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WATER BINDING AND PHASE STRUCTURES FOR DIFFERENT *ACHOLEPLASMA LAIDLAWII* MEMBRANE LIPIDS STUDIED BY DEUTERON NUCLEAR MAGNETIC RESONANCE AND X-RAY DIFFRACTION

ÅKE WIESLANDER [a], JAN ULMIUS [b], GÖRAN LINDBLOM [b] and KRISTER FONTELL [b]

[a] *Department of Microbiology, University of Lund, Sölvegatan 21, S-223 62 Lund, and* [b] *Division of Physical Chemistry 2, Lund Institute of Technology, Chemical Centre, P.O. Box 740, S-220 07 Lund 7 (Sweden)*

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Summary

Water binding capability and phase structures for different lipid species extracted from *Acholeplasma laidlawii* A membranes have been studied using deuteron nuclear magnetic resonance and low-angle X-ray diffraction.

The dominating membrane lipids are monoglucosyldiglyceride and diglucosyldiglyceride and each of them takes up limited amounts of water (bound plus trapped), i.e., up to 13% (w/w), whereas the phospholipids and phosphoglycolipids have larger hydration capacities.

Addition of magnesium and calcium ions, but not sodium ions, to the diglucosyldiglyceride increases the hydration capability. This increase is accompanied by the formation of a metastable liquid crystalline phase and a hysteresis effect for the transition temperature.

Large differences in water deuteron quadrupole splitting were observed between mono- and diglucosyldiglyceride. Both ^2H nuclear magnetic resonance and low-angle X-ray diffraction studies on lipids containing biosynthetically incorporated ω-d_3-palmitic acid clearly indicate the existence of a reverse hexagonal phase structure for the monoglucosyldiglyceride and lamellar structures for the diglucosyldiglyceride and the other membrane lipids.

The low hydration capability of the large diglucosyldiglyceride polar head is discussed in terms of polar head configuration.

Both mono- and diglucosyldiglyceride have several physical properties similar to those of phosphatidylethanolamine.

Introduction

For a better understanding of the function of biological membranes there is a need for studies of the properties of their different components. We have

focused our attention on the role played by the lipid component. For these studies the membranes of *Acholeplasma laidwii* are very suitable, since defined changes in the molecular structure of the lipid components can be conveniently obtained without seriously affecting the living cell [1,2]. Studies of these membranes [2–4] indicate that the physical properties of the individual lipids (glycolipids, phospholipids and phosphoglycolipids) are important for different membrane functions. Important problems in this area concern the contribution of the uncharged glycolipids to the properties of the membrane surface. Furthermore, variations in membrane surface properties and lipid dynamics caused by biosynthetic changes in the relative proportions of individual lipids [2–4] are probably of great importance. The lateral packing properties of the lipids, i.e., lipid methylene subcell areas, can be modified by loosely-bound molecules at the membrane bilayer surface [5,6].

When analyzing these problems a physical method that introduces negligible perturbations to the system should be preferred [1,7]. Nuclear magnetic resonance has proved to be a useful technique for studying both structural [8–11] and dynamic [12,13] properties of membranes and of amphiphilic liquid crystals composed of lipid and water. Here we report deuteron NMR studies of lipid/heavy water samples and of lipid samples containing biosynthetically incorporated deuterated fatty acids for several different polar lipid species extracted from the membranes of *A. laidlawii* A.

Materials and Methods

Organism and growth conditions. A. laidlawii A, strain EF22 [2], was grown in a lipid-depleted bovine serum albumin/tryptose medium (for composition and extraction procedure, see ref. 14) adjusted to pH 8.5. The medium was supplemented with either 0.075 mM oleic acid (Sigma Chemical Co., 99% pure) plus 0.075 mM ω-d_3-palmitic acid (Larodan Lipids, Malmö, Sweden) or 0.150 mM elaidic acid (Sigma Chemical Co., 99% pure) added as sterile ethanol solutions. Growth was followed by absorbance measurements at 540 nm. The cells were harvested by centrifugation after 24 h growth at 37°C. Membranes were prepared by osmotic lysis, washed and freeze-dried [14].

Purification of lipids. Lipids were removed from the dried membranes by four chloroform/methanol (2 : 1, v/v) extractions with agitation: three for 1 h at 23°C and one for 1 h at 45°C. The combined centrifuged extracts were freed from non-lipid contaminants by passage over a Sephadex G-25 Fine (Pharmacia) column [15]. Typically, a 20 l batch of cells yielded approximately 1050 mg of total lipids after this purification step. The different lipid classes were separated by silicic acid column chromatography [2]. Both this chromatography step and the purification step above were performed with slight positive N_2 pressure on the columns. Individual lipid species were isolated by preparative thin-layer chromatography on silica gel H (Merck) in chloroform/methanol/water (65 : 25 : 4, v/v), solvent A [2], on plates previously washed by empty runs in the same solvent system. Lipid zones were visualized by spraying the plates with deionized water. Methods for identification of the lipids have been described previously [2]. Single lipid zones combined with silica gel were scraped into a glass column and the lipids eluted at 4°C by

chloroform/methanol (2 : 1, v/v) followed by methanol. The extracts were concentrated with a rotary evaporator at less than 40°C. The lipids were stored in concentrated solutions in chloroform/methanol (2 : 1, v/v) at −70°C and were stable for more than a year.

Sample preparation. For NMR studies 75—100 mg of a lipid, or a lipid mixture applied as a film in a NMR tube, was thoroughly dried (≤0.2 mmHg overnight) in a vacuum evaporator. By weight analysis of the tube a specified amount of either (a) ^2H_2O (99.8% isotopic enrichment, Norsk Hydro or Ciba/Geigy) or a salt solution of ^2H_2O, or (b) deionized H_2O was added to the tube followed by a smooth flush with N_2 before flame sealing. The components were thoroughly mixed in the tube by repeated centrifugation ($3700 \times g_{ave}$) at extended periods (1—2 days) above the appropriate lipid phase transition temperature. The tubes were kept at room temperature for several days in order to assure complete equilibration before being analyzed. The water content of a sample was assumed to be that of the initial mixture. For the X-ray diffraction study specimens (lipid plus known amounts of water) were taken either from the NMR tubes or from samples especially prepared and equilibrated in ampoules, and placed in thin-walled glass capillary tubes (glass thickness 0.01 mm, diameter 0.7 mm) which were subsequently flame sealed.

Physical and analytical methods. Deuteron NMR investigations were done with a modified Varian XL-100-15 NMR spectrometer in the Fourier-transform mode as described previously [8]. Quadrupole splittings, $\Delta = |\frac{3}{4} \nu_Q \cdot S|$ [9], were recorded for both water deuterons and specifically-labelled deuterons in the lipid acyl chains. Here, ν_Q is the quadrupole coupling constant and S the order parameter.

X-ray samples were studied with a "long spacing" pinhole camera [16]. The distance between the specimen and film was 200 mm, and the camera was evacuated to reduce backgroud. The X-ray radiation used was nickel-filtered copper K (λ = 0.1542 nm) and the exposure time was 20 h. Temperature was kept constant throughout the measurements (40 ± 1°C) by a thermostated circulating water system. Exposures in the wide-angle region were made using a standard Debye-Scherrer camera with a diameter of 114.6 mm.

Lipids were analyzed for composition and constancy both before and after NMR and X-ray analysis. This was done by thin-layer chromatography of the polar lipids on silica gel H in solvent A [2], and in petroleum ether/diethylether/acetic acid (80 : 20 : 1, v/v) [2]. These solvent systems will detect any polar and apolar contaminants or break-down products. Fatty acid composition of individual lipids was determined by gas-liquid chromatography as described earlier [2].

Results

Lipid and fatty acid composition. Polar membrane lipids in *A. laidlawii* A are made up of monoglucosyldiglyceride, diglucosyldiglyceride, apolar monoglucolipid, phosphatidylglycerol, glycerophosphoryldiglucosyldiglyceride and glycerophosphorylmonoglucosyldiglyceride. The relative amounts of these lipids species vary as a consequence of induced variations in membrane viscosity properties [2—4]. Fatty acid composition and amounts of these lipids used

TABLE I

RELATIVE AMOUNT AND FATTY ACID COMPOSITION OF DIFFERENT MEMBRANE LIPID SPECIES IN *A. LAIDLAWII* A

Fatty acid composition of individual lipids was determined by gas-liquid chromatography after transmethylation both before and after NMR and X-ray analysis. Lipid amounts were estimated by weight analysis of large batches (see Materials and Methods).

Added acid	Relative amounts of lipids (mol/100 mol)					
	Monoglucosyl-diglyceride	Diglucosyl-diglyceride	Phosphatidyl-glycerol	Glycerophosphoryl-monoglucosyl-diglyceride	Glycerophosphoryl-diglucosyl-diglyceride	Glucolipid X
A. Elaidic ($18:1_t$)	18	48	22	2	10	— *
B. (Palmitic$_{\omega\text{-}d_3}$ (16 : 0) + Oleic ($18:1_c$)	34	31	19	3	8	5
Acid found (mol/100 mol)						
A. Elaidic	92	95	96	93	90	— *
Lauric (12 : 0) / Myristic (14 : 0) / Palmitic	8	5	4	7	10	—
B. Palmitic$_{\omega\text{-}d_3}$	52	43	37	53	54	74
Oleic	46	56	61	44	45	23
Lauric / Myristic	2	1	2	3	1	3

* This lipid is not synthesized with the elaidic acid supplement (see ref. 2).

here are shown in Table I. Among the fatty acids that can be incorporated into *A. laidlawii* membrane lipids [17], elaidic acid was chosen for this study primarily because physical investigations of membranes and lipids from *Escherichia coli* fatty acid auxotrophs yielded comparable results with different techniques and laboratories with this acid [7]. In order to increase growth rate and cell yields [1] to the same level as with elaidic acid [2] oleic acid was added when ω-d_3-palmitic acid was used as the fatty acid supplement.

X-ray diffraction findings. As will be shown in the next section both the water-deuteron and ω-d_3-palmitic acid, NMR quadrupole splittings differ considerably for the mono- and diglucosyldiglyceride systems. This might indicate differences in phase structure between the aqueous systems of these structurally similar lipids. The inner phase structures may be determined with low-angle X-ray diffraction [16,18,19]. X-ray diffractograms for non-aqueous samples show for the mono- and diglucosyldiglyceride (containing elaidic acid) fundamental repeats of 5.7 and 3.5 nm at 40°C, respectively. In the wide-angle region there was some diffuse "semi-crystalline" spacings in the 0.4 nm region. Between 10 and 13% (w/w) water content both lipids form a narrow zone of a homogenous phase with liquid crystalline properties. This was evident from the appearance in the polarizing microscope and of the X-ray diffraction patterns in the low-angle region. However, the appearance of the X-ray diffractograms differed as the spacings for the aqueous monoglucosyldiglyceride system followed the sequence to be expected for a two-dimensional hexagonal liquid crystalline structure (middle phase, ratio 1: $1/\sqrt{3}$: $1/\sqrt{4}$:) while the spacings for the diglucosyldiglyceride system followed the sequence of a lamellar liquid crystalline structure (neat phase, ratio 1 : 1/2 : 1/3). This held for both the elaidic acid and the palmitic plus oleic acid supplements. The spacings were 6.54 and 5.69 nm for the monoglycosyldiglyceride and diglucosyldiglyceride systems at 40°C, respectively. Maximum hydration plus excess water yielded the same spacings (Fig. 1). The only feature in the wide-angle region was a diffuse reflection at about 0.45 nm indicating a liquid state of the hydrocarbon chains of the lipids. In addition, all the other polar lipids plus an "in vivo" mixture (containing 20%, w/w, water) showed lamellar phase structures with lamellar repeat distances ranging from 4.6 to 6.3 nm.

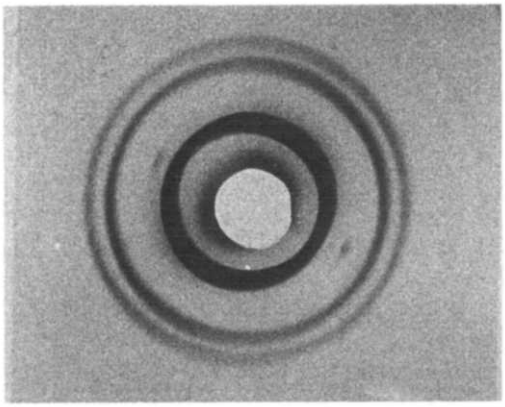
Due to the narrowness of the homogenous phase region of the glycolipids it was not possible to detect any variance in the obtained spacings with different amounts of water present. No variance in the spacings was obtained by lowering the temperature to 20°C.

Addition of sodium ions had negligible influence on the diffraction patterns.

^2H_2O nuclear magnetic resonance. Water deuteron NMR spectra for lipid-^2H_2O samples (anisotropic liquid crystals) show quadrupole splittings which can give information about the degree of water orientation at the amphiphilic surfaces. Orientation of the water molecules, and hence the water splitting, are often different in different phases. Transitions like the gel to liquid crystalline phase transitions occurring in many lipids, can thus be easily detected [8,20]. The maximum hydration, i.e., trapped plus bound water, in the liquid crystalline phases can be calculated by measuring the areas of the isotropic and quadrupole split peaks in the ^2H NMR spectra (Fig. 2 and ref. 8).

The main polar membrane lipids of *A. laidlawii*, containing elaicid or ω-d_3-

A



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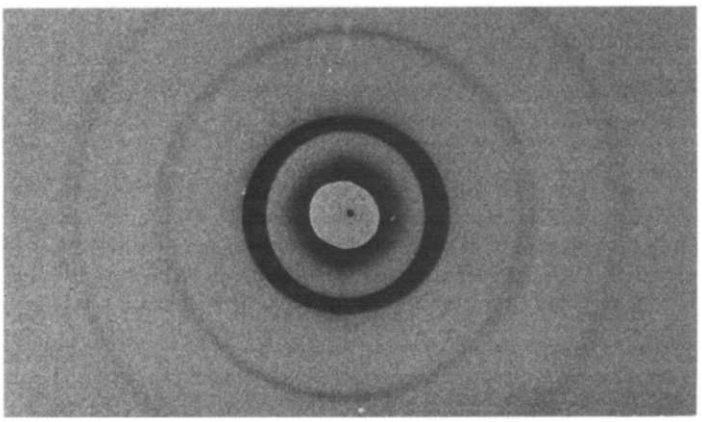

Fig. 1. Low-angle X-ray diffraction of lipid-water dispersions from (A) monoglucosyldiglyceride and (B) diglucosyldiglyceride (both containing elaidic acid, see Table I) at 40°C. Water content of samples 20% (w/w). See Materials and Methods for technical measurement data.

Equivalent Bragg spacings of low-angle diffraction

Monoglucosyldiglyceride			Diglucosyldiglyceride		
Band number	Band spacing (nm)	Ratio	Band number	Band spacing (nm)	Ratio
1	6.54	1/1	1	5.69	1/1
2	3.74	$1/\sqrt{3}$	2	2.82	1/2
3	3.27	$1/\sqrt{4}$	3	1.84	1/3
Structure indicated	Hexagonal			Lamellar	

palmitic plus oleic acid, have been studied either at a constant (heavy) water concentration of 20% (w/w) or as a function of temperature and (heavy) water concentration. The effect on the quadrupole splitting of different biologically important ions (Na^+, Ca^{2+}, Mg^{2+}) added as chloride salt solutions in ^2H_2O has also been investigated. In systems like these with limited amounts of water,

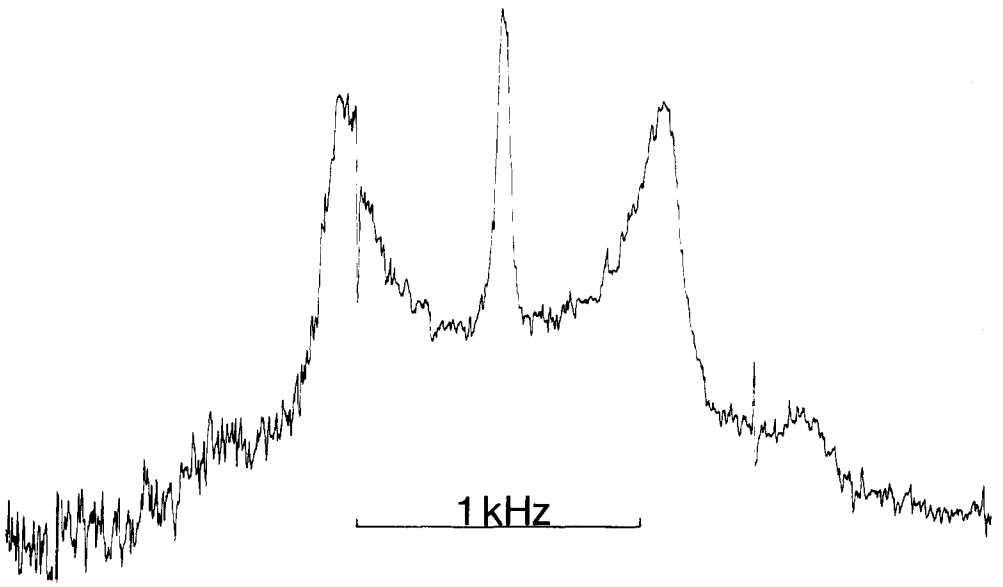

Fig. 2. 15.4 MHz deuteron NMR spectrum of heavy water in an *A. laidlawii* diglycosyldiglyceride-^2H_2O sample at 28°C and 13% (w/w) heavy water content. The central narrow peak is due to free water.

larger splittings imply a higher degree of water orientation in the lipid polar head area. The results obtained are summarized in Table II. Mono- and diglucosyldiglyceride showed similar maximum hydration properties both with elaidic and palmitic plus oleic acid. Furthermore, hydration abilities rose slightly with increased temperature. The charged lipids, phosphatidylglycerol and glycerophosphoryldiglucosyldiglyceride, appeared to have larger hydration capacities than the glycolipids. Sodium produced no significant increase in hydration for mono- and diglucosyldiglyceride, whereas divalent cations, calcium and magnesium, increased the amount of water in diglucosyldiglyceride. Transition temperatures for mono- and diglucosyldiglyceride containing the same fatty acids were very similar. Quadrupole splittings for water in the polar head area were constant above the transitions. There were large differences between mono- and diglucosyldiglyceride in splittings, which were also dependent on the fatty acid composition.

In addition to increased hydration, both calcium and magnesium had a significant influence on the temperature dependence of the water-deuteron quadrupole splitting for diglucosyldiglyceride. These ions resulted in the large splittings normally occurring above the transition being maintained upon cooling. Splitting returned to pretransition levels approximately 15—20°C below the "normal" transition (Fig. 3). This hysteresis effect was quantitatively similar for both calcium and magnesium at a molar ratio diglucosyldiglyceride/ion of approx. 4.35.

ω-d_3-palmitic acid ^2H NMR. In order ot obtain a more fully detailed picture of individual lipid physical behaviour, the medium was supplemented with ω-d_3-palmitic acid. This fatty acid, which can probe hydrocarbon chain properties of the lipids causing almost no perturbation, was biosynthetically incorporated into difference membrane lipids without being modified (Table I

TABLE II

QUADRUPOLE SPLITTINGS AND WATER BINDING FOR DIFFERENT MEMBRANE LIPIDS MEASURED BY ^2H_2O NMR

The ^2H_2O NMR spectra were recorded at 15.4 MHz with an acquisition time of 0.1 s and number of transients approx. 1000.

Lipid	Temperature (°C)	Maximum hydration (bound + trapped water, mol/mol lipid)	Δ (kHz)	Transition temperature (°C)
Monoglucosyldiglyceride (18 : 1_t)	35	7.3	0.23	30—35
	50	9.8	0.23	
Monoglucosyldiglyceride + 1.0 M NaCl	35	8.0	0.27	30—35
	50	9.3	0.27	
Diglucosyldiglyceride (18 : 1_t)	35	6.9	1.00	30—35
	50	8.6	1.00	
Diglucosyldiglyceride + 1.0 M NaCl	35	7.6	1.00	30—35
	50	9.0	1.00	
Diglucosyldiglyceride + 1.0 M $CaCl_2$ or $MgCl_2$	35	10.1	0.80	30—35 + hysteresis
Phosphatidylglycerol (18 : 1_t)	60	>10	0.125	50—60
Phosphatidylglycerol + 1.0 M NaCl	60	>10	no splitting observed	>60
Glycerophosphoryldiglycosyldiglyceride (18 : 1_t)	50	13	0.80	35—50
Monoglucosyldiglyceride (ω-d_3-16 : 0 + 18 : 1_c)	17	6.0	0.25	20—25 *
	27	7.6	0.25	
	35	8.0	0.25	
Diglucosyldiglyceride (ω-d_3-16 : 0 + 18 : 1_c)	17	5.4	1.25	15—20
	27	7.0	1.30	
	35	8.9	1.30	

* Phase transition determined from quadrupole splittings of ω-d_3-16 : 0, since water deuteron splittings are insensitive to the transition here.

and ref. 2). Fig. 4 shows typical ω-d_3-palmitic acid ^2H spectra obtained from mono- and diglucosyldiglyceride. Splittings for the four main lipids in the liquid crystalline phase and the effect of temperature on splittings for mono- and diglucosyldiglyceride are tabulated in Table III. In the gel phase no splittings could be observed. All of the lipids studied except monoglucosyldiglyceride showed similar quadrupole splittings, while monoglucosyldiglyceride had a splitting about one-half that of the other lipids. For mono- and diglucosyldiglyceride, the gel to liquid crystalline phase transition occurred

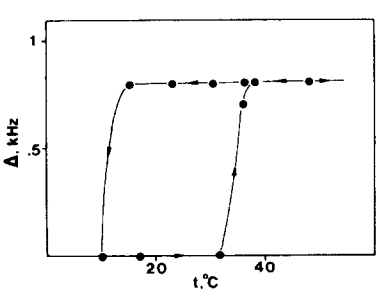

Fig. 3. Hysteresis effect in *A. laidlawii* diglucosyldiglyceride-^2H_2O-Ca^{2+} sample observed by the water-deuteron splitting. Water content 20% (w/w) and molar ratio diglycosyldiglyceride : Ca^{2+} = 4.35.

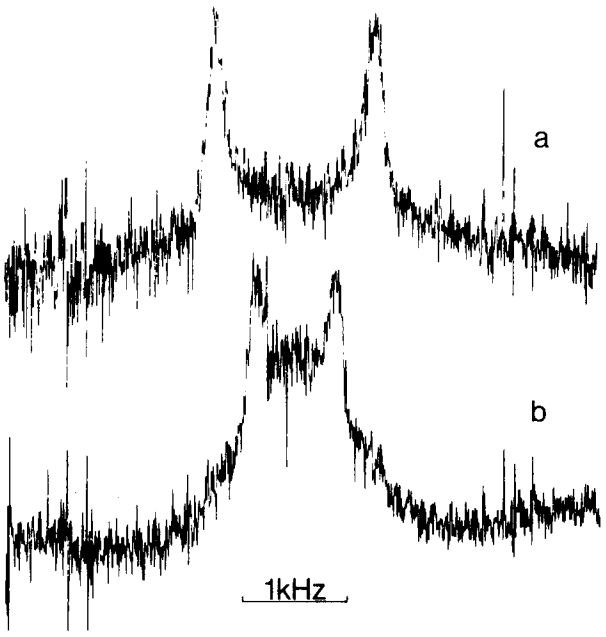

Fig. 4. 15.4 MHz NMR spectra of ω-d_3-palmitic acid labelled lipids from *A. laidlawii* at 41°C and 20% (w/w) water content. (a) Diglucosyldiglyceride and (b) monoglucosyldiglyceride. Acquisition time 0.1 s. Number of transients 20 000.

TABLE III

^2H NMR SPLITTINGS FROM DIFFERENT MEMBRANE LIPIDS CONTAINING ω-d_3 PALMITIC ACID

See Fig. 4 for technical measurement data. Water content of samples 20% (w/w).

Lipid	Temperature (°C)	Δ (kHz)
Monoglucosyldiglyceride *	20	no splitting observed
	30	0.88
	41	0.76
Diglucosyldiglyceride	20	1.80
	30	1.66
	41	1.52
Phosphatidylglycerol	41	1.54 + isotropic peak
Glycerophosphoryldiglucosyldiglyceride	25	1.60

* See Table I for actual lipid fatty-acid composition.

between 20—25°C, and 15—20°C, respectively (Table II). Phosphatidylglycerol was heated above 40°C, and glycerophosphoryldiglucosyldiglyceride to 25°C, before liquid crystalline properties, i.e., splittings, were observed. Both mono- and diglucosyldiglyceride showed decreased splittings with increased temperature (cf. heavy water splittings).

Discussion

Several workers have shown that information about binding and orientation of water in lipid-water systems can be conveniently obtained from ^2H_2O NMR

[20,21]. Recently a detailed phase diagram for the dipalmitoyl phosphatidylcholine-water systems was constructed from such measurements [8]. The same techniques have been used here with the main membrane lipids from *A. laidlawii* A. Although the di- and the monoglucosyldiglyceride are structurally similar, the polar head of diglucosyldiglyceride contains two glucose residues and monoglucosyldiglyceride one glucose residue [2]. Furthermore, monoglucosyldiglyceride is considered to be the biosynthetic precursor of diglucosyldiglyceride [22].

The concept of hydration includes both bound and possibly trapped water [23]. As can be seen in Table II, mono- and diglucosyldiglyceride have about the same hydration capacities, i.e., bound plus trapped water, but the splittings, i.e., water orientation, are less for monoglucosyldiglyceride than for diglucosyldiglyceride. These results are probably due in part to the different phase structures of the lipids. Both these lipids, which are the dominant lipids in the membranes [2], take up water to a much lesser extent than do the phosphatidylcholines [23]. The hydration properties of these two lipids, approximately 13% (w/w), are much more similar to those of phosphatidylethanolamines [23]. On the other hand, the negatively charged membrane lipids phosphatidylglycerol and glycerophosphoryldiglucosyldiglyceride have much higher water binding capacities.

The structurally similar mono- and digalactosyldiglyceride from Pelargonium leaves both have substantially higher hydration capacities, i.e., 22% (w/w) each [19], than have the glycolipids from *A. laidlawii*. The differences are probably due to the fact that the galactolipids contain large amounts ($\geqslant$70 mol/100 mol) of polyunsaturated fatty acids, mainly linolenic acid [24]. These acids strongly affect the physical properties of the galactolipids. At comparable surface pressures these galactolipids both occupy significantly larger surface areas per molecule in monolayers [25] than does elaidic acid containing *A. laidlawii* glycolipid [26]. Furthermore, the gel to liquid crystalline phase transition occurs at least 50°C below that of the *A. laidlawii* glycolipids (Table II and ref, 24). These inherent physical properties most likely affect the packing, and hence hydration properties, of the galactolipids in their different phase structures by allowing more water binding sites to be exposed. Differences in amount of trapped and bound water will probably also occur as a function of the dissimilar sugar/sugar and sugar/glycerol bond in the galactolipids [24] and the *A. laidlawii* glycolipids [26]. Interestingly, there is no variation in hydration between the mono- and disugar derivates for these lipids.

The gel to liquid crystalline phase transition temperatures for mono- and diglucosyldiglyceride, containing elaidic acid (Table II), are approximately 30°C higher than that of phosphatidylcholine containing the same acid [27]. The transition temperatures are approximately the same as those for synthetic dielaidoyl phosphatidylethanolamine [28] and phosphatidylethanolamine from *E. coli* fatty acid auxotrophs grown with elaidic acid [29,30]. Finally the different acyl chains of these glycolipids produce no large difference in hydration capacity, whereas the transition temperatures are affected (Table II).

The effect of sodium chloride (lipid : ion molar ratio between 3 and 5) on both hydration and phase transition temperature for mono- and diglucosyldiglyceride was found to be negligible (Table II). However, the divalent cations

calcium and magnesium (lipid : ion molar ratio approx. 4.4) strongly affected the hydration capacity of diglucosyldiglyceride. Furthermore, the temperature dependence of the splittings exhibited a hysteresis effect with these ions (Fig. 3). Below the gel to liquid crystalline phase transition, the calcium or magnesium chloride salts are probably concentrated to regions with exess water. The amount of water bound in the gel phase is probably very small, since we have not been able to observe a water quadrupole splitting for these phases, cf. the splittings obtained for gel phases fo dipalmitoyl phosphatidylcholine-water systems [8]. At temperatures above the transition interval, however, a large amount of water is intercalated between the lipid bilayers. If divalent ions are present, the hydration capacity of the liquid crystalline phase increases (Table II). Monovalent ions increase the hydration capacity to a much lesser extent. This change in hydration capacity may be due either to the fact that divalent ions are highly hydrated or that the ions are causing a conformational change of the polar head group allowing a larger water uptake (cf. ref. 21). Thus, a "new" metastable liquid crystalline phase with higher water binding capacity is created by the presence of divalent ions, and the transition point for this phase is changed to a lower temperature. It is very likely that both calcium and magnesium interact with the sugar groups of the glycolipid [31], since it has been shown that these ions can interact with sugars [32]. Furthermore, the ion concentrations in the samples studied are quite small compared to the physiological concentration occurring in *E. coli* [33] and in *A. laidlawii* membranes [34], since the microbial cell is highly organized with little intracellular free water.

It has been shown [35] that deuteron quadrupole splittings for a lamellar and a hexagonal phase should differ by a factor of two, the lamellar phase having the larger splitting, provided that the local environments of the lipids are the same. This is the case of the splittings observed for mono- and diglucosyldiglyceride containing a covalently bound ^2H NMR label in the acyl chains (Table III). Low-angle X-ray diffraction (Fig. 1) verified that monoglucosyldiglyceride has a hexogonal phase structure and that the other lipids, including diglucosyldiglyceride, have lamellar structures. Since diglucosyldiglyceride, being lamellar, has a more bulky head than monoglucosyldiglyceride, and both lipids contain similar fatty acids and are both uncharged [2], the hexagonal structure of monoglucosyldiglyceride is considered to be of the reversed or H_{II} type [18]. Furthermore, this hexagonal structure of monoglucosyldiglyceride can occur at such small water concentrations that the amount of water is not sufficient to fill the space between lipid rod structures of the H_I type [18]. Similar phase appearances, i.e., reversed hexagonal and lamellar, have been observed for the mono- and digalactosyldiglyceride from Pelargonium leaves [24].

The water deuteron splitting for monoglucosyldiglyceride is significantly lower than a factor of two for that from diglucosyldiglyceride (Table II). This indicates that monoglucosyldiglyceride, despite a different phase structure, has less water orientation than diglucosyldiglyceride. Recent experiments have indicated a large physical inequality between the two fatty acid chains in a lipid molecule. In short, a lipid molecule can be viewed as having the glycerol backbone perpendicular to the bilayer plane and one of the two fatty acid

chains running parallel to this plane of a length of approximately two carbon atoms [36,37]. For the *A. laidlawii* glycolipids, water may bind to the hydroxyl groups of the sugar residues. Since monoglucosyldiglyceride takes up at least as much water as does diglucosyldiglyceride (Table II), several of the more abundant hydroxyl groups of diglucosyldiglyceride are probably shielded from water binding. Because of the type and specificity of intramolecular bonds in diglucosyldiglyceride, the exact structure of which is 1,2-diacyl-[*O*-α-D-glucopyranosyl-(1′ → 2′)-*O*-α-D-glucopyranosyl (1′ → 3)]-*sn*-glycerol, several configurations of the polar head area seems not to agree with low water binding. Studies with molecule models indicate that one way to decrease exposure of hydroxyl groups in diglucosyldiglyceride is with a polar head structure more or less parallel to the bilayer plane. Such a configuration can also explain how different fatty acid residues can induce angular movement of the glycerol backbone and thus affect the stiffness of the polar head. Of all *A. laidlawii* lipids investigated, diglucosyldiglyceride has the highest phase transition enthalphy as measured by differential scanning calorimetry [26]. Recent results on interaction between concanavalin A and monolayers of mono- and diglucosyldiglyceride support a parallel head configuration [38]. Here, diglucosyldiglyceride was not able to interact with concanavalin A after a moderate increase in surface pressure from 30 to 35 dynes/cm, whereas monoglucosyldiglyceride still showed a strong interaction. The physical dissimilarities of these lipids are further strengthened by the finding that a critical excess of monoglucosyldiglyceride over diglucosyldiglyceride in the membrane of an *A. laidlawii* mutant seems to make these cells resistant to reactive complement lysis [39].

In conclusion, the dominating glycolipids in *A. laidlawii* membranes have similar properties to those of phosphatidylethanolamine, a common lipid in most biological membranes and especially in bacteria. These properties are (1) low hydration capacities, (2) relatively high transition temperatures, and (3) presence of a reversed hexagonal phase structure. Changes in the phase structure of phosphatidylethanolamine from *Pseudomonas fluorescens* caused by changes in temperature or fatty acid composition [19] are probably of the same importance for membrane properties as are the physiological variations between the physically dissimilar mono- and diglucosyldiglyceride in this *A. laidlawii* A [2—4].

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