Quantitation of the Phase Preferences of the Major Lipids of the *Acholeplasma laidlawii* B Membrane†

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ABSTRACT: We have quantitated the phase preferences of all of the quantitatively significant lipids of fatty acid-homogeneous *Acholeplasma laidlawii* membranes by determining the effect of small amounts of each lipid on the lamellar/reversed hexagonal phase transition temperature of a phosphatidylethanolamine matrix of identical fatty acid composition using differential scanning calorimetry. We find that the incorporation of small amounts of these lipids produce effects ranging from a moderate depression to a marked elevation of the lamellar/reversed hexagonal phase transition temperature of the corresponding phosphatidylethanolamine. Thus, although the total membrane lipids from this organism form only lamellar phases under physiological conditions, the individual membrane lipids appear to exhibit a wide range of phase preferences. Phosphatidylglycerol and diglucosyldiacylglycerol seem to have relatively strong and weak preferences for the lamellar liquid-crystalline phase, respectively, while monoglucosyldiacylglycerol, and especially acyl polyprenyl glucoside, strongly prefers the reversed hexagonal phase. Most notable in this regard is the phase preference of glycerylphosphoryldiglucosyldiacylglycerol, which strongly destabilizes the reversed hexagonal phase and which we show in the accompanying paper [Lewis, R. N. A. H., & McElhaney, R. N. (1995) *Biochemistry 34*, 13818−13824] actually prefers the normal micellar phase in isolation. The presence of normal, lamellar, and reversed phase-preferring lipids in a single membrane has important implications for understanding the physical basis of lipid organization and biosynthetic regulation in this and possibly in other organisms. We also show that the characteristic effect of the individual *A. laidlawii* membrane lipids on the lamellar/reversed hexagonal phase transition temperature of the phosphatidylethanolamine matrix is not well correlated with their polar headgroup intrinsic volumes. This result indicates that the effective cross-sectional area of the polar headgroups of these lipid species must be strongly influenced by factors such as charge, hydration, orientation, and motional freedom, as well as by intrinsic headgroup volume.

The mixture of lipids present in all biological membranes studied to date appears to exist exclusively in the liquid-crystalline lamellar phase under physiologically relevant conditions of temperature and hydration. However, individual membrane lipids can potentially form a variety of liquid crystalline normal, lamellar, or reversed phases when dispersed in water, depending primarily on their effective molecular shapes. For these rod-like amphiphilic lipid molecules, the relative effective sizes of their polar and nonpolar regions are important elements in determining their molecular shapes, in particular, the relative cross-sectional areas occupied by their polar headgroups and nonpolar hydrocarbon chains. The effective cross-sectional area of a lipid polar headgroup appears to depend primarily on headgroup volume while the effective cross-section area of the hydrocarbon chain depends primarily on its length and degree of unsaturation. If the effective cross-sectional area of the polar headgroup exceeds that of the nonpolar region, then the lipid molecule will have a conical shape and will tend to aggregate in water to form normal micelles or related structures. Conversely, if the relative cross-sectional of the polar headgroup is less than that of the hydrocarbon chains, the lipid will have an "inverted" conical shape and will tend to aggregate in water to form a reversed cubic or hexagonal phase. If, however, the relative areas occupied by the polar headgroup and the hydrocarbon chains are roughly equal, the molecules will be cylindrical in shape and will tend to form a lamellar or bilayer phase. Since the effective area of the hydrocarbon chains in the liquid-crystalline state increases to a much greater extent with temperature than does that of the polar headgroup, increases in temperature favor the formation of lamellar over normal and reversed over lamellar phases (Israelachvili *et al.*, 1980; Rilfors *et al.*, 1984; Cullis *et al.*, 1985; Gruner, 1992).

It is relatively straightforward to determine the types of phases formed by aqueous dispersions of individual membrane lipids over a range of temperature and thus to infer something about their overall effective shape. In fact, the structures of the various phases formed by the individual lipids of the membrane *Acholeplasma laidlawii* have been extensively studied (see Discussion). It is difficult, however, to quantitate the relative strengths of the phase preferences of a series of different lipids, since the effective shapes of the lipid molecules cannot be directly determined in their various liquid-crystalline phases. However, Epand (1985) has shown that small amounts of lipids with small (large) polar headgroups decrease (increase) the liquid-crystalline lamellar−reversed hexagonal phase transition temperature

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(T_h)[1] of the host DEPE or POPE bilayer, and Janes and coworkers (Lee *et al.*, 1993) have recently shown that the intrinsic headgroup volumes of seven synthetic dioleoyl glycerolipids correlate well with the ability of these lipids to alter the T_h of a POPE matrix. In fact, both groups have presented evidence that T_h probably varies linearly with the effective size of the lipid polar headgroup at the lipid/water interface. This approach thus seems suitable for quantitating the relative phase preferences of any series of lipids based on differences in their effective shapes, which will be largely determined by effective headgroup size when the structures of their fatty acyl chains are identical. However, this method would generally not be applicable to the lipids of most biological membranes, since the fatty acid compositions of the individual lipids are usually quite different. However, the ability to manipulate the fatty acid composition of the membrane lipids of the simple, cell wall-less prokaryote *A. laidlawii* B has permitted us to determine the relative effective headgroup sizes, and thus the relative strength of the phase preferences, of all of the quantitatively significant membrane lipids of this organism, by determining the effect of the incorporation of small amounts of these lipids on the T_h of a PE matrix of identical fatty acid composition.

The matrix lipid selected for these studies was DEPE. We selected a PE molecular species in order to facilitate the comparison of our results with the earlier studies of Epand (1985), Lee *et al.* (1993), and Perillo *et al.* (1994), who all utilized either DEPE, POPE, or DOPE as the matrix phospholipid. DOPE could not be used as a matrix in this study since *A. laidlawii* cells are incapable of growth in exogenous oleic acid in the presence of avidin. In principle, a POPE matrix could have been used, since this organism does grow well in an equimolar mixture of exogenous palmitic and oleic acids under these conditions. However, some variation in the palmitate/oleate ratios of the individual *A. laidlawii* membrane lipids is observed in this case, possibly complicating the interpretation of our results. Thus, we chose DEPE as the matrix lipid, since the T_h of this well-studied lipid occurs at an experimentally convenient temperature and *A. laidlawii* grows well with exogenous elaidic acid in the presence of avidin. We were thus able to study a series of fatty acid-homogeneous *A. laidlawii* membrane lipids whose fatty acid compositions were essentially identical to one another and to the PE matrix employed.

MATERIALS AND METHODS

A. laidlawii B cells were grown at 37 °C in a lipid-poor growth medium and harvested at mid-log phase as described previously (Silvius & McElhaney, 1978a,b; Silvius *et al.*, 1980). Avidin, an inhibitor of *de novo* fatty acid biosynthesis and exogenous fatty acid chain elongation in this organism, was added to the growth medium, as was exogenous elaidic acid. Under these conditions, the fatty acyl groups of the lipids from these "fatty acid-homogeneous membranes" essentially consist only of elaidoyl residues (>98 mol %). The polar lipids were extracted from isolated membranes and the individual lipid components purified, separated, and quantitated as already reported (Monck *et al.*, 1992). The DEPE used for the phospholipid matrix was from Avanti Polar Lipids (Alabaster, AL) and was >99% pure by thin-layer chromatography. DSC heating thermograms were recorded with a Microcal MC-2 high-sensitivity instrument (Microcal Inc., Northampton, MA) operating at scan rates of 30 °C/h, and ^{31}P-NMR spectra were recorded with a Varian Unity 300 instrument (Varian Instruments, Palo Alto, CA) operating at 121.42 MHz for ^{31}P. The conditions for acquiring the NMR spectra were the same as previously used in this laboratory (Lewis *et al.*, 1988, 1990). The lipid mixtures were dispersed by vigorous vortexing in a buffer composed of 50 mM Tris, 100 mM NaCl, and 10 mM EDTA (pH 7.4) at temperatures near 50 °C. The total lipid concentrations were about 5 and 10 mg/mL for the DSC and NMR analyses, respectively, providing full hydration for the lipid samples. The T_h values were defined as the temperature of 50% conversion from the lamellar (or reversed cubic) to the H_{II} phase as estimated from the areas under the peaks of the various DSC thermograms.

Table 1: The Polar Headgroup Composition of the Membrane Lipids from *A. laidlawii* B Cells Grown in the Presence of Exogenous Elaidic Acid and Avidin[a]

membrane lipid	quantity present (mol %)	membrane lipid	quantity present (mol %)
APG	1.0 ± 0.5	PG	27.3 ± 6.0
MGDG	43.1 ± 6.3	GPMGDG	0.5 ± 0.2
DGDG	20.7 ± 4.7	GPDGDG	7.2 ± 2.0

[a] Values presented are the arithmatic means (averages) and standard deviations from the mean of three independent experiments.

RESULTS

The polar headgroup composition of the membrane lipids from *A. laidlawii* B cells grown in exogenous elaidic acid and avidin are shown in Table 1. The three major lipid components of such elaidic acid-homogeneous membranes are the neutral glycolipids MGDG and DGDG and the anionic phospholipid PG. The glycerylphosphorylated glycolipid GPDGDG is present in smaller quantities, while the APG and GPMGDG are quite minor components in cells cultured under these conditions. However, cultured under other conditions, GPDGDG and APG (but not GPMGDG) can be major lipid components of the *A. laidlawii* B membrane (Lewis & McElhaney, 1995; Bhakoo *et al.*, 1987). Thus, the relative strengths of the phase preferences of the three major and of the first two minor membrane lipids listed above were determined as described below; the very small quantities of GPMGDG present precluded its inclusion in this study at any rate. As noted above, the overall fatty acid composition of these lipids is essentially identical, since elaidic acid makes up at least 98 mol % of their fatty acyl groups in all cases.

A DSC thermogram of synthetic DEPE alone in excess water is presented in Figure 1. The DSC instrument essentially measures the heat absorbed when lipid aggregates transform from one phase to another as the sample and an inert reference are both heated at identical, predetermined rates. For DEPE alone, two cooperative endothermic phase

[1] T_h, liquid-crystalline lamellar to reversed hexagonal phase transition temperature; DOPE, dioleoylphosphatidylethanolamine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; DEPE, dielaidoylphosphatidylethanolamine; DEPC, dielaidoylphosphatidylcholine; DEPS, dielaidoylphosphatidylserine; DSC, differential scanning calorimetry; PG, phosphatidylglycerol; MGDG, monoglucosyldiacylglycerol; DGDG, diglucosyldiacylglycerol; GPMGDG, glycerylphosphorylmonoglucosyldiacylglycerol; GPDGDG, glycerylphosphoryldiglucosyldiacylglycerol; APG, acyl polyprenyl glucoside.

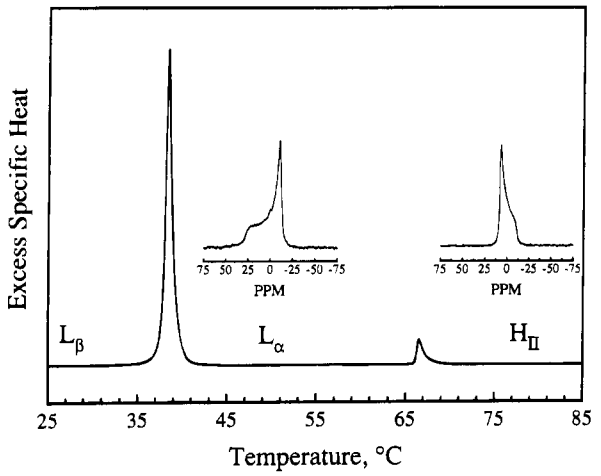

FIGURE 1: High-sensitivity DSC thermogram of an aqueous suspension of pure DEPE. All DSC analyses were performed using a scan rate of 30 °C/h in the heating mode. Identification of the phases formed in all experiments was confirmed by ^{31}P-NMR spectroscopy, and representative ^{31}P-NMR spectra at 50 and 75 °C, and representing the lamellar liquid-crystalline (L_α) and reversed hexagonal phase (H_{II}), are illustrated, whereas the broader lamellar-type spectra of the gel (L_β) phase is not.

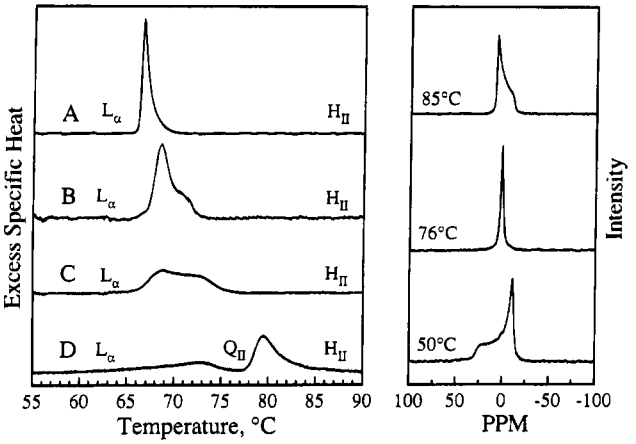

FIGURE 2: High-sensitivity DSC thermograms of aqueous dispersions of DEPE containing (A) 0 mol %, (B) 2.5 mol %, (C) 5.0 mol %, and (D) 10.0 mol % of PG from elaidic acid-homogeneous *A. laidlawii* B membranes. Only the lamellar/nonlamellar phase transition is illustrated. Representative ^{31}P-NMR spectra from the 10.0 mol % PG sample taken at 50, 76, and 80 °C represent the lamellar (L_α), reversed cubic (Q_{II}), and reversed hexagonal (H_{II}) phases, respectively.

transitions occur. The more energetic phase transition centered at 38 °C arises from a transition from the solid-like, lamellar gel phase to the liquid-crystalline lamellar phase, and the less energetic transition at 66.4 °C arises from a transition from the lamellar liquid-crystalline to the reversed hexagonal phase. In this study, we focus exclusively on the latter transition.

Figure 1 also shows that the structural changes occurring at the lamellar/reversed hexagonal phase transition temperature of DEPE result in distinct changes in the ^{31}P-NMR spectrum of this lipid. At temperatures below the T_h, DEPE exhibits axially symmetric powder patterns consisting of an upfield peak and a downfield shoulder. Such powder patterns are characteristic of liquid-crystalline lamellar phases (Tilcock *et al.*, 1986). At temperatures above the T_h, the powder pattern narrows significantly and the asymmetry of its absorption intensity reverses. Such powder patterns are characteristic of the reversed hexagonal phases of phospholipid assemblies (Tilcock *et al.*, 1986). With a few of lipid mixtures used, we also found that the conversion of the lamellar liquid-crystalline to the inverted hexagonal phase proceeded via a stable intermediate phase which exhibits a ^{31}P-NMR spectrum consisting of a relatively sharp isotropic peak near 2 ppm downfield (see Figure 2). Under the conditions used in this study, the appearance of this isotropic peak is generally correlated with the formation of one or more reversed cubic phases (Tilcock *et al.*, 1986). Because of the distinctiveness of the ^{31}P-NMR spectroscopic signatures of the various types of lipid phases, we were able to use ^{31}P-NMR spectroscopy to determine the structural basis of the transitions observed by DSC.

A series of DSC endotherms of DEPE containing small but progressively increasing amounts of PG from elaidic acid-homogeneous *A. laidlawii* B membranes are shown in Figure 2. Note that the presence of PG results in two-component endotherms, both of which are elevated in temperature relative to DEPE alone. At lower temperatures, the DEPE–PG mixture exists exclusively in the lamellar phase, and at higher temperatures, exclusively in the reversed hexagonal phase, according to ^{31}P-NMR spectroscopy. However, in the temperature range between the two endotherms, the DEPE–PG mixture exhibits a ^{31}P-NMR spectrum characteristic of the reversed cubic phase. Thus, the lower temperatures endotherm represents a lamellar/cubic and the higher temperature endotherm a cubic/reversed hexagonal phase transition. Nevertheless, the addition of PG clearly results in a progressive increase in the T_h, here defined as the midpoint temperature for the formation of the reversed hexagonal phase, indicating that PG differentially stabilizes the lamellar as compared to the reversal hexagonal phase of DEPE. Thus the effective size of the polar headgroup of PG appears to be considerably larger than that of PE. Interestingly, the effect of this PG on increasing the T_h of the DEPE matrix is comparable to that of DEPC and greater than that of DEPS, the major lamellar phase-forming zwitterionic and anionic glycerophospholipids of eukaryotic plasma membranes, respectively (data not presented).

A series of DSC endotherms of DEPE containing small but progressively increasing amounts of DGDG from elaidic acid-homogeneous *A. laidlawii* B membranes are presented in Figure 3. The progressive introduction of DGDG into the DEPE matrix results in the appearance of two or more endotherms at slightly elevated temperatures in comparison of DEPE alone. In this case, however, ^{31}P-NMR spectroscopy provided no evidence for the formation of cubic phases. Thus, these broad, multicomponent DSC endotherms all appear to be overlapping lamellar/reversed hexagonal phase transitions. The increase in the T_h, observed upon the addition of DGDG, is slight in comparison with the addition of comparable amounts of *A. laidlawii* elaidic acid-homogeneous PG (or in comparison to synthetic DEPC or DEPS). The effective polar headgroup size of DGDG thus seems only slightly greater than that of the DEPE molecules making up the matrix, since this lipid only weakly stabilizes the lamellar and weakly destabilizes the reversed hexagonal phase of the latter.

A series of DSC endotherms of DEPE dispersions containing small but progressively increasing amounts of MGDG from elaidic acid-homogeneous *A. laidlawii* B membranes are shown in Figure 4. Except at 5.0 mol %,

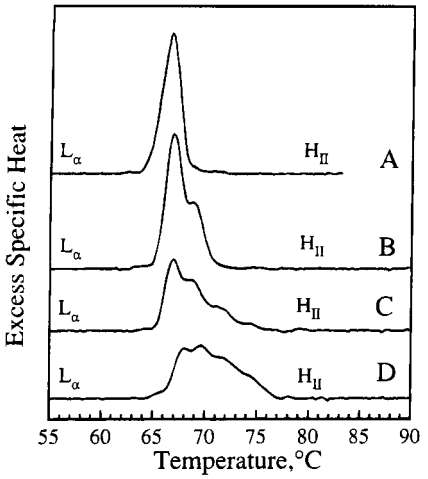

FIGURE 3: High-sensitivity DSC thermograms of aqueous dispersions of DEPE containing (A) 0 mol %, (B) 2.5 mol %, (C) 5.0 mol %, and (D) 10.0 mol % of the DGDG from elaidic acid-homogeneous *A. laidlawii* B membranes.

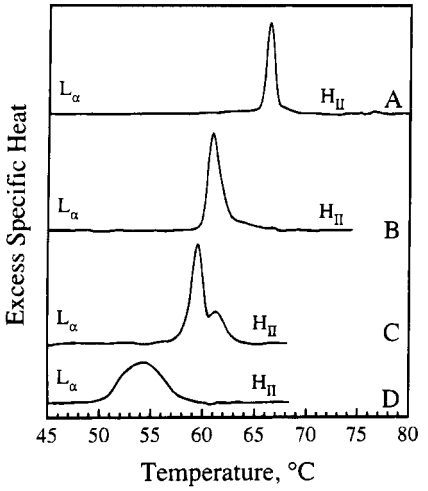

FIGURE 4: High-sensitivity DSC thermograms of aqueous dispersions of DEPE containing (A) 0 mol %, (B) 2.5 mol %, (C) 5.0 mol %, and (D) 10.0 mol % of MGDG from elaidic acid-homogeneous *A. laidlawii* B membranes.

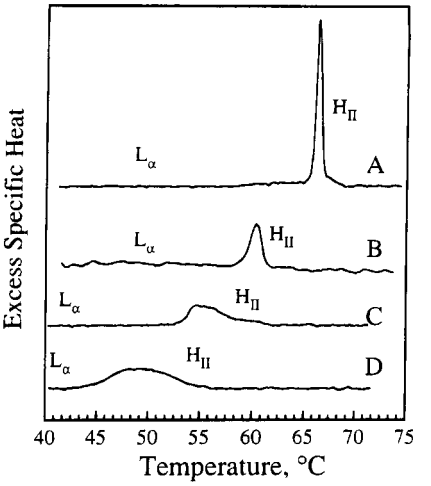

FIGURE 5: High-sensitivity DSC thermograms of aqueous dispersions of DEPE containing (A) 0 mol %, (B) 2.5 mol %, (C) 5.0 mol %, and (D) 10.0 mol % of APG from elaidic acid-homogeneous *A. laidlawii* B membranes.

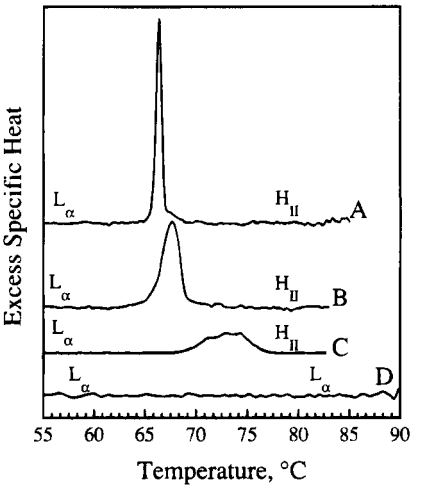

FIGURE 6: High-sensitivity DSC thermograms of aqueous dispersions of DEPE containing (A) 0 mol %, (B) 2.5 mol %, (C) 5.0 mol %, and (D) 10.0 mol % of the GPDGDG from elaidic acid-homogeneous *A. laidlawii* B membranes.

the addition of MGDG results in one-component DSC endotherms corresponding to lamellar/reversed hexagonal phase transitions. Again, no evidence for the existence of stable cubic phases was obtained by ^{31}P-NMR spectroscopy. The addition of MGDG results in a progressive and fairly substantial decrease in the T_h, indicating that MGDG destabilizes the lamellar as compared to the reversed hexagonal phase of DEPE. Thus, the effective size of the MGDG polar headgroup appears to be somewhat smaller than that of PE.

A series of DSC endotherms of DEPE containing small but progressively increasing amounts of APG from elaidic acid-homogeneous *A. laidlawii* B membranes are presented in Figure 5. The progressive introduction of APG results in the appearance of broad, apparently single-component endotherms at considerably lower temperatures in comparison to pure DEPE. Again, no evidence of the formation of stable cubic phase intermediates was found using ^{31}P-NMR spectroscopy. Since AGP strongly destabilizes the lamellar and stabilizes the reversed hexagonal phase of the DEPE matrix, even in comparison to MGDG, it would appear that the polar headgroup of APG has an even smaller effective size.

Finally, a series of DSC endotherms of DEPE containing small but progressively increasing amounts of GPDGDG from elaidic acid-homogeneous *A. laidlawii* B membranes are shown in Figure 6. Although smaller amounts (2.5 mol %) of added GPDGDG produce an increase in the T_h of the DEPE matrix roughly comparable to that of PG, moderate amounts (5.0 mol %) produce much larger increases in this parameter. Moreover, at still higher levels (7.5 and 10.0 mol %), the DEPE matrix does not undergo a lamellar/reversed hexagonal phase transition at experimentally accessible temperatures but remains exclusively in the lamellar phase at temperatures of at least 85−90 °C. This very marked stabilization of the lamellar and destabilization of the reversed hexagonal phase of the DEPE matrix over most of the concentration range tested indicates that the effective size of the GPDGDG polar headgroup must be much larger than that of PG (or of other synthetic phospholipids such as DEPC or DEPS).

A summary of the results of the DSC analyses just described is presented in Figure 7 and Table 2. In Figure 7 the change in the T_h of the DEPE matrix is plotted as a function of the concentration of each of the individual *A.*

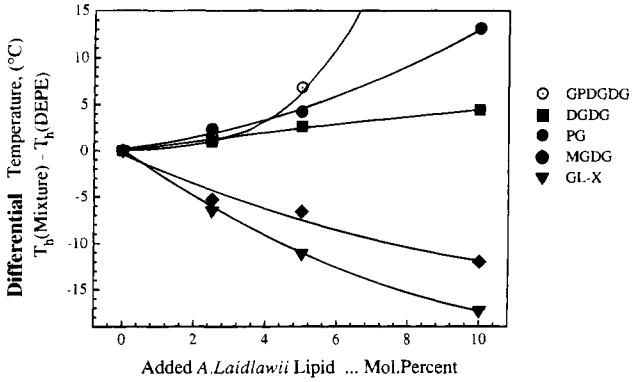

FIGURE 7: A plot of the difference in T_h values between aqueous dispersions of pure DEPE and mixtures of DEPE containing increasing quantities of the dielaidoyl species of each of the major lipids of the *A. laidlawii* B membrane. The symbol (⊙) indicates GPDGDG, (●) indicates PG, (■) indicates DGDG, (♦) indicates MGDG, and (▼) indicates APG.

Table 2: A Comparison of the Direction and Magnitude of the T_h Shifts Induced in DEPE by the Various *A. laidlawii* Membrane Lipids[a]

A. laidlawii lipid	slope of T_h curve	*A. laidlawii* lipid	slope of T_h curve
GPDGDG	+3.95	MGDG	−1.14
PG	+1.29	APG	−1.74
DGDG	+0.45		

[a] The slopes of the experimental curves from Figure 7 are given for the *A. laidlawii* lipids present at a concentration of 5 mol % in 95 mol % DEPE.

laidlawii B membrane lipids tested. Lipids with larger effective polar headgroup sizes than DEPE should exhibit positive slopes in Figure 7, with the magnitude of the size difference increasing with the slope of the line relating the increase in the T_h to the amount of *A. laidlawii* lipid present. Conversely, lipids with smaller polar headgroups than DEPE should exhibit negative slopes, the magnitude of which should increase with the relative difference in effective headgroup size. In Table 2 the "average" slopes of the curves presented in Figure 7 are quantified by measuring the slope of a tangent to these experimental curves at a concentration of added lipid of 5.0 mol %. This treatment of the data was adopted because most of the curves in Figure 7 are nonlinear and values for GPDGDG cannot be determined at higher concentrations. Our major conclusions are listed below:

(1) Three of the *A. laidlawii* membrane lipids (GPDGDG, PG, and DGDG) apparently have polar headgroups with larger effective sizes than PE and therefore should exhibit a stronger preference for forming either normal micellar or lamellar phases.

(2) The relative effective polar headgroup sizes of these three lipids are, however, quite different, with GPDGDG ≫ PG > DGDG.

(3) The ability of GPDGDG to increase the T_h of the DEPE matrix is extraordinarily high, such that the reversed hexagonal phase does not even form at temperatures of less than 100 °C when more than 5 mol % of this lipid is present.

(4) Two of the *A. laidlawii* membrane lipids (MGDG and APG) have polar headgroups of smaller effective size than PE and should therefore strongly prefer a reversed hexagonal phase, since PE itself prefers this phase at higher temperatures.

(5) The relative effective size of the polar head group of APG appears to be smaller than that of MGDG (but see below), and thus APG appears to have a stronger propensity to form reversed phases than does MGDG.

DISCUSSION

The results obtained in this study are generally compatible with, but considerably extend, those of previous studies of the phase preferences of the individual *A. laidlawii* membrane lipids. Thus PG and DGDG have been identified as lamellar phase-preferring lipids based on the fact that aqueous dispersions of these lipids form only gel or liquid-crystalline bilayer phases between 0 and 100 °C, irrespective of fatty acid composition, whereas MGDG and APG have been identified as nonlamellar phase-preferring lipids since they form reversed cubic and/or reversed hexagonal phases over a considerable region of this temperature range, especially when enriched in unsaturated fatty acids (Wieslander *et al.*, 1978, 1981; Lindblom *et al.*, 1986; Bhakoo *et al.*, 1987; Lewis *et al.*, 1990; Eriksson *et al.*, 1991). Moreover, based on the lower lamellar/reversed hexagonal phase transition temperature of APG compared to MGDG, we have argued previously that the former lipid should have a stronger tendency to form reversed cubic or hexagonal phases than the latter lipid at physiological temperatures (Bhakoo *et al.*, 1987; Lewis *et al.*, 1990), a prediction confirmed in the present study. However, previous studies of GPDGDG have identified this lipid as exclusively bilayer-forming under physiologically relevant conditions of temperature and hydration (Wieslander *et al.*, 1978, 1981; Lindblom *et al.*, 1986). However, the extremely marked reversed hexagonal phase-destabilizing effect of this compound on DEPE bilayers in comparison to PG and DGDG suggests that GPDGDG may actually be a normal micellar phase-preferring lipid. Indeed, we demonstrate in the accompanying paper that aqueous dispersions of this lipid do form micelles at physiologically relevant temperatures (Lewis & McElhaney, 1995). To our knowledge, this is the first identification of a major micellar-forming lipid in any prokaryotic biological membrane (GPDGDG may compose up to 30 mol % of the *A. laidlawii* membrane lipids), although the gangliosides of eukaryotic cells also form micelles (Maggio, 1985; Maggio et al., 1988; Acquotti et al., 1994) and strongly destabilize the reversed hexagonal phase of PE at physiological levels (Perillo *et al.*, 1994). However, gangliosides are typically minor components of most eukaryotic plasma membranes and are confined to the outer surface of the membrane. Nevertheless, these compounds could also have major effects on the lipid phase preference in eukaryotic plasma membranes.

In their studies of the effect of small amounts of a diacylglycerol and of six different phospholipids on the T_h of a POPE matrix, James and co-workers (Lee *et al.*, 1993) found an excellent correlation (correlation coefficient 0.95) between the T_h and intrinsic lipid headgroup molecular volume, indicating that effects such as charge, hydration, and conformation play only secondary roles in determining relative polar headgroup effective sizes or cross-sectional areas at the lipid bilayer surface in this particular group of lipids. However, in this study of PG and various glycolipids, we find a much poorer correlation between these two parameters. Thus, the unhydrated polar headgroup volumes, calculated from headgroup atomic composition and covalent

radii, decrease in the following order: GPDGDG (58.2 Å^3) > DGDG (38.9 Å^3) > MGDG (20.0 Å^3) ≥ APG (19.9 Å^3) > PG (19.4 Å^3). In contrast, from the effects of small amounts of the dielaidoyl species of these lipids on the T_h of DEPE, the effective headgroup sizes decrease in the order GPDGDG > PG > DGDG > MGDG > APG. Therefore, the strongly lamellar-preferring nature of PG and the weakly lamellar-preferring nature of DGDG are not explainable on the basis of intrinsic headgroup volume alone, nor is the significantly stronger reversed micellar phase preference of APG as compared to MGDG. However, the effective polar headgroup size, the really relevant parameter, is known to depend not only on the volume of the polar headgroup but also on its shape, charge and degree of hydration, as well as on its orientation and motional freedom at the bilayer interface (Mannock *et al.*, 1992; Trouard *et al.*, 1994; Lewis *et al.*, 1995). Moreover, the nature and relative strength of the interactions between adjacent polar headgroups on the bilayer surface may also influence lipid phase preference (Mannock *et al.*, 1992; Trouard *et al.*, 1994; Lewis *et al.*, 1995). We therefore suggest that the stronger lamellar phase preference of PG compared to DGDG, and particularly to MGDG, can be explained by its greater degree of hydration (Wieslander *et al.*, 1978, 1981; Lindblom *et al.*, 1986; Eriksson *et al.*, 1991), by its charged character (Lewis *et al.*, 1995), and by its greater degree of conformational freedom at the bilayer surface (Jarrell *et al.*, 1986, 1987a,b; Renou *et al.*, 1989; Carrier *et al.*, 1989). Moreover, the PG headgroup is aligned with its long axis nearly parallel to the bilayer surface whereas the sugar headgroups of MGDG and DGDG extend further away from the bilayer surface (Jarrell *et al.*, 1986, 1987a,b; Renou *et al.*, 1989; Carrier *et al.*, 1989). As well, the strength of polar headgroup interactions at the polar surface of PG is clearly less than that of MGDG or DGDG as shown by the much lower gel to liquid-crystalline phase temperature of the former when the fatty acid composition of these two lipids are identical (Silvius & McElhaney, 1978a,b; Silvius *et al.*, 1980). On the other hand, we propose that the greater reversed hexagonal phase preference of APG as compared to MGDG is not due primarily to differences in the intrinsic volumes of the polar headgroups at the lipid/water interface (which are at least formally almost the same), but rather to differences in the nature of their hydrocarbon chains. The APG contains a highly branched polyprenyl chain as well as an unbranched elaidoyl chain attached directly to the glucose headgroup, whereas MGDG contains two elaidoyl chains attached to the glycerol backbone to which the single glucose headgroup is, in turn, attached. The larger cross-sectional area of the polyprenyl chain imparts a more inverted conical shape to the APG molecule in comparison to MGDG. The absence of a glycerol backbone in APG may also shift the pivotal plane of the molecule toward the polar headgroup, which would also accentuate the inverted conical shape of APG (Gruner *et al.*, 1992). However, the absence of a free hydroxyl group at C2 of the glucose moiety of APG but not of MGDG could also contribute to the stronger reversed phase-forming tendency of the former, since in general a reduction in the number of free hydroxyl groups in carbohydrate-based amphiphiles tends to promote reversed hexagonal phase formation (van Doren & Wingert, 1994).

A considerable amount of work has been done on the biosynthetic regulation of overall lipid phase preference in *A. laidlawii* strains A (see Rilfors *et al.*, 1984, 1993) and B (see McElhaney, 1984, 1989, 1992). In these previous studies, the individual lipids of the *A. laidlawii* membrane have been considered to be either exclusively lamellar- or reversed phase-preferring lipids, and it has been assumed that the strengths of their characteristic phase preferences are equivalent. However, this work reveals that normal- as well as lamellar- and reversed phase-forming lipids exist in the membrane of this organism and that the relative strengths of the characteristic phase preferences of the various phospho- and glycolipids vary considerably. Thus these results have important implications for the interpretation of past and future studies of overall membrane lipid organization and biosynthetic regulation in *A. laidlawii* and possibly in other organisms.

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