Biochimica et Biophysica Acta 812 (1985) 816–826
Elsevier

BBA 72469

Phase equilibria of mixtures of plant galactolipids. The formation of a bicontinuous cubic phase

Ingvar Brentel [a], Eva Selstam [b] and Göran Lindblom [a]

[a] *Department of Physical Chemistry and* [b] *Department of Plant Physiology, University of Umeå, S-901 87 Umeå (Sweden)*

(Received July 9th, 1984)
(Revised manuscript received October 26th, 1984)

Key words: Phase equilibrium; Galactolipid; Chloroplast lipid; ^{2}H-NMR; Monogalactosyldiacylglycerol; Digalactosyldiacylglycerol; Cubic phase

The chloroplast galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) were isolated from wheat leaves. The phase equilibria of galactolipid-water systems with MGDG/DGDG molar ratios equal to 0:1, 1:2, 1.2:1, 2:1 and 1:0 were investigated, using nuclear magnetic resonance (NMR) methods. MGDG and DGDG form reversed hexagonal and lamellar phases, respectively, at temperatures between 10 and 40°C at all water contents studied (up to about 14 mol ^2H_2O per mol lipid). The galactolipid mixtures show a complex phase behaviour forming reversed hexagonal, lamellar and reversed cubic phases, depending on water content and temperature. It was found that the water hydration is similar for the lamellar and hexagonal phases formed by DGDG and MGDG, respectively. The non-lamellar phase areas increase with increasing content of MGDG. Small-angle X-ray measurements show that the cubic phase belongs to the *Ia3d* space group. From translational diffusion studies by NMR it is concluded that the structure of this cubic phase is bicontinuous.

Introduction

The chloroplast membrane of higher plants has a specific lipid composition of galactolipids, phospholipids and a sulfolipid [1]. The galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) amount to 80% of these lipids. Therefore, the chloroplast membrane is dominated by two non-ionic amphiphilic lipids in contrast to most other cell membranes where phospholipids are the dominating ones. Due to the different size of the mono- and digalactosyl groups, MGDG and DGDG differ in molecular shape, MGDG having a conical and DGDG a cylindrical shape. The variation in the molecular shape between MGDG and DGDG [2,4] leads to formation of different phases. Previously, Shipley et al. [3] showed in a brief study of the phase diagrams that MGDG forms a reversed hexagonal and that DGDG forms a lamellar phase with water.

In the chloroplast membrane the proportion of the wedge-like MGDG amounts to about 50% of the amphiphilic lipids [1]. This high content of a conical shaped lipid may be important for the incorporation and activity of membrane proteins [2,4]. Thus, the activity of Ca^{2+}-dependent ATPase [5], chlorophyllase [6] and light energy transfer from the light-harvesting complex to the reaction center [7] has so far been found to be especially dependent on MGDG. It may be noted however, that Triton X-100 or chlorophyll *a* was present in these experiments, making it difficult to draw consistent conclusions about the role played by MGDG. Triton X-100 and chlorophyll *a* have molecular shapes that might compensate for that of MGDG. Also, the lateral heterogeneity of Pho-

tosystem I and Photosystem II in exposed and appressed regions of the thylakoid is accompanied by a lateral heterogeniety of lipids [8,9]. In the exposed regions, chlorophyll-protein complexes of Photosystem I, ATPase and ferredoxin-$NADP^+$ reductase are located, while chlorophyll-protein complexes of Photosystem II and the light-harvesting complex are located in the appressed regions [10,11]. In the exposed and appressed regions the proportion of MGDG is approx. 35 and 45%, respectively [8,9]. Besides the lateral heterogeneity in membrane proteins, the protein/lipid ratio is higher in the appressed than in the exposed regions [8]. Thus, MGDG might be specifically associated to Photosystem II and the light-harvesting complex or unspecifically associated to the appressed region, due to the high protein to lipid content.

The phase equilibria of MGDG and DGDG have been briefly studied previously by low-angle X-ray diffraction. In the binary mixtures MGDG forms a reversed hexagonal and DGDG forms a lamellar phase at all water contents studied between -10 and $+80°C$ [3]. The structure of MGDG and DGDG dispersed in water can also be distinguished on electron micrographs of freeze-fracture replicas [12,13]. This technique was also used for studies of the structures formed by MGDG and DGDG mixtures [14,15] as well as mixtures of total chloroplast lipids [16] (see also review by Quinn and Williams [17]). Besides the reversed hexagonal and lamellar phases, a structure described as 'inverted micelles sandwiched within lipid bilayers' was also found in the mixtures. The identification of this structure was based on its similarities with structures formed by mixtures of phospholipids forming reversed hexagonal and lamellar phases [18–20]. However, this interpretation is still debated by several authors [21–24] and it is obvious that further studies are needed. Here we have used ^{2}H-NMR spectroscopy to determine the phase equilibria of MGDG and DGDG and mixtures thereof. In all lipid mixtures of MGDG and DGDG an isotropic cubic liquid crystalline phase was observed. The structure of this phase was further studied by small-angle X-ray diffraction and by NMR diffusion measurements.

Materials and Methods

Purification of lipids. Green seedlings of wheat (*Triticum aestivum L.*) were used as the source for galactolipids. Leaf lipids were extracted with chloroform/methanol [25] and fractionated by silicic acid (Silicar CC-7, Mallinkrodt) column chromatography [26,27]. The galactolipid fractions were separated on activated silica gel plates (0.5 Kieselgur 60 H, Merck) with the eluting solvent chloroform/methanol/acetic acid/water (170 : 30 : 20 : 7, by vol.) [28]. Monogalactosyldiacylglycerol and digalactosyldiacylglycerol were chromatographed a second time on activated silica gel plates eluted with chloroform/methanol/7 N ammonium (65 : 25 : 4, by vol.) [29]. The plates used for the second chromatography were washed with chloroform/methanol/water (65 : 25 : 4, by vol.) before activation. After purification of the galactolipids the lipid extracts were washed three times with distilled water. The acyl group composition of monogalactosyldiacylglycerol and diagalactosyldiacylglycerol was analysed after transmethylation with a Varian (model 3700) gas chromatograph on a 10% SP 2230 column. Both lipids have a high degree of unsaturation and no 16 : 3 (Table I).

Sample preparation. 100 mg of freshly purified lipids were vacuum dried for 2–4 days. After drying the sample, the vacuum was reduced by addition of N_2 gas to the vacuum chamber. The lipids were equilibrated with ^2H_2O at 6°C for a fortnight. The samples, which had a light yellow colour, were kept in N_2 atmosphere and in darkness. Despite this treatment trace amounts of the galactolipids were oxidized; this could not be completely avoided. However, no free fatty acids were detected in the samples and the TLC showed only one spot.

Determination of phase equilibria. The NMR techniques for determining the phase equilibria of lipid-^2H_2O mixtures have been described in many previously published works [30–39]. ^{2}H-NMR spectra were recorded at 13.70 MHz on a Jeol FX 90 Q Fourier transform NMR Spectrometer equipped with an external lithium lock. The temperature of the probe was controlled by a variable temperature control unit and it was measured with a thermometer. The samples were thermally equi-

TABLE I

ACYL GROUP COMPOSITION OF MONOGALACTOSYLDIACYLGLYCEROL AND DIGALACTOSYLDIACYLGLYCEROL

$n = 6$, t, trace.

Lipid	Acyl groups (mol%)					
	15:0	16:1	18:0	18:1	18:2	18:3
MGDG	1.0	t	t	t	3.6	3.695.0
DGDG	8.0	t	0.7	1.0	2.3	87.6

librated in a water bath for at least 1 h before the spectra were taken. Depending on the water contents of the samples, the number of transients to get an acceptable signal-to-noise ratio were between 500 and 10000. The temperature range studied was between 10 and 40°C.

Measurements of translational diffusion coefficients. The diffusion coefficient of the galactolipids in the cubic liquid crystalline phase was determined with the pulsed magnetic field gradient technique as described previously [40–42]. A Bruker 322 s pulsed NMR spectrometer equipped with a home-built digitalized pulsed magnetic field gradient unit was used. The 60 MHz proton spin echo was obtained by the 90°-τ-180° standard radio-frequency pulse sequence. The pulsed field gradients were applied at each side of the 180° pulse and the molecular diffusion coefficient is obtained from the equation:

$$\ln\frac{E}{E_0} = -(\gamma g\delta)^2\left(\Delta - \frac{\delta}{3}\right)D$$

where E/E_0 is the echo attenuation, γ is the gyromagnetic ratio, D the diffusion coefficient, g is the magnitude of the gradient pulses, Δ is the time spacing between these pulses and δ is the duration of the gradient pulses. In a typical experiment Δ was kept constant at 80 ms and δ was varied between 0 and 9 ms, while g was set to 2 $T \cdot m^{-1}$ and τ was 50 ms. The diffusion coefficient, D, was calculated from the slope of a plot of $\ln(E/E_0)$ against $\delta^2(\Delta - \delta/3)$. The magnetic field gradients were calibrated by measurements on glycerol with a known diffusion coefficient [43]. The probe temperature was controlled by a heated airflow around the sample tube and measured by using a copper/constantan thermocouple placed in a tube of the same type as the sample tubes. The samples were thermally equilibrated for at least 1 h before measurements were performed. 325 mg of a mixture of mono- and digalactosyldiacylglycerol forming a cubic phase at the molar raio 1.2 : 1 was investigated at 50°C.

Small-angle X-ray diffraction investigations. Since the structures for the hexagonal and the lamellar phases of MGDG and DGDG, respectively, have been determined in a previous paper by Shipley [3] et al., using X-ray diffraction we have concentrated our efforts here on the structure of the cubic phase, formed by a mixture of MGDG and DGDG. The X-ray diffraction measurements were performed with a standard technique developed by Luzzati and co-workers [44–45]. One sample of the cubic phase in the narrow phase area of the system, composed of a mixture of mono- and digalactosyldiacylglycerol at the molar ratio of 1.2 : 1, was investigated at 30°C. The water content was 3 mol ^2H_2O per mol total lipid.

Results and Discussion

The phase equilibria of the lipid/^2H_2O mixtures were investigated, using ^{2}H-NMR spectroscopy. The ^{2}H-NMR spectrum of heavy water is dominated by the interaction of the deuteron quadrupole moment with the electric field gradients at the nucleus. For an anisotropic liquid crystalline sample, like the lamellar and hexagonal phases, this quadrupole interaction generates a spectrum with two equally intense peaks. In an isotropic solution or a cubic liquid crystal, on the other hand, this interaction is averaged to zero as a result of rapid and isotropic molecular motions,

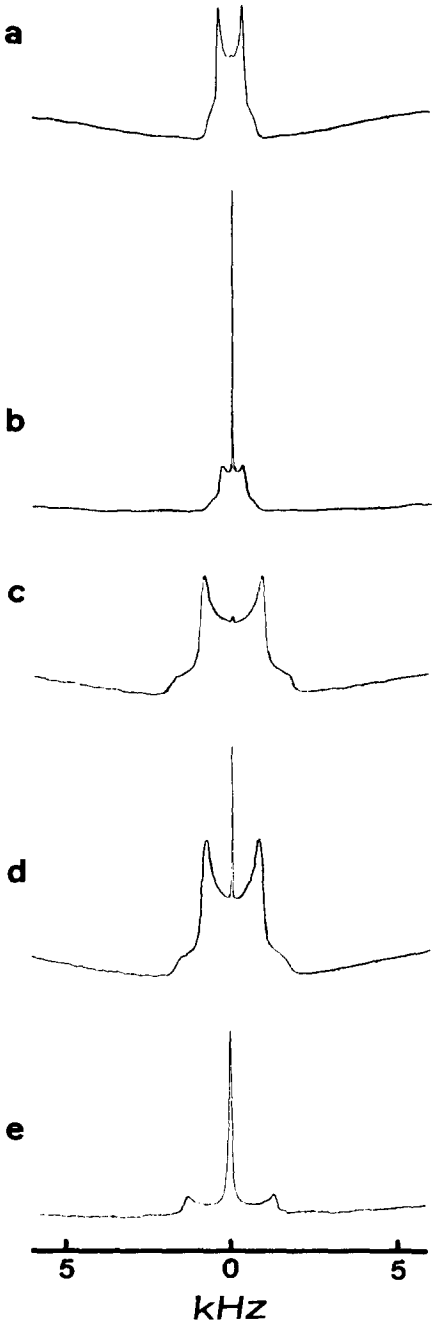

Fig. 1. ^{2}H-NMR spectra showing quadrupole splittings of different samples composed of heavy water and plant galactolipids at 10°C; a, reversed hexagonal phase of 10 mol ^2H_2O per mol monogalactosyldiacylglycerol; b, reversed hexagonal phase in equilibrium with free ^2H_2O formed by 13 mol ^2H_2O per mol monogalactosyldiacylglycerol; c, lamellar phase of 14 mol ^2H_2O per mol digalactosyldiacylglycerol; d, lamellar phase in equilibrium with free ^2H_2O formed by 15 mol ^2H_2O per mol digalactosyldiacylglycerol; e, lamellar and cubic phases in equilibrium formed by 5 mol ^2H_2O per mol mono- and digalactosyldiacylglycerol with molar ratio 1.2:1.

with the result that the spectrum consists of a rather sharp singlet. An observed quadrupole splitting, $\Delta(^2H)$, depends on the fraction of deuterons in one or more anisotropic sites, the quadrupole coupling constant, and the average molecular ordering of water molecules in the sites. A detailed theoretical treatment of ^{2}H quadrupole splittings in lyotropic liquid crystalline phases has been reported by several authors [47,48]. It has been shown previously that ^{2}H-NMR of deuterated water can conveniently be used to study the phase equilibria of lipid/water systems [30–39].

For a system with two or more phases, it is expected that the ^{2}H-NMR spectrum consists of a superposition of spectra characterizing the phases, provided that the deuteron exchange between the phases is slow. Thus, for a system containing a mixture of a hexagonal and a cubic liquid crystalline phase, a quadrupole splitting and a single peak will be observed.

For a three-phase system, consisting of two anisotropic liquid crystalline phases and one isotropic phase, the ^{2}H-NMR spectrum will contain two doublets and a central singlet, while for a two-phase system with two anisotropic phases two doublets will be observed.

The correctness of the phases observed was confirmed by studies using polarizing microscopy as described previously [36]. Furthermore, the study by Shipley et al. [3] was used as a basis for the determination of the various phase structures obtained.

^{2}H-NMR spectra were recorded for lipid samples with different water contents and at varying temperatures. The appearance of the spectra varied with the composition and temperature as illustrated in Fig. 1. The presence of an isotropic phase is directly seen from the appearance of a sharp singlet and that of an anisotropic phase from a doublet of broad peaks as described above. The identification of lamellar and hexagonal phases was achieved by the prediction of a larger quadrupole splitting for the lamellar phase than for the hexagonal phase. Theoretically, the splitting of a lamellar phase should be twice that of the

hexagonal phase [47]. It was possible, with a systematic variation of composition and temperature and using the simple principles outlined above, to establish, with relatively good precision, the phase equilibria of the galactolipid/water systems, except for the part with very low water content. This part is difficult to study because low water contents result in large splittings giving low peak amplitudes, and very long times are required to obtain phase equilibria.

Phase equilibria

In the study of liquid crystalline phases of lipid/water systems there is often a practical problem in separating the individual phases from each other, in particular if they have similar densities and/or are very viscous. Here, the ^{2}H-NMR method is very convenient for determination of phase diagrams by simply following the deuteron spectra of different samples. The technique is non-destructive and does not require a physical separation of the individual phases of the mixture. Usually it leads to a facile and rapid establishment of most features of the phase diagram.

The phase equilibria of the systems MGDG/^2H_2O and DGDG/^2H_2O were examined between 10 and 40°C. Since both these systems are binary ones, if we disregard the small heterogeneity in the acyl chains of the lipids, the phase equilibria can, according to Gibbs' phase rule, be represented by a standard temperature-composition diagram. It was found that, between the temperatures studied, MGDG and DGDG formed reversed hexagonal and lamellar phases, respectively, up to ^2H_2O contents at the maximal hydration of the liquid crystalline phases. For MGDG the maximum uptake of water in the reversed hexagonal phase was equal to about 12–13 mol ^2H_2O per mol lipid and, for DGDG in the lamellar phase, it was about 14–15 mol ^2H_2O per mol lipid. The association of water to the liquid crystalline phases was found to decrease slightly with increasing temperature. The results obtained are in good agreement with those published by Shipley et al. [3].

Recently, we performed an investigation of the phase equilibria of the two dominating lipids of the membrane of the bacterium *Acholeplasma laidlawii,* namely monoglucosyldiacylglycerol and diglucosyldiacylglycerol (Ref. 4 and Lindblom et al., unpublished results). In this study both the lipids had oleoyl acyl chains. As for the chloroplast lipid, DGDG, it was found that the diglucosyldiacylglycerol also formed only a lamellar phase over a large range of temperature and composition. The dioleoylmonoglucosyldiacylglycerol, on the other hand, formed three different phases, namely reversed hexagonal, cubic and lamellar phases, in contrast to chloroplast MGDG which formed a reversed hexagonal phase only. The underlying reasons for this difference in phase behaviour can be understood on the basis of the theory of self-assembly of lipids developed by several authors (see for example the review by Israelachvili et al. [2]. According to this theory, the molecular shape of the lipids play an important role in determining the phase structure, as was briefly discussed in the introduction. Thus, chloroplast MGDG, containing almost exclusively two linolenoyl (18 : 3*c*) acyl chains, has a more pronounced wedge-like shape than *Acholeplasma* dioleoylmonoglucosyldiacylglycerol, and therefore forms a reversed hexagonal phase only. For a lipid with a saturated acyl chain, on the other hand, as for distearoylmonogalactosyldiacylglycerol, the shape of the molecule will be more cylindrical-like and a lamellar phase is formed in excess water [12,13]. The structure of the sugar group (glucose

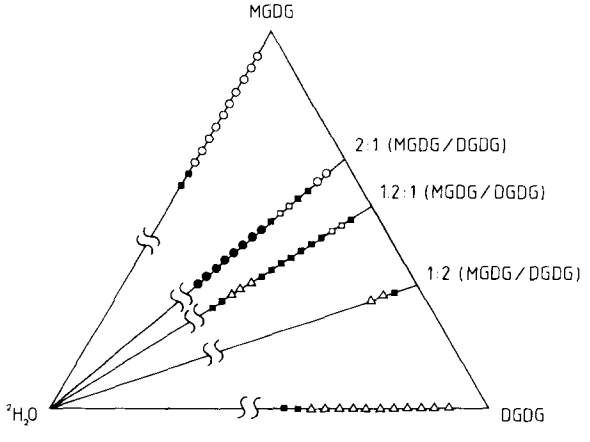

Fig. 2. Scheme of part of a ternary phase diagram for the system MGDG, DGDG and heavy water at about 40°C. △, lamellar phase; ○, hexagonal phase; □, cubic phase; ■, two phases; ●, three phases. Compositions are given in molar ratios and the lines have been intersected to magnify the area of interest of the phase diagram.

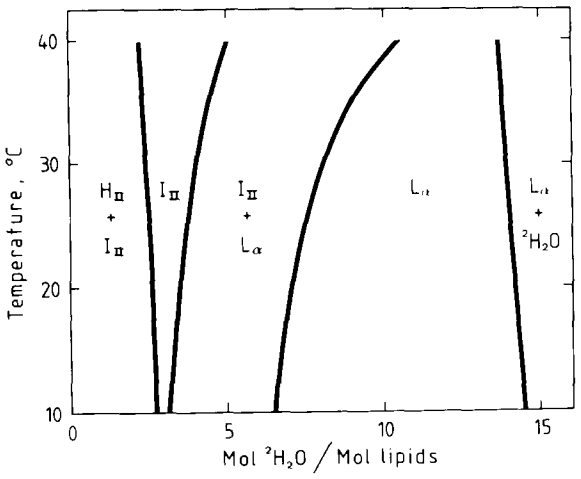

Fig. 3. Temperature-composition phase equilibria for the system composed of heavy water and MGDG/DGDG (1.2:1, mol/mol). H_{II}, reversed hexagonal liquid crystalline phase; I_{II}, reversed cubic liquid crystalline phase; L_α, lamellar liquid crystalline phase.

or galactose) is probably less important than the structure of the acyl chains in determining the phase structure.

Concerning the maximal uptake of water for the galacto- and glucolipids, it is interesting to note that both the MGDG and the corresponding DGDG lipids take up about the same amount of water irrespective of the phase structure formed. These experimental findings are unexpected when

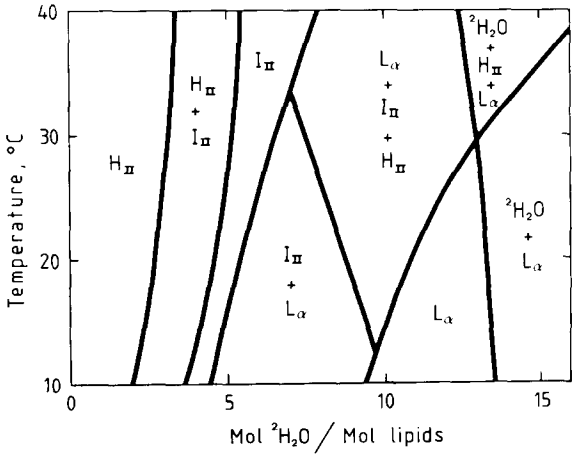

Fig. 4. Temperature-composition phase equilibria for the system composed of heavy water and MGDG/DGDG (2:1, mol/mol). For abbreviations, see Fig. 3.

compared to what has been observed in other similar systems containing so-called swelling amphiphiles like, for example, phosphatidylethanolamine [49]. A probable explanation for these observations may be that the hydration of the polar head group is less for the digluco- and digalactolipids, due to an unfavorable conformation of the sugar groups, than for the monogluco- and monogalactolipids. Such a difference may then in turn result in the so-called 'hydration force' [50] in the DGDG lamellar phase being less than in the MGDG hexagonal phase, so that the lamellar phase swells less than predicted in one theory [51]. To settle this point, however, further expeirmental support is needed.

Part of the phase diagram of mixtures of MGDG, DGDG and water has also been investigated. According to Gibbs' phase rule, such a three-component system (again neglecting the small heterogeneity in the lipid acyl chains) should be represented by a triangular composition diagram, one at each temperature. To perform such a determination is both costly and extremely time-consuming and therefore a simplification of the procedure has been utilized in this study. Thus, investigations of the phase equilibria have been undertaken for three molar ratios between MGDG and DGDG as a function of water content and temperature. Fig. 2 shows a schematic ternary phase diagram, where three lines of compositions studied at a constant temperature have been depicted. Such an illustration of the phase behaviour is, however, less useful in a study like the one performed here. A more surveyable representation of the phase equilibria can be obtained by considering the lipid mixture of MGDG and DGDG as one component and by displaying the phase equilibria as an ordinary temperature-composition diagram for a binary system. Figs. 3 and 4 show such diagrams for MGDG/DGDG molar ratios of 1.2 and 2.0, respectively. The molar ratio 0.5 was also investigated but gave only one lamellar phase up to 14–15 mol ^2H_2O, except at very low water contents (2 mol ^2H_2O/mol total lipid), where a two-phase region appeared with a lamellar phase in equilibrium with a cubic phase between 30 and 40°C. The pseudo binary phase equilibria of the mixtures of MGDG and DGDG exhibit increasing one-phase areas of non-lamellar phases (hexag-

onal and cubic ones) with increasing molar ratios between MGDG and DGDG (cf. Figs. 3 and 4). The molar ratio MGDG/DGDG equal to 1.2 (Fig. 3) yields a reversed hexagonal, reversed cubic and a lamellar phase with increasing water content. The diagram also shows that the cubic phase area broadens with increasing temperature. In analogy with this, the lipid mixture with molar ratio MGDG/DGDG equal to 2 also shows reversed hexagonal, cubic and lamellar phases with increasing water concentration. Similarly, also the cubic phase region widens slightly with increasing temperature. However, the overall phase diagram is more complicated in Fig. 4, since also three-phase areas appear. This is, of course, due to the fact that the system is not a true binary system, in which only three-phase lines are occurring according to Gibbs' phase rule. This should, however, not create any problem when studying the phase diagram of these lipid system, provided some care is taken. In the two three-phase areas in Fig. 4 it can be inferred that, between about 7 and 13 mol ^2H_2O per mol lipid, lamellar, hexagonal and cubic phases are in equilibrium with each other and that about 13.5 mol water per mol lipid and above about 28°C, lamellar, hexagonal and free water are in equilibrium. Thus, the diagrams in Figs. 3 and 4 clearly show that for mixtures of MGDG and DGDG a lamellar phase in equilibrium with free water forms at physiological temperatures.

Water dispersions of MGDG and DGDG with a molar ratio equal to 2 : 1 have been extensively studied by electron microscopy of freeze-fracture replicas [14,15,17,52]. When these dispersions were quenched at 20°C a number of arrangements of 'lipidic particles' as well as vesicles and 'tubular liposomes' were observed. The 'lipidic particles' were interpreted to represent 'inverted micelles sandwiched within bilayers' [17].

From the phase diagram in Fig. 4 it can be inferred that, for such a mixture, only a lamellar phase should be observed. This strongly implies that the samples used for the freeze-fracture studies [14,15,17,52] were not in equilibrium.

The cubic phase

An isotropic liquid crystalline phase is easily identified by ^{2}H-NMR spectroscopy, since it gives rise to a single peak in the spectrum as described above, and also by inspection of the sample between two crossed polarizers. From the reflections obtained by X-ray diffraction on a cubic phase sample containing 3 mol ^2H_2O per mol lipid and an MGDG/DGDG mixture with the molar ratio equal to 1.2, the space group was determined as belonging to *Ia3d*, i.e., the same group as has been observed for a number of different cubic liquid crystalline phases (Refs. 42, 44, and Lindblom et al., unpublished results, and Gulik-Krzywicki, T. and Rilfors, L., 'Phosphatidylethanolamine isolated from *Bacillus megaterium*, with 3 mol ^2H_2O per mol lipid forms a cubic phase also belonging to the space group *Ia3d*.' unpublished results). The length of the unit cell was determined to be 102 Å.

It has been shown in a number of previous studies that in order to establish the structure of cubic phases, translational diffusion coefficients determined by NMR are very helpful [24,40–42, 53]. The advantages of this method are that the diffusion coefficient can be measured directly, i.e., no probe molecule has to be incorporated into the system studied, and, no model-dependent assumptions have to be made. Moreover, the diffusion time can be varied, which is very important when studying cubic phases. Thus, the molecular displacements can be measured over distances that are much larger than the dimension of a single micelle. From a comparison of translational diffusion coefficients in cubic and lamellar liquid crystalline phases, it is possible to differentiate between two fundamentally different types of cubic phases [40,41]: (1) structures with continuous regions of both water and hydrocarbon chains [42,45], and (2) structures, either with discontinuous hydrocarbon regions but with continuous water regions ('oil-in-water' structure), or with discontinuous water regions but with continuous hydrocarbon regions ('water-in-oil' structure) [54,55]. In cubic phases of the first type, translational diffusion of lipid molecules can occur over macroscopical distances without polar groups passing through hydrocarbon regions, or without hydrocarbon chains passing through water regions. The measured lipid diffusion coefficient for such phases is therefore of the same order of magnitude as that obtained for the corresponding lamellar phase [42]. For cubic phases of the second type, composed of closed aggregates, the lipid molecules can move

freely within the aggregates, but it is very unlikely that they exchange between adjacent aggregates [40]. Although the local molecular diffusion probably is the same as in lamellar aggregates, the measured diffusion coefficient is much lower (between one and two orders of magnitude) for such

TABLE II

EXPERIMENTAL WATER DEUTERON QUADRUPOLE SPLITTINGS [$\Delta(^2H)$] FOR SELECTED SAMPLES AT DIFFERENT TEMPERATURES IN GALACTOLIPID-WATER SYSTEMS

H_{II}, reversed hexagonal phase, L_α, lamellar phase, I_{II}, reversed cubic phase.

Composition	(mol/mol)	Temperature (°C)	$\Delta(^2H)$ (kHz)	Phase designation
MGDG/^2H_2O	1:2	30	1.8	H_{II}
	1:4		1.4	
	1:5		1.1	
	1:6		1.1	
	1:7		0.87	
	1:8		0.77	
	1:9		0.74	
	1:10		0.72	
	1:11		0.65	
	1:12		0.64	
	1:13		0.61	
DGDG/^2H_2O	1:3	40	3.2	L_α
	1:4		3.0	
	1:5		2.6	
	1:6		2.5	
	1:7		2.2	
	1:7.5		2.1	
	1:8		2.0	
	1:8.5		2.0	
	1:9		1.9	
	1:10		1.9	
	1:11		1.7	
	1:12		1.7	
	1:13		1.6	
	1:14		1.6	
	1:15		1.5	
MGDG/^2H_2O	1:8	10	0.82	H_{II}
		20	0.81	
		30	0.77	
		40	0.77	
DGDG/^2H_2O	1:8	10	2.3	L_α
		20	2.3	
		30	2.2	
		40	2.0	
MGDG/DGDG/^2H_2O	1.2:1:9	10	2.4	L_α
		20	2.4	
		30	2.3	
		40	2.2	
MGDG/DGDG/^2H_2O	2:1:9	10	2.4	$L_\alpha + I_{II}$
		20	2.1; 0.94	$L_\alpha + H_{II} + I_{II}$
		30	2.0; 0.87	$L_\alpha + H_{II} + I_{II}$
		40	1.9; 0.85	$L_\alpha + H_{II} + I_{II}$

cubic phases than for the corresponding lamellar phase [40,41]. For a detailed discussion of the NMR diffusion method in studies of cubic phases, see Refs. 40 and 42. The translational diffusion coefficient of the lipids for a cubic phase at 50°C with the molar ratio MGDG/DGDG equal to 1.2 was determined to be equal to $1.1 \cdot 10^{-12}$ $m^2 \cdot s^{-1}$. This value is of the same order of magnitude as the diffusion coefficient of many different membrane lipids in the lamellar phase [56,57]. It can therefore be concluded that the galactolipids can diffuse over macroscopical distances in the cubic phase. The structure of the cubic phase is thus bicontinuous and consequently cannot be built up of closed inverted micelles (cf. Refs. 14 and 22).

It is interesting to note that the molar ratio MGDG/MGDG is somewhat higher in the prolamellar body than in the prothylakoid membranes [58,59]. Figs. 3 and 4 show that an increase in this ratio shifts the phase equilibria towards non-lamellar structures (cubic and hexagonal phases). This might be of importance for the formation of the characteristic structure of the prolamellar body [60,61]. This structure is very similar to the bicontinuous cubic structure previously proposed by us [42,62,63].

^2H quadrupole splittings and lipid hydration

Water quadrupole splittings contain information on the hydration of lipid aggregates in terms of the fraction, p, of water molecules appreciably oriented at the aggregate surfaces and their average degree of orientation, characterized by an order parameter, S. The quadrupole splitting may be written [64]:

$$\Delta(^2H) = p\nu_Q S = \left(nX_A / X_{^2H_2O}\right)\nu_Q S \qquad (1)$$

where ν_Q is the quadrupole coupling constant, n the average hydration number of the lipid, X_A the total mole fraction of lipid and $X^2_{H_2O}$ the mole fraction of water.

In order to determine a phase diagram a large number of samples have to be investigated at many temperatures. In this work about 500 ^2H-NMR spectra have been recorded and it is, of course, not possible to publish all the quadrupole splittings obtained. Here, only the general trends observed will be given and Table II summarizes some of the data obtained. Generally, it is found that the deuteron quadrupole splitting decreases with increasing water content and temperature. This is also an expected result, since usually pS (cf. Eqn. 1) decreases upon the aforementioned alterations [64]. As can be inferred from Table II, the values of $\Delta(^2H)$ at 40°C for the lamellar phase of the DGDG/water system and the hexagonal phase of the MGDG/water system decrease strongly with increasing water content. However, a plot of $\Delta(^2H)$ against molar ratio of lipid to water is not linear [64]. This shows that the splitting does not follow the simple 'two-site' model with constant hydration proposed for the water binding to amphiphile aggregates [64]. Furthermore, the splittings observed in the lamellar phase (DGDG) is more than twice those observed in the hexagonal phase (MGDG). An assumption of the same hydration in the two phases (and a lamella of no curvature) should give a splitting in the lamellar phase which is twice as large as the one in the hexagonal phase. Since, at high water contents the splitting may be as much as 3–4-times larger for DGDG than MGDG it is tempting to interprete this finding to be due to a larger hydration for DGDG in the lamellar phase. This should lead to a larger maximal hydration for the lamellar phase than for the hexagonal phase. However, this is not observed, instead, the hydration of the two phases is very similar, i.e., in spite of the fact that DGDG has two sugar groups, compared to one for MGDG in the polar head-group, the hydration is about the same. A possible interpretation of this finding might be that the molecular orientation of water (i.e., the order parameter) is different for the two lipids. This probably has consequences for the so called 'hydration force', as discussed above.

For the lipid mixtures, analogous results are obtained. However, for the plot of $\Delta(^2H)$ vs. molar ratio of total lipid to water at 10, 20 and 30°C and with [MGDG]/[DGDG] equal to 1.2, it was found that part of the plot is linear for $X_A/X_{^2H_2O} < 0.1$ and that the 'two-site' model can be used to describe the hydration in this part of the lamellar phase of this system (see Table II).

Acknowledgements

We are grateful to Dr. T. Gulik for performing

the X-ray measurements. This work was supported by the Swedish Natural Science Research Council.

References

1 Harwood, J.L. (1980) in The Biochemistry of Plants, Vol. 4 (Stumpf, P.K., ed.), pp. 2–55, Academic Press, New York
2 Israelachvili, J.N., Marčelja, S. and Horn, R.G. (1980) Q. Rev. Biophys. 13, 121–200
3 Shipley, G.G., Green, J.P. and Nichols, B.W. (1973) Biochim. Biophys. Acta 311, 531–544
4 Rilfors, L., Lindblom, G., Wieslander, Å. and Christiansson, A. (1984) in Membrane Fluidity, Biomembranes, Vol. 12 (Kates, M. and Manson, B., eds.), pp. 205–246, Plenum Press, New York
5 Navarro, J., Toivio-Kinnucan, M. and Racker, E. (1984) Biochemistry 23, 130–135
6 Terpasta, W. and Lambers, J.W.J. (1983) Biochim. Biophys. Acta 746, 23–31
7 Siefermann-Harms, D., Ross, J.W., Kaneshiro, K.H. and Yamamoto, H.Y. (1982) FEBS Lett. 149, 191–195
8 Murphy, D.J. and Woodrow, I.E. (1983) Biochim. Biophys. Acta 725, 104–112
9 Gounaris, K., Sundby, C., Andersson, B. and Barber, J. (1983) FEBS Lett. 156, 170–174
10 Andersson, B. and Anderson, J.M. (1980) Biochim. Biophys. Acta 593, 427–440
11 Anderson, J.M. (1981) FEBS Lett. 124, 1–10
12 Sen, A., Williams, W.P. and Quinn, P.J. (1981) Biochim. Biophys. Acta 663, 380–389
13 Sen, A., Mannock, D.A., Collins, D.J., Quinn, P.J. and Williams, W.P. (1983) Proc. R. Soc. B. 218, 349–364
14 Sen, A., Williams, W.P., Brain, A., Dickens, M.J. and Quinn, P. (1981) Nature 293, 488–490
15 Sen, A., Williams, W.P., Brain, A.P.R. and Quinn, P.J. (1982) Biochim. Biophys. Acta 685, 297–306
16 Gounaris, K., Sen, A., Brain, A.P.R., Quinn, P.J. and Williams, P. (1983) Biochim. Biophys. Acta 728, 129–139
17 Quinn, P.J. and Williams, W.P. (1983) Biochim. Biophys. Acta 737, 223–266
18 De Kruijff, B., Verkleij, A.J., Van Echteld, C.J.A., Gerritsen, W.J., Mombers, C., Noordam, P.C. and De Gier, J. (1979) Biochim. Biophys. Acta 555, 200–209
19 Verkleij, A.J., Mombers, C., Gerritsen, W.J., Leunissen-Bijvelt, J. and Cullis, P.R. (1979) Biochim. Biophys. Acta 555, 358–361
20 Verkleij, A.J., Van Echteld, C.J.A., Gerritsen, W.J., Cullis, P.R. and De Kruijff, B. (1980) Biochim. Biophys. Acta 600, 620–624
21 Hui, S.W. and Stewart, T.P., and reply by Verkleij, A.J. and De Kruijff, B. (1981) Nature 290, 427–428
22 Verkleij, A.J. (1984) Biochim. Biophys. Acta 779, 43–63
23 Hui, S.W. and Boni, L.T., and reply by Williams, W.P., Sen, A., Brain, A.P.R. and Quinn, P.J. (1982) Nature 296, 175–176
24 Rilfors, L., Eriksson, P.-O., Arvidson, G. and Lindblom, G. (1985) Biochemistry, in the press
25 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917
26 Rouser, G., Kritchevsky, G., Simon, G. and Nelson, G.J. (1967) Lipids 2, 37–40
27 Selldén, G. and Selstam, E. (1976) Physiol. Plant 37, 35–41
28 Nichols, B.W., Harris, R.V. and James, A.T. (1965) Biochem. Biophys. Res. Commun. 20, 256–267
29 Nichols, B.W. (1964) in New Biochemical Separations (James, A.T. and Morris, L.J., eds.), pp. 321–337, Van Nostrand, London
30 Ulmius, J., Wennerström, H., Lindblom, G. and Arvidson, G. (1977) Biochemistry 16, 5742–5745
31 Persson, N.-O., Fontell, K., Lindman, B. and Tiddy, G.J.T. (1975) J. Colloid. Interface Sci. 53, 461–466
32 Khan, A., Söderman, O. and Lindblom, G. (1980) J. Colloid Interface Sci. 78, 217–224
33 Khan, A., Fontell, K. and Lindblom, G. (1982) J. Phys. Chem. 86, 383–386
34 Khan, A., Rilfors, L., Wieslander, Å. and Lindblom, G. (1981) Eur. J. Biochem. 116, 215–220
35 Wieslander, Å., Ulmius, J., Lindblom, G. and Fontell, K. (1978) Biochim. Biophys. Acta 512, 241–253
36 Khan, A., Fontell, K., Lindblom, G. and Lindman, B. (1982) J. Phys. Chem. 86, 4266–4271
37 Wieslander, Å., Rilfors, L., Johansson, L.B.-Å. and Lindblom, G. (1981) Biochemistry 20, 730–735
38 Rilfors, L., Khan, A., Brentel, I., Wieslander, Å. and Lindblom, G. (1982) FEBS Lett. 149, 293–298
39 Gutman, H., Arvidson, G., Fontell, K. and Lindblom, G. (1984) in Surfactants in Solution, Vol. 1 (Mittal, K.L. and Lindman, B., eds.), pp. 143–152, Plenum Press, New York
40 Lindblom, G. and Wennerström, H. (1977) Biophys. Chem. 6, 167–171
41 Eriksson, P.-O., Khan, A. and Lindblom, G. (1982) J. Phys. Chem. 86, 387–393
42 Lindblom, G., Larsson, K., Johansson, L.B.-Å., Fontell, K. and Forsén, S. (1979) J. Am. Chem. Soc. 101, 5465–5470
43 Tomlinson, D.J. (1973) Mol. Phys. 25, 735–739
44 Luzzati, V. and Spegt, P.A. (1967) Nature 215, 701–704
45 Luzzati, V., Gulik-Krzywicki, T. and Tardieu, A. (1968) Nature 218, 1031–1034
46 Gulik-Krzywicki, T., Aggerbeck, L.P. and Larsson, K. (1984) in Surfactants in Solution (Mittal, K.L. and Lindman, B., eds.), Vol. 1, pp. 237–257, Plenum Press, New York
47 Wennerström, H., Lindblom, G. and Lindman, B. (1974) Chem. Ser. 6, 97–103
48 Davies, J.H. (1983) Biochim. Biophys. Acta 737, 117–171
49 Luzzati, v. and Husson, F. (1962) J. Cell. Biol. 12, 207–219
50 Rand, R.P. (1981) Annu. Rev. Biophys. Bioeng. 10, 277–314
51 Kirk, G.L., Gruner, S.M. and Stein, D.L. (1984) Biochemistry 23, 1093–1102
52 Sen, A., Brain, A.P.R., Quinn, P.J. and Williams, W.P. (1982) Biochim. Biophys. Acta 686, 215–224
53 Wieslander, Å., Rilfors, L., Johansson, L.B.-Å. and Lindblom, G. (1981) Biochemistry 20, 730–735
54 Tiddy, G.J.T. (1980) Phys. Rep. 57, 1–46
55 Fontell, K. (1981) Mol. Cryst. Liq. Cryst. 63, 59–82
56 Eriksson, P.-O., Johansson, L.B.-Å. and Lindblom, G. (1984)

in Surfactants in Solution (Mittal, K.L. and Lindman, B., eds.), Vol. 1, pp. 219–236, Plenum Press, New York
57 Lindblom, G., Johansson, L.B.-Å. and Arvidson, G. (1981) Biochemistry 20, 2204–2207
58 Ryberg, M., Sandelius, A.S. and Selstam, E. (1983) Physiol. Plant. 57, 555–560
59 Selstam, E. and Sandelius, A.S. (1984) Plant Physiol., in the press
60 Ikeda, T. (1968) Bot. Mag. Tokyo 81, 517–527
61 Gunning, B.E.S. and Jagoe, M.P. (1967) in Biochemistry of chloroplasts (Goodwin, T.W., ed.), pp. 655–676, Academic Press, London
62 Larsson, K. and Lindblom, G. (1982) J. Dispersion Sci. Technol. 3, 61–66
63 Selstam, E., Lindblom, G., Brentel, I. and Ryberg, M. (1982) in Biochemistry and Metabolism of Plant Lipids (Wintermans, J.F.G.M. and Kuiper, P.J.C., eds.), pp. 389–392, Elsevier Biomedical Press, Amsterdam
64 Wennerström, H., Persson, N.-O. and Lindman, B. (1975) ACS Symp. Ser. 9, 253–269