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Reversed Cubic Phase with Membrane Glucolipids from *Acholeplasma laidlawii*. ^1H, ^2H, and Diffusion Nuclear Magnetic Resonance Measurements†

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ABSTRACT: Monoglucosyl diglyceride and diglucosyl diglyceride are the dominant lipids of the *Acholeplasma laidlawii* membrane. Diglucosyl diglyceride forms a lamellar liquid crystalline phase with water while monoglucosyl diglyceride forms a reversed hexagonal phase. Depending on the amounts of unsaturated acyl chains of the lipids, a *mixture* of monoglucosyl diglyceride and diglucosyl diglyceride forms lamellar or reversed cubic phases at physiological temperatures. A high degree of cis unsaturation favors formation of the cubic phase with increasing monoglucosyl diglyceride content. The structure of the cubic phase is composed of aggregates, where the lipids can diffuse over macroscopical distances. A structure containing close-packed spherical *micelles* is therefore ruled out, and the NMR diffusion data are compatible with other previously proposed cubic bicontinuous structures [Luzzati, V., & Spegt, P. A. (1967) *Nature (London) 215*, 701; Scriven, L. E. (1976) *Nature (London) 263*, 123; Lindblom, G., Larsson, K., Johansson, L. B.-Å., Fontell, K., & Forsén, S. (1979) *J. Am. Chem. Soc. 101*, 5465]. Monoglucosyl diglyceride/diglucosyl diglyceride ratios forming cubic phases have not been observed in vivo. It is concluded that formation of the cubic phase is strongly dependent on the molecular shape of the lipids. The results are significant for the physiological regulation of the lipid composition in *A. laidlawii* membranes as well as for the function and organization of biological membranes in general.

All biological membranes contain a number of different lipids (Ansell et al., 1973), most of which form lamellar liquid crystalline phases together with water (Luzzati & Tardieu, 1974). However, exceptions to formation of the bilayer structure have been found for some lipids. Hence it has been shown that phosphatidylethanolamine (PE)[1] from various organisms (Shipley, 1973) and monoglucosyl diglyceride (MGDG) from *Acholeplasma laidlawii* (Wieslander et al., 1978) form reversed hexagonal (H_{II}) mesophases. The only lipid structure compatible with a nonleaky and functioning membrane is the lamellar phase, and lipid bilayer stability thus is of crucial importance for the living cell. It cannot be excluded, however, that 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 (Lucy, 1975), exo- and endocytosis, and transbilayer movement of lipids (Cullis & De Kruijff, 1979). The very rapid translocation of PE from the inner to the outer leaflet in *Bacillus megaterium* membranes has been shown to be independent of the synthesis of lipid and protein and of sources of metabolic energy, probably excluding a "flip-flop" mechanism (Langley & Kennedy, 1979). Instead, lateral diffusion along transient lipid "hairpin" structures in the vicinity of hydrophilic transmembrane channels is suggested. Since the hairpin bend has a small radius of curvature, it must be stabilized by lipids forming highly curved aggregates (Langley & Kennedy, 1979). However, large amounts of such lipids destabilize the membrane, eventually leading to a membrane disrupture. Therefore, the balance between lipids forming lamellar and nonlamellar phases can only vary within certain limits in order to

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[1] Abbreviations used: MGDG, monoglucosyl diglyceride; DGDG, diglucosyl diglyceride; NMR, nuclear magnetic resonance; H_{II}, reversed hexagonal; PE, phosphatidylethanolamine.

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keep the bilayer stable (Wieslander et al., 1980).

In bacteria the membrane often has to cope with various factors affecting the bilayer stability. An increase in temperature might lead to the formation of an isotropic phase and, on the other hand, a decrease in temperature may induce a lipid crystalline (gel) phase. At constant temperatures, an increase in the amount of "nonlamellar lipids" above a critical concentration destabilizes the membrane. Such events must be counteracted by the cell, and mechanisms must exist regulating lipid properties in the membrane so that optimal stability always is achieved. These regulations occur in the membrane of *A. laidlawii*, and recently we showed that lipid composition in this membrane to a large extent is determined by the shape or the molecular geometry of the various lipids (Wieslander et al., 1980).

In this work we have studied the liquid crystalline phase behavior of different mixtures of the two dominant polar lipids in *A. laidlawii*, MGDG, and diglucosyl diglyceride (DGDG). It is found that the amount of unsaturated acyl chains in the lipids is of utmost importance for the phase structure formed. Here we report the first observed cubic lyotropic liquid crystalline phase for a mixture of membrane lipids at *low temperatures*. Previously, cubic phases for lecithin (Small, 1967; Luzzati, 1968) and for chloroplast galactolipids at high temperatures (Rivas & Luzzati, 1969) have been reported. The structure of the amphiphilic aggregates building up cubic phases have been determined for some systems by using X-ray (Luzzati & Spegt, 1967; Lindblom et al., 1979) and NMR techniques (Lindblom et al., 1979). Here is shown that the structure of the cubic phase observed for the mixture of *A. laidlawii* glucolipids consists of continuous hydrocarbon regions.

Materials and Methods

Membrane Lipids. Acholeplasma laidlawii A, strain EF 22, was grown statically in a lipid-depleted bovine serum albumin–tryptose medium (Wieslander et al., 1978). To obtain lipids containing only cis-unsaturated acyl chains, the growth medium was supplemented with 150 μM oleic acid (Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1980) and the cells were harvested after growth for 36 h at 30 °C. This temperature has the effect of increasing the relatively low amounts of MGDG normally obtained with oleic acid supplementation (Christiansson & Wieslander, 1978). Lipids containing approximately equal amounts of an unsaturated and a saturated acyl chain were obtained by supplementing the medium with 75 μM oleic acid and 75 μM palmitic acid and growing the cells for 24 h at 37 °C (Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1980). Preparation of membranes, purification, and analysis of single lipid species were done as described previously (Wieslander et al., 1978). Typically, a 10-L batch yielded 525 mg of total lipids.

Sample Preparation. Different mixtures of MGDG and DGDG, approximately 90 mg of total lipids, were prepared by careful weight analysis. The solvents were removed by N_2 and by drying the samples overnight at a pressure less than 0.1 mmHg. Dissociable protons in the lipid polar head groups were exchanged for deuterons by vigorously mixing the dried samples with excess ^2H_2O in an atmosphere of N_2 and thereafter removing the heavy water by drying as described above. This procedure was carried out three times. Finally 11 or 13% (w/w) of ^2H_2O was added, followed by a flush of N_2, and mixed with the lipids by extended centrifugation. The heavy water concentrations used are slightly lower than the maximum hydration capacities of these lipids (Wieslander et al., 1978). Water concentrations above this limit were avoided since two-phase samples may complicate the interpretation of the NMR spectrum. Moreover, MGDG and DGDG form H_{II} and lamellar phase structures, respectively, in excess water (Wieslander et al., 1978), and cubic liquid crystalline phases have been shown to be in equilibrium with very dilute aqueous solution phases (Fontell, 1978). Preparation and the macroscopic alignment (De Vries & Berendsen, 1969; Lindblom, 1972) of the lamellar samples between cleaned glass plates were performed on a heated metal plate in a plastic box containing N_2 and ^2H_2O vapor at 35 °C.

^1H and ^2H NMR. ^2H NMR spectra were recorded at 15.351 MHz on a Varian XL 100 NMR spectrometer operating in the Fourier transform mode. For an oriented lamellar sample, the ^2H NMR spectrum consists of a doublet, a quadrupole splitting given by (Wennerström et al., 1974)

$$\Delta(\theta) = |\nu_Q S(3\cos^2\theta - 1)|$$

where θ is the angle between the external magnetic field, B_0, and the axis of alignment (the director), ν_Q is the effective quadrupole coupling constant, and S is the order parameter, describing the average orientation of the water molecules in the mesophase. Thus, if the lamellar phase is aligned along the glass plates, the splitting is largest when the plates are perpendicular to the magnetic field ($\theta = 0°$) and Δ has half this value when the plates are parallel with the magnetic field ($\theta = 90°$). This was also observed as illustrated in Figure 1. The temperature dependence of the splittings was 3% between 30 and 45 °C. For a H_{II} phase aligned between glass plates, the deuteron NMR spectrum will have quite a different pattern (Mely et al., 1975; Söderman et al., 1980). For an isotropic phase, like a cubic phase, no splitting is usually observed (Figure 1d). As a complement, proton NMR spectra were recorded at 100 MHz (Figure 1e).

NMR Diffusion. The lipid diffusion coefficients were measured at 61 MHz on a Bruker 322s spin-echo NMR spectrometer using a pulsed magnetic field gradient technique (Stejskal & Tanner, 1965). The spectrometer was equipped with a home-built pulsed magnetic field gradient unit with digital settings for all experimental parameters used. A schematic illustration of the experiment is presented in Figure 2. The diffusion coefficient is obtained from the attenuation of E_g/E_0 of a spin echo by varying the strength of the magnetic field gradients (δ or g) or the distance, Δ, between them according to

$$\ln \frac{E_g}{E_0} = -(\gamma g \delta)^2 D(\Delta - \delta/3)$$

where δ is the width and g the height of the magnetic gradients, γ is the gyromagnetic ratio, and D is the diffusion coefficient. Typical settings for a lamellar sample were Δ = 12 ms, δ = 2 ms, and g varied between 0 and 3.5 T m^{-1}. For a cubic mesophase sample, δ and Δ were chosen larger, 5 and 30 ms, respectively. The temperature for all samples was 45 °C. All measurements were performed relative to glycerol reference with a known diffusion coefficient (Tomlinson, 1973).

For determination of the lateral diffusion coefficient of a bilayer, the lamellar sample has to be macroscopically aligned and put at the so-called "magic angle" in the magnetic field ($\theta = 54.7°$), as illustrated in Figure 2. This technique for measurement of lipid lateral diffusion has been shown to be very useful and has been practiced in several previous studies by us (Lindblom & Wennerström, 1977; Wennerström & Lindblom, 1977; Lindblom et al., 1977, 1979; Arvidson et al., 1978; Söderman et al., 1980) and others (Tiddy, 1977; Kuo & Wade, 1979). For details of the method, see Lindblom & Wennerström (1977).

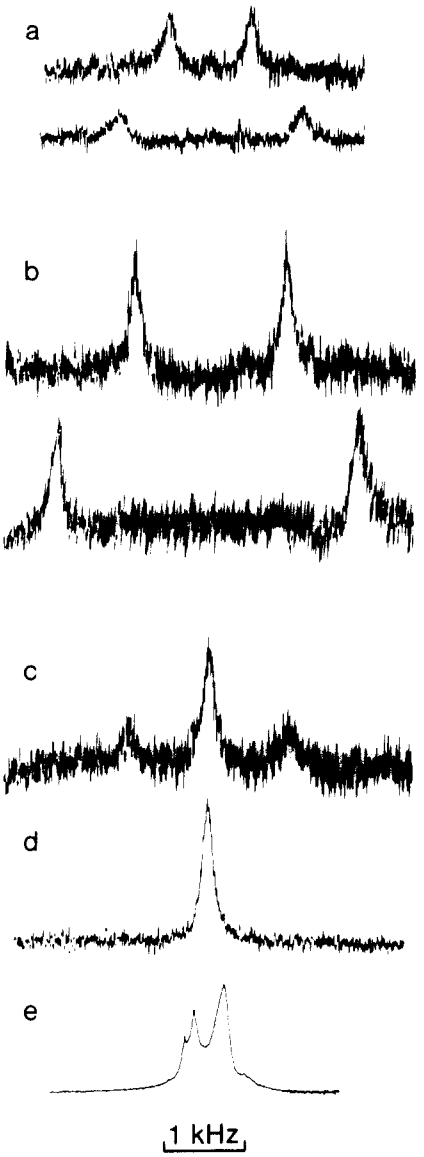

FIGURE 1: (a–d) Deuteron NMR spectra; (e) proton NMR spectrum. (a) Macroscopically aligned lamellar sample of DGDG (16:0/18:1c). Upper spectrum: the magnetic field, B_0, is parallel with the glass plates. Lower spectrum: B_0 is perpendicular to the glass plates. Note the difference in the quadrupole splitting equal to 2. (b) Macroscopically aligned lamellar sample of DGDG (18:1c/18:1c). Upper and lower spectra as in (a). (c) Macroscopically aligned two-phase sample containing lamellar (splitting) and cubic (central peak) mesophases. Molar ratio MGDG/DGDG (18:1c/18:1c) is equal to 1.2. (d) Cubic liquid crystalline phase with molar ratio MGDG/DGDG (18:1c/18:1c) equal to 2.5. (e) Proton NMR spectrum of the sample in d. All spectra were recorded at 30 °C. Reproducibility of the spectral shapes was complete when investigated at intervals of a month. The standard deviation for the quadrupole splittings is better than ±5%.

Results

The composition of the samples studied are summarized in Table I. Two series of samples were employed having (a) variations in the molar ratio between MGDG and DGDG from 0 to 2 (in this series about half of the acyl chain content was cis unsaturated) and (b) similar changes as in (a) of the ratio MGDG/DGDG but all (>95%) of the lipid acyl chains were cis unsaturated. A glucolipid composition like that in samples I and IV has not been observed in vivo. The composition of samples II and III occurs in vivo, but only with this acyl chain composition (Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1978, 1980; Wieslander et al., 1979). For lipids containing oleic acyl chains only, the MGDG/DGDG ratio in sample V was slightly higher, and in sample VI it was much higher than what has been observed in vivo.

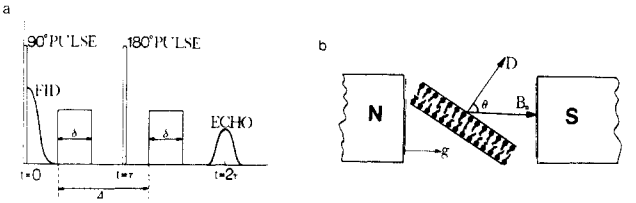

FIGURE 2: (a) Schematic picture of the NMR diffusion method with 90–τ–180 rf pulses and pulsed magnetic field gradients. δ is the width and Δ the spacing between the field gradient pulses. FID, free induction decay. (b) Illustration of the orientation of the macroscopically aligned lamellar sample relative to the external magnetic field, B_0, and the magnetic field gradient, g.

Table I: Composition of Mixtures Made from *A. laidlawii* Membrane Glucolipids

sample	MGDG/DGDG[a]	acyl chain composition		
			16:0[b]	18:1c[b]
I	0:1.0	MGDG	54	46
II	1.0:1.0	DGDG	45	55
III	2.0:1.0			
IV	0:1.0	MGDG and DGDG		
V	1.2:1.0	18:1c[b] ≥ 95		
VI	2.5:1.0			

[a] MGDG and DGDG: mono- and diglucosyl diglyceride; molar relationships in samples. Total amounts of lipids approximately 90 mg. [b] 16:0, palmitic acid; 18:1 c, oleic acid; amounts in mol %.

As can be inferred from Figure 1 the magnitude of the deuteron quadrupole splitting was larger for the samples containing ~100% unsaturated acyl chains than for the corresponding samples with ~50% of the acyl chains being unsaturated. For samples I, II, III, and IV it was found that the deuteron NMR spectra obtained at $\theta = 0°$ and 90° are compatible with lamellar phases (cf. Methods) as shown for samples I and IV in Figure 1. Sample VI showed no quadrupole splitting, but only a narrow single NMR peak (Figure 1d). In sample V, however, a splitting of very low intensity was observed, together with the central high intensive peak (Figure 1c). The splitting was twice as large when the sample was rotated 90° in the magnetic field. The proton NMR spectrum of the sample VI exhibits quite narrow peaks (Figure 1e), indicating that it consists of an isotropic phase. A lamellar phase would give a broad and unressolved proton NMR spectrum. Such spectra were obtained from samples I, II, III, and IV. Sample VI was found to be optically isotropic when studied between crossed polarizers, but sample V showed both anisotropic and isotropic regions, i.e., V is a two-phase sample. The macroscopic viscosity was considerably higher for samples V and VI compared to the other samples. A common feature for optical isotropic liquid crystalline phases is their very stiff consistency (Small, 1967; Fontell, 1978). The hexagonal phases are characterized by a somewhat lower apparent viscosity, and the lamellar phase is much less viscous (Fontell, 1978). All these data taken together lead to the conclusion that samples I, II, III, and IV consist of lamellar liquid crystalline phases, while sample VI is an isotropic cubic liquid crystal and sample V consists of two phases, lamellar and cubic.

In order to gain further information about the structure of the amphiphilic aggregates in the isotropic phase, the diffusion coefficient for some of the samples was determined. The data obtained are given in Table II. Lipid diffusion in lamellar and cubic phases differs by about one order of magnitude (compare the experimentally determined coefficients).

Table II: Experimental and Calculated Values for Lipid Diffusion Coefficients[a]

sample[b]	$D \times 10^{11}$ (m^2/s)	$D_L \times 10^{11}$ (m^2/s)	$D_{\parallel}^{cub}$ (m^2/s)
III	0.8	1.2	
IV	2.6	3.9	
V[c]	0.1		0.3
VI	0.1		0.3

[a] D_L represents the lateral diffusion coefficient in a lipid bilayer, obtained by correcting the experimentally determined diffusion coefficient, D, for the "magic angle". $D_{\parallel}^{cub}$ is the estimated diffusion coefficient for translational motion along an assumed rodlike aggregate unit in a cubic phase. Temperature 45 °C. The standard deviation for D is ±30%. [b] See Table I for sample composition. [c] This sample formed a pure cubic phase at 45 °C.

Discussion

In previous work (Lindblom & Wennerström, 1977; Arvidson et al., 1978; Lindblom et al., 1979) it has been shown that from a comparison between diffusion coefficients for lamellar and cubic phases, determined by the NMR diffusion technique, information about the aggregate structure in the cubic phase can be obtained. Conclusions made are based on the fact that the value of the apparent diffusion coefficient depends strongly on the overall structural geometry. For a discussion of the method in detail the reader is referred to the works cited above. Here only a brief account will be given. For a cubic phase composed of closepacked spherical aggregates, the lipid translational motion is confined to the globular micelle (exchange between aggregates can be neglected), and therefore the measured diffusion coefficient will be very low. It has been found that this coefficient for such a cubic phase is more than two orders of magnitude lower than for the corresponding lamellar phase. For a cubic phase based on rod network systems (Luzzati & Spegt, 1967), lipid diffusion can occur over macroscopial distances, and the measured lipid diffusion coefficient for such a phase is approximately of the same order of magnitude as the value obtained for the lamellar phase. A quantitative comparison between measured and calculated diffusion coefficients in the different phases is often possible to make.

From the experimental findings obtained for the lamellar samples III and IV, it can be inferred (Table II) that the lateral diffusion coefficients are of the same order of magnitude, being about a factor three lower for sample III. This is in line with previous results where it was found that the lipid lateral diffusion depends on the polar head group area at the water–lipid interface, i.e., on the packing properties of the bilayer (Lindblom & Wennerström, 1977). Thus the more saturated lipids in sample III can pack together much tighter than the cis-unsaturated lipids in sample IV. An increase of the polar head group area with increasing amounts of cis-unsaturated acyl chains of the lipids is supported by the water deuteron NMR data in Figure 1. The larger splitting obtained for the more unsaturated lipids is most likely due to an increase in the water binding to the head-group region of the bilayers. Such an increase will occur because of the increase in space between the lipid molecules.

Recently we studied the lateral diffusion of some different lecithins (Lindblom et al., 1980), making it possible here to consider also the effect of a change in the polar head group on the translational lipid motion in a bilayer. The lateral diffusion coefficients of palmitoyloleoyllecithin and dioleoyllecithin were found to be nearly one order of magnitude lower than for the glucolipid lamellar samples with corresponding acyl chain composition. The most likely explanation of this is, in our opinion, that the packing and electrostatic properties are very different in the phospho- and glucolipid systems in question. It should be noted that the gel to liquid crystalline phase transition temperature is approximately 20 °C higher for the *A. laidlawii* glucolipids (Wieslander et al., 1978) than for the corresponding lecithins.

Table II shows that for samples V and VI, containing cubic phases, the experimentally determined diffusion coefficient is not more than one order of magnitude lower than for the lamellar samples III and IV. It is obvious from a comparison between the diffusion coefficients obtained for the lamellar and the cubic mesophases that the latter phase cannot be composed of spherical aggregates (cf. above), but must contain *continuous hydrocarbon regions*. Our results are compatible with the cubic phase structure proposed by Luzzati & Spegt (1967). Since the water content is only 11% (w/w), the cubic phase most likely is of the reversed type. It can be pictured as two distinct three-dimensional networks of water rods in a lipid matrix, which are unconnected but mutually interwoven [see Figure 7 in Fontell (1978)]. However, other types of cubic phases containing continuous hydrocarbon regions cannot be totally ruled out. A cubic phase formed by a network of hexagon-shaped lamellar bilayer units has been found in a mixture of monoolein and water (Lindblom et al., 1979), and Scriven (1976) has described some additional continuous cubic lattices.

The phase structures formed by the glucolipids can be compared to those formed by the structurally close related galactolipids. With galactolipids isolated from wheat endosperm flour, monogalactosyl diglyceride/digalactosyl diglyceride ratios ranging from 0.6 to 2.5 resulted in a two-phase region at low water contents, consisting of a lamellar and a H_{II} phase (Larsson & Puang-Ngern, 1979). Higher and lower ratios gave pure H_{II} and lamellar phases, respectively. Rivas & Luzzati (1969) investigated a galactolipid mixture isolated from maize chloroplasts. This mixture formed a H_{II} phase from −20 to 100 °C when the water content was below 10% (w/w) and a cubic phase, consisting of two interwoven three-dimensional rod networks, with water concentrations between 10 and 20% (w/w) and at temperatures between 60 and 100 °C. However, this lipid preparation also contained some sulfolipids, which form a lamellar phase with water (Shipley et al., 1973). Moreover, the degree of unsaturation was different in the two lipid preparations, which has been shown in this work to influence the phase structures formed by mixtures of the glucolipids.

The cubic liquid crystalline phase can form with a mixture of MGDG and DGDG when (a) MGDG is present in excess and (b) the content of cis-unsaturated acyl chains is approximately 100%. The area occupied by the polar head group of MGDG is smaller than that occupied by DGDG. Morever, a cis-unsaturated acyl chain cannot extend to the same length as a saturated one (Träuble & Haynes, 1971). The above-mentioned factors thus affect the *mean* polar head group area at the hydrocarbon–water interface and the hydrocarbon chain length in the lipid aggregates. According to the theory of self-assembly of amphiphiles developed by Israelachvili et al. (1976; Israelachvili 1977), these quantities determine the shape of the lipid molecules and consequently the structure of the amphiphilic aggregates that are formed. MGDG has a more or less pronounced wedge-like molecular shape with different acyl chain contents, and forms a H_{II} phase structure with water. DGDG, having a rodlike molecular shape, forms a lamellar phase (Wieslander et al., 1978). In 1:1 and 2:1

mixtures of MGDG and DGDG with about 50% of the acyl chains being cis unsaturated, lamellar phases were formed. DGDG thus has the capacity to stabilize the lamellar phase with this acyl chain composition. When the content of cis-unsaturated acyl chains was increased to nearly 100%, a lamellar *and* a cubic mesophase was formed with the MGDG/DGDG ratio being 1.2:1, and a *pure* cubic phase was formed with the 2.5:1 MGDG/DGDG mixture. An increase in the fraction of cis-unsaturated acyl chains will increase the hydrophobic bulkiness of both the glucolipid molecules, leading to more wedgelike molecular shapes and enhanced tendencies to form nonlamellar phases (Wieslander et al., 1980). The bilayer stabilizing property of DGDG is diminished, and when MGDG is present in great excess the wedge shape properties are accentuated to such an extent that the lamellar phase is totally abolished.

The results obtained are highly relevant to the regulation of membrane lipid composition observed in *Acholeplasma laidlawii* grown under different conditions. In particular, the ratio between MGDG and DGDG is altered depending on configuration of incorporated fatty acids, growth temperature, and membrane cholesterol content (Wieslander & Rilfors, 1977; Christiansson & Wieslander, 1978, 1980). These factors affect the molecular geometry of the membrane lipids (Israelachvili et al., 1976, Israelachvili, 1977; Wieslander et al., 1980). Incorporation of different ratios of saturated/unsaturated fatty acids in *A. laidlawii* membranes causes a successive change in the hydrophobic geometry of the lipids. When the amount of cis-unsaturated acyl chains in the membrane increases, the organism maintains the membrane stability by substantially decreasing the amount of MGDG, the polar lipid exerting the greatest bilayer destabilizing effect in *A. laidlawii* membranes. The MGDG/DGDG ratio has a maximum value of 0.8 in membranes, being totally enriched in oleic acid (Christiansson & Wieslander, 1978), a value which is somewhat *lower* than that in sample V, giving a mixture of a cubic and a lamellar phase. A larger hydrophobic bulkiness of the lipid molecules is likewise generated by raising the temperature. The effect is counteracted in *A. laidlawii* by supressing the wedge shape properties of these molecules in two ways: (1) reduced incorporation of unsaturated fatty acids and (2) decreased synthesis of lipids with a small polar head group like MGDG. Finally, the MGDG content in the *Acholeplasma* membrane containing oleic acyl chains is strikingly reduced by incorporation of cholesterol. The latter molecule destabilizes the bilayer structure in the presence of lipids, forming a H_{II} phase, because of its marked wedge shape (Carnie et al., 1979). The effects of temperature and cholesterol on the phase structure of MGDG/DGDG mixtures have also been investigated, and the results further support the conclusion that the MGDG/DGDG ratio has a bilayer stability regulating function (A. Khan, Å. Wieslander, L. Rilfors, and G. Lindblom, unpublished results).

In conclusion, samples with MGDG/DGDG ratios and an acyl chain composition occurring in vivo (II, III) clearly revealed stable lamellar phases. The sample with a composition just outside the range found in vivo (V) gave a two-phase system consisting of a lamellar and a cubic phase. When the composition was far removed from that observed in vivo (VI), a pure cubic phase was obtained. Since the other polar lipid species isolated from the membrane of *A. laidlawii* strain A form lamellar phases (Wieslander et al., 1978), it can be assumed that the cells, at the prevailing growth conditions, actively avoid lipid compositions resulting in nonlamellar phases of the bulk membrane lipids. It is conceivable, however, that such phases can form temporarily and/or within local regions of biological membranes, since several observations indicate that the lipid species are not uniformly distributed in this organelle: (1) the activities of a limited number of membrane associated enzymes are dependent on a specific lipid surrounding (Sandermann, 1978); (2) there are strong indications of an asymmetrical distribution of lipid species between the bilayer halves in procaryotic as well as eucaryotic cells (Op den Kamp, 1979; Gross & Rottem, 1979); and (3) lipids with specific molecular geometries may be required in order to minimize packing defects around the irregular surfaces of integral membrane proteins (Israelachvili, 1977; Sandermann, 1978). Stepwise selective solubilization of *A. laidlawii* membranes with Tween 20 has shown that a specific protein is enriched together with MGDG (Å. Wieslander and K.-E. Johansson, unpublished results). Indications of the existence of nonlamellar phases within plasma membranes of both eucaryotic and procaryotic cells derives from some ^{31}P NMR investigations (Stier et al., 1978; Cullis & De Kruijff, 1979; Burnell et al., 1980). Most biological membranes contain at least one lipid species having the capacity to form a hexagonal phase. These observations, together with the fact that the synthesis of MGDG never ceases in *A. laidlawii*, favor the proposal that certain amounts of a lipid forming a nonlamellar phase must be beneficial for membrane bilayer properties and some membrane-mediated cell processes. Exo- and endocytosis, membrane fusion, and transbilayer movement of lipids are easier to reconcile with a transient of regional formation of nonlamellar phases than with an invariable preservation of the bilayer structure. Recently it has also been suggested that the transport of nucleic acid into the recipient cell at the early stage of genetic transformation, conjugation, transfection, and viral infection involves a rearrangement of the phospholipid bilayer and the formation of a transmembrane channel (Grinius, 1980).

In this work it has been demonstrated that knowledge about the phase structures formed by membrane lipids in simple lipid–water systems is important in order to understand the molecular organization of biological membranes. As shown for *A. laidlawii*, the biological significance of these studies is particularly evident when physical and physiological investigations are connected to each other.

Acknowledgments

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Kinetics of Formation of Deoxyribonucleic Acid Cross-Links by 4′-(Aminomethyl)-4,5′,8-trimethylpsoralen†

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ABSTRACT: If a mixture of T4 deoxyribonucleic acid (DNA) and 4′-(aminomethyl)-4,5′,8-trimethylpsoralen is irradiated with two closely spaced pulses of long-wave UV laser light, the resulting cross-linking is dependent on the time delay between the pulses. As the delay lengthens to 1 μs, a rise in the number of cross-links is observed which follows first-order kinetics. This delay, the time required for most monoadducts to be able to absorb a second photon and thereupon form a cross-link, is interpreted in terms of a conformational change in the DNA at the psoralen intercalation site which may occur upon monoadduct formation.

Psoralens are planar heterocyclic molecules (furocoumarins) which photosensitize skin erythema and are used to treat psoriasis and vitiligo. In addition to this clinical utility, they have been used to locate nuclease-protected regions of deoxyribonucleic acid (DNA) in chromatin and regions of secondary structure in single-stranded DNA and ribonucleic acid (RNA), to study DNA repair mechanisms, and in other applications [for recent reviews, see Scott et al. (1976), Song & Tapley (1979), and Hearst (1979)]. The chemical and at least a part of the biological activity of psoralens results from their ability to intercalate between the base pairs of nucleic acids and, upon absorption of long-wave UV light (320–380 nm), form mono- and diadducts to pyrimidine bases.

A good deal of evidence (Musajo et al., 1967a, b; Krauch et al., 1967) suggests that monoaddition involves the formation of a cyclobutane bridge between the 5,6 double bond of a pyrimidine and either the 4′,5′ or the 3,4 double bonds of psoralen. Photoreaction at both ends of the psoralen forms

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