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Lipids in total extracts from *Acholeplasma laidlawii* A pack more closely than the individual lipids. Monolayers studied at the air–water interface

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Abstract

Pressure–area curves were obtained at 25, 35 and 45°C for total lipid extracts and four individual glucolipids isolated from *Acholeplasma laidlawii* strain A-EF22. The glucolipids are 1,2-diacyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol (MGlcDAG), 1,2-diacyl-3-*O*-[α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl]-*sn*-glycerol (DGlcDAG), 1,2-diacyl-3-*O*-[α-D-glucopyranosyl-(1 → 2)-*O*-(6-*O*-acyl-α-D-glucopyranosyl)]-*sn*-glycerol (MADGlcDAG), and 1,2-diacyl-3-*O*-[glycerophosphoryl-6-*O*-(α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl)]-*sn*-glycerol (GPDGlcDAG). The total lipid extracts were obtained from *A. laidlawii*, grown at 37°C with fatty acids of varying degrees of unsaturation and chain length. The mean surface area per molecule was obtained from these pressure–area curves at surface pressures equal to 10, 20, 30 and 40 mN/m. It was found that the interfacial area of the lipids increases with increasing degree of unsaturation, but is nearly independent of the acyl chain length at constant unsaturation. The surface charge density varied between 4.7×10^{-3} $e^-/\text{Å}^2$ and 9.4×10^{-3} $e^-/\text{Å}^2$ for the total lipid extracts studied, but did not exhibit any consistent dependence on variations in degree of unsaturation or acyl chain length. The mean area per molecule was found to be smaller for the total lipid extracts than for the individual lipids. It is concluded that the bacterium strives to regulate its lipid composition in such a way that the packing of the lipids in the membrane is appropriately tight, and/or to keep a slight negative spontaneous curvature of the lipid bilayer of the cell membrane (''optimal packing''). This is in accordance with the physico-chemical model for the regulation of the lipid composition in the membrane of *A. laidlawii* previously presented by us (see e.g. Andersson, A.-S., Rilfors, L., Bergqvist, M., Persson, S. and Lindblom, G. (1996) Biochemistry 35, 11119–11130). © 1998 Elsevier Science B.V.

Keywords: Monolayer; Molecular area; Chain length; Unsaturation; Lipid; *(Acholeplasma laidlawii)*

1. Introduction

It is well established that membrane lipids generally can self-assemble into different aggregate structures, such as those found in normal (L_1) and reversed (L_2) micellar solutions, and in liquid crystalline phases, like the lamellar (L_α), cubic, normal hexagonal (H_I), and reversed hexagonal (H_{II}) phases [1,2]. Moreover, several studies indicate that membrane lipids are involved in the function of the cell membranes more extensively than previously thought

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[1,3–7]. It is also well known, from a large number of investigations on plasma membranes from different organisms, that they adapt their lipid composition to the prevailing environmental and physiological conditions [8–22]. Much of this information has been accumulated from investigations of bacterial membranes, in particular the plasma membrane of the parasitic organism *Acholeplasma laidlawii* [8,9,19–21] and membranes of one of the foremost prokaryotic model organisms, namely *Escherichia coli* [7,10–12,22,23]. Almost two decades ago, we presented a physico-chemical model which states that the lipid composition in the cell membrane is determined by the balance between lipids forming lamellar and nonlamellar phases ([19] and references therein). Obviously, the physico-chemical properties of the membrane lipids play an important role, but *how* these are coupled to the different membrane functions pursued by the lipids, or *why* the lipid regulation in terms of this lamellar/nonlamellar balance is needed, are questions that still remain unanswered. It has been observed that the activity of some membrane proteins does depend on the presence of lipids forming nonlamellar structures, but the underlying mechanisms remain to be found. Several workers have speculated that the curvature stress or "frustration" created by these lipids may trigger some membrane enzymes. Recently, another interesting approach was taken by Cantor, who suggested that a lateral pressure *gradient* across the membrane might affect the activity of a protein [24,25]. A related idea from studies on *A. laidlawii* and *E. coli* was put forward by us, suggesting that an "optimal" packing of the lipids in the cell membrane plays a crucial role [1,9,12,19,26]. This simple idea arose from the experimental findings that the total lipid extracts from the bacterial plasma membrane always show a phase transition between a lamellar and a nonlamellar state at a defined temperature range above the growth temperature ([9,12,21,27,28], Andersson et al., submitted). Furthermore, both *A. laidlawii* and *E. coli* always seem to prefer to grow in such a way that the phase state of the membrane lipids is confined to a "window", bounded by the gel phase and nonlamellar liquid crystalline phases [1,9,12]. Since membrane curvature, lateral pressure and acyl chain ordering are dependent on the packing of the lipid molecules, it is of great interest to get further, and possibly more detailed, information about the packing properties of the membrane lipids. Armed with such data, we will then hopefully be able, at least qualitatively, to understand the mechanical features and physico-chemical properties of a lipid bilayer at the molecular level. Unfortunately, packing properties of lipids are difficult to measure accurately in a direct and quantitative way for a cell membrane, and we will have to rely on estimates and more or less indirect determinations. However, from a study of monomolecular layers of lipids at the air–water interface information about lipid packing densities and lipid–lipid interactions may be obtained. In this work, we present such data for lipids extracted from the membrane of *A. laidlawii*.

2. Materials and methods

2.1. Growth of A. laidlawii

All the purified lipids and the total lipid extracts were isolated from membranes of *Acholeplasma laidlawii* strain A-EF22. The total lipid extracts used in this study are those prepared by Andersson et al. [19]. However, not all of the extracts from that study were utilized. The preparations of 1,2-diacyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol (MGlcDAG), 1,2-diacyl-3-*O*-[α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl]-*sn*-glycerol (DGlcDAG), 1,2-diacyl-3-*O*-[α-D-glucopyranosyl-(1 → 2)-*O*-(6-*O*-acyl-α-D-glucopyranosyl)]-*sn*-glycerol (MADGlcDAG), and 1,2-diacyl-3-*O*-[glycerophosphoryl-6-*O*-(α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl)]-*sn*-glycerol (GPDGlcDAG) were also the ones obtained by Andersson et al. [19]. *A. laidlawii* was grown in media which force the cells to incorporate the supplemented fatty acids into its membrane lipids. The supplemented fatty acids are presented in Tables 1 and 2.

2.2. Lipid extraction and purification of lipids

The lipids were extracted from the cell pellets and the nonlipid contaminants were removed [19]. In order to isolate the lipids from the total lipid extracts, column chromatography and preparative thin-layer chromatography (TLC) were used [19]. The purity was determined to be ≥ 97% for MGlcDAG, ≥ 99%

Table 1
Acyl chain composition (mol%) in total lipid extracts from *Acholeplasma laidlawii* strain A-EF22 grown at 37°C

Lipid extract	Fatty acid [a] supplement	Acyl chain composition											
	(μM/μM)	12:0	13:0	14:0	15:0	16:0	16:1c	18:0	18:1c	20:0	n.d. [b]	C_n [c]	UAC [d]
1	14:0/16:1c (110/40)	—	—	56.8	—	3.0	33.3	3.1	—	1.4	2.5	15.0	33.3
2	14:0/16:1c (75/75)	0.6	—	49.2	—	1.5	45.4	0.7	2.4	—	0.2	15.1	47.8
3	14:0/16:1c (75/75)	1.0	0.6	46.3	0.4	2.6	44.6	0.4	4.0	—	—	15.1	48.6
4	16:0/16:1c (120/30)	—	—	0.2	—	68.2	29.6	0.8	1.0	—	0.1	16.0	30.6
5	16:0/16:1c (120/30)	—	—	0.9	0.8	71.9	25.2	0.4	0.7	—	—	16.0	26.0
6	16:0/16:1c (75/75)	0.3	—	0.4	—	38.0	57.8	0.3	3.1	—	—	16.1	61.0
7	16:0/16:1c (30/120)	—	—	0.4	—	24.3	71.9	0.5	3.0	—	—	16.1	74.8
8	16:1c (150)	1.6	0.7	5.2	0.6	5.5	29.7	0.4	56.2	—	—	16.9	86.0
9	18:0/18:1c (120/30)	1.7	0.9	2.2	0.6	1.6	—	58.1	34.9	—	—	17.7	34.9
10	18:0/18:1c (120/30)	1.4	1.1	3.6	2.0	3.8	—	45.4	42.1	—	0.7	17.6	42.1
11	18:0/18:1c (75/75)	1.4	1.7	5.3	2.4	6.0	—	38.5	43.9	—	0.8	17.4	43.9
12	18:1c (150)	0.6	0.6	2.3	0.9	2.3	—	0.3	92.0	—	1.0	17.8	92.0
13	18:1c (150)	0.8	1.0	3.9	2.7	8.0	—	0.5	79.6	—	3.3	17.5	79.6
14	20:0/18:1c (75/75)	1.8	1.3	3.9	1.3	3.4	—	1.0	54.3	32.9	0.1	18.2	54.3

[a] Fatty acids and acyl chains are denoted as $n:k$, where n is the number of carbons and k the number of double bonds.
[b] Not determined, or acyl chains in minor amounts.
[c] Average acyl chain length.
[d] Unsaturated acyl chains (mol%).

for DGlcDAG, ≥ 98% for MADGlcDAG, and ≥ 97% for GPDGlcDAG, as judged by TLC. Divalent cations were removed and exchanged for sodium ions by a modified version [21] of the procedure described by Smaal et al. [29]. This procedure was performed on all the total lipid extracts and purified lipids used in this study.

2.3. Composition of total lipid extracts and purified lipids

The acyl chain compositions in the total lipid extracts and the purified lipids (Tables 1 and 2), and the polar head group compositions in the total lipid extracts (Table 3), were analyzed by gas–liquid chromatography (GLC) and high performance liquid chromatography (HPLC), respectively [19]. We would like to correct an error in Table 1 in Andersson et al. [19]. When the growth medium was supplemented with 75 μM arachidic acid (20:0) and 75 μM oleic acid (18:1c), the average acyl chain length should be 18.2 instead of 18.8. The correct value is presented in Table 1 in this work. There was too little material remaining of the lipid extract from the cells that were supplemented with 110 μM myristic acid (14:0) and 40 μM palmitoleic acid (16:1c) from the study by

Table 2
Acyl chain composition (mol%) in purified lipids isolated from *Acholeplasma laidlawii* strain A-EF22 grown at 30°C. The growth medium was supplemented with 124–150 μM α-deuterated oleic acid (18:1c–d_2)

Lipid	Acyl chain [a] composition										
	12:0	13:0	14:0	15:0	16:0	17:0	18:0	18:1c–d_2	n.d. [b]	C_n [c]	UAC [d]
MGlcDAG	—	1.7	9.5	7.7	19.1	1.2	0.8	58.3	1.7	16.9	58.3
DGlcDAG	0.1	0.8	5.0	3.7	8.8	0.5	0.4	78.9	1.8	17.5	78.8
MADGlcDAG	10.0	5.7	12.5	4.0	6.1	0.1	0.3	59.7	1.6	16.3	59.7
GPDGlcDAG	5.3	3.4	10.9	4.6	10.4	—	0.3	65.1	—	16.7	65.1

[a] Acyl chains are denoted as $n:k$, where n is the number of carbons and k the number of double bonds.
[b] Not determined.
[c] Average acyl chain length.
[d] Unsaturated acyl chains (mol%).

Table 3
Polar head group composition (mol%) in total lipid extracts from *Acholeplasma laidlawii* strain A-EF22 grown at 37°C

Lipid extract	DAG [a]	MGlcDAG	MAMGlcDAG	DGlcDAG	MADGlcDAG	PG	GPDGlcDAG	MABGPDGlcDAG	n.d. [b]
1	15.6	35.4	—	3.6	9.0	11.6	3.5	20.8	0.4
2	—	25.0	—	12.1	13.0	11.4	19.0	18.4	1.1
3	0.2	30.2	—	13.6	18.8	14.3	11.7	9.9	1.2
4	13.7	35.3	1.6	21.4	2.2	10.3	14.1	1.5	—
5	8.2	22.1	4.4	21.5	3.5	24.6	9.7	5.9	—
6	—	21.6	—	26.1	10.4	27.3	8.4	6.2	—
7	0.7	4.3	—	30.8	21.4	21.9	11.5	9.4	—
8	0.8	5.1	—	35.7	4.2	35.9	13.6	4.3	0.4
9	14.6	15.8	3.7	33.2	1.1	16.0	14.9	0.6	0.2
10	5.4	8.2	0.5	38.0	3.1	34.4	8.8	1.3	0.3
11	3.4	5.6	7.3	45.2	4.7	20.6	12.7	0.5	—
12	—	6.6	—	40.2	—	22.1	31.2	—	—
13	—	4.6	—	54.4	3.2	15.1	21.2	—	1.4
14	3.0	4.1	0.3	45.0	0.8	23.4	20.9	0.8	—

[a] Lipid abbreviations, see main text.
[b] Not determined.

Andersson et al. [19] to perform the monolayer experiments. Consequently, a new extract had to be prepared with this specific fatty acid combination. This extract was prepared as described previously [19], and the cations were exchanged by sodium ions as described earlier.

2.4. Monolayer technique

Interfacial measurements were performed in a thermostatically controlled box at the appropriate temperatures with an accuracy better than ± 0.1°C [30,31]. The surface pressure was measured with the Wilhelmy plate method using a Cahn 2000 electrobalance.

Monomolecular layers were spread from a chloroform–methanol (8:2) solution into a subphase of milli-Q-water. Thereafter, 50 nmol of lipid were spread on a surface area of 613.28 cm^2. The compression rate was 86.50 cm^2/min. The reproducibility of the area determinations was better than 0.01 nm^2 per molecule. The stability of the monolayer films for both GPDGlcDAG and some total lipid extracts were investigated at different surface pressures as a function of time.

3. Results

The average molecular areas were obtained from experimental pressure–area (π–A) curves of membrane lipids from *A. laidlawii* grown with fatty acids of different chain length and degrees of unsaturation. Investigations were performed on total lipid extracts, as well as on the individual membrane lipids MGlcDAG, DGlcDAG, MADGlcDAG and GPDGlcDAG. The measurements on the monolayer films were performed at 25, 35 and 45°C and the surface pressures were 10, 20, 30 and 40 mN/m. The π–A-curves for all the lipids and lipid mixtures are characteristic for monolayers in a liquid expanded phase.

3.1. Individual lipids

The values of the molecular areas for the individual lipids decreased in the following order: MADGlcDAG > DGlcDAG > GPDGlcDAG > MGlcDAG at all the surface pressures and temperatures studied (Fig. 1(A) and (B)). At 35°C and 30 mN/m, the area per molecule is 0.85, 0.76, 0,72 and 0.65 nm^2, respectively.

3.2. Total lipid extracts

The average molecular areas of the total lipid extracts are plotted in Fig. 1(A) as a function of the degree of unsaturation in the acyl chains, and in Fig. 1(B) as a function of the average acyl chain length. It can be inferred from these figures that the mean molecular area increases with increasing unsaturation,

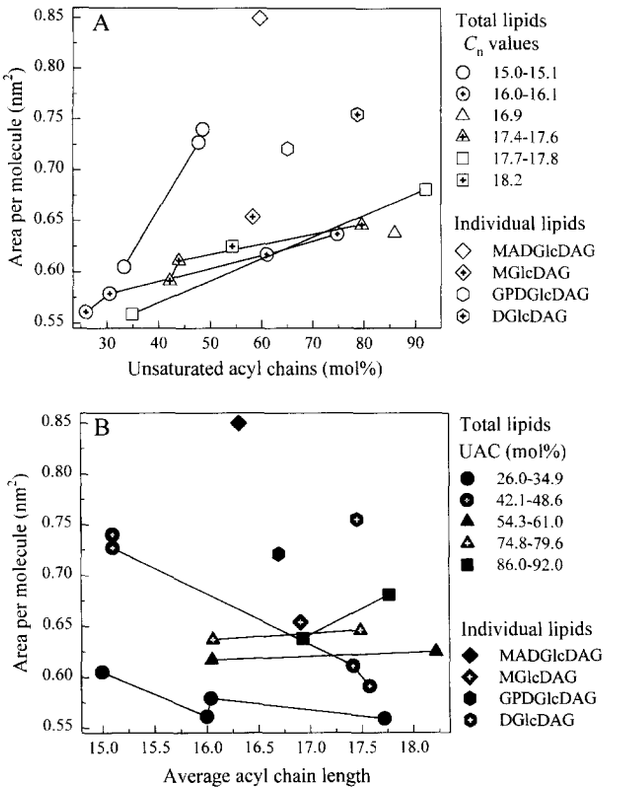

Fig. 1. Monolayer studies performed with total lipid extracts and individual purified lipids from *A. laidlawii* strain A-EF22. The measurements were performed at a surface pressure of 30 mN/m and at 35°C: (A) – The mean area per molecule expressed as a function of the fraction of unsaturated acyl chains (UAC); and (B) – The mean area per molecule expressed as a function of the average acyl chain length (C_n).

while it is relatively independent of the chain length at constant unsaturation. The data shown in Fig. 1(A) and (B) were obtained at 35°C and a surface pressure of 30 mN/m. The same results were obtained in this kind of plots at all temperatures and pressures studied. Two total lipid extracts were also investigated with a subphase containing 10 mM Tris, pH 7.4; however, no significant differences were observed.

Blume [32] established for phospholipids that ≈ 30 mN/m is the surface pressure at which monolayer systems behave in a similar way as bilayer systems. Furthermore, the best agreement between monolayers and bilayers, as judged from the function of anesthetics and phosholipases, is found at a surface pressure of 30–35 mN/m [33,34]. Two of the total lipid extracts have similar acyl chain compositions as two of the *A. laidlawii* total lipid extracts examined by the monolayer technique by Christiansson et al. [35]. Their measurements were performed at 22°C and at a pressure of 35 mN/m and can be compared with the present results obtained at 25°C and 30 mN/m. The average area 0.63 nm^2 per molecule for extract 13 in Table 1 is compared to the area 0.64–0.65 nm^2 for the extract with an average acyl chain length (C_n) ≈ 17.5 and ≈ 74 mol% unsaturated acyl chains (UAC). A similar comparison can be made between extract 12 in Table 1 and an extract in the former study with $C_n \approx 17.7$ and ≈ 87 mol% UAC, for which the areas are 0.66 and 0.68–0.69 nm^2, respectively. The values are almost identical, even though the neutral fractions had been removed from the total lipid extracts in the former study.

It can also be seen in the figures that there seems to be a difference in the packing properties below a chain length of about 16 carbons, which is difficult to explain. Since all the lipid extracts were in the liquid expanded phase at all temperatures and pressures, the effect is not due to the fact that the lipids were in different monolayer phases.

3.3. Mixtures of MGlcDAG and MADGlcDAG

It was also investigated how the molecular area varied with the composition for a mixture of MADGlcDAG/MGlcDAG at the molar ratios 1/0; 3/1; 1/1; 1/3; and 0/1. From these data, summarized in Fig. 2, it can be seen that the area/lipid molecule increases with an increasing MADGlcDAG content in the monolayer film, but that the area per acyl chain *decreases* with an increasing MADGlcDAG concentration.

3.4. Surface charge densities

From the fractions of anionic lipids (Table 3) and the average molecular areas for the total lipid extracts, an average surface charge density of 7.3 ± 1.5 (S.D.) × 10^{-3} electronic charges (e^-) per $Å^2$ could be calculated assuming that the anionic groups are fully ionized. The surface charge densities varied between 4.7 × 10^{-3} $e^-/Å^2$ and 9.4 × 10^{-3} $e^-/Å^2$ for the total lipid extracts studied. In a previous study [35], the average surface charge density has been reported to be 5.7 ± 0.2 (S.D.) × 10^{-3} $e^-/Å^2$ for total lipid extracts from *A. laidlawii*. Our results do

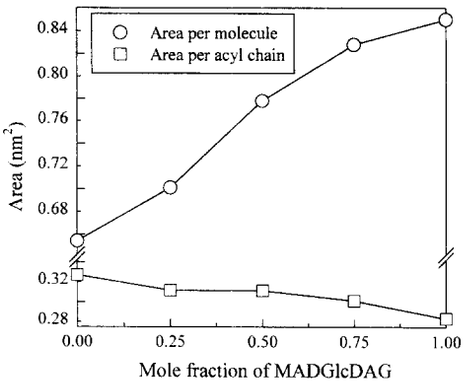

Fig. 2. Monolayer studies performed with mixtures of MGlcDAG and MADGlcDAG. The measurements were performed at a surface pressure of 30 mN/m and at 35°C: (○) – the mean molecular areas as a function of the mole fraction of MADGlcDAG; and (□) – the mean areas per acyl chain as a function of the mole fraction of MADGlcDAG.

not support their conclusion that the surface charge density is maintained at a constant value for different total lipid extracts. It should be noted that, since the early study by Christiansson et al. [35], the chemical structures of 1,2-diacyl-3-*O*-[glycerophosphoryl-6-*O*-(α-D-glucopyranosyl-(1 → 2)-monoacylglycerophosphoryl-6-*O*-α-D-glucopyranosyl)]-*sn*-glycerol (MABGPDGlcDAG), and of other lipids, from *A. laidlawii* A-EF22 have been determined by two-dimensional high resolution NMR spectroscopy [36–38]. Thus, the charges on one of the anionic lipids were not known at the time of that study, which may be one reason for the observed discrepancy. Moreover, the data set on which Christiansson et al. [35] based their conclusion was rather limited.

4. Discussion

The most striking result from the present study is that, under the same external conditions, and with similar acyl chain compositions, the molecular area for the four individual lipids studied is larger than the average molecular area in the total lipid extracts (Fig. 1(A) and (B)). The area per molecule for DGlcDAG is 0.76 nm^2 while the average area for extract 13 in Table 1 is 0.65 nm^2 (cf. Table 2 and Fig. 1). Similar comparisons can be made between the areas per molecule for MGlcDAG, MADGlcDAG and GPDGlcDAG and for extract 6 in Table 1, for which the areas are 0.65, 0.85, 0.72 and 0.62 nm^2, respectively. Finally, in a previous study the area per molecule for 1,2-diacyl-3-*O*-[6-*O*-acyl-(α-D-glucopyranosyl)]-*sn*-glycerol (MAMGlcDAG) was determined to be 0.78 nm^2 at 40°C and 30 mN/m [30]. The MAMGlcDAG preparation had $C_n \approx 16$ and > 95% saturated acyl chains but no total lipid extract in this study contains such a high degree of acyl chain saturation. However, extrapolation in Fig. 1 indicates that the area for MAMGlcDAG is much larger than the average molecular area of a total lipid extract, even if the temperature difference of the measurements is taken into account. Therefore, we conclude that, in a lipid bilayer, the lipids in *A. laidlawii* total lipid extracts are more tightly packed than the individual lipid species.

The finding that the area increases with increasing degree of unsaturation is in line with previous investigations [35,39]. In the present study, it was found that, upon an increase in the unsaturation between 26 and 92 mol%, the change in the molecular area was ca. 0.56–0.74 nm^2 per molecule or 0.26–0.34 nm^2 per acyl chain at 35°C and 30 mN/m; the latter area values are given since three *A. laidlawii* lipids contain three acyl chains. However, if only acyl chain lengths above 16 carbons are considered, the increase was from 0.56 nm^2 to 0.68 nm^2 per lipid molecule. In contrast, the area/lipid molecule does not vary significantly upon variation of the acyl chain length, provided the unsaturation is kept within a narrow range and that the average acyl chain length is above 16. Thus, it seems as if the bacterium is able to compensate the interfacial surface area, or the molecular packing in the lipid monolayer, for changes in the acyl chain length, but it has to accept and cope with a slight change in the packing due to variations in lipid unsaturation.

A comparison of the molecular area for some of the individual lipids shows that it decreases in the following order: MADGlcDAG > DGlcDAG > GPDGlcDAG > MGlcDAG. MADGlcDAG has the largest area due to the fact that it contains three acyl chains. The relatively large difference in molecular area between the two major glucolipids, DGlcDAG and MGlcDAG, is mainly attributed to the smaller polar head group of MGlcDAG than that of DGlcDAG. The higher fraction of palmitic acid (16 : 0) in MGlcDAG (Table 1), can also contribute to

the difference. In the work by Demel et al. [30], the area per molecule for MGlcDAG and DGlcDAG at 30 mN/m and 22°C was determined to be 0.53 and 0.69 nm^2, respectively. At the same pressure, but at 25°C, the measurements in this study gave the values 0.62 and 0.70 nm^2, respectively. The acyl chain compositions of the two different DGlcDAG preparations are very similar; the larger area obtained for MGlcDAG in the present study is probably due to an 11 mol% higher degree of unsaturation of the acyl chains. The surprising result that GPDGlcDAG, being a charged lipid with a relatively large head group, has a smaller area than DGlcDAG is most probably an artefact. Recently, it has been shown that GPDGlcDAG forms rod-like micelles in a highly diluted water solution [40]. This is probably the cause for the difficulty to obtain a sufficiently stable film over time with GPDGlcDAG, so that an accurate molecular area could be determined. Most probably, a fraction of this lipid forms micelles in the subphase, resulting in a decrease in the lipid monolayer concentration and an underestimation of the area per molecule.

In Fig. 2, the variation of the mean molecular area at 30 mN/m and 35°C is expressed as a function of the fraction of MADGlcDAG in an MGlcDAG monolayer. The observed increase in the average molecular area with an increasing MADGlcDAG content is simply due to the fact that MADGlcDAG contains three acyl chains. However, the area per acyl chain with an increasing MADGlcDAG concentration in the monolayer decreases, i.e. the lipid molecules pack more closely as the MADGlcDAG content increases. Such a behaviour is adequate since MADGlcDAG is synthesized by the bacterium preferentially, when it is fed with short acyl chains [19], where it probably has to cope with an increasing permeability across the relatively thin membrane. A tighter packing of the lipid molecules will counteract such an unfavourable increase in the permeability of the cell membrane.

The molecular packing or the area per acyl chain of lipid molecules with different polar head groups may be a useful parameter in the comparison of the phase behaviour of the lipids, provided lipids with similar acyl chain composition are compared. For lipid molecules with a large polar head group compared to the hydrocarbon region (lipids forming *normal* nonlamellar aggregates), the area at the air–water interface will be determined preferentially by the head group and the area per acyl chain will be large. On the other hand, for molecules with a small polar head group and a relatively bulky hydrocarbon region (lipids forming *reversed* nonlamellar aggregates), the interfacial area will be determined by the acyl chains and the area per acyl chain will be much smaller. From an earlier study on MAMGlcDAG [30] and from the study on MADGlcDAG, MGlcDAG and DGlcDAG in this work, it is seen that the areas per acyl chain at 35°C, or 40°C for MAMGlcDAG, and a surface pressure of 30 mN/m are equal to 0.26 [30], 0.28, 0.33, and 0.38 nm^2, respectively. MAMGlcDAG, MADGlcDAG and MGlcDAG can form reversed nonlamellar phases [9,19,41] and their areas per acyl chain are smaller than that of DGlcDAG, or of a phosphatidylcholine (PC) molecule with saturated acyl chains [30], which form only lamellar phases. For comparison, it might be interesting to note that the molecular areas for the lamellar-forming lipids dipalmitoyl-PC and dioleoyl-PC, and for the nonlamellar-forming lipids dipalmitoyl-phosphatidylethanolamine (PE) and dioleoyl-PE, at 40°C and 30 mN/m are equal to 0.60, 0.67, 0.43, and 0.60 nm^2, respectively ([42,43], and Demel, unpublished results).

How can we understand that the lipids in the total extracts can pack more closely than the individual lipids, considering also the fact that there is always a large fraction of anionic lipids (varying between $\approx$ 26–54 mol%) in the cell membrane? It should be remembered that the total lipid extracts exhibit a transition from a lamellar to a nonlamellar liquid crystalline phase within a suitable temperature interval above the growth temperature of the bacterium at rather low degrees of hydration ([9,21,27,28], and Andersson et al., to be submitted). Such a transition demands that the membrane spontaneous curvature is negative [2,44,45] which is achieved by the balance between the differently positioned attractive and repulsive forces between the lipid molecules. Since the lipids in the monolayer of the total extracts are more closely packed, it seems reasonable to assume that there is an attractive force existing between the polar head groups, which is not present for the individual lipids. A similar interpretation has been presented previously to explain the formation of an H_{II} phase

when mixing two L_α phases consisting of dioleoyl-PC and monoacylglycerol [46,47]. Intuitively, one would expect a strong repulsive interaction to occur between the polar head groups of the ionic lipids. However, since the ionic lipids are also closely packed, and since they do not significantly affect the lamellar-to-nonlamellar phase transition as observed in previous investigations of the phase behaviour of these lipids [9,27], a possible interpretation might be that the repulsive forces between the ionic head groups are reduced or eliminated. It can be speculated that extensive hydrogen bonding may occur between the polar head groups of the anionic lipids and the sugar moieties of the various glucolipids. The glycerol of the head group of PG may be a good candidate for such hydrogen bonding and the sugar groups of the other anionic glucolipids, GPDGlcDAG and MABG-PDGlcDAG, may be involved in such interactions.

Our earlier results from studies on *A. laidlawii* lipids have mainly been based on NMR spectroscopy and X-ray diffraction. In this work, we have used a new approach to get insight into the packing properties of *A. laidlawii* lipids and the interactions among them. In summary, it is shown that *A. laidlawii* regulates its lipid composition so that a relatively close lipid packing is accomplished which is independent on the chain length when $C_n > 16$. On the other hand, the average area per lipid molecule increases with the unsaturation of the acyl chains. Finally, in contrast to earlier studies [35], the surface charge density was found to vary with the chain length and with the degree of acyl chain unsaturation.

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