New Aspects on Membrane Lipid Regulation in Acholeplasma laidlawii A and Phase Equilibria of Monoacyldiglucosyldiacylglycerol †

ARTICLE *in* BIOCHEMISTRY · SEPTEMBER 1996

Impact Factor: 3.02 · DOI: 10.1021/bi960561w · Source: PubMed

New Aspects on Membrane Lipid Regulation in *Acholeplasma laidlawii* A and Phase Equilibria of Monoacyldiglucosyldiacylglycerol†

Ann-Sofie Andersson,*,‡ Leif Rilfors,‡ Magnus Bergqvist,§ Stefan Persson,‡ and Göran Lindblom‡

Department of Physical Chemistry, Umeå University, S-901 87 Umeå, Sweden, and Department of Analytical Chemistry, Stockholm University, S-106 91 Stockholm, Sweden

Received March 7, 1996; Revised Manuscript Received May 29, 1996⊗

ABSTRACT: A new membrane lipid, monoacyldiglucosyldiacylglycerol (MADGlcDAG), was recently discovered in *Acholeplasma laidlawii* strain A-EF22, demanding a new study of the biosynthetic regulation, and the phase behavior, of the glucolipids in this organism. The only liquid-crystalline phase formed by MADGlcDAG is a reversed hexagonal phase. *A. laidlawii* A-EF22 synthesizes four lipids that have the ability to induce the formation of reversed nonlamellar phases: MADGlcDAG, monoglucosyldiacylglycerol (MGlcDAG), monoacylmonoglucosyldiacylglycerol (MAMGlcDAG), and diacylglycerol (DAG). A C_n value of approximately 16 seems to be a critical value for the fractions of these lipids in the membrane: the fractions of MADGlcDAG and MGlcDAG are largest when the C_n values are lower than 16, while the fractions of MAMGlcDAG and DAG are largest when the C_n values are higher than 16. The fraction of nonlamellar-forming lipids was 55 mol % when the C_n value was 14.8 and the degree of unsaturation was 33 mol %. This fraction was reduced to 7 mol % when the C_n value and the degree of unsaturation were increased to 17.8 and 92 mol %, respectively, i.e., at conditions that markedly favor the formation of reversed nonlamellar phases. These observations convincingly show that a balance between lamellar- and nonlamellar-forming lipids is maintained in the membrane and strongly support the validity of the lipid regulation model proposed by us. From earlier biochemical data, obtained with short acyl chains, that were difficult to reconcile with our regulation model, it could be predicted that a lipid ought to be synthesized that assists MGlcDAG to maintain the nonlamellar-forming properties with the short chains. It is shown in the present work that this lipid is MADGlcDAG and that the regulation of the balance between lamellar- and nonlamellar-forming lipids is even more complex and sophisticated in *A. laidlawii* A-EF22 than previously proposed.

Acholeplasma laidlawii is a well-studied organism concerning the regulation of its lipid composition and the physicochemical properties of its membrane lipids (McElhaney, 1992; Rilfors et al., 1993). This cell-wall-less prokaryotic organism is a parasite and must have the ability to adapt to different growth conditions. A common way to respond to varying growth temperatures is to regulate the degree of unsaturation of the acyl chains of the lipids (Hazel & Williams, 1990; Morein et al., 1996; Suutari & Laakso, 1994). *A. laidlawii* lacks the ability to produce unsaturated fatty acids (Panos & Rottem, 1970), and saturated fatty acids are synthesized in very limited amounts when the organism is grown in a thoroughly lipid-depleted medium. Thus, at least one fatty acid must be supplemented to the growth medium in order for the cells to grow. *A. laidlawii* exploits another mechanism to adapt the physicochemical properties of the membrane lipid bilayer to changes in the environmental conditions, namely, altering the proportion between the polar head groups of the lipids (McElhaney, 1992; Rilfors et al., 1993).

It has been hypothesized and experimentally supported (Lindblom et al., 1986; Wieslander et al., 1980) that *A. laidlawii* strain A-EF22 regulates the membrane lipid composition in order to maintain a proper balance between the lipids forming a lamellar liquid-crystalline (L_α)[1] phase and the lipids forming reversed nonlamellar phases. The only lipid known at that time to have the property to form nonlamellar phases was 1,2-diacyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol (MGlcDAG) (**1** in Figure 1) (Lindblom et al., 1986; Wieslander et al., 1978). The other dominant lipid

† Supported by the Swedish Natural Science Research Council and The Knut and Alice Wallenberg Foundation.

* To whom correspondence should be addressed. Tel: +46-90-16-65-76. FAX: +46-90-16-77-79. E-mail: annsofie.andersson@chem.umu.se.

‡ Umeå University.

§ Stockholm University.

⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1996.

[1] Abbreviations: MGlcDAG, 1,2-diacyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol; DGlcDAG, 1,2-diacyl-3-*O*-[α-D-glucopyranosyl(1→2)-*O*-α-D-glucopyranosyl]-*sn*-glycerol; MAMGlcDAG, 1,2-diacyl-3-*O*-[6-*O*-acyl(α-D-glucopyranosyl)]-*sn*-glycerol; MADGlcDAG, 1,2-diacyl-3-*O*-[α-D-glucopyranosyl(1→2)-*O*-(6-*O*-acyl-α-D-glucopyranosyl)]-*sn*-glycerol; DAG, 1,2-diacylglycerol; GPDGlcDAG, 1,2-diacyl-3-*O*-[glycerophosphoryl-6-*O*-(α-D-glucopyranosyl(1→2)-*O*-α-D-glucopyranosyl)]-*sn*-glycerol; MABGPDGlcDAG, 1,2-diacyl-3-*O*-[glycerophosphoryl-6-*O*-(α-D-glucopyranosyl(1→2)monoacylglycerophosphoryl-6-*O*-α-D-glucopyranosyl)]-*sn*-glycerol; PAGlc, 1-*O*-polyprenyl-2-*O*-acyl-α-D-glucopyranoside; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; 14:0, myristic acid; 16:0, palmitic acid; 18:0, stearic acid; 20:0, arachidic acid; 16:1c, palmitoleic acid; 18:1c, oleic acid; 18:1c-d_2, α-deuterated oleic acid; C_n, average acyl chain length; ^1H_2O, deuterium-depleted water; L_1, normal micellar solution phase; L_2, reversed micellar solution phase; L_α, lamellar liquid-crystalline phase; I_{II}, reversed cubic liquid-crystalline phase; H_{II}, reversed hexagonal liquid-crystalline phase; T_{NL}, temperature for a lamellar to nonlamellar phase transition; T_m, temperature for the transition between lamellar gel and L_α phases; GLC, gas−liquid chromatography; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography.

S0006-2960(96)00561-2 CCC: $12.00

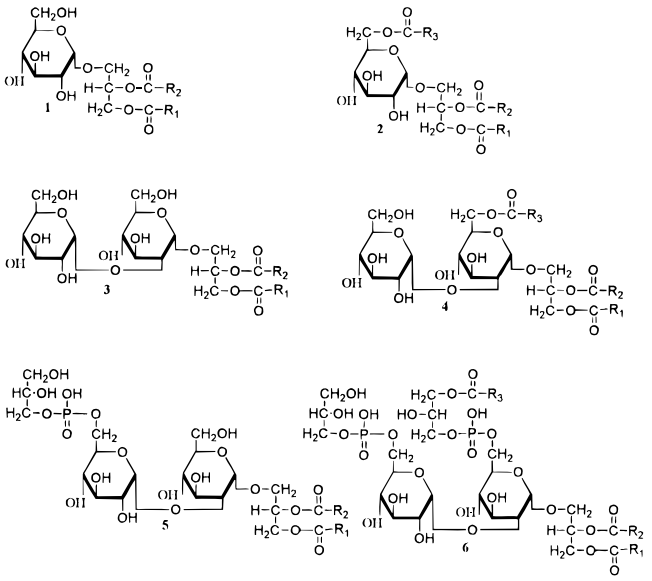

FIGURE 1: Structures of the glucolipids and the phosphoglucolipids synthesized by *A. laidlawii*, strain A-EF22. **1**, MGlcDAG; **2**, MAMGlcDAG; **3**, DGlcDAG; **4**, MADGlcDAG; **5**, GPDGlcDAG; **6**, MABGPDGlcDAG.

in *A. laidlawii* is 1,2-diacyl-3-*O*-[α-D-glucopyranosyl(1→2)-*O*-α-D-glucopyranosyl]-*sn*-glycerol (DGlcDAG) (**3** in Figure 1), which forms solely lamellar gel and L_α phases at all temperatures and with all acyl chain compositions (Eriksson et al., 1991; Lindblom et al., 1986; Wieslander et al., 1978). It was early shown that *A. laidlawii* A-EF22 varies the fractions of MGlcDAG and DGlcDAG in relation to the prevailing growth conditions [see Rilfors et al. (1993)]. Studies of the phase properties of one of the triacylglucolipids in *A. laidlawii* A-EF22, 1,2-diacyl-3-*O*-[6-*O*-acyl(α-D-glucopyranosyl)]-*sn*-glycerol (MAMGlcDAG) (**2** in Figure 1), revealed that this lipid forms a reversed micellar solution (L_2) phase when its acyl chains are in the melted state (Lindblom et al., 1993). The fraction of MAMGlcDAG is largest under conditions when MGlcDAG has a lower ability to form reversed nonlamellar phases, and MAMGlcDAG therefore seems to assist MGlcDAG in maintaining the balance between bilayer- and nonbilayer-forming lipids. Thus, the basic idea in our lipid regulation model still appeared to be valid.

An analogue of MAMGlcDAG was recently discovered in *A. laidlawii* A-EF22, and its structure was determined to be 1,2-diacyl-3-*O*-[α-D-glucopyranosyl(1→2)-*O*-(6-*O*-acyl-α-D-glucopyranosyl)]-*sn*-glycerol (MADGlcDAG) (**4** in Figure 1) (Hauksson et al., 1995). Neither the phase behavior of MADGlcDAG nor the metabolic regulation of the fraction of this lipid has been investigated before. Moreover, MADGlcDAG has probably contaminated MGlcDAG in several of our earlier biochemical as well as physicochemical investigations (Hauksson et al., 1995). The main purposes of the present work are therefore to determine how the incorporation of exogenously fed fatty acids of different lengths and degrees of unsaturation influences the relative amounts of MADGlcDAG and MGlcDAG synthesized by *A. laidlawii* A-EF22 and to study the phase equilibria of the two lipids. The present work is of crucial importance for our proposed lipid regulation model and further emphasizes the complexity of the regulation mechanism in this simple bacterium. It is now established that this organism synthesizes four lipids having the ability to form, or to induce the formation of, reversed nonlamellar structures, and the role of each one of these lipids in the regulation mechanism will be discussed. In particular, it is argued that the results obtained for MADGlcDAG strengthen the validity of the lipid regulation model.

Table 1: Degree of Unsaturation and Average Acyl Chain Length of Total Lipid Extracts from *A. laidlawii* Strain A-EF22 Used for the Study of Regulation of the Membrane Lipid Composition

fatty acids supplemented to the growth medium	μM/μM	UFA[a]	C_n[b]
14:0/16:1c	75/75	47.8	15.0_6
14:0/16:1c	75/75	48.6	15.1_0
14:0/16:1c	110/40	33.1	14.8_2
16:0/16:1c	0/150	85.3	16.6_4
16:0/16:1c	0/150	86.0	16.9_3
16:0/16:1c	10/140	79.8	16.1_5
16:0/16:1c	30/120	74.8	16.0_6
16:0/16:1c	75/75	43.8	16.0_2
16:0/16:1c	75/75	61.0	16.0_5
16:0/16:1c	75/75	56.6	16.0_2
16:0/16:1c	100/50	29.3	16.0_2
16:0/16:1c	120/30	30.6	16.0_4
16:0/16:1c	120/30	26.0	16.0_0
18:0/18:1c	0/150	92.0	17.7_7
18:0/18:1c	0/150	79.6	17.4_9
18:0/18:1c	0/150	77.6	17.3_8
18:0/18:1c	75/75	50.5	17.6_7
18:0/18:1c	75/75	43.9	17.4_2
18:0/18:1c	75/75	42.6	17.3_1
18:0/18:1c	120/30	34.9	17.7_2
18:0/18:1c	120/30	42.1	17.5_8
20:0/18:1c	75/75	54.3	18.8_2
20:0/18:1c	75/75	62.0	18.2_5

[a] Unsaturated fatty acids (mol %). [b] Average acyl chain length.

MATERIALS AND METHODS

Organism and Growth Conditions. A. laidlawii A-EF22 was grown in a lipid-depleted bovine serum albumin/tryptose medium (Rilfors, 1985), where the tryptose had been prepared with an improved lipid-depletion procedure (Niemi et al., 1995). This further reduced the endogenous production of saturated fatty acids by the organism. For the lipid regulation studies the saturated fatty acids, myristic (14:0), palmitic (16:0), stearic (18:0), and arachidic (20:0) acid, and the unsaturated fatty acids, palmitoleic (16:1c) and oleic (18:1c) acid, were supplemented to the medium from sterile ethanol stock solutions. The acids were supplemented in the proportions shown in Table 1. The cultures were grown at 37 °C and were adapted to the different fatty acid supplementations by at least five consecutive daily inoculations. The growth of the cultures was followed by measuring the turbidity at 540 nm to make sure that each one of the cultures was harvested in the late logarithmic growth phase. It has earlier been observed that the growth phase influences the polar head group composition of the membrane lipids (Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1978). In the two cultures that included large fractions of saturated fatty acids, namely, 16:0/16:1c (120/30, μM/μM) and 18:0/18:1c (120/30, μM/μM), cell lysis occurred at OD_{540} values of about 0.3−0.4. Therefore, we ensured that these cultures were harvested before lysis occurred. All of the cultures were studied in a phase contrast light microscope to ensure that no contaminants were present. The volume of each culture was 300−400 mL, and the growth time varied

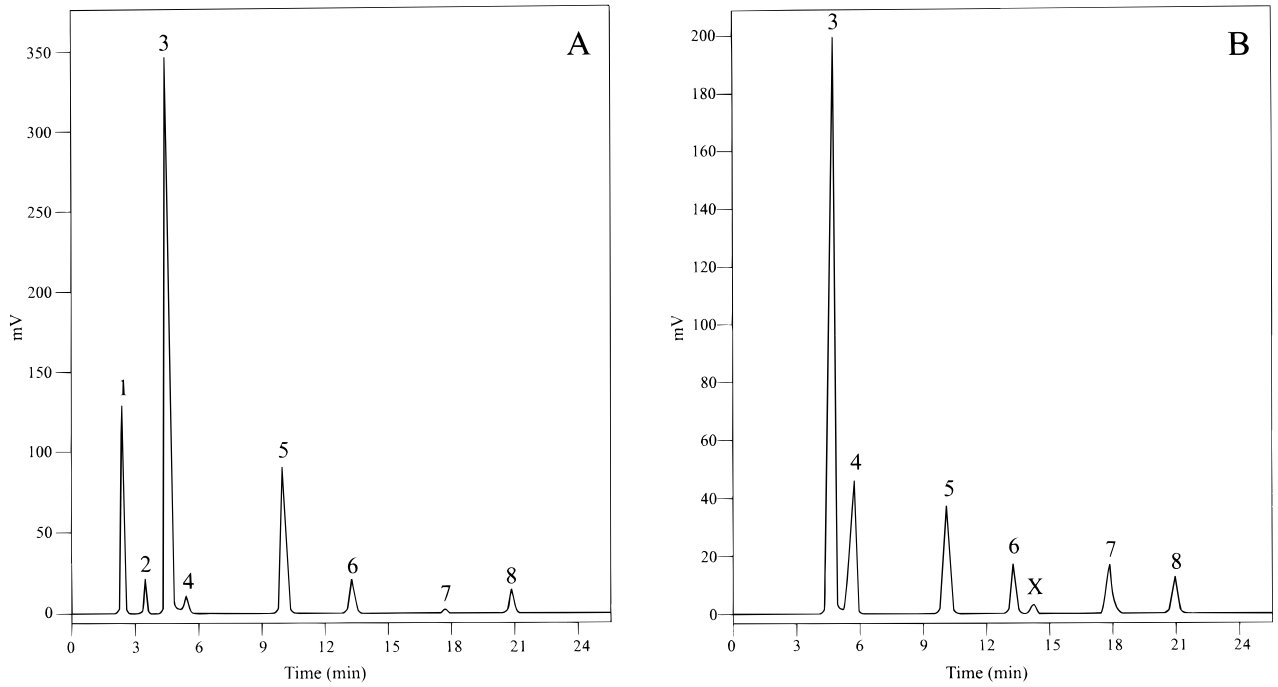

FIGURE 2: HPLC chromatograms of two total lipid extracts from *A. laidlawii* A-EF22. Chromatographic conditions were as described under Materials and Methods. (A) Cells grown with 16:0/16:1c (120/30, μM/μM), giving C_n values of 16.0 and 30.6 mol % unsaturated acyl chains. (B) Cells grown with 14:0/16:1c (75/75, μM/μM), giving C_n values of 15.1 and 47.8 mol % unsaturated acyl chains. The peaks correspond to the following lipids: 1, DAG; 2, MAMGlcDAG; 3, MGlcDAG; 4, MADGlcDAG; 5, DGlcDAG; 6, PG; 7, MABGPDGlcDAG; 8, GPDGlcDAG. The peak X in chromatogram is an unidentified lipid.

from 16 to 24 h depending on the fatty acid mixture. The cultures were harvested by centrifugation at 11 300g_{max} and 5 °C for 45 min and washed once with β-buffer (Christiansson & Wieslander, 1978), and either the cells were stored at −40 °C or the membrane lipids were immediately extracted from the cells.

For the purification of MGlcDAG, DGlcDAG, MADGlcDAG, and 1,2-diacyl-3-*O*-[glycerophosphoryl-6-*O*-(α-D-glucopyranosyl(1→2)-*O*-α-D-glucopyranosyl)]-*sn*-glycerol (GPDGlcDAG) (**5** in Figure 1), *A. laidlawii* A-EF22 was grown in the same medium as described previously. The medium was supplemented with 124−150 μM α-deuterated oleic acid (18:1-d_2). 18:1c-d_2 was synthesized according to Tulloch (1977). The cell cultures were grown at 30 °C and were adapted to growth with 18:1c-d_2 by five consecutive daily inoculations; these cultures were studied in a microscope as described previously. The volume of each culture was 5−7.5 L, and the growth time was 22−24 h. The cells were harvested and washed as described previously and stored at −40 °C. The cell pellets were pooled to achieve two batches, each corresponding to 30 L of cell culture.

Lipid Extraction. A. laidlawii has all of its lipids located in its cytoplasmic membrane (Razin, 1978). The frozen cell pellets were thawed and extracted twice with chloroform/methanol (2:1 by volume) and once with methanol, by agitation at room temperature. The lipid extracts were then applied to a Sephadex G-25 Fine column to remove all nonlipid contaminants (Wells & Dittmer, 1963).

Purification of MGlcDAG, DGlcDAG, MADGlcDAG, and GPDGlcDAG. The two batches of lipid extracts were each applied to a silica gel (Bio-Sil HA$^-$ 325 mesh, Bio-Rad Laboratories, Richmond, CA, or silica gel 60, 230−400 mesh, Merck, Darmstadt, Germany) column. Pigments, neutral lipids, and free fatty acids were eluted with chloroform, the glucolipids were eluted with acetone, and the anionic lipids were eluted with methanol. Preparative thin-layer chromatography (TLC) was further used to separate the glucolipids (Hauksson et al., 1995) and GPDGlcDAG [chloroform/methanol/water (55:35:5 by volume)]. The lipids were eluted from the gel as described previously (Lindblom et al., 1986). Divalent cations were removed and exchanged for sodium ions by a modified version (Rilfors et al., 1994) of the procedure described by Smaal et al. (1985). The purity of the lipids was checked on TLC plates and was determined to be ≥97% for MGlcDAG, ≥99% for DGlcDAG, ≥98% for MADGlcDAG, and ≥97% for GPDGlcDAG by the use of a charring agent (Ziminski & Borowski, 1966) and proper references. The preparative TLC purification method was checked by high-performance liquid chromatography (HPLC), and the same, or a higher, degree of purity was obtained.

Polar Head Group and Acyl Chain Compositions. The acyl chain compositions of the lipid extracts were quantified by converting the acyl chains to their methyl ester derivatives and by analyzing them with gas−liquid chromatography (GLC) as described previously (Rilfors et al., 1978; Rilfors, 1985). The GLC analyses were performed with a Varian Instruments, model 3700, apparatus connected to an integrator and equipped with a polar SP-2330 column (length, 180 cm; inner diameter, 0.13 cm) from Supelco Inc. (Bellefonte, PA). The average chain lengths and the degrees of unsaturation were calculated after the response factors had been obtained from standard reference mixtures (Larodan Fine Chemicals, Malmö, Sweden).

The polar head group compositions were analyzed by HPLC as described in Arnoldsson and Kaufmann (1994). The peaks in the chromatogram were assigned by comparing their retention times with those of purified *A. laidlawii* lipids. A good separation was obtained between all the lipids synthesized by *A. laidlawii* A-EF22 (Figure 2). The analyses

were performed with a LiChrosphere 100 DIOL (250 mm × 4.6 mm, 5 μm particle size) column (dihydroxypropyl-silica, Merck, Darmstadt, Germany). The column was enclosed in a water jacket connected to a circulating thermostated water bath held at 75 °C. The system consisted of an HPLC pump from LDC (model CM 4000, Milton Roy, Riviera Beach, FL) and an injector, model 7125, with a 10 μL injection loop from Rheodyne Inc. (Cotati, CA). The detector (evaporative light-scattering principle) used was a Sedex 45 (S.E.D.E.R.E., Vitry/Seine, France). The flow rate was 1 mL/min with a linear gradient from 100% of eluent A (hexane/isopropanol/butanol/tetrahydrofuran/isooctane/water, 64:20:6:4.5:4.5:1 by volume, including 180 mg of NH_4Ac/L) to 100% of eluent B (isopropanol/butanol/tetrahydrofuran/isooctane/water, 75:6:4.5:4.5:10 by volume, including 180 mg of NH_4Ac/L). The detector temperature was 97 °C, and the inlet nebulizer pressure was set to 1.6 bar. Processing of chromatographic data was done with the Gynkosoft Chromatography Data System (Gynkotek, Germering b. München, Germany). The polar head group composition of each lipid extract was quantified by at least four analyses.

A mixture of MAMGlcDAG, DGlcDAG, MADGlcDAG, 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG), and GPDGlcDAG was analyzed in order to determine their molar response factors. POPG was obtained from Avanti Polar Lipids (Bimingham, AL), and a preparation of MAMGlcDAG from Lindblom et al. (1993) was used. When the area % values were plotted versus the lipid concentrations (total lipid concentration, 0.5−12.7 mg/mL), a linear relationship with $r = 0.98$, or better, was obtained for each of the five lipids. The molar response factors for MGlcDAG and 1,2-diacyl-3-*O*-[glycerophosphoryl-6-*O*-(α-D-glucopyranosyl(1→2)-monoacylglycerophosphoryl-6-*O*-α-D-glucopyranosyl)]-*sn*-glycerol (MABGPDGlcDAG) (**6** in Figure 1) were interpolated from a plot of response factors versus retention times. The average molar response factors were calculated to be 0.49 ± 0.056 ($n = 6$) for MAMGlcDAG, 0.51 ± 0.045 ($n = 6$) for MGlcDAG, 0.93 ± 0.058 ($n = 6$) for MADGlcDAG, 1.0 for DGlcDAG, 2.1 ± 0.17 ($n = 6$) for POPG, 3.1 ± 0.14 ($n = 6$) for MABGPDGlcDAG, and 4.0 ± 0.27 ($n = 5$) for GPDGlcDAG. Since the fraction designated DAG also includes other molecules (see Results), this fraction is presented as area % values.

Sample Preparation for ^2H NMR Studies. The lipids (20−25 mg) were dried in an 8 mm outer diameter glass tube in a stream of N_2 and then dried to constant weight in vacuum. After the addition of 5%, 10%, 15%, or 20% (wt/wt) deuterium-depleted water (^1H_2O) (Fluka, Chemika-Bio-Chemika, Buchs, Switzerland) the tubes were flame-sealed, and lipids and water were mixed by extended centrifugation. The samples were freeze−thawed for ten cycles to ensure that they were completely equilibrated.

^2H NMR Spectroscopy. ^2H NMR spectroscopy was carried out on the lipid samples at the frequency 76.77 MHz on a Bruker AMX2-500 spectrometer. A selective high-power probe, tuned to deuterium with an 8 mm horizontal solenoid coil (500/8/X, Cryomagnetic Systems Inc., Indianapolis, IN) was used. A phase-cycled quadrupolar echo pulse sequence (Davis et al., 1976) was used, having a $\pi/2$ pulse length of 6.4 μs and a 40−50 μs pulse separation. Between 40 000 and 70 000 scans were collected for each temperature with a recycle time of 0.15 s. The temperature was controlled by a Eurotherm B-VT 2000 unit. A temperature calibration curve was made on the standard setting from which the desired temperatures were calculated. At each temperature the sample had at least 30 min of equilibration time before the acquisition started.

Computational Procedures. Data files were converted with a home-written Fortran program into a format suitable for the program Felix (Hare Research Inc., Woodinwill, WA). The free induction decays (FIDs) in this format were fractionally left shifted by the use of a home-written program FRLS. The FIDs were processed according to the following scheme: an appropriate left shift was performed; the file size was set to 512 points (or more depending on the lifetime of the echo) and was followed by a zero-filling to 2048 points; a line-broading of 50 Hz was applied, and the FID was finally Fourier transformed. Care was taken to start the Fourier transform exactly at the top of the quadrupole echo.

X-ray Diffraction. The X-ray measurements of MADG-lcDAG and MGlcDAG were performed at Station 8.2 at the Daresbury Laboratory (Cheshire, U.K.) using a monochromatic beam of wavelength 1.5 Å. The small-angle powder diffraction patterns were recorded electronically by means of a quadrant detector (Towns-Andrews et al., 1989) situated 1.5 m from the sample position. Immediately before the diffraction experiment the samples were brought from flame-sealed glass tubes and placed between then mica sheets held by a copper spacer. The sample temperatures were thermostatically controlled by mounting the samples on a modified microscope cryostage (Linkam, U.K.) and monitoring the temperature by a thermocouple embedded in the sample adjacent to the beam.

RESULTS

Acyl Chain Length and Unsaturation. The degree of incorporation of the exogenously supplied fatty acids into the membrane lipids of *A. laidlawii* was generally 80−98 mol %. Exceptions to this high degree of incorporation were obtained when the organism was grown with 150 μM 18:1c or 150 μM 16:1c; the lowest degrees of incorporation were 77 and 30 mol %, respectively. In the case with 16:1c this acid was elongated to 18:1c. In Table 1 the range of the acyl chain lengths and the degrees of unsaturation of the lipid extracts used in the lipid regulation studies are presented.

The acyl chain compositions of the two lipid batches used to purify the glucolipids and GPDGlcDAG are shown in Table 2, and it is seen that the compositions are very similar. The acyl chain compositions of the purified glucolipids are also presented. It can be noted that MADGlcDAG contains a larger fraction of short saturated chains (lauric acid, tridecanoic acid, and myristic acid) compared to MGlcDAG and DGlcDAG. If *A. laidlawii* synthesizes MADGlcDAG from DGlcDAG, the third acyl chain probably consists preferentially of the short saturated chains. DGlcDAG contains the largest fraction of 18:1c and has the highest average acyl chain length. These differences in the incorporation of different fatty acids are in accordance with earlier results (Hauksson et al., 1995).

Polar Head Group Composition. A. laidlawii is forced to change its polar head group composition according to the physicochemical properties of the fatty acids supplied to the growth medium. The cells incorporate the acids into

Table 2: Acyl Chain Composition of Total Lipid Extracts and Purified Lipids from *A. laidlawii* Strain A-EF22 Used for the Investigation of Phase Equilibria

acyl chain	12:0	13:0	14:0	15:0	16:0	17:0	18:0	18:1c	nd[a]	SFA[b]	UFA[c]	C_n[d]
batch 1	0.7	1.0	5.8	3.8	9.8	0.5	0.6	75.9	1.9	22.2	75.9	17.3_6
batch 2	2.6	1.7	6.7	3.4	7.1	0.3	0.3	76.0	1.9	22.1	76.0	17.2_4
MGlcDAG[e]	0	1.7	9.5	7.7	19.1	1.2	0.8	58.3	1.7	40.0	58.3	16.9_1
DGlcDAG[e]	0.1	0.8	5.0	3.7	8.8	0.5	0.4	78.9	1.8	19.3	78.8	17.4_6
MADGlcDAG[e]	10.0	5.7	12.2	3.5	5.1	0	0	61.7	1.8	36.5	61.7	16.3_6
MADGlcDAG[f]	10.0	5.7	12.5	4.0	6.1	0.1	0.3	59.7	1.6	38.7	59.7	16.3_2

[a] nd, not determined. [b] Saturated fatty acids (mol %). [c] Unsaturated fatty acids (mol %). [d] Average acyl chain length. [e] Purified from batches 1 and 2. [f] Purified from batch 1.

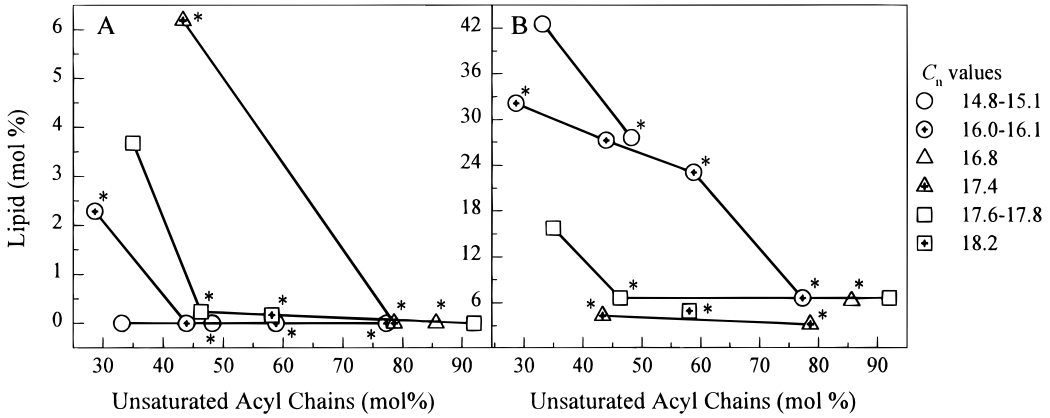

FIGURE 3: Fractions of (A) MAMGlcDAG and (B) MGlcDAG in *A. laidlawii* membranes as a function of the degree of unsaturation of the acyl chains. Data points in the graphs representing lipid extracts with similar average acyl chain length (C_n) are connected with lines. The data points marked with "*" represent an average value obtained from two or more lipid extracts with similar acyl chain compositions.

phosphatidic acid. This lipid is situated in the branchpoint in the lipid biosynthetic pathway where one competing pathway yields phosphatidylglycerol (PG) and the other pathway yields the glucolipids and the phosphoglucolipids (Rilfors et al., 1993). The glucolipids are MGlcDAG and DGlcDAG and their monoacyl derivatives MAMGlcDAG and MADGlcDAG, respectively. This pathway most probably also produces two glycerophosphoryl derivatives of DGlcDAG, namely, GPDGlcDAG and MABGPDGlcDAG (Hauksson et al., 1994a, 1995). The polar head group composition of the lipids in *A. laidlawii* A-EF22 has not been examined by HPLC before. In earlier publications it has been quantified with TLC in combination with scintillation counting or GLC (Wieslander et al., 1995; Rilfors, 1985). Moreover, the newly discovered triacylglucolipid MADGlcDAG has not been quantified before, and the conditions under which it is synthesized are thus established here.

1,2-Diacylglycerol. Due to its large hydrophobic volume 1,2-diacylglycerol (DAG) can induce the formation of reversed nonlamellar phases when it is incorporated into membrane lipid bilayers (Das & Rand, 1986; De Boeck & Zidovetzki, 1989; Siegel et al., 1989). In this study the fraction designated DAG also includes free fatty acids and pigments. According to previous studies (Wieslander et al., 1995), and to new TLC experiments (data not shown), this fraction constitutes about 75−95 mol % of DAG. The synthesis of DAG in *A. laidlawii* is negligible when the degree of unsaturation is >60 mol %. The largest fraction appears when the average acyl chain length (C_n) is ≥16 and the degree of unsaturation is <40 mol %. The fraction of DAG constitutes up to 10−15 area % (data not shown).

MGlcDAG and DGlcDAG. The largest fraction of MGlcDAG appears when $C_n \leq 16$ and when the degree of saturation is high (Figure 3B). In an earlier study (Wieslander et al., 1995) the dependence of MGlcDAG on the degree of unsaturation was not as clear cut as it is here. This is probably because MADGlcDAG was included in the analyses of MGlcDAG in the previous work. The fraction of DGlcDAG generally increases with both the acyl chain unsaturation and the C_n value (Figure 4B).

MAMGlcDAG and MADGlcDAG. These two lipids have a third acyl chain linked to the hydroxyl group in position 6 on the glucosyl moiety (Hauksson et al., 1994b, 1995); in MADGlcDAG the acyl chain is linked to the glucosyl moiety closest to the glycerol backbone. The third acyl chain gives the lipids a very large hydrophobic volume. MAMGlcDAG is only synthesized when the acyl chains are unsaturated up to a degree of about 50 mol % and $C_n \approx 16-18$ (Figure 3A), which is in agreement with earlier observations (Wieslander et al., 1995). The fraction of MADGlcDAG reaches the highest values when $C_n \leq 16$ (Figure 4A). In this range the fraction increases with an increasing degree of unsaturation. With longer acyl chain lengths the fraction of MADGlcDAG is around 1−5 mol % and is approximately constant when the degree of unsaturation changes; however, no synthesis of MADGlcDAG seems to occur when 18:1c constitutes nearly all the acyl chains. It is noteworthy that MADGlcDAG is synthesized with almost all acyl chain compositions investigated here. This is in contrast to the syntheses of DAG and MAMGlcDAG which are practically shut off above 50−60 mol % of acyl chain unsaturation irrespectively of the chain length.

PG, GPDGlcDAG, and MABGPDGlcDAG. The fraction of PG varies in a complex way (Figure 5A); with increasing degree of unsaturation the fraction increases when $C_n \approx 16-17$, but it decreases when $C_n > 17$. The fraction of GPDGlcDAG seems to increase slightly with the C_n value (Figure 5B); the largest fractions are observed with long and

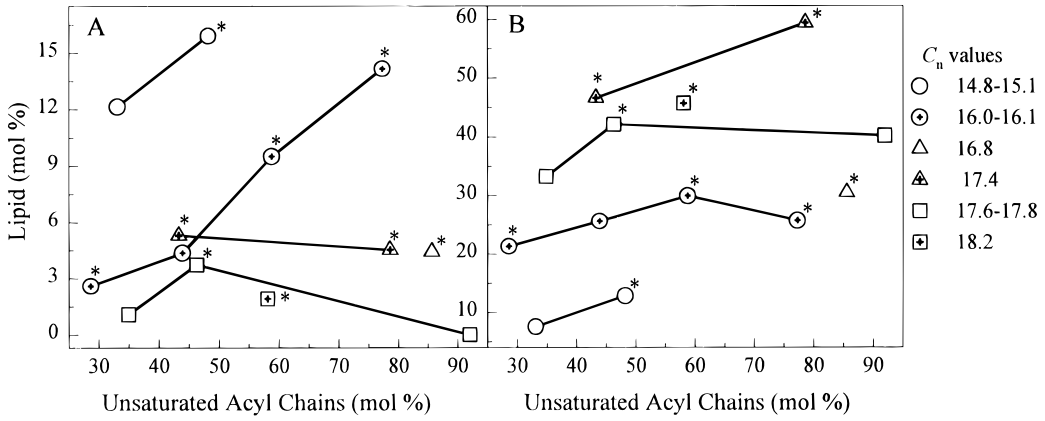

FIGURE 4: Fractions of (A) MADGlcDAG and (B) DGlcDAG in *A. laidlawii* membranes as a function of the degree of unsaturation of the acyl chains. Data points in the graphs representing lipid extracts with similar average acyl chain length (C_n) are connected with lines. The notation * is as described in the legend of Figure 3.

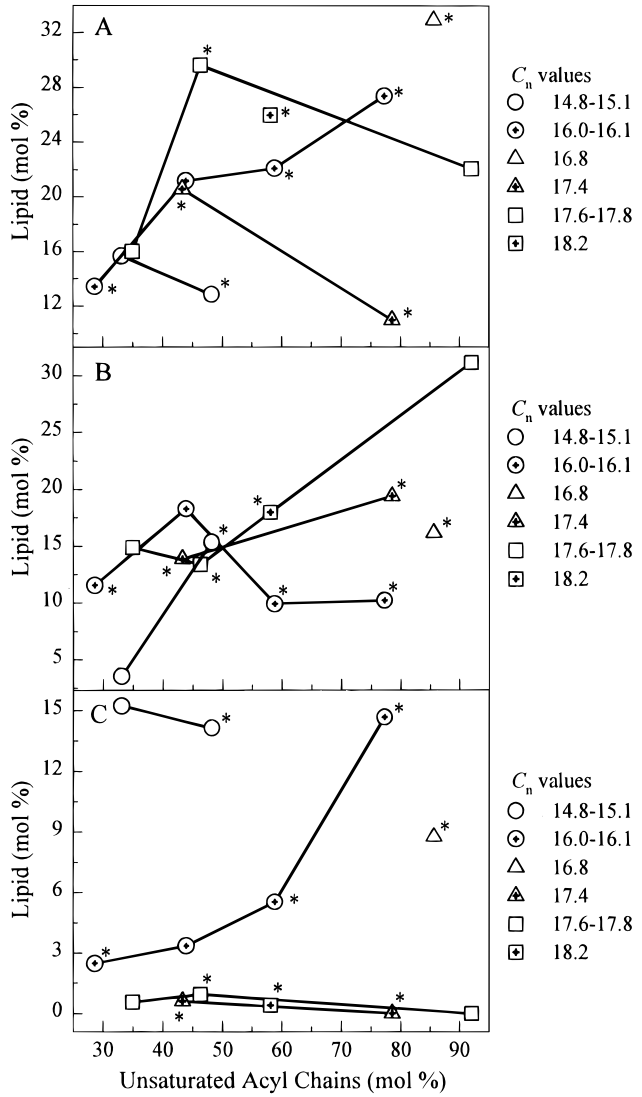

FIGURE 5: Fractions of (A) PG, (B) GPDGlcDAG, and (C) MABGPDGlcDAG in *A. laidlawii* membranes as a function of the degree of unsaturation of the acyl chains. Data points in the graphs representing lipid extracts with similar average acyl chain length (C_n) are connected with lines. The notation * is as described in the legend of Figure 3.

unsaturated acyl chains, i.e., at conditions that cause a decrease in the fraction of PG. The fraction of GPDGlcDAG is often larger than that of MABGPDGlcDAG. The fraction of the latter lipid is only significant when $C_n < 17$, and the fraction increases when the C_n value decreases and the degree of unsaturation increases (Figure 5C). The total fraction of anionic lipids has in earlier studies been found to increase with the degree of unsaturation (Christiansson et al., 1985; Wieslander et al., 1980, 1995), and essentially the same feature is observed in this work.

Nonlamellar-Forming Lipids. In an *A. laidlawii* cell all the lipids are most probably mixed to make up a functional membrane. Therefore, it is of interest to know how the fraction of all the lipids that form nonlamellar structures varies according to the fatty acid supplementation. In Figure 6 it is shown that the largest fraction of these lipids is observed when the acyl chains are short and the degree of unsaturation is low, and the fraction is progressively reduced when the acyl chains are longer and more unsaturated. The fractions of MADGlcDAG, MAMGlcDAG, and DAG, the three lipids beside MGlcDAG having the ability to form, or to induce the formation of, reversed nonlamellar structures, all have different acyl chain length dependencies. The fraction of MADGlcDAG is enhanced by short chains ($C_n \leq 16$); MAMGlcDAG is synthesized when $C_n \approx 16-18$, constituting a maximal fraction at $C_n \approx 17-17.5$; and the fraction of DAG increases with an increasing chain length.

Lipids Synthesized by A. laidlawii Strain B-PG9. According to preliminary results from our laboratory we find evidence for the existence of MADGlcDAG and MABGP-DGlcDAG also in strain B-PG9, which have not been reported before. The B strain was grown in the lipid-depleted bovine serum albumin/tryptose medium supplemented with 14:0/16:1c (75/75, μM/μM). A lipid extract was prepared as described under Materials and Methods. The extract was analyzed by TLC using the solvent system chloroform/methanol/25% ammonia (91:35:10 by volume), and the lipids were visualized by the use of a charring agent (Ziminski & Borowski, 1966).

Phase Equilibria of Glucolipids. To determine the phase equilibria of MGlcDAG and MADGlcDAG, seven samples were prepared. Three contained MGlcDAG with 5%, 10%, and 15% (w/w) ^1H_2O, corresponding to 2.2, 4.7, and 7.4 mol of ^1H_2O/mol of lipid, and four contained MADGlcDAG with 5%, 10%, 15%, and 20% (w/w) ^1H_2O, corresponding to 3.3, 7.1, 11.2, and 15.9 mol of ^1H_2O/mol of lipid. The quadrupolar splittings, $\Delta\nu_Q$, depend on the molecular ordering of the glucolipid acyl chains and provide structural information about the membrane system examined. The quadrupolar

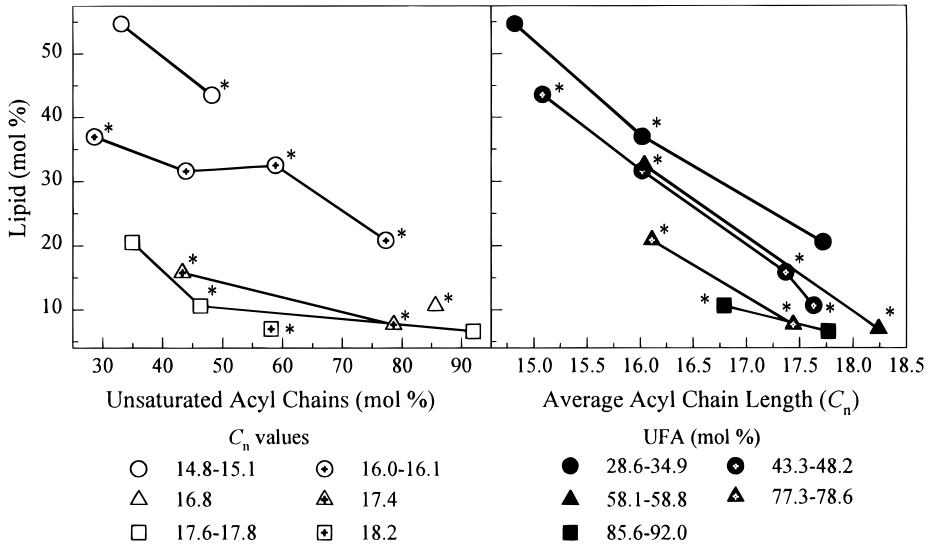

FIGURE 6: Fraction of *A. laidlawii* lipids able to form reversed nonlamellar phases as a function of the degree of unsaturation of the acyl chains (UFA) and of the average acyl chain length. The total fraction consists of MGlcDAG, MAMGlcDAG, and MADGlcDAG. Data points in the graphs representing lipid extracts with similar average acyl chain length (C_n) or degree of unsaturation are connected with lines. The notation * is as described in the legend of Figure 3.

splittings are reduced by approximately a factor of 2 when the system changes from an L_α phase to a reversed hexagonal (H_{II}) phase, due to the change in the axis of symmetry around which motion is averaged (Lindblom, 1996). In an isotropic phase, such as a cubic phase, there is fast isotropic molecular motion which leads to a rather narrow signal.

In the present study the C-2 position on the oleic acid is labeled with deuterium. In the case where both the *sn*-1 and *sn*-2 positions of a membrane phospholipid contain acyl chains labeled in the C-2 position, three quadrupolar splittings appear in the spectrum (Seelig & Seelig, 1980). Rance et al. (1983) studied the glucolipid fraction of *A. laidlawii* strain B. The glucolipids contained approximately 50% α-deuterated *cis*-9,10-methylene-9-octadecenoic acid, and less than 10% of this fatty acid was situated in position *sn*-1 (Saito et al., 1977). Rance et al. (1983) could see only two splittings of unequal intensity in their de-Paked spectra, but by using simulation an additional splitting had to be added to account for the unequal intensities. Rance and co-workers concluded that the two major splittings resulted from the deuterons in the C-2 position of the *sn*-2 chain and that the third, minor component, overlapping the outer splitting of the *sn*-2 chain, resulted from the *sn*-1 chain. In spectra recorded from a sample with DGlcDAG containing 20% (w/w) ^1H_2O, corresponding to 12.9 mol of ^1H_2O/mol of lipid, we can only find two splittings (Figure 7), but in a de-Paked spectrum recorded at 70 °C a third component, severely overlapped by the outer splitting, may be revealed (data not shown). The reason for the small difference in the quadrupolar splittings of the *sn*-1 and *sn*-2 acyl chains could be the large heterogeneity in the acyl chain composition (Table 2). Moreover, the low intensity of the largest splitting, originating from the *sn*-1 chain, is probably due to the facts that the incorporation of 18:1c-d_2 is only 79 mol % and that oleic acid is preferentially incorporated into the *sn*-2 position (Saito et al., 1977). In Figure 7 it is seen that DGlcDAG forms an L_α phase up to at least 70 °C. A water content of 12.9 mol of ^1H_2O/mol of lipid is above the maximum hydration for DGlcDAG (Lindblom et al., 1986).

As can be expected, the third acyl chain in MADGlcDAG gives the lipid completely different phase properties as

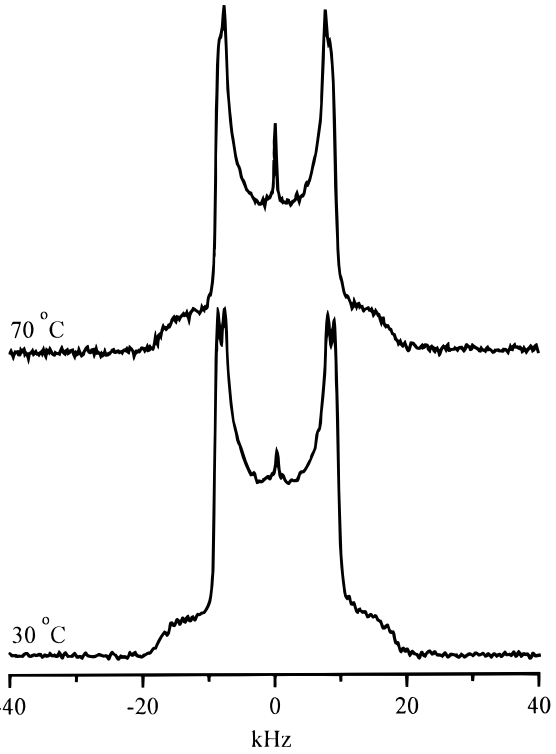

FIGURE 7: ^2H NMR spectra of DGlcDAG containing 18:1c-d_2 recorded at 30 and 70 °C. The water content of the sample was 20% (w/w) ^1H_2O.

compared to DGlcDAG. It was proven by ^2H NMR and X-ray diffraction that MADGlcDAG forms an H_{II} phase when its acyl chains melt. Figure 8A shows a ^2H NMR spectrum of MADGlcDAG recorded at 50 °C. In the same figure spectra of a total lipid extract from *A. laidlawii* are included; in Figure 8C the lipids form an L_α phase, while in Figure 8B the lipids form both an L_α phase and an H_{II} phase. The magnitude of the quadrupolar splitting indicates that MADGlcDAG forms an H_{II} phase. It was conclusively established by X-ray diffraction that an H_{II} phase was formed at 20 °C, and up to at least 60 °C, in samples with 10% (Figure 9) and 20% (w/w) water. The lattice parameters in the H_{II} phase formed at 20 °C with 10% and 20% (w/w)

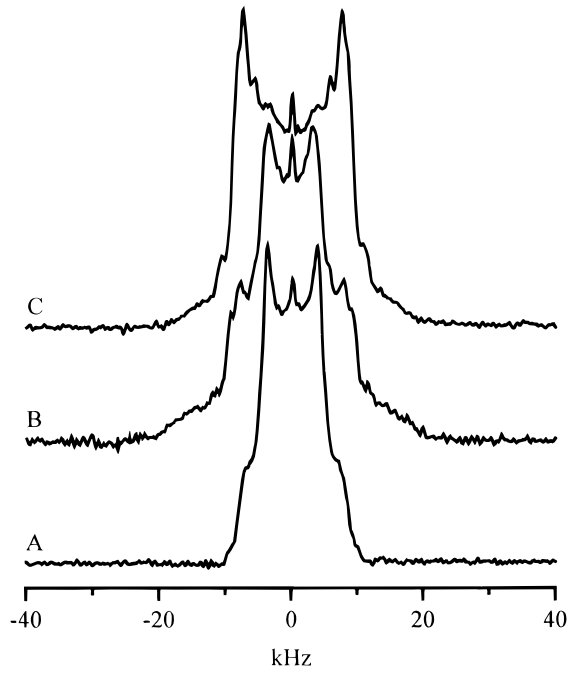

FIGURE 8: ^2H NMR spectra of MADGlcDAG and of a total lipid extract from *A. laidlawii*, both containing 18:1c-d_2. (A) MADGlcDAG with 15% (w/w) ^1H_2O, recorded at 70 °C; (B) total lipid extract with 20% (w/w) ^1H_2O, recorded at 50 °C; and (C) total lipid extract with 40% (w/w) ^1H_2O, recorded at 60 °C.

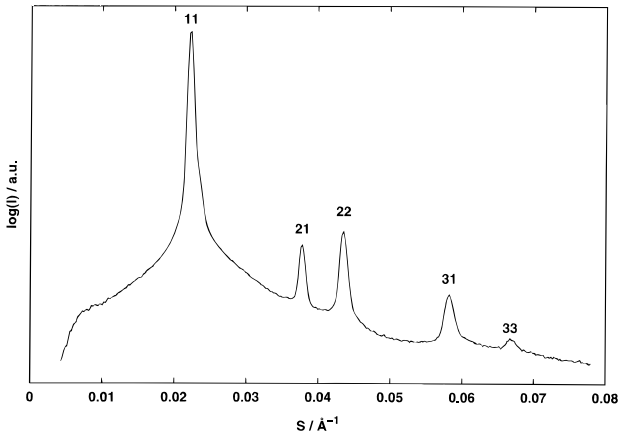

FIGURE 9: Small-angle X-ray powder diffraction pattern obtained from an MADGlcDAG−water mixture, 10% (w/w) ^1H_2O, at 25 °C. The position of the diffraction peaks indicates a hexagonal phase with a Bragg spacing of 45 Å, which gives a lattice parameter of 52 Å.

water are 52 and 57 Å, respectively. X-ray diffraction also revealed that a lamellar gel phase started to form in these samples at about 20 °C upon a decrease in temperature. The exact temperature for the disappearance of the gel phase when raising the temperature seemed to depend on the thermal history of the samples. Consequently, an H_{II} phase is the only liquid-crystalline phase formed by this preparation of MADGlcDAG up to 60 °C with water contents between 5% and 20% (w/w). DGlcDAG and MGlcDAG have their maximum hydration at 7−11 mol of ^2H_2O/mol of lipid, depending on the degree of acyl chain unsaturation (Lindblom et al., 1986). The maximum hydration of MAMGlcDAG is at the most 5 mol of ^2H_2O/mol of lipid (Lindblom et al., 1993). This clearly shows that the loss of a hydroxyl group drastically decreases the maximum hydration for a glucolipid, and MADGlcDAG is probably fully hydrated at the two highest water concentrations studied.

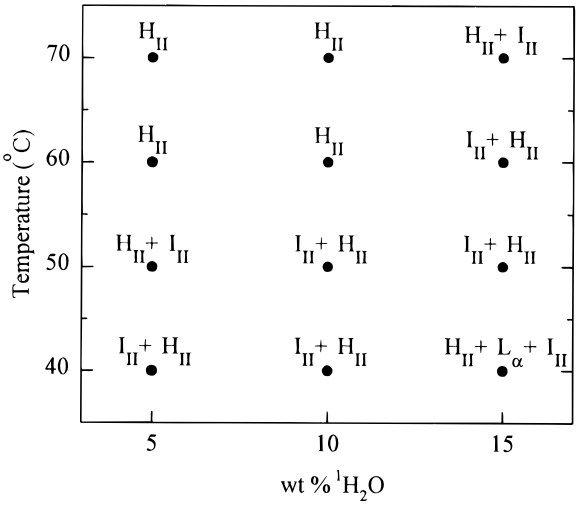

FIGURE 10: Tentative phase diagram of the system MGlcDAG-^1H_2O. The phase diagram was deduced by ^2H NMR spectroscopy and X-ray diffraction: L_α, lamellar liquid-crystalline phase; H_{II}, reversed hexagonal phase; I_{II}, reversed cubic phase. When two or three phases are in equilibrium, the notations are written in the order of a decreasing fraction of the different phases.

In most of the earlier studies of MGlcDAG this lipid has probably been contaminated by MADGlcDAG. For that reason it is of great importance to check the validity of those results (Eriksson et al., 1991; Lindblom et al., 1993; Niemi et al., 1995; Wieslander et al., 1978, 1981). Our data on the newly purified MGlcDAG are presented in a simplified phase diagram (Figure 10). When the water concentration is 5% and 10% (w/w) there is a reversed cubic (I_{II}) phase in equilibrium with an H_{II} phase at 40 °C, and when the temperature is raised the I_{II} phase disappears and only an H_{II} phase is formed at 60 °C. X-ray diffraction of the sample with 10% (w/w) water also revealed that an I_{II} phase is in equilibrium with an H_{II} phase at temperatures between 40 and 60 °C; at 60 °C the lattice parameter of the H_{II} phase is 37 Å. Moreover, X-ray diffraction showed that a lamellar gel phase is formed at temperatures lower than 40 °C. At the highest water content (15%, w/w) a mixture of H_{II}, L_α, and I_{II} phases is present at 40 °C (Figure 11). At 50 °C the L_α phase has disappeared, and the component originating from the I_{II} phase dominates the spectra recorded at this temperature and at 60 °C. However, the equilibrium is shifted back toward the H_{II} phase between 60 and 70 °C. A mixture of I_{II} and H_{II} phases was also observed by X-ray diffraction at temperatures between 40 and 60 °C; the lattice parameter of the H_{II} phase formed at higher temperatures is 49 Å. It is rather surprising that the fraction of the H_{II} phase in the sample first decreases, and then increases again, when the temperature is raised. One explanation for this behavior could be the presence of several lipid species due to the large heterogeneity of the acyl chains (Table 2). It can also be seen in Figure 10 that the I_{II} phase remains in the MGlcDAG−water samples at gradually higher temperatures when the water concentration is raised.

DISCUSSION

The present study of the regulation of the membrane lipid composition in *A. laidlawii* A-EF22 was initiated by the recent discovery of the triacylglucolipid MADGlcDAG (Hauksson et al., 1995). The combined results regarding the phase behavior, and the regulation of the synthesis, of

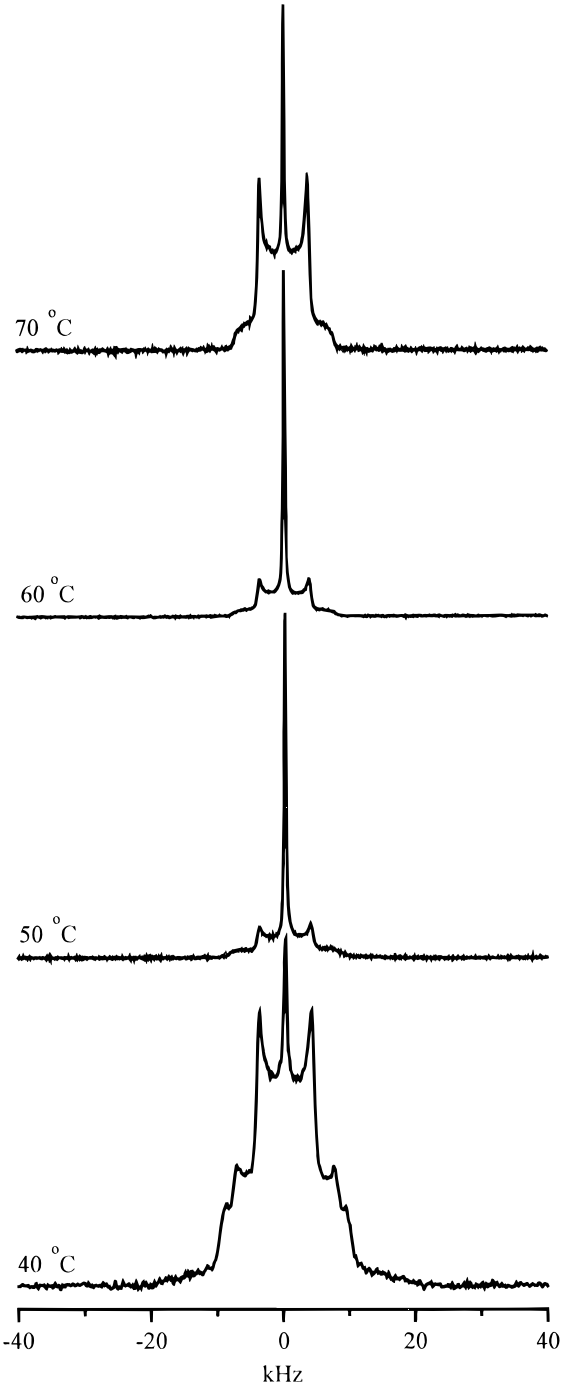

FIGURE 11: ^2H NMR spectra of MGlcDAG containing 18:1c-d_2, recorded at 40, 50, 60, and 70 °C. The water content of the sample was 15% (w/w) ^1H_2O.

MADGlcDAG resolve some earlier observations that were difficult to explain. The new results constitute an important and necessary extension of our model for membrane lipid regulation, and the validity of the model is further strengthened.

Phase Equlibria of Glucolipids. It is evident that the addition of an acyl chain to the polar head group of DGlcDAG drastically alters the phase behavior of the lipid. DGlcDAG forms an L_α phase up to at least 70 °C (Figure 7), which is in accordance with previous results (Eriksson et al., 1991; Rilfors et al., 1993). On the other hand, MADGlcDAG forms an H_{II} phase when the acyl chains melt in spite of the facts that this lipid contains nearly 20 mol % more saturated acyl chains and that the chains are on the average one carbon atom shorter (Table 2); both of these conditions generally shift the phase equilibria of membrane lipids toward the L_α phase (Rilfors et al., 1984). Therefore, the decisive difference between the lipids is that an acyl chain has been substituted for an hydroxyl group in the polar head group of DGlcDAG, which increases the hydrophobic volume and decreases the hydrophilic characteristics of MADGlcDAG. A comparison can be made with the difference in phase behavior between MGlcDAG and MAMGlcDAG; the former lipid can form L_α, I_{II}, or H_{II} phases (Figure 10) (Eriksson et al., 1991; Lindblom et al., 1986, 1993; Mannock et al., 1990; Wieslander et al., 1978), while its monoacyl derivative forms only an L_2 phase with melted acyl chains (Lindblom et al., 1993). MAMGlcDAG thus has a greater tendency than MGlcDAG to form reversed nonlamellar structures. Moreover, the lipid 1,2-di-*O*-tetradecyl-3-*O*-(3-*O*-methyl-*β*-D-glucopyranosyl)-*sn*-glycerol also forms an L_2 phase with melted acyl chains (Trouard et al., 1994), which implies that the loss of a hydroxyl group, not the addition of a large hydrophobic moiety, is the most important cause for shifting the phase equilibria of these glucosyldiacylglycerol lipids toward reversed nonlamellar structures.

A phase diagram has been presented for dioleoyl-MGlcDAG (Lindblom et al., 1986). Fortunately, this phase diagram is most probably correct since MADGlcDAG is absent in this range of degrees of unsaturation and lengths of the acyl chains according to the present study (Figure 4A). Dioleoyl-MGlcDAG forms I_{II} and H_{II} phases above 30 °C in the presence of 5%, 10%, and 15% (w/w) water. These phases are formed also by the MGlcDAG preparation studied in the present work (Figure 10). However, the I_{II} phase remains at higher temperatures with the new MGlcDAG, above all at the highest water content studied. It is therefore concluded that the ability of MGlcDAG to form reversed nonlamellar phases increases with longer and more unsaturated acyl chains. In most of the other previous studies of MGlcDAG (Eriksson et al., 1991; Lindblom et al., 1993; Niemi et al., 1995; Wieslander et al., 1978, 1981) this lipid was probably contaminated by MADGlcDAG; the fraction of MADGlcDAG could have been approximately 10%−30% in the old MGlcDAG preparations (Figures 3B and 4A) (Hauksson et al., 1995). One interesting comparison can be made between the new MGlcDAG preparation and the one examined by Lindblom et al. (1993). The two preparations have practically identical acyl chain compositions. It is noticed that the phase equilibria for the lipid preparations are very similar, and the differences are restricted to small displacements of the phase transition temperatures. Moreover, a property that MGlcDAG and MADGlcDAG, prepared from the same cell batch, have in common is that both lipids form reversed nonlamellar liquid-crystalline phases. It is therefore possible that the results obtained with the old MGlcDAG preparations are on the whole still valid.

Lipid Regulation. It is now clear that *A. laidlawii* A-EF22 synthesizes three lipids that can form reversed nonlamellar structures (MGlcDAG, MAMGlcDAG, and MADGlcDAG), and one lipid that can induce the formation of nonlamellar phases when mixed with membrane lipids (DAG). The fractions of these lipids all have a characteristic dependence on the fatty acids that are incorporated into the membrane lipids, and the regulation of the fractions of these lipids can be closely related to the phase behavior of the lipids.

MGlcDAG is synthesized under all growth conditions that have been studied so far (Figure 3B) (Rilfors et al., 1993; Wieslander et al., 1995). The fraction is kept on a comparatively low and constant level with acyl chains having $C_n > 16$ and degrees of unsaturation larger than about 40 mol %. MGlcDAG species with such acyl chains have a pronounced ability to form I_{II} and H_{II} phases [see Figure 7 in Wieslander et al. (1995)]. Moreover, for $C_n = 16-20$ the L_α to nonlamellar phase transition temperature (T_{NL}) does not change significantly for the α-anomers of MGlcDAG (Mannock et al., 1990). When $C_n \leq 16$, and the degree of saturation is higher than approximately 30 mol %, the formation of MGlcDAG is increased drastically (Figure 3B) (Thurmond et al., 1994). The cells most likely try to compensate for the increased T_{NL} value obtained for MGlcDAG with such acyl chains (Lindblom et al., 1993; Mannock et al., 1990). However, this compensation is not sufficient and the cells synthesize above all MADGlcDAG and also DAG and small amounts of MAMGlcDAG; all of these lipids lower the T_{NL} value of membrane lipids (Das & Rand, 1986; De Boeck & Zidovetzki, 1989; Niemi et al., 1995; Siegel et al., 1989).

The finding that MADGlcDAG is synthesized with short acyl chains (Figure 4A) makes it considerably easier to interpret the biochemical data obtained with these chains by means of our model for lipid regulation; the nonbilayer-forming tendency of total lipid extracts from *A. laidlawii* A-EF22 (Lindblom et al., 1986; Rilfors et al., 1994; Österberg et al., 1995) is probably sustained by MADGlcDAG under these conditions. MADGlcDAG has a greater ability than MGlcDAG to form nonlamellar phases since it forms only an H_{II} phase when its acyl chains are melted. This implies that MGlcDAG is assisted, or replaced, by a potent nonlamellar-forming glucolipid also when short, unsaturated acyl chains are incorporated into the lipids; the syntheses of DAG and MAMGlcDAG is in effect shut off with these chains (Figure 3A). However, it can only be speculated about the importance of this assistance or replacement, since the phase behavior of MGlcDAG with short, unsaturated chains is not known. The regulation of the fraction of MADGlcDAG explains why no consequent changes in the fraction of MGlcDAG were observed when the extent of acyl chain unsaturation was varied in a previous study (Wieslander et al., 1995). The apparent *increase* in the fraction of MGlcDAG with acyl chains having $C_n \approx 15-16$ depended on MGlcDAG and MADGlcDAG being quantified together in that work.

The fractions of MAMGlcDAG and DAG are increased concomitantly with the reduction of the fraction of MADGlcDAG. Among the *A. laidlawii* lipids MAMGlcDAG has by far the most pronounced ability to form nonlamellar structures since it does not form any liquid-crystalline phase at all but only an L_2 phase with melted acyl chains (Lindblom et al., 1993). At low concentrations, MAMGlcDAG is far more capable than MGlcDAG to induce nonlamellar phases in *A. laidlawii* lipids (Niemi et al., 1995). MAMGlcDAG and DAG thus help MGlcDAG to sustain the nonbilayer-forming tendency when the degree of acyl chain saturation is higher than 40−50 mol % and $C_n \geq 16$. The high T_{NL} values of MGlcDAG with long, saturated acyl chains (Mannock et al., 1990) most probably necessitate this assistance.

When discussing the lipid regulation in *A. laidlawii* A-EF22, and in particular the fraction of nonlamellar-forming lipids, it is evident that the sum of the fractions of MAMGlcDAG, MGlcDAG, and MADGlcDAG must be taken into consideration. A graph of this total fraction of nonlamellar-forming lipids shows that it is reduced from 55 to 7 mol % when the average acyl chain length and the degree of unsaturation are increased (Figure 6), i.e., at conditions that favor reversed nonlamellar phases (Rilfors et al., 1984). These plots convincingly show that a balance between lamellar- and nonlamellar-forming lipids is maintained in the membrane and strongly support the validity of our lipid regulation model. This regulation of the balance between lamellar- and nonlamellar-forming lipids appears even more complex and sophisticated after the discovery of MADGlcDAG, since four lipids (including DAG) act together to maintain the balance.

It should be mentioned that GPDGlcDAG, which is synthesized by both A and B strains of *A. laidlawii*, can by itself also form a nonlamellar phase. However, this lipid forms a *normal* micellar solution phase (L_1). The results from two different studies of GPDGlcDAG are not fully consistent: one study shows that only micelles form at water contents of 95% (w/w) and higher (Lewis & McElhaney, 1995), while the other study shows that micelles are in equilibrium with an L_α phase at these water contents (D. Danino, A. Kaplun, G. Lindblom, L. Rilfors, G. Orädd, J. B. Hauksson, and Y. Talmon, submitted for publication). Among the membrane lipids occurring in strain B-PG9 of *A. laidlawii* GPDGlcDAG has the strongest ability to transform an H_{II} phase formed by dielaidoylphosphatidylethanolamine to an L_α phase (Foht et al., 1995). This conclusion most probably holds also for strain A-EF22 since the other glycerophosphoryl derivative of DGlcDAG synthesized by the A strain, MABGPDGlcDAG, does not form an L_1 phase (Danino et al., submitted for publication). If both strain A-EF22 and strain B-PG9 are considered, it does not appear that the fraction of GPDGlcDAG is consistently regulated in relation to the phase behavior of the lipid. On one hand, the elevated fraction of GPDGlcDAG with long and cis-unsaturated acyl chains (Figure 5) (Lewis & McElhaney, 1995) might be interpreted as a regulatory response to the increased tendencies to form reversed nonlamellar phases. On the other hand, the progressively larger fractions of GPDGlcDAG present when the B strain is grown with iso methyl-branched acyl chains of decreasing length (Lewis & McElhaney, 1995) cannot be explained by our lipid regulation model.

Comparison of A and B Strains. The regulation of the membrane lipid composition has also been studied in two B strains, B-JU and B-PG9, and differences and similarities between A and B strains have been discussed in the literature [for reviews, see McElhaney (1992) and Rilfors et al. (1993)]. Strain B-JU seems to consistently regulate its lipid composition according to the hypothesis proposed by us. However, the experiments were performed before it was known that MAMGlcDAG forms an L_2 phase and before the discovery of MADGlcDAG. Results from strain B-PG9 are not fully compatible with the regulation hypothesis (Bhakoo & McElhaney, 1988; McElhaney, 1992; Silvius et al., 1980). McElhaney and colleagues have concluded that neither the bilayer−nonbilayer phase preference of its membrane lipids nor the fraction of anionic lipids is biochemically regulated

in a coherent manner in this B strain; it should be pointed out, however, that the anionic lipid fraction included only PG in these studies. On the other hand, when strains A-EF22 and B-PG9 were grown in the two media used for these bacteria, in most cases, it was observed that both strains regulate the balance between bilayer-forming and nonbilayer-forming lipids in a similar way but that the B strain exhibited a weaker response in the anionic lipid fraction (Wieslander et al., 1994). The striking feature of strain A-EF22, to synthesize several nonbilayer-forming lipids that complement each other in a subtle way, seems also to be a property of strain B-PG9. This strain produces large quantities of 1-*O*-polyprenyl-2-*O*-acyl-α-D-glucopyranoside (PAGlc), mainly at the expense of MGlcDAG, when it is grown in media enriched in high-melting-point fatty acids (Lewis et al., 1990). Both the lamellar gel phase−L_α and the L_α−H_{II} phase transition temperatures of PAGlc are lower than those of MGlcDAG, implying that the B strain utilizes PAGlc to regulate these quantities. Furthermore, strain B-PG9 synthesizes MADGlcDAG, and the fraction of this lipid might be regulated also in the B strain.

Lipid Regulation in Other Organisms. Changes in the polar head group structure and changes in the acyl chain structure are two strategies used by organisms to adapt their membrane lipid composition to various environmental and physiological conditions (Hazel & Williams, 1990; Suutari & Laakso, 1994). Besides *A. laidlawii* two species of the bacterial genus *Clostridium* have been shown to regulate the polar head group composition in response to the fatty acids that are incorporated into the lipids, and it was suggested that the *Clostridium* species also maintain a certain balance between bilayer-forming and nonbilayer-forming lipids (Goldfine et al., 1987a,b; Johnston & Goldfine, 1992). In contrast to *A. laidlawii* and the *Clostridium* species, some bacterial genera have a considerably simpler setup of lipids. For example, the membrane lipids in *Escherichia coli*, *Bacillus megaterium*, and *Pseudomonas fluorescens* are phosphatidylethanolamine (PE), PG, and diphosphatidylglycerol. PE is the predominant lipid in all three species: its fraction does not change significantly with the growth temperature, and it has the strongest propensity to form I_{II} and H_{II} phases. When the growth temperature is increased, the acyl chain structure is changed in such a way that the ability of PE to form I_{II} and H_{II} phases is reduced (Koynova & Caffrey, 1994; Rilfors et al., 1994) and the effect of the increased temperature is thus counteracted (Cullen et al., 1971; Morein et al., 1996; Rilfors et al., 1994). It was recently shown that wild-type cells of *E. coli* regulate the degree of acyl chain unsaturation in order to avoid the formation of both a lamellar gel phase and reversed nonlamellar phases (Morein et al., 1996).

A. laidlawii has also been shown to be subjected to the restrictions described for *E. coli* (Lindblom et al., 1986; Lindblom & Rilfors, 1989), and in fact all living organisms are. A difference between *E. coli*, *B. megaterium*, and *P. fluorescens* on one hand and *A. laidlawii* and the *Clostridium* species on the other hand is that the first group of organisms regulates the temperature for the transition between lamellar gel and L_α phases (T_m) and the T_{NL} values, in the same direction due to changes in the acyl chain structure (Morein et al., 1996), while the second group of organisms often regulate the T_{NL} value in the direction expected from the regulation model but concomitantly change the T_m value in a nonfunctional direction (MacDonald & Goldfine, 1991; Rilfors et al., 1993, 1994; Wieslander et al., 1986).

Eukaryotic organisms, like fungi, higher plants, and poikilothermic animals, often change their membrane lipid composition in response to changes in the ambient temperature (Hazel & Williams, 1990; Hazel, 1995). It has been suggested that the lipid regulation in these organisms take place according to a model that has the same features as the regulation model suggested for *E. coli* and *A. laidlawii*; it is implied that the temperature acclimation or adaptation modifies both the T_m and the T_{NL} values so that the ambient temperature remains at a suitable interval above T_m but yet below T_{NL} (Hazel, 1995).

ACKNOWLEDGMENT

Susann Haraldsson and Seija Holma are acknowledged for performing the large-scale cultivations of *A. laidlawii* on α-deuterated oleic acid and for preparing a total glucolipid fraction from this organism. We thank Gösta Arvidson for making the α-deuterated oleic acid and Eva Selstam for putting her GLC equipment at our disposal. Eva Wikström is acknowledged for preparing Figure 1. We also thank Patrick Williams, Department of Biochemistry, Kings College, London, for his assistance during the operation of the SAXS machine at the Daresbury Laboratory. Marie South-Wångdahl at Scotia LipidTeknik, Stockholm, Sweden, is acknowledged for performing the HPLC analyses for determination of the molar response factors.

REFERENCES

Arnoldsson, K. C., & Kaufmann, P. (1994) *Chromatographia 38*, 317−324.
Bhakoo, M., & McElhaney, R. N. (1988) *Biochim. Biophys. Acta 945*, 307−314.
Christiansson, A., & Wieslander, Å. (1978) *Eur. J. Biochem. 85*, 65−76.
Christiansson, A., Eriksson, L. E. G., Westman, J., Demel, R., & Wieslander, Å. (1985) *J. Biol. Chem. 260*, 3984−3990.
Cullen, J., Phillips, M. C., & Shipley, G. G. (1971) *Biochem. J. 125*, 733−742.
Das, S., & Rand, R. P. (1986) *Biochemistry 25*, 2882−2889.
Davis, J. H., Jeffrey, K. R., Bloom, M., Valic, M. I., & Higgs, T. P. (1976) *Chem. Phys. Lett. 44*, 390−394.
De Boeck, H., & Zidovetzki, R. (1989) *Biochemistry 28*, 7439−7446.
Eriksson, P.-O., Rilfors, L., Wieslander, Å., Lundberg, A., & Lindblom, G. (1991) *Biochemistry 30*, 4916−4924.
Foht, P. J., Tran, Q. M., Lewis, R. N. A. H., & McElhaney, R. N. (1995) *Biochemistry 34*, 13811−13817.
Goldfine, H., Johnston, N. C., Mattai, J., & Shipley, G. G. (1987a) *Biochemistry 26*, 2814−2822.
Goldfine, H., Rosenthal, J. J. C., & Johnston, N. C. (1987b) *Biochim. Biophys. Acta 904*, 283−289.
Hauksson, J. B., Lindblom, G., & Rilfors, L. (1994a) *Biochim. Biophys. Acta 1214*, 124−130.
Hauksson, J. B., Lindblom, G., & Rilfors, L. (1994b) *Biochim. Biophys. Acta 1215*, 341−345.
Hauksson, J. B., Rilfors, L., Lindblom, G., & Arvidson, G. (1995) *Biochim. Biophys. Acta 1258*, 1−9.
Hazel, J. R. (1995) *Annu. Rev. Physiol. 57*, 19−42.
Hazel, J. R., & Williams, E. E. (1990) *Prog. Lipid Res. 29*, 167−227.
Johnston, N. C., & Goldfine, H. (1992) *J. Bacteriol. 174*, 1848−1853.
Koynova, R., & Caffrey, M. (1994) *Chem. Phys. Lipids 69*, 1−34.
Lewis, R. N. A. H., & McElhaney, R. N. (1995) *Biochemistry 34*, 13818−13824.

Lewis, R. N. A. H., Yue, A. W. B., McElhaney, R. N., Turner, D. C., & Gruner, S. M. (1990) *Biochim. Biophys. Acta 1026*, 21−28.
Lindblom, G. (1996) in *Advances in Lipid Methodology* (Christie, W. W., Ed.) Vol. 3, pp 133−209, The Oily Press Ltd., Dundee, Scotland.
Lindblom, G., & Rilfors, L. (1989) *Biochim. Biophys. Acta 988*, 221−256.
Lindblom, G., Brentel, I., Sjölund, M., Wikander, G., & Wieslander, Å. (1986) *Biochemistry 25*, 7502−7510.
Lindblom, G., Hausson, J. B., Rilfors, L., Bergenståhl, B., Wieslander, Å., & Eriksson, P.-O. (1993) *J. Biol. Chem. 268*, 16198−16207.
MacDonald, D. L., & Goldfine, H. (1991) *Appl. Environ. Microbiol. 57*, 3517−3521.
Mannock, D. A., Lewis, R. N. A. H., & McElhaney, R. N. (1990) *Biochemistry 29*, 7790−7799.
McElhaney, R. N. (1992) in *Mycoplasmas: Molecular Biology and Pathogenesis* (Maniloff, J., McElhaney, R. N., Finch, L. R., & Baseman, J. B., Eds.) pp 113−155, American Society for Microbiology, Washington, DC.
Morein, S., Andersson, A.-S., Rilfors, L., & Lindblom, G. (1996) *J. Biol. Chem. 271*, 6801−6809.
Niemi, A., Rilfors, L., & Lindblom, G. (1995) *Biochim. Biophys. Acta 1239*, 186−194.
Österberg, F., Rilfors, L., Wieslander, Å., Lindblom, G., & Gruner, S. M. (1995) *Biochim. Biophys. Acta 1257*, 18−24.
Panos, C., & Rottem, S. (1970) *Biochemistry 9*, 407−412.
Rance, M., Smith, I. C. P., & Jarrell, H. C. (1983) *Chem. Phys. Lipids 32*, 57−71.
Razin, S. (1978) *Microbiol. Rev. 42*, 414−470.
Rilfors, L. (1985) *Biochim. Biophys. Acta 813*, 151−160.
Rilfors, L., Wieslander, Å., & Ståhl, S. (1978) *J. Bacteriol. 135*, 1043−1052.
Rilfors, L., Lindblom, G., Wieslander, Å., & Christiansson, A. (1984) *Biomembranes 12*, 205−245.
Rilfors, L., Wieslander, Å., & Lindblom, G. (1993) in *Subcellular Biochemistry* (Rottem, S., & Kahane, I., Eds.) Vol. 20, pp 109−166, Plenum Press, New York.
Rilfors, L., Hauksson, J. B., & Lindblom, G. (1994) *Biochemistry 33*, 6110−6120.
Saito, Y., Silvius, J. R., & McElhaney, R. N. (1977) *Arch. Biochem. Biophys. 182*, 443−454.
Seelig, J., & Seelig, A. (1980) *Q. Rev. Biophys. 13*, 19−61.
Siegel, D. P., Banschbach, J., & Yeagle, P. L. (1989) *Biochemistry 28*, 5010−5019.
Silvius, J. R., Mak, N., & McElhaney, R. N. (1980) *Biochim. Biophys. Acta 597*, 199−215.
Smaal, E. B., Romijn, D., Geurts van Kessel, W. S. M., De Kruijff, B., & De Gier, J. (1985) *J. Lipid Res. 26*, 634−637.
Suutari, M., & Laakso, S. (1994) *CRC Crit. Rev. Microbiol. 20*, 285−328.
Thurmond, R. L., Niemi, A. R., Lindblom, G., Wieslander, Å., & Rilfors, L. (1994) *Biochemistry 33*, 13178−13188.
Towns-Andrews, E., Berry, A., Bordas, J., Mant, G. R., Murray, P. K., Roberts, K., Sumner, I., Worgan, J. S., & Lewis, R. (1989) *Rev. Sci. Instrum. 60*, 2346−2349.
Trouard, T. P., Mannock, D. A., Lindblom, G., Rilfors, L., Akiyama, M., & McElhaney, R. N. (1994) *Biophys. J. 67*, 1090−1100.
Tulloch, A. P. (1977) *Lipids 12*, 92−98.
Wells, M. A., & Dittmer, J. C. (1963) *Biochemistry 2*, 1259−1263.
Wieslander, Å., & Rilfors, L. (1977) *Biochim. Biophys. Acta 466*, 336−346.
Wieslander, Å., Ulmius, J., Lindblom, G., & Fontell, K. (1978) *Biochim. Biophys. Acta 512*, 241−253.
Wieslander, Å., Christiansson, A., Rilfors, L., & Lindblom, G. (1980) *Biochemistry 19*, 3650−3655.
Wieslander, Å., Rilfors, L., Johansson, L. B.-Å., & Lindblom, G. (1981) *Biochemistry 20*, 730−735.
Wieslander, Å., Rilfors, L., & Lindblom, G. (1986) *Biochemistry 25*, 7511−7517.
Wieslander, Å., Rilfors, L., Dahlqvist, A., Jonsson, J., Hellberg, S., Rännar, S., Sjöström, M., & Lindblom, G. (1994) *Biochim. Biophys. Acta 1191*, 331−342.
Wieslander, Å., Nordström, S., Dahlqvist, A., Rilfors, L., & Lindblom, G. (1995) *Eur. J. Biochem. 227*, 734−744.
Ziminski, T., & Borowski, E. (1966) *J. Chromatogr. 23*, 480−482.

BI960561W