# LIPID PHASE STRUCTURE GOVERNS THE REGULATION OF LIPID COMPOSITION IN MEMBRANES OF *ACHOLEPLASMA LAIDLAWII*

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## 1. Introduction

A great variety of lipid molecules are present in biological membranes – a mammalian cell membrane may contain 100 different lipids including intramolecular variants. Many of these form lamellar liquid crystalline phases together with water [1]. However, most biological membranes contain at least one major lipid species forming a non-lamellar phase. Monogalactosyldiglyceride and monoglucosyldiglyceride (MGDG) form a reversed hexagonal ($H_{II}$) phase [2,3], while phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol and diphosphatidylglycerol (cardiolipin) adopt a lamellar or a $H_{II}$ phase depending on temperature, acyl chain content, pH and the presence of divalent cations [4–7]. Lysolipids form normal hexagonal ($H_I$) and cubic phases [8–10].

The 'fluid mosaic model' disregards membrane lipid heterogeneity and stresses the two-dimensional nature of the bilayer and the independence of proteins for membrane structure [8,11]. No attempt is made to explain why certain lipids form bilayers while others do not, even though the lipid matrix is proposed to constitute the structural framework of a biological membrane. This was considered in discussing the mechanism of lipid and protein assembly in membranes [8,11–13]. The tendencies for amphiphiles to form various aggregate structures can be explained by a theory linking interaction free energy, molecular geometry and thermodynamics [12,13]. Both attractive and repulsive interactions are involved in determining the shape of the lipid aggregate. Each lipid molecule can be visualized as a building brick having a certain hydrocarbon–water interfacial area $a$, hydrocarbon chain length $l$, and hydrocarbon chain volume $v$. The following aggregates are formed for different values of $v/al$: $v/al < 1/3$, spherical micelle; $v/al = 1/3–1/2$, rod-like micelle; $v/al = 1/2–1$, lamellae [8]. A $H_{II}$ phase will form when $v/al > 1$. It follows that different lipids may be accommodated in a bilayer as long as they can pack together to form a stable lamellar structure [8,14]. Thus, the geometry of the lipid molecules is a very important factor when discussing the stability of a bilayer.

The polar lipid composition in membranes of *Acholeplasma laidlawii* is extensively regulated as a response to environmental changes. The ratio between the dominating lipids monoglucosyldiglyceride and diglucosyldiglyceride is altered depending on temperature, configuration of incorporated fatty acids, and membrane cholesterol content. In this work we apply the self-assembly theory [8,12] to explain the observed physiological regulation [15–18] of the different major lipids in *A. laidlawii*. It is found that the response in lipid metabolism following external and internal stimuli can be predicted by the theory. Studies of the phase structures formed by in vitro lipid mixtures further support the proposed mechanism for regulation of the lipid composition in the membranes.

## 2. Membrane lipids in *Acholeplasma laidlawii*

The following major lipids occur in *A. laidlawii* A (EF22) [18]: MGDG, diglucosyldiglyceride (DGDG) and phosphatidylglycerol. Further, glycerophosphorylderivatives of MGDG and DGDG and small amounts of an apolar monoglucolipid are present.

Three distinct ways for regulation of lipid composition have been discovered [15–18]: (A) changes in the incorporation of different fatty acids into membrane lipids; (B) variation in the relative synthesis of MGDG and DGDG; (C) alteration in the balance between ionic and non-ionic lipids. These changes play a central role concerning the relation between lipid molecular shape and membrane stability.

### 3. Lipid molecular shape

Fig.1 shows a schematic representation of the packing of lipid molecules in various amphiphilic aggregates. The hydrocarbon chain length ($l$), hydrophobic volume ($v$) and polar head group area ($a$) will determine the overall shape of the molecules. The phase structure formed by different lipids is in turn determined by the molecular shape.

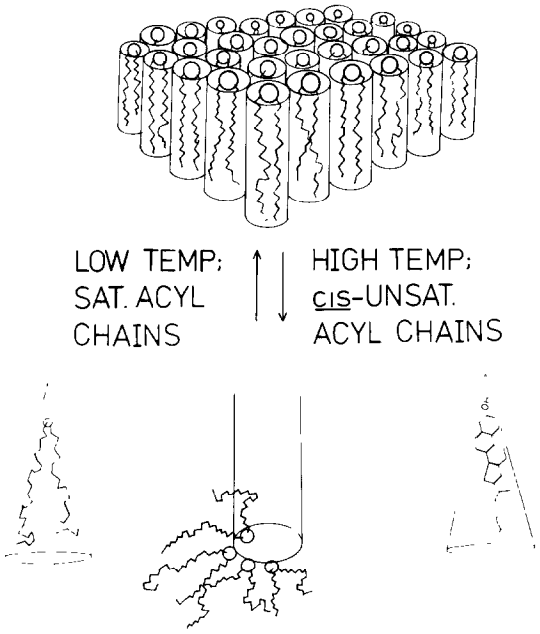


Fig.1. The structure of the amphiphilic aggregates formed strongly depends on the molecular shape of the lipid molecules. The upper part of the figure illustrates the molecular packing of rod-like amphiphiles in a bilayer. By increasing the temperature and/or the degree of *cis*-unsaturation in the acyl chains the lipid molecules adopt a more wedge-shaped geometry. The amphiphiles may then form a reversed hexagonal or cubic liquid crystalline phase structure. Similarly cholesterol, having a wedge-like geometry, may induce non-lamellar structures. These effects are shown at the lower part of the figure.

(i) With a given acyl chain composition, i.e., constant values of $v$ and $l$, any change in the size (bulkiness, charge, hydration) of the polar head group will affect the optimal surface area $a$ of the lipid molecule. Modifications of polar head structure can thus alter the packing properties ($v/al$) of an individual lipid in the bilayer [14].

(ii) For lipids with a given polar head group, i.e., constant value of $a$, the molecular shape depends on several factors. A substitution of unsaturated acyl chains (fig.1) for saturated ones, especially with cis double bonds, will reduce the length of the hydrocarbon chains ($l$), and increase the width of the hydrocarbon space almost without any change in the hydrophobic volume [13]. By increasing the temperature, enhanced thermal motions of the hydrocarbon chains will yield the same result as mentioned above.

(iii) Cholesterol has a pronounced wedge shape [19] (fig.1). Therefore cholesterol and MGDG have similar effects on a lipid bilayer.

### 4. Phase structures of in vitro *A. laidlawii* lipid mixtures and the lipid regulation in membranes

MGDG and DGDG constitute >50% (mol/mol) of the amphiphilic lipids in *A. laidlawii* [18]. The phase structures of different MGDG/DGDG mixtures have been studied with $^2$H NMR, NMR diffusion techniques and polarizing light microscopy [20,21]. The results are here compared to the physiological regulation of fatty acid incorporation and MGDG/DGDG ratios. *A. laidlawii* has been shown to vary the composition of the polar head groups of the membrane lipids upon: (1) incorporation of fatty acids with different degree of saturation; (2) changes in temperature; (3) incorporation of cholesterol [15–18]. Table 1 shows the phases formed by MGDG/DGDG mixtures with different acyl chain contents. The effect of variations in the temperature and in the cholesterol concentration has also been investigated.

#### 4.1. *Acyl chain saturation*

In table 1 MGDG/DGDG mixtures with two hydrocarbon chain compositions have been employed:

(i) Lipids with approximately equal amounts of palmitoyl chains and oleoyl chains;

(ii) Lipids having 95% oleoyl chains [20,21].

According to the theory, increased amounts of *cis*-

Table 1
Phase structures formed by MGDG/DGDG mixtures between 0–60°C

| Acyl chain content | MGDG/DGDG (molar ratio) | % Cholesterol (mol/mol) | Phase structures formed at different temp. (°C) |
|---|---|---|---|
| 16:0/18:1c | 1.0/1 | 0 | 0–60, L[a] |
| | 1.0/1 | 27 | 0–50, L |
| | 1.0/1 | 50 | 0–45, L[d] |
| | 2.0/1 | 0 | 0–60, L |
| | 2.0/1 | 27 | 0–50, L |
| | 2.0/1 | 50 | 0–45, L[d]; 45–60, L + $H_{II}$[b] |
| 18:1c/18:1c | 1.2/1 | 0 | 0–20, L; 20–45, L + $Q_{II}$[c]; 45–60, $Q_{II}$ |
| | 1.2/1 | 27 | 0–10, L; 10–35, L + $H_{II}$; 35–60, $H_{II}$ |
| | 2.5/1 | 0 | 0–15, L + $Q_{II}$; 15–60, $Q_{II}$ |
| | 2.5/1 | 27 | 0–10, L + $H_{II}$; 10–60, $H_{II}$ |

[a] L, lamellar phase; [b] $H_{II}$, reversed hexagonal phase; [c] $Q_{II}$, reversed cubic phase; [d] Samples containing free cholesterol

Different mixtures of MGDG, DGDG and cholesterol were prepared. Dissociable protons in the polar head groups were exchanged for deuterons and $^2H_2O$ was added to a final concentration of 11 or 13% (w/w). $^2H$ NMR spectra were recorded at 15.351 MHz to establish the various phases [20,21]. The samples were thermally equilibrated for ≥1 h before the spectra were taken. Polarizing light microscopy was also used to elucidate the phase structures formed. Fatty acid composition of the glucolipids isolated from cells grown with equal amounts of palmitic (16:0) and oleic (18:1c) acid was: MGDG, 54 mol% 16:0 and 46 mol% 18:1c; DGDG, 45 mol% 16:0 and 55 mol% 18:1c. Glucolipids isolated from cells grown with oleic acid only contained at least 95 mol% of this fatty acid. The limits of the phase temperature intervals were determined within ±3°C

unsaturated hydrocarbon chains will increase the hydrophobic bulkiness of the molecules (fig.1), leading to a more accentuated wedge-shape. If large amounts of such lipids are present in the mixture, curved aggregates (e.g., a $H_{II}$ phase and/or a reversed cubic ($Q_{II}$) phase) not fitting in a membrane may be formed. This is illustrated in table 1. In the absence of cholesterol both 1/1 and 2/1 mixtures of MGDG/DGDG with 50% acyl chain saturation formed a lamellar phase at 37°C, whereas mixtures with lipids having 95% oleoyl chains formed a $Q_{II}$ phase with increasing MGDG/DGDG ratios. The cubic phase is probably built up of two distinct networks of water rods in a lipid matrix [20]. Thus the pronounced wedge shape of oleoyl-containing MGDG promotes the formation of a cubic phase. Increased amounts of unsaturated acyl chains in the lipids can therefore be expected to destabilize the bilayer in vivo, unless compensated for. Table 2 shows the MGDG/DGDG ratio in membranes from *A. laidlawii* grown at 37°C with different amounts of palmitic and oleic acids. The molar ratio MGDG/DGDG decreases upon an increase in *cis*-unsaturation leading to a stabilization of the bilayer structure. The extent of variation of MGDG was 60% larger than that of DGDG, indicating that MGDG is the most actively regulated compound [14]. The MGDG/DGDG ratio has a maximum value of 0.8 in *A. laidlawii* membranes being totally enriched in oleoyl chains [15]. Note that a somewhat higher ratio (1.2/1 sample in table 1) gives a mixture of $Q_{II}$ and lamellar phases. However, in cells grown with equal amounts of palmitic and oleic acids MGDG/DGDG ratios approaching 2.0 have been found in vivo [15]. An in vitro MGDG/DGDG mixture with this composition gives a lamellar phase (table 1).

### 4.2. *Temperature*

An increase in temperature leads to a decrease in the molecular ordering of the acyl chains, enhancing the hydrophobic bulkiness (fig.1). A similar reasoning explains the effect of temperature and *cis*-unsaturation. Table 1 shows that an increase in temperature from 0–60°C leads to a transition from a lamellar to a $Q_{II}$ phase for lipid mixtures containing oleoyl chains only. On the contrary, mixtures containing 50% palmitoyl chains remain lamellar.

Table 2
Glucolipid composition in membranes from *A. laidlawii* A grown with different amounts of saturated and unsaturated fatty acids (18 h, 37°C)

| | Supplementation to the growth medium (μM palmitic/μM oleic acid) | | | | | |
|---|---|---|---|---|---|---|
| | 120/30 | 90/60 | 75/75 | 30/120 | 0/150 | 0/150 + 20 μM cholesterol[a] |
| + Oleic acid in lipids (mol/mol) | 30.3 | 44.4 | 49.6 | 55.2 | 95 | 95 |
| Ratio MGDG/DGDG | 2.37 | 1.21 | 0.80 | 0.75 | 0.57 | 0.27 |

[a] The ratio cholesterol/lipid was 0.29 (mol/mol), corresponding to 15% (w/w), which is the maximum incorporable amount in *A. laidlawii* [17]

*A. laidlawii* A was grown in a lipid-depleted basal medium [15] supplemented with palmitic plus oleic acid, oleic acid only or oleic acid plus cholesterol. Menbrane lipids were labeled by adding 30 μCi [$^3$H]palmitic acid/l and/or 10 μCi [$^{14}$C]oleic acid/l culture medium. Membranes were prepared and lipids isolated as in [15]. Fatty acid compositions and total amount of each lipid were determined by liquid scintillation counting

*A. laidlawii* regulates its lipid composition to maintain optimal membrane stability at the growth temperature. A decrease in temperature will diminish the wedge-shape properties of the lipids, and thus must be compensated for. This can be achieved in two ways: an increased incorporation of unsaturated fatty acids and increased synthesis of lipids with small polar head groups like MGDG [14]. Both effects occur in vivo [15]. Fig.2 shows how the MGDG/DGDG ratio at the time of shift-down (12 h) differs strongly (fig.2A,B) depending on the difference in fatty acyl chain composition (cf. section 4.1.). This ratio diminishes during growth at 37°C [15–18]. However, at 17°C the ratio rises due to a largely enhanced synthesis of MGDG [15]. An optimal packing of the

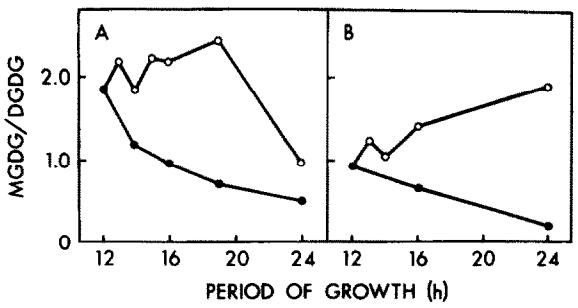


Fig.2. Changes in the MGDG/DGDG ratio after a temperature shift: (A) cells grown with 75 μM palmitic acid and 75 μM oleic acid; (B) cells grown with 150 μM oleic acid; (•) growth at 37°C; (○) cells shifted to 17°C after growth for 12 h at 37°C. The experimental conditions are described in [15].

membrane components will be re-established due to the increase in the amounts of MGDG.

### 4.3. *Cholesterol*

All *Mycoplasma* species have a strict demand for cholesterol which constitutes up to 50 mol% of the total membrane lipids [22]. The *Acholeplasma* species lack the growth requirement for cholesterol but have reduced capacity for cholesterol incorporation [23]. The mechanism responsible for this difference [22] as well as the role played by sterols in biological membranes is poorly known. Physicochemical properties of cholesterol in model membranes have been extensively studied. In [24,25] the lipid lateral diffusion in a bilayer was almost unaffected by incorporation of cholesterol. However, the order parameter of the hydrocarbon chains, which reflects the average chain conformation, was markedly increased [24].

The effect of adding cholesterol to in vitro mixtures of MGDG and DGDG is shown in table 1. It is evident that the glucolipid mixtures containing 95% oleyol chains are much more sensitive to the bilayer destabilizing effect of the sterol than are the mixtures with equal amounts of palmitoyl and oleoyl chains. The latter mixtures maintain the lamellar phase in the growth temperature range of *A. laidlawii* even in the presence of 50 mol% cholesterol. However, cholesterol forms a separate phase above 27 mol% which corresponds approximately to the maximum incorporable amount in *A. laidlawii* membranes. A free cholesterol phase is probably fatal for a biological

membrane. When the glucolipids contain 95% oleoyl chains the wedge-shape properties are so pronounced that the lamellar phase cannot form in the presence of cholesterol except at very low temperatures (table 1).

Addition of cholesterol to an *A. laidlawii* culture grown with an equimolar mixture of palmitic and oleic acid yields higher incorporation of oleic acid both at 37°C and after a shift to 17°C [15]. This response probably counteracts the increased molecular ordering caused by cholesterol. The MGDG amount is not decreased in these membranes and a response is not motivated by lipid-phase structural demands (table 1). However, when cholesterol is added to an *A. laidlawii* culture grown with oleic acid only the relative amount of MGDG is reduced by 40% (table 2). As can be seen in table 1 lower MGDG/DGDG ratios supress the tendencies to form nonlamellar phases. Thus, the regulation of:

(i) The amount of cholesterol incorporated;
(ii) The lipid composition in response to cholesterol incorporation;

both preserve an isolating and stable lipid bilayer. Compared to the *Acholeplasma* species the *Mycoplasma* species contain:

(i) Smaller amounts of lipids forming a $H_{II}$ phase;
(ii) Larger amounts of ionic lipids;
(iii) Small amounts of lysolipids [26] which form normal hexagonal ($H_I$) phases [8].

All these factors neutralize the wedge-shape properties of cholesterol and allow concentrations of this lipid to reach higher levels in *Mycoplasma* species as compared to *Acholeplasma* species. However, due to the phylogenic distance between the two groups of species [27] the cholesterol tolerance of *Mycoplasma* might involve still other mechanisms.

## 5. Conclusions

A comparison of the results in table 1 with the lipid composition in *A. laidlawii* membranes clearly reveals that the MGDG/DGDG ratios, cholesterol contents and acyl chain compositions occuring in vivo lead to the formation of a lamellar phase. Lipid compositions just outside the range found in vivo gave a two-phase system consisting of a lamellar and a $Q_{II}$ or $H_{II}$ phase in the physiological temperature interval. When the composition was far from that observed in vivo a $Q_{II}$ or $H_{II}$ phase was obtained. Since the other amphiphilic lipids isolated from the membrane of *A. laidlawii* strain A form lamellar phases [3] it is assumed that the cells actively avoid lipid compositions resulting in non-lamellar phases of the bulk membrane lipids. However, other mesophase structures may form within the membrane during short time periods or in the proximity of integral membrane proteins. Local regions forming such structures have been proposed to be advantageous to cell functions like membrane fusion, exo- and endocytosis, and transbilayer movement of lipids [28].

The reduced capacity for cholesterol incorporation into *Acholeplasma* membranes most likely depends on:

(i) The low solubility of cholesterol in the glucolipids;
(ii) The induction of non-lamellar phases in the presence of lipids forming a $H_{II}$ phase (e.g., MGDG).

The latter effect is especially pronounced when the membrane lipids contain unsaturated acyl chains only. Moreover, in biological membranes the effect of the static properties of cholesterol are of greater importance than its influence on lipid bilayer dynamics.

A qualitative application of the theory developed in [8,12] can be used to describe the phase structures formed by different in vitro mixtures of lipids isolated from *A. laidlawii* membranes. The results obtained from these mixtures are hightly relevant to the regulation of membrane lipid composition observed in *A. laidlawii* grown under different conditions. It is inferred that the molecular geometry of the lipids is of vital importance for preservation of the membrane stability. Although this investigation has dealt with the membrane of *A. laidlawii,* the conclusions drawn can nevertheless be expected to hold also for other biological membranes.

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