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Packing of a triacylglucolipid from the membrane of *Acholeplasma laidlawii* strain A at the air/water interface

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Abstract

Pressure–area curves were generated at 22°C and 40°C for three 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), and 1,2-diacyl-3-*O*-[3-*O*-acyl-(α-D-glucopyranosyl)]-*sn*-glycerol (MAMGlcDAG). The curves for MGlcDAG and DGlcDAG are characteristic for monolayers in a liquid phase at both temperatures. MGlcDAG has a smaller molecular area at all surface pressures compared to DGlcDAG. At 22°C MAMGlcDAG shows a phase transition at 13 mN/m. However, at 40°C the pressure–area curve for this lipid is characteristic for a monolayer in a liquid state. Mixed MAMGlcDAG-DGlcDAG and MGlcDAG-DGlcDAG monolayers showed no significant deviation from the additivity rule at 40°C. The area per acyl chain is nearly the same for MAMGlcDAG and MGlcDAG. Our study supports our previous results that aqueous dispersions of these lipids form non-lamellar, reversed aggregates.

Key words: Triacylglucolipid; Membrane; Air/water interface; (*A. laidlawii*)

1. Introduction

In recent years convincing evidence has been obtained showing that the membrane lipids participate in the function of the cell membrane in a much more active way than previously thought [1–5]. Much of this information has been obtained from investigations of bacterial membranes, and in particular from studies of the plasma membrane of the parasitic organism *Acholeplasma laidlawii* [5]. Therefore, it is of great interest to study the physical chemistry of lipids, like phase equilibria [4,6,7], inter-lipid interactions [8], lipid translational diffusion [4,9,10], rotational dynamics [11], and properties at the air/water interface of the dominant glucolipids of the membrane of *A. laidlawii*. Monomolecular layers can yield information about packing densities and lipid–lipid interactions.

The membrane lipid composition in several strains of the bacterium *A. laidlawii* is similarily regulated upon changes in the surrounding milieu [5,7,12–15]. The two glucolipids 1,2-diacyl-3-*O*-(α-D-glucopyranosyl)-*sn*-glycerol (MGlcDAG) and 1,2-diacyl-3-*O*-[α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl]-*sn*-glycerol (DGlcDAG) constitute a major fraction of the membrane lipids in *A. laidlawii*.

In a large number of studies we have shown (see Refs. [5,7] for recent reviews) that the phase behaviour of the lipids plays a crucial role in the regulation of the membrane lipid composition. The potential ability of the lipids to form a variety of non-bilayer liquid crystalline phases is of great importance. Qualitatively, the lipid regulation can be understood in terms of changes in the molecular shape of the lipids in the membrane [7,13]. Thus, an increase in the growth temperature can be predicted to induce a decrease in the ratio MGlcDAG/DGlcDAG, since a stable bilayer cannot incorporate large amounts of MGlcDAG, a molecule which has a tendency to induce non-bilayer aggregate structures. In the same way, introduction of hydrophobic molecules like alkanes in the membrane also leads to a decrease in this ratio [14,16].

A third glucolipid is synthesized by *A. laidlawii* strain A-EF22 when large amounts of saturated, straight-chain fatty acids (16 to 18 carbon atoms) are

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Table 1
Acyl chain composition (mol%) of the MGlcDAG-d_{31}, DGlcDAG-d_{31} and MAMGlcDAG preparations as determined by gas–liquid chromatography

Acyl chain	12:0	13:0	14:0	15:0	16:0	16:0-d_{31}	17:0	18:0	18:1c9	SFA [1]	UFA [2]
MGlcDAG-d_{31}	4.2	2.7	11.2	4.3	14.1	15.6	–	1.3	46.6	53.4	46.6
DGlcDAG-d_{31}	0.4	0.5	3.0	1.9	8.1	8.2	–	0.9	77.0	23.0	77.0
MAMGlcDAG	1.7	1.8	4.0	0.4	85.9	–	0.3	2.4	3.5	96.5	3.5

[1] Saturated fatty acids.
[2] Unsaturated fatty acids.

incorporated into the lipids [12,17,18]. The structure of this lipid was determined to be 1,2-diacyl-3-*O*-[3-*O*-acyl-(α-D-glucopyranosyl)]-*sn*-glycerol (MAMGlcDAG) [19]. Consequently, growth of this organism in the presence of certain high-melting fatty acids induces the synthesis of a membrane lipid with a large hydrophobic part and a relatively small hydrophilic headgroup.

In a recent investigation [20] we have compared the phase equilibria of MGlcDAG and MAMGlcDAG isolated from *A. laidlawii* strain A-EF22. It was shown that hydrated MAMGlcDAG does not form any (thermodynamically stable) liquid crystalline phases, but a gel/crystalline phase at physiological temperatures and only a *reversed micellar* (L_2) phase is formed at higher temperatures.

2. Materials and methods

Cell growth

For preparation of MGlcDAG and DGlcDAG, *A. laidlawii* strain A-EF22 [17] was grown in a medium containing lipid-depleted tryptose and fatty acid poor bovine serum albumin as described in Ref. [21]. For preparation of MAMGlcDAG, the cells were grown in the above mentioned medium supplemented with 120 μM palmitic acid and 30 μM oleic acid. A significant synthesis of MAMGlcDAG occurs only when *A. laidlawii* strain A-EF22 incorporates a large fraction of saturated, straight-chain fatty acids into the membrane lipids. The cells were grown, harvested, and washed as described in Refs. [20,21].

Isolation and purification of lipids

MGlcDAG and DGlcDAG containing a small fraction of perdeuterated palmitic acid (MGlcDAG-d_{31} and DGlcDAG-d_{31}) were used in this investigation. These two lipids and MAMGlcDAG were isolated and purified as described in Refs. [20,21]. The acyl chain composition of the lipid preparations are shown in Table 1.

Monolayer techniques

Interfacial measurements were performed in a thermostatically controlled box at 22 or 40 ± 0.1°C [22]. The surface pressure was measured with the Wilhelmy plate method using a Cahn 2000 electrobalance.

Monomolecular layers were spread from a chloroform-methanol solution into a subphase of double distilled water. 50 nmol of lipid was spread at a surface area of 613.28 cm^2. The compression rate was 86.50 cm^2/min. The reproducibility was better than 0.01 nm^2 per molecule.

3. Results and discussion

The pressure–area curves for MGlcDAG, DGlcDAG and MAMGlcDAG at 22°C are compared in Fig. 1. The curves for MGlcDAG and DGlcDAG are characteristic for monolayers in a liquid phase. MGlcDAG has a smaller molecular area at all surface pressures compared to DGlcDAG. At a surface pressure of 30 mN/m the molecular area is 0.533 nm^2 for MGlcDAG and 0.689 nm^2 for DGlcDAG. The relatively large difference in molecular area between these glucolipids can be partially attributed to the higher amount of palmitic acid (16:0) in MGlcDAG (Table 1), but the main difference is due to the smaller polar headgroup

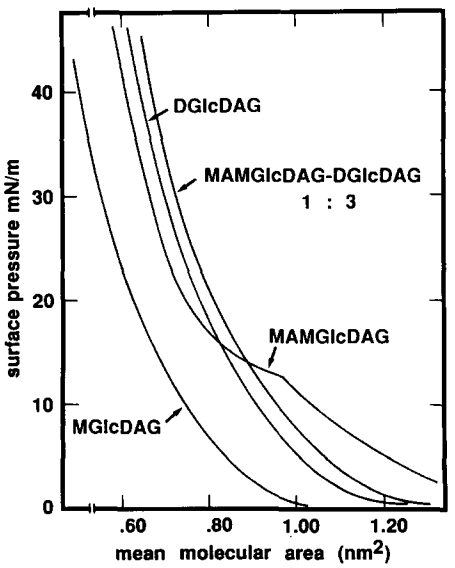

Fig. 1. Pressure–area curves at 22°C for MGlcDAG, DGlcDAG and MAMGlcDAG and mixed monolayers of MAMGlcDAG/DGlcDAG at the molar ratio 1:3.

of MGlcDAG than that of DGlcDAG. Qualitatively, the difference is in line with that between e.g. the dilinolenoyl derivatives of DGalDAG (0.691 nm^2/molecule) and MGalDAG (0.647 nm^2/molecule) [23] and the difference between phosphatidylcholine (PC) and phosphatidylethanolamine (PE). For comparison, the molecular areas for dipalmitoyl-PC, dioleoyl-PC, dipalmitoyl-PE, and dioleoyl-PE under these conditions are 0.47, 0.61, 0.41, and 0.57 nm^2, respectively (Refs. [24,25], and Demel, unpublished results). MAMGlcDAG demonstrates a phase transition at 13 mN/m, and a molecular area of 0.96 nm^2, from an expanded state with high compressibility to a state with a compressibility similar to that of DGlcDAG (Fig. 2). Most probably, the phase transition is primarily initiated by the acyl chains on the glycerol moiety, and eventually the third chain becomes oriented. At high surface pressures the molecular area of MAMGlcDAG reaches a value that is expected for three condensed acyl chains. At pressures lower than 13 mN/m the molecular area of MAMGlcDAG is larger than for DGlcDAG, and at pressures higher than 18 mN/m the area is slightly smaller than for DGlcDAG. At 30 mN/m and 22°C the molecular area of MAMGlcDAG is 0.658 nm^2. No phase transition is found for the mixed monolayer of MAMGlcDAG/DGlcDAG with a 1:3 molar ratio. This results in a larger mean molecular area at surface pressures higher than 18 mN/m for this lipid mixture than for the pure components.

In Fig. 2, the variation of the mean molecular area is expressed as a function of monolayer composition. The larger mean molecular area at 22°C of the mixed MAMGlcDAG/DGlcDAG monolayer (molar ratio 1:3) is seen as a deviation from the additivity rule. Mixtures of MGlcDAG-DGlcDAG showed no significant deviation from the additivity rule at 22°C.

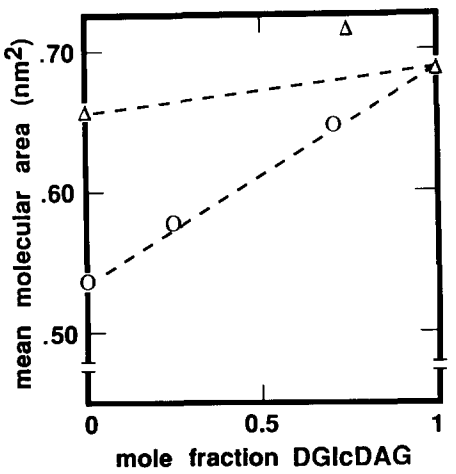

Fig. 2. Variation of the mean molecular area as a function of the composition for mixed monolayers of MGlcDAG/DGlcDAG (○) and MAMGlcDAG-DGlcDAG (△) at a surface pressure of 30 mN/m and at 22°C.

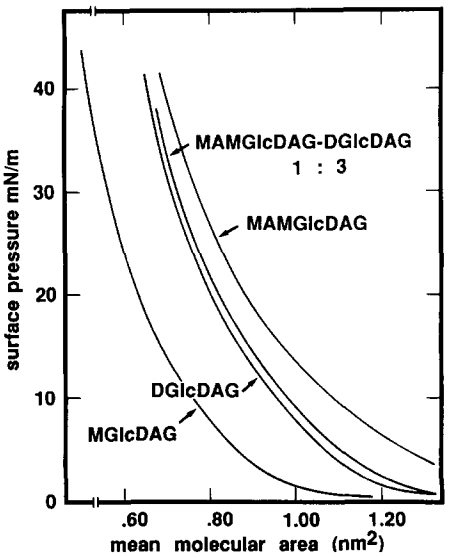

Fig. 3. Pressure–area curves at 40°C for MGlcDAG, DGlcDAG and MAMGlcDAG and mixed monolayers of MAMGlcDAG/DGlcDAG at the molar ratio 1:3.

The pressure–area curves of MGlcDAG and DGlcDAG show only a small increase in the molecular area when the temperature is increased to 40°C (Fig. 3). At a surface pressure of 30 mN/m the molecular area for MGlcDAG and DGlcDAG is 0.547 nm^2 and 0.705 nm^2, respectively. MAMGlcDAG shows no phase transition at 40°C and the molecular area at 30 mN/m has therefore significantly increased to 0.768 nm^2. The molecular areas for dipalmitoyl-PC, dioleoyl-PC, dipalmitoyl-PE, and dioleoyl-PE at 40°C at a surface pressure of 30 mN/m are 0.60, 0.67, 0.43, and 0.60 nm^2, respectively [24, 25, Demel, unpublished results].

Mixed monolayers of MGlcDAG and DGlcDAG at 40°C exhibit mean molecular areas, which are slightly higher than expected by the additivity rule (Fig. 4). However, the mean molecular area of a MAMGlcDAG-DGlcDAG mixture (molar ratio 1:3) at this temperature is exactly the one that is predicted by the additivity rule.

The area per acyl chain of lipid molecules with different polar headgroups 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 headgroup compared to the hydrocarbon region (lipids forming *normal*, nonlamellar aggregates), the area at the air/water interface, as measured by the monolayer technique, will be determined preferentially by the headgroup and the area per acyl chain will be large. On the other hand, for molecules with a small polar headgroup and a relatively bulky hydrocarbon region (lipids forming reversed, nonlamellar aggregates), the

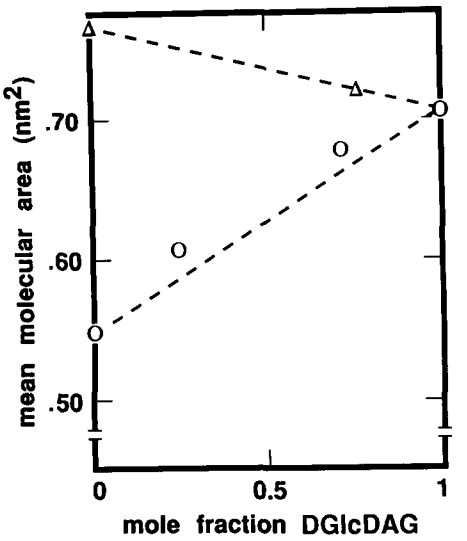

Fig. 4. Variation of the mean molecular area as a function of the composition for mixed monolayers of MGlcDAG/DGlcDAG (○) and MAMGlcDAG/DGlcDAG (△) at a surface pressure of 30 mN/m and at 40°C.

interfacial area will be determined by the acyl chains and the area per acyl chain will be much smaller. The area per acyl chain for MAMGlcDAG, DPPE, and DPPC at 40°C and a surface pressure of 30 mN/m is 0.26, 0.22, and 0.30 nm^2, respectively. The first two lipids can form reversed, nonlamellar phases [20,26] and their area per acyl chain is smaller than that of DPPC which forms only lamellar phases.

The MGlcDAG and DGlcDAG preparations used in this investigation contain about 25–50 mol% saturated fatty acids (the rest being oleic acid, see Table 1), and the area per acyl chain is 0.274 and 0.353 nm^2, respectively, at 40°C and a surface pressure of 30 mN/m. MGlcDAG forms nonlamellar phases of the reversed type while DGlcDAG forms lamellar phases [6]. Moreover, the area per acyl chain is approximately the same for MGlcDAG and MAMGlcDAG. Therefore, the difference in molecular area between these lipids can be roughly accounted for by the presence of two acyl chains in MGlcDAG and three acyl chains in MAMGlcDAG.

Finally, the area per acyl chain can be compared for MGalDAG and DGalDAG molecules having mainly linolenoyl chains; the area is 0.324 och 0.346 nm^2, respectively, at 40°C and a surface pressure of 30 mN/m [23,27]. As with the corresponding glucolipids, MGalDAG forms reversed, nonlamellar phases, while DGalDAG forms lamellar phases [28,29]. However, in this example the comparison is not as clear-cut as in the GlcDAG's, since linolenoyl chains occupy a rather large area at the air/water interface. Hence, the area per acyl chain is comparatively large, even for a lipid with a small polar headgroup like MGalDAG, since the acyl chains, due to the high degree of unsaturation, cannot be tightly pressed together.

The presence of 10 mM Ca^{2+} in the subphase had no significant effect on the compression curves of MGlcDAG and DGlcDAG.

Lipid regulation

The monolayer properties of MAMGlcDAG investigated in this work, together with previous studies of the phase equilibria [20], are relevant for the regulation of the membrane lipid composition in *A. laidlawii* strain A-EF22. As outlined in the introduction, changes in the molecular shape encompass the driving forces behind the alterations in the lipid composition in the plasma membrane of this organism. In a hypothesis put forward earlier [4,13], we stated that the cells strive to maintain a balance between the membrane lipids constituting a bilayer structure and those forming reversed non-lamellar aggregates. In this simple model, there is a requirement of a predetermined packing or curvature in the membrane, which strongly depends on the physico-chemical properties of the lipids. The main idea behind the hypothesis is that for the membrane to function these packing and curvature properties, determined by the lipids, have to be kept approximately constant. If there is a perturbation of the molecular packing in the membrane, there will be corresponding or compensating changes in the lipid composition, which strive to maintain the balance between the cylindrical-like (such as DGlcDAG) and the wedge-shaped (such as MAMGlcDAG and MGlcDAG) lipids.

The synthesis of MAMGlcDAG is induced by low growth temperatures and especially by incorporation of a large fraction of saturated fatty acids of medium chain length into the lipids, i.e. at conditions that decrease the ability of MGlcDAG to form non-bilayer structures [5]. The effect of esterfying one of the hydroxyl groups of the sugar ring of the lipid with a saturated (16:0) fatty acid is that the polar headgroup will be less hydrophilic and that the hydrophobic volume of the molecule will be enhanced. The only aggregate structure formed by MAMGlcDAG, when its acyl chains are in a melted state, is the reversed micelle, which, like the aggregate of an H_{II} phase, has a negative curvature. MAMGlcDAG may therefore be considered to be strongly wedge-shaped, which will affect the packing properties and intermolecular forces, assisting MGlcDAG to maintain the potential ability of the ensemble lipids to form non-bilayer structures. The model for the regulation of the membrane lipid composition for *A. laidlawii*, has also been used to interpret the lipid regulation mechanisms observed in the bacteria *Pseudomonas fluorescens* [30], *Clostridium butyricum* [31], *Clostridium acetobutylicum* [32], *Escherichia coli* [33] and *Bacillus megaterium* [34,35].

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