1971), who obtained areas of 62 and 65 Å^2·molecule^{-1}, respectively, when studying DSPC and 18-1,2-PC at room temperature.

Even if one takes the experimental errors into account, the packing properties of the ether glucolipids definitely appear to be more similar to those of the phosphatidylethanolamines than the phosphatidylcholines.

The previous comparison of 14-1,2-G and the C_{16} and C_{18} lipids is meaningful, because the lipids with different alkyl chains are well below their T_{m2} values at the temperature of the Π–A measurements.

The situation at higher areas per molecule is more complex. Comparing the 22 °C isotherms, given by Phillips & Chapman (1968), for DMPE with our data for 14-1,2-G shows that the liquid-expanded state of the glucolipid becomes detectable at about 102 ± 4 Å^2·molecule^{-1}, while the area for DMPE is only about 82 Å^2·molecule^{-1}.

A simple interpretation of the result comes from model-building studies. It can be visualized that, at low surface pressure, the practically unhindered rotation around the glycosidic bond permits the glucose head group to assume conformations with larger space requirement than at high packing densities. A more speculative explanation is the following. According to heat capacity measurements of aqueous solutions of saccharides (Kawaizumi et al., 1981), the sugars have to be classified as "structure forming" in an analogous sense as hydrophobic compounds. Supporting evidence for the "non-hydrophilic" nature of the glucose head group comes from studies of Sen et al. (1981) and Shipley et al. (1973) and from our observation that it is extremely difficult to form stable aqueous dispersions of saturated galacto- and glucolipids, respectively. Thus, the apparently conflicting findings of low space requirement at high surface pressure and large space requirement at low surface pressure could be rationalized by the assumption that the glucose head groups form bulky hydrophobic type of hydration structures (Hvidt, 1983) at low surface pressure, which are destructed with increasing pressure. This interpretation gains also support from the results of Hui et al. (1975), who observed that the drying effect in bilayers is very similar to the pressure effect in monolayers, both resulting in transition temperature elevation and intermolecular spacing reduction.

Microcalorimetric Studies on Bilayer Suspensions. (*A*) *Transition Temperatures.* Variations in the head group such as replacement of PC by PE have been known to increase transition temperatures by 17–30 °C, depending on the lengths of the saturated acyl chains (dilauroyl and distearoyl species, respectively) (Simon et al., 1975; Mabrey & Sturtevant, 1976; van Dijck et al., 1976; Jackson & Sturtevant, 1977; Yang et al., 1979; Wilkinson & Nagle, 1981).

The increased stability of disaturated PE in the gel state has been ascribed both to the smaller size of the PE as compared to the PC head group and to the ability of the protonated amino group of PE to additionally stabilize the very compact head-group lattice by strong electrostatic interaction and hydrogen bonds between the ammonium nitrogen and the phosphate oxygens (Eibl & Wooley, 1979; Wilkinson & Nagle, 1981; Nagle, 1976; Hauser et al., 1981).

On the basis of the limiting area values, A_0, determined by our monolayer studies, the space requirement of the glucose moiety can be assumed to be comparable to that of the PE head group. Also the hydration properties of PE lipids (Hauser et al., 1981) and the glucolipids studied by us appear to be qualitatively very similar. Therefore, in the absence of any favorable electrostatic interaction the high stability of the structures formed by the glucolipids can be understood, when strong direct intermolecular hydrogen bonds between the OH groups of the sugar moieties are assumed. This interpretation gains some indirect support from results of various studies. Wieslander et al. (1978), when studying the water binding capability of glyceroglycolipids by X-ray diffraction, found that glycolipids took up less water than phospholipids. Also Iwamoto et al. (1982) suggested, on the basis of micropolarity measurements, a lower hydration capacity for glyceroglycolipids than for DPPC.

Furthermore, X-ray studies of Moews & Knox (1976) on decyl α-D-glucopyranoside showed that this compound occurred in a bilayer-like structure of interdigitated hydrocarbon chains, stabilized by hydrogen-bonded layers of glucose rings, each glucose participating in two hydrogen bonds. Thus, the comparatively high T_m values of the glucolipids are probably a result of both optimal packing of the alkyl chains, unperturbed by the head groups, and inter-head-group hydrogen bonding of the glucose.

High transition temperatures and enthalpies have been found previously with cerebrosides (Ruocco et al., 1981; Freire et al., 1980), and the magnitude of the thermodynamic parameters has been partially rationalized by the assumption of an inter-head-group network of hydrogen bonds. However, a direct comparison with the present results on glyceroglycolipids appears to be difficult due to the structural differences between the sphingosine and the glycerol moieties, which very likely result in different energetic contributions to the transition parameters.

(*B*) *Transition Enthalpies.* In general transition temperatures can be determined with much higher accuracy than transition enthalpies. The relatively high standard deviations, given in Table I for the ΔH values of the glucolipids, do not reflect intrinsically low precision of the calorimetric measurements (repetitions with the same sample show practically

identical ΔH values) but rather the low accuracy of the concentration determination. In our hands the various methods of sugar analysis did not give better accuracy than approximately ±10%. This was mainly due to the strong aggregation tendency of the glucolipids, which prevented quantitative hydrolysis of the sugar moieties. Therefore, concentration was determined by exact weight of the dry samples, and the suspensions were then treated as described under Materials and Methods.

As mentioned before, it is hardly possible to recognize a systematic variation among the transition enthalpies such as increase or decrease with particular variations of the head group or with the nature of the hydrocarbon chain link. There is some general agreement in the literature that the energetics of ether or ester lipids should not differ significantly. Inspection of Table I shows, however, that the existing data are probably not accurate enough to prove or disprove that conjecture. One conclusion can be drawn, however, when comparing the transition enthalpies of lipids having identical hydrocarbon chains but different head groups. The C_{16} and C_{18} glucolipids apparently exhibit the highest ΔH values among corresponding lipids. The strikingly low ΔH value determined for 16-1,2-α,β-G (20.9 kJ·mol^{-1}) by Endo et al. (1982) constitutes an exception, which remains to be resolved by further studies.

The striking phenomenon of a highly cooperative transition approximately 5 deg above the main transition was only observed with 14-1,2-G. The transition cannot be ascribed to an impurity contaminating the lipid preparation. Therefore, we assume that the posttransition reflects some specific but yet unknown structural change of the 14-1,2-G. The enthalpy is approximately 20% of that involved in the main transition, but its cooperativity is considerably higher than that of the main transition. The experiments on 14-1,2-G with all glucose OH protons exchanged by deuterium show that the posttransition is no longer well separated from the main transition but is shifted to lower temperature.

The overall ΔH value is, within error limits, identical with the sum of ΔH values of main transition and posttransition of undeuterated 14-1,2-G. We consider this finding as indicative of involvement of the glucose head group in the posttransition. We cannot, however, exclude the possibility that the posttransition reflects a bilayer to inverted hexagonal phase transition, as was found, e.g., with 1,2-ditetradecyl-*rac*-glycero-3-phosphoethanolamine (DTPE) by Harlos & Eibl (1981) and Seddon et. al. (1983). It is also known that natural unsaturated monogalactosyldiglycerides form a hexagonal phase at high concentration (Shipley et al., 1973).

Since, however, the hexagonal transition of DTPE has been studied at 1000 times higher lipid concentrations (100–250 mg mL^{-1}) and has been found to occur at temperatures considerably higher (almost 40 deg) than the gel to liquid-crystalline transition temperature (Harlos & Eibl, 1980, 1981; Boggs et al., 1981; Seddon et al., 1983), we consider the occurrence of a hexagonal transition in the glucolipid bilayer a less likely possibility. Positive identification can, however, only come from the results of X-ray studies.

(*C*) *Apparent Specific Heat Capacities.* Single phase region specific heat capacities of phospholipid systems have recently found increasing interest as characteristic thermodynamic parameters which are more likely to be interpretable in molecular terms than, e.g., transition enthalpies or entropies. Wilkinson & Nagle (1982) were the first to report c_p values for dispersions of saturated DPPC and DPPE, and Blume (1983) provided a collection of c_p data of various lipids with palmitoyl chains. A comparison of the c_p values obtained for the glucolipid dispersions with the published data on phospholipids reveals that the apparent specific heat capacity of the glucolipids is larger than that of the phospholipids.

It is common procedure to approximate partial molar heat capacities at infinite dilution from group parameters on the basis of additivity schemes as suggested by Nichols et al. (1976), Roux et al. (1978), and Cabani et al. (1977). For simple compounds there is usually excellent agreement between experimental and calculated values. In order to calculate partial molar heat capacities of the glucolipids, we assumed that the partial molar heat capacities of the glucose head group are the same as that of free glucose [331 J·K^{-1}·mol^{-1} (Kawaizumi et al., 1981)] minus an OH group contribution [+9 J·K^{-1}·mol^{-1} (Nichols et al., 1976)] plus an ether-linked O contribution [−57 J·K^{-1}·mol^{-1} (Nichols et al., 1976)]. Then the residual partial molar heat capacity of the saturated ditetraalkylglyceryl moiety is 1546 + 9 + 57 − 331 = 1281 J·K^{-1}·mol^{-1}. This heat capacity has been approximated by assuming that in the bilayer each of the first 3-CH_2 groups of the two alkyl chains is accessible to hydration while the residual 22 CH_2 groups contribute to molar heat capacity like two liquid *n*-undecane chains at 298.16 K [345 J·mol^{-1}·undecane^{-1} (Finke et al., 1954)].

The partial molar heat capacity calculated in this manner deviates only by approximately 3% from the experimental value. The result can be interpreted as providing additional circumstantial evidence for the existence of some hydration of the hydrophobic region contiguous to the glycerol moiety (Büldt et al., 1978; Zaccai et al., 1979; Blume, 1983; Lawaczek, 1979; Nichols & Deamer, 1980; Rossignol et al., 1982) while the residual parts of the alkyl chains behave like ordinary *n*-alkanes.

(*D*) *Comparison of Mono- and Bilayer Studies.* The low limiting area observed for the glucolipids is almost identical with that of corresponding saturated PE lipids. Therefore, packing properties and configuration of alkyl chains should be similar if not identical. Recent X-ray studies by McIntosh (1980) provided evidence for the absence of a chain tilt in DPPE. Just as ethanolamine lipids, glucolipids do not exhibit pretransitions. The absence of a pretransition in PE lipids has been attributed to the absence of tilt of the hydrocarbon chains (McIntosh, 1980). Therefore, we infer that the alkyl chains of the glucolipids are also approximately perpendicularly oriented to the bilayer plane.

The orientation approximately normal to the bilayer ought to result in optimal van der Waals contacts between the alkyl chains associated with high transition enthalpies. While this conclusion appears to be supported by the high ΔH values for 16-1,2-G and 18-1,2-G, the relatively low transition enthalpies of DPPE and DSPE do not reflect the expected high interaction energy due to optimal packing. Thus, although the transition temperatures, which are proportional to the Gibbs energies, indicate similarity in the overall stability of PE and glucolipid bilayers, the ΔH values suggest differences in the energetic interactions.

It has been suggested by Hui et al. (1975) and Nagle (1980) that in first approximation lipid bilayers are back to back lipid monolayers at surface pressures between 45 and 50 mN·m^{-1}. Therefore, in order to detect similarities between the mono- and bilayer studies on 14-1,2-G, the microcalorimetric results must be compared with monolayer data obtained from the high pressure part of the isotherms. Inspection of Figure 2 under this aspect shows that a change in shape of the isotherms can be seen between 49.4 and 51 °C, which has to be ascribed to

the occurrence of the phase transition in the monolayer. This transition temperature is in excellent agreement with the temperature observed in the DSC studies, which supports the idea that mono- and bilayer results can be correlated when referring to the proper conditions.

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Registry No. 14-1,2-G, 81281-23-4; 16-1,2-G, 86363-39-5; 18-1,2-G, 86363-40-8.

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Chemical Synthesis of Peptide Fragments of the Hormone-Specific β-Subunit of Human Follicle-Stimulating Hormone†

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ABSTRACT: In order to determine the specific antigenic determinants of human follicle-stimulating hormone (hFSH), hFSH-β peptides with amino acid residues 33-49 (V2), 95-118 (V3), 76-118 (V3 + $^1/_2$C2), 1-33 (V1 + C1), 22-33 ($^1/_2$C1), and 95-107 (V3 + $^1/_4$C2) according to the nomenclature of Stewart and Stewart [Stewart, M., & Stewart, F. (1977) *J. Mol. Biol. 116*, 175] as well as additional peptides with the residues 93-107, 91-107, 89-107, 87-107, and 85-107 were chemically synthesized. The peptides were examined in radioimmunoassay systems of FSH, luteinizing hormone (LH), or human chorionic gonadotropin (hCG). V3 + $^1/_2$C2 and V1 + C1 showed immunological activity, whereas the other peptides did not. Antibodies were raised in rabbits against these peptides and examined for specific binding with hFSH, LH, thyroid-stimulating hormone (TSH), and hCG. V3 + $^1/_2$C2 as well as V1 + C1 produced antisera, which specifically bound hFSH, hLH, and hTSH, indicating that the amino acid sequences contained in hFSH-β peptides V3 + $^1/_2$C2 and V1 + C1 share common antigenic sites with hLH and hTSH. Antisera were produced in rabbits against hFSH-β, against reduced and S-aminoethylated hFSH-β (AE-FSH-β), and against AE-FSH-β coupled to hemocyanin. Reduced and S-aminoethylated β-subunit of FSH-β coupled with hemocyanin produced antisera in rabbits that specifically bound only hFSH and not hLH, hTSH, or hCG.

The primary amino acid sequences of the α- and β-subunits of human pituitary follicle-stimulating hormone (hFSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG) have been elucidated (McKerns, 1978). The amino acid sequences of the α-subunit of these glycoprotein hormones are identical, whereas those of the hormone-specific β-subunits are different. The structural similarities among these hormones do not permit an easy recognition of the hormone-specific antigenic sites and create difficulties in the production of specific antisera by the use of either native hormones or their hormone-specific β-subunits as antigens. The importance of the conformation of the antigen in producing specific antibodies has been well recognized. For example, antibodies against the C-terminal peptides of the hCG-β subunit show specific binding with hCG but fail to neutralize the biological activity of the intact hormone in vivo and thus fail to compete with hCG at the receptor site (Louvet et al., 1976). Therefore, on the basis of (1) the immunological activity of hFSH peptides recovered during amino acid sequence determination, (2) the location of the disulfide bonds (Rathnam et al., 1982), (3) the location of the carbohydrate moieties (Tolvo et al., 1982), (4) the location of the "variable" and "constant" regions (Stewart & Stewart, 1977), and (5) the location of the disulfide bonds that are essential for the molecular conformation, we selected various regions of the hFSH-β amino acid sequence for chemical synthesis. The synthetic peptides were used as antigens to produce antibodies in rabbits. The synthetic peptides and antibodies against them were examined for immunological

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