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Phase properties of binary mixtures of monogalactosyldiacylglycerols differing in hydrocarbon chain substituents dispersed in aqueous systems

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Monogalactosyldiacylglycerols isolated from spinach leaves contain a high proportion of polyunsaturated fatty acyl substituents and form hexagonal-II structures when dispersed in excess water. Catalytic hydrogenation of the lipid in the presence of Adam's catalyst completely saturates the hydrocarbon chains and the lipid forms typical open sheet bilayer structures in water at 20°C. Binary mixtures of the native and hydrogenated lipid tend to phase separate at 20°C. Freeze-fracture electron microscopy reveals lamellar phase lipid indispersed with regions of hexagonal-II structure and the proportions of each reflect the composition of the mixture. X-ray diffraction in both wide- and low-angle regions show that the saturated lipid forms the typical stable gel-phase structure in mixtures that are allowed to equilibrate over three days at 20°C. The phase transition behaviour of binary mixtures of the two galactolipids was investigated by differential scanning calorimetry and fluorescence probe methods. Thermal studies indicate that the phase-separated gel structure undergoes an anomalous transition compared with the saturated pure lipid in that the transition temperature is reduced from about 57°C to 41°C and the enthalpy of the transition is also somewhat reduced. Furthermore, the transition appears to involve the conversion of the completely phase-separated system into bilayer coexisting with phases intermediate between bilayer and hexagonal-II. A homogeneous hexagonal-II phase is presumably formed at higher temperatures. The thermal and structural studies were consistent with fluorescence polarization measurements of 1,6-diphenyl-1,3,5-hexatriene interpolated into the hydrocarbon domain of the structure.

Introduction

One of the characteristic features of most biological membranes is the heterogeneous composition of the fatty acyl chains of the membrane lipids. The fatty acids vary in both length and number of *cis* unsaturated bonds. In general, reconstitution and other studies have shown that a wide variety of membrane functions can be performed with a single molecular species of lipid. The reason for the great complexity observed in membrane lipid composition is therefore not immediately obvious. Secondly, it is remarkable that, although complex, the particular composition of molecular species of lipid present in each morphologically distinct membrane and the ratio of lipid to membrane protein is preserved within relatively narrow limits. One must presume that biochemical mechanisms are responsible for maintaining membrane lipid composition and that control of these processes is exercised in a way that is responsive to some physical parameter associated with the membrane itself.

Many studies of pure lipids dispersed in aqueous systems have shown that the particular phase adopted by the lipid at a given temperature and water concentration depends on the length of the associated hydrocarbon chains [1,2] and the num-

ber and location of *cis* unsaturated bonds in the chains [3,4]. Examination of the phase properties of the molecular species of lipids found in biological membranes shows that, at the growth temperature, some lipids form lamellar structures, mostly in the liquid-crystalline chain conformation, while others tend to form hexagonal-II structures. In membranes like the photosynthetic membrane of higher plant chloroplasts the hexagonal-II forming lipid, monogalactosyldiacylglycerol indeed, dominates the lipid composition of the membrane. A particular feature of this lipid species is that it is present in a highly unsaturated form and, in certain varieties of bean and in spinach [5], the dilinolenoyl derivative constitutes the major form of the lipid. As in other hexagonal-II forming lipids, such as cardiolipin in the presence of calcium [6–8] and phosphatidylethanolamines [9], the tendency to form the hexagonal-II phase depends markedly on the extent of saturation of the associated fatty acyl chains. It has already been reported, for example, that the distearoyl derivative of monogalactosyldiacylglycerol forms bilayers at the growth temperature in excess water [10,11]. Furthermore, hydrogenation studies of the polyunsaturated monogalactosyldiacylglycerol from broad bean has shown that the extent of unsaturation determines the conditions under which the lipid is transformed from hexagonal-II to a bilayer configuration [12].

Phase separations in binary mixtures of membrane lipids differing in their hydrocarbon chain composition have been reported in a number of systems. Mixtures of distearoyl- and dioleoylphosphatidylcholines have been shown, by differential scanning calorimetry, to segregate into domains during cooling which subsequently undergo endothermic transitions upon heating that correspond to the individual components of the mixture [3]. Similar observations have been reported using freeze-fracture electron microscopic methods which also show evidence for phase separations in binary mixtures of these two phosphatidylcholines [13,14]. Fluorescence probe methods have been used to construct phase diagrams of binary mixtures of 1-palmitoyl-2-docosahexaenoyl- and 1,2-dipalmitoylphosphatidylcholines and the phase separation is shown to take place over a wide range of temperatures and in varying proportions of the two lipids [15]. Phase separations of bovine brain sphingomyelins in mixed dispersions with egg phosphatidylcholine have been demonstrated to occur at low temperature on the basis of X-ray diffraction and calorimetry [16] but binary mixtures of pure molecular species of sphingomyelin do not show evidence of any phase separation [17]. Other experimental [18,19] and theoretical studies [20,21] of binary mixtures of pure saturated phosphatidylcholines have indicated that the ability of phospholipids differing only in the type of hydrocarbon chain substituent to separate into two phases depends, in general, on the difference in gel to liquid-crystalline phase transition temperatures of the individual species in the mixture.

All of these previous studies have been performed on lipids which form bilayer structures over the range of temperatures investigated. Phase separations of hexagonal-II-forming monogalactosyldiacylglycerols from total polar lipid extracts of chloroplasts have been reported, however, and this has been shown to depend on the ability of the lipid to assume a hexagonal-II structure [12]. We have undertaken a study of the phase behaviour of binary mixtures of monogalactosyldiacylglycerols differing in the extent of saturation of the hydrocarbon chain substituents and hence their tendency to form hexagonal-II structures on the one hand and lamellar gel-phase on the other.

Materials and Methods

Preparation of lipid extracts. Total polar lipid extracts of fresh spinach leaves were prepared essentially using the method of Kates [22]. Briefly 100 g of washed leaf tissue was homogenised in 300 ml chloroform/methanol (1 : 2, by vol.). The homogenate was filtered to remove particulate matter and the residue washed sequentially with 400 ml chloroform/methanol/water (2 : 1 : 0.8, by vol.) and 150 ml chloroform/methanol (2 : 1, by vol.). To the combined filtrates were added 250 ml chloroform and 290 ml water and the mixture shaken and allowed to partition in a separating funnel. The lower organic phase containing the total polar lipids of the spinach leaves was evaporated to dryness in vacuo and stored in chloroform solution at −20°C in the presence of antioxidant until required for further purification.

Isolation of monogalactosyldiacylglycerol. The monogalactosyldiacylglycerols were separated from the total polar lipids by fractionation on an acidified Florisil column. The total polar lipid extract was applied to the column and eluted with 4 bed volumes of chloroform. This procedure eluted the neutral lipids including most of the pigments from the column. The monogalactosyldiacylglycerols were eluted using a solvent consisting of an equal volume of chloroform : acetone and collected at a flow rate of 3 ml · min^{-1} in 5 bed volumes of the solvent. The column was subsequently eluted with acetone and methanol to remove the digalactosyldiacylglycerols and phospholipids, respectively. The chloroform-acetone fraction was evaporated to dryness and applied to a preparative TLC plate which was subsequently developed with a solvent system consisting of acetone/benzene/water (91 : 30 : 8, by vol.). The band migrating at the same rate as a standard monogalactosyldiacylglycerol was scraped off the plate and the lipid eluted with a solvent consisting of methanol/chloroform (2 : 1, by vol.). The purity of the lipid was checked against the standard using several solvent systems on TLC and was essentially free of pigment contamination.

Hydrogenation of monogalactosyldiacylglycerol. Dry benzene saturated with N_2 was added to a dry residue of the native lipid preparation and the solution was sparged with N_2 for 20 min. Adam's catalyst (Johnson-Matthey Chemicals, Ltd., U.K.) was added in an amount equal by weight to that of the original lipid. The mixture was bubbled with N_2 for a further 10 min and then with H_2. The hydrogenation was performed at 30°C. After 24 h the reaction was centrifuged to remove the catalyst and the lipid again separated from traces of catalyst and any degradation products by preparative TLC as described above. Complete hydrogenation was confirmed by gas chromatography.

Lipid analyses. Analyses of the fatty acids of the native and hydrognated lipids were performed by gas-liquid chromatography of the methyl ester derivatives as described previously [23]. Table I shows the fatty acyl composition of the monogalactosyldiacylglycerol before and after hydrogenation.

Lipid dispersions. Samples of native and hydrogenated monogalactosyldiacylglycerol or mixtures of the two lipids in chloroform were evaporated to dryness under N_2 and vacuum desiccated overnight to remove traces of solvent. The dried lipid was dispersed in distilled water under anoxic conditions by irradiation for 5 min at 60°C in an ultrasonic bath.

TABLE I

FATTY ACID COMPOSITION OF MONOGALACTOSYLDIACYLGLYCEROL FROM SPINACH LEAF TISSUE BEFORE AND AFTER HYDROGENATION IN THE PRESENCE OF ADAM'S CATALYST

$\bar{N}$, average number of double bonds per molecule.

Sample	Fatty acid composition (mol%)						$\bar{N}$
	16:0	16:1	18:0	18:1	18:2	18:3	
Native lipid	18.9	7.0	–	1.0	21.5	51.8	4.14
Hydrogenated lipid	25	–	75	–	–	–	0

Electron microscopy. The monogalactosyldiacylglycerol dispersions were equilibrated at the required quench temperature for at least 15 min prior to freezing in a slurry of nitrogen. The frozen samples were fractured at −115°C in a Poleron freeze-fracture device and shadowed with platinum and carbon immediately after fracture. The replicas were washed with chloroform/methanol (2 : 1, by vol.) and examined in a Philips EM 301 electron microscope.

X-ray diffraction. Wide-angle X-ray diffraction patterns of dispersions of monogalactosyldiacylglycerols were obtained using a Philips PW 1024 Debije Scherrer powder camera. X-rays were produced from a Philips generator fitted with a fine-focus stationary anode tube. Freeze-dried lipids were dispersed in excess water saturated with N_2 and sealed in thin-wall (0.01 mm) glass capillaries (1 mm diameter). Exposure times were approximately 1 h.

Wide-angle X-ray diffraction measurements were also performed at the small angle scattering station of the Daresbury Synchrotron. Exposure times of 100 s to X-rays of wavelength 0.15 nm were recorded using a linear detector.

Differential scanning calorimetry. Freeze-dried preparations of monogalactosyldiacylglycerols were placed in aluminium pans and hydrated with a 4-fold excess by weight of water and sealed in the pan. Full hydration was ensured by maintain-

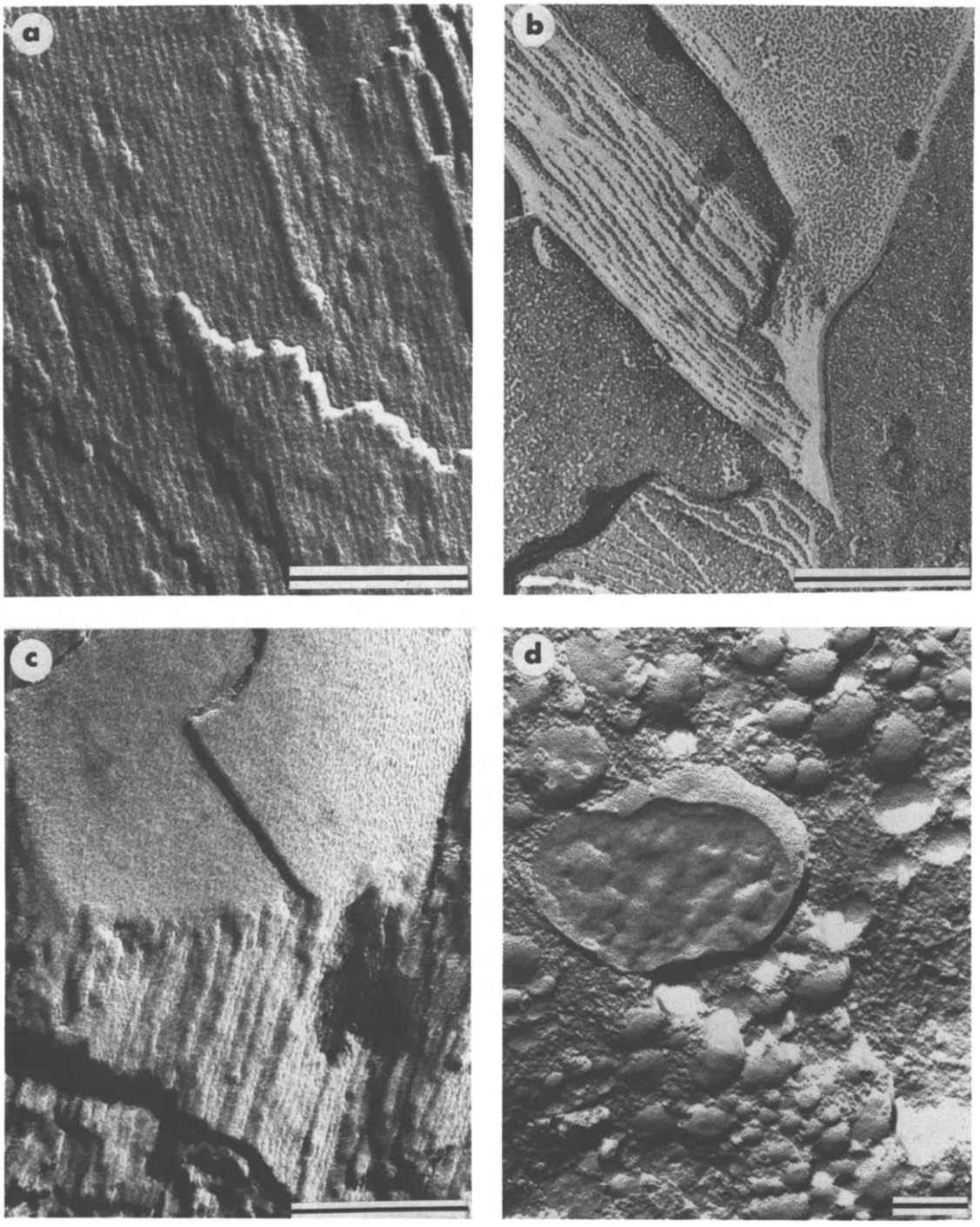

Fig. 1. Electron micrographs of freeze-fracture replicas prepared from aqueous dispersions of (a) native monogalactosyldiacylglycerol, (b) fully saturated monogalactosyldiacylglycerol, (c) an equimolar mixture of the native and saturated monogalactosyldiacylglycerols. All samples were prepared in excess water, equilibrated for 3 days and thermally quenched from 20°C. (d) Same preparation as (c) but heated to 80°C and thermally quenched from 50°C. Bars, 150 nm.

ing the hydrated specimens at 85°C for 15 min. Heating and cooling thermograms in the temperature range 0–85°C were recorded with a reference consisting of an empty pan or a pan containing the same amount of water as the sample at a scan rate of 5 Cdeg · min^{-1} at a sensitivity of 2 mcal · s^{-1}. Calorimetry was performed in a Perkin Elmer DSC-2 instrument. Enthalpy values were determined by estimation of peak areas relative to those obtained from recorder tracings of thermograms of indium. The lipid content of the pans was determined by quantitive gas chromatography of the fatty acid methyl esters using an internal standard of pentadecanoyl methyl ester.

Fluorescence probe studies. Monogalactosyldiacylglycerols were dried from chloroform solution under N_2 and dispersed in 3 ml water at 60°C by ultrasonic irradiation. The fluorescence probe, 1,6-diphenyl-1,3,5-hexatriene (DPH), was added to the dispersions in a solvent of tetrahydrofuran to give a molar ratio of 1 : 250, DPH/lipid. Fluorescence measurements were made using a Perkin Elmer MPF-44A fluorescence spectrometer. The value of fluorescence polarization, P, was calculated using the equation:

$$P = \frac{I_{\parallel\parallel} I_{\perp\perp} - I_{\parallel\perp} I_{\perp\parallel}}{I_{\parallel\parallel} I_{\perp\perp} + I_{\parallel\perp} I_{\perp\parallel}}$$

where I is the intensity of fluorescence emission and the subscripts refer to the orientations of the polarizer and analyser, respectively. A lipid dispersion without added diphenylhexatriene was used to correct for light scatter in the dispersions.

Results

Phase properties of monogalactosyldiacylglycerols

Aqueous dispersions of pure dilinolenoyl and polyunsaturated natural molecular species of monogalactosyldiacylglycerols are known to form typical hexagonal-II structures at least in the range of temperatures 0°–20°C [24,25]. The distearoyl and fully saturated native molecular species of these lipids by contrast form bilayer structures under these conditions [11,24]. The bilayers are not of the conventional multibilayer liposome type but exist in flat sheets which stack one upon the other. These structural forms are confirmed for the monogalactosyldiacylglycerol from spinach leaves and its fully saturated counterpart in Fig. 1. This shows freeze-fracture electron micrographs of the two lipid species dispersed in water and thermally quenched from 20°C. Fig. 1a shows typical hexagonal-II structures of the native lipid and Fig. 1b shows sheet-like lamellae of the saturated monogalactosyldiacylglycerol. When the two lipids are codispersed in water in equimolar proportions (Fig. 1c) and examined under the same conditions extensive phase separation of saturated and unsaturated lipids appears to have taken place. Smooth sheet-like structures presumably consisting of the saturated lipid are juxtaposed with regions of hexagonal-II structure formed by the polyunsaturated lipid. The presence of hexagonal-II structure is observed in such dispersions when thermally quenched from temperatures down to −15°C suggesting that the hexagonal-II to lamellar phase transition of the native lipid occurs at a lower temperature and this is consistent with other studies of polyunsaturated molecular species of monogalactosyldiacylglycerols [25]. Examination of replicas obtained from mixtures of native and saturated monogalactosyldiacylglycerols in proportions of 75 : 25 and 87.5 : 12.5, respectively, also showed evidence of phase separation but with the proportion of sheet-like bilayer reduced in extent and in a way predicted from the composition of the mixture. A replica obtained from the same preparation shown in Fig. 1c, i.e. an equimolar mixture of the two species of lipid but after heating to 80°C and quenching from 55°C is shown in Fig. 1d. Smooth vesicle-type structures can be seen indispersed with non-bilayer arrangments of lipid. No evidence for the existence of hexagonal-II structures is observed. Thermal quenching from temperatures greater than 55°C were unreliable with the device used in these studies so the formation of an hexagonal-II phase at higher temperatures could not be confirmed directly by freeze-fracture methods.

The structure of mixed dispersions was also examined by X-ray diffraction methods. The results obtained for low- and wide-angle scattering are presented in Fig. 2. A diffuse diffraction maxima centred at 0.46 nm was obtained from a dispersion of the native monogalactosyldiacylglycerol (Fig. 2a) indicating the hydrocarbon chains

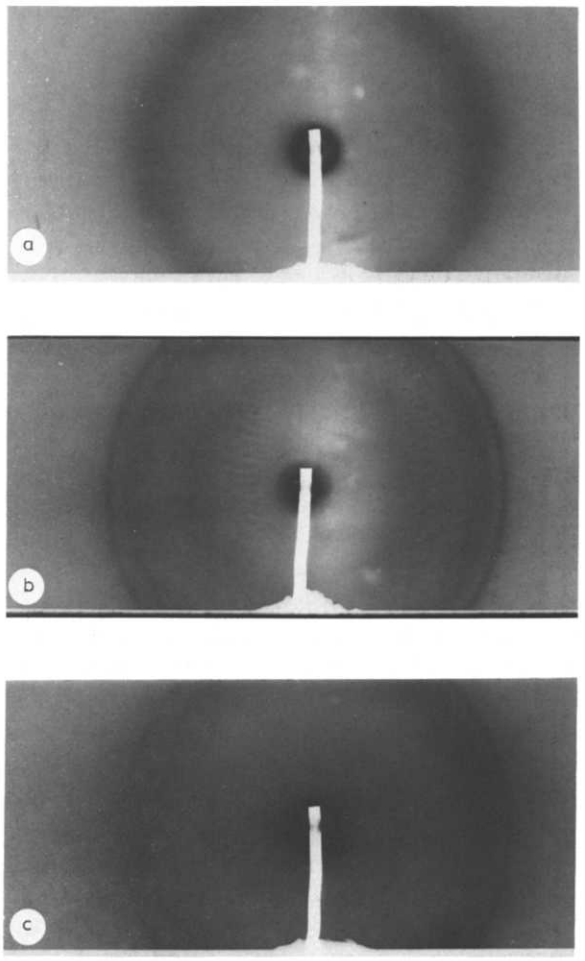

Fig. 2. X-ray diffraction patterns obtained from aqueous dispersions of samples of (a) native monogalactosyldiacylglycerol (b) fully saturated monogalactosyldiacylglycerol (c) an equimolar mixture of the native and saturated monogalactosyldiacylglycerols. All dispersions had been equilibrated for 2 weeks at 20°C and the diffraction patterns were obtained during 1 h exposure at the same temperature.

are in a disordered state consistent with a hexagonal-II configuration of the lipid. Sharp wide-angle diffraction bands were always observed with the saturated derivative indicative of a gel phase, however, the number and spacing of bands depended on the thermal history of the dispersion. The behaviour of the fully saturated lipid is consistent with that characterised in the distearoyl derivative of the lipid [11] which forms a metastable lamellar gel-phase with a single sharp reflection at about 0.42 nm which becomes transformed at 20°C into a stable crystalline lamellar phase characterised by three wide-angle diffraction bands centred at 0.40, 0.44 and 0.48 nm, respectively. Fig. 2b is a diffraction pattern obtained from a dispersion of the fully saturated derivative of the monogalactosyldiacylglycerol that had been equilibrated for 2 weeks at 20°C prior to examination. It is consistent with the existence of a stable gel phase. Measurements of the same specimen, taken immediately after cooling from 80°C (not shown) had only a single sharp wide-angle diffraction band centred at 0.42 nm indicating that a transition takes place between the two gel phases. The pattern obtained from an aqueous dispersion of an equimolar mixture of native and saturated lipid that had been equilibrated for 2 weeks at 20°C (Fig. 2c) shows two sharp reflections centred at about 0.40 and 0.44 nm, respectively, superimposed on a diffuse scattering lattice of about the same spacing. This indicates the coexistence of gel and liquid-crystalline phases in the lipid mixture. A similar pattern, albeit with weaker diffraction intensity from the sharp bands, was observed in a mixture of the two lipids in a proportion of 75 : 25 native/saturated lipid, respectively, that had been subjected to the same thermal history.

Low-angle X-ray data is summarised in Table II. The spacings are consistent with an hexagonal-II and lamellar arrangement of the native and fully saturated monogalactolipids, respectively. The low-angle region is less distinct in the mixed lipid dispersion but it appears that the lamellar orders dominate this region of the diffraction pattern. The diffraction bands tend to be broader and evidence for a hexagonal-II structure, which is clearly visible by freeze-fracture electron microscopy (Fig. 1c), is obscured.

Wide-angle diffractions of the dispersions were also examined using a synchrotron X-ray source and the results are presented in Fig. 3. An aqueous dispersion of the saturated lipid equilibrated for 3 days at 20°C gives a dispersion shown as curve (a). Diffraction maxima at 0.40 nm and 0.49 nm with a shoulder at 0.44 nm in this region again confirms that the gel phase is that obtained from the stable crystalline packing of the hydrocarbon chains. A dispersion containing an equimolar mixture of the saturated and unsaturated lipids also equilibrated

TABLE II

LOW-ANGLE X-RAY DIFFRACTION SPACINGS OF AQUEOUS DISPERSIONS OF NATIVE AND FULLY SATURATED MONOGALACTOSYLDIACYLGLYCEROL AND EQUIMOLAR MIXTURES OF THE TWO LIPIDS

Lipid	Diffraction spacing (nm)			
	1st	2nd	3rd	4th
Native	5.31	3.05	–	–
Saturated	5.89	2.95	2.02	1.47
Equimolar mixture	5.89	2.95	1.96	–

for 3 days at 20°C is given as curve (b) and again confirms that the phase-separated lipid forms the stable gel-phase of the type formed by equilibration of the fully saturated lipid dispersed alone. The origin of the differences in intensities observed in these samples and those shown in Fig. 2 are presently unknown. Heating both types of dispersion to temperatures above 60°C results in a loss of the sharp reflections and their replacement

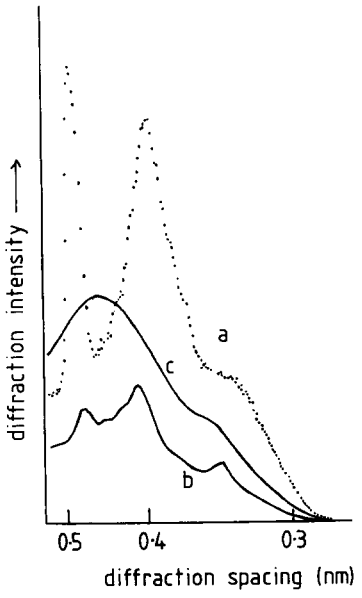

Fig. 3. Diffraction intensities recorded using a linear detector to record scattering of synchrotron X-rays (0.15 nm) from aqueous dispersions of (a) fully saturated monogalactosyldiacylglycerol, (b) an equimolar mixture of native and saturated monogalactosyldiacylglycerol, (c) same as sample (b) but recorded at a temperature of 63°C. Measurements in (a) and (b) were performed at 7°C and 25°C, respectively.

by a broad band centred about 0.46 nm consistent with a disordering of the hydrocarbon chains (curve c).

Dynamics of phase behaviour of mixed monogalactosyldiacylglycerols

The dynamic aspects of the phase mixing and separation in binary mixtures of unsaturated and saturated monogalactosyldiacylglycerols in aqueous systems was examined by differential scanning calorimetry. In the temperature range investigated in this study, 0–85°C, there was no evidence of any enthalpic phase changes in the polyunsaturated native lipid. Thermograms of a dispersion of saturated monogalactosyldiacylglycerol between 30° and 85°C are presented in Fig. 4. A sample that had been equilibrated at 20°C for three days when first heated (scan a) shows a rather broad endotherm with a transition temperature of about 57°C. Upon subsequent cooling an exotherm is observed at about 41°C (scan b). Immediate heating of this sample (scan c) produces an exotherm consistent with a phase transition of the metastable lamellar gel-phase of the lipid. This behaviour is analogous to the distearoyl and other saturated derivatives of monogalactosyldiacylglycerol [11,12] and again reflects the conversion of the equilibrated stable crystalline phase to the metastable gel on heating and cooling. Continual cycling of the sample shows no significant change in transition temperature or enthalpy of the transitions indicating that there is little or no hysteresis in the transition between the metastable lamellar gel phase and hexagonal-II phase in the dispersion of the fully saturated lipid.

Corresponding thermograms of a binary mixture of the native and saturated monogalactosyldiacylglycerols dispersed in excess water in an equimolar proportion are also shown in Fig. 4. In the first heating of a sample thermally equilibrated at 20°C for 3 days (scan d) there is again a broad endotherm but in this case it takes place at only about 40°C. The subsequent cooling scan (e) shows a very broad exotherm centred at about 26°C. If the sample is again heated (scan f). There is no shift in the transition temperature but there is some decrease in transition enthalpy.

A summary of the thermal data is presented in Table III. The temperature of the transition of the

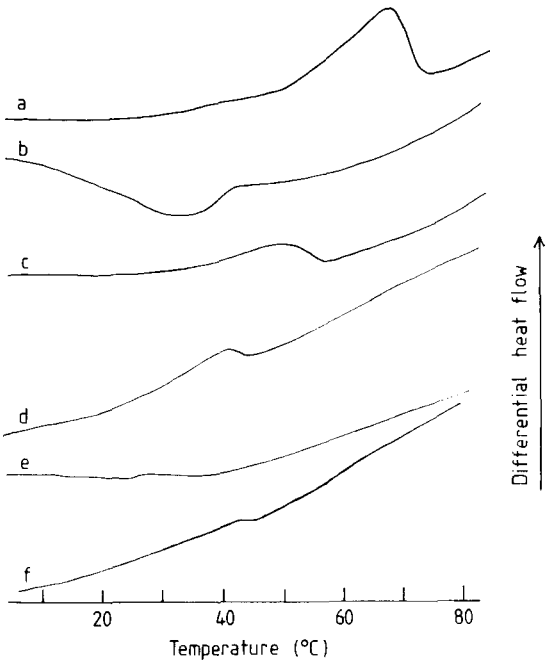

Fig. 4. Differential scanning calorimetric curves of aqueous dispersions of fully saturated monogalactosyldiacylglycerol (a) heating thermogram measured after equilibration of the sample at 20°C for 3 days; subsequent cooling (scan b) and heating (scan c) thermograms were obtained immediately after scans a and b were recorded. Similar initial heating, cooling and subsequent heating scans for an aqueous dispersion of an equimolar mixture of native and saturated monogalactosyldiacylglycerols are shown in scans d, e and f, respectively. All thermograms were recorded at a rate of temperature change of 5 Cdeg·min^{-1} and a sensitivity of 2 mcal·s^{-1}.

equilibrated gel-phase of the fully saturated monogalactosyldiacylglycerol and the molar enthalpy value are less than reported previously for the distearoyl derivative [11] but this is expected in view of the significant proportion of palmitoyl substituent esterified to the lipid. This also explains the lower values for the transition temperature and enthalpy of the metastable lamellar-gel to hexagonal-II liquid-crystalline transition. The different phase behaviour of the mixed lipid dispersion is evident from the characteristics of the phase transitions observed. The transition temperature upon heating the thermally equilibrated sample is some 17°C lower than in the fully saturated lipid. If only the saturated lipid contributes to the transition, as predicted from the absence of any transitions in the native lipid over this temperature range, the molar enthalpy value should be doubled

TABLE III

TRANSITION TEMPERATURES AND ENTHALPIES OF AQUEOUS DISPERSIONS OF FULLY SATURATED MONOGALACTOSYLDIACYLGLYCEROL AND EQUIMOLAR MIXTURES WITH NATIVE LIPID

Sample	Thermal history	T_c (°C)	ΔH (kJ·mol^{-1})
Saturated lipid	1st heating [a]	57	39.8
	1st cooling	41	14.4
	2nd heating	41	10.8
	2nd cooling	40	12.4
	3rd heating	40	10.3
	3rd cooling	39	12.6
Saturated/native (50/50)	1st heating [a]	40	34.5 [b]
	1st cooling	26	5.5
	2nd heating	40	7.2
	2nd cooling	25	2.5
	3rd heating	40	1.8
	3rd cooling	–	–

[a] Samples equilibrated 3 days at 20°C.

[b] Enthalpy values are X2 measured heats of transition as the unsaturated lipid component shows no phase change over the temperature range 0–80°C.

giving a value of 34.6 kJ · mol^{-1} of saturated lipid. It should be noted that all enthalpy values in the mixed lipid dispersion presented in Table III have been doubled on the assumption that only the saturated lipid undergoes a transition in the temperature range of the scan. The enthalpy value of the mixture is therefore not dissimilar from the enthalpy of the first transition observed in the fully saturated lipid and considerably more than the enthalpy of the metastable lamellar-gel to hexagonal-II transition. This suggests firstly that nearly all of the lipid in the mixture is segragated into phases consisting of lamellar gel and liquid-crystalline hexagonal-II and secondly that despite the lower transition temperature the structure of the lamellar gel phase is consistent with an equilibrated stable form characteristic of the fully saturated monogalactosyldiacylglycerol. The other feature evident from this data is that recycling the mixed dispersion through the transition causes a progressive loss of transition enthalpy suggesting that mixing of the two component lipids is taking place and that phase separations require some

time to form at temperatures below the phase transition temperature of the fully saturated lipid.

Polarization of the fluorescent probe diphenylhexatriene intercalated into aqueous dispersions of the polyunsaturated and saturated monogalactosyldiacylglycerols and binary mixtures of the two lipids as a function of temperature are presented in Fig. 5. As expected polarization values decrease in all dispersions with increasing temperature reflecting the increasing rotational motion of the probe. There is a marked decrease in the polarization value of diphenylhexatriene in the saturated lipid centred about 52°C indicating a significant change in the constraints on probe motion about this temperature. Lower polarization values are obtained from a mixed dispersion of the saturated and polyunsaturated lipid in equimolar proportions and the midpoint of the change in phase shifts to about 42°C. No obvious inflections in a mixed dispersion containing a mole ratio of 25 : 75, saturated/polyunsaturated lipid was observed (data not shown). A parallel curve at lower P values can be seen with the native lipid dispersion at lower temperatures, however, the values obtained for the native lipid became more variable at temperatures greater than 30°C; the origin of this variation is unknown.

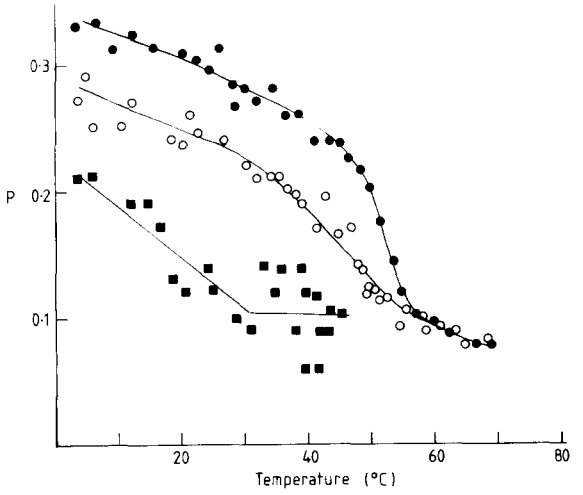

Fig. 5. Fluorescence polarization of diphenylhexatriene interpolated into aqueous dispersions of native monogalactosyldiacylglycerol (■), fully saturated monogalactosyldiacylglycerol (●) and an equimolar mixed dispersion of the native and saturated lipid (○) plotted as a function of sample temperature.

Discussion

The ability of membrane lipids to mix homogeneously in aqueous dispersions appears to depend not only on the gel to liquid-crystalline phase transition temperature within the bilayer phase of the particular lipids in the mixture but also the tendency of lipids to assume an hexagonal-II, rather than a lamellar structure. Clearly the difference in phase properties of the native and fully saturated monogalactosyldiacylglycerols examined in this work are suffiicent to drive an almost complete phase separation of the two constituents when dispersed in excess water at 20°C as judged by the molar enthalpy values. Indeed the behaviour of the saturated lipids in mixed dispersions is, in most respects, including transition between stable and metastable phases, closely related to that of the saturated monogalactosyldiacylglycerol dispersed alone in water.

One of the most interesting features of the phase behaviour of the mixture of monogalactolipids is the formation of a mixed liquid-crystalline lamellar-phase in association with intermediate phases consisting of what appear, from the freeze-fracture data, to be inverted micelles possibly sandwiched between the leaflets of the bilayer or packed into a quasi-cubic lattice. No evidence for hexagonal-II structure was observed. This is rather unexpected since the liquid-crystalline lamellar phase does not appear to be a stable structure formed by long-chain saturated monogalactosyldiacylglycerols in aqueous systems [11] nor the dipalmitoyl-derivative of this lipid [26]. Direct transitions from the gel phase to hexagonal-II have, however, been observed in certain derivatives of phosphatidylethanolamine and in binary mixtures of phosphatidylcholine and fatty acids [27]. The formation of a liquid-crystalline phase cannot be excluded as an intermediate structure between the gel-phase lamellar and hexagonal-II structure but the kinetics and mechanisms of such transitions have not yet been studied in any detail [28]. Furthermore, fewer studies have concentrated on the transition from hexagonal-II to lamellar phase in these systems and intermediate phases may differ considerably in their stability.

The formation of liquid-crystalline lamellar and other intermediate phases implies that on chain

melting the saturated monogalactosyldiacylglycerol diffuses into the hexagonal-II structure of the native lipid phase and breaks down the ordered hexagonal lattice. The sequence of events observed upon heating also indicates that there must be almost complete phase separation between the native and fully saturated lipids because the hexagonal-II phase is formed at lower temperatures.

The extent to which lipid phase separation is driven by differences in hydrocarbon chain composition of molecular species of lipid in biological membranes is problematic at the present time. Previous studies in our laboratory [12] have suggested that the gel phase lamellar structure is formed by chloroplast lipids which possess relatively unsaturated fatty acyl substituents. Clearly further studies are required to establish the phase behaviour of more closely related molecular species of the type that are present within the photosynthetic membrane.

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