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Thermotropic characterization of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside isolated from palmitate-enriched *Acholeplasma laidlawii* B membranes

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The thermotropic phase behavior of a monoacylated neutral glucolipid (2-*O*-acyl,polyprenyl α-D-glucopyranoside), isolated from palmitate-enriched *Acholeplasma laidlawii* B membranes, was studied by differential scanning calorimetry, infrared spectroscopy and X-ray diffraction. When equilibrated at low temperatures, aqueous dispersions of this lipid form an ordered, crystal-like lamellar gel phase which transforms to an inverted hexagonal phase at temperatures near 65 °C upon heating. However, upon cooling from high temperatures, the inverted hexagonal phase remains stable down to temperatures near 45 °C. Further cooling first results in the formation of a metastable lamellar liquid crystalline phase at temperatures near 35 °C and then a metastable gel phase at lower temperatures. The metastable gel phase, if immediately reheated at a fast scan rate, undergoes a gel/liquid-crystalline phase transition at temperatures near 33 °C. These results indicate that this monoacylated glucolipid exhibits its gel/liquid-crystalline phase transition and its lamellar/non-lamellar phase transition at considerably lower temperatures than does the monoglycosyldiacylglycerol formed under the same conditions. When cultured in media enriched in 'high-melting' fatty acids, *Acholeplasma laidlawii* B synthesizes large quantities of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside (up to 60 mol%) mainly at the expense of the monoglucosyldiacylglycerol (the only other nonbilayer-forming liquid normally found in the cell membrane of this organism). We thus suggest that the biosynthesis of this novel glucolipid, in response to the biosynthetic incorporation of high-melting exogenous fatty acids, is an adaptive response designed to maintain a predominantly liquid-crystalline membrane lipid bilayer at the growth temperature, while retaining the high proportion of nonbilayer-forming glucolipid species characteristic of *A. laidlawii* B cells cultured under these conditions.

Introduction

The polar lipid composition of the cell membranes of many organisms is under active study in many laboratories as workers investigate the functional basis of the diversity of lipid polar headgroups found in natural membranes The organism *Acholeplasma laidlawii* has been particularly useful in such studies, since the fatty acid composition of its membrane can be widely manipulated [1,2] This enables one to study the changes in the lipid polar headgroup composition of this microorganism in response to the changes in membrane phase state and fluidity and other parameters dependent on fatty acid composition The *A laidlawii* membrane usually contains five polar lipid components, three of which (MGDG, DGDG and PG) typically comprise more than 85% of the total [2] In a recent report, this microorganism was shown to synthesize large quantities of an additional lipid component when supplemented with palmitic acid and cultured under conditions of glucose limitation stress [3] This lipid was identified as a 2-*O*-acyl,polyprenyl α-D-glucopyranoside From an examination of its structure (see Ref 4 for a diagram), in particular the relatively small size of its polar headgroup in relation to its apolar hydrocarbon chains,

Abbreviations DSC, differential scanning calorimetry, CH_2, methylene, C=O, carbonyl, FTIR, Fourier transform infrared, MGDG, monoglucosyldiacylglycerol, DGDG, diglucosyldiacylglycerol, PG, phosphatidylglycerol

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it was expected to be a nonbilayer-forming lipid [3,4] The possibility that this unusual lipid may form nonlamellar phases is important when viewed from the perspective of the many studies which are currently addressing the issue of whether or not the levels of bilayer- and nonbilayer-forming lipids in *A laidlawii* are finely regulated (4–9 and references cited therein) In our initial studies of this unusual glucolipid [3], we reported on the chemical, enzymatic and spectroscopic studies used to identify this lipid and presented a preliminary differential scanning calorimetric study of its thermotropic phase behavior In this paper we present a more detailed study of its thermotropic phase properties using differential scanning calorimetry, Fourier-transform infrared spectroscopy and X-ray diffraction In particular, we show that 2-*O*-palmitoyl,polyprenyl α-D-glucopyranoside is indeed a potent nonbilayer-forming lipid and that it has a relatively low gel/liquid-crystalline phase transition temperature when compared with the other nonbilayer-preferring lipid species present in *A laidlawii* membrane

Materials and Methods

The material used for this study was isolated from the cell membrane of *A laidlawii* B, which was cultured in a palmitate-enriched medium that had been previously extracted with chloroform and prepared as described by Silvius and McElhaney [1] Total membrane lipids were extracted using the method developed by Bligh and Dyer [10], and the polar lipid fraction was separated from the membrane carotenoids by silicic acid chromatography [1] The neutral glycolipid, 2-*O*-acyl,polyprenyl α-D-glucopyranoside, was subsequently purified by chromatography on a column of silica gel The polar lipid fraction was dissolved in dry methylene chloride and applied to a silica gel column slurried in the same solvent The column was developed by a gradient of acetonitrile in methylene chloride, and the fractions containing the pure lipid were pooled and concentrated in vacuo The pure sample was subsequently dissolved in benzene and lyophylized Differential scanning calorimetric measurements were performed in a Perkin Elmer DSC-2C scanning calorimeter equipped with a thermal analysis data station and the data anlyzed using TADS software and other computer programs developed in this laboratory Samples were prepared for the DSC measurements as follows About 3–4 mg of the dry lyophylized lipid was loaded into a large, stainless-steel sample capsule which was then placed on a heated stage and allowed to warm up to temperatures near 90 °C to facilitate the absorption of water by the sample At this point 50 ml of distilled water was added, the capsule was sealed, and then repeatedly heated and cooled at 10 C°/min to insure complete hydration The sample capsule containing the hydrated lipid was then rapidly centrifuged in a microcentrifuge to ensure good contact between the lipid and the bottom of the capsule, whereupon it was checked by reheating in the calorimeter At the end of the DSC measurements, the sample was quantified by gas chromatography as described by Lewis and McElhaney [11] Samples for FTIR spectroscopy were prepared by squeezing a paste of the lipid in D_2O (1–2 mg lipid in 50 ml D_2O) between barium fluoride plates to make a 25 μm film which was mounted in the cell holder The lipid sample was then hydrated in situ by three cycles of heating to temperatures near 85 °C and cooling The infrared spectra were then recorded with a Digilab FTS-40 Fourier transform infrared spectrometer using the data acquisition and data processing parameters previously reported [12] Small-angle X-ray scattering patterns were recorded with the Princeton SIV X-ray beam lines using the sample preparation and data acquisition methodologies previously described [13]

The concentration of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside dispersion in H_2O (or D_2O) ranged from about 4 wt % in the FTIR experiments to about 40 wt % in the X-ray diffraction studies However, in all cases this glycolipid was fully hydrated, i e , the glycolipid molecules contained their full complement of bound water molecules and an excess water phase was present Under these circumstances the thermotropic phase behavior of this material as observed by all three physical techniques should be, and in fact is comparable, when allowances are made for the different 'effective scan rates' characteristic of each technique Since many of the thermal events observed by calorimetry are broad and scan-rate dependent (i e , are kinetically limited under the conditions employed), an exact correspondence between the phase transition temperatures observed by DSC, which utilizes relatively high rates of temperature change (1–5 C°/min), and X-ray diffraction and FTIR spectroscopy, where temperature changes between determinations are much slower, cannot be expected

Results

Differential scanning calorimetry

The thermotropic phase behavior of aqueous dispersions of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside isolated from palmitate-enriched *A laidlawii* B membranes is complex in that several thermotropic events are observed in each heating or cooling scan Moreover, the thermotropic phase behavior observed is markedly different in the heating and cooling modes and, in the former, depends also on the thermal history of the sample This complex behavior is illustrated by the representative DSC heating and cooling curves presented in Fig 1 Considering first the heating scans, the thermogram in Fig 1A was recorded from a sample

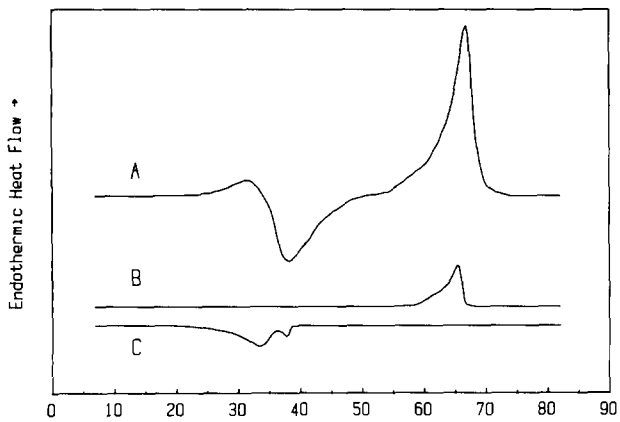

Fig 1 DSC thermograms of aqueous dispersions of the 2-*O*-acyl, polyprenyl α-D-glucopyranoside isolated from palmitate-enriched *A laidlawii* B membranes The curves shown are not drawn to the same scale (A) A 5 C°/min heating scan obtained immediately after cooling from high temperature (B) A 1 C°/min heating scan obtained after prolonged incubation at low temperature (C) A 1 C°/min cooling scan

which was first rapidly cooled from a high temperature and the DSC heating scan immediately begun using a relatively high scan rate (5 C°/min) In this case a broad endothermic transition of relatively low enthalpy occurs at about 33°C followed by a relatively major exothermic event centered between 35–40°C, which is in turn followed by a sharper but asymmetric endothermic transition centered at 67°C In rapidly cooled samples immediately reheated, decreases in the heating scan rate employed lead to a progressive decrease in the areas under the lower temperature endothermic and exothermic peaks and a progressive increase in the area under the higher temperature endothermic peak (data not presented) Moreover, if the glycolipid dispersion is rapidly cooled to a temperature below 15°C and held at this temperature for a sufficient period of time, the lower temperature thermal events disappear entirely from the thermogram and only the higher temperature endotherm remains, which is now clearly bimodal, and exhibits an enthalpy change of 8 5 kcal/mol (see Fig 1B), even if very high heating scan rates are employed Since an exothermic event in a calorimetric heating scan can be observed only under conditions where the sample is not initially in thermodynamic equilibrium, it follows that the exotherm observed upon the rapid heating of unannealed samples of this glycolipid is due to the temperature-induced conversion of a higher-energy metastable state, which was kinetically trapped upon rapid cooling, to another lower energy stable state, which in turn is converted to another phase in an endothermic process at higher temperatures Interestingly, quite similar calorimetric phase behavior is also exhibited by synthetic disaturated α- and β-MGDG's, the former also being constituents of the *A laidlawii* membrane (see Ref 14 and unpublished observations)

In the case of these glyceroglycolipids, the stable, high-melting phase is a lamellar crystalline gel phase (the L_c phase) and the low-melting metastable phase is another less ordered lamellar gel phase (the L_β phase) Moreover, these MGDG's convert at higher temperatures to cubic or inverted hexagonal liquid-crystalline phases As we shall see below, the overall thermotropic phase behavior of this 2-*O*-acyl,polyprenyl α-D-glucopyranoside dispersion is generally similar to that of these glyceroglycolipid dispersions

The cooling behavior of aqueous dispersions of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside is illustrated in Fig 1C. In the cooling mode, two exotherms are observed, a relatively sharper, lower enthalpy (about 1 kcal/mol) transition centered at 38°C and a considerably broader, higher enthalpy (4 kcal/mol) transition centered at 33°C The fact that both the temperature and the enthalpy change of the higher temperature thermal event observed upon cooling are considerably lower than those observed upon the heating of either the annealed or unannealed sample, suggests that there are differences in at least one of the polymorphic states formed during the heating and cooling runs In particular, the marked hysteresis observed for the bimodal, higher temperature heating transition indicates that it is not a 'pure' chain-melting transition from the lamellar gel to the lamellar liquid-crystalline state, as such processes do not exhibit such hysteresis [11,14–18] Rather, such pronounced hysteresis is characteristic of phospho- [11,15–18] and glyco- [14] lipids which melt directly from an L_c phase to either a lamellar or non-lamellar liquid-crystalline phase However, the correspondence between the temperatures of the lower temperature endothermic event observed upon the rapid heating of the unannealed sample and the lower temperature exothermic event upon cooling from high temperature does suggest that this thermal event is a lamellar chain-melting transition in both cases, the lower enthalpy observed upon heating being due to the fact that a portion of the lower-melting metastable gel state has already converted to the higher-melting stable gel state before or during the calorimetric heating scan Again, similar thermotropic phase behavior has been observed calorimetrically upon the cooling of α- and β-MGDG dispersions as well (Ref 14 and unpublished observations) In the case of these glyceroglycolipids, the higher temperature exotherm observed upon cooling is due to the conversion of a cubic or reversed hexagonal phase to a lamellar liquid-crystalline phase and the lower temperature endotherm to the conversion of the lamellar liquid-crystalline to the metastable lamellar gel phase As will be shown below, essentially similar phase behavior is exhibited by the 2-*O*-acyl,polyprenyl α-D-glucopyranoside dispersion studied here

In summary, these DSC results indicate that aqueous dispersions of this 2-*O*-acyl,polyprenyl α-D-gluco-

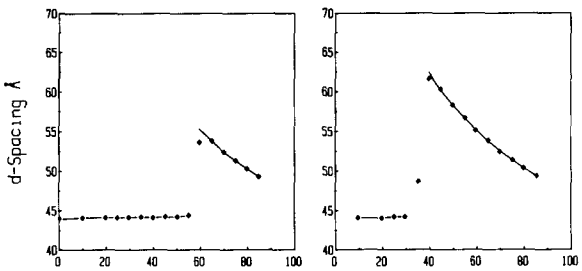

Fig 2 Effect of temperature on the *d*-spacing of an aqueous dispersion of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside isolated from *A laidlawii* B membranes Data are shown for measurements taken in the heating mode (left panel) and in the cooling mode (right panel)

pyranoside can exist in any of at least four different phase states (two gel and two liquid-crystalline states), depending upon the temperature and thermal history of the sample The structures of these four phase states were determined by the X-ray diffraction and FTIR spectroscopic studies described below

X-ray diffraction

In these studies, low-angle X-ray diffraction patterns were recorded as a function of temperature in both the heating and cooling modes The phase transitions exhibited by this lipid were readily detected by the temperature-dependent changes in the *d*-spacings (Fig 2) and coincided with the thermotropic events exemplified by the calorimetric traces B and C in Fig 1 In the heating mode it is clear that no thermotropic phase changes occur at temperatures between 0 and 55 °C Upon heating to temperatures above 55 °C, there is first a discontinuous increase in the *d*-spacings, which then gradually decrease with further increases in temperature In the heating experiment, the small-angle diffraction patterns exhibited at temperatures below 55 °C are illustrated in Fig 3D and are characteristic of a highly ordered lamellar gel phase This diffraction pattern changes abruptly at the calorimetrically determined phase transition to one exemplified by Fig 3A, which is characteristic of the liquid-crystalline inverted hexagonal phase Thus, these X-ray data establish that the major endothermic transition exhibited by fully equilibrated samples of this glucolipid is a conversion from its stable lamellar gel phase to an inverted hexagonal phase

The thermotropic phase behavior observed by X-ray diffraction upon cooling this unusual glycolipid is somewhat more complex When cooled from high temperature, the diffraction pattern of the inverted hexagonal phase persists to temperatures near 40 °C (see Fig 2), at which temperature a more complex diffraction pattern emerges (Fig 3B) The complex pattern which is observed at temperatures just above the onset of the first

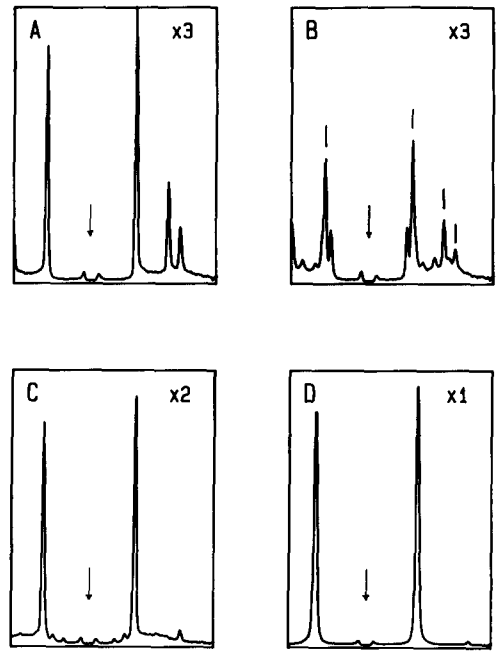

Fig 3 Radially integrated X-ray powder diffraction patterns of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside isolated from *A laidlawii* B membranes The diffraction patterns were acquired in the cooling mode at the following temperatures (A) 45 °C, (B) 40 °C, (C) 35 °C, (D) 20 °C

of the two cooling exotherms observed by DSC (see Fig 1C), suggests that a mixture of cubic and inverted hexagonal phases may be present This observation seems consistent with current ideas which suggest that cubic phases may be intermediates between lamellar and reversed hexagonal phases (Refs 19–21 and references cited therein) Upon further cooling to temperatures near 35 °C, there is an abrupt change in diffraction pattern to that indicative of a liquid-crystalline lamellar phase (Fig 3C), and this clearly identifies the weakly energetic exothermic event observed upon cooling (see Fig 1C) as a non-bilayer/bilayer transition The lamellar phase formed upon cooling of these lipids to temperatures near 35 °C does not exhibit the strong wide-angle reflections which would be indicative of long range order (data not shown) Thus, these observations are consistent with the formation of a lamellar phase with melted hydrocarbon chains (i e , a liquid-crystalline phase) when the sample is cooled to temperatures near 35 °C At lower temperatures, the diffraction patterns indicate that the lipid remains in a lamellar phase (Fig 3D) However, at the completion of the main cooling exotherm observed calorimetrically, there is a sharp decrease in the d-spacing (Fig 2) and the appearance of wide angle reflections indicative of long range order (data not shown) These observations are consistent with the formation of a solid phase with ordered polymethylene chains and identify the broad cooling exotherm centered at 33 °C as a lamellar liquid-crystalline/gel phase transition

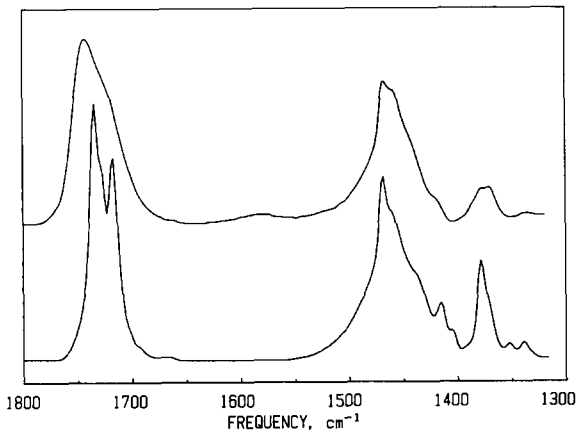

Fig 4 FTIR spectra of the high-temperature (upper trace) and low-temperature (lower trace) phases of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside isolated from *A laidlawii* B membranes The data shown are absorbance spectra between 1800 and 1300 cm^{-1}

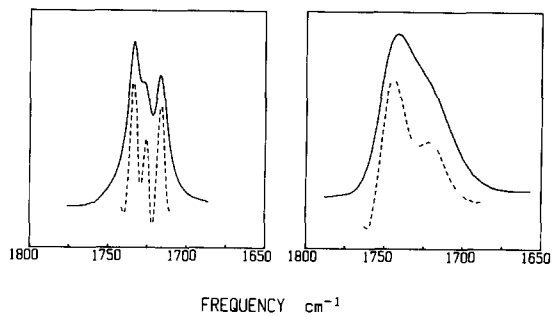

Fig 5 Infrared spectra of the carbonyl stretching region of the low-temperature (left panel) and high-temperature (right panel) phases of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside isolated from *A laidlawii* B membranes The normal spectra are shown by the solid lines while the dashed lines show the same spectra after resolution enhancement by Fourier self-deconvolution

Fourier-transform infrared spectroscopy

In these infrared spectroscopic studies high quality data were obtained only for the reversed hexagonal phase observed at high temperature and the stable low-temperature gel state obtained upon equilibration of this lipid at low temperatures We found that the metastable gel and liquid-crystalline phases detected in the DSC cooling experiments are too unstable to be characterized in a pure state under our experimental conditions When cooled from high temperatures to temperatures in the range of the cooling exotherms observed calorimetrically, complex spectra that were obviously of mixtures of many phases were obtained (data not shown) This is attributable to the fact that, unlike the samples used for the DSC measurements, FTIR samples could not be cooled quickly enough to kinetically trap pure forms of any of the metastable phases identified in the DSC and X-ray experiments

The spectra of the reversed hexagonal phase and the stable lamellar gel phase of this lipid showed significant differences in the region encompassing the methylene stretching vibrational modes (2800–3000 cm^{-1}, not shown here) as well as in the region of the spectrum encompassing the C=O stretching and the methylene deformation modes (1800–1300 cm^{-1}, Fig 4) The stable gel exhibits a very sharp methylene symmetric stretching band at 2849 cm^{-1}, and upon heating to temperatures above 65 °C, the band broadens and shifts to a higher frequency (2852 cm^{-1}) This type of change typifies the increase in conformational disorder which occurs when all-*trans* polymethylene chains melt [15,16], and indicates that the thermotropic events occurring near 65 °C include a chain-melting transition Fig 4 also shows that the conversion of the stable gel phase to the reversed hexagonal phase also involves considerable broadening of the C=O ester stretching bands (1700–1750 cm^{-1}), the CH_2 scissoring band (1468 cm^{-1}), the α-CH_2 bending band (1418 cm^{-1}) and the CH_3 umbrella bands (1378 cm^{-1}) These changes are all indicative of increases in the mobility of both the hydrophobic and interfacial domains of this lipid and are consistent with the conversion from a highly ordered solid state to a melted state Of added interest here is the fact that, at the transition temperature, there are no significant changes in the frequency of the CH_2 scissoring band, even though this phase transition evidently involves the melting of a highly ordered crystalline structure With most diacyl glycerolipids, one can usually obtain information about the type of chain packing and about the type of lateral interactions between the hydrocarbon chains from changes in the frequency of this band [22,23] With this particular lipid, such frequency shifts were not observed because the highly branched chain of the aglycone moiety (see Refs 3,4) probably cannot interact with the unbranched fatty acyl chain in ways which would give rise to either factor group splitting of the scissoring band or to increase in its frequency The latter changes are typical of orthorhombic and triclinic packing, respectively [24,25], and have been observed in gel state bilayers formed from phospho- and glycolipids containing two linear saturated polymethylene chains

A more detailed look at the C=O stretching bands between 1700 and 1800 cm^{-1} (Fig 5) reveals other interesting aspects of this unusual glucolipid In the high-temperature phase, a broad asymmetric carbonyl stretching band is observed Upon resolution enhancement, two components with maxima at 1743 and 1720 cm^{-1} are clearly resolved (Fig 5) Unlike the more common diacyl glycerolipids, however, these two components cannot be ascribed to the presence of two distinct ester carbonyl groups, since this particular glucolipid only contains one fatty acyl group per molecule [3,4] Here, it should also be noted that the frequencies

of the two components are lower than 1748 cm^{-1}, the C=O stretching frequency of neat anhydrous triacetin [26] Since hydrogen bonding to the carbonyl oxygen is known to shift the C=O stretching band to lower frequencies [27], it seems reasonable to suggest that the two components observed may arise either from separate populations of free and hydrogen-bonded ester carbonyls, or even from populations of hydrogen ester carbonyls differing in the type of hydrogen bond formed Fig 5 also shows that the formation of the stable gel phase of this lipid coincides with the appearance of three narrow bands centered at 1738, 1726 and 1714 cm^{-1} The formation of these very sharp C=O stretching bands, coupled with the general shift to lower frequencies, indicates that the formation of the low-temperature phase involves considerably stronger hydrogen bonding of the ester carbonyl along with an effective immobilization of that carbonyl ester group These changes are consistent with the formation of a highly ordered crystalline structure Moreover, the fact that there are three hydrogen-bonded carbonyl ester bands in the infrared spectrum also suggests that the lipid has crystallized into a complex structure in which there are at least two vibrationally inequivalent lipid molecules per unit cell The multiplicity of C=O stretching bands observed in the stable crystalline phase is probably a reflection of the inequivalence in the hydrogen bonding interactions between the ester carbonyl group and proton donor arising from the solvent phase and/or hydroxyl groups on the sugar headgroup

Discussion

The results presented here demonstrate that the thermotropic phase behavior exhibited by aqueous dispersion of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside isolated from palmitate-enriched *A laidlawii* B membranes depends critically on the thermal history of the sample If dispersions are annealed at low temperature, a highly ordered lamellar gel phase is formed which undergoes chain-melting and concomitant conversion to the liquid-crystalline reversed hexagonal phase only at relatively high temperatures In contrast, if dispersions of this glycolipid are not exposed to low temperature, a less ordered metastable gel is formed which converts first to a liquid-crystalline lamellar and then to a reversed hexagonal phase at much lower temperatures The question then arises as to whether the thermotropic phase behavior of the stable or metastable gel state is more relevant to the physical properties of this glycolipid in palmitate-enriched *A laidlawii* membranes We believe that the thermotropic phase properties of the metastable gel phase are most relevant in this regard for several reasons One reason is that the *A laidlawii* culture from which this lipid was derived was grown at a constant temperature of 37°C (the optimal growth temperature for palmitic acid-supplemented cultures) Therefore, the low-temperature nucleation required to initiate the formation of the stable gel state cannot occur Another reason is the presence of the other polar lipids normally present in the *A laidlawii* membrane, which would be expected to inhibit the formation of highly crystalline gel states by introducing compositional heterogeneity into the membrane lipid bilayer For these reasons we consider only the thermotropic phase behavior of dispersions which have not been exposed to low-temperature annealing in our discussion of the physiological relevance of the presence of this lipid in the *A laidlawii* membrane

In this study and in previous work in which this lipid was first isolated and identified [3], we found that when *A laidlawii* B is cultured in palmitate-supplemented media at 37°C, it synthesizes large quantities of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside mainly at the expense of the MGDG normally found in the membrane Our physical studies with pure synthetic α-D-glucosyl diacylglycerols (unpublished experiments from this laboratory) indicate that the gel/liquid-crystalline phase transition of the MGDG which would be synthesized under these conditions (mainly dipalmitoyl-α-D-glucosyldiacylglycerol) occurs near 57°C Clearly, the accumulation of such a high-melting MGDG component in the membrane could be deleterious to the microorganism, since it would result in the formation of appreciable amounts of gel-state lipid at the optimal growth temperature 37°C, which would in turn inhibit cell growth [28,29] However, in this study we demonstrate that the metastable gel phase of the 2-*O*-acyl,polyprenyl α-D-glucopyranoside melts at temperatures near 33°C Thus, the biosynthesis of this lipid in preference to the higher-melting MGDG would enable this organism to maintain a predominantly liquid-crystalline membrane at its growth temperature, while at the same time maintaining, or even increasing, the levels of nonbilayer-forming lipids which may be essential for its normal growth under these conditions [5,6] We, therefore, suggest that the partial replacement of the MGDG with the 2-*O*-acyl,polyprenyl α-D-glucoside may be an adaptive biochemical response to the biosynthetic incorporation of higher melting fatty acids from the growth medium As was observed in the initial studies of this glucolipid, this facility is not limited to *A laidlawii* B cultured in palmitate-enriched media, since the appropriate 2-*O*-acyl,polyprenyl α-D-glucopyranoside is also synthesized when the organism is cultured in media supplemented with high-melting isoacyl and ω-cyclohexyl fatty acids, where a predominantly liquid-crystalline membrane can also be maintained at 37°C (unpublished experiments from this laboratory)

In the initial studies, in which this lipid was first isolated and characterized [3], we found that high levels

of this lipid were only synthesized when the organism was cultured under conditions of glucose-limitation stress We now find that when cultured in palmitate-enriched media, the *A laidlawii* B membrane contains up to 60 mol% of this glucolipid even when the organism is cultured with normal levels of glucose This obvious difference between these and our earlier observations may either be the result of some metabolic changes in the organism itself or of undefined changes in the culturing conditions Of the two possibilities, we suspect that the latter is the more likely, since we find no difference in the behaviour of 'old' seed cultures and the ones we currently use

The unambiguous demonstration that this glucolipid is indeed a potent nonbilayer-forming lipid is relevant to the question of whether or not the relative amounts of bilayer- and nonbilayer-forming lipids in natural cell membranes are regulated so as to produce a constant 'nonbilayer-forming tendency' It has been postulated that all biological membranes must maintain a certain balance of bilayer-preferring and nonbilayer-preferring lipids in order to ensure the optimization of bilayer stability and membrane functionality [5–8] Our observation that when cultured in media enriched in high-melting fatty acids, *A laidlawii* B synthesizes this glucolipid mainly at the expense of MGDG is generally compatible with such ideas when viewed from two perspectives Firstly, the 2-*O*-acyl,polyprenyl α-D-glucopyranoside is a more potent nonbilayer former than the MGDG it replaces, since its bilayer/nonbilayer transition is a bilayer/H_{II} transition occurring at 39°C, whereas the bilayer/nonbilayer transition of the dipalmitoyl MGDG is a bilayer/cubic transition occurring at 79°C (unpublished experiments from this laboratory) Thus, its relative increase upon supplementation with high-melting fatty acids, which tend to increase the bilayer/nonbilayer transition temperature, is correctly predicted Secondly, the biosynthetic function of this glucolipid occurs mainly at the expense of the MGDG component, the only other nonbilayer-forming lipid normally found in *A laidlawii* B membrane This also seems compatible with the postulated regulation of lipid phase tendency, since in this case one nonbilayer-preferring lipid is partially replaced by another, albeit a more potent one Nevertheless, the question of whether or not the actual bilayer/nonbilayer transition temperature of the total membrane lipid mixture is maintained constant under these circumstances remains to be determined However, the fact that palmitate-enriched *A laidlawii* B can grow and function normally even when its membrane contains as much as 70 mol% of two nonbilayer-forming lipids (this lipid and MGDG), whereas membranes from oleate-enriched cells contain only about 30 mol% MGDG and no monoacylated glycolipid, does indicate that this organism has considerable flexibility as regards the structures, absolute amounts and relative potencies of the nonbilayer-forming lipids that it can safely accommodate in its membrane

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