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THE PHASE BEHAVIOR OF MONOGALACTOSYL, DIGALACTOSYL, AND SULPHOQUINOVOSYL DIGLYCERIDES

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SUMMARY

The phase behavior in water of mono- and digalactosyl diglycerides from pelargonium leaves and sulpholipid from an algal source have been defined by X-ray diffraction methods.

The monogalactosyl diglyceride forms an hexagonal lipid–water phase (water cylinders in a lipid matrix) over an extended part of its phase diagram. Incorporation of water into the hexagonal lattice is limited (about 22 weight %), at which point the cylinder-to-cylinder separation, at 20 °C, is 60.5 Å. The rod-like structure of this phase is confirmed by freeze–etch electron microscopy and calculations of the cylinder separation are in good agreement with those shown by X-ray diffraction. The addition of a second galactose unit completely alters the phase behavior and digalactosyl diglyceride, over an equivalent temperature–composition range, forms only a lamellar lipid bilayer phase, again with limited uptake of water (about 22 weight %). At 20 °C the lamellar repeat distance, at maximum water uptake, is 54 Å with a lipid thickness of 41.6 Å and a surface area per molecule of 75 $Å^2$, these values being similar to those of hydrated phospholipids. Algal sulpholipids, with much higher contents of saturated fatty acids, show a more complex behavior in that the lamellar phase formed appears to exhibit limited swelling behavior at low temperatures, whereas raising the temperature results in a gradual increase in interbilayer water uptake. At high temperatures the swelling behavior resembles that of phospholipids with a net charge (*e.g.* phosphatidylserine) where all the water added is incorporated between the lipid bilayers.

The phase behavior of glycolipids and phospholipids are compared and considered in terms of their respective roles in plant and animal cell membranes.

INTRODUCTION

Considerable information exists on the phase behavior in water of numerous biologically important lipids, particularly phospholipids, and several liquid crystal phases, with lamellar, hexagonal and cubic symmetry have been described[1]. Since

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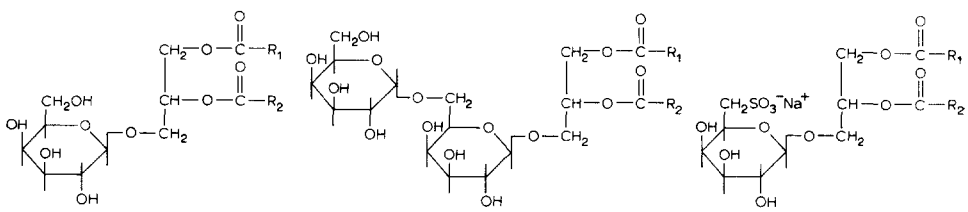
phospholipids are major constituents of cellular organisms, located principally in the membrane of the cell and cell organelles, phospholipid–water systems have been used extensively as models of natural membranes[2,3]. Furthermore, as revealed by X-ray diffraction, certain structural features of simple phospholipid–water systems seem to be recognizable in the more complex membrane systems[4].

The structure of a different group of lipids important in plant cell membranes, the glycolipids, has received much less attention. Rivas and Luzzati[5] have examined the lipid–water phase diagrams of both the polar lipids and the galactolipids from maize chloroplasts but so far little structural information exists on the different classes of glycolipids. As part of a systematic investigation of the structure of chloroplasts, Kreutz[6] has obtained X-ray diffraction patterns from dried preparations of the monogalactosyl diglyceride, digalactosyl diglyceride and phospholipid components of the thylakoid membrane.

This paper describes X-ray diffraction studies of the phase behavior in water of the uncharged glycolipids monogalactosyl diglyceride and digalactosyl diglyceride extracted from pelargonium leaves. The more complex phase behavior of two preparations of a charged glycolipid, sulphoquinovosyl diglyceride from an algal source, is also described.



EXPERIMENTAL

Preparation and analysis of glycolipids

Mono- and digalactosyl diglycerides. Pelargonium leaves were macerated with approximately 20 vol. of isopropanol and after filtering the residue was re-extracted at room temperature with chloroform–methanol (2:1, v/v). The isopropanol and chloroform–methanol extracts were then combined, dried *in vacuo*, and water-soluble contaminants removed from the extract by the method of Folch *et al.*[7]. Crude mono- and digalactosyl diglycerides were isolated from this extract by column chromatography on DEAE-cellulose according to the method of Nichols and James[8]. Monogalactosyl diglyceride was purified from contaminating pigment by preparative thin-layer chromatography on silicic acid employing chloroform–methanol–water (100:25:2, by vol.) as the mobile phase and water as the detection reagent. The digalactosyl diglyceride was purified by a similar technique employing chloroform–methanol–water (100:30:2, by vol.) as the mobile phase.

Sulphoquinovosyl diglyceride (sulpholipid). Two sulpholipid preparations were obtained from lipid extracts of a mixed algal colony by column chromatography and preparative thin-layer chromatography as described above, the mobile phase used to separate the sulpholipid from phosphatidylglycerol and phosphatidylinositol in the

latter operation comprising chloroform–methanol–acetic acid–water (85:30:5:3, by vol.).

Fatty acid compositions. Samples of the purified lipids were refluxed for 90 min with benzene–methanol–conc. H_2SO_4 (20:10:1, by vol.). After water-washing and drying, the methyl esters were analyzed by gas–liquid chromatography employing polyethyleneglycol adipate as stationary phase.

X-ray diffraction

Homogeneous dispersions of glycolipids were prepared by repeated centrifugation at 22 °C of the required quantities of lipid and water through a narrow constriction in a sealed glass capillary. Glycolipid–water samples were then sealed in a variable temperature sample holder and their low-angle X-ray diffraction patterns recorded photographically as a function of temperature within the range −10 °C to 80 °C. A Rigaku-Denki camera using CuKα nickel filtered radiation and a sample to recording plane distance of 200 mm gave diffraction patterns in the range $1/200 < 1/d < 1/15$ ($Å^{-1}$). Wide-angle diffraction patterns, $1/8 < 1/d < 1/3.6$ ($Å^{-1}$) were obtained using a special film holder with a sample to recording plane distance of 50 mm.

Electron microscopy

Electron microscopy was carried out using a Jeol JEM 7A microscope. Samples were prepared on a Polaron freeze–etching unit at −100 °C and etched for 15 s. Magnifications were of the order of 100000 ×.

RESULTS

The fatty acid compositions of the mono- and digalactosyl diglycerides and two sulpholipids used in this study are shown in Table I. Of particular interest is the extremely high content of polyunsaturated fatty acids, particularly 18:3, in both monogalactosyl diglyceride and digalactosyl diglyceride, with almost no saturated fatty acids present in monogalactosyl diglyceride. However, in the algal sulpholipids palmitic acid (16:0) is the major fatty acid in both sulphoquinovosyl diglyceride I (48%) and sulphoquinovosyl diglyceride II (71%).

Monogalactosyl diglyceride

The phase behavior of monogalactosyl diglyceride was examined in the concentration range $c = 1.0$ to 0.5, where c = g lipid/(g lipid + g water), and within the temperature range −15 to 80 °C. The phase diagram is shown in Fig. 1 and indicates the presence of an hexagonal phase over much of the concentration and temperature region studied. In the concentration range $c = 0.9$ to 0.78 a liquid crystalline lipid–water phase is observed characterized by X-ray diffraction spacings in the ratio $1 : 1/\sqrt{3} : 1/\sqrt{4} : 1/\sqrt{7} : 1/\sqrt{9} \ldots$ corresponding to the lattice planes 10, 11, 20, 21, 30, ... of an hexagonal lattice, the dimension of the phase increasing with decreasing c. At concentrations $c < 0.78$ a two-phase system, the hexagonal lipid–water phase in excess water, is present. At all concentrations a diffuse X-ray diffraction line in the wide-angle region centered at 4.6 Å confirms that the phase is liquid crystalline. Differential scanning calorimetry indicates that at the concentration $c = 0.5$ there is a liquid-crystalline to gel transition at about −30 °C.

TABLE I

FATTY ACID COMPOSITIONS OF GALACTOSYL AND SULPHOQUINOVOSYL DIGLYCERIDES

Compound	*16:0*	*16:1*	*16:2*	*18:0*	*18:1*	*18:2*	*18:3*	*18:4?*	*Unknown*	*Saturated/unsaturated fatty acid ratio*
Monogalactosyl diglyceride	<1	<1	—	—	<1	3	86	10	—	—
Digalactosyl diglyceride	17	2	—	2	3	5	61	8	2	1:4
Sulphoquinovosyl diglyceride I	48	2	—	2	10	16	19	2	—	1:1
Sulphoquinovosyl diglyceride II	71	2	—	—	3	4	21	—	—	1:0.4

TABLE II

STRUCTURAL PARAMETERS OF THE HEXAGONAL PHASE OF MONOGALACTOSYL DIGLYCERIDE–WATER

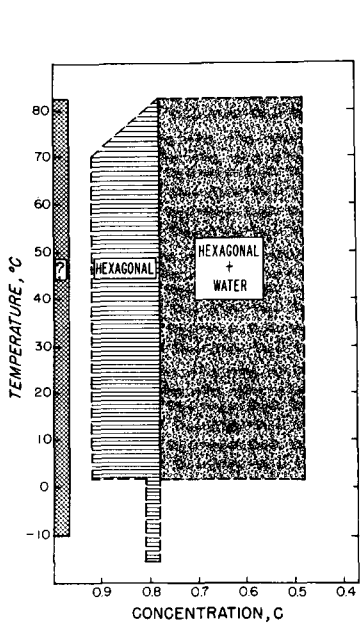
c, concentration; d_a, distance between cylinder axes (Å); S_I and S_{II}, surface area/molecule at interface (Å^2); r_{II}, radius of water cylinder (Å).

c	20 °C				0 °C			
	d_a (Å)	H_I	H_{II}		d_a (Å)	H_I	H_{II}	
		S_I (Å^2)	S_{II} (Å^2)	r_{II} (Å)		S_I (Å^2)	S_{II} (Å^2)	r_{II} (Å)
0.900	52.5	99.0	33.0	8.7	53.7	96.9	32.3	8.9
0.850	56.9	94.1	39.6	11.6	58.3	91.8	38.6	11.9
0.834	55.4	97.5	43.5	11.9	56.6	95.5	42.6	12.1
0.820	56.7	96.2	45.0	12.6	58.1	94.0	44.0	12.9
0.790	59.1	93.9	48.4	14.2	61.6	90.2	46.5	14.8
0.667*	60.5	—	49.0	14.9	62.4	—	47.6	15.4
0.500*	61.2	—	48.5	15.1	63.5	—	46.7	15.6

* See Results section.

X-ray diffraction from anhydrous monogalactosyl diglyceride, $c = 1.0$ exhibits only one diffraction spacing in the low angle region varying from 35.1 Å at −10 °C to 33.9 Å at 80 °C. As several orders of the primary diffraction spacing are required to distinguish unequivocally between possible structures the structure in this region of the phase diagram remains uncertain. The wide-angle region was characterized by a broad diffuse line at 4.6 Å at all temperatures.

Luzzati and coworkers (for a review see ref. 1) have shown that two different structural arrangements are possible for the hexagonal phase of lipid–water systems, one in which there are cylinders of lipid in a water matrix (hexagonal I), and the other in which there are cylinders of water in a lipid matrix (hexagonal II). In both cases the parallel packing of the cylinders forms a two-dimensional hexagonal lattice. In order to differentiate between the two types of structure, the dependence of the lattice parameter S, the surface area per molecule at the lipid–water interface, must be considered as a function of the lipid concentration c. In the case of hexagonal I, S should remain constant on decreasing c whereas for hexagonal II, S should increase on decreasing c. Assuming the partial specific volumes of both the lipid and water to be 1.0 ml/g. the surface areas were calculated for both structural models at 0 and 20 °C (see Table II). In Fig. 2 the surface areas at 20 °C calculated for the two types of



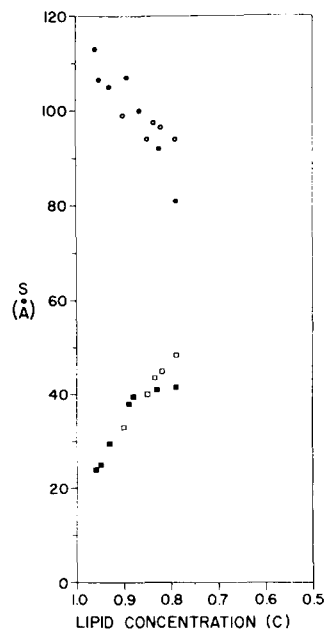


Fig. 1. The phase diagram of monogalactosyl diglyceride–water. Dashed lines indicate boundaries of the phase diagram studied. Area with horizontal lines, hexagonal phase H_{II}; pebbled area, H_{II} + excess water; cross-hatched area, phase unknown.

Fig. 2. A comparison of the concentration dependence of the surface area per molecule (S) for monogalactosyl diglyceride and egg yolk phosphatidylethanolamine (data from Reiss-Husson[9]) calculated assuming the two types of hexagonal phase. Monogalactosyl diglyceride at 20 °C: ○, assuming hexagonal Type I; □, assuming hexagonal Type II. Egg yolk phosphatidylethanolamine at 55 °C; ●, assuming hexagonal Type I; ■, assuming hexagonal Type II.

hexagonal structure are plotted as a function of lipid concentration c, together with the equivalent data obtained by Reiss-Husson[9] for egg yolk phosphatidylethanolamine at 55 °C. Although the decrease in S assuming the hexagonal I structure is not so convincing for the monogalactosyl diglyceride, the two sets of calculations and their agreement with the phosphatidylethanolamine data indicate that the hexagonal phase exhibited by monogalactosyl diglyceride is Type II.

Fig. 3 illustrates the behavior of various lattice parameters at 0 and 20 °C as a function of concentration. At 0 °C the distance between the cylinder axes d_a increases to a limiting value of 62.5 Å at an estimated concentration $c=0.78$, the lipid being fully hydrated at this point. The lipid concentrations plotted for $c<0.78$ are theoretical in the sense that in the two-phase region the sampling process for the X-ray experiment is unlikely to select a representative sample from the bulk mixture. Thus the intended concentration c is plotted in Fig. 3 and the diffraction data obtained are used to more carefully define both the limits of swelling and the structural dimensions at maximum hydration. Calculations of the structural parameters (S_I, S_{II}, r_{II}) are based upon a lipid concentration $c=0.78$. At this limiting dimension the calculated surface area per molecule at the lipid–water interface approaches 47 Å^2. On increasing the temperature to 20 °C a small decrease in the limiting dimension d_a is observed, from 62.5 to 60.5 Å with a concomitant small increase in the surface area S to 48.7 Å^2.

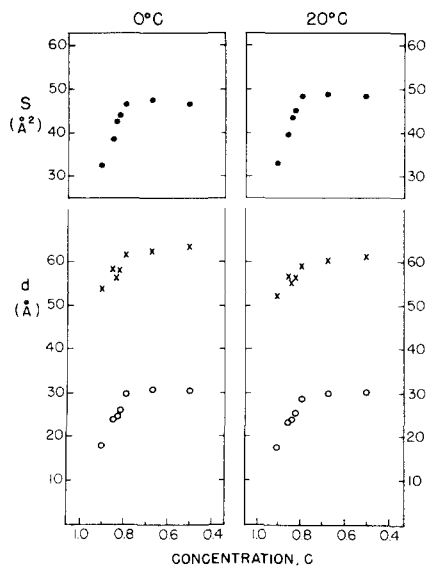


Fig. 3. Lattice parameters of the hexagonal phase H_{II} of monogalactosyl diglyceride–water at 0 and 20 °C. ×, distance between cylinder axes; ○, diameter of water cylinders; ●, surface area per molecule (S) at the lipid–water interface.

It has been shown recently[10] that rapid freezing of liquid-crystalline lipid–water phases apparently preserves their structure, in particular lamellar and hexagonal phases. A typical electron micrograph of a freeze-etched preparation of the hexagonal II phase of monogalactosyl diglyceride in excess water is shown in Fig. 4. The fracture faces consist of long parallel lines with the occasional appearance of areas which

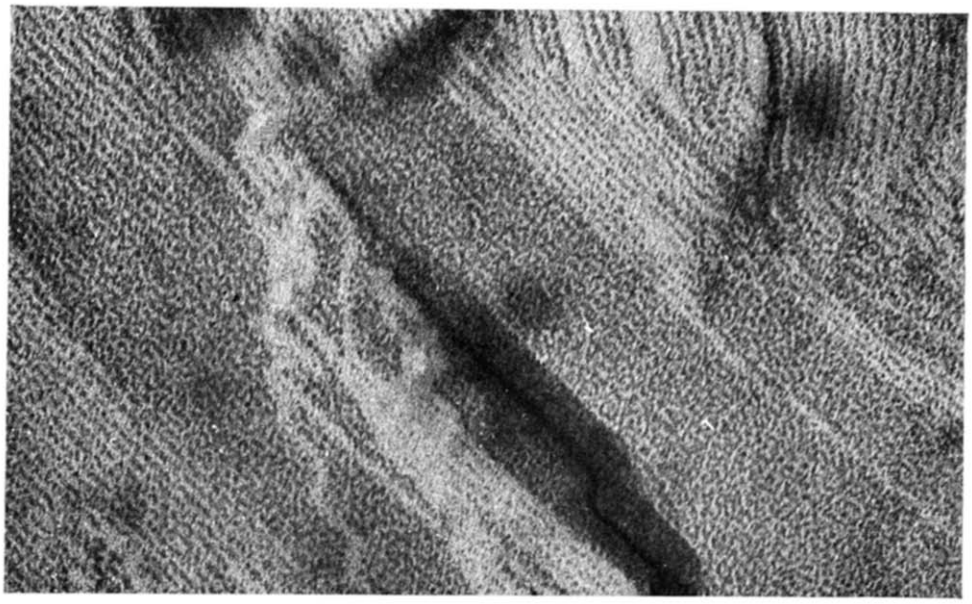


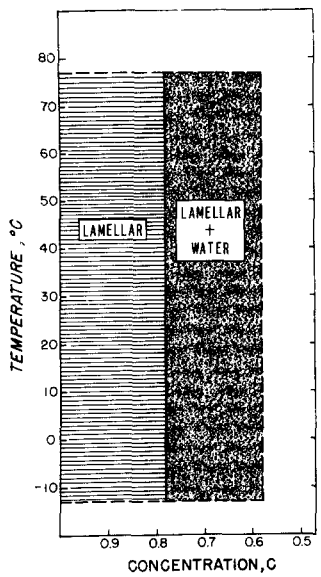
Fig. 4. Electron micrograph of freeze-etched monogalactosyl diglyceride–water at $c=0.50$. Magnification 250000×.

apparently represent cross-sections through the hexagonal arrays of rods. The micrographs are extremely similar to those obtained by Deamer *et al.*[10] for calcium cardiolipin which also exhibits a hexagonal II phase over much of its phase diagram. A mean value for the cylinder axis-to-cylinder axis distance of 65 Å was obtained from measurements of the electron micrograph, in reasonable agreement with the limiting dimension of the hexagonal phase determined by X-ray diffraction.

Digalactosyl diglyceride

Digalactosyl diglyceride was examined over the concentration range $c=1.0$ to 0.6 and over the temperature range −10 to 80 °C. The phase behavior, illustrated in Fig. 5, indicates the presence of a liquid-crystalline lamellar lipid–water phase over the complete range of concentration and temperature studied. This phase is characterized by low-angle X-ray diffraction spacings in the ratio 1:1/2:1/3:1/4 . . . . and diffuse scattering in the wide-angle region centered at 4.6 Å. Differential scanning calorimetry shows a transition from the gel to the liquid-crystalline phase at about −50 °C.

For concentrations $c<0.78$ the digalactosyl diglyceride exists as a lamellar phase in excess water the system having reached full hydration at $c=0.78$ whilst for $c>0.78$ the lamellar phase expands with increasing water content or decreasing c. The problem of sampling at $c<0.78$ is similar to that described above and the calculations of d_l and S are made assuming a lipid concentration $c=0.78$. The lipid bilayer thickness (d_l) and the surface area (S) were calculated from the primary diffraction spacing d_{100} making the same assumption about the partial specific volumes as described above. Table III lists these parameters at two temperatures (0 and 20 °C) and Fig. 6 illustrates their behavior as a function of concentration. At 0 °C the primary



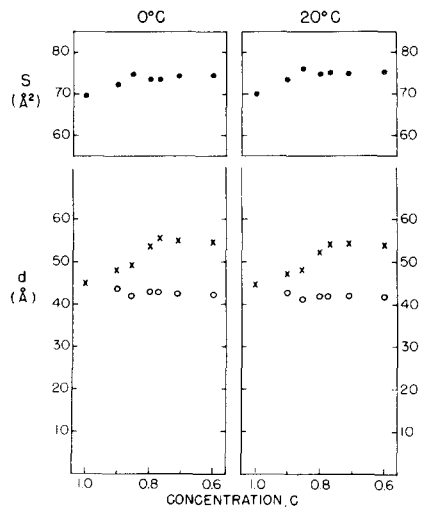


Fig. 5. The phase diagram of digalactosyl diglyceride–water. Dashed lines indicate boundaries of the phase diagram studied. Area with horizontal lines, lamellar phase L; pebbled area, L + excess water.

Fig. 6. Lattice parameters of the lamellar phase L of digalactosyl diglyceride–water at 0 and 20 °C. ×, lamellar diffraction spacing d_{100}; ○, lipid thickness d_1; ●, surface area per molecule at the lipid–water interface S.

TABLE III

STRUCTURAL PARAMETERS OF THE LAMELLAR PHASE OF DIGALACTOSYL DIGLYCERIDE–WATER

c, concentration; d_1, lipid bilayer thickness (Å); S, surface area molecule at interface (Å²).

c	20 °C			0 °C		
	d (Å)	d_1 (Å)	S (Å²)	d (Å)	d_1 (Å)	S (Å²)
1.000	44.8	44.8	69.9	45.0	45.0	69.5
0.900	47.3	42.6	73.5	48.2	43.4	72.1
0.853	48.4	41.3	75.8	49.3	42.0	74.4
0.796	52.6	41.9	74.7	53.7	42.7	73.2
0.770	54.1	41.7	75.1	55.4	42.7	73.3
0.712*	54.4	41.9	74.7	55.0	42.4	73.9
0.600*	54.0	41.6	75.2	54.6	42.0	74.4

* See Results section.

spacing d_{100} increases from 45 Å at $c = 1.0$ to a limiting value of 55 Å at $c = 0.78$ with the bilayer thickness approaching a limiting value of 42.5 Å in the region $0.9 < c < 0.85$. At this limiting dimension the mean surface area per molecule at the lipid–water interface is of the order of 74 Å². At 20 °C a small decrease is observed in the limiting

dimension d_{100} from 55 to 54 Å; d_1 decreases from 42.3 to 41.6 Å whilst a small increase in the surface area S to 75 Å^2 occurs. Freeze-etched preparations of digalactosyl diglyceride in excess water showed typical multilamellar structures when viewed in the electron microscope (see Fig. 7).

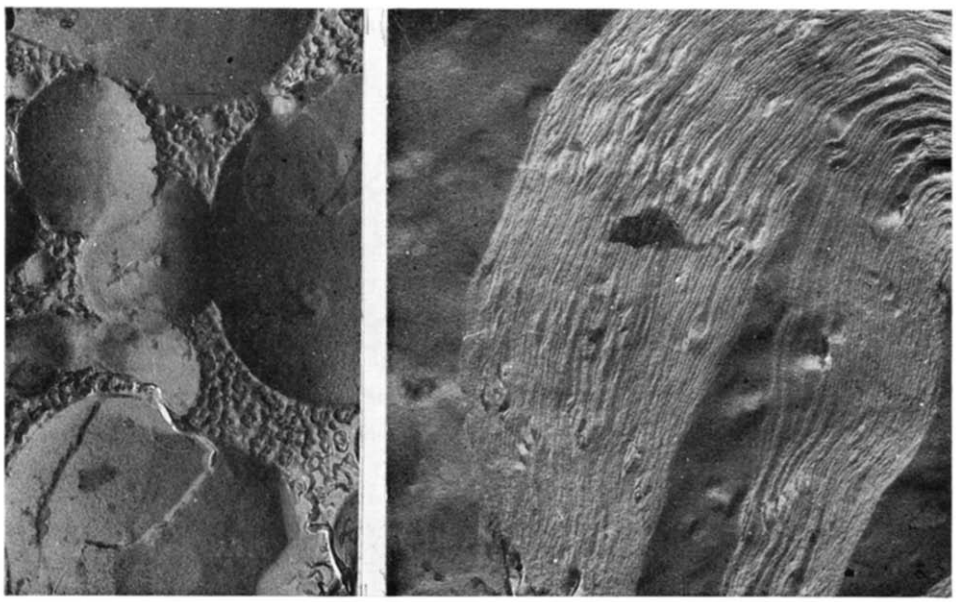


Fig. 7. Electron micrograph of freeze-etched digalactosyl diglyceride-water at $c=0.50$. Magnification 50000×.

From the temperature dependence of the lattice spacings the negative coefficient of linear expansion may be calculated, a typical value, for monogalactosyl diglyceride at $c=0.85$, being $\alpha=1.9\cdot10^{-3}$. However, since for all the monogalactosyl diglyceride and digalactosyl diglyceride preparations the lattice dimensions vary linearly with temperature, the expansion rate, in the units Å/°C, may be plotted as a function of lipid concentration. As shown in Fig. 8, as the water content is increased the expansion

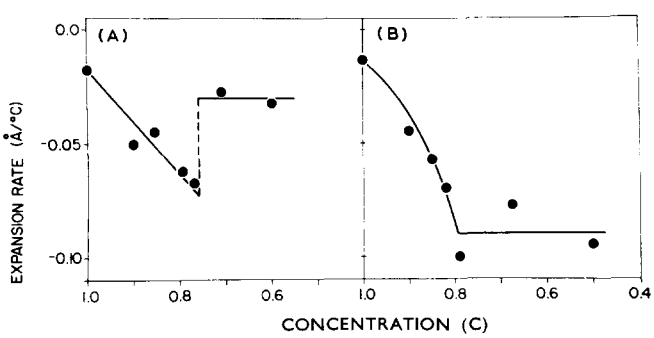


Fig. 8. Plot of expansion rate (Å/°C) as a function of lipid concentration c for (A) monogalactosyl diglyceride-water; (B) digalactosyl diglyceride-water.

rate decreases within the range -0.01 to -0.10 Å/°C with, for both glycolipids, a discontinuity in the curves occurring at lipid concentrations close to the point of maximum hydration. At this point for monogalactosyl diglyceride there is some evidence that a decrease in the negative expansion rate occurs with a levelling-off at an expansion rate of approximately -0.03 Å/°C. A possible explanation for this is that the maximum hydration level is slightly temperature dependent. For digalactosyl diglyceride there is no significant increase in the negative expansion rate and the levelling-off occurs at approximately -0.09 Å/°C.

Sulphoquinovosyl diglyceride

The fatty acid composition of the two sulphoquinovosyl diglyceride preparations are listed in Table I, an interesting feature being the high content of palmitic acid, 48% for sulphoquinovosyl diglyceride I and 71% for sulphoquinovosyl diglyceride II. The phase behavior in water of both preparations was examined over the concentration range $c=0.8$ to 0.35 and a temperature range of -10 to 80 °C. Sulphoquinovosyl diglyceride I and II show a similar behavior overall, the differences in detail presumably relating to their different fatty acid compositions.

Sulphoquinovosyl diglyceride I. Above 20 °C the sulphoquinovosyl diglyceride I–water system exists as a liquid-crystalline lamellar phase characterized by X-ray diffraction spacings in the ratios 1:1/2:1/3:1/4 .... The dimensions of this phase show a dependence on both concentration and temperature. Wide-angle X-ray diffraction indicates that below 20 °C the liquid-crystalline phase, characterized by a diffuse 4.5 Å spacing, coexists with a hydrated gel phase giving a sharp reflection at 4.1 Å. With decreasing temperature the liquid-crystalline phase is gradually replaced by the gel until, at -10 °C, the system consists entirely of the latter phase. However, interestingly, in the low-angle region only one continuous set of lamellar diffraction lines was observed over the temperature range 20 to -10 °C. On this basis the partial phase diagram shown in Fig. 9A was derived.

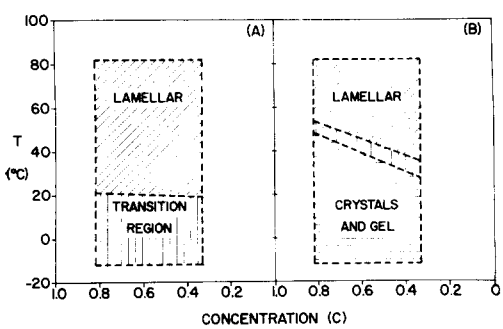


Fig. 9. The phase diagrams of sulphoquinovosyl–water systems. (A) sulphoquinovosyl diglyceride I–water; (B) sulphoquinovosyl diglyceride II–water. Area with diagonal lines, lamellar phase L; area with vertical lines, transition region; area with horizontal lines, crystals or gel.

However, the swelling behavior of the sulphoquinovosyl diglyceride I–water lamellar phase is apparently complex as illustrated by the unusual temperature dependence of the lattice parameter, d, on concentration shown in Fig. 10A. At high water contents, $c=0.5$ and 0.35, the anomalous behavior of increasing lamellar

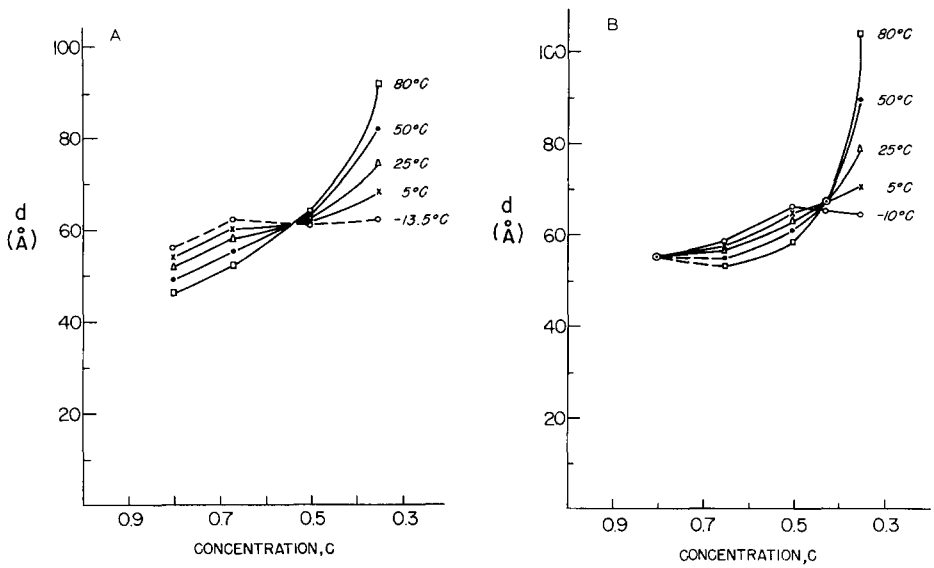


Fig. 10. Temperature dependence of the swelling behavior of (A) sulphoquinovosyl diglyceride I–water, (B) sulphoquinovosyl diglyceride II–water.

spacings with increasing temperature is observed. It is assumed that this lattice expansion observed above 25 °C reflects an increase in the water trapped between the liquid-crystal lipid bilayers the extra water coming from excess water present at these concentrations. If this is the case, then within the lamellar liquid-crystalline phase shown in Fig. 9A a temperature- and concentration-dependent phase boundary, as yet undefined, separating a two-phase lamellar liquid crystal–excess water region from the expanding lamellar phase region must be present. At high lipid concentrations the observed decrease in d as the temperature is raised is due to the gradual melting of the gel phase to the liquid-crystal phase, this transition always producing a reduction in the lipid thickness d_l. The further decrease observed when all the lipid is in the liquid crystal phase presumably reflects the usual negative coefficient of linear expansion of the lipid bilayer region.

Thus at -10 °C, where the gel phase predominates, the system incorporates water only over the concentration range $0.8 > c > 0.67$ thereafter producing a hydrated gel phase in excess water. At 80 °C, where only the liquid-crystalline phase is present, there appears to be a hydrating lamellar phase incorporating more water as the temperature is raised. At intermediate temperatures an intermediate type of swelling behavior is observed.

Sulphoquinovosyl diglyceride II. The sulphoquinovosyl diglyceride II–water system shows a similar phase behavior to sulphoquinovosyl diglyceride I but with a relatively narrow transition region between the gel and liquid-crystalline states. The partial phase diagram (Fig. 9B) shows that at $c = 0.35$ this transition occurs between 25–35 °C and between 45–55 °C at an increased lipid concentration of $c = 0.8$. Above this intermediate region a liquid-crystalline lamellar phase exists whilst below it an apparently concentration-dependent demixed system of two phases occurs. This

consists of a crystal phase characterized by a primary diffraction spacing of 51.5 Å which is independent of both temperature and concentration, and a lamellar gel phase characterized by a primary spacing which is dependent upon both of these variables. The two phases are also distinguishable in the wide-angle diffraction region, the gel phase represented by a single sharp diffraction line of 4.15 Å and the crystal phase by several sharp lines of which 4.69 Å and 4.52 Å are the most intense. The diffracted intensities of the two phases indicate that the crystal phase extends over the whole concentration range although apparently quantitatively decreasing with increasing water content, there being a parallel increase in the amount of gel present. At the high lipid concentration, $c=0.8$, the sulphoquinovosyl diglyceride did not disperse in water and X-ray diffraction showed that below 45 °C this sample consisted entirely of the crystal form.

On heating through the transition region the gel converts to a liquid-crystalline lamellar phase with no discontinuous change in the lattice dimensions but the intensities of all the diffraction lines originally associated with the gel increase. At the same time there is a decrease in intensity of the diffraction lines from the crystal and these lines disappear completely just before the liquid-crystalline region is reached.

There is a complex temperature dependence of the diffraction spacings similar to that observed for sulphoquinovosyl diglyceride I. Again at high lipid concentrations d decreases on increasing the temperature whereas at low lipid concentrations an increase in d is observed (see Fig. 10B). At $c \simeq 0.43$ the diffraction spacings of this phase are virtually independent of temperature. This results, as before, in a system, the gel, which is apparently fully hydrated at low temperatures transforming into a continuously hydrating system as the temperature is raised.

DISCUSSION

Clearly the three isolated glycolipids exhibit different swelling behavior in the presence of water. The monogalactocyl diglyceride with a single galactose unit linked to the C-3 of glycerol favors the hexagonal structure of aqueous cylinders in a lipid matrix. This phase extends over the complete temperature and concentration range studied with the possible exception of the dry material. The single X-ray diffraction line obtained from the dry material shows no discontinuity over the temperature range -10 to 80 °C, the dimension at room temperature, 34.5 Å, agreeing with that obtained by Kreutz[6] for monogalactosyl diglyceride isolated from thylakoid chloroplasts. The presence of only one diffraction line does not permit an unequivocal distinction between the lamellar and hexagonal types of structure.

In terms of the swelling behavior monogalactosyl diglyceride incorporates water into the aqueous channel only to the concentration $c=0.78$. At higher water contents a two phase turbid system being formed. This limited swelling behavior in the hexagonal phase is identical to that shown by two isolated phospholipids, the phosphatidylethanolamines from egg yolk[9] at 55 °C and beef liver (Williams, R. M., private communication) at 23 °C and in both cases the boundary of the two phase system occurs at $c \simeq 0.78$. The cylinder axis separation lies between 60 and 68 Å and, perhaps significantly, the diameter of the water cylinder in all three cases is 30 Å. This structural relationship between the two lipid classes, phosphatidylethanolamine and monogalactosyl diglyceride, presumably arises from similarities in their relative bulkiness

of the hydrophilic head groups compared to their lipid chains and perhaps suggests that their structural role in animal and plant membranes, respectively, may be similar. Although the phosphatidylethanolamine lipids tend to form mixed lamellar–hexagonal phases at lower temperatures, presumably the high degree of unsaturation of the monogalactosyl diglyceride prevents this transition at least down to −15 °C. Differential scanning calorimetry shows a phase transition, at −30 °C presumably into a gel phase but it has not been established whether a transition into a lamellar liquid-crystal phase occurs between −15 and −30 °C.

The addition of a further galactose unit to the hydrophilic part of the molecule producing digalactosyl diclyceride completely alters the swelling behavior in water. For the digalactosyl diglyceride only a lamellar phase is observed, water being incorporated between the lipid bilayers to a lipid concentration $c=0.78$, a hydration value identical to that observed for the hexagonal phase of monogalactosyl diglyceride. Again we may compare its swelling behavior with that of one of the phospholipid classes, the phosphatidylcholines. In this case the digalactosyl diglyceride incorporates only 22% (weight) of water with a limiting lipid–water repeat distance of 54 Å whereas egg yolk phosphatidylcholine, for example, hydrates to approximately 40% (by weight) with a corresponding increase in the lamellar repeat to 62 Å[10]. For the dry material, in this case lamellar, there is no evidence of a phase change in the range −10 to 75 °C. At 20 °C we obtain a lamellar repeat unit of 44.7 Å in good agreement with the value quoted by Kreutz[6].

The two isolated sulpholipids show a similar swelling behavior in water, the differences being due to the presence in sulphoquinovosyl diglyceride II of an apparently concentration-dependent crystal phase. It seems probable that the high content of palmitic acid results in a partial separation of the molecular species of sulphoquinovosyl diglyceride II, with perhaps a higher melting dipalmitoyl (16:0, 16:0) component separating from the usual single-phase mixed-chain system. We may then account for the presence of crystals in the aqueous mixtures since the higher melting component would have its mesomorphic transition temperature higher than the equilibration temperature, thus the prerequisite for the dispersion of lipids in water would not be met[11].

However, the most interesting feature of the two sulpholipid–water systems is the temperature dependence of the swelling behavior. It might be expected that the sodium salt of sulphoquinovosyl diglyceride, at least when above the gel–liquid crystal transition would form the continuously expanding lamellar phase shown by other charged lipid species[12,13]. However, both sulphoquinovosyl diglyceride I and sulphoquinovosyl diglyceride II show a gradual shift from a non-expanding gel system at low temperatures through to an expanding system at higher temperatures, although it would appear, from a comparison with other continuously-swelling lipid–water systems, that even at 80 °C not all of the available water is being incorporated.

Gulik-Krzywicki *et al.*[13] have shown, by incorporating varying amounts of ionizable lipids, both anionic and cationic, with non-ionizable lipids, that the hydration behavior of lipid–water phases may fall between the extremes of systems such as the "infinite swelling" phosphatidylserine and those that reach a limiting hydration level after which the lamellae exist in excess water, *e.g.* digalactosyl diglyceride. In the light of these observations an explanation of the behavior of the sulpholipid–water systems may be found in considering how the effective charge could be limited

at lower temperatures. A possible effect may be the shielding of the charged group at lower temperatures due to its orientation, and a lack of rotational freedom, at low surface area per molecule (S) at the lipid–water interface.

It is tempting to suggest that the monogalactosyl diglyceride, digalactosyl diglyceride and sulphoquinovosyl diglyceride may play, respectively, similar structural roles in plant cell membranes to phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine, major lipid components of animal cell membranes. In any event this information on the phase behavior of the single glycolipid components is necessary if we are to understand the important factors determining lipid mixing, and perhaps demixing, phenomena which may occur in lipid regions of both animal and plant membranes.

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