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The effect of variations in growth temperature, fatty acid composition and cholesterol content on the lipid polar head-group composition of *Acholeplasma laidlawii* B membranes

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We have systematically investigated the effect of variations in growth temperature, fatty acid composition and cholesterol content on the membrane lipid polar headgroup composition of *Acholeplasma laidlawii* B. Two important lipid compositional parameters have been determined from such an analysis. The first parameter studied was the ratio of the two major neutral glycolipids of this organism, monoglucosyldiacylglycerol (MGDG) and diglucosyldiacylglycerol (DGDG). As the former lipid prefers to exist in a reversed hexagonal phase at higher temperatures, with unsaturated fatty acyl chains or in the presence of cholesterol, the ratio of these two lipids reflects the phase state preference of the total *A. laidlawii* membrane lipids. Although we find that the MGDG/DGDG ratio is reduced in response to an increase in fatty acid unsaturation, increases in growth temperature or cholesterol content reduce this ratio only in cells enriched in a saturated but not an unsaturated fatty acid. The second parameter studied was the ratio of these neutral glycolipids to the only phosphatide in the *A. laidlawii* membrane, phosphatidylglycerol (PG); this parameter reflects the relative balance of uncharged and charged lipids in the membrane of this organism. We find that the MGDG + DGDG/PG ratio is lowest in cells enriched in the saturated fatty acid even though these cells already have the highest lipid bilayer surface charge density. Moreover, this ratio is not consistently related to growth temperature or changes in cholesterol levels, as expected. We therefore conclude that *A. laidlawii* strain B, apparently unlike strain A, does not possess coherent regulatory mechanisms for maintaining either the phase preference or the surface charge density of its membrane lipid constant in response to variations in growth temperature, fatty acid composition or cholesterol content.

Introduction

Acholeplasma laidlawii is a simple, cell wall-less procaryotic microorganism that possesses a number of features which make it an attractive system for studying the roles of lipids in biological membranes [1,2]. An especially useful feature of this organism as a model membrane system is the

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Abbreviations: MGDG, monoglucosyldiacylglycerol; DGDG, diglucosyldiacylglycerol; PG, phosphatidylglycerol.

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ability to dramatically alter its membrane lipid fatty acid composition and cholesterol content by appropriate manipulation of the lipid composition of the growth medium. Utilizing this ability, Lindblom, Rilfors, Wieslander and co-workers [3–11] have shown that alterations in membrane lipid fatty acid composition and cholesterol content, as well as in growth temperature, induce marked changes in the quantitative distribution of the major polar lipids on the limiting membrane of *A. laidlawii* strain A. These alterations in the lipid polar headgroup composition are of two types. The first type of alteration involves a change in the ratio of neutral glycolipids (monoglycosyldiacylglycerol (MGDG) plus diglucosyl/diacylglycerol (DGDG)) to anionic lipids (primarily phosphatidylglycerol (PG)), so as to maintain a constant surface charge density in response to the lipid fatty acid composition-induced changes in the average cross-sectional areas occupied by the lipid molecules in the bilayer or ionic strength-dependent changes in the effective surface charge of the lipid bilayer [12]. The second type of alteration involves a change in the ratio of the two neutral glycolipids, MGDG and DGDG. In particular, the MGDG/DGDG ratio of the *A. laidlawii* A membrane is reported to decrease with an increase in the degree of unsaturation of the membrane lipids fatty acyl chains, with an increase in cholesterol incorporation into the membrane, and with an increase in growth temperature.

The lipid compositional- and temperature-induced changes observed in the MGDG/DGDG ratio of *A. laidlawii* A were subsequently related to the phase preferences of the various membrane polar lipids by Lindblom, Rilfors, Wieslander and co-workers [5,7,9,11]. These workers studied the lyotropic and thermotropic phase behavior of single lipids and lipid mixtures by X-ray diffraction and NMR spectroscopy and the effect of cholesterol on the phase preferences of the neutral glycolipids. Aqueous dispersions of MGDG were found to prefer the reversed hexagonal phase structure at higher temperatures, particularly if the fatty acyl chains are unsaturated or cholesterol is present. In contrast, the other major polar lipids, DGDG and PG, as well as the minor lipids, exist exclusively in the lamellar state at physiological temperatures, irrespective of fatty acid composition or cholesterol content. The MGDG presumably prefers to form a reversed phase under certain conditions because its relatively small, uncharged and poorly hydrated headgroup occupies a smaller cross-sectional area than do its hydrocarbon chains, particularly at higher temperatures or when these chains are unsaturated (see Refs. 13 and 14). This imparts an inverted cone shape to the MGDG molecule, which in turn results in a tendency for monolayers of this molecule to curve into reversed phase structures. Cholesterol molecules presumably accentuate the tendency of MGDG because of their similar inverted wedge shape. In contrast, the other lipid molecules, because of a relatively good match between the cross-sectional areas occupied by their larger, more hydrated polar head groups and their hydrocarbon chains, are cylindrically shaped and thus form only planar bilayer phases (see Refs. 13 and 14).

Lindblom, Rilfors, Wieslander and co-workers have postulated that in all biological membranes a certain balance of bilayer-preferring and nonbilayer-preferring lipids must be maintained in order to ensure an optimal degree of lipid stability and functionality [6,8,11]. For *A. laidlawii*, this requires maintaining an optimal balance between the amount of MGDG and the other membrane lipids present in the face of alterations in growth temperature or in the fatty acid composition or cholesterol content of the growth medium. Specifically, since an increase in temperature, an increase in the degree of unsaturation of the fatty acyl chains or an increase in cholesterol incorporation increases the tendency of MGDG to induce a reversed hexagonal phase, the reduced MGDG/DGDG ratio observed in cells grown at high temperature or with unsaturated fatty acids or cholesterol is viewed as an attempt by this organism to maintain its lipids at least primarily in the bilayer state, and thus to preserve the osmotic integrity of its membrane. On the other hand, the increase in the MGDG/DGDG ratio observed with a decrease in growth temperature or with a decrease in fatty acyl chain unsaturation or cholesterol content, is viewed as an attempt by this organism to maintain a certain degree of nonbilayer-forming ability, since the transient formation of local regions of non-lamellar lipid may be advantageous for certain membrane functions

(see Refs. 13–15). Thus these workers postulate that the necessary dynamic balance between the tendency of the lipid components to form lamellar and non-lamellar structures is maintained in *A. laidlawii* A by appropriate alterations in the polar headgroup composition of the membrane lipids [6,8,11,13].

Although the hypothesis of Lindblom, Rilfors, Wieslander and co-workers is relatively well supported by their studies with *A. laidlawii* strain A (see also Refs. 17–19), there is a considerable amount of incidental work with the generally more extensively studied B strain which does not appear fully compatible with this hypothesis (Refs. 1 and 2). As a first step in determining the generality of the shape-regulation concept, we have carried out a systematic investigation of the effect of variations in growth temperature, fatty acid composition and cholesterol content on the membrane lipid polar headgroup composition of the closely related *A. laidlawii* strain B. Although some of the results obtained are similar to those already reported for the A strain, other results are not easily accounted for simply on the basis of the effective surface charge density or lamellar-non-lamellar phase preferences of the membrane lipids of this organism.

Materials and Methods

Fully acclimated *A. laidlawii* B was innoculated into 1 litre of delipidated growth media (Bacto-Heart Infusion Broth, 12 g/l; Bactopeptone, 5 g/l; Bactoyeast, 5, fatty acid-poor bovine serum albumin, 4 g/l; glucose, 2.5 g/l; and penicillin, 105 I.U./l) as previously described [20]. The growth medium contained 0.12 mM palmitic (16:0), elaidic (18:1,Δ9) or oleic (18:1,Δ9) acids and either 12.5 or 25.0 mg/l of cholesterol, if required. The fatty acids, with or without cholesterol, were added to the growth medium as ethanolic solutions before innoculation. Growth of the culture was followed turbidometrically (OD_{450nm}) until the late logarithmic phase when cells were collected by centrifugation and washed twice with α-buffer (0.15 M NaCl, 0.01 M $MgSO_4$, 0.05 Tris (pH 7.8)). Since the ratios of the membrane lipids change with the age of the culture [1,2], care was taken to harvest cells in comparable phases of their growth cycle, whatever the growth temperature or lipid composition of the growth medium.

The total membrane lipids were extracted from washed cells with chloroform/methanol (2:1, v/v) and purified on a silica gel column as described elsewhere [21]. The polar lipid fraction was then separated into the individual lipid classes by thin-layer chromatography on Silica gel G by development in a chloroform/ methanol/ water (70:25:3, v/v/v) solvent system. After a visualization of the separated lipid components by a brief exposure to iodine vapor, the Silica gel G bands containing the separated lipid classes were scraped into a small column and the individual glyco- or phospholipids eluted with methanol/ chloroform (9:1, v/v). The fatty acids from each diacylglycerolipid were then converted to their methyl esters by treatment with anhydrous methanol (2.0 ml) and concn. H_2SO_4 at 70°C for 1 h; 250 μg of heptadecanoic acid was added to each lipid before methylation as an internal standard. The μmols of glyco- or phospholipid present in the original sample was then determined by gas-liquid chromatography, since each μmol of diacylglycerol lipid should produce 2 μmol of fatty acid methyl esters, all as described previously [22]. The MGDG/DGDG and (MGDG + DGDG)/PG mol ratios were then calculated from this data. Cells grown in the presence of exogenous palmitic, elaidic or oleic acids were enriched to average levels of 69.3, 77.4 and 67.3 mol%, respectively, of the added fatty acids, irrespective of the presence or absence of cholesterol or of variations in the growth temperature (see Table I). In contrast, the amount of cholesterol incorporated depended upon the fatty acid supplement. Cells supplemented with palmitic, elaidic or oleic acids and 25 mg/l of cholesterol incorporated approximately 12, 15 and 20 mol of cholesterol per 100 mol total glycerolipid, respectively.

The differential scanning calorimetric studies of the lipid thermotropic phase behavior of isolated membranes of *A. laidlawii* B, and of the MGDG components, were performed on a MicroCal MC-1 differential scanning microcalorimeter operating at a heating scan rate of 30 C°/h.

Results

The effect of variations in fatty acid composition and cholesterol content on membrane lipid polar headgroup composition at a constant growth temperature

The MGDG/DGDG and (MGDG + DGDG)/PG ratios of *A. laidlawii* B cells, grown in the presence of different exogenous fatty acids and amounts of cholesterol at a constant temperature of 35°C (the average optimum growth temperature), are presented in Table I. Considering first the MGDG/DGDG ratio, which reflects the ratio of nonbilayer/bilayer phase-preferring neutral glycolipids in the membrane, we note that in the absence of cholesterol this ratio decreases progressively in the order palmitic > elaidic > oleic acid-enriched cells. A similar decrease in MGDG/DGDG ratio as one proceeds from linear saturated to *trans* -monounsaturated to *cis*-monounsaturated fatty acid supplementation has been reported previously in both the A [3,6,8,11] and B [22] strains. Although the incorporation of increasing amounts of cholesterol reduces the MGDG/DGDG ratio in all cases, the magnitude of its effect depends greatly on the membrane lipid fatty acid composition. Cells enriched in palmitic, elaidic and oleic acids exhibit a marked, a moderate and only a slight reduction in their MGDG/DGDG ratios, respectively. In fact, at the higher level of cholesterol incorporation, the MGDG/DGDG ratio of the palmitic acid-enriched membrane falls to a value below that of the *A. laidlawii* B membranes enriched in the unsaturated fatty acids. Interestingly, these results are the opposite to those recently reported for *A. laidlawii* strain A, where it was found that cholesterol incorporation had little effect on the MGDG/DGDG ratio in palmitic acid-enriched membranes but reduces this ratio substantially in oleic or linoleic acid-supplemented cells [18,19].

Considering next the (MGDG + DGDG)/PG ratio, which represents the ratio of uncharged glycolipid to negatively charged phospholipid in the *A. laidlawii* B membrane, we note that in the absence of cholesterol this ratio is lower in palmitic acid- than in elaidic or oleic acid-grown cells. Essentially opposite results have been reported in the A strain, since the mol fraction of charged lipids (inversely related to the (MGDG + DGDG)/PG ratio) increases as palmitic acid is replaced by oleic, linoleic or linolenic acids [12,18,19]. The incorporation of increasing

TABLE I

FATTY ACID COMPOSITION OF THE TOTAL MEMBRANE LIPIDS OF *A. LAIDLAWII* B CELLS GROWN IN THE PRESENCE OF VARIOUS EXOGENEOUS FATTY ACIDS BUT IN THE ABSENCE OF CHOLESTEROL AT 35°C

The fatty acids are designated by the number of carbon atoms followed by the number of double bonds, if any, present in the molecule; the subscripts *c* and *t* denote the *cis* or *trans* configuration, respectively, of the double bond. The results, which are the averages of five experiments, are expressed in mol per cent.

Membrane lipid fatty acids	Exogenous fatty acid added		
	palmitic (16:0)	elaidic ($18:1_t$)	oleic ($18:1_c$)
12:0	13.3	4.5	2.0
14:0	10.8	6.5	6.1
16:0	69.3	11.1	20.3
18:0	3.0	trace	4.1
18:1	2.5	77.4	67.3
18:2	0.9	0.5	trace

TABLE II

EFFECT OF VARIATIONS IN FATTY ACID COMPOSITION AND CHOLESTEROL CONTENT ON THE MEMBRANE LIPID POLAR HEAD GROUP COMPOSITION OF *A. LAIDLAWII* B CELLS GROWN AT 35°C

The values presented are the averages of five independent determinations. Although we found considerable variability from experiment to experiment in the absolute values measured, the trends revealed by these average values were consistently observed in the various individual experiments.

Exogenous lipid additions		MGDG	MGDG+DGDG
fatty acid (12 mM)	cholesterol (mg/l)	DGDG	PG
16:0	0.0	3.0	2.6
16:0	12.5	1.9	2.1
16:0	25.0	0.92	1.3
$18:1_t\Delta9$	0.0	2.0	4.2
$18:1_t\Delta9$	12.5	1.6	4.0
$18:1_t\Delta9$	25.0	1.5	4.5
$18:1_c\Delta9$	0.0	1.4	4.0
$18:1_c\Delta9$	12.5	1.5	4.0
$18:1_c\Delta9$	25.0	1.2	3.9

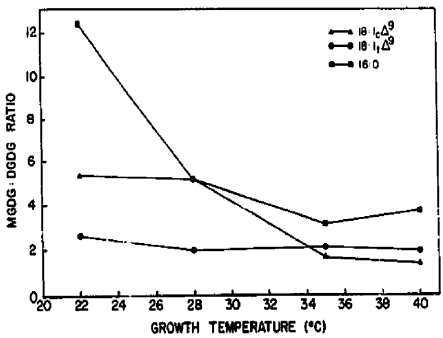

Fig. 1. A plot of the MGDG/DGDG ratio versus the growth temperature of membranes from *A. laidlawii* B cells grown in the presence of exogeneous palmitic, elaidic or oleic acid but in the absence of cholesterol. Cells were cultured from about 24–72 h at the temperature indicated and all were harvested in comparable phases of their growth cycles (late log phase). The results presented are the averages of five independent experiments.

amounts of cholesterol into the *A. laidlawii* B membranes results in a substantial reduction of the (MGDG + DGDG)/PG ratio only in palmitic acid-enriched cells; cells enriched in elaidic or oleic acids show no consistent variation in this ratio. Again, these results contrast with those previously reported for *A. laidlawii* A, where it has been reported that the incorporation of cholesterol has no effect on the mol fraction of charged lipids in membranes enriched in palmitic acid but increases the fraction of anionic phospholipids present in membrane enriched in oleic, linoleic or linolenic acids [18,19].

The effect of variations in fatty acid composition on membrane lipid polar headgroup composition as a function of variations in growth temperature

The effect of different growth temperatures on the MGDG/DGDG and (MGDG + DGDG)/PG ratios in membranes of *A. laidlawii* B cells grown on several different exogenous fatty acids in the absence of cholesterol are presented in Figs. 1 and 2, respectively. As can be clearly seen in Fig. 1, a decrease in the growth temperature below the optimal growth temperature of 35 °C increases the MGDG/DGDG ratio in all cases. However, this increase is very marked for palmitic acid-enriched cells, is modest for oleic-enriched cells, and is slight or nonexistent for cells supplemented with elaidic acid. The present results are qualitatively similar to those previously presented for *A. laidlawii* A [4,6], where the MGDG/DGDG ratio is increased in cells shifted from 37 to 17 °C in comparison to cells maintained at 37 °C, and where cells supplemented with equimolar ratios of palmitic and oleic acids exhibit a larger relative increase in this ratio than do cells supplemented with oleic acid alone. However, in both the A and B strains a substantial increase in the MGDG/DGDG ratio occurred upon continued growth in the presence of oleic acid at 17 °C. Growing *A. laidlawii* B cells at 40 °C has little if any effect on the MGDG/DGDG ratio relative to growth at 35 °C, whatever the fatty acid composition of the membrane lipids.

The effect of growth temperature variations on the (MGDG + DGDG)/PG ratio of *A. laidlawii* B cells supplemented with various exogenous fatty acids is illustrated in Fig. 2. Cells enriched in palmitic, elaidic or oleic acid all appear to exhibit a minimum in this ratio in the region of the average optimal growth temperature of 35 °C, with a small increase in the relative proportions of the neutral glycolipids being observed at the lower growth temperatures. However, the variations in the (MGDG + DGDG)/PG ratio with growth temperature are not great and may in fact not be statistically significant. In contrast, the growth of *A. laidlawii* A at 17 °C for 24 h results in a substantial increase in the (MGDG +

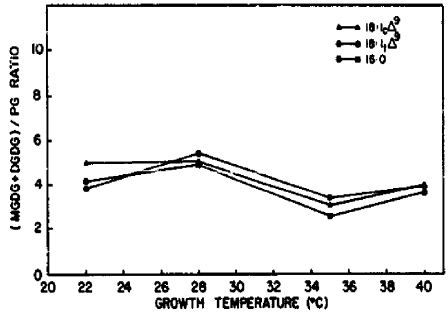

Fig. 2. A plot of the (MGDG+DGDG)/PG ratio versus growth temperature of membranes from *A. laidlawii* B cells grown in the presence of palmitic, elaidic or oleic acid but in the absence of cholesterol. (The experimental details are presented in the legend of Fig. 1.)

DGDG)/PG ratio relative to cells growth at 37 °C, particularly for cells grown in the presence of palmitic and oleic acids and in the absence of cholesterol [4,6].

Discussion

The differences in the fluidity and phase state of the lipids in the membranes of *A. laidlawii* B cells grown with each of the three exogenous fatty acid examined should be borne in mind when analyzing the temperature-, fatty acid and cholesterol composition-induced changes in the lipid polar head group distribution presented here. Membranes enriched in palmitic acid exhibit a gel/liquid-crystalline phase transition extending over the temperature range 15–45 °C, with the midpoint temperature at about 37 °C, as determined by differential scanning calorimetry. For elaidic and oleic acid-enriched membranes, the lipid phase transition ranges are 5–35 °C and −20 to 0 °C, respectively, and the phase transition midpoint temperatures are 22 and −10 °C. Thus, at the average optimal growth temperature of 35 °C, palmitic acid-enriched membranes consist of a roughly equal proportion of gel and liquid-crystalline lipid while all of the lipid in the *A. laidlawii* B membranes enriched in elaidic or oleic acids will be in the liquid-crystalline state. However, at growth temperatures below 35 °C, elaidic acid-enriched membranes will also contain an increasing proportion of gel state lipid, whereas all of the lipids in membranes enriched in oleic acid will remain liquid-crystalline. Thus lipid 'fluidity', defined either as rates of molecular motion or the amplitude of these motions, and the average cross-sectional area occupied by the glyco- and phospholipids, will increase in the order palmitic < elaidic < oleic acid-enriched membranes (see Refs. 1 and 2). Moreover, the addition of cholesterol will have little effect on the fluidity or average cross-sectional area of the glycerolipids of palmitic acid-enriched membranes at the optimal growth temperature, since the incorporation of cholesterol has little if any effect on the phase transition midpoint temperature of these membranes but only broadens the gel liquid-crystalline phase transition and reduces its enthalpy. However, at 35 °C the addition of cholesterol will decrease lipid fluidity and the effective cross-sectional area of the glycerolipids in elaidic and oleic acid-enriched membranes, since this sterol is known to condense or order liquid-crystalline lipid [1,2]. In all cases, however, the incorporation of the uncharged cholesterol molecule will decrease the surface charge density of the lipid bilayer, although its effect on membranes enriched in the two unsaturated fatty acids will be partially compensated for by its condensing effect on the adjacent liquid-crystalline glycerolipid molecules (see Refs. 1 and 2).

Also of relevance to a discussion of the present results is the effect of variations in fatty acid composition and cholesterol content on the thermotropic phase behavior of the MGDG, the only nonbilayer-forming lipid in the *A. laidlawii* membrane. The lamellar liquid-crystalline/reversed hexagonal phase transition temperature of the MGDG from the saturated to the *trans*-unsaturated to the *cis*-monounsaturated fatty acid-enriched membranes should decrease in the above order. More specifically, our calorimetric studies of the relevant MGDGs indicate that this temperature decreased from about 80 °C to 40 °C to 10 °C as one proceeds from palmitic and through the elaidic acid- to the oleic acid-enriched MGDG. Although we have not specifically determined the effect of the presence of cholesterol on the lamellar liquid-crystalline/reversed hexagonal phase transition of these MGDGs, Khan et al. [7] have shown that the addition of 27 mol% cholesterol to mixtures of MGDG and DGDG highly enriched in oleic acid results in a lowering of the bilayer to non-bilayer phase transition by roughly 5–10 °C. In the present experiments, where smaller incorporations of cholesterol were obtained, the effect of cholesterol on the phase preference of the MGDG in the *A. laidlawii* B membranes should actually be fairly small, at least in the case of enrichment with the unsaturated fatty acids. Moreover, although cholesterol additions have a greater effect on reducing the lamellar/reversed hexagonal phase transitions of mixtures of MGDG and DGDG containing saturated fatty acids as compared to those mixtures containing only unsaturated fatty acids [7], it should be remembered that smaller amounts of cholesterol are incorporated into palmitic acid-enriched membranes.

In the absence of cholesterol, the MGDG/DGDG ratio of *A. laidlawii* B cells grown at 35°C was found to decrease in the order palmitic > elaidic > oleic acid-enriched membranes. Moreover, the incorporation of increasing amounts of cholesterol and higher growth temperatures generally tended to decrease this ratio. These results are qualitatively those predicted by the hypothesis of Lindblom, Rilfors and Wieslander, since lipid compositional or temperature changes which tend to promote the formation of nonlamellar lipid phases result in a decrease in the relative proportion of nonbilayer-forming MGDG component in the membrane. However, the large decrease in the MGDG/DGDG ratio of palmitic acid-enriched membranes observed upon cholesterol incorporation, the smaller decrease in oleic acid-enriched membranes, and the even smaller changes observed in elaidic acid-enriched membranes, do not appear to be in accord with this concept, since the non-bilayer promoting tendency of elaidic acid is intermediate between that of palmitic and oleic acids. Similarly, the increases in the MGDG/DGDG ratio of palmitic acid- and oleic acid-enriched membranes observed with decreases in growth temperature and the small or nonexistence decrease in elaidic acid-enriched membranes are difficult to explain for the same reason. It seems clear from the present results that *A. laidlawii* B does not in fact maintain the lamellar/nonlamellar phase transition temperature of its total membrane lipids constant in the face of all lipid compositional and temperature variations, as is apparently the case with the A strain.

In the absence of cholesterol, the (MGDG + DGDG)/PG ratio of *A. laidlawii* B cells grown at 35°C was found to be lower in palmitic acid-enriched than in membranes enriched in elaidic or oleic acids. This result is incompatible with the results of Christiansson et al. [12] on surface charge density regulation in the A strain, since the smaller cross-sectional areas occupied by the more saturated glyco- and phospholipids should in fact result in a bilayer with a higher surface charge density in the absence of any change in lipid polar head group distribution. We would therefore predict a (MGDG + DGDG)/PG ratio which would decrease in the order palmitic > elaidic > oleic acid-enriched membranes if the surface charge density of the bilayer is to be maintained constant. Although the reduction in the (MGDG + DGDG)/PG ratio with increases in cholesterol incorporation observed in the palmitate-enriched membranes is in the right direction, the observed increase of 55–60% in the amount of negatively charged PG in the membrane is much more than would be required to compensate for an incorporation of roughly 12 mol% cholesterol. Moreover, the lack of any effect of cholesterol incorporation on the (MGDG + DGDG)/PG ratio of elaidate- or oleate-enriched membranes is not expected if surface charge density is to be maintained. Although the small increase in this ratio observed with decreases in growth temperature below the optimum would have the effect of buffering the increase in surface charge density which would accompany a decrease in effective cross-sectional areas of the glyco- and phospholipids in the *A. laidlawii* B membrane, the increase in this ratio observed at temperatures above the optimum would have the opposite effect.

In summary, the present results indicate that *A. laidlawii* B, unlike the closely related A strain, does not consistently and coherently regulate either the bilayer/nonbilayer phase preference or the surface charge density of its membrane lipids by making appropriate alterations in the proportions of the major glyco- and phospholipids in response to changes in growth temperature, fatty acid composition or cholesterol content. It is possible that such regulatory mechanisms once existed in this strain but have somehow been lost or become at least partially defective upon culturing this organism under laboratory conditions over many years. It is also possible that other, unrecognized factors may be modulating or overriding the 'normal' membrane lipid polar headgroup regulatory response of this particular strain to variations in growth temperature and lipid composition. However, in view of the fact that *A. laidlawii* strain B appears to grow well upon as wide a variety of environmental conditions as the A strain, it is also possible that the phase preference and surface charge density regulatory mechanisms described

by Wieslander and colleagues are not required for successful membrane functions in all organisms (see Refs. 1 and 2).

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