Metabolic Changes of Membrane Lipid Composition in *Acholeplasma laidlawii* by Hydrocarbons, Alcohols, and Detergents: Arguments for Effects on Lipid Packing†

Åke Wieslander,*,‡ Leif Rilfors,§ and Göran Lindblom§

Departments of Biochemistry and of Physical Chemistry, University of Umeå, S-901 87 Umeå, Sweden

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ABSTRACT: The packing of lipids into different aggregates, such as spheres, rods, or bilayers, is dependent on (i) the hydrophobic volume, (ii) the hydrocarbon–water interfacial area, and (iii) the hydrocarbon chain length of the participating molecules, according to the self-assembly theory [Israelachvili, J. N., Marčelja, S., & Horn, R. G. (1980) *Q. Rev. Biophys. 13*, 121–200]. The origin of the participating molecules should be of no importance with respect to their abilities to affect the above-mentioned parameters. In this investigation, *Acholeplasma laidlawii*, with a defined acyl chain composition of the membrane lipids, has been grown in the presence of three different classes of foreign molecules, known to partition into model and biological membranes. This results in an extensive metabolic alteration in the lipid polar head group composition, which is expressed as changes in the molar ratio between the lipids monoglucosyldiglyceride (MGDG) and diglucosyldiglyceride (DGDG), forming reversed hexagonal and lamellar phases in excess water, respectively. The formation of nonlamellar phases by *A. laidlawii* lipids depends critically upon the MGDG concentration [Lindblom, G., Brentel, I., Sjölund, M., Wikander, G., & Wieslander, Å. (1986) *Biochemistry* (preceding paper in this issue)]. The foreign molecules tested belong to the following groups: nonpolar organic solvents, alcohols, and detergents. Their effects on the gel to liquid crystalline phase transition temperature (T_m), on the order parameter of the acyl chains, and on the phase equilibria between lamellar and nonlamellar liquid crystalline phases in lipid–water model systems are known in several instances. The observed alterations in the ratio MGDG/DGDG can only be explained as a response to changes of the geometrical packing parameters i–iii caused by the foreign molecules, and hence as a response to changes of the equilibria between lamellar and nonlamellar phases. No consistent correlation was found between the lipid ratio and the T_m or the order parameter. Our conclusions lend further support to the applicability of the self-assembly theory for explaining lipid regulation in *A. laidlawii* membranes.

The relative amount of membrane lipids is metabolically altered in many organisms in response to various changes of the environmental conditions. The aim of these regulations most probably is to maintain one or more physicochemical properties of the lipid bilayer within certain limits. The exact nature of these properties is still only partly defined.

It has been shown for some bacterial membranes that at least 10–50% of the lipids must be in a liquid crystalline state in order for the organisms to grow and divide (McElhaney, 1984b). The maintenance of a minimal fraction of liquid crystalline lipids may thus in some cases be the reason for a regulation of the membrane lipid composition. However, many organisms grow at temperatures that are well above the gel to liquid crystalline phase transition interval (T_m).[1] Despite this, such organisms regulate their lipid composition in response to changes of the environmental conditions, even to those changes having a minimal effect on T_m. Besides the lamellar gel (L_β) and liquid crystalline (L_α) phases, certain membrane lipids can form cubic and hexagonal phases, usually of the reversed type (I_{II} and H_{II}, respectively) (Luzzati, 1968; Gulik et al., 1985). The transition between lamellar and nonlamellar phases in lipid–water model systems is affected by, e.g., the temperature, the hydration, cations, pH, additives such as steroids, alcohols, local anestethics, and detergents, and the structure of the lipid molecules (Rilfors et al., 1984). Extended formation of nonlamellar phases in biological membranes cannot be allowed since such phases lack the barrier properties of the L_α phase. Thus, just as a complete transition from an L_α to an L_β phase is forbidden, so is a transition from an L_α to a nonlamellar phase.

The factors governing the formation of different lipid aggregate structures have been discussed by Tanford (1980), Israelachvili et al. (1976, 1980), and Mitchell et al. (1983). From these theoretical calculations, it is concluded that the effective geometry of the lipid molecules determines the shape of the aggregates. More specifically, the packing of the lipids into different aggregates is dependent on the hydrophobic volume, the hydrocarbon–water interfacial area, and the hydrocarbon chain length of the participating molecules. According to this approach, a lamellar phase is built up of cylindrical-like molecules. A decrease in the hydrocarbon–water interfacial area, an increase in the hydrophobic volume, or a simultaneous shortening and broadening of the hydrophobic region with no volume change causes the lipid molecules to occupy a space with the effective shape of a truncated cone (Wieslander et al., 1980). This, in turn, will favor the for-

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‡ Department of Biochemistry.

§ Department of Physical Chemistry.

[1] Abbreviations: L_α, lamellar liquid crystalline phase; L_β, lamellar gel phase; I_{II}, reversed cubic phase; H_I, normal hexagonal phase; H_{II}, reversed hexagonal phase; L_I, micellar solution phase; T_m, temperature (interval midpoint) for the L_β to L_α transition; T_{LH}, temperature (interval midpoint) for the L_α to H_{II} transition; MGDG, monoglucosyldiglyceride; DOMGDG, dioleoyl-MGDG; DGDG, diglucosyldiglyceride; DODGDG, dioleoyl-DGDG; PE, phosphatidylethanolamine; DOPE, dioleoyl-PE; PC, phosphatidylcholine; DOPC, dioleoyl-PC; DPPC, dipalmitoyl-PC; C_nEO_m, n is the number of carbons in the alkyl chain and m is the number of oxyethylene units in the polar head of poly(oxyethylene) alkyl ether detergents, respectively; in vivo lipid mixture, lipid mixture of all *A. laidlawii* polar membrane lipid species from a specific growth condition and at their natural percentage.

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mation of nonlamellar aggregates of the reversed type (water in oil). The opposite changes of the above-mentioned quantities will accordingly yield nonlamellar aggregates of the normal type (oil in water) (Israelachvili et al., 1980). The different aggregate structures, such as spheres, rods, and lamellae, then build up different liquid crystalline phase structures (Rilfors et al., 1984). We have previously shown that the ideas of lipid molecular geometry can be used to explain the regulation of membrane lipid composition occurring in the cell wall less bacterium *Acholeplasma laidlawii* upon manipulation of the growth temperature and of the membrane acyl chain and cholesterol composition (Wieslander et al., 1980, 1981a; Rilfors, 1985).

If the above-mentioned approach is of general validity when discussing lipid regulation mechanisms, the origin of the molecules present in the membrane should be of no importance. Thus, several classes of foreign molecules that are incorporated into the membrane should affect the lipid composition in accordance to their ability to change the effective hydrophobic volume, hydrocarbon–water interfacial area, and hydrocarbon chain length, in the membrane of the living cell. These changes will shift the phase equilibria of the membrane lipids in model systems. In this work we show that the ideas of lipid molecular geometry are able to explain why an extensive metabolic alteration of membrane lipid composition is induced upon the presence of different classes of foreign molecules in the *A. laidlawii* membrane.

Materials and Methods

Organism and Growth Medium. Strain A of *Acholeplasma laidlawii* (Wieslander & Rilfors, 1977) was grown in a thoroughly lipid-depleted bovine serum albumin–tryptose medium (Christiansson & Wieslander, 1980). In order to be able to grow in this medium, this strain is forced to use selectively supplemented fatty acids as precursors for membrane polar lipid synthesis. Exogenous fatty acids are thus incorporated to a very high extent in the membrane lipids (≥95 mol %). Oleic acid was added from an ethanolic stock solution (20 mg/mL). The cells were adapted to this fatty acid supplement by five to seven consecutive daily inoculations.

The Shift Technique. The alterations of membrane lipid composition caused by nonpolar organic solvents, alcohols, and detergents were analyzed by a shift technique. Suitable concentrations of the additives were selected in the following way. Cells were grown at 30 °C (2% v/v inoculum) in a medium supplemented with 150 μM oleic acid. After approximately 12–13 h, with a cell density corresponding to a turbidity of 0.20 at 520 nm, the cultures were divided into 10-mL aliquots (in screw-capped tubes), each supplemented with a specified amount of the molecule to be tested. The ability of the cells to maintain integrity and continue to grow for another 6–8 hours was analyzed by turbidity measurements and by phase-contrast light microscopy. Concentrations of the additives that did not impair growth severely were selected (Figure 1). At these concentrations the effect of the additives on membrane lipid metabolism was then studied in separate shift experiments by harvesting 25-mL cultures 6 h after the shift. Such media were also supplemented with 10 μCi/L [^{14}C]oleic acid.

Additives in Shift Cultures. The following additives were used: (nonpolar organic solvents) benzene, cyclohexane, diethyl ether, and *n*-dodecane; (alcohols) phenethyl alcohol (2-phenylethanol), ethanol, 1-butanol, 1-hexanol, 1-octanol, 1-decanol, 1-dodecanol, 1-tetradecanol, and 1-hexadecanol; (detergents) the poly(oxyethylene) alkyl ether derivatives (C_nEO_m) $C_{12}EO_3$, $C_{12}EO_4$, $C_{12}EO_6$, $C_{12}EO_8$, $C_{12}EO_{12}$, $C_{16}EO_3$, $C_{16}EO_4$, $C_{16}EO_6$, and $C_{16}EO_8$ [cf. Mitchell et al. (1983)].

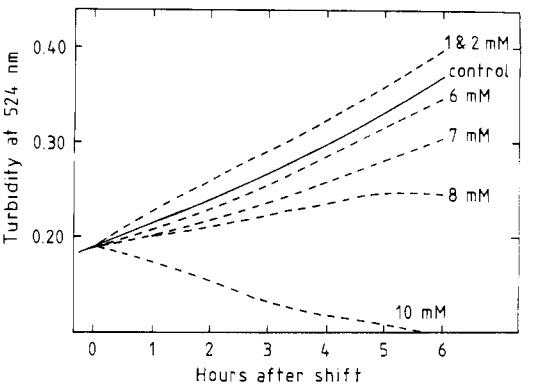

FIGURE 1: Growth of *A. laidlawii* during exposure toward nonpolar organic solvents, alcohols, and detergents. Cells were grown approximately 12 h at 30 °C in a medium with 150 μM oleic acid. The cultures were then divided into several aliquots, each supplemented with different amounts of the molecules to be tested. Growth was allowed to proceed at 30 °C after the shift and was monitored by turbidity measurements and by light microscopy. The experiments shown held for 1-hexanol. Note that low amounts of the alcohol stimulated growth. This was the case for most supplements tested (data not shown). At high concentrations, the cells became swollen, stopped to grow, and eventually started to lyse. Concentrations that did not severely impaired growth were chosen for lipid composition experiments (see Tables I–III).

Lipid Analysis. Harvest of cells, purification of membrane lipids by Sephadex column chromatography, and separation of polar species by thin-layer chromatography were done as described (Christiansson & Wieslander, 1980). Radioactively labeled lipids were quantified by liquid scintillation counting in a Packard PLD Tri-Carb using Pico-Fluor 30 cocktail.

Polarized Light Microscopy. The ability of several additives to change the phase eqiulibria of *A. laidlawii* membrane lipids was analyzed by polarizing light microscopy. Different phase structures have typical textures that are easily recognized; cf., e.g., Rosevear (1954) and Fontell (1978). A mixture of dioleoylmonoglucosyldiglyceride (DOMGDG) and dioleoyldiglucosyldiglyceride (DODGDG) (molar ratio 1.2:1) with water was prepared by centrifugation; excess water was then withdrawn (Wieslander et al., 1981b; Lindblom et al., 1986). This mixture forms a lamellar phase at room temperature with excess water (Lindblom et al., 1986). A sample was deposited on a clean microscope slide and covered with a glass cover slip. The void space was then filled with the solvent to be used and the cover slip sealed to the slide. Samples were observed with a Zeiss Standard WL microscope, equipped with polarization optics and a temperature stage.

Materials. Tryptose was obtained from Difco Laboratories, Detroit, MI. Bovine serum albumin, fatty acids, *n*-dodecane, and penicillin were from Sigma Chemical Co., St. Louis, MO. Poly(oxyethylene) detergents were from Nikko Chemicals Co., Tokyo, Japan, or from Dr. Gordon Tiddy, Unilever Research, Port Sunlight Laboratory, Merseyside, U.K. Sephadex (G-25 fine) was from Pharmacia Fine Chemicals, Uppsala, Sweden, and *n*-alcohols were from Larodan Fine Chemicals, Malmö, Sweden. Thin-layer silica gel (H60), solvents, and bulk chemicals (analytical grade) were from Merck, Darmstadt, FRG and radioactive isotopes were from Amersham International, U.K.

Results and Discussion

Membrane Lipid Regulation

The following amphiphilic lipids occur in the membrane of *A. laidlawii* strain A (Wieslander & Rilfors, 1977): the nonionic lipids monoglucosyldiglyceride (MGDG), di-

Table I: Nonpolar Organic Solvents: Effect on Growth of *A. laidlawii* and Calculated Concentrations in the Cell Membrane

organic solvent	largest concn permitting growth after shift (mM)	concn used for lipid composition expt (mM)	lipid–buffer partition coeff[a]	mol of solvent/100 mol of lipids in membranes[b]
n-dodecane	≫max solubility[c]	≤50		
cyclohexane	15	≤10	60	52
benzene	25	≤20	51	44
diethyl ether	250	100[d]	1.7	15

[a] Partition coefficients for DPPC or DOPC (mol of solute per L of lipid/mol of solute per L of water) from Hill (1974) and Simon et al. (1982). [b] Calculated by using partition coefficients from footnote *a*, a lipid specific volume of 0.97 cm^3/g (Gulik-Krzywicki et al., 1967; Rivas & Luzzati, 1969), a mean molecular weight of *A. laidlawii* oleoyl lipids of 900, and lipid amounts in *A. laidlawii* membranes after the shift period, respectively. Generally, partition into saturated lipid bilayers is slightly lower than that into unsaturated lipid bilayers (Simon et al., 1982). [c] Solubility for *n*-dodecane 1.66×10^{-5} mM. [d] The anesthetic concentration of diethyl ether is 50 mM (Seeman, 1972). All solvents used, *except* *n*-dodecane, are anesthetic.

glucosyldiglyceride (DGDG), and a minor glucolipid X, and the ionic lipids phosphatidylglycerol, glycerophosphomonoglucosyldiglyceride, and glycerophosphodiglucosyldiglyceride. MGDG forms an H_{II} phase in excess water at physiological temperatures, and the other lipids form a lamellar phase under the same conditions (Wieslander et al., 1978; Lindblom et al., 1986).

The molar ratio between MGDG and DGDG is of particular interest when investigating the regulation of lipid composition in the *A. laidlawii* membrane. These are the major lipids and make up between 55 and 75% (mol/mol) of the polar lipids in this membrane. The two lipids form different phase structures (see above), and the phase equilibria in mixtures of these lipids are affected by several factors (Wieslander et al., 1981b; Khan et al., 1981; Lindblom et al., 1986): (1) the ratio between the two lipids; (2) the degree of unsaturation of the acyl chains; (3) the temperature; (4) the hydration; (5) the presence of cholesterol. In vivo mixtures of all polar *A. laidlawii* membrane lipids also form lamellar and nonlamellar (I_{II} or H_{II}) phase structures. The phase equilibria in these mixtures are affected by the same factors as the MGDG–DGDG mixtures (Lindblom et al., 1986). Since MGDG is the only lipid that alone with water can form nonlamellar phase structures, it is a key component in determining the phase equilibria of the in vivo mixtures. The ratio MGDG/DGDG in the *A. laidlawii* membrane is extensively regulated upon alterations of the growth conditions (Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1978, 1980; Christiansson et al., 1981; Rilfors, 1985; Clementz et al., 1986). The regulations can be explained by the phase equilibria in MGDG–water, MGDG–DGDG–water, and in vivo total polar lipid–water mixtures, and hence by the ideas of lipid molecular geometry (Wieslander et al., 1980, 1981a; Rilfors, 1985; Lindblom et al., 1986). When the conditions in the membrane are altered in such a way that the phase equilibria of the lipids are shifted toward nonlamellar phases (see introduction), *A. laidlawii* alters the lipid composition so that the phase equilibria are shifted in the opposite direction. In this investigation, the variations of the ratio MGDG/DGDG have been followed after incorporation of various hydrophobic and amphiphilic molecules into the *A. laidlawii* membrane.

Nonpolar Organic Solvents

The following solvents were selected to represent this group of molecules: *n*-dodecane, cyclohexane, benzene, and diethyl ether. The ether is slightly more polar than the hydrocarbons; the dielectric constant at 20 °C is 4.34 for diethyl ether and 2.01, 2.02 and 2.28 for *n*-dodecane, cyclohexane, and benzene, respectively (Weast, 1971). The corresponding value for water is 80.1.

Model Systems. The interactions of nonpolar organic molecules with model lipid–water systems have been investigated. All the four solvents used in this investigation lower the T_m of lipid–water model systems. At the solvent concentrations used (Table I), the lowering of T_m for dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine was 1–3 °C with *n*-dodecane, benzene, and diethyl ether and 8 °C with cyclohexane (McIntosh et al., 1980; McDaniel et al., 1982; Janoff & Miller, 1982).

The nonpolar organic solvents used partition more or less deeply into the hydrophobic interior of the bilayer (McIntosh et al., 1980; White et al., 1981; Simon et al., 1982; Nagarajan et al., 1984; Pope et al., 1984). These solvents are able to transform a lamellar phase into nonlamellar phases of the reversed type. For example this has been shown by addition of *n*-dodecane to the three systems egg phosphatidylethanolamine (PE)–water (Hornby & Cullis, 1981), dioleoyl-PE (DOPE)–dioleoylphosphatidylcholine (DOPC)–water (Kirk & Gruner, 1985), and DOPC–water (M. Sjölund, G. Lindblom, L. Rilfors, and G. Arvidson, unpublished results). Under conditions where these systems form an L_α phase, the presence of *n*-dodecane induces the formation of an H_{II} phase in the first two systems and the formation of an I_{II} or an H_{II} phase in the third system. Likewise, the 20-carbon alkane *n*-eicosane lowers the temperature for the transition from an L_α to an H_{II} phase (T_{LH}) in the systems dielaidoyl-PE–water and 1-palmitoyl-2-oleoyl-PE–water (Epand, 1985). Finally, benzene and cyclohexane induce the formation of an H_{II} phase in DPPC at low water content (McDaniel et al., 1982).

In this study it was determined by polarized light microscopy that a lamellar phase of the *A. laidlawii* model system DOMGDG–DODGDG–water is transformed to an H_{II} phase by addition of *n*-dodecane (see Materials and Methods). Moreover, benzene and cyclohexane induce the formation of cubic and H_{II} phases in the DOMGDG–DODGDG–water system.

Physiological Response in A. laidlawii. Table I presents data concerning the largest concentrations of the solvents that permit growth of *A. laidlawii*, the solvent concentrations used in the lipid composition experiments, the lipid–buffer partition coefficients, and the calculated fraction of solvent molecules in the membranes. An upper concentration limit permitting growth of *A. laidlawii* seems not to exist for *n*-dodecane; at a concentration of 50 mM in the growth medium the organism grew equally well as in the control culture. Diethyl ether, the most polar of the four molecules, has a lower lipid–buffer partition coefficient than cyclohexane and benzene and has to be used in higher concentrations in order to reduce the growth rate of *A. laidlawii*.

The effect on the lipid composition of incorporation of the nonpolar organic solvents into the *A. laidlawii* membrane is shown in Figure 2. All solvents cause a decrease in the ratio MGDG/DGDG. For cyclohexane and benzene, the decrease is dependent on the concentration of the solvent in the growth

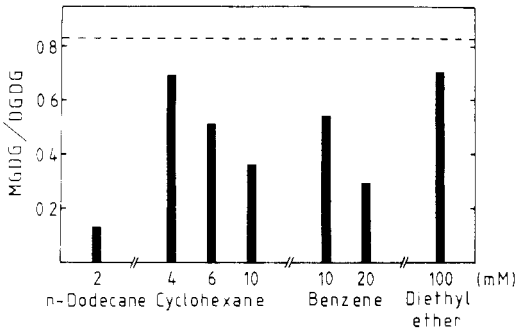

FIGURE 2: Effect of nonpolar organic solvents on membrane lipid regulation in *A. laidlawii*. The lipid composition was determined after 6-h exposure to the solvents as described in Figure 1. The membrane lipids contained ≥95 mol % oleoyl chains. Broken horizontal line indicates MGDG/DGDG ratio in control culture devoid of solvent supplements.

medium. The concentration dependence was not studied with *n*-dodecane and diethyl ether.

When the value of the ratio MGDG/DGDG is lowered, *A. laidlawii* reduces the fraction of membrane lipids able to form an H_{II} phase and increases the fraction forming a lamellar phase. The organism in this way counteracts the nonbilayer-promoting ability of the nonpolar organic molecules, and the stability of the lipid bilayer is thus maintained.

It is unlikely that the variation of the ratio MGDG/DGDG can be explained as an effort of the organism to regulate the T_m. The effect of these solvents on the T_m of model systems is small (see above) and probably is of little importance for the organism since the T_m of *A. laidlawii* membrane lipids enriched in oleoyl chains already is about 45 °C below the growth temperature (McElhaney, 1984a). The T_m for DOMGDG and DODGDG are −15 and −20 °C, respectively (Lindblom et al., 1986), and a reduction of the ratio MGDG/DGDG thus causes a small additional decrease of the T_m. Furthermore, there is no strict connection between the alteration of the T_m and the regulation of the ratio MGDG/DGDG in the *A. laidlawii* membrane. An increase in the chain length of the fatty acids incorporated into the membrane lipids will *increase* the T_m of the lipids (Silvius et al., 1980). However, the organism responds by lowering the ratio MGDG/DGDG (Silvius et al., 1980; Rilfors, 1985).

Alcohols

This group of molecules is represented by the *n*-alcohols with an even number of carbon atoms from ethanol to hexadecanol and by phenethyl alcohol. The latter alcohol has been shown to affect the posttranslational processing of membrane proteins (Halegoua & Inouye, 1979). Alcohols are anchored with the hydroxyl group near the hydrocarbon–water interface, and the hydrocarbon chain extends into the hydrocarbon region of the lipid bilayer (Ekwall, 1975; Ebihara et al., 1979; Pope et al., 1984).

Model Systems. Investigations have been carried out concerning the effects of the *n*-alcohols, phenethyl alcohol, and benzyl alcohol (homologue to phenethyl alcohol) in lipid–water model systems. Alcohols up to 1-octanol lower the T_m for saturated PC and PE species (Lee, 1976; Richards et al., 1978). The T_m for these lipids is either raised or lowered by 1-decanol and 1-dodecanol depending on the acyl chain length and the polar head group structure; 1-tetradecanol and 1-hexadecanol increase the T_m (Pringle & Miller, 1979), and benzyl alcohol decreases the T_m for DPPC (Ebihara et al., 1979). In *A. laidlawii* 1-pentanol (50–200 mM) and benzyl alcohol (≤120 mM) decrease the T_m of the membrane lipids in a dose-dependent manner (MacDonald & Cossins, 1983).

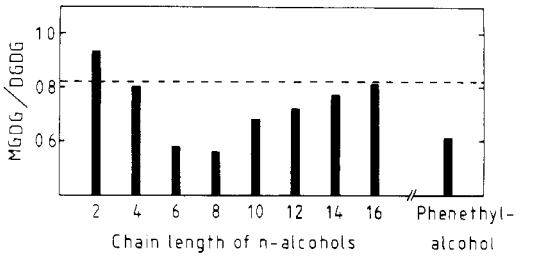

FIGURE 3: Lipid composition in *A. laidlawii* membranes after incorporation of alcohols of different chain lengths. Broken horizontal line indicates MGDG/DGDG ratio in control culture with no alcohol. See Figures 1 and 2 and Table II for experimental details.

Different techniques have given somewhat different results concerning the effect of *n*-alcohols on the order parameter of the acyl chains in membranes. In summary, alcohols with short hydrocarbon chains (≤octanol) seem to lower the order parameter (Boigegrain et al., 1984; Zavoico et al., 1985), whereas the longer chain alcohols slightly decrease, have no effect on, or slightly increase the order parameter (Lawrence & Gill, 1975; Pringle et al., 1981; Thewalt et al., 1985; Zavoico et al., 1985). Benzyl alcohol decreases the acyl chain order parameter of dimyristoyl-PC (Turner & Oldfield, 1979), and phenethyl alcohol decreases this order parameter in *Escherichia coli* membranes (Halegoua & Inouye, 1979).

The phase equilibria in the system egg PE–water are influenced by the *n*-alcohols. Ethanol and 1-butanol raise the T_{LH}, and 1-hexanol, 1-octanol, 1-decanol, and 1-dodecanol lower the T_{LH} (Hornby & Cullis, 1981). Phase diagrams of the systems *n*-alcohol–sodium octanoate–water, with the *n*-alcohol ranging from methanol to decanol, have been constructed (Ekwall, 1975). Sodium octanoate–water systems form a micellar solution, and an hexagonal phase of the normal type (H_I), with increasing soap concentration. Methanol is not able to induce the formation of a lamellar phase when added to this soap–water system, and ethanol induces the formation of a very limited phase area representing a lamellar phase. However, when the *n*-alcohols ranging from propanol to decanol are added to the sodium octanoate–water system, gradually larger phase areas representing a lamellar phase are formed.

It has been shown in this study by polarized light microscopy that phenethyl alcohol induces the formation of cubic and H_{II} phases when added to a lamellar phase of the *A. laidlawii* DOMGDG–DODGDG–water model system.

Physiological Response in A. laidlawii. In Table II, some data for the alcohols are given. The largest concentrations permitting growth of *A. laidlawii* and the concentrations used in the lipid composition experiments decrease, and the lipid–buffer partition coefficients increase, with increasing chain length of the *n*-alcohols. With respect to these properties, phenethyl alcohol can be placed between 1-butanol and 1-hexanol.

Figure 3 displays the effect on the ratio MGDG/DGDG of incorporation of the alcohols into the *A. laidlawii* membrane. Ethanol increases the value of this ratio, and 1-butanol causes practically no change in it. The largest decrease in the lipid ratio is achieved by 1-hexanol, 1-octanol, and phenethyl alcohol. This decrease becomes gradually smaller when the chain length of the *n*-alcohols increases from decanol to hexadecanol.

The effect of the alcohols on the ratio MGDG/DGDG can be explained by their influence on the volume of the hydrocarbon region and on the hydrocarbon–water interfacial area, and thus the influence on the phase equilibria in lipid–water

Table II: Effect of Different Alcohols on *A. laidlawii* and Calculated Membrane Concentrations

alcohol	largest concn permitting growth after shift (mM)	concn used for lipid composition expt (mM)	lipid–buffer partition coeff[a]	saturated H_2O concn (mM)[a]	mol of solvent/100 mol of lipids in membranes[b]	anesthetic concn in tadpole (mM)[a]
ethanol	430	350	0.2	13 800	6	120
1-butanol	100	60	3.2	960	17	54
1-hexanol	8.0	6.0	50	61	26	0.7
1-octanol	1.0	0.8	387	4.5	27	0.06
1-decanol	0.5	0.3	2000	0.32	52	0.013
1-dodecanol	0.15	0.10	5500	2.19×10^{-2}	11 (48)[d]	0.005
1-tetradecanol	0.10	0.075	2000	1.46×10^{-3}	0.3 (13)[d]	*e*
1-hexadecanol	0.075	0.050	11 500	1.7×10^{-4}	0.2 (49)[d]	*e*
phenethyl alcohol	20	12	14[c]		15	*c*

[a] From Pringle et al. (1981) and Janoff and Miller (1982). Partition coefficients for lipid bilayers are usually slightly higher than those for biological membranes. [b] Calculated as described in Table I. [c] For benzyl alcohol. Anesthetic concentration for this alcohol is 20 mM (Seeman, 1972), which lowers T_m for DPPC 5 °C (Ebihara et al., 1979). [d] Figures within parentheses denote the concentration that maximally could be achieved if *all* alcohol were accessible for partition into the membrane, using the same partition coefficients. [e] *Not* anesthetic, see references sited in footnote *a*.

systems. Solubilization of alcohols in the bilayer increases the hydrocarbon–water interfacial area (Pope et al., 1984). Ethanol probably causes a negligible increase in the bilayer hydrocarbon volume and raises the T_{LH} for egg PE–water systems (Hornby & Cullis, 1981). *A. laidlawii* responds to ethanol incorporation by increasing the ratio MGDG/DGDG. This leads to a reduction of the hydrocarbon–water interfacial area since MGDG has a smaller polar head group. 1-Butanol has a larger hydrocarbon volume than ethanol and has opposite effects on the phase equilibria in two different lipid–water model systems (see above). It is therefore reasonable that this alcohol has a small effect on the ratio MGDG/DGDG in the *A. laidlawii* membrane. A noticeable increase in the bilayer hydrocarbon volume is likely to be obtained by solubilization of 1-hexanol and 1-octanol. These alcohols shift the phase equilibria in two lipid–water model systems from an H_I phase to an L_α phase and from an L_α phase to an H_{II} phase, respectively (see above). The ratio MGDG/DGDG is decreased, which leads to an increase in the hydrocarbon–water interfacial area that counteracts the increase in the hydrocarbon volume caused by the alcohols. The *n*-alcohols ranging from 1-decanol to 1-hexadecanol all increase the bilayer hydrocarbon volume and thus should reduce the MGDG/DGDG ratio. However, the solubility of 1-hexadecanol in the growth medium is about 2000 times lower as compared to that of 1-decanol, while the lipid–buffer partition coefficient is just about 6 times higher (Pringle et al., 1981, cf. Table II). Thus, the fraction of the long-chain alcohol molecules in the *A. laidlawii* membrane is gradually diminished with increasing chain length, and growth in the presence of 1-hexadecanol has no effect on the ratio MGDG/DGDG. The hydrocarbon region of phenethyl alcohol extends like those of 1-pentanol or 1-hexanol into the bilayer, and the ratio MGDG/DGDG is decreased by phenethyl alcohol nearly as much as with 1-hexanol.

The regulation of the ratio MGDG/DGDG cannot be explained as a regulation of the T_m or a regulation of the order parameter of the acyl chains. For example, ethanol and 1-hexanol both lower the T_m and decrease the acyl chain order parameter in a lipid bilayer (see above), but the two alcohols have opposite effects on the ratio MGDG/DGDG. Moreover, 1-hexanol and 1-octanol lower the T_m for DPPC, while 1-tetradecanol raises the T_m for this lipid (see above), but the ratio MGDG/DGDG is decreased in all cases.

Detergents

Two series of poly(oxyethylene) alkyl ether surfactants were chosen: one series with dodecyl chains and with 3, 4, 6, 8, or 12 oxyethylene units and one series with hexadecyl chains and with 3, 4, 6, or 8 oxyethylene units. When these molecules are present in a bilayer, the oxyethylene units are located in the hydrocarbon–water interfacial region and the alkyl chain extends into the hydrocarbon region.

Table III: Concentration of Poly(oxyethylene) Alkyl Ether Detergents Used in Shift Experiments with *A. laidlawii*

detergent[a]	largest concn permitting growth after shift (mM)	critical micellar concn (mM at 20 °C)[b]	concn used for lipid composition expt (mM)
$C_{12}EO_3$	0.126	0.063	0.095
$C_{12}EO_4$	0.110		0.085
$C_{12}EO_6$	0.088	0.080	0.070
$C_{12}EO_8$	0.074	0.100	0.055
$C_{12}EO_{12}$	0.112	0.210	0.070
$C_{16}EO_3$	0.107		0.053
$C_{16}EO_4$	0.084		0.049
$C_{16}EO_6$	0.064		0.049
$C_{16}EO_8$	0.067	0.001	0.051

[a] C_{12} and C_{16}, number of carbon atoms in alkyl chain. EO_3 to EO_{12}, number of oxyethylene units in polar head. [b] From Schick (1967) and Dr. G. Tiddy.

Model Systems. The critical micelle concentration, the largest surfactant concentrations permitting growth of *A. laidlawii*, and the surfactant concentrations used in the lipid composition experiments are listed in Table III.

The phase diagrams for the poly(oxyethylene)–water systems have been determined (Mitchell et al., 1983). With a growing number of oxyethylene units, the hydrocarbon–water interfacial area of these compounds increases. Thus, the $C_{12}EO_3$ and $C_{12}EO_4$ molecules have a small interfacial area and form either no ($C_{12}EO_3$), or just a small ($C_{12}EO_4$), phase area representing a micellar solution (L_1). An L_α phase forms at higher surfactant concentrations. When the dodecyl derivatives have 6, 8, or 12 oxyethylene units, the L_1 phase area grows larger; cubic and H_I phases are formed, and the L_α phase gradually vanishes. It has also been shown that $C_{12}EO_8$ has the ability to induce the formation of an H_I phase when mixed with a lamellar phase consisting of egg PC and water (Beyer, 1983). These results are in line with the ideas of lipid molecular geometry. The phase diagrams for the hexadecyl derivatives are changed in a similar way when the number of oxyethylene units is increased (Mitchell et al., 1983). However, elongation of the alkyl chain shifts the phase equilibria toward the L_α phase.

Physiological Response in A. laidlawii. The effect on the ratio MGDG/DGDG of incorporation of the surfactants into the *A. laidlawii* membrane is shown in Figure 4. With $C_{12}EO_3$, the glucolipid ratio is lower than in membranes from

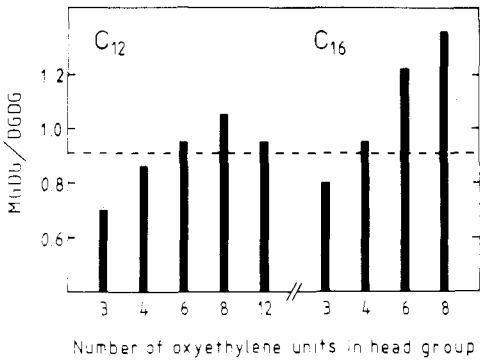

FIGURE 4: MGDG/DGDG ratio in *A. laidlawii* membranes after incorporation of poly(oxyethylene) alkyl ethers with carbon chain lengths of 12 and 16 and with a varying number of oxyethylene units in the head group. Broken horizontal line indicates MGDG/DGDG ratio in control culture devoid of detergents. See Figures 1 and 2 and Table III for experimental details.

the control culture. The ratio increases when the number of oxyethylene units is increased from three to eight, and it then decreases slightly for the $C_{12}EO_{12}$ surfactant. Incorporation of $C_{16}EO_3$ again gives a ratio MGDG/DGDG that is lower than in membranes from the control culture; $C_{16}EO_4$ causes practically no change in the glucolipid ratio, and $C_{16}EO_6$ and $C_{16}EO_8$ gradually increase this ratio.

With a growing number of oxyethylene units in the polar head group, the surfactants acquire an increasing tendency to shift the phase equilibrium of the membrane lipids toward nonlamellar phases of the normal type. *A. laidlawii* responds to this influence by raising the ratio MGDG/DGDG when the number of oxyethylene units is increased from three to eight in both surfactant series. In this way the hydrocarbon–water interfacial area of the membrane lipids is decreased, and the phase equilibrium is shifted toward a lamellar phase. Since the head group of $C_{12}EO_{12}$ is larger than that of $C_{12}EO_8$, the glucolipid ratio should be further increased when $C_{12}EO_{12}$ is incorporated into the *A. laidlawii* membrane. However, this effect is not observed. Thus, factors besides the phase behavior of the $C_{12}EO_{12}$–water system must be important when discussing the effect of this surfactant molecule on the glucolipid ratio.

Conclusions

The membrane lipid composition in *Acholeplasma laidlawii* is regulated in response to changes of several environmental conditions. The conditions chosen in previous investigations were mainly of the kind that the organism will meet in its biological surroundings, such as changes in the growth temperature and the presence of cholesterol and different fatty acids used in membrane lipid synthesis (Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1978, 1980; Rilfors, 1985). One important aim of this regulation is to maintain a stable lipid bilayer structure during various environmental conditions (Wieslander et al., 1980, 1981a; Rilfors et al., 1984; Rilfors, 1985). The regulation principally mainifests itself as a variation of the ratio between the two dominating membrane lipids MGDG and DGDG; these lipids form reversed hexagonal and lamellar phases in excess water, respectively (Wieslander et al., 1978, 1981b; Khan et al., 1981). The phase equilibria of in vivo mixtures of all polar *A. laidlawii* membrane lipids is mainly determined by the ratio MGDG/DGDG in these mixtures (Lindblom et al., 1986).

According to the above-mentioned explanation of the lipid regulation mechanism, the origin of molecules present in the membrane should be of no importance for their ability to affect the phase equilibria of the membrane lipids. Therefore, in this work *A. laidlawii* has been grown in the presence of several foreign hydrophobic and amphiphilic compounds, whose influence on the T_m, on the order parameter of the acyl chains, and on the phase equilibria between lamellar and nonlamellar liquid crystalline phases of lipid–water model systems is known in several cases. The incorporation of these molecules into the *A. laidlawii* membrane affects the ratio MGDG/DGDG: (1) all nonpolar organic solvents decrease this ratio; (2) *n*-alcohols increase or decrease the ratio depending on the chain length of the alcohol; (3) poly(oxyethylene) alkyl ether detergents increase the ratio as a function of the size of the polar head group. The foreign molecules thus affect the packing of the membrane lipids (Israelachvili et al., 1980), and the organism attempts to restore the lipid packing by altering the lipid composition. The changes in the glucolipid ratio can throughout be explained by the influence of the foreign molecules on the equilibrium between a lamellar liquid crystalline phase and cubic or hexagonal phases. This interpretation is based on studies of the influence of the foreign molecules on the phase equilibria of both synthetic membrane lipids (PC and PE) and *A. laidlawii* membrane lipids. The regulation of the ratio MGDG/DGDG cannot be explained by an effect of the foreign molecules on the T_m or the order parameter of the acyl chains.

Thus, when discussing the physicochemical basis for the membrane lipid composition per se, as well as the regulation of this composition in *A. laidlawii*, it is of great importance to understand the phase equilibria of membrane lipid–water systems, in particular the transition between lamellar and nonlamellar liquid crystalline phases.

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Registry No. $C_{12}EO_3$, 3055-94-5; $C_{12}EO_4$, 5274-68-0; $C_{12}EO_6$, 3055-96-7; $C_{12}EO_8$, 3055-98-9; $C_{12}EO_{12}$, 3056-00-6; $C_{16}EO_3$, 4484-59-7; $C_{16}EO_4$, 5274-63-5; $C_{16}EO_6$, 5168-91-2; $C_{16}EO_8$, 5698-39-5; Et_2O, 60-29-7; EtOH, 64-17-5; BuOH, 71-36-3; $Bu(CH_2)_2OH$, 111-27-3; $Bu(CH_2)_4OH$, 111-87-5; $Bu(CH_2)_6OH$, 112-30-1; $Bu(CH_2)_8OH$, 112-53-8; $Bu(CH_2)_{10}OH$, 112-72-1; $Bu(CH_2)_{12}OH$, 36653-82-4; $Ph(CH_2)_2OH$, 60-12-8; cyclohexane, 110-82-7; benzene, 71-43-2.

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