Lipid bilayer stability in membranes. Regulation of lipid composition in Acholeplasma laidlawii as governed by molecular shape

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Lipid Bilayer Stability in Membranes. Regulation of Lipid Composition in *Acholeplasma laidlawii* As Governed by Molecular Shape†

Åke Wieslander,* Anders Christiansson, Leif Rilfors, and Göran Lindblom

ABSTRACT: The polar lipid composition in membranes of *Acholeplasma laidlawii* is extensively regulated as a response to environmental changes. In particular, the ratio between the dominating lipids monoglucosyldiglyceride and diglucosyldiglyceride is altered depending on temperature, configuration of incorporated fatty acids, and membrane cholesterol content. Synthesis of monoglucosyldiglyceride is stimulated by low temperature and saturated fatty acids but diminished by the presence of cholesterol. These factors are likely to affect the molecular geometry of the membrane lipids. Monoglucosyldiglyceride and diglucosyldiglyceride have wedge- and rodlike molecular shapes, respectively, that are modifiable to a certain extent. The packing constraints of lipids in amphiphilic aggregates, i.e., hydrocarbon–water interfacial area, hydrocarbon chain volume, and hydrocarbon chain length, are very important in determining the aggregate structure [Israelachvili, J. N., Mitchell, D. J., & Ninham, B. W. (1976) *J. Chem. Soc., Faraday Trans. 2 72*, 1525]. Pure monoglucosyldiglyceride forms a reversed hexagonal (H_{II}) phase structure with different fatty acid contents, while diglucosyldiglyceride forms a lamellar phase. However, the only lipid structure compatible with a functional biological membrane is the lamellar phase. Consequently, the balance between lipids forming lamellar and other mesophase structures must keep within certain limits. Here we show that the response in *A. laidlawii* lipid metabolism following external and internal stimuli can be predicted on the basis of molecular shapes and is necessary for the cell in order to maintain optimal membrane stability. Furthermore, the reduced capacity of *Acholeplasma* membranes to incorporate cholesterol is another consequence of this regulation, aiming at preservation of bilayer stability.

The functional state of biological membranes involves a dynamic cooperation between the physiologically active proteins and the lipid matrix. Most physicochemical investigations of these membranes have focused on the gel to liquid crystalline phase transition and its influence on lipid lateral packing (Engelman, 1971), growth temperature adaptation (McElhaney, 1974), permeability characteristics of water, ions, and other species (Razin, 1975a), enzyme and transport activities (Read & McElhaney, 1975; Silvius et al., 1978), and protein disposition (Verkleij, 1975).

Concerning the contribution of lipid polar head groups to membrane function and stability, little is known. However, the maintenance of a critical balance between lipids with different anionic polar head groups has been observed in *Escherichia coli* (Raetz, 1978). Recent findings also show that the activities of a very limited number of membrane-associated enzymes are dependent on a specific lipid surrounding (Sandermann, 1978).

Our previous investigations have focused on the relationships between physiological regulation mechanisms and physical

† From the Department of Microbiology, University of Lund, S-223 62 Lund, Sweden (Å.W., A.C., and L.R.), and the Division of Physical Chemistry 2, Chemical Centre, S-220 07 Lund, Sweden (G.L.). *Received February 14, 1980.* This work was supported by the Swedish Natural Science Research Council.

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properties of membrane lipids from the wall-less procaryote *Acholeplasma laidlawii* strain A (Carlemalm & Wieslander, 1975; Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1978, 1980; Wieslander et al., 1978, 1979). Three distinct ways for regulating the membrane lipid composition have been discovered in this strain: (1) incorporation of fatty acids into membrane lipids is governed by growth temperature as well as the presence of cholesterol; (2) the relative syntheses of the dominating polar lipids monoglucosyldiglyceride (MGDG)[1] and diglucosyldiglyceride (DGDG) are related to each other; they are influenced by changes in the apparent molecular ordering of the hydrocarbon chains caused by growth temperature, fatty acid content, and the presence of cholesterol; (3) the total balance between ionic and nonionic lipids seems to depend on the average lateral packing area occupied by the lipid molecules in the membrane. Such drastic changes in polar head group composition and in the ratio ionic/nonionic lipids have not been observed in other membranes, cf. *E. coli* wild type (Raetz, 1978). Investigations on lipid packing properties in the dry crystalline state (Carlemalm & Wieslander, 1975; Carlemalm, 1976), water binding capacities, transition temperatures, phase structures (Wieslander et al., 1978), and lateral diffusion (Å. Wieslander, L. Rilfors, L. B.-Å. Johansson, and G. Lindblom, unpublished results) of membrane lipids from this *A. laidlawii* strain have also been performed.

In this paper we will show how the physical properties of the membrane lipids of *A. laidlawii* can be related to membrane stability. The only lipid structure compatible with a functional biological membrane is the lamellar one. However, it has been found that often this phase is not the thermodynamically stable one formed by a single membrane lipid–water system at physiological temperatures. Typical membrane lipids forming hexagonal mesophases are phosphatidylethanolamine (PE) (Cullis & De Kruijff, 1978b), diphosphatidylglycerol (DPG) (Rand & Sengupta, 1972), monogalactosyldiglyceride (Shipley, 1973), MGDG (Wieslander et al., 1978), and sphingomyelin (Yeagle et al., 1978). Mixtures of MGDG and DGDG can form a cubic mesophase (Å. Wieslander, L. Rilfors, L. B.-Å. Johansson, and G. Lindblom, unpublished results).

The tendencies for amphiphiles to form different structures (micelles and lamellar or hexagonal phases) can be understood from a unified theory that links interaction free energy, molecular geometry, and thermodynamics (Israelachvili et al., 1976, 1977). Two opposing forces contribute to the interaction free energy. The attractive interactions are the hydrophobic effect (Tanford, 1973), which tends to eliminate the contact between water and the hydrophobic core. Repulsive interactions arise from electrostatic and steric head group repulsion. Steric acyl chain repulsion is also involved. These interactions aim at keeping the total interaction free energy per lipid molecule at a minimum, resulting in an optimal surface area per head group, i.e., the area occupied by the polar head at the hydrocarbon–water interface. Thus, the packing constraints of the lipids in the amphiphilic aggregate are important factors in determining the aggregate shape (Israelachvili et al., 1976, 1977). These authors derived geometric expressions relating the hydrocarbon–water interfacial area a, the hydrocarbon chain volume v, and the hydrocarbon chain length l to the aggregate structure. The following results were obtained for various aggregate shapes: spherical micelle, $v/al = 1/3$; rodlike micelle, $v/al = 1/2$; lamellae, $v/al = 1$. It follows that v/al must be larger than 1 for an aggregate building up a reversed hexagonal (H_{II}) phase, i.e., water cylinders in a hydrocarbon matrix (Ekwall, 1975).

Recently, Wennerström (1979) showed that the theory by Israelachvili et al. is of great importance for the understanding of the phase diagrams of soap–water systems. He concludes that the formation of a lamellar phase on addition of alcohol to a micellar solution or a hexagonal (H_I) phase is due to the fact that the hydrocarbon volume v is enhanced, i.e., v/al approaches unity. Similarly, the lipid organization in a bilayer is a delicate balance between various packing constraints, which determine the membrane stability. All other mesophase structures but the lamellar phase are prohibited. Thus, in a multicomponent bilayer system the geometrical properties of the individual lipid molecules are very important factors. In this work we apply the self-assembly theory by Israelachvili et al. to explain the observed physiological regulation 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.

Results and Discussion

Synthesis and Physical Properties of Membrane Lipids. A. laidlawii has an obligate demand for unsaturated fatty acids that it cannot synthesize (Pollack & Tourtellotte, 1967). Saturated fatty acids are synthesized by the malonyl-coenzyme A pathway (Rottem & Panos, 1970). This synthesis is inhibited by exhaustion of pantethine, a precursor to coenzyme A and acyl carrier protein, from the growth medium (Christiansson & Wieslander, 1980). An absolute control of fatty acid incorporation from exogenous sources can thus be achieved (Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1978, 1980).

In *A. laidlawii* strain A (EF22) the following polar lipids occur (Wieslander & Rilfors, 1977): MGDG, DGDG, an apolar monoglucolipid, phosphatidylglycerol (PG), and glycerophosphoryl derivatives of MGDG and DGDG. Under certain growth conditions DPG is synthesized (Christiansson & Wieslander, 1978). Although uncharged at physiological pH, the glucolipids are cationic selective (Hopper et al., 1970) and calcium ion binding (Wieslander et al., 1978) in resemblance to PE. PG, DPG, and the phosphoglucolipids each carry one negative charge. The tentative lipid biosynthetic pathways are shown in Figure 1.

^2H NMR on heavy water and ^2H-labeled acyl chains together with low-angle X-ray diffraction for lipid–water samples reveal a reversed hexagonal (H_{II}) phase structure for MGDG with different fatty acid contents, whereas DGDG and the other lipids form lamellar phases (Wieslander et al., 1978). DPG can alter between the two phase structures (Rand & Sengupta, 1972). Maximum hydration (7–9 mol of H_2O/polar head group) and phase transition temperatures for MGDG and DGDG are very similar to those of both synthetic and naturally occurring PE (Wieslander et al., 1978). The transition temperatures are about 20 °C above that of phosphatidylcholine with the corresponding fatty acid content (Wieslander et al., 1978).

Lipid Molecular Shape. The various lipid molecules in the bilayer can be visualized as building blocks with different geometries (Figure 2). The hydrocarbon chain length, hydrophobic volume, and polar head group area will determine the overall shape of the blocks (see also introductory statement). The phase structure formed by the different lipids is determined by this molecular shape.

[1] Abbreviations used: MGDG, monoglucosyldiglyceride; DGDG, diglucosyldiglyceride; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; NMR, nuclear magnetic resonance.

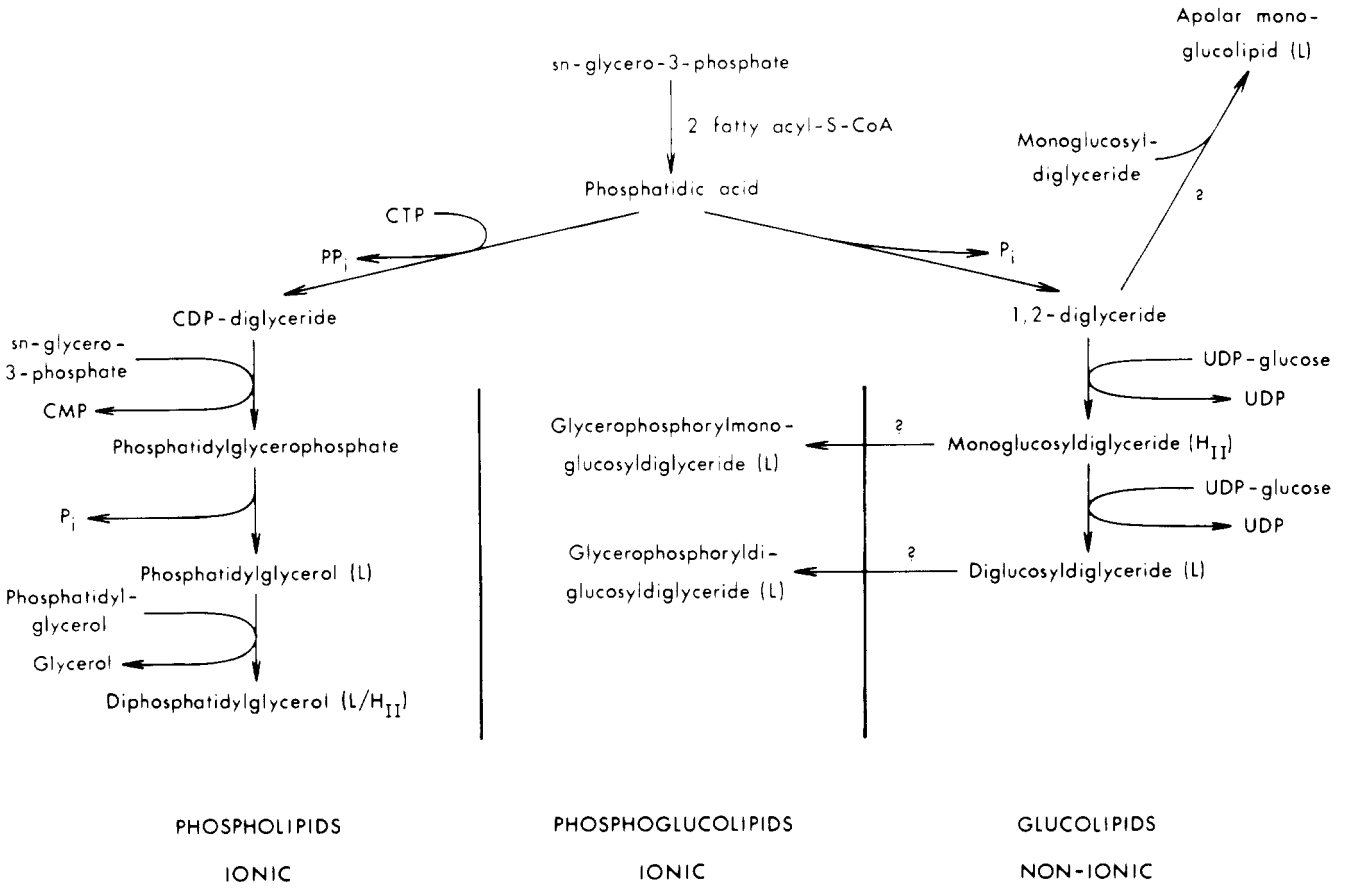

FIGURE 1: Tentative pathways for membrane polar lipid biosynthesis in *Acholeplasma laidlawii* strain A. Abbreviations used: CMP, cytidine 5′-monophosphate; CDP, cytidine 5′-diphosphate; CTP, cytidine 5′-triphosphate; UDP, uridine 5′-diphosphate; P_i, inorganic orthophosphate; PP_i, inorganic pyrophosphate; CoA, coenzyme A; L, lamellar phase structure; H_{II}, reversed hexagonal phase structure; ?, suggested pathway.

(i) With a given *fatty acid 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 (Figure 2A).

(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 fatty acids (Figure 2B, left) for saturated ones, especially with cis double bonds (Figure 2B, right), 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 (v) (Träuble & Haynes, 1971; Israelachvili et al., 1977). By increasing the temperature, trans–gauche isomerizations and 2g1 kinks are introduced into the hydrocarbon chains (Mendelsohn & Taraschi, 1978; Träuble & Haynes, 1971), yielding the same effects as mentioned above (Figure 2B).

(iii) *Cholesterol* has a pronounced wedge shape (Carnie et al., 1979; Figure 2B). Furthermore, cholesterol slightly diminishes the lateral packing area of an individual lipid in a monolayer, since the observed mean molecular areas for cholesterol–lipid mixtures are smaller than those obtained by theoretical calculations (Demel & De Kruijff, 1976). However, the presence of cholesterol will nevertheless largely increase the distances between all the other lipid molecules.

Lipid Regulating Mechanisms in Acholeplasma laidlawii. (*A*) *Fatty Acid Incorporation.* The potential capacity of *A. laidlawii* to regulate membrane fatty acid incorporation is best evoked by applying a temperature-shift technique (Christiansson & Wieslander, 1978). Cells grown in an equimolar mixture of palmitic and oleic acid incorporate more oleic acid when shifted to 17 °C than cells maintained at 37 °C. This increase of oleic acid takes different courses with respect to different lipid species and time after the temperature decrease. The most rapid response occurs in MGDG, the polar lipid having the highest content of palmitic acid. In PG the oleic acid content rises after an initial lag period, whereas DGDG remains nearly unchanged. Thus, in order to maintain an optimal packing of the various lipids in the bilayer, according to the self-assembly theory, the cell can "afford" to incorporate unsaturated fatty acids into MGDG, although this lipid forms a reversed hexagonal (H_{II}) phase. In fact, since the temperature is lowered, it can be expected that the MGDG content is responsible for about the same packing constraint at the low temperature as it was at the higher temperature with more saturated fatty acids (Figure 2B), giving an optimal packing in the bilayer.

(*B*) *Regulation of Polar Head Group Composition.* Three different ways to change the polar head group composition have been exploited in *A. laidlawii* strain A (Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1978, 1980): (i) variations in structure of incorporated *fatty acids*; (ii) changes in growth *temperature*; (iii) incorporation of *cholesterol*. The predominant response in polar lipid metabolism is a large change in the ratio between the structurally related glucolipids MGDG and DGDG (Figure 1). Furthermore, the ionic lipid fraction varies profoundly. These changes in *A. laidlawii* play a central role concerning the relation between lipid molecular shape and membrane bilayer stability.

(i) Successive changes in the hydrophobic geometry of the lipids caused by different incorporated ratios of saturated/unsaturated *fatty acids* yield correlated responses in the molar ratio MGDG/DGDG (Table I). Increased amounts of cis-

Table I: Polar Lipid 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
% ionic lipids of total (mol/mol)	13.5	17.2	22.9	25.5	35.9	43.1

[a] The ratio cholesterol/total lipids is 0.29 (mol/mol), corresponding to about 15% (wt/wt), which is the maximum incorporable amount in *A. laidlawii*.

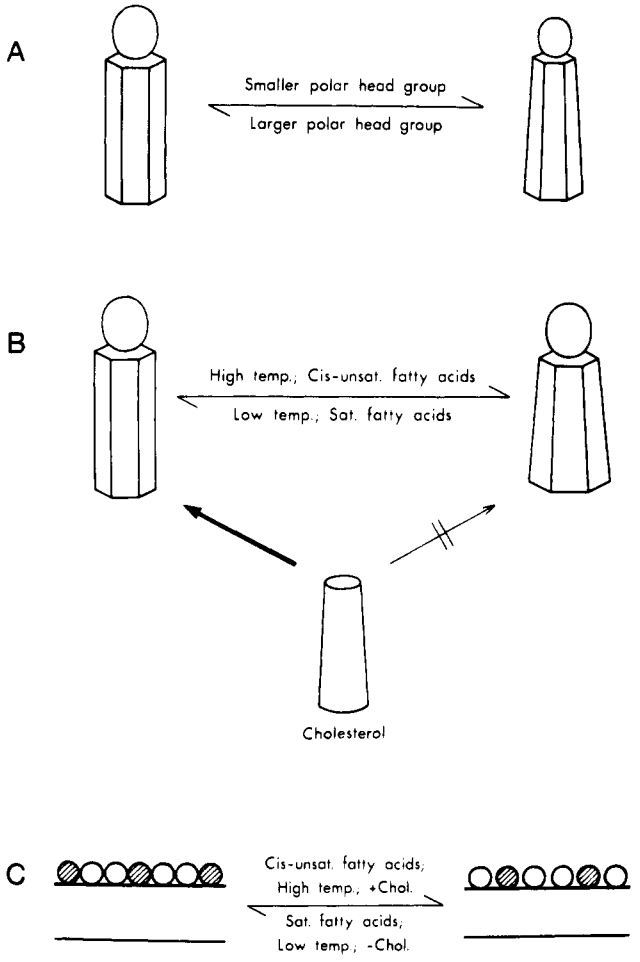

FIGURE 2: Molecular shapes of lipids. The geometrical properties of the lipid molecules determine their ability to pack together into a bilayer structure. (A) Molecules with identical fatty acid compositions. Left: geometry of lipids having a moderately large polar head group and a tendency to form a lamellar phase structure. Right: geometry of lipids having a small polar head group and thus a tendency to form a reversed hexagonal (H_{II}) phase structure due to the reduced hydrocarbon–water interfacial area. Note the pronounced wedge shape of the molecule. (B) Molecules with identical polar head groups. Left: geometry of lipids at temperatures below the phase transition temperature (T_t) and/or with saturated fatty acids only. Right: geometry of lipids at temperatures above T_t and/or containing cis unsaturated fatty acids. The shortening and broadening of the hydrophobic part of the molecule result in an increased tendency to form a reversed hexagonal (H_{II}) phase structure. Lower middle: molecular geometry of cholesterol. The arrows denote the possibilities for cholesterol to copack with lipids having different geometries and still preserve a stable bilayer structure. The dimensions of the molecules are based on literature values. (C) Membrane surface charge density. Left: polar head group density at temperatures below T_t and/or with lipids containing saturated fatty acids only. Right: polar head group density at temperatures above T_t and/or with lipids containing cis unsaturated fatty acids and/or cholesterol. Hatched circles denote anionic lipid molecules.

unsaturated fatty acids will increase the hydrophobic bulkiness of the lipid molecules (Figure 2B), leading to a more accentuated wedge shape, especially of MGDG, which fits better in a curved aggregate than in a bilayer structure. Consequently, the membrane stability is maintained by *decreasing* the ratio MGDG/DGDG upon an increase of cis unsaturation. The extent of variation of MGDG is 60% larger than that of DGDG, indicating that MGDG is the most actively regulated component. Similar alterations in the ratio MGDG/DGDG occur when the endogenous saturated fatty acid synthesis is stimulated by stepwise additions of the coenzyme precursor pantetheine (Christiansson & Wieslander, 1980).

By decreasing the molar fraction of cis-unsaturated fatty acid in the membrane from 95 to 30%, the ionic lipid fraction is reduced by almost a factor of three (Table I). This might be due partly to a compensation for the enhanced surface charge density caused by the decreased lateral packing area of the lipid (Figure 2C). Since the presence of divalent cations in the culture medium raises the gel to liquid crystalline transition temperature for the ionic lipids (Träuble, 1977), a reduction in the amount of these lipids may also be necessary to prevent the molecular ordering of the acyl chains from becoming too high.

(ii) A decrease in *temperature* leads to an increase in molecular order of the acyl chains, diminishing the hydrophobic bulkiness of the lipids (Figure 2). The magnitude of this effect depends on the existing fatty acid composition before the temperature shift (Figure 2B). Most biological membranes contain a certain fraction of wedge-shaped polar lipids. The presence of these lipids is of importance for bilayer stability, and, consequently, a decrease in temperature must be neutralized by accentuating the wedge shape properties. This can be achieved in two ways: (1) an enhanced incorporation of unsaturated fatty acids (section i above) and (2) increased synthesis of lipids with a small polar head group like MGDG (Figure 2A). The latter effect is demonstrated in Figure 3, where it is shown how the ratio MGDG/DGDG is altered with cell growth after a temperature shift and/or with different membrane fatty acid compositions. At the time of shift-down (12 h), the ratio of MGDG/DGDG differs significantly depending on the difference in fatty acid composition as shown in Table I. With both fatty acid supplementations this ratio is diminished during growth at 37 °C (Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1978). However, at 17 °C the ratio rises due to a largely *enhanced* synthesis of MGDG (Christiansson & Wieslander, 1978). This effect is observed also when *A. laidlawii* is grown at a constant low temperature, 10 °C. Because of the shape of the molecule, MGDG will reestablish an optimal packing of the membrane components in the bilayer.

When passing the phase transition interval a decrease in temperature will decrease the lateral packing area of the lipids

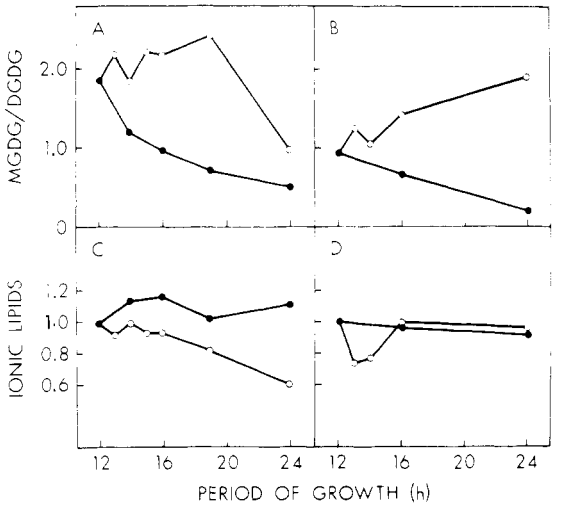

FIGURE 3: Regulation of polar lipid composition in *A. laidlawii* after a temperature shift: (A and B) ratio MGDG(H_{II})/DGDG(L); (C and D) relative amount of ionic lipids; the amount at 12 h is assigned the value of 1.0; (A, C) cells grown with 75 μM palmitic acid and 75 μM oleic acid; (B, D) cells 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 by Christiansson & Wieslander (1978).

and consequently increase the surface charge density (Träuble, 1977; Träuble & Haynes, 1971; Figure 2C). With membranes containing palmitic and oleic acid, a shift from 37 °C to a temperature within the phase-transition interval, e.g., 17 °C (Christiansson & Wieslander, 1978; Wieslander et al., 1978) yields a strong reduction in the biosynthesis of ionic lipids (Figure 3C). The reasons for this may be the same as mentioned in section i. With membranes containing oleic acid only, 17 °C is well above the phase-transition interval (cf. Figures 3C and 3D).

(iii) 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 (Christiansson & Wieslander, 1978). Obviously cholesterol causes an increase in the ordering of the fatty acyl chains resulting in a more rodlike type of molecular geometry of the surrounding lipids. This is counteracted by an increased incorporation of unsaturated fatty acids in all lipids. Furthermore, since cholesterol has a significant wedge shape (Carnie et al., 1979; Figure 2B), insertion of this molecule into the membrane destabilizes the bilayer structure, especially in the presence of lipids forming a reversed hexagonal (H_{II}) phase. In *A. laidlawii* the formation of such a phase in the membrane is prevented by a relative *reduction* of the amount of MGDG by 40% (Table I). Incorporation of cholesterol into a membrane also dilutes the surface charge density (Figure 2C). *A. laidlawii* compensates for this by increasing the ionic lipid fraction (Table I).

Thus, according to the presented theory, an increase in the number of cis double bonds, an increase in temperature, as well as the presence of cholesterol destabilize the lamellar phase structure, which the cell must compensate for. This indeed occurs by a change in the polar head composition: a lowering of the MGDG lipid content and an increase in the amount of ionic lipids.

Concluding Remarks

It has been shown that for most purposes a qualitative application of the theory developed by Israelachvili et al. (1976, 1977) can be used for a sufficiently good description of the lipid bilayer stability in *Acholeplasma laidlawii* membranes. Thus, it can be concluded that the molecular geometry of the lipids is of vital importance for the stability. Although the theory has been applied to the membrane of *A. laidlawii*, it can nevertheless be expected to hold also for other biological membranes. For example, it has been found that *E. coli* counteracts the effect of a drop in the growth temperature by an increase in the degree of cis unsaturation in the lipids (Cronan & Gelmann, 1975), which changes the geometry of the dominating polar lipid PE to a more pronounced wedge shape (Cullis & De Kruijff, 1978a). In *Bacillus megaterium* this temperature adaptation is accomplished by regulation of the fatty acid chain length and by the ratio between two different types of branched fatty acids (Rilfors et al., 1978), which influence the hydrophobic volume.

A proposal can be made to explain the occurrence of several different polar lipid species in the membranes of most organisms, and why their relative amounts must be actively regulated. These membrane properties most certainly would have been lost during evolution were they not of crucial importance for survival to the cell. An optimal packing of the lipid molecules is essential for obtaining a stable bilayer structure. This demand cannot be fulfilled by a single lipid species; it necessitates a mixture of lipids having tendencies to form lamellar and other mesophase structures (e.g., hexagonal, cubic). As shown in this work, the balance between such lipids must keep within certain limits, and this probably explains why some lipid species are not coexisting in biological membranes (Demel et al., 1977; Carnie et al., 1979). Furthermore, it has been proposed that local regions of lipids forming transitory nonlamellar phases may be advantageous to some membrane functions such as movement of membrane components between bilayer halves, fusion, exo- and endocytosis, and facilitated transport (Cullis & Verkleij, 1979). Nonlamellar phases have been reported for microsomal membranes (De Kruijff et al., 1978; Stier et al., 1978). It has also been suggested that packing constraints impose a structural coupling between membrane proteins and their surrounding lipids, and thus a heterogeneous composition of lipids with different molecular geometries may be required in order to minimize packing defects around the irregular surfaces of proteins (Israelachvili, 1977; Sandermann, 1978). This suggestion is strongly supported by protein–lipid interaction studies performed on *A. laidlawii* (K.-E. Johansson, C. Jägersten, A. Christiansson, and Å. Wieslander, unpublished experiments).

The balance between ionic and nonionic lipids is actively regulated in *A. laidlawii* membranes (Table I). The great capability of *A. laidlawii*, compared to *E. coli*, to regulate the ionic lipid fraction in response to environmental changes seems logical considering that the *A. laidlawii* membrane directly faces the surrounding physical milieu, while the *E. coli* membrane is enclosed by a cell envelope.

Our results also show the significance of knowing the phase diagram of the individual as well as mixed compositions of the various membrane lipids. Hitherto, few such diagrams have been reported in the literature. Recently, we discovered a cubic phase consisting of a mixture of MGDG and DGDG (Å. Wieslander, L. Rilfors, L. B.-Å. Johansson, & G. Lindblom, unpublished experiments). This phase is formed by introducing surplus amounts of MGDG with pronounced wedge shape (i.e., containing cis unsaturated fatty acids) in the lamellar phase of DGDG. The observation further supports the self-assembly theory used.

It should be noted that the theory also nicely pertains to the effect of cholesterol on biological membranes. Monosugar diglycerides, forming a reversed hexagonal (H_{II}) phase

structure, are present in significant amounts in the membranes of all *Acholeplasma* species (Smith, 1979). The genus *Acholeplasma* is also characterized by having no requirement for cholesterol (Razin, 1978). Moreover, in resemblance to cell wall enclosed bacteria, *A. laidlawii* has a reduced capacity for cholesterol incorporation (Razin, 1975b). The presence of this molecule in the membrane diminishes the MGDG content (Table I). In light of the observed phase behavior of MGDG/DGDG mixtures (see above), it can be anticipated that large amounts of the wedge-shaped cholesterol molecule must induce a nonlamellar phase just as MGDG does. Hence, the *simultaneous* presence of large amounts of MGDG and cholesterol must be avoided. This finding is supported by the observation that cholesterol destabilizes the bilayer structure of dioleoylphosphatidylethanolamine as well as a mixture of soy phosphatidylethanolamine and egg yolk phosphatidylcholine (Cullis & De Kruijff, 1978a). Previously, the results obtained usually were discussed in terms of cholesterol as a moderator of "fluidity" or "microviscosity". This explanation has recently been questioned (G. Lindblom, L. B.-Å. Johansson, & G. Arvidson, unpublished experiments), since it was observed that the lipid lateral diffusion (a *dynamic* parameter) was practically unaffected by the presence of cholesterol in the bilayer. We propose that cholesterol, due to its molecular shape, exerts its greatest influence on bilayer structures by disturbing the packing of the lipid molecules.

Acknowledgments

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