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LIPID AND PROTEIN COMPOSITION AND THERMOTROPIC LIPID PHASE TRANSITIONS IN FATTY ACID-HOMOGENEOUS MEMBRANES OF *ACHOLEPLASMA LAIDLAWII* B

JOHN R. SILVIUS, NANETTE MAK and RONALD N. McELHANEY

Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7 (Canada)

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Summary

The membrane composition and lipid physical properties have been systematically investigated as a function of fatty acid composition for a series of *Acholeplasma laidlawii* B membrane preparations made homogeneous in various fatty acids by growing cells on single fatty acids and avidin, a potent fatty acid synthetic inhibitor. The membrane protein molecular weight distribution is essentially constant as a function of fatty acid composition, but the lipid/protein ratio varies over a 2-fold range when different fatty acid growth supplements are used. The membrane lipid head-group composition varies somewhat under these conditions, particularly in the ratio of the two major neutral glycolipids. Differential thermal analytical investigations of the thermotropic phase transitions of various combinations of membrane components suggest that these compositional changes are unlikely to result in qualitative changes in the nature of lipid-protein or lipid-lipid interactions, although lesser changes of a quantitative nature probably do occur. The total lipids of membranes made homogeneous in their lipid fatty acyl chain composition exhibit sharper than normal gel-to-liquid-crystalline phase transitions of which midpoint temperatures correlate very well with the phase transition temperatures of synthetic hydrated phosphatidylcholines with like acyl chains. Our results indicate that using avidin and suitable fatty acids to grow *A. laidlawii* B, it is possible to manipulate the position and the sharpness of the membrane lipid phase transition widely and independently without causing major modifications in other aspects of the membrane composition. This fact makes the fatty acid-homogeneous *A. laidlawii* B membrane a very useful biological

Abbreviation: SDS, sodium dodecyl sulfate.

membrane preparation in which to study lipid physical properties and their functional consequences.

Introduction

We have recently reported [1] that when cultures of *Acholeplasma laidlawii* B are grown in the presence of the biotin-binding protein avidin and a suitable exogenous fatty acid, the cells become incapable of fatty acid synthesis yet continue to grow normally, incorporating the exogenous acyl species into their membrane lipids to levels approaching homogeneity (i.e., to 95 + % of the total lipid acyl chains). We have found that any of a variety of exogenous fatty acids of various hydrocarbon chain structures and chain lengths, which we have termed 'Class I' fatty acids, can support good growth of *A. laidlawii* B when incorporated to such high levels in the cell membrane lipids. The fatty acid-homogeneous membranes obtainable from *A. laidlawii* B grown with avidin and a Class I fatty acid have great potential experimental utility as a biological membrane preparation of relatively simple and readily manipulable lipid composition. To assess the usefulness of avidin/fatty acid treatment of *A. laidlawii* B for producing controlled and selective modifications of the membrane lipid acyl groups, and thereby of the lipid physical properties, we have addressed two questions in the present study:

(1) Does *A. laidlawii* B grown with avidin and various Class I fatty acids show changes only in its lipid fatty acyl groups, or are other aspects of the membrane composition changed as well?

(2) How greatly can changes in the nature of the lipid fatty acyl groups in fatty acid-homogeneous membranes alter the physical properties of the membrane lipids, and how greatly can other types of compositional variations in different fatty acid-homogeneous membranes affect the overall physical properties of the membrane lipids?

To answer these questions, we have combined direct determinations of the lipid and protein composition of fatty acid-homogeneous *A. laidlawii* B membranes with differential thermal analytical investigations of the thermotropic phase transitions of such membranes and of isolated membrane components.

Materials and Methods

Cell culture and membrane isolation

The growth medium and conditions used for culturing *A. laidlawii* B with avidin and fatty acids have been described elsewhere [1]. Membranes were prepared from late log phase cultures of this organism by osmotic lysis essentially as previously described [2].

Membrane compositional measurements

The protein content of isolated membranes was determined by a modification of the method of Hartree [3] as we have previously described [2]. To separate membrane proteins on the basis of molecular weight, membranes were dissolved to 2 mg protein/ml in a buffer containing 50 mM NaH_2PO_4/Na_2HPO_4,

1% SDS, 1% β-mercaptoethanol and 6 M urea (pH 7.0), then heated to 45°C for 1 h, and 50 μg samples of protein were applied to the long edge of a 6 × 20 × 0.3 cm slab gel of 8% polyacrylamide prepared in a buffer containing 200 mM NaH_2PO_4/Na_2HPO_4, 0.2% SDS and 6 M urea, pH 7.0. Gels were electrophoresed for 6—8 h at a current of 150 mA/gel, using bromophenol blue as a tracking dye. The gels were stained with Coomassie blue and destained as described by Weber and Osborn [4], then scanned at 596 nm on a Gilford model 250 spectrophotometer equipped with a gel-scanning accessory.

Total membrane lipids (which constitute essentially all of the cellular lipids in *A. laidlawii* B [5]) were extracted and purified as described elsewhere [6]. For analysis of the lipid head-group distribution, the total lipids were applied to 100 × 50 × 0.5 mm plates of Silica gel G, which were then developed with chloroform/methanol/water (70 : 25 : 4, v/v/v). After drying, the developed plates were charred by heating after spraying with 3% CrO_3 in 60% H_2SO_4, or, if lipid recovery was desired, the plates were briefly exposed to I_2 vapor to very lightly stain the lipid spots. In the latter case, the lipid spots of interest were scraped into small chromatographic columns, and the lipids were eluted from the gel with 10 ml of 10% chloroform in methanol under slight positive nitrogen pressure, then recovered by removal of the solvent in vacuo.

To estimate the amount of glycerolipids present in a purified lipid sample, the lipid and a known weight of a standard fatty acid not present in the sample (which served to calibrate the mass response of the gas chromatograph flame-ionization detector) were reacted with 5% H_2SO_4 in methanol at 60°C for 2 h, and the methyl esters were isolated and analyzed by gas-liquid chromatography as described elsewhere [6]. The μmol of glycerolipid present in the original sample could then be calculated as one-half of the μmol of lipid-derived methyl esters determined from the gas-chromatographic results.

Sample preparation for differential thermal analysis

Particular lipid fractions were prepared for differential thermal analysis as follows. Total membrane lipids were isolated from the crude lipid extract by applying the lipid extract to a column of 5—10 g of Bio-Sil packed in chloroform, then eluting with 50—100 ml of methanol. Total polar lipids were obtained by eluting the column first with 100 ml of chloroform, which was discarded, followed by 100 ml of methanol, which was retained. Total neutral glycolipids and total phosphate-containing lipids were obtained by successively eluting the column with 20 vols. each of chloroform, acetone and methanol according to the procedure of Vorbeck and Marinetti [7]. Individual lipid species were obtained from the appropriate fractions from this last procedure by thin-layer chromatography on 200 × 50 × 0.8 mm plates of Silica gel H, using chloroform/methanol/water, (75 : 20 : 4, v/v/v) for glycolipids and (65 : 25 : 5, v/v/v) for phosphate-containing lipids, as the developing solvent. The separated lipids were recovered from the plates as described above.

Purified lipid fractions were equilibrated with various ionic solutions by a Folch washing procedure [8]. In most cases, a buffer of roughly physiological ionic composition (0.15 M NaCl, 50 mM Tris, 5 mM $MgSO_4$, pH 8.0) was used for the equilibration. The lipid was then dried down in vacuo and redissolved in a small amount of benzene, which was rapidly frozen and lyophilized to give

the lipids in a powdery or waxy form. The dried lipid was packed into capillary tubes and hydrated with 50% (w/w) water or 70% (w/w) of 30% ethylene glycol in water by the procedure of Ladbrooke et al. [9]. The hydrated lipid dispersions or whole packed membranes were subjected to differential thermal analysis on a DuPont model 900 Thermal Analyzer, using heating and cooling rates of 5 degrees/min for most samples. Glass beads were used as the inert thermal reference.

Results

Membrane protein composition

To test the effects of exogenous fatty acids on the membrane protein composition of *A. laidlawii* B grown with avidin, cultures were grown with elaidic acid with or without avidin, and with isopalmitic or anteisopentadecanoic acid plus avidin. The cell membranes were isolated, and the molecular weight distribution of the membrane proteins was determined by urea/SDS polyacrylamide gel electrophoresis as described in Materials and Methods. A representative set of gel scans is shown in Fig. 1, illustrating the basic similarity of the protein molecular weight distribution in the membranes of cells grown under the varying conditions described above. The protein molecular weight distribution in membranes of cells grown without avidin or fatty acid supplementation (scan not shown) was not clearly different from those of the membrane preparations whose gel scans are shown in Fig. 1. While some variations in the staining intensities of individual bands may be seen in Fig. 1, variations of a comparable magnitude were also observed in membrane preparations from different cultures grown under identical conditions of supplementation. In general, the overall molecular weight distribution of the membrane proteins, and the positions of the major bands, do not appear to be significantly influenced by the presence of avidin or by the nature of the exogenous fatty acid added to the cell culture medium. In contrast, we found that the total amount of membrane protein relative to lipid is strongly affected by the nature of the exogenous fatty acid when cells are grown in the presence of avidin. In Table I, the membrane lipid/protein ratio (as μmol lipid per mg protein) for membranes prepared from various fatty acid-homogeneous cell cultures and from unsupplemented (avidin-untreated) cell cultures are given. The effect of an exogenous Class I fatty acid on the membrane lipid/protein ratio depends strongly on its structure; thus *trans*-unsaturated or -cyclopropane fatty acids give a near-normal lipid/protein ratio while *cis*-unsaturated species increase it by as much as 2-fold and branched-chain fatty acids have an intermediate effect. The range of membrane lipid/protein ratios observed in avidin-treated cells is comparable to that previously observed in cells grown with various fatty acids but without avidin [2]. Therefore, avidin itself seems to have little if any effect on the rates of synthesis of membrane proteins or glycerolipids in this organism, but the nature of the exogenous fatty acid supplement supplied along with avidin can significantly affect the relative rates of synthesis of these two classes of membrane components.

Membrane lipid head-group composition

The polar lipids of *A. laidlawii* B consist mainly of five diacyl glycerolipid

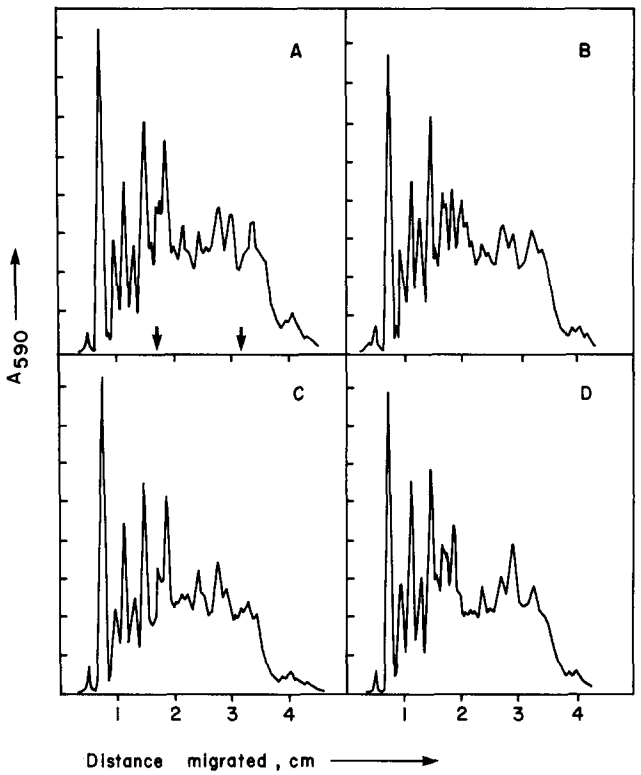

Fig. 1. Coomassie blue staining profiles after urea/SDS polyacrylamide gel electrophoresis for membrane proteins of *A. laidlawii* B grown under various conditions of fatty acid and avidin supplementation: A, elaidic acid (0.12 mM); B, elaidic acid plus avidin (2 mg/ml); C, isopalmitic acid (0.12 mM) plus avidin; D, anteisopentadecanoic acid (0.12 mM) plus avidin. Details of the electrophoretic and staining procedures are described in the text. All scans are plotted to the same vertical scale (absolute values are, however, unimportant and are not indicated). The arrows along the distance axis in scan A represent the distance migrated by bovine serum albumin (mol. wt. 63 000) and DNAase I (mol. wt. 31 000) in these gels.

species, of which two neutral glycolipids, mono- and diglucosyl diacylglycerol and one phospholipid, phosphatidylglycerol, constitute the major membrane lipid components [10,11]. Since changes in the relative proportions of these lipids accompanying variations in their fatty acyl composition could affect the physical properties of the membrane lipids in fatty acid-homogeneous cells, we investigated the extent of such changes in cells grown with avidin plus a variety of Class I fatty acids. The total lipids of these fatty acid-homogeneous cells were extracted, isolated and separated by thin-layer chromatography as described above. A set of developed and charred chromatoplates is shown in Fig. 2, from which two major effects of the exogenous fatty acid on the lipid head-group distribution can be identified. First, branched-chain fatty acids, particularly those of shorter chain length, cause a decrease in the intensity of the diglucosyl diacylglycerol spot relative to that of the monoglucosyl diacylglycerol spot. Secondly, a few of the shorter-chain Class I fatty acids (isomyristic, anteisopentadecanoic and palmitelaidic) significantly increase the amount of one of the minor lipids, glycerolphosphoryl diglucosyl diacyl-

TABLE I

MEMBRANE LIPID/PROTEIN RATIOS FOR CELLS GROWN WITH AVIDIN (2 mg/ml) AND VARIOUS CLASS I FATTY ACIDS (0.12 mM)

Lipid/protein ratios given were determined as described in the text, and are the averages of values determined in at least two separate experiments.

Fatty acid supplement *	Lipid/protein (μmol/mg)
14:0*i*	0.83
15:0*i*	0.79
16:0*i*	0.77
17:0*i*	0.88
(±)15:0*ai*	0.84
(±)17:0*ai*	0.70
16:1$t\Delta^9$	0.57
18:1$t\Delta^9$	0.68
18:1$t\Delta^{11}$	0.63
18:1$c\Delta^4$	0.98
18:1$c\Delta^{11}$	1.01
19:*cp*,$t\Delta^9$	0.57
None (— avidin)	0.56
16:0 (— avidin)	0.75

* Fatty acids are represented by the following symbols: The carbon number is followed, after a colon, by the number of double bonds or, if a cyclopropane ring is present, by the letters '*cp*'. This term is followed by the letters '*i*' or '*ai*' if the fatty acid is iso- or anteisobranched, or by the letters '*c*' or '*t*' if a double bond or cyclopropane ring of *cis*- or *trans*-configuration is present. Finally, if necessary, the symbol Δ plus a superscript number may be added to indicate the position of a double bond or cyclopropane group.

glycerol. To more quantitatively examine the effect of the exogenous fatty acid on the proportions of the three most abundant lipid species in fatty acid-homogeneous membranes, the developed chromatoplates for certain lipid samples prepared as described above were lightly stained with I_2 vapor, and the major lipids were recovered and quantified as described in Materials and Methods. The results of a number of such experiments are summarized in Table II. It can be seen from the data shown in this table that the molar ratio of monoglucosyl diacylglycerol to diglucosyl diacylglycerol varies over a very wide range depending on the nature of the exogenous fatty acid, while the ratio of total neutral glycolipids to phosphatidylglycerol shows a much smaller variation. This result suggests that the relative rates of synthesis of phosphatidylglycerol and glycolipid precursors are not greatly sensitive to the lipid fatty acid composition, while the monoglucosyl diacylglycerol to diglucosyl diacylglycerol conversion [10] is quite sensitive to the nature of the lipid fatty acyl chains. In general, growth of *A. laidlawii* B in the presence of avidin plus various Class I fatty acids will result in very significant variations in the relative levels of the two major neutral glycolipids but will lead to much more modest variations in the ratio of total neutral glycolipids to phosphate-containing lipids (of which phosphatidylglycerol is the major component) or, except in the case of a few short-chain fatty acids, in the relative proportions of the various phosphate-containing lipids.

Membrane compositional effects on lipid thermotropic behaviour

Since different fatty acid-homogeneous membranes can clearly vary in

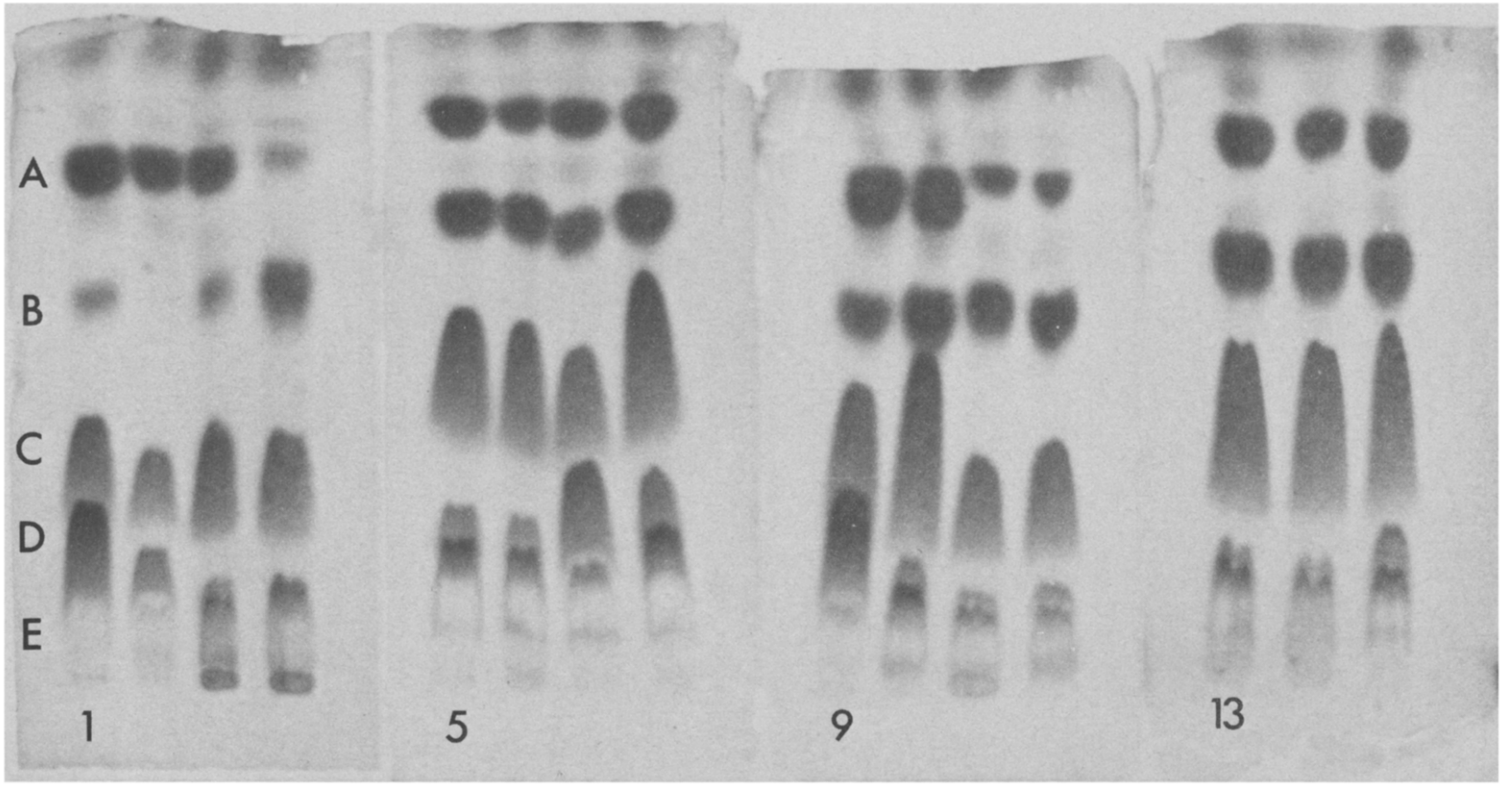

Fig. 2. Sample chromatoplates showing the distribution of glycerolipids of various head-groups in the membranes of *A. laidlawii* B grown with avidin plus various Class I fatty acids. The major polar lipids are designated as follows: A, monodiglucosyl diacylglycerol; B, diglucosyl diacylglycerol; C, phosphatidylglycerol; D, glycerophosphoryl diglucosyl diacylglycerol; and E, 3′-*O*-aminoacyl (mainly *O*-alanyl) phosphatidylglycerol. Experimental details are described in the text. The samples are numbered according to the fatty acid used to grow the cells, as follows (in the shorthand nomenclature described in the footnote to Table I): 1—4, 14 : 0*i*, 15 : 0*i*, 16 : 0*i*, 17 : 0*i*; 5—8, 18 : 1*t*Δ^9, 18 : 1*t*Δ^{11}, 16 : 1*t*Δ^9, 18 : 1*t*Δ^6; 9—12, (±) 15 : 0*ai*, (±) 17 : 0*ai*, (±) 19 : *cp*,*t*Δ^9, (±) 19 : *cp*,*t*Δ^{11}; 13—15, 18 : 1*c*Δ^4, 18 : 1*c*Δ^{11}, 18 : 1*c*Δ^{15}.

TABLE II

MOLAR RATIOS OF THE MAJOR POLAR LIPID SPECIES IN *A. LAIDLAWII* B MEMBRANES FROM CELLS GROWN WITH AVIDIN (2 mg/ml) AND VARIOUS CLASS I FATTY ACIDS (0.12 mM)

Fatty acid supplement *	Monoglycosyl diacylglycerol / Diglucosyl diacylglycerol	Monoglycosyl diacylglycerol + diglucosyl diacylglycerol / Phosphatidylglycerol
14:0*i*	18.1	2.7
16:0*i*	5.6	3.7
(±)15:0*ai*	4.8	3.9
(±)15:0*ai*	2.4	2.4
18:1*t*Δ^9	0.82	2.9
18:1*c*Δ^4	0.54	2.3
18:1*c*Δ^{11}	0.53	3.1
19:*cp*,*t*Δ^9	0.15	2.1
None (— avidin)	2.3	3.6

* See footnote to Table I.

aspects of their composition other than their lipid acyl chains, we sought to evaluate the effects of various membrane components, and of changes in their membrane levels, on the thermotropic behaviour of the membrane lipids. Differential thermal analysis was used to monitor the thermotropic phase transitions of various membrane components. Our first series of experiments focused on the effects of membrane proteins, carotenoids and divalent cations on the thermotropic behaviour of the total membrane polar lipids. To evaluate these effects, we analyzed and compared the phase transitions of the following preparations, obtained as described in Materials and Methods: whole membranes, hydrated total lipids, and hydrated total polar lipids equilibrated with one of three buffers (50 mM EDTA, 50 mM $MgCl_2$ or 150 mM NaCl/5 mM $MgCl_2$, all including 50 mM Tris, pH 8.0). A set of differential thermograms for these preparations derived from isopalmitate-homogeneous cells is shown in Fig. 3. The membrane and total lipid endotherms are quite comparable in shape, position and width, indicating that the membrane proteins do not greatly alter the phase transition of the bulk lipid phase. This result was also obtained with elaidate-homogeneous membranes and agrees with previous findings [12,13] with membranes of heterogeneous fatty acid composition. The smaller size of the membrane endotherm is largely attributable to the fact that the membrane pellet is a relatively dilute lipid sample. The transition endotherm for the total membrane lipid sample, which includes the membrane carotenoids and was isolated under nitrogen and in semidarkness to minimize their degradation, shows a slightly greater skewing toward lower temperatures than does the total polar (carotenoid-free) lipid sample, but the transition peak and upper boundary are only very slightly lowered in the presence of carotenoids. With elaidic acid-homogeneous lipids, no effect of carotenoids on the lipid phase transition could be detected. The generally low levels of carotenoids present in the *A. laidlawii* B membrane thus only slightly modify the lipid phase transition, and variations in carotenoid levels in fatty acid-homogeneous membranes (which, judging from the rather consistent color intensity we observed for various membrane samples dissolved in SDS solutions, are not

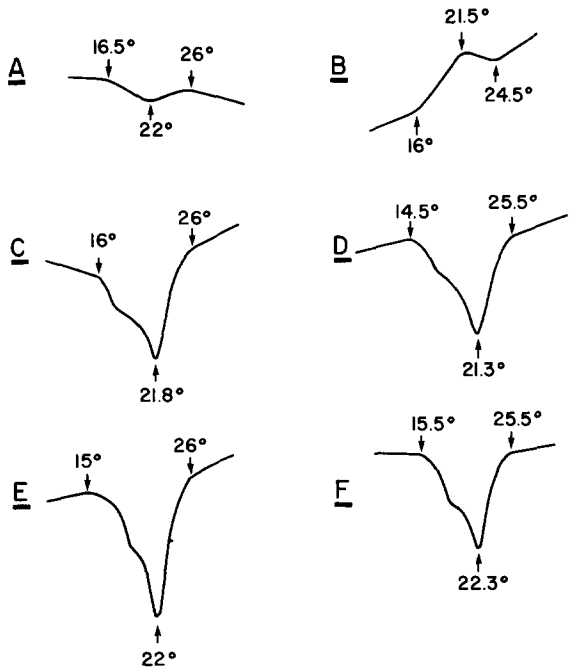

Fig. 3. Representative differential thermograms (differential vs. absolute temperature) for hydrated dispersions of *A. laidlawii* B lipids made homogeneous in isopalmitic acid, or of whole membranes of like lipid acyl chain composition. The various membrane and lipid fractions were obtained and analyzed as described in the text, and are as follows: A, membranes (heating); B, membranes (cooling); C, total lipids; D, total polar lipids; E, total polar lipids equilibrated with 50 mM $MgCl_2$; F, total polar lipids equilibrated with 50 mM EDTA. The vertical axis (differential temperature) is unscaled in this and subsequent figures showing differential thermograms, as the size of the vertical deflection cannot be accurately calibrated in terms of any basic physical quantity from one sample to the next.

great) are unlikely to significantly influence the membrane lipid thermotropic behaviour. Finally, in Fig. 3 we show the transition endotherms for total isopalmitate-homogeneous membrane polar lipids equilibrated with EDTA, high-Mg^{2+} or high-Na^+/low-Mg^{2+} (physiological) buffers. Within the limits of normal experimental variation, these endotherms are identical, a result which is somewhat surprising in view of the fact that the membrane polar lipids include substantial amounts of anionic lipids (phosphatidylglycerol and glycerophosphoryl diglucosyl diacylglycerol), whose thermotropic properties are known to be sensitive to divalent cation levels [14,15]. This result was also obtained using elaidate-homogeneous lipids, and using 1 M solutions of EDTA, NaCl or $MgCl_2$ to rehydrate the lipids after lyophilization from benzene. It seems most likely that the neutral glycolipids in the *A. laidlawii* B membrane dilute out the anionic lipids sufficiently to prevent the formation of higher-melting phases of the latter in the presence of Mg^{2+}.

To evaluate the possible effects of lipid head-group variations on the lipid thermotropic behaviour, we isolated various lipid fractions from isopalmitate- or elaidate-homogeneous membranes, equilibrated them with a buffer of physiological ionic composition (150 mM NaCl, 50 mM Tris, 5 mM $MgCl_2$, pH 8.0), and monitored the phase transitions of the hydrated lipids by differen-

tial thermal analysis. The results from a series of experiments using isopalmitate-homogeneous lipids are illustrated in Fig. 4. Of the three major membrane lipids, phosphatidylglycerol shows a reversible thermotropic transition most nearly resembling that of the total membrane lipids. The total phosphate-containing lipids exhibit a phase transition very similar to that of pure phosphatidylglycerol, which is reasonable in view of the predominance of phosphatidylglycerol in this lipid fraction. When heated from 0 to 70°C, hydrated isopalmitate-homogeneous diglucosyl diacylglycerol shows a series of transition endotherms, all occurring at temperatures above the midpoint of the total lipid phase transition, while on subsequent cooling, the sample shows a single exothermic transition at a temperature (15.5°C) considerably below those of the endothermic transitions seen on heating. Wieslander et al. [16] have also noted this rather complex behaviour for diglucosyl diacylglycerol from *A. laidlawii* A highly enriched in elaidic acid, using X-ray diffraction and ^2H magnetic resonance. Our differential thermal analysis results with elaidate-homogeneous diglucosyl diacylglycerol were quite consistent with our results for isopalmitate-homogeneous diglucosyl diacylglycerol and with the results of Wieslander et al. (data not shown). In contrast to diglucosyl diacylglycerol,

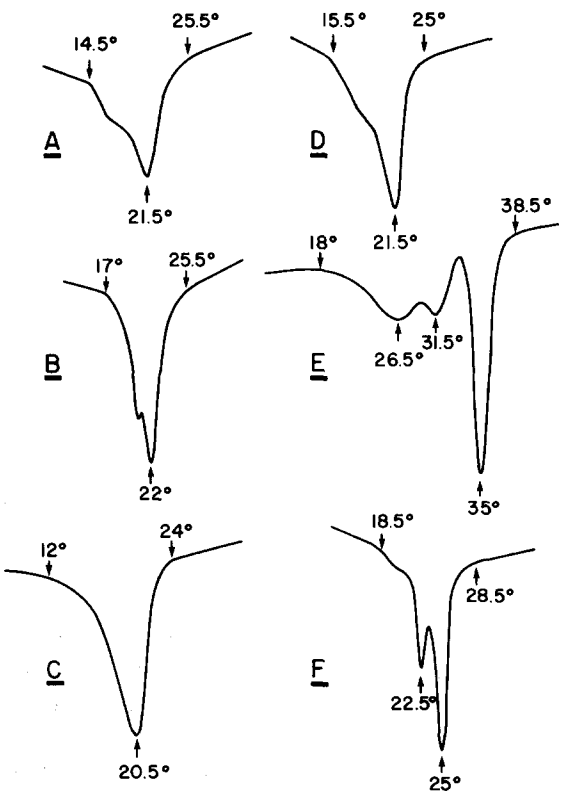

Fig. 4. Representative differential thermograms for various pure isopalmitate-homogeneous lipids and lipid mixtures from *A. laidlawii* B membranes. The lipid fractions, which were isolated and analyzed as described in the text, are as follows: A, total lipids; B, total neutral glycolipids; C, total phosphate-containing lipids; D, phosphatidylglycerol; E, diglucosyl diacylglycerol; F, monoglucosyl diacylglycerol.

monoglucosyl diacylglycerol from isopalmitate-homogeneous membranes shows a completely reversible double transition at a temperature somewhat above that of the total lipid phase transition; a single slightly broader transition was seen with monoglucosyl diacylglycerol from elaidate-homogeneous membranes. The nature of the monoglucosyl diacylglycerol transitions is unclear, for Wieslander et al. [16] have reported X-ray evidence that monoglucosyl diacylglycerol exists in a nonlamellar (probably hexagonal) phase both above and below the temperature of the phase transition detected by ^2H_2O- and ω-d_3-palmitic acid ^2H nuclear magnetic resonance. As conventional hexagonal lipid phases exist only with the lipid acyl chains in a liquid-crystalline and not a highly ordered (gel) state, it would thus be rather rash to attribute the monoglucosyl diacylglycerol phase transition to a melting of the acyl chains without further experimental evidence. Mixtures of monoglucosyl diacylglycerol with diglucosyl diacylglycerol exhibit a fully reversible phase transition, indicating that the complex thermotropic behaviour of isolated diglucosyl diacylglycerol is characteristic of this lipid only in the pure state. This result was obtained both with elaidate-homogeneous lipid extracts, in which diglucosyl diacylglycerol is the dominant neutral glycolipid, and with isopalmitate-homogeneous membranes, in which monoglycosyl diacylglycerol predominates. Results obtained with these and other preparations indicate that the total neutral glycolipids from fatty acid-homogeneous membranes exhibit phase transitions at temperatures near or somewhat (up to 10 degrees) above the total lipid transition midpoint temperature (T_{AL}), while the total phosphate-containing lipids exhibit phase transitions centered on or slightly below T_{AL}.

Thermotropic transitions of total lipids of fatty acid-homogeneous membranes

The production of membranes whose lipids are homogeneous in various fatty acyl chains will be most useful for membrane studies if the lipid physical properties (e.g., the phase transition temperature) can be widely manipulated in such membranes without sacrificing good cell culture yields, and if the various membrane lipid components behave sufficiently similarly that the bulk membrane lipid phase can be treated to a (reasonable) first approximation as homogeneous. To study these points, we cultured *A. laidlawii* B with all of the Class I fatty acids in the presence of avidin, extracted and purified the total membrane lipids and monitored the thermotropic behaviour of the hydrated lipids by differential thermal analysis. A representative set of differential thermograms for the total membrane lipids of cells grown with various fatty acids in the presence or absence of avidin is shown in Fig. 5. The most dramatic feature of the endotherms for fatty acid-homogeneous membrane lipids is their greater sharpness in comparison with the transition endotherms for the lipids of cells grown without avidin. We have previously reported a similar result using a more limited number of fatty acids and a less potent antilipogenic agent with *A. laidlawii* B [17]. Integration of differential thermograms such as those shown in Fig. 5 allows us to calculate the approximate fraction of the total lipids in the gel and liquid-crystalline states at any given temperature, although this calculation will not be rigorously correct if the lipid components undergoing the transition at higher temperatures have different molar heats of transi-

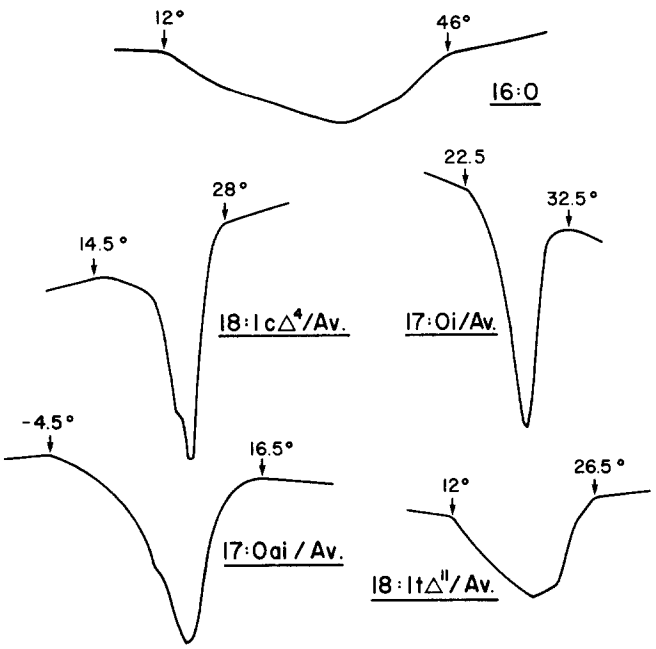

Fig. 5. Differential thermograms for preparations of total *A. laidlawii* B membrane lipids made homogeneous in various Class I fatty acids. Details of lipid preparation and analysis are described in the text. Fatty acid supplements are represented by the shorthand nomenclature described in the footnote to Table I, and the letters Av. indicate that the cells from which the lipids were derived were grown in the presence of avidin.

tion from those passing through the transition at lower temperatures. We have used such calculations to assign the phase transition midpoint, T_{AL}, and the temperature range required for the transition to pass from 10% to 90% of completion, $\delta T(10 \rightarrow 90)$, for the measured phase transitions of the lipids from membranes made homogeneous in each of the Class I fatty acids. The results of these calculations are summarized in Table III, from which two points are evident. First, it is possible to vary the T_{AL} value for fatty acid-homogeneous membrane lipids over a range of more than 50 degrees, from well below the freezing point of water to slightly above the normal *A. laidlawii* B optimum growth temperature of 36°C, by choosing appropriate Class I fatty acid supplements. Secondly, it is possible to increase the sharpness of the lipid phase transition by more than 4-fold over control values by growing *A. laidlawii* B with avidin and certain branched-chain fatty acids, and by at least 2-fold over control values by using avidin plus any of a variety of fatty acids of widely varying structures. By selecting the proper growth supplements, it is thus possible to obtain biological membranes exhibiting sharp lipid phase transitions ($\delta T(10 \rightarrow 90)$<5—8 degrees) in a physiological range of temperatures (10—40°C), a fact which should make this system a very useful one for studying membrane lipid phase changes and their effects on membrane functions.

Our ability to obtain membrane lipids whose acyl chains consist essentially entirely of any of a number of single acyl species affords a unique opportunity to carefully compare the effects of acyl chain structure on the gel-to-liquid-

TABLE III

MIDPOINT TEMPERATURES (T_{AL}) AND TRANSITION WIDTHS FOR THE GEL-TO-LIQUID-CRYSTALLINE PHASE TRANSITION OF TOTAL *A. LAIDLAWII* B MEMBRANE LIPIDS MADE HOMOGENOUS IN VARIOUS FATTY ACIDS

$\delta T(10\rightarrow90)$ is defined as the temperature range required for the transition to pass from 10% to 90% at completion.

Fatty acid *	% of total fatty acids	T_{AL} (°C)	$\delta T(10\rightarrow90)$ (degrees)
15:0	92	36.7	10.6
14:0*i*	96	10.1	5.1
15:0*i*	98	14.8	9.0
16:0*i*	99	21.8	6.1
17:0*i*	98	28.8	4.8
(±)14:0*ai*	95	−14.9	7.6
(±)15:0*ai*	95	−4.7	8.3
(±)16:0*ai*	100	4.1	11.1
(±)17:0*ai*	96	8.2	9.4
(±)18:0*ai*	99	21.8	6.1
16:1*t*Δ^9	98	6.7	8.9
18:1*t*Δ^6	96	22.2	11.6
18:1*t*Δ^9	98	20.1	10.4
18:1*c*Δ^{11}	99	20.0	8.4
18:1*c*Δ^4	91 **	24.1	5.0
18:1*c*Δ^{11}	96	−8.3	27.1
18:1*c*Δ^{15}	91	27.6	8.9
19:*cp*,*t*Δ^9	97	23.8	10.9
16:0 (− avidin)	67	31.2	17.8
18:1*t*Δ^9(−avidin)	72	32.7	12.9
16:0*i* (−avidin)	76	25.8	11.2

* See footnote to Table I.

** These fatty acids were only about 93% pure as they were added to the culture medium.

crystalline phase transition in the heterogeneous mixture of lipids found in a biological membrane with the corresponding effects of the acyl chains on the phase transition in a simpler model membrane system, the hydrated diacyl phosphatidylcholines. We have recently synthesized a wide variety of novel diacyl phosphatidylcholines, including the phosphatidylcholine derivatives of all Class I fatty acids save petroselaidic acid, and we have determined the transition temperatures, T_c, for these lipids in their fully hydrated state [18—21]. We have plotted the transition midpoint temperature, T_{AL}, for *A. laidlawii* B lipids homogeneous in various fatty acids vs. the T_c values of the corresponding diacyl phosphatidylcholines (plot not shown). An excellent correlation ($r = 0.94$) is seen between T_{AL} and T_c, indicating that the same fatty acid structural features determine the phase transition temperatures in the two systems. The very strong correlation of T_{AL} and T_c also suggests that variations in the lipid head-group distribution in *A. laidlawii* B membranes made homogeneous in various Class I fatty acids do not have a major effect on the total lipid T_{AL} values. However, since the slope of the regression line relating T_{AL} and T_c is significantly different from unity (T_c varies somewhat more widely than T_{AL}), it seems that the nature of the lipid head-group(s) (and possibly the heterogeneity) of a given lipid system can influence the magnitude of the

shift in the lipid phase transition temperature which results when the fatty acyl composition is changed. Consideration of the published T_c values for a series of diacyl phosphatidylcholines and the corresponding phosphatidylethanolamines [23] leads to a similar conclusion. Therefore, while it would seem to be possible to make predictions regarding the direction of the shift in the gel-to-liquid-crystalline transition temperature of any given membrane lipid system when its fatty acid composition is altered, predictions of the magnitude of the shift would require a consideration of the lipid head-group composition as well.

Discussion

The results of our compositional studies indicate that while avidin itself does not cause any significant changes in the membrane composition of *A. laidlawii* B, the nature of the fatty acid used together with avidin as a growth supplement can significantly affect the lipid/protein ratio and the lipid head-group distribution. The former effect has been noted previously in cultures of this organism grown with various fatty acids but without avidin [2], but the lipid head-group distribution has been previously reported to be essentially invariant with changes in the fatty acid composition of cells grown under these conditions [22]. This latter study, however, used only straight-chain fatty acids as growth supplements, and different fatty acids of this type cause relatively small changes in the lipid head-group distribution even when the lipids are made fatty acid-homogeneous (see Table II). The major influence of exogenous fatty acids on the distribution of the lipid head groups is exerted on the ratio of the two major neutral glycolipids, monoglucosyl diacylglycerol and diglucosyl diacylglycerol. This result is in agreement with the findings of Wieslander and Rilfors [11] for the related A strain of *A. laidlawii*. However, the observed variation of the monoglucosyl diacylglycerol/diglucosyl diacylglycerol ratio with the fatty acid composition of the membranes of *A. laidlawii* B is not consistent with the proposal of these workers that this ratio is primarily a function of lipid fluidity. For example, *cis*-vaccenate- and *cis*-4-octadecenoate-homogeneous membranes have a very similar monoglucosyl diacylglycerol/diglucosyl diacylglycerol ratio, yet their lipid phase transition midpoint temperatures differ considerably, while anteisopentadecanoate-homogeneous membranes, with a transition midpoint very near that of *cis*-vaccenate-homogeneous membranes, have a nearly 10-fold higher monoglucosyl diacylglycerol/diglucosyl diacylglycerol ratio.

How are fatty acid-dependent changes in membrane composition in aspects other than lipid acyl chains likely to affect the membrane lipid physical properties? Our differential thermal analysis studies of the thermotropic behaviour of various membrane components suggest that changes in the levels of carotenoids or divalent cations have little if any effect on the lipid phase transition, and that membrane proteins do not appreciably influence the thermotropic behaviour of the bulk lipid phase. However, as a certain fraction of the membrane lipid is directly associated with membrane protein [24] and does not take part in the bulk lipid phase transition [12], variations in the lipid/protein ratio induced by different fatty acid supplements will result in changes in the ratio of 'boundary' to 'free' (bulk) lipid, a fact which may be of significance in

the design or interpretation of certain types of experiments. Since the molecular weight distribution of membrane proteins was found to be nearly unchanged by avidin and fatty acid supplementation of *A. laidlawii* B, no qualitative changes in the nature of lipid-protein interactions in the membrane are likely to occur when cells are made homogeneous in different fatty acids. Fatty acid-dependent variations in the lipid head-group (especially the glycolipid) distribution are likewise unlikely to cause changes of a qualitative nature in the overall behaviour of the membrane lipids. In general, the rather complex thermotropic behaviour exhibited by certain single fatty acid-homogeneous lipid species reduces to a rather simple behaviour when mixtures of the various species are examined. However, because pure monoglucosyl diacylglycerol can form hexagonal phases in excess water, it is possible that membranes with a high monoglucosyl diacylglycerol/diglucosyl diacylglycerol ratio might show an enhancement in the rates of certain processes (such as lipid 'flip-flop' or membrane fusion) which are thought to proceed through the transient formation of small domains of lipid in a hexagonal phase [25,26]. As well, because the different lipid fractions from a given fatty acid-homogeneous membrane exhibit thermotropic phase transitions at different temperatures, fatty acid-dependent changes in the lipid head-group distribution can potentially alter the membrane phase transition midpoint temperature, T_{AL}, from that which would be observed if the head-group composition was invariant. However, in view of the very good correlation of the T_{AL} values for various fatty acid-homogeneous membrane lipid samples from *A. laidlawii* B and the T_c values for the corresponding phosphatidylcholines, the effect of lipid head-group variation (which bears no discernible relationship to T_{AL}) on the total *A. laidlawii* lipid T_{AL} value appears to be small. In general, we conclude that while the fatty acid-dependent variations observed in the lipid head-group and protein compositions of fatty acid-homogeneous membranes from *A. laidlawii* B should not be ignored in designing or interpreting experiments using this membrane system, the variations observed are unlikely to cause changes of a qualitative nature in the lipid-lipid or lipid-protein interactions in the membrane. If it is desirable to maintain a constant membrane lipid head-group or protein composition while varying the lipid acyl chains, it should be possible in many cases to select a set of Class I fatty acids which will give substantial variations in the membrane lipid properties yet maintain a fairly constant lipid head-group and/or protein composition.

Measurements of the thermotropic phase transitions of the total lipids from various fatty acid-homogeneous *A. laidlawii* B membranes leads us to two conclusions. First, it is possible to vary the lipid gel-to-liquid-crystalline transition midpoint, T_{AL}, and presumably the lipid fluidity as well, very widely in fatty acid-homogeneous membranes by growing *A. laidlawii* B in the presence of avidin plus appropriate Class I fatty acids. This finding supports previous suggestions [6] that lipid physical properties need not be rigorously maintained within narrow limits in order to support proper membrane function (and cell growth) in this organism. Secondly, the lipids of fatty acid-homogeneous membranes exhibit a considerably sharper lipid phase transition than do the lipids of cells grown without avidin and with or without an exogenous fatty acid. The enhanced phase transition sharpness in fatty acid-homogeneous

membanes suggests that the different lipid species in such membranes more reasonably approximate a homogeneous lipid pool than do the lipids in fatty acid-heterogeneous cells. In some cases, the transition widths for the total membrane lipids of fatty acid-homogeneous cells approach those observed by differential thermal analysis for pure diacyl phosphatidylcholines (which generally require 1—2 degrees for passage from 10% to 90% of completion of their phase transitions under the conditions we used), suggesting that the various lipid species in fatty acid-homogeneous *A. laidlawii* B membranes mix very well with one another. It is possible, by appropriate choices of Class I fatty acid supplements, to vary the sharpness and the midpoint temperature of the membrane phase transition independently over very wide limits in our system. This high degree of manipulability of the characteristics of the membrane lipid phase transition offers an excellent opportunity to study the effects of lipid phase changes on membrane function. We have recently exploited this potential to elucidate the physical basis of the temperature dependence of the membrane-bound ($Na^+ + Mg^{2+}$)-ATPase activity (Silvius, J.R. and McElhaney, R.N., unpublished results.) in a manner that was not previously possible. When these possibilities are considered along with the rather specific nature of the membrane compositional modifications that can be achieved, and the immensely reduced number of individual lipid species that are present in fatty acid-homogeneous as compared to normal *A. laidlawii* B membranes, it is clear that the fatty acid-homogeneous *A. laidlawii* B membrane can be a very useful one for studies of the physical properties of membrane lipids and their functional consequences in a native biological membrane preparation.

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