Biochimica et Biophysica Acta 817 (1985) 289–298
Elsevier

BBA 72659

The phase behaviour of 1,2-diacyl-3-monogalactosyl-*sn*-glycerol derivatives

D.A. Mannock [a,*], A.P.R. Brain [b] and W.P. Williams [a]

[a] *Department of Biophysics and* [b] *Electron Microscopy Unit, Chelsea College, University of London, London SW3 6LX (U.K.)*

(Received November 13th, 1984)
(Revised manuscript received April 10th, 1985)

Key words: Monogalactosyldiacylglycerol; Phase transition; Differential scanning calorimetry; X-ray diffraction; Freeze-fracture; Electron microscopy

Monogalactosyldiacylglycerol was isolated from the blue-green alga *Anacystis nidulans*. Part of this lipid, which is rich in the 1-16:1/2-16:0 derivative, was hydrogenated to yield a lipid fraction rich in the 1-16:0/2-16:0 derivative. The phase behaviour of the two fractions were studied using differential scanning calorimetry, wide-angle X-ray diffraction and freeze-fracture electron microscopy. Both fractions exhibited complex polymorphic behaviour. Two distinct gel phases were identified; a stable form ($MGDG_I$) and a metastable form ($MGDG_{II}$). The transition temperatures for the two forms were 345 K and 325 K for the 1-16:0/2-16:0 fraction and 311 K and 279 K for the 1-16:1/2-16:0 fraction, respectively. The corresponding enthalpy values were 59.3 and 24.5 kJ · mol^{-1} and 51.4 and 11.5 kJ · mol^{-1}. Inverted hexagonal (H_{II}) phases were seen at higher temperatures. The transition to the H_{II} phase appears to occur directly from the $MGDG_I$ gel phase, but may involve the formation of a lamellar liquid -crystalline phase existing between the melting points of the two gel phases in the case of the transitions from the $MGDG_{II}$ gel phase.

Introduction

The phase behaviour of membrane lipids, particularly of phospholipids and sphingolipids, has been an area of considerable interest for many years [1,2]. In contrast, there have been relatively few investigations of the phase behaviour of glycolipids isolated from plants or microorganisms [3–5].

To date, the majority of studies of the phase behaviour of plant glycolipids have employed samples with a heterogeneous acyl chain composition. In the case of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), the two main chloroplast lipids, these samples consisted predominantly of polyunsaturated derivatives. Sen and co-workers [6,7] isolated the dilinolenoyl (di-18:3) derivatives of these lipids from *Vicia faba* and prepared the distearoyl (di-18:0) derivatives by catalytic hydrogenation. This initial work concentrated on the structural phases formed by these two MGDG derivatives and showed that the unsaturated derivative formed inverted hexagonal (H_{II}) structures when dispersed in water at room temperature, while the saturated derivative formed bilayer structures under similar conditions. More recent studies have shown that the di-18:3 derivative also forms bilayer structures at about −28°C [8] and that the di-18:0 derivative forms H_{II} phases at high temperatures (Gounaris, K., personal communication). The phase behaviour of the di-18:0 MGDG derivative has recently been the subject of an extensive investigation using calorimetry and X-ray diffraction techniques [9]. This study has demon-

* To whom reprint requests should be sent at (present address): Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

strated the existence of two distinct gel phases. The first, termed $MGDG_I$, appears only on storage at low temperatures and is characterized by higher transition temperature and enthalpy values, while the second, termed $MGDG_{II}$, is characterized by lower transition temperature and enthalpy values. Recent studies of phosphatidylethanolamine have revealed a similar pattern of phase behaviour [10,11].

In the present study, the physical properties of dispersions of both 1,2-dipalmitoyl (di-16:0) and 1-palmitoleoyl-2-palmitoyl (1-16:1/2-16:0) MGDG derivatives are investigated using differential scanning calorimetry (DSC), wide-angle X-ray diffraction and freeze-fracture electron microscopy.

Materials and Methods

Lipid preparation. Total polar lipids were extracted from *Anacystis nidulans* and separated into their individual lipid classes on a column of acid-washed Florisil [8]. The MGDG fraction was further purified by TLC (chloroform/methanol/water, 65:25:4, v/v) and hydrogenated with Adams' catalyst using the procedure of Gounaris et al. [8]. The hydrogenated lipid was then separated from the catalyst, and possible degradation products, by thin-layer chromatography. Saturation of the fatty acyl residues was verified by gas chromatography of the methyl ester derivatives [12]. Pentadecanoic acid was added to samples prior to methylation as an internal reference to permit quantitative estimations of the galactolipid concentrations.

Differential scanning calorimetry. Samples for calorimetry were dispersed in distilled water or aqueous ethylene glycol (1:1, v/v), as described by Sen and co-workers [9]. Thermal analysis was performed using a Perkin-Elmer DSC-2 calorimeter operated under conditions specified in the figure legends. Lipid contents of the pans were estimated by gas chromatography and the areas of the thermotropic transitions measured from recorder tracings with the aid of an Apple II microcomputer, using a graphics tablet. The values of transition enthalpies were estimated against an indium standard. The characteristic transition temperatures, T_c, were measured from the intercept of the estimated baseline and an extrapolation of the leading edge of the individual traces.

Wide-angle X-ray diffraction. Samples for wide-angle X-ray diffraction were prepared as described by Sen and co-workers [9]. Wide-angle X-ray diffraction patterns were obtained with a Philips PW1024 Debije-Scherrer powder camera fitted with a variable-temperature sample holder. X-rays were produced from a Philips generator fitted with a fine-focus stationary anode tube. Exposure times were typically 1 h.

Electron microscopy. Samples for freeze-fracture electron microscopy consisted of 6 mg lyophilized lipid dispersed in 0.5 ml distilled water. Aliquots of the lipid dispersion were equilibrated at the required quench temperature for 10 min prior to freezing in a slurry of nitrogen. The frozen samples were fractured at −115°C in a Polaron freeze-fracture unit and shadowed with platinum-carbon immediately after fracture. The replicas were washed with chloroform/methanol (2:1, v/v) and examined in a Philips EM301 electron microscope.

Results

Fatty acid analysis

The fatty acyl composition of native and catalytically hydrogenated samples of MGDG isolated from *Anacystic nidulans* is shown in Table I. The acyl chains of the native lipid were about 85% palmitoyl or palmitoleoyl residues, and about 15% stearoyl or oleyl residues. Murata et al. [13] have shown that most of the MGDG in this alga is present as the 1-16:1/2-16:0 derivative. As the monoenoic acids in this alga are almost exclusively in the C-1 position, the native lipid must also contain 25–30% of the 1-18:1/2-16:0, 1-18:1/2-18:0 derivative together with small amounts of the 1-16:1/2-18:0 derivative and the hydrogenated samples corresponding amounts of the di-18:0 and 1-16:0/2-18:0 derivatives. The phase behavior of the two samples will, however, be determined primarily by their respective 1-16:1/2-16:0 and di-16:0 constituents and for the sake of convenience these samples will be referred to by their bulk components.

Differential scanning calorimetry

The thermal properties of aqueous dispersions

TABLE I

THE FATTY ACID COMPOSITION OF MONOGALACTOSYLDIACYLGLYCEROL ISOLATED FROM *ANACYSTIS NIDULANS*, BEFORE AND AFTER HYDROGENATION WITH ADAMS' CATALYST

Hydrogenation time (h)	Fatty acid composition (mol%)				Double bond index [a]
	16:0	16:1	18:0	18:1	
0	44.3	41.3	2.2	12.2	1.07
2	83.7	–	15.7	–	0

[a] The average number of double bonds per molecule given as DBI = Σ(fatty acid %)×(number of double bonds in the fatty acid)×0.02.

of native and catalytically hydrogenated samples of MGDG isolated from *Anacystis nidulans* were examined by DSC.

The thermogram obtained on heating a sample of fully hydrated di-16:0 MGDG that had been pre-equilibrated at 290 K for three days following initial cooling from the liquid-crystalline state is shown in Fig. 1a. It consists of a single sharp endotherm at about 345 K. The subsequent cooling thermogram (Fig. 1b) shows a single narrow exotherm at about 328 K, which is considerably smaller in area than the endotherm in Fig. 1a. On immediate reheating of the sample, a small endotherm of similar area to the cooling exotherm is observed at about 325 K (Fig. 1c). The initial endotherm at 345 K is virtually absent with only minor traces of a possible peak at about 345 K remaining. Little or no further change was seen in subsequent thermal cycles. Re-equilibration at 290 K, however, led to the restoration of the pattern seen in Fig. 1a.

This pattern of phase behaviour is similar to that reported by Sen and co-workers [9] for the di-18:0 MGDG derivative. The endotherm at 345 K appears to represent a transition from the $MGDG_I$ gel phase which is formed on storage at 290 K to the liquid-crystalline phase ($MGDG_{LC}$), while the endotherm at 325 K marks the transition from the $MGDG_{II}$ gel phase to $MGDG_{LC}$. The similarities in enthalpy values and transition temperatures suggest that the 328 K exotherm observed on cooling the samples corresponds to the formation of $MGDG_{II}$ from $MGDG_{LC}$. A summary of the interconversions between these different forms of di-16:0 MGDG based on the scheme put forward by Sen and co-workers [9] for the di-18:0 derivative is presented in Fig. 2. These workers also observed a broad exotherm at 320 K in the initial heating thermograms of their di-18:0 MGDG derivative which was interpreted as re-

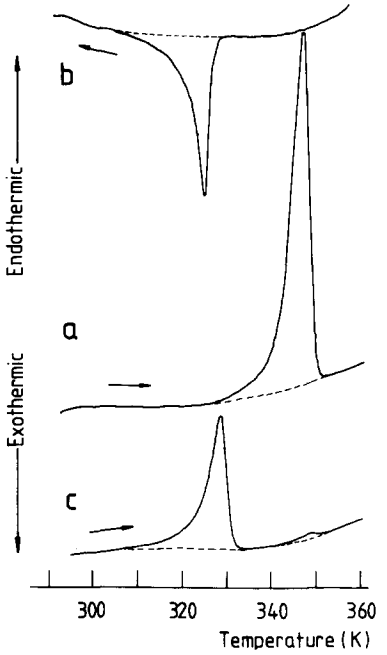

Fig. 1. DSC thermograms of di-16:0 MGDG dispersed in a 4-fold excess by weight of water. (a) heating curve after storage at 290 K for 3 days; (b) and (c) subsequent cooling and heating curves. All measurements were performed at 10 $K \cdot min^{-1}$, 1 $mcal \cdot s^{-1}$.

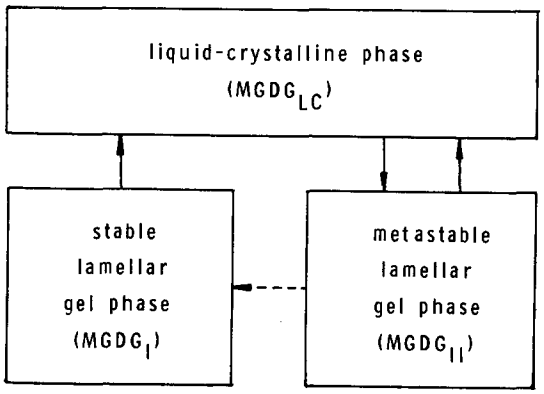

Fig. 2. Scheme showing relationship of different thermotropic phases of di-16:0 MGDG. The dashed arrows indicate non-cooperative changes in phase, the rates of which depend on temperature.

flecting the existence of a second low temperature form of $MGDG_I$. No comparable transitions were observed for the di-16:0 derivative, possibly because of the presence of appreciable amounts of the di-18:0 derivative as an impurity in the sample.

Sen and co-workers [9] showed the interconversion of di-18:0 MGDG between the $MGDG_I$ and $MGDG_{II}$ forms was dependent on water content. Thermograms of a sample of di-16:0 MGDG with a limited water content are presented in Fig. 3. The heating thermogram (Fig. 3a) clearly shows that reducing the water content of the sample leads to a shift in the equilibrium between the $MGDG_I$ and $MGDG_{II}$ gel phases in favour of the higher melting point $MGDG_I$ form, adding support to earlier suggestions that the $MGDG_I$ gel phase is less hydrated than the $MGDG_{II}$ gel phase [9].

In order to test whether the polymorphism seen in fully saturated MGDG derivatives extends to MGDG derivatives containing double bonds, the thermal properties of predominantly 1-16:1/2-16:0 MGDG isolated from *Anacystis nidulans* were also investigated.

Thermograms obtained for a sample of the 1-16:1/2-16:0 MGDG dispersed in aqueous ethylene glycol (1 : 1, v/v) are shown in Fig. 4. On initial heating after pre-equilibration at 277 K for 24 h (Fig. 4a), a large single endotherm at 310 K was obtained, closely resembling that observed for the di-16:0 derivative. On cooling, a small sharp exotherm was seen at about 311 K, with a larger,

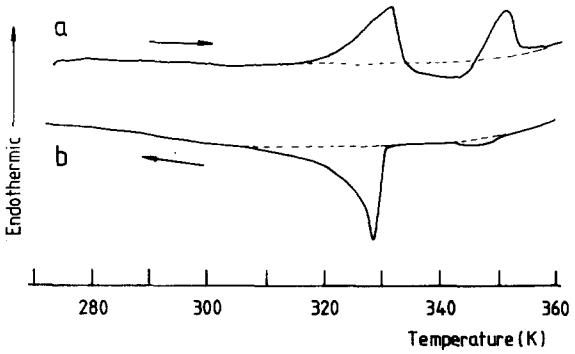

Fig. 3. DSC curves of a sample of di-16:0 MGDG, containing 4 moles of water per mole of lipid. (a) heating, and (b) cooling thermograms obtained after repeated thermal cycling. Measurements were performed at 10 $K \cdot min^{-1}$, 2 $mcal \cdot s^{-1}$.

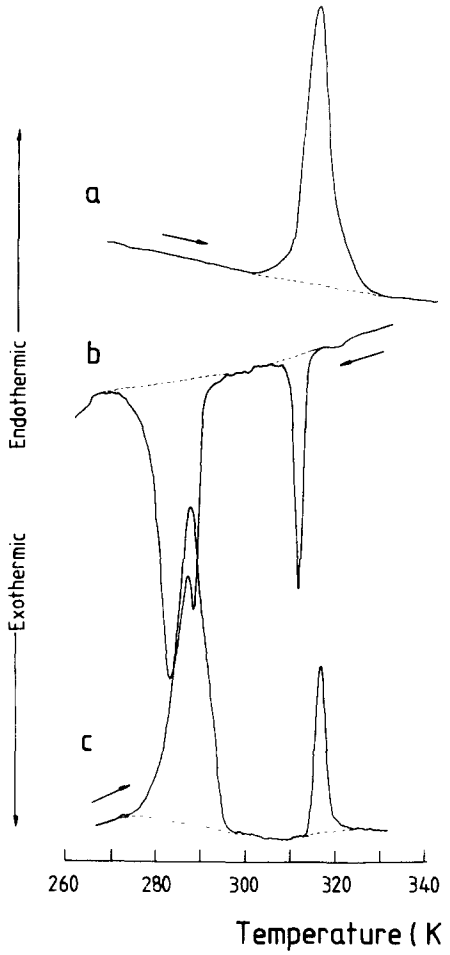

Fig. 4. Thermograms of a sample of 1-16:1/2-16:0 MGDG dispersed in aqueous ethylene glycol (1:1, v/v). (a) initial heating thermogram after storage at 277 K for 24 h, (b) and (c) subsequent cooling and heating thermograms. Measurements were performed at 10 $K \cdot min^{-1}$ and (a) 1 $mcal \cdot s^{-1}$, (b) and (c) 0.2 $mcal \cdot s^{-1}$.

broader exotherm at about 290 K (Fig. 4b). The subsequent heating thermogram shows corresponding endotherms at 279 K and 313 K (Fig. 4c). The endotherm at 279 K and its corresponding exotherm at 290 K both show considerable signs of broadening, possibly due to the presence in the sample of significant proportions of molecular species containing 18-carbon acyl residues. It is clear, however, that the same general pattern of phase behaviour exists in the native MGDG derivative as exists in the fully saturated lipids. The transition temperatures and enthalpy values for both di-16:0 and 1-16:1/2-16:0 MGDG are listed in Table II.

TABLE II

THERMAL PHASE TRANSITIONS OF DISPERSIONS OF DI-16:0 AND 1-16:1/2-16:0 MONOGALACTOSYL-GLYCEROLS

(a) Endotherm of sample equilibrated at 290 K; (b) second heating endotherm; (c) cooling exotherm; (d) endotherm of sample equilibrated at 277 K; (e) second heating endotherms; (f) cooling exotherms. Transition temperatures are means ± S.E. of 10 scans of at least two independent samples. Enthalpy values are based on at least two independent samples.

Lipid	Transition temperature (K)	Enthalpy ΔH (kJ·mol^{-1})
di-16:0 MGDG	(a) 344.8 ± 0.4	59.3 ± 2.4
	(b) 324.6 ± 0.3	24.5 ± 0.3
	(c) 328.1 ± 0.2	−26.4 ± 0.7
1-16:1/2-16:0 MGDG	(d) 310.6 ± 0.5	51.5 ± 2.7
	(e) 278.7 ± 0.5	11.5 ± 0.2 [a]
	312.8 ± 0.4	2.3 ± 0.1
	(f) 289.7 ± 0.4	−13.2 ± 0.4
	310.7 ± 0.3	−2.3 ± 0.1

[a] Value increases to 12.0 ± 0.2 kJ·mol^{-1} if the 313 K endotherm is attributed to $MGDG_I$.

Wide-angle X-ray diffraction

Further evidence supporting the idea that these lipids are characterized by two distinct gel phases is provided by wide-angle X-ray diffraction. Typical diffraction patterns obtained from samples of di-16:0 MGDG which had been stored at 277 K for 7 days and then measured over the temperature range 295–350 K are shown in Fig. 5. Diffraction patterns measured at 295, 323, 339 and 345 K all showed three sharp lines at 0.41, 0.44 and 0.50 nm of the type shown in Fig. 5a. This pattern of diffraction spacings is similar to that reported for the $MGDG_I$ gel phase of di-18:0 MGDG [9].

Heating the sample to 350 K led to the replacement of these lines by a single diffuse band centred at 0.47 nm (Fig. 5b). This band, which is characteristic for hydrocarbon chains undergoing rapid motion [14,15], reflects the presence of a liquid-crystalline state. Subsequent cooling of the sample of di-16:0 MGDG below 333 K resulted in the replacement of the 0.47 nm band by a single sharp line corresponding to an interplanar spacing of 0.42 nm (Fig. 5c), typical of lamellar gel phases in which the hydrocarbon chains are packed on a regular hexagonal lattice [14].

Wide-angle X-ray diffraction patterns of a sample of 1-16:1/2-16:0 MGDG dispersed in aqueous ethylene glycol (1 : 1, v/v) are presented in Fig. 6. The phase behaviour of this lipid shows a dependence on the thermal history of the sample similar to that seen for the fully saturated lipid. Samples stored at 277 K for 7 days and then measured at temperatures below 313 K, the temperature at which $MGDG_I$ melts to form the liquid-crystalline phase, are characterized by three sharp diffraction maxima corresponding to spacings of 0.41, 0.45 and 0.50 nm. Above 313 K these lines disappear, and are replaced by a diffuse band centred at 0.47 nm typical of a liquid-crystalline phase. Typical examples of such patterns are shown in Figs. 6a and 6b.

The diffraction pattern of a sample preheated to 323 K and subsequently cooled to a tempera-

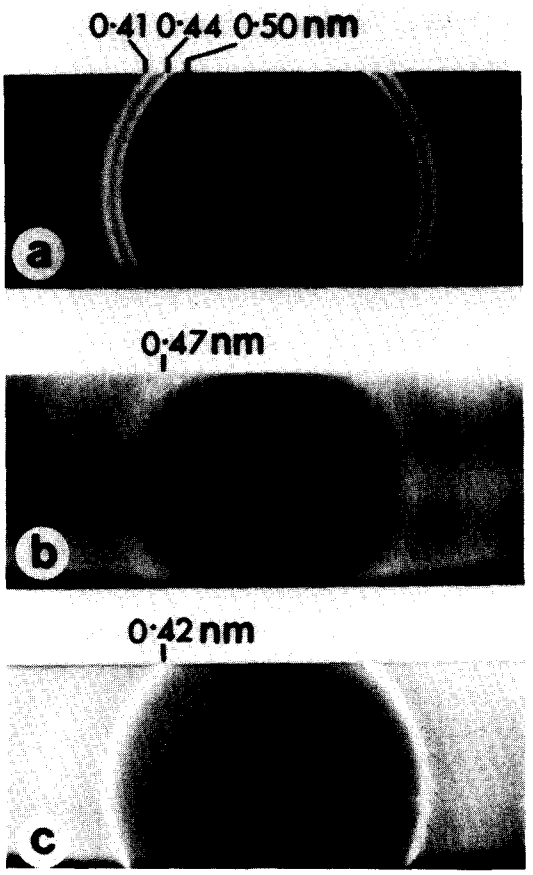

Fig. 5. Wide-angle X-ray diffraction patterns of di-16:0 MGDG dispersed in excess water at different temperatures: (a) 323 K, (b) 350 K, (c) heated to 353 K then cooled to 294 K.

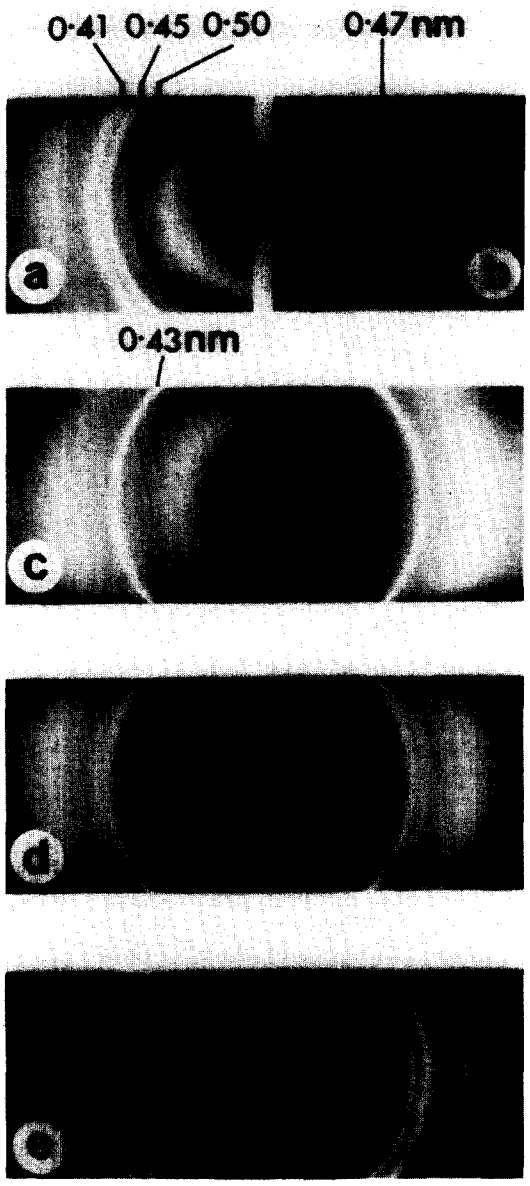

Fig. 6. Wide-angle X-ray diffraction patterns of samples of 1-16:1/2-16:0 MGDG dispersed in aqueous ethylene glycol (1 : 1, v/v). Samples (a) and (b) were pre-equilibrated at 277 K for 7 days and then measured at (a) 287 K, (b) 321.5 K, whereas samples (c)–(e) were first heated to 323 K, then cooled to 273 K and finally measured at (c) 278 K, (d) 282 K and (e) 288 K. Exposure times were 1 h 15 min.

ture below the onset of the melting endotherm of the $MGDG_{II}$ gel phase is shown in Fig. 6c. The single sharp line centred at 0.43 nm coincides with the diffraction spacings reported for the $MGDG_{II}$ gel phases of both the di-16:0 and di-18:0 MGDG derivatives (see Fig. 5 and Ref. 9). It is interesting to note, however, that if the preheated sample is first cooled to 273 K and the diffraction patterns are then measured at 282 or 288 K (i.e., at temperatures above the melting point of $MGDG_{II}$) the pattern changes from a single line, first to an intermediate pattern (Fig. 6d) and then to a pattern characteristic of the $MGDG_{I}$ phase (Fig. 6e). No diffraction pattern characteristic of a liquid-crystalline phase is observed. The time required for the reversion of $MGDG_{II}$ to $MGDG_{I}$ in this temperature range thus appears to be of the order of only a few minutes. This is again consistent with earlier reports based on calorimetric data indicating that slow heating of the di-18:0 derivative through the $MGDG_{II}$ transition range favours the rapid formation of the $MGDG_{I}$ phase [9]. Craievich and co-workers [16] have observed a similar pattern of behaviour in weakly hydrated crystals of dipalmitoylphosphatidylcholine, which they attribute to the melting of an L_{β} phase and the subsequent formation of a crystalline phase which melts at a higher temperature.

Freeze-fracture electron microscopy

Sen and co-workers [6] reported that di-18:0 MGDG tends to form planar lamellar sheets rather than the closed vesicles normally associated with phospholipid dispersions. However, the high gel to liquid-crystalline transition temperature of this lipid prevented detailed structural investigations of the different thermotropic phases. The lower transition temperatures of the di-16:0 and 1-16:1/2-16:0 MGDG derivatives were more conducive to this type of study.

Samples of di-16:0 MGDG and 1-16:1/2-16:0 MGDG were dispersed in distilled water and examined over the temperature range 275–350 K and 273–328 K, respectively. In both cases, samples quenched from below the $MGDG_{I}$ phase transition temperature showed only lamellar sheets (Fig. 7a, b) of the type seen for the di-18:0 derivative. At high magnifications these appear as flat sheets but at lower magnifications the lamellae of the 1-16:1/2-16:0, but not the di-16:0 derivative, show a slight curvature similar to that shown in Fig. 7c, possibly reflecting the increased flexibility of the partly unsaturated derivatives. In both cases

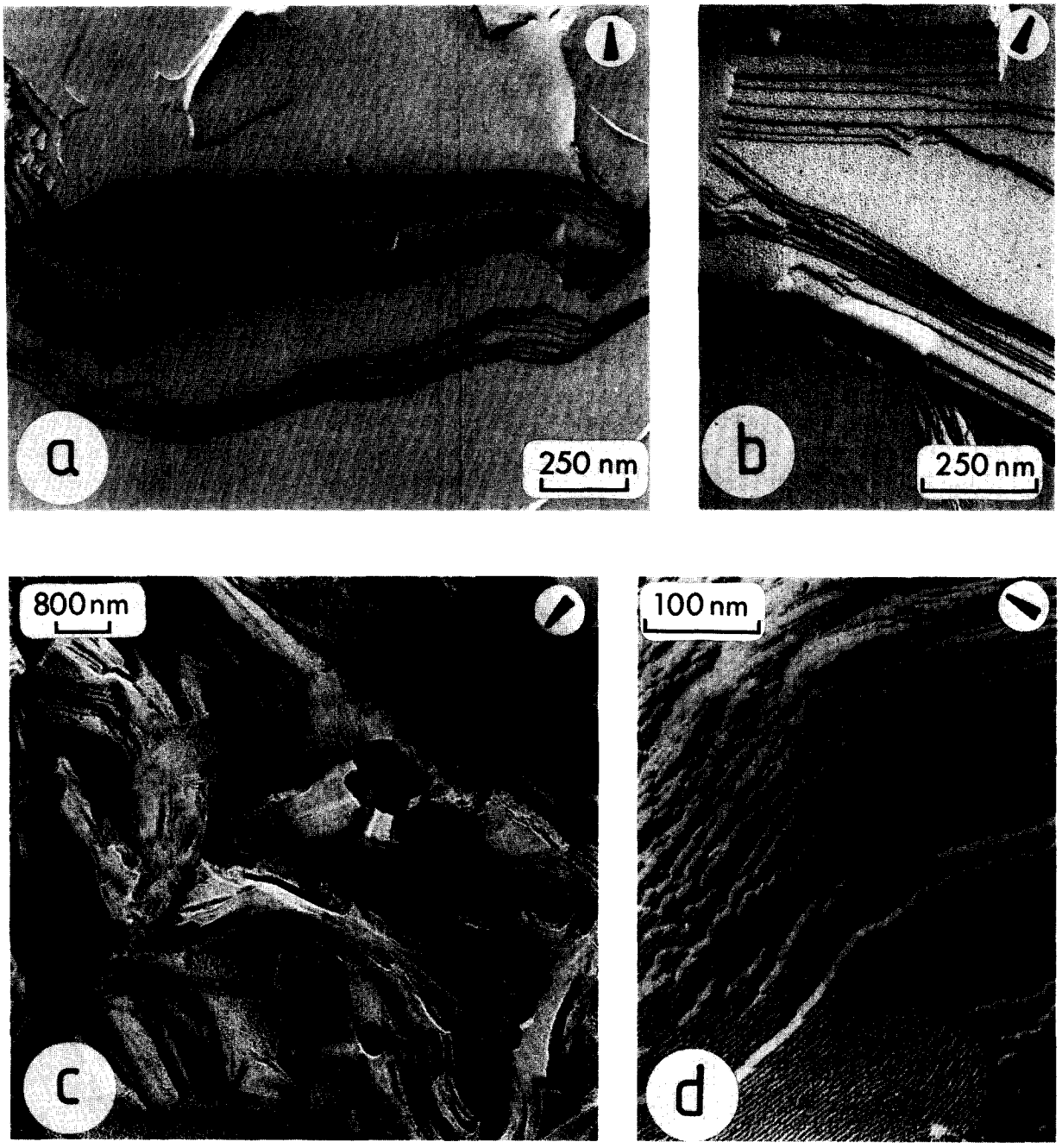

Fig. 7. Freeze-fracture electron micrographs of aqueous dispersions of (a) di-16:0 MGDG quenched from 343 K, (b) 1-16:1/2-16:0 MGDG quenched from 273 K, (c) 1-16:1/2-16:0 MGDG quenched from 303 K, (d) 1-16:1/2-16:0 MGDG quenched from 328 K. Samples (a) and (b) were pre-equilibrated at 277 K for 24 h to ensure that they were in the $MGDG_I$ phase.

there was no visible difference between samples which had been pre-equilibrated at 277 K for 24 h and those which had been preheated to 350 K, suggesting that the $MGDG_I$ and $MGDG_{II}$ gel phases are morphologically similar. Attempts at quenching samples of di-16:0 MGDG directly from

the liquid-crystalline state proved difficult because of excessive water loss.

Sample of 1-16:1/2-16:0 MGDG thermally quenched from above the $MGDG_I$ phase transition (Fig. 7d) formed a H_{II} phase of the type reported by Shipley et al. [4], Sen et al. [6], and Gounaris et al. [8] for polyunsaturated MGDG derivatives. This suggests that the endotherm seen at about 310 K in 1-16:1/2-16:0 MGDG may represent a transition from the $MGDG_I$ gel phase directly to a H_{II} phase. A pattern characteristic of a H_{II} phase was also observed in samples which had been first heated to 328 K, then cooled to 273 K and finally reheated and quenched from 328 K. Samples which were heated to 328 K and cooled to 303 K, a temperature between the $MGDG_{II}$ and $MGDG_I$ transitions, in contrast, showed lamellar sheets (Fig. 7c). It is not clear, however, whether this represents the formation of an intermediate lamellar liquid-crystalline phase at temperatures between the melting points of the $MGDG_{II}$ and $MGDG_I$ forms or simply a reversion of the liquid-crystalline phase to $MGDG_I$ on annealing at high temperatures prior to quenching the sample in liquid nitrogen.

Discussion

The pattern of gel phases existing below the gel to liquid-crystalline phase transition in aqueous dispersions of di-16:0 and 1-16:1/2-16:0 MGDG closely resembles that reported for the di-18:0 derivative [9]. Similar patterns of phase behaviour have also been observed in several phospholipids and glycolipids, most notably those reported for phosphatidylglycerol derivatives at low pH, or in the presence of divalent cations [17–21], for phosphatidylethanolamine [10,11,22–24] and for sphingomyelin [25] and cerebroside [26]. In the case of the MGDG derivatives, two distinct lamellar gel phases are present: a metastable gel phase in which the hydrocarbon chains are packed on a hexagonal lattice ($MGDG_{II}$) and a more stable gel phase with a more complex hydrocarbon chain packing ($MGDG_I$). The equilibrium between these two phases is determined by the thermal history (Figs. 1 and 4) and also by the water content of the sample (Fig. 3). Formation of the $MGDG_I$ gel phase appears to be favoured by long-term storage at a temperature below the $MGDG_{II}$ transition and also by low hydration.

On the basis of the evidence obtained in the present study, the endotherm seen at 345 K for samples of the di-16:0 MGDG (Fig. 1a) and at 311 K for the 1-16:1/2-16:0 MGDG (Fig. 4a) stored at low temperatures, appears to correspond to transitions from the $MGDG_I$ gel phase direct to a H_{II} phase. The interpretation of the significance of the smaller endotherm, and its corresponding exotherm, seen at about 313 K in the second heating curves of the 1-16:1/2-16:0 MGDG sample (Figs. 4b and 4c), is less certain. It is possible that they reflect the presence of a small proportion (5%) of $MGDG_I$ in the samples. The X-ray data presented in Fig. 6 indicates that equilibration of $MGDG_{II}$ samples at temperatures above about 285–290 K leads to their rapid reversion to the $MGDG_I$ form. Earlier studies on di-18:0 MGDG [9] have also shown that $MGDG_I$ is formed from $MGDG_{II}$ in the course of measurement if slow scan rates are employed in measuring DSC thermograms. Against this possibility, however, must be set the fact that the 313 K endotherm shows a corresponding exotherm on cooling. Whilst the formation of $MGDG_I$ from $MGDG_{II}$ might be possible under the conditions of measurement, the direct formation of $MGDG_I$ from $MGDG_{LC}$ on cooling would seem to be most unlikely.

Another possibility is that these transitions reflect the presence of a lamellar liquid-crystalline phase. The lower temperature exotherm and endotherm seen on cooling and susbequent reheating of the 1-16:1/2-16:0 MGDG samples (Figs. 4b and 4c) would, on this basis, correspond to transitions between this unstable lamellar liquid-crystalline form and the $MGDG_{II}$ form. This suggests that the small endotherm and exotherm at about 313 K may correspond to a transition from a lamellar liquid-crystalline phase to H_{II}. A similar interpretation may account for the small endotherms and exotherms see in samples of the di-16:0 MGDG (Figs. 1b and 1c) and di-18:0 MGDG derivatives [9]. Transitions from the gel phase to H_{II} commonly take place in two stages in this way, with an initial transition from a gel to liquid-crystalline lamellar state, followed by a low-enthalpy transition to a H_{II} phase at rather higher temperatures.

It should be noted, however, that no such transitions were observed for partially hydrogenated samples of MGDG isolated from higher plant chloroplasts [8], and that the enthalpy change associated with the 313 K endotherm seen in 1-16:1/2-16:0 MGDG is higher than that of corresponding transitions in phosphatidylethanolamines [27]. It is noteworthy, however, that there is considerable variation in the enthalpy change associated with the liquid-crystalline to Hex_{II} phase transition among phosphatidylethanolamine derivatives [28].

Direct transitions from the gel phase to H_{II} of the type seen with MGDG in this study are less common. They have, however, been reported to occur in certain phosphatidylethanolamine derivatives and in fatty acid-phosphatidylcholine mixtures [29].

Nagle [30] has suggested that the enthalpy value associated with a gel to liquid-crystalline phase transition can be divided into two components: ΔH_m, originating from the melting of the hydrocarbon chains, and ΔH_h, originating from the changes in headgroup interaction. Recent studies have shown that the difference in enthalpy values for the endotherms associated with the melting of the stable and metastable forms of a series of phosphatidylethanolamine derivatives is about 40 $kJ \cdot mol^{-1}$, independent of chain length [10,11,23]. Assuming the same values of ΔH_m for the two forms of a given lipid derivative, these results can be interpreted as a reflection of the increased headgroup interactions existing in the stable as opposed to the metastable forms. Similar calculations can be performed using the enthalpy values for the di-18:0, di-16:0 and 1-16:1/2-16:0 MGDG derivatives listed in Table II. The resulting values of ΔH_m and ΔH_h are set out in Table III. While the values of ΔH_m range from 11.5 $kJ \cdot mol^{-1}$ for the 1-16:1/2-16:0 MGDG to 30.5 $kJ \cdot mol^{-1}$ for the di-18:0 MGDG, the estimated values for ΔH_h are consistently close to 36 $kJ \cdot mol^{-1}$, lending considerable support to the earlier suggestions [9] that the $MGDG_I$ phase is stabilized by the existence of strong interactions, probably as a result of hydrogen bond formation between neighbouring headgroups.

TABLE III

A COMPARISON OF ENTHALPY VALUES OBTAINED FOR THE DIFFERENT GEL PHASE TRANSITIONS OF THREE MONOGALACTOSYLDIACYLGLYCEROL DERIVATIVES

MGDG derivative	Gel phase enthalpy value		Enthalpy difference ΔH_h ($kJ \cdot mol^{-1}$)
	$MGDG_I$ ΔH_{m+h} ($kJ \cdot mol^{-1}$)	$MGDG_{II}$ ΔH_m ($kJ \cdot mol^{-1}$)	
di-18:0 [b]	66.8	30.8	36.3
di-16:0	59.3	24.5	34.8
1-16:1/2-16:0	51.4	11.5 [a]	39.9

[a] See footnote to Table II.
[b] Taken from Sen et al. [9].

Acknowledgements

This work was supported by grants from the Science and Engineering Research Council and the Central Research Fund of London University. We also thank Dr. A. Sen and Dr. R.N. McElhaney for reading the manuscript and for their helpful comments.

References

1 Ladbrooke, B.D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304–367
2 Boggs, J.M. (1980) Can. J. Biochem. 58, 755–770
3 Rivas, E. and Luzzati, V. (1969) J. Mol. Biol. 41, 261–275
4 Shipley, G.G., Green, J.P. and Nichols, B.W. (1973) Biochim. Biophys. Acta 311, 531–544
5 Wieslander, A., Ulmius, J., Lindblom, G. and Fontell, K. (1978) Biochim. Biophys. Acta 512, 241–253
6 Sen, A., Williams, W.P. and Quinn, P.J. (1981) Biochim. Biophys. Acta 663, 380–389
7 Sen, A., Mannock, D.A., Williams, W.P. and Quinn, P.J. (1981) Biochem. Soc. Trans. 9, 134–135
8 Gounaris, K., Mannock, D.A., Sen, A., Brain, A.P.R., Williams, W.P. and Quinn, P.J. (1983) Biochim. Biophys. Acta 732, 229–242
9 Sen, A., Mannock, D.A., Collins, D.J., Quinn, P.J. and Williams, W.P. (1983) Proc. R. Soc. Lond. B218, 349–364
10 Chang, H. and Epand, R.M. (1983) Biochim. Biophys. Acta 728, 319–324
11 Mantsch, H.H., Hsi, S.C., Butler, K.W. and Cameron, D.G. (1983) Biochim. Biophys. Acta 728, 325–330
12 Restall, C.J., Williams, W.P., Percival, M.P., Quinn, P.J. and Chapman, D. (1979) Biochim. Biophys. Acta 555, 119–130
13 Murata, N., Ono, T. and Sato, N. (1979) in Low Temperature Stress in Crop Plants (Lyons, J.M., Graham, D. and

Raison, J.K., eds.), pp. 337–345, Academic Press, New York
14 Luzzati, V. (1968) in Biological Membranes (Chapman, D., ed.), pp. 71–123, Academic Press, New York
15 Charvolin, J. and Tardieu, A. (1978) Solid State Phys. Suppl. 14, 209–257
16 Craievich, A.F., Levelut, A.M., Lambert, M. and Albon, N. (1978) J. Phys. 39, 377–388
17 Harlos, K. and Eibl, H. (1980) Biochemistry 19, 595–599
18 Van Dijck, P.W.M., Ververgaert, P.H.J.T., Verkleij, A.J., Van Deenen, L.L.M. and De Gier, J. (1975) Biochim. Biophys. Acta 406, 465–478
19 Van Dijck, P.W.M., De Kruijff, B., Verkleij, A.J., Van Deenen, L.L.M. and De Gier, J. (1978) Biochim. Biophys. Acta 512, 84–96
20 Sacre, M.M., Hoffman, W., Turner, M., Tocanne, J.F. and Chapman, D. (1979) Chem. Phys. Lipids 25, 69–83
21 Findlay, E.J. and Barton, P.G. (1978) Biochemistry 17, 2400–2405
22 Harlos, K. (1978) Biochim. Biophys. Acta 511, 348–355
23 Wilkinson, D.A. and Nagle, J.F. (1981) Biochemistry 20, 187–192
24 Seddon, J.M., Harlos, K. and Marsh, D. (1983) J. Biol. Chem. 258, 3850–3854
25 Estep, T.N., Calhoun, W.I., Barenholz, Y., Biltonen, R.L., Shipley, G.G. and Thompson, T.E. (1980) Biochemistry 19, 20–24
26 Ruocco, M.J., Atkinson, D., Small, D.M., Skarjune, R.P., Oldfield, E. and Shipley, G.G. (1981) Biochemistry 20, 5957–5966
27 Cullis, P.R. and De Kruijff, B. (1978) Biochim. Biophys. Acta 513, 31–42
28 Cullis, P.R. and De Kruijff, B. (1979) Biochim. Biophys. Acta 559, 339–420
29 Marsh, D. and Seddon, J.M. (1982) Biochim. Biophys. Acta 690, 117–123
30 Nagle, J.F. (1976) J. Membrane Biol. 27, 233–250